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APPLICATION OF BIOTECHNOLOGY FOR CONSERVATION AND UTILISATION OF ANDEAN ROOT AND TUBER CROPS AND BIOSAFETY GUIDELINES

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ABSTRACT

The International Potato Centre (CIP) recognises the value of biotechnology in the conservation and use of solanum potato and sweet potato germplasm. Biotechnological development and utilisation at CIP is currently at the transgenic plant production and gene mapping stages. CIP complies with international standards regarding biosafety guidelines to ensure biotechnology products without adverse environmental effects, and to prevent unintentional release of hazardous organisms. CIP preserves thousands of potato and sweet potato accessions and other Andean root and tuber crops *in-vitro*, a biotechnology technique, as opposed to risky field conditions. The paper highlights the value of the *in-vitro* technique in storing and distributing pathogen-tested clonal accessions of potato and sweet potato. Aspects of germplasm characterisation, genome modification and manipulation, and biosafety guidelines are discussed.

***Key Words*: Biosafety guidelines, gene mapping, germplasm, potato, transgenic crops**

RÉSUMÉ

Le Centre International de la Pomme de terre (CIP) reconnait l'importance de la biotechnologie dans la conservation et l'utilisation des germoplasmes de la pomme de terre et de la patate douce. Le développement biotechnologique et son utilisation au CIP est actuellement à l'étape de production de plantes transgéniques et de définition structurelle des gènes. Le CIP applique les standards internationaux en ce qui concerne les réglementations sur la biosécurité pour garantir des produits de la biotechnologie sans effets environnementaux adverses et pour prévenir la libération invonlontaire d'organismes dangereux. Le CIP conserve *in-vitro* des milliers d'accessions de pommes de terre et de patates douces et d'autres cultures andéenes à racines et à tubercules, une technique biotechnologique contraire aux conditions en champs à risque. Ce papier met l'accent sur l'importance de la technique *in-vitro* dans la conservation et la distribution d'accessions clonales de pommes de terre et de patates douces testées contre les pathogènes. Les aspects de caractérisation du germoplasme, de modification et manipulation du génome et les directives de la biosécurité sont discutés.

***Mots Clés*: Les directives de la biosécurité, germoplasme, définition structurelle des gènes, la pomme de terre, cultures transgéniques**

INTRODUCTION

The International Potato Centre (CIP) has become an internationally acknowledged centre of excellence through its more than 20 years of working to conserve and use potato and sweet potato germplasm. Recently, CIP has also assumed the responsibility for preserving genetic resources of other Andean root and tuber crops (ARTC). CIP's genetic resources have been used by

breeding programmes worldwide to provide national agricultural research systems (NARS) with sources of improved materials.

Biotechnology has simplified conservation and has expanded the possibilities for using CIP's germplasm. The biotechnological techniques used at CIP range from relatively simple procedures for conserving germplasm through alternative evaluation schemes for wild species, to more complex techniques such as developing transgenic plants and gene mapping.

CIP's policy statement summarises the need for biotechnological approaches and the guidelines that regulate their application in the institution thus: CIP is convinced that the application of biotechnology will have a direct beneficial impact on its crop improvement programmes, and on their contribution to improved welfare in developing countries. Therefore, CIP emphasises the application of appropriate biotechnological techniques in its research, training, and collaborative activities. In accordance with international standards, CIP's Biosafety Guidelines are designed to ensure that the products of biotechnology will not have adverse effects on the environment and agriculture, to prevent the unintentional release of hazardous organisms, and to protect the surrounding communities as well as employees and researchers involved in the use of such products.

GERMPLASM MAINTENANCE

Conservation. Maintaining germplasm in the field is expensive and risks the loss of germplasm due to infectious diseases or unfavourable climatic conditions. Biotechnology through *in vitro* methods offers a valuable alternative which helps to safeguard these genetic resources.

In CIP's case, clonal collections of potato, sweet potato, and other Andean roots and tuber crops are maintained both under field conditions and *in-vitro.* CIP has actively researched many *in-vitro* methods to effectively maintain its germplasm collections (Lizarraga *et al.*, 1989). Presently, CIP maintains under *in-vitro* conditions 5,566 accessions of potato, 4,641 of sweet potato, and 2,260 of other Andean root and tuber crops (olliico, oca, mashua, yacon). Maintaining these large *in-vitro* germplasm collections requires a long-term maintenance system. By using both growth restriction media and a reduced incubation temperature it is possible to maximise the interval between transfers (subcultures) *of in-vitro* plantlets. CIP can maintain potato germplasm for an average of two years between transfers by applying this system. Preliminary results have shown that it is possible to maintain in storage some sweet potato genotypes for one year. However, the focus of future assays is to apply long-term storage to the entire sweet potato collection (Espinoza *et al.*, 1992; Lizarraga *et al.*, 1992).

At CIP, using osmotic stress and a low temperature of 6°C for potato and 18°C for sweet potato appears to be the best and least costly way to maintain the germplasm collections in the long term.

Distribution. In addition to its important role in genetic resource storage, *in-vitro* methods play a major role in the international distribution of clonal genetic resources. Thermotherapy and meristem culture are being applied to potato and sweet potato germplasm to eradicate pathogens and produce *in-vitro* germplasm which can be defined as "virus free" or "pathogen tested", thus allowing the worldwide distribution of clonal material without quarantine risks. CIP has 746 "pathogen tested" clonal accessions of potato and 218 of sweet potato in its collection. These accessions are freely distributed to national seed and breeding programmes in the form of *in-vitro* plantlets or tubers.

GERMPLASM CHARACTERISATION

The evaluation and characterisation of germplasm collections facilitate the efficient use of genetic resources by breeding programmes and direct users. In the beginning, morphological markers were used to evaluate genotypes. However, when germplasm collections include several thousand accessions, applying these markers became a very difficult and slow process for evaluating accessions for their uniqueness, estimating their diversity, or eliminating redundant introductions.

Sensitive biochemical methods, are useful

tools for determining the genetic structure of a particular genotype. Methods such as isozyme analysis, RFLP, or random amplified polymorphic DNA (RAPD) can simplify this work and add efficiency to the breeder's work.

Genetic diversity can be effectively evaluated by analysing polymorphism in DNA. Analysis of DNA polymorphism by RFLP markers allows the assessment of a greater proportion of the total genome and can easily provide interpretable results. Another alternative for studying DNA polymorphism, RAPD, is now available because of the existence of the polimerase chain reaction (PCR). The most exciting use of RAPD and RFLP techniques is in the mapping of the polygenic source of variation (Klein-Lankhorst *et al.*, 1991).

The International Potato Centre (CIP) is using isozyme analysis for the characterisation of its germplasm. They are used to identify duplicate accessions in potato and sweet potato, estimate genetic variability and assemble a "core collection" of potato, and verify hybrids from germplasm enhancement research. RFLP and RAPD techniques will also be used to accelerate the fingerprinting of individual accessions and consequently to eliminate duplicate accessions in the germplasm collections.

GENOME MODIFICATION

The most convenient technique of genome modification and plant transformation is the vector system, in which the natural plant transformation capability of the soil bacterium, *Agrobacterium*, is used to introduce foreign genes into plants. The use of *Agrobacterium* to transform plants is determined by the presence of two regions: the virulence region (vir) and the t-DNA region on either the Ti or Ri plasmid. Several plant vectors have been developed from Ti or Ri plasmids of *Agrobacterium*. Host specificity is dependent both on the nature of the plasmid harboured by the vector bacteria and the plant chromosomal background (An *et al.*, 1986).

The transformation technique offers a good alternative for resolving some classical breeding problems, specifically in crops whose ploidy level does not facilitate the production of hybrids with desirable traits, and also in those cases where a natural source of desirable traits is not available. The International Potato Centre (CIP) is using these techniques to transfer into potato and sweet potato genomes those genes necessary to provide resistance to diseases and pests without modifying other clonal characters. In the case of potato, genetic engineering techniques are used to transfer coat protein genes of three viruses (PLRV, PVX, and PVY) to breeding lines for providing virus resistance. The introduction of quimeric genes to produce antibacterial proteins will improve bacterial disease resistance in potato (Janes *et al.*, 1987). *Bacillus thuringiensis* toxin genes (Vaeck *et al.*, 1987) are being incorporated into important potato varieties to aid in combating potato tuber moth.

In sweet potato, CIP has developed a simple transformation method using either *Agrobacterium tumefaciens* or *A. rhizogenes* containing a binary plasmid. By using this technique, foreign marker genes (Kanamycin resistance and GUS introngenes) have been successfully introduced and expressed in sweet potato genotypes. In order to combat sweet potato weevil, the most important sweet potato pest, CIP will use this technique to provide weevil resistance using cowpea trypsin inhibitor, or *Bacillus thuringiensis* genes.

GENOME MANIPULATION

Genome manipulation involves using the biotechnology techniques developed to facilitate enhancement efforts. Breeding programmes are applying genetic markers in basic studies of taxonomy, population variability, and gene mapping and introgression. The genetic markers (RFLPs and RAPDs), offer a powerful tool for establishing the relationship between plants and for augmenting classical breeding methods when used to transfer genes from wild germplasm. They provide an efficient means for the selection and introgression of desired genetic traits (Tanksley *et al.*, 1989; Debener *et al.*, 1990). By using RFLP and RAPD, it is possible to detect chromosomal fragments of exotic origin and estimate their size and map position; and they can help isolate genes of interest. The use of RFLP markers in the construction of genetic maps is another useful tool for breeders. These maps provide a more efficient method for selecting

desirable genes via their linkage to easily detectable RFLP markers (Beckmann and Soller, 1985).

The construction of a genetic linkage or RFLP map initially involves selecting important genotypes (parents), and producing a mapping population of an F_1, F_2, or backcross. Final scoring of this population by polymorphism screening is used in the linkage analysis.

The International Potato Centre (CIP) is using RFLP markers to map genes for resistance to bacterial and virus diseases. In future, RFLP techniques must be simplified and modified using RAPDs produced by a polymerase chain reaction. Finally, these techniques can be combined to survey useful genes, monitor the genes in introgression, and identify and synthesise genes into target potato and sweet potato genotypes by transformation or other gene transfer methods.

BIOSAFETY GUIDELINES

Biosafety guidelines were published by CIP in June 1993 and approved by the Peruvian Government on October 23, 1994 through Ministerial Resolution No. 0682-94-AG. CIP Biosafety issues are dealt with by a Committee that includes a representative from the Ministry of Agriculture in Peru, four CIP scientists and a representative from a leading institution having previous experience with biosafety issues. CIP guidelines have been developed in such a way that they can be applied to its laboratories, greenhouses and field facilities at headquarters and its regional stations. Distribution of genetically modified organisms from CIP headquarters to NARS are dealt with on a case-by-case basis. If distribution is approved, CIP will provide genetically modified organisms to NARS in a manner consistent with its general policy for distribution of germplasm, which ensures that CIP has thoroughly tested all materials released through its international system. This includes testing for agronomic value, true-to-type character, expression of particular genetic components, and biosafety parameters. NARS wishing to introduce any of the genetically modified organisms available at CIP should make a request through its Regional Representative.

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