JARQ 55 (SI), 405-418 (2021) https://www.jircas.go.jp

REVIEW

# Potato Germplasm Enhancement with Genetic Resources and Biotechnology Applications and Related Achievements in the Early Years of the International Potato Center (CIP)

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## Abstract

The aim of this review was to give an overview regarding key points of potato germplasm enhancement associated with the International Potato Center and its global research-for-development partners including feedback from technology and germplasm recipients. Highlights were addressed on the participation by Japanese scholars to CIP. Also, specific impacts were raised on the Japanese research contribution on potato research for technology application and development for the partner countries by co-working with key Japanese organizations. Special reference was made to research on genetic underpinnings and technology development for enhancing the introgression and incorporation of traits that were not available in the primary potato breeding pools. The use of genetic engineering was also judged and in particular for its potential to address global uses. Future outlook was also provided for hinting Japanese participation to the international agriculture.

**Discipline:** Biotechnology
**Additional key words:** breeding, ploidy manipulation, tuber-bearing Solanum species, 2n gametes

## Introduction

### 1. CIP beginnings

The International Potato Center (CIP, Centro Internacional de la Papa) was established in 1971 through a decree from the Government of Perú (https://cipotato.org/about/). Initial efforts on international potato improvement began in 1947 within the Mexican Agriculture Project (MAP) by the Government of Mexico in partnership with the Rockefeller Foundation, which provided the funding. This potato project was led by the late Dr. John S. Niederhauser with the aim to breed host plant resistance to *Phytophtora infestans,* the fungal pathogen causing late blight, which has been the main global problem on potato production (https://rockfound.rockarch.org/biographical/-/asset_publisher/6ygcKECNI1nb/content/john-s-niederhauser?inheritRedirect=false). As a precursor for CIP, MAP formed an Inter-American Potato Program in 1961, which evolved into the International Potato Improvement Program in 1966. This program was a co-organizer of CIP in 1971, whose founding Director General was the late Dr. Richard L. Sawyer. The MAP contributed both pathogen management and breeding resources for the subsequent research-for-development tasks CIP undertook. Although the South American Andes is both the center of origin and main center of diversity of potato, which underwent a complex evolutionary process (Hardigan et al. 2017), the *Solanum* genetic resources originated in Mexico also contributed to potato breeding and genetics research (Table 1). Many wild *Solanum* species from Mexico and its Meso-American neighbors are potential sources of genes for resistance to late blight –which remains the main global constraint for potato production. Some of these species were rated as resistance sources to late blight such as those diploid species in the tuberous *Solanum* series Bulbocastana and Cardiophylla (Graham & Dionne 1961).

The difficulty in interspecific crossing of the tetraploid potato cultigen pool with these species was

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Received 24 February 2021; Accepted 20 July 2021.

**Table 1. Examples regarding the contribution to potato breeding of *Solanum* genetic resources originated from Mexico and its neighbors.**

| Germplasm | Ploidy/EBN[1] | Trait | Contribution | Ref. |
|---|---|---|---|---|
| *Solanum bulbocastanum* | 2*x*/1 | Late blight resistance | Race specific genes (*Rpi*) added to potato germplasm | Racosy-Tican et al. (2020) |
| *Solanum demissum* | 6*x*/4 | Late blight resistance | Eleven race specific *R* genes used: Global contribution to resistance at an early stage of potato breeding | Malcolmson & Black (1966) |
| *Solanum jamesii* | 2*x*/1 | Drought stress tolerance | New sources of potato germplasm | Watanabe et al. (2011) |
| *Solanum stoloniferum* | 4*x*/2 | Potato virus Y resistance | Extreme resistance ($Ry_{sto}$) used in many cultivars worldwide | Ross (1986) |
| *Solanum tarnii* | 2*x*/1 | Potato virus Y resistance + late blight resistance | Combined resistances already in wild species and relevant expression of resistance traits | Thieme et al. (2008) |

[1] Endosperm Balance Number

mainly due to interploidy and genetic barriers. Now with a systematic approach based on the knowledge about reproductive biology, genetics and biotechnology tool applications, potato germplasm enhancement with these wild species could be facilitated (Racosy-Tican et al. 2020). Indeed, the durability of late blight resistance derived from wild species, mainly *S. demissum,* had supported potato production in the past (Grünwald et al. 2002). By using such materials, CIP further catalyzed the transfer of this germplasm into potato breeding programs through its global potato network (Ortiz et al. 1994). Earlier achievements can be read in reports by CIP (1996, 1998).

## 2. Evolving and enlarged mandate

With the increasing food security concern and local demands on specific crops under multilateral international discussions, CIP has been expanding its mandate from potato to sweet potato, and then to Andean root and tuber crops with an international genebank function on them. Likewise other CGIAR (formerly known as the Consultative Group for International Agricultural Research) Research Center, CIP also has its focus on the 2030 agenda for the Sustainable Development Goals (SDGs).

The key points on CIP's initial approach for organizing and operating elsewhere were: a small center facility investment for its headquarters in Peru, but with a broad networking worldwide wherever potatoes are grown with the secondment of CIP's staff for in hosting institutes in the developing world, and contract research with top notch potato institutes in the North, particularly from the US Land Grant University System.

## 3. Research networking

CIP enhanced the potato research community participation both in North America and Europe through The Potato Association of America (PAA, https://potatoassociation.org) and The European Association for Potato Research (EAPR, https://www.eapr.net). PAA's journal, as the token of the interest, keeps a Spanish version of the abstract as its requirement to encourage Latin American participation. CIP also urged complementary research tasks at various top notch potato research institutes in areas such as taxonomy, genetics, breeding, agronomy, and plant health management as well as human resources development for partner nations in the South.

Regional networking was set up to empower research-for-development cooperation through technology transfer, particularly of breeding materials and "seed" systems to ensure clean planting propagules. Examples are, *inter alia*, the Regional Cooperative Potato Program in Mexico, Central America, and the Caribbean (PRECODEPA), the African Potato Association or the Asian Potato Association. With the decrease of public funding from development investors at the turning point with the end of the Cold War at the beginning of 1990s, such regional cooperation and associations now face difficulty for continuing although the potato production interest remains due to food and nutrition security concerns. However, with both autonomy growth of nations and human resources empowerment, a network such as the Latin American Potato Association (ALAP, http://www.papaslatinas.org/?lang=en) has been becoming strong due to the capacity shown by participating potato program members, ALAP has research partnerships with the North. Among such a cooperative framework, significant achievements are noticed in breeding, genetics, germplasm and biotechnology for both CIP's clients and the global research community engaged in the genetic

enhancement of potato.

**4. Japan's contribution in brief**

CIP invited experts through bilateral research arrangements with different nations to enrich its capacity. Research staff from Japan were employed directly by CIP, as well as specialists were seconded from other organizations such as Japan International Cooperation Agency (JICA) and the Tropical Agricultural Research Center (TARC), which was reorganized in 1993 as the Japan International Research Center for Agricultural Sciences (JIRCAS). TARC scientists were seconded to CIP either in short- or long-term-assignments at CIP headquarters or its regional offices. Their expertise included plant pathology, genetic resources, anthropology, seed technology and breeding. Citizens of Japan were also involved in CIP governance by being members of its Board of Trustees. Collaborative shuttle research and joint expedition of genetic resources were implemented between CIP and research institutes from Japan, particularly after sweetpotato was included in CIP's mandate. The sweetpotato research community of Japan contributed their knowledge and germplasm with CIP's headquarters in Peru and its regional office in China.

**5. A case on multilateral partnerships with Japanese participation**

Indonesia is one of the countries in Southeast Asia that benefits from CIP. The most valuable merit is the utilization of a system for the multiplication and distribution of certified potato seed tubers, which is still running today. This system was developed in 1992 when the Government of Indonesia initiated a technical cooperation project with JICA in West Java to set up certified potato seeds system. CIP followed up with the continuation and further supported human resources development by relying the JICA settlement.

Potato genetic resources in the form of advanced tetraploid clones introduced from the CIP headquarters in Lima, Peru, also support potato breeding programs in Indonesia. Several selected clones have been used as parental clones in sexual crossing, whereas other advanced clones have been directly released as new cultivars following the regulations for variety release in Indonesia. Seed tuber system helped speeding up processes to distribute healthy seed tubers of these new cultivars. Voluntary funds from Japanese sources once supported Asian Potato Association to promote the exchange of experiences and potentially germplasm in the neighboring nations. LBR 40 (CIP 387164.4), which is a late blight resistant clone, has been intensively used as the key parental clone in late blight resistance breeding program for developing several new cultivars with late blight resistance or tolerance. Through the participatory breeding program in 2001–2003 (Basuki et al. 2005), the Indonesian Vegetable Research Institute (IVegRI) registered several cultivars in the 2005, namely, 'Tenggo', 'Balsa', 'Erika', 'Fries' and 'Krespo', which were previously known as advanced breeding clones CIP 390584.3, TS-2, I-1085, MF-II, and FBA-4, respectively.

**6. The focus of this review article**

Our aim is to highlight past achievements of CIP research and the subsequent utilization on the ensuing outputs related to potato. A review of recent CIP research has been given by Campos & Ortiz (2020). We prefer to focus herein on previous achievements related to potato genetic resources, genetics and breeding, which include various types of research-for-development partnerships.

## Potato Genetic Resources

Tuber-bearing *Solanum* consists of nearly 200 wild and cultivated species according to various views among taxonomists (Hawkes 1990). We use the classification by Hawkes (1990). While majority of the tuber-bearing *Solanum* are diploids, their ploidy levels range from diploid to hexaploid, having a wide ecogeographic distribution from Nebraska (USA) to the coastal edge of southern Chile. The South American Andes is the center of origin and main center of diversity for this crop. The cultigen pool includes diploid to pentaploid species, while most cultivars in the world are tetraploids. Wild species and landraces have been used as sources for host plant resistance to pathogens and pests, as well as tolerance to abiotic stresses (Machida-Hirano 2015). Major databases with such trait information, have been enriched through cooperation among CIP, USDA-ARS' NRSP-6 and European potato genebanks. We have been engaged in evaluating, capturing and manipulating the diversity available in these tuber-bearing species to obtain hybrids with the objective to transfer valuable traits to the potato cultigen pool.

The classification of potato species could be simplified pragmatically by assessing their crossability for further breeding use. Incompatibility, incongruity, male-sterility and hybrid death are noticed after crossing with potato wild relatives. Ploidy difference is key for the success on crossbreeding, but on the *Solanum* species, a genic factor known as the endosperm balance number (EBN) (Johnston et al. 1980, Carputo et al. 1999) also determines the success of hybrid seed production after intraspecific, interploidy or interspecific crossing (Ortiz & Ehlenfeldt 1992; Ehlenfeldt & Ortiz 1995). Male-sterility, self-incompatibility in diploids, occurrence of 2*n* gametes (which allows sexual polyploidization), haploidization

(particularly from tetraploids) and poor flowering in tetraploid cultivars with sterility are reproductive biology issues that assist potato germplasm enhancement with diverse genetic resources (Watanabe 2015).

## Tetraploid genetics, haploidation and 2*n* gametes

Comprehensive reviews were provided by Ortiz (1998, 2003) and Ortiz & Mihovilovich (2020). The focus of this article is on the integration of quantitative polyploid genetics and 2*n* gamete uses for trait transmission to the filial generations in tetraploid cultivar selection.

### 1. Tetraploid genetics

Most potato cultivars are tetraploids. Hence, key genetic and technical issues for potato breeding are polyploid genetics and ploidy manipulation with germplasm at different ploidy levels. The relation between potato breeding and polyploid genetics was explained by Ortiz and Watanabe (2004). The most important potato cultivars are tetraploids showing polysomic genetics. Tetrasomic teteraploids have more complex segregation than diploids even at a single locus, requiring 10 times larger population size to have a successful selection as indicated by Allard (1960). Potatoes are also highly heterozygous, thus the main challenges of potato breeding are estimating and predicting the selection outcome that may be affected by the complexity of tetrasomic genetics. The positive side of potato breeding is that the selected genetic combination can be conserved clonally and used as a cultivar without further progeny selection by backcrossing and fixation.

The quantitative genetics approach assisted effectively to improve traits with continuous variation in the potato cultigen pool at the tetraploid level (Mendoza & Haynes 1973, 1974). Also population improvement approach was once promoted together with the use of true potato seed as propagules (Thompson et al. 1983, Mendoza 1989). With such an approach, short-day adapted potato cultivars were used for developing long-day adapted potato germplasm through selection (Plaisted et al. 2019), which was further facilitated with the aid of DNA markers (Hosaka and Sanetomo 2020).

### 2. Haploids from tetraploids and diploid genetics for analytic breeding

Haploids are produced from tetraploid potatoes by crossing them with some diploid germplasm that acts as haploid inducers (Hermsen & Verdenius 1973; Hougas & Peloquin 1958). Germplasm enhancement with potato haploids was proposed (Hermundstad & Peloquin 1985) and further demonstrated extensively involving often research and breeding partnerships with CIP (Peloquin et al. 1989a,b, Jansky et al. 1990, Ortiz et al. 1993b).

There may be still controversy on who was the first to use the haploid concept, but without doubt at least two research groups contributed significantly to study and use potato haploids derived from the tetraploid cultigen pool, namely at the University of Wisconsin (Madison, USA) and at Wageningen University (The Netherlands). Their contributions to both potato genetics research and on capacity building through education and training of human resources are worth highlighting. CIP employed and catalyzed their research outputs and provided funding to capacity building, particularly through PhD fellowships in the US Land Grant University System.

### 3. Occurrence, genetics and use of 2*n* gametes

Analytic breeding can be made at the diploid to capitalize on its simpler genetics than that at the tetraploid level (Ortiz 2003). But the question stays how to transmit the useful traits, especially those being multigenic, to the tetraploid potato cultivars. With the evolutionary studies of polyploidization on potatoes, there are various research articles on the occurrence of 2*n* gametes over tuber-bearing Solanum species both on male and female gametes. Ortiz et al. (1994) gives the largest summary based on screening of cultigen pool and related wild species at the University of Wisconsin. Cytological mechanisms had been examined in microsporogenesis (Mok & Peloquin 1975a, Iwanaga & Peloquin 1982, Watanabe & Peloquin 1993) and megasporogenesis (Iwanaga & Peloquin 1980, Werner & Peloquin 1990, 1991) and both first division restitution (FDR) and second division restitution (SDR) mechanisms were observed across various species in the tuber-bearing *Solanum*. The genetic mechanism of 2*n* gamete formation was also examined by Mok & Peloquin (1975b). A single recessive gene control with the *ps* (*parallel spindles*) is regarded as the most important for producing 2*n* pollen during microsporogenesis. The gene frequency of the *ps* over tuber-bearing *Solanum* species is high (Iwanaga & Peloquin 1982, Watanabe & Peloquin 1989, 1991), and this enables to identify diploid breeding clones with 2*n* pollen to transfer valuable traits to the tetraploids by crossing them. The genetic mechanism related to *ps* is a FDR, so that 80% of the diploid genome can be transmitted without its reshuffling. The evaluation of the genetic consequence of 2*n* eggs could be more tedious than that for 2*n* pollen, but half-tetrad analysis facilitated it and led to identifying SDR as the most frequent (Werner et al. 1992).

Efficiency has been demonstrated on the use of 2*n* gametes following a 4*x*–2*x* crossing approach (Ortiz & Peloquin 1991a,b, 1994, Ortiz et al. 1991a,b, 1993a,b, 1994). The general occurrence of 2*n* gametes in the tuber-

bearing *Solanum*, strongly supports quantitative trait transmission efficiency after 4*x*–2*x* crossing (Watanabe et al. 1991). Obtaining a high frequency of even combined quantitative host plant resistances was further proven to be possible in tetraploid hybrid offspring (Watanabe et al. 1999a, Watanabe & Watanabe 2000, Watanabe 2015).

Overall, the innovation for novel potato breeding lies on the amalgamation of the knowledge over tetrasomic genetics, haploidization, germplasm enhancement at the diploid level, and transfer of traits by 2*n* gametes to the tetraploid cultigen pool. It is worth highlighting that academic research throughout the world facilitated significantly potato germplasm enhancement and with CIP as catalyst and disseminator of the knowledge and breeding materials. The research was undertaken competitively by diverse groups in the global potato research community, but we shall acknowledge the leadership of Campbell-Bascom Professor Dr. Stanley J. Peloquin, particularly in the theory and practice of ploidy manipulations for potato germplasm enhancement with haploids, wild species, 2*n* gametes and EBN (Ortiz et al. 2005, 2009).

## Examples of Germplasm Enhancement

Potatoes can be attacked by over 500 different taxa causing pests such as insects, nematodes, fungi, bacteria, viruses and other pathogens (Stevenson et al. 2001). On the other hand, potato wild relatives and exotic landraces are superb sources of host plant resistance against these pests and pathogens. Databases regarding screening for host plant resistance to these pests and pathogens are available in various potato genebanks. However, how would breeders make the choice of these sources of genetic variation? Any genetic enhancement approach should consider both breeding targets and end-user demands. It is also important to access to this germplasm, thus dealing with various issues such as, inter alia, ownership, quarantine, crossability, adaptation, segregation, or combining ability.

CIP and research-for-development partners made significant advances for incorporating a desired trait such as host plant resistance from wild species or landrace germplasm to the tetraploid cultigen through ploidy manipulation and related knowledge ensuing from research (Watanabe et al. 1995a, Table 2). CIP bred its own diploid breeding clones (Watanabe et al. 1994a, 1996a,b), as well as with research partner such as the University of Wisconsin-Madison (Watanabe et al. 1995b). The ideas and germplasm ensuing from this work innovated the potato crossbreeding elsewhere. For example, CIP pre-breeding germplasm –obtained after several years of painstaking work involving partners from Japan and USA that shared their diploid germplasm for crossing– was further used for developing potato cultigens, one of which was released in Burundi as a new red-skin potato cultivar named 'Nemared' due to its root knot nematode resistance and desired agronomic traits (Ortiz & Mihovilovich 2020, Figure 1). Table 3 lists some of the traits bred in CIP diploid breeding population.

Methods for using disomic tetraploid species such as *S. acaule* (Iwanaga et al. 1991, Watanabe et al.1992b, 1994b) and distantly related diploid species (Watanabe et al. 1995c) –based on EBN knowledge and simple embryo culture– also became available from research at CIP. With the advances, almost all species in tuber-bearing *Solanum* and some of non-tuber bearing taxa can be used through

**Table 2. Examples of incorporation of pest and disease resistance with potato genetic resources that can be transmitted effectively by 2*n* gametes to the tetraploid cultigen pool after breeding at the diploid level.**

| Pathogen/Pest | Popular name | Genetics/Mechanism | Gene | Ref. |
|---|---|---|---|---|
| *Globodera pallida* | White cyst nematodes | Quantitative<br>Race specific genes | *Grp* | Ortiz et al. (1997) |
| *Globodera rostochiensis* | Cyst nematodes | Race specific gene | *H1* | Scurrah et al. (1973) |
| *Meloidogyne incognita* | Root-knot nematode | Quantitative | | Watanabe et al. (1994a) |
| *Phthorimaea operculella* | Potato tuber moth | Quantitative | | Ortiz et al. (1990)<br>Watanabe et al. (1999c) |
| *Ralstonia solanacearum* | Bacterial wilt | Quantitative | | Watanabe et al. (1992a)<br>Watanabe et al. (1999a)<br>Watanabe & Watanabe (2000) |
| *Potato virus A* | PVA | Extreme resistance | *Ra* | Reviewed in Valkonen et al. (1996) |
| *Potato virus X* | PVX | Extreme resistance<br>Hypersensitivity | *Rx*<br>*Nx* | Reviewed in Valkonen et al. (1996) |
| *Potato virus Y* | PVY | Extreme resistance<br>Hypersensitivity | *Ry*<br>*Ny* | Reviewed in Valkonen et al. (1996) |
| *Potato leaf roll virus* | PLRV | Quantitative | | Valkonen et al. (1995b) |

**Table 3. Breeding of host plant resistance in CIP diploid breeding population and potential for transferring to the tetraploid cultigen pool**

| Disease or pest | Remarks | Source |
|---|---|---|
| Late blight | Haploids of tetraploid cultivar Atzimba (México) with 2*n* pollen due to synaptic mutants, which also provide a very efficient method of transferring diploid germplasm with target traits to the tetraploid cultigen pool | Iwanaga (1984) |
| Early blight | Additivity was the most important type of gene action for determining resistance to early blight at the diploid level, thus suggesting that host plant resistance can be used in 4*x*×2*x* crossings to obtain resistant tetraploid offspring to this fungal disease | Ortiz et al. (1993c) |
| Bacterial wilt | Resistance found in tuber-bearing *Solanum* species was bred into a diploid potato breeding population, which was further transmitted to the tetraploid cultigen pool through 4*x*×2*x* crossings | Ortiz et al. (1994)<br>Watanabe et al (1992a) |
| Potato tuber moth | Relatively simple inheritance resistance derived from *Solanum sparsipilum* transmitted, undiminished, into an advanced 2*x* population using simple phenotypic selection, which suggests that 4*x* × 2*x* crossings could be used to transfer the resistance to the tetraploid cultigen pool | Ortiz et al. (1990) |
| Cyst nematode | Resistance from *Solanum vernei, S. sparsipilum* and haploids of *S. tuberosum* group Andigena was introgressed into tetraploid cultigen pool using first division restitution (FDR) 2*n* gametes from diploid parents with similar breeding values as advanced resistant tetraploid clones that were bred after several cycles of selection against this pest | Ortiz et al. (1997) |
| Root-knot nematode | Resistance from *Solanum sparsipilum* and *S. chacoense* transmitted into an advanced diploid breeding population from which FDR 2*n* pollen producing clones were used to transfer this resistance to 18% of the tetraploid offspring after 4*x* × 2*x* crossings | Iwanaga et al. (1989) |
| *Potato virus X*<br>*Potato virus Y*<br>*Potato leaf roll virus* | Haploids derived from tetraploid potato breeding lines with virus resistance after pseudogametic parthenogenesis using 4*x* × 2*x* crossings. They were further used for breeding virus resistance in the diploid breeding population and obtaining 2*n* pollen producing diploid breeding clones for crossing with the tetraploid cultigen pool | Watanabe et al. (1994a) |

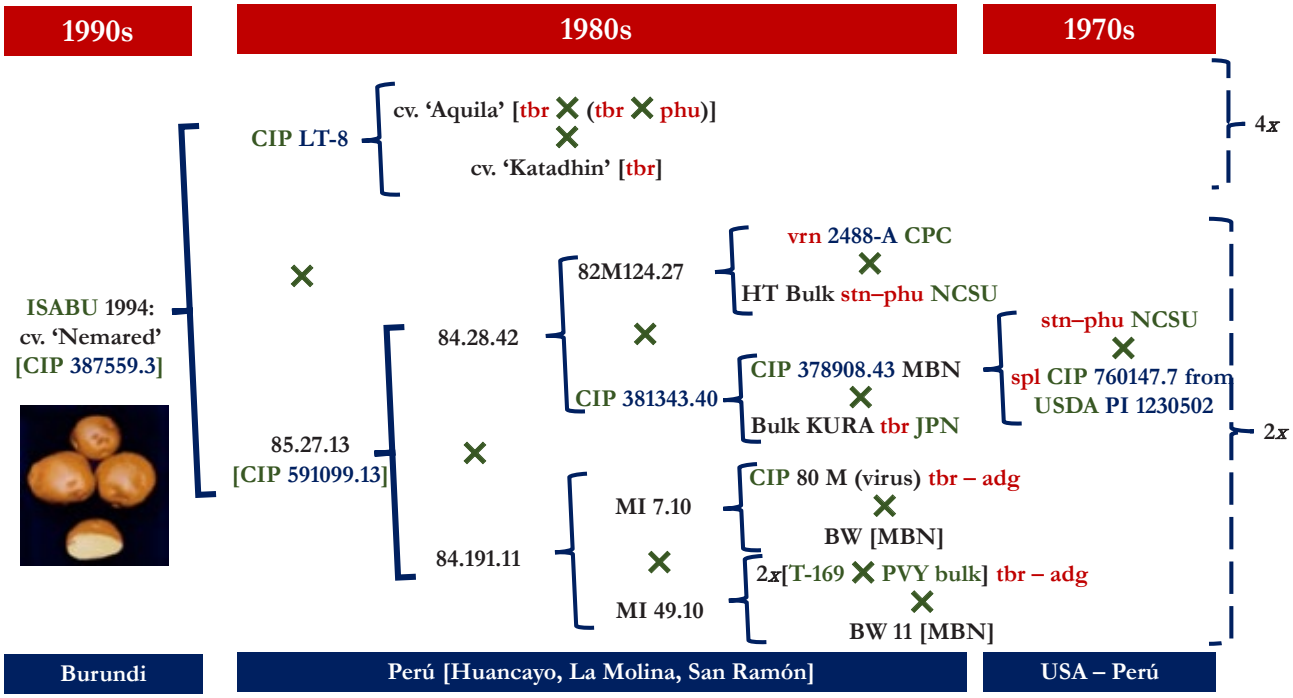


**Fig. 1. Long-term germplasm enhancement at diploid (2*x*) and tetraploid (4*x*) levels by the Internatinal Potato Center for developing the tetraploid cultivar 'Nemared', which was released by Institut des Sciences Agronomiques du Burundi (ISABU) in Burundi due to its high tuber yield, desired quality and host plant resistance to root knot nematode and other pests.**

crossbreeding in potato. These methods are also used in breeding programs elsewhere, especially in Europe.

## Biotechnology applications

### 1. Beginning

Both potato germplasm conservation and tuber "seed" production had been boosted with tissue culture. *In vitro*-based seed tuber production was spread worldwide by CIP, including the cleaning up of vegetative propagules through pathogen eradication. Likewise, mass tuber "seed" propagation was facilitated by micro-tuber technology. Many potato growers benefitted from mass propagation of tuber "seed" and sometimes used a farm kitchen to make media and culturing *in vitro* propagules in Asia and Latin America. The local potato industry came up with such a technology transfer at various nations (Watanabe et al. 1995d). Producing and mass-propagating clean planting material after tissue culture may be regarded among the initial CIP impacts. CIP continues supporting national potato programs for developing a healthy potato tuber "seed" industry and enhancing potato production with clean planting propagules.

### 2. Adoption of modern biotechnology

Biotechnology contributed to potato research in early phase on germplasm management, pathogen identification and breeding at CIP (Dodds & Watanabe 1990). Starting from late 1980s, CIP observed the development of genetic markers and genetic engineering and accessed both through research partnerships with Cornell University, Max Planck Institute for Plant Breeding Research, Wageningen University and the then Scottish Crop Research Institute –(today's The James Hutton Institute), among others (Watanabe et al. 1995d, 1997). Gradually, CIP incorporated DNA markers to assist genetic resources management and trait introgression or incorporation in its breeding populations (Watanabe 1994, Celebi-Toprak et al. 2005b, Ghislain & Douches 2020).

### 3. Genetic markers and genome-led breeding

Two distinctive groups had initiated and collaborated with CIP on developing baselines on potato nucleic acid-based markers, starting with genetic mapping using restriction fragment length polymorphisms (RFLP)) (Bonierbale et al. 1988, Gebhardt et al. 1989). Later on mapping paved the way for identifying DNA markers for further used as indirect selection aids in potato breeding (Gebhardt et al. 1991). The development of new DNA markers such as amplified fragment length polymorphisms (AFLP) (van Eck et al. 1995, Rouppe van der Voort et al. 1998) provide a further foundation for both genetic linkage research and breeding in potato. Thereafter simple sequence repeats (SSR) or microsatellites, sequence-related amplified polymorphisms (SRAP), conserved ortholog sets (COS) (Lindqvist-Kreuze et al. 2013), and single nucleotide polymorphisms (SNPs) became available (Watanabe 2015 and references therein).

The potato genome was decoded about 10 years ago (The Potato Genome Consortium 2011). This research enterprise was facilitated by using diploid potato haploid-species hybrids. The availability of the first draft genome allows identifying new genetic markers for potato breeding and research (Slater et al. 2014). Now, potato genetic research and breeding are further accelerated by genome-wide association analysis (GWAS) and genomic prediction of breeding values (GEBV) for selection (Klaassen et al. 2019, Ortiz 2020; Ortiz et al. 2020, Selga et al. 2021). Systematic diversity assessments of gene expression were undertaken using transcriptome analyses (Shan et al. 2013, Petek et al. 2020, Tiwari et al. 2020). These historical landmarks have been linked with CIP's in-house research and facilitated the potato breeding for global needs.

### 4. An example of international and interdisciplinary cooperation: *Potato virus Y* (PVY) extreme resistance gene $Ry_{adg}$ identification

This should be regarded as an example on a series of joint research on PVY resistance between CIP and its partners at an early time when genetic markers began becoming available to assist potato breeding. In the late 1980s, CIP initiated in-house research on nucleic acid-based genetic markers. Since the phenotypic evaluation of target traits was essential, there was a close interaction between geneticists, molecular biologists and virologists. The first author of this article (then at CIP), and partners at both Cornell University and University of Helsinki initiated germplasm assessments for virus resistance. We found new resistances sources for PVY (Valkonen et al. 1994, 1995b) and *Potato leaf roll virus* (PLRV) (Watanabe et al. 1994b, 1995c) in new genetic resources yet to be used in potato breeding. In addition, further resistance identification was achieved for *Cucumber mosaic virus* (Valkonen et al. 1995a) and *Potato mop-top virus* (Sandgren et al. 2002).

Based on the strong foundation of phenotypic evaluation, benchmark progress was made on identifying and refining DNA markers for the PVY resistance gene $Ry_{adg}$. An initial trial was based on RFLP markers (Hamalainen et al. 1997, 1998), and thereafter we moved to PCR-based cleaved amplified polymorphic sequence (CAPS) marker system (Sorri et al. 1999) to enable an easy selection and increase accuracy for validation (Shiranita et al. 1999). A PCR-based sequence characterized amplified

region (SCAR) marker was also developed for tagging *Ry*$_{adg}$ (Kasai et al. 2000, Watanabe et al. 2003), while further marker refinement continued by CIP (Herrera et al. 2018) and other research groups (Elison et al. 2020 and references therein). Although genomics-based marker systems following a whole genomic approach are possible presently, this early achievement in the development and use of DNA markers for potato genetic research and breeding deserves its highlighting.

**5. Genetic engineering**

CIP established in the late 1980s and early 1990s research partnerships with public institutions on genetic engineering to search for alternative genetic variation that was not available in potato germplasm held in its genebank or breeding populations. The main aim was to examine the potential of speeding up potato breeding by inserting target transgenes of interest into potato cultivars while avoiding large undesired genetic variation while doing crossbreeding. Through fast negotiations and implementation considering newly emerging concerns brought up by the Cartagena Protocol on Biosafety (CPB) of the Convention on Biological Diversity (CBD) since late 1995, simultaneously, the entire CGIAR placed attention on how to work with modern biotechnology, and especially for using a transgenic approach for plant breeding. After doing the technological feasibility assessment on different trait uses by genetic engineering (Dodds & Watanabe 1990, Watanabe et al. 1995d), CIP also had pursued an overall risk assessment and management (RA&RM) on transgenic potatoes with adverse effect to the environment with the deliberate release of such genetically engineered potatoes. Its collaborating partners had made field assessment independently, and they have provided key advice on the field assessment of potato in the center of origin of this crop (Celis et al. 2004). At present, there are approved transgenic potato for growing in USA, pending application approval in Japan, and proactive testing in the United Kingdom (Lazebnik et al. 2017), but it is yet to reach a consensus on how to apply RA&RM for decision making regarding transgenic potatoes. The first author of this article has been very proactive since 1996 to elucidate the environmental and food aspects of the risk assessments on transgenic plants including potatoes besides conducting trait incorporation and evaluation on transgenic potatoes (Iwaki et al. 2013, Shimazaki et al. 2016). Having such experience, we recommend that CIP takes one step forward to provide technical advice to its partner potato programs working regarding CBD's CPB.

**6. Example of molecular biology research contribution from Japan: Tuberization**

It is obvious that the main aim of growing potato crop is the harvest of its tubers. The mechanism of tuberization was fully elucidated after several years of research on this subject. Tuberization is influenced by multiple internal physiological factors and external conditions such as daylength and external application of stimulant chemicals (Jackson 1999). The tuberization factor was found to bind with florigen, which was originally thought to be a substance that induces flowering (Taoka et al. 2011). It also triggers tuberization (Navarro et al. 2011). Daylength signal is directly associated with tuberigen, and the transported signal from leaves leads to tuberization. Genetics of tuberigen could alter the tuberization under different daylengths (Teo et al. 2017). These recent findings could drastically support the control of tuber production under variable weather conditions and market requirements.

**7. More challenges on tolerance to abiotic stresses: anthropogenic climate change**

The main impacts of climate change on any crop production relate to abiotic stresses. They are overwhelming, and combinations of alternative solutions are essential to tackle their related production constraints in agriculture. Research on plant stress responses could facilitate sorting out potential technology applications as well as plant breeding. Abiotic stress responses in plants are complex, and many pathways are possible with the same type of stress (Hancock et al. 2014). Environmental stresses alter whole metabolic pathways, thus using transcriptomic, proteomic and metabolomic research is relevant (Batista et al. 2017), especially for investigating secondary metabolites (Yang et al. 2018). Potato genetic resources with diverse abiotic stress tolerance provide means for both understanding mechanisms and their further use in breeding (Watanabe et al. 2011, Handayani et al. 2019). Moreover, stress response research can contribute to host plant resistance management such as noticed on bacterial wilt in potato (Watanabe et al. 1999b), which calls for an integrated production management approach for this crop under a changing climate.

While it may be tedious to address abiotic stress tolerance with an orthodox approach (Handayani & Watanabe 2020), transgenic breeding can improve promptly the potato crop. There has been an accumulation of knowledge across multiomics platforms, especially for gene expression on potato (Resink et al. 2005, Gong et al. 2015, Pieczynski et al. 2018). Altering these gene functions using a genetic engineering approach can generate potato cultivars that are tolerant to unfavorable environments prone to abiotic stress(es) (Table 4).

**Table 4. Genes enhancing abiotic stress tolerance in potatoes. (After Handayani et al. 2019)**

| Abiotic stress | Gene | Source | Function | References |
|---|---|---|---|---|
| Heat | *nsLTP1* | *Solanum tuberosum* | Enhance cell membrane integrity under stress conditions<br>Enhance activation of antioxidative defense mechanisms<br>Regulate expression of stress-related genes | Gangadhar et al. (2016) |
| | *HSP17.7* | *Daucus carota* | Improve membrane stability | Ahn & Zimmerman (2006) |
| | *CuZnSOD; APX; NDPK2* | *Manihot esculenta, Pisum sativum, Arabidopsis thaliana* | Increase levels of the anti-oxidants superoxide dismutase, ascorbate peroxidase and catalase, which are responsible for ROS scavenging | Kim et al. (2010) |
| | *CBF3* | *Arabidopsis thaliana* | Induce expression of genes involved in photosynthesis activities and antioxidant defense | Dou et al. (2014) |
| Drought | *CBF1* | *Arabidopsis thaliana* | Modulate the abiotic stress-responsive genes expression, maintain high photosynthetic activity | Storani et al. (2015) |
| | *DREB 1B* | *Arabidopsis thaliana* | Preserve cell water content | Movahedi et al. (2012) |
| | *BZ1* | *Capsicum annuum* | ABA-sensitive stomata closure and reduce water loss, up-regulate stress related genes | Moon et al. (2015) |
| | *MYB1R-1* | *Solanum tuberosum* | Reduce water loss<br>transcription factor involved in drought-related genes activation | Shin et al. (2011) |
| | *BADH* | *Spinacia oleracea* | Membrane stabilization | Zhang et al. (2011) |
| | *DHAR1* | *Arabidopsis thaliana* | Maintain membrane integrity, protecting chlorophyll against degradation, allowing faster removal of $H_2O_2$ | Eltayeb et al. (2011) |
| | *codA* | *Arthrobacter globiformis* | Maintain the osmotic equilibrium of cells by inducing glycine betaine production as osmoregulator | Cheng et al. (2013a) |
| Salinity | *DREB1* | *Solanum tuberosum* | Activate stress-inducible genes, accumulate proline osmoprotectant | Behnam et al. (2006)<br>Bouaziz et al. (2013) |
| | *DREB1A* | *Arabidopsis thaliana* | Transcription factor involved in abiotic stress-related genes activation | Celebi-Toprak et al. (2005a),<br>Shimazaki et al. (2016) |
| | *MYB1* | *Ipomoea batatas* | Regulate the metabolism of secondary metabolites | Cheng et al. (2013b) |
| | *SOD; APX* | *Potentilla atrosanguinea, Rheum australe* | Enhance lignin deposition and scavenging capacity | Shafi et al. (2017) |
| | *BADH* | *Spinacia oleracea* | Membrane stabilization | Zhang et al. (2011) |
| | *NHX1* | *Arabidopsis thaliana* | Enhance the capacity of vacuolar compartmentation of extra $Na^+$ | Wang et al. (2013) |
| | *DHAR1* | *Arabidopsis thaliana* | Membrane integrity, protect chlorophyll against degradation, allowing faster removal of $H_2O_2$ | Eltayeb et al. (2011) |
| Freeze | *DREB1A* | *Arabidopsis thaliana* | Transcription factor involved in abiotic stress-related genes activation | Behnam et al. (2007)<br>Kikuchi et al. (2015) |
| Combined stresses | *DREB1A* | *Arabidopsis thaliana* | Transcription factor involved in abiotic stress-related genes activation | Watanabe et al. (2011) |

The first author and his research team have been collaborating with JIRCAS on testing transcription factors such as *DREB1A* and *rd29* promoters from *Arabidopsis*. This research found that single copy of the gene in tetraploid potato increased salt stress tolerance (Celebi-Toprak et al. 2005a). Likewise, freezing tolerance and multiple stress tolerance to both salt and freezing could be obtained with the same gene insertion (Behnam et al. 2006, 2007). Furthermore previously selected transgenic tetraploid potatoes showed dehydration stress tolerance (Huynh et al. 2014). Table 4 provides more examples on abiotic stress tolerance in potato through genetic engineering. Overall, this transgenic approach could significantly improve the stress responses, thus bringing increased tolerance in potato. Field assessments are necessary under different environments to determine its potential in potato breeding.

These testing experiments provide the foundation for future breeding application. While environmental biosafety risk assessment processes are ”hurdles” to overcome under any national legal regimes along with general social acceptance, the genetic engineering approach for potato breeding should not be ignored due to its potential for altering genetic variation of traits that are

not available.in the cultigen pool or its wild relatives, and for shortening the breeding process.

## Conclusion

After reflecting on our research and showing as evidence the ensuing publications, some of which are cited in this review article, we think that a proactive use of *Solanum* genetic resources along with genetic understanding allows potato germplasm enhancement for further breeding with other biotechnology applications. We recognize, however, that with the healthy cooperation through research partnerships in science, the overall potato research community could act effectively using these and other findings for increasing sustainably potato production worldwide. CIP has been, as noted herein, both the facilitator and catalyst for disseminating research findings through both knowledge and germplasm sharing with the global potato research community and other end users. At the earlier time of CIP, Japanese researchers and technical experts had participated or collaborated with CIP with a diversity of research and technical disciplines. Eminent interaction has not been seen for a decade, while potato academic research and breeding is active in Japan. More independent capacity allows Japanese potato research community run in a bilateral way with the partner research groups and nations without CIP. As seen in the past, the multilateral partnerships could proceed competitive, but productive and problem-solving approaches on the tasks to tackle. It would be encouraged to promote reexamination of the mode and modality of collaboration by Japanese communities for the upcoming decades, although financial resources are limited elsewhere, human wisdom is infinite for good.

## Acknowledgements:

The authors thank Dr. K. V. Raman and Dr. P. J. Gregory, International Program, Cornell University, Ithaca, USA for checking the content of this manuscript as former senior research managers at CIP. They also acknowledge Dr. Keiji Kainuma as a former CGIAR Science Council Member and Director General of JIRCAS. We recognize the review of this manuscript by today's CIP staff. This manuscript was made in part as an activity of the Plant Transgenic Design Initiative (PtraD) at Tsukuba Plant Innovation Research Center, University of Tsukuba, Japan.

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