Jagesh K Tiwari, Poonam, Sapna Devi, Nilofer Ali, S K Luthra, R K Singh, Vinod Kumar, Vinay Bhardwaj and S K Chakrabarti

Interspecific somatic hybrids enriching potato gene pool

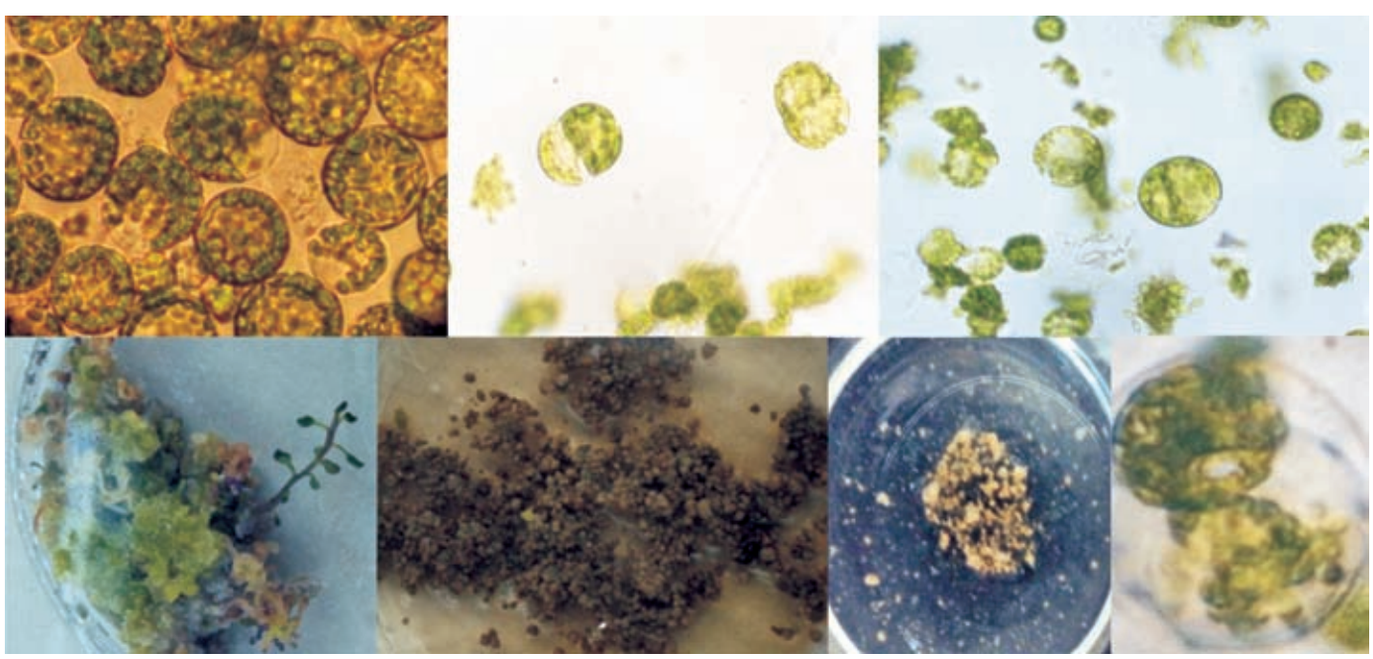
The scientists of our Shimla-based ICAR institute have produced interspecific potato somatic hybrids via protoplast fusion between cultivated tuber-bearing *Solanum tuberosum* dihaploid clone 'C-13' and non-tuberous diploid wild *S. etuberosum* species for resistance to potato virus Y (PVY). These somatic hybrids are tetraploid, male fertile, PVY resistant, having diverse cytoplasm type wider genetic base. Therefore, these somatic hybrids have immense potential for uses in potato breeding via conventional-cum-molecular breeding methods to widen the gene pool of cultivated potato by introgression of genes from wild *Solanum* species, says Dr S K Chakrabarti, Director, CPRI, Shimla.

POTATO is most popular non-cereal food crop of the world and third most important food crop worldwide after rice and wheat. Wild and cultivated species of potato have been effectively used in potato breeding but represent only a tiny fraction of available potato biodiversity. Utilization of wild diploid species has been remained untapped potential source for transferring resistant trait into common potato. *Solanum* species are widely distributed from south-western USA to central Argentina and Chile. This extensive geographical range has resulted in types adapted to a broad range of climatic and soil conditions. In the course of evolution, these plants have also developed resistance/tolerance to different pathogens and pests.

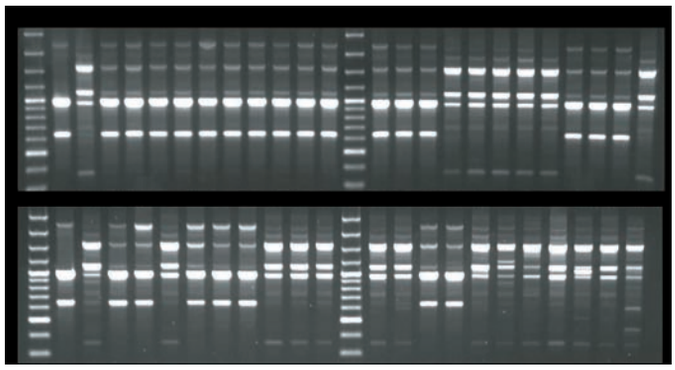
Important wild tuber-bearing 1 endosperm balance number (EBN) diploid species such as *S. capsicibaccatum* (CGN 22388), *S. circaefolium* subsp. *quimense* (CGN 18158), *S. huancabambense* (CGN 17719), *S. jamesii* (CGN 18349), *S. polyadenium* (CGN 17749), *S. pinnatisectum* (CGN 17743; CGN 23011) possess resistance to late blight. This trait is particularly attractive to breeders to widen the potato genetic base, but the barrier between the cultivated potato and many wild species has proved a difficult task, even when unconventional crossing methods are used.

Interspecific Somatic Hybrids

Many useful genes derived from wild sources cannot be transferred through conventional technique because of sexual incompatibilities are primarily due to difference in ploidy and endosperm balance number (EBN). It is extremely difficult to cross 1 EBN wild species directly with common cultivated 4 EBN potato. Limited success has been obtained by utilizing bridging species but incompatibility of 1 EBN wild species has generally prevented the use of this particularly valuable trait.



Technique of symmetric protoplast fusion in potato



RAPD profiles generated by primer OPAC-13 on 1.6% agarose gel. M = 100 bp ladder, P_1 (Parent 1) = C-13, P_2 (Parent 2) = *S. etuberosum*, P_1+P_2 = pooled parental DNA, clones (No. 1-12, 18-20, 22, 24-26, 32, 33) are confirmed as somatic hybrid, whereas, clones (No. 13-17, 21, 23, 27-31, 34-40) are not somatic hybrids.

However, modern research and new techniques have made it possible to expand considerably the genetic resources available for use in breeding programmes. A few methods have now become available to overcome this problem.

These methods include manipulation of ploidy and endosperm balance number (EBN), bridge crosses, mentor pollination and embryo rescue, hormone treatment, and reciprocal crosses. Among all, somatic hybridization, which removes prezygotic and some postzygotic barriers, can likewise surmount the barrier between cultivated and wild species. Somatic hybridization can provide a means of bypassing sexual incompatibility between *Solanum* species, leading to fertile plants that can be used directly in breeding programs.

Somatic hybridization generates functional combinations of large sets of genetic material, which makes it similar to sexual hybridization. This method can also be used to overcome limitations of genetic transformation. Many of the important traits are predominantly polygenic such as late blight resistance and thus unavailable as isolated and characterized sequences that are ready for genetic transformation. Therefore, efficient methods of transformation are yet to be available for multiple genes that are expressed in a coordinated manner. On the other hand, somatic hybrids obtained directly after fusion contain all organelles from the cytoplasm of both parents.

Application of Technology

Globally, symmetric protoplast fusion approaches involving diploid *Solanum* species in combination with

Somatic Hybridization

Somatic hybridization via protoplast isolation, electrofusion and regeneration is a useful tool to transfer polygenic traits such as late blight resistance in a single step. It enables a development of tetraploid somatic hybrid between diploid wild species and dihaploid of common potato. As a result, tetraploid somatic hybrids may be utilized in conventional breeding for late blight resistance and improvement of other traits. Thus, production of somatic hybrids between tetraploid 4EBN *S. tuberosum* and diploid 1EBN wild species has been envisaged for imparting durable resistance to late blight. In consequence, aim of this somatic fusion technology is creditable to enrich the cultivated potato gene pool by incorporating genes from a new exotic wild species, in order to enhance resistance to late blight disease.

C-13 | *S. etuberosum* | *Somatic hybrid (C-13+S. etuberosum)*

Phenotypic appearance of plants of somatic hybrid and their fusion parents (C-13 and *S. etuberosum*)

C-13 *S. etuberosum* Somatic hybrid

Morphology (leaf, flower and tubers) of somatic hybrid (clone E1-3) and their fusion parents (C-13 and *S. etuberosum*) (Somatic hybrid E1-3 is registered with NBPGR Reg. No. INGR 11050).

dihaploid *S. tuberosum* have been essentially used to develop somatic hybrids potato having desirable introgression from wild relatives. Application of this technology has been observed widely for the production of multiple resistant somatic hybrids.

Somatic Hybrids

The somatic hybrids *S. tuberosum* dihaploid 'C-13' (+) *S. etuberosum* are tetraploid, male fertile, and having resistance for potato virus Y. Somatic hybrids were characterized for hybridity by molecular markers (RAPD, SSR, and cytoplasm (chloroplast/mitochondrial genomes)), ploidy analysis by flow cytometry, cytology and phenotypic characterization. These somatic hybrids have diverse genetic base based on cytoplasm types consisted predominantly of T-, W-, and C, with a few A-and S-types chloroplast. One potential clone, E1-3 (*INGR 11050*), has been registered with NBPGR, New Delhi. Though elongated type tubers of somatic hybrids are not desirable appearance type because one of the fusion parents was non-tuberous *S. etuberosum*, a donor parent for PVY resistance and another was recipient tuber bearing C-13. However, male fertility of somatic hybrids has added advantages besides wider genetic base to be used in breeding as male parents.

First Ever Success

In India, to the best of our knowledge, no other research group/institution in India is presently working on somatic hybridization. And this is first ever successfully developed interspecific somatic hybrid between *Solanum tuberosum* dihaploid 'C-13' and non-tuberous diploid wild species *Solanum etuberosum* for Potato Virus Y (PVY) resistance. Somatic hybrids were produced following optimized protocol of protoplast isolation, electrofusion and regeneration of post-fusion products.

For further information contact:
Drs Jagesh Kumar Tiwari, Poonam, Sapna Devi, Nilofer Ali, S K tuthra, R K Singh, Vinod Kumar and **Vinay Bhardwaj** (Scientists), **Dr S K Chakrabarti** (Director), Central Potato Research Institute, Shimla 171 001, Himachal Pradesh, India. *E-mail:* jageshtiwari@gmail.com