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2017 FINAL REPORT

ADVANCING ACHIEVEMENTS IN BREEDING FOR EARLY, RESILIENT, AND NUTRITIOUS POTATO AND SWEETPOTATO

31 October 2017

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October 2015–September 2017

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ACRONYMS

ABMs	Accelerated breeding methods
ABS	Accelerated breeding scheme
AFLP	Amplified fragment length polymorphisms
APC	Arid Pacific coast
AUDPC	Area under the disease progress curve
BW	Bacterial wilt
CIP	International Potato Center
CR_NDVI	Canopy reflectance normalized difference vegetation index
CTCRI	Central Tuber Crops Research Institute
CV	Cross validation
D	Diploid accession
DAP	Days after planting
DM	Doubled monoploid
DTI	Drought tolerance index
DW	Dry weight
ELISA	Enzyme linked immune-sorbent assay
ER	Extreme resistance
Fe	Iron
FW	Fresh weight
GCA	General combining ability
GEBV	Genomic-estimated breeding values
GS	Genomic selection
GTDMS	Global trial data management system
GWAS	Genome-wide association studies
HIDAP	Highly Interactive Data Analysis Platform
HTA	Humid tropics of the Amazon Basin
ILETRI	Indonesian Legumes and Tuber Crops Research Institute
LB	Late blight
LBHT	LB-resistant, Heat Tolerant potato population
LTVR	Lowland tropic virus-resistant potato population
MAS	Marker-assisted selection
NAM	Nested association mating
NARS	National agriculture research system
NDVI	Normalized difference vegetation index
NIRS	Near-infrared reflectance spectroscopy

OFSP	Orange-fleshed sweetpotato
PaO	Pheophorbide A oxygenase
PCR	Polymerase chain reaction
PLRV	Potato leafroll virus
PVS	Participatory varietal selection
PVY	Potato Virus Y
QTL	Quantitative trait locus
RCRDC	Root Crop Research and Development Center
RRD	Red River Delta
RSA	Root system architecture
RTB	CGIAR Research Program on Roots, Tubers and Bananas
SASA	Semi-arid savanna of Southern Africa
SASHA	Sweetpotato Action for Security and Health in Africa
SC	Self-compatibility
SCA	Specific combining ability
SI	Self-incompatible
Sli	S-locus inhibitor
SNP	Single-nucleotide polymorphism
SPCSV	Sweet potato chlorotic stunt virus
SPFMV	Sweetpotato feathery mottle virus
SPVD	Sweet potato virus disease
SSA	Sub-Saharan Africa
SSR	Simple sequence repeat
TGA	Total glycoalkaloids
TP	Training population
TS	True seed
UPLC	Ultra performance liquid chromatography
USAID	United States Agency for International Development
VP	Validation population
Zn	Zinc

EXECUTIVE SUMMARY

This is the final report for the 2-year (Oct. 2015–Sept. 2017) project "Advancing Achievements in Breeding for Early, Resilient, and Nutritious Potato and Sweetpotato," funded by the United States Agency for International Development (USAID). The report addresses the progress and achievements that the International Potato Center (CIP) made in its potato and sweetpotato breeding programs.

The main achievements in the breeding work supported by USAID at CIP can be organized as follows:

- Preparing CIP breeding programs to deliver novel, more effective breeding approaches
- Expanding sources of resistance and selection to main pests, diseases, and traits affecting end-users' preferences
- Enabling partners in developing countries to enhance and speed up the delivery of improved varieties benefitting smallholders.

Report organization

The "Advancing Achievements in Breeding for Early, Resilient, and Nutritious Potato and Sweetpotato" is necessarily a highly technical project that advances targeted and complex breeding programs for both crops. As a final project report, this document draws on the many components, activities, and results discussed in earlier project deliverables, and discusses progress since the 2016 annual report. This wealth of data helps to illuminate the science behind breeding research and its potential for delivery and impact on end-users.

Section 2 summarizes the achievements of both the potato (section 2.2) and sweetpotato (2.3) breeding programs by outputs. The two sections are organized by individual project output, and the set of deliverables and milestones for that output. For the potato breeding program, there are four outputs; the sweetpotato program comprises five outputs.

The report concludes with supporting appendix material.

Preparing CIP breeding programs to deliver novel, more effective breeding approaches

An emergent breeding trend within the domain of root and tubers breeding is the systematic exploitation of heterosis as an overall approach to address the limited genetic gain for yield observed to date in potato. Limited genetic gains are attributed to low recombination, long generation cycle, polyploidy, and inbreeding depression. Progenies derived from crossing of selected parents from CIP's independent Lowland tropic virus-resistant potato population (LTVR) and late blight-resistant/heat tolerance (LBHT) populations displayed highly significant levels of heterosis over the control variety 'Unica': up to 53%, 37%, and 17% for economically relevant traits such as tubers/plant, tuber weight, and dry matter, respectively. The best clones display high to moderate resistance to LB, early maturing with a growing period of 80–90 days, and yields ranging 15–50 t/ha under warm heat conditions. On the other hand, control varieties 'Desiree' and 'Unica' yielded 12–14 t/ha and 24–27 t/ha, respectively. These clones will be introduced to *in-vitro* conditions at the beginning of 2018.

The global development of hybrid potato varieties at the diploid level hinges on the use of the gene *Sli* from a wild potato (*S. chacoense)* accession to break the natural self-incompatibility of this crop. Progress

was made towards developing novel, more effective sources to develop self-compatible genetic stocks, from *S. tuberosum Phureja* and *Stenotomum* groups of germplasm, and the genetic studies conducted suggest the existence of a novel S-locus inhibitor *(Sli*) gene. This gene, upon genetic confirmation, could be deployed at CIP and beyond as an improved source of self-compatibility to develop diploid hybrid varieties of potato.

In sweetpotato, a full hybrid breeding scheme has been implemented, which builds on the heterosis observed between genetically diverse groups such as PJ and PZ. The first hybrid progenies were tested in Peru and, subject to funding availability, true hybrid seed would be shipped to Bangladesh, Nepal, The Philippines, and Vietnam during 2018.

Progress was also attained as a multiplex polymerase chain reaction assay, which enabled the simultaneous selection for potato virus Y (PVY) and potato leafroll virus (PLRV) resistance to achieve its final validation step before wide deployment. In addition, a single-nucleotide polymorphism marker linked to the extreme resistance for LB in the LBHT population, one of CIP's main breeding pools, was advanced to its final validation step. It can now be deployed in the forward selection of highly LB-resistant individuals in B3 population intercrosses. An additional LB resistance gene from *S. demissum* was mapped and its DNA sequence characterized.

Under the project CIP's breeding program made significant progress at deploying an accelerated breeding scheme (ABS) in potato, which cuts the time needed to develop a candidate variety to be submitted to registration trials by 20–30%. Furthermore, data collection from the ABS potato breeding program was partially implemented in HIDAP (Highly Interactive Data Analysis Platform), the software developed at CIP to manage data flows in roots and tubers breeding programs from crossing to advanced yield trials.

Expanding sources of resistance and selection to main pests, diseases, climate resilience, and traits affecting end-users' preference and sustained genetic progress

In potato, PLRV can become a severe disease in tropical regions as high populations of its aphid vector are frequent. Through selection, clones exhibiting much less PLRV than control varieties and more tuber yield/plant were identified and also carried heat tolerance and PVY extreme resistance. These clones will undergo *in-vitro* cleaning before distribution as parental clones to breeders in CIP's regional programs pursuing agile potatoes adapted to the tropical lowland areas of target countries in Asia. Potato breeding families of botanical seed have been developed that combine LB resistance, tuber yield under conditions of high temperatures, resistance to PVX and PVY, precocity, and tuber yield. These progenies will be distributed to regions and partners at different national agricultural research systems (NARS) to select new varieties using ABS.

Tetraploid biofortified potato populations were intercrossed to generate more than 140 families in a second cycle of recurrent selection. They demonstrated higher yield, earliness, tolerance to abiotic stress, combined resistances to LB and virus (PVY), higher iron and zinc concentrations, and higher dry matter content. Twenty of these families were dispatched to Yunnan Province, China, in August 2017, for selection and evaluation under ABS. In the coming months 40 families will be dispatched to Bangladesh, Nepal, and Bhutan.

The breeding program made progress with developing near-infrared reflectance spectroscopy. This technique is used to rapidly and cheaply assess total glycoalkaloids, secondary metabolites from potato (which must be kept at very low levels), and to explore diversity for bitterness and flavor intensity- related compounds in raw, freshly harvested, boiled, and boiled potatoes stored at 4°C. In both cases expected

progress was achieved within the limits of sample sizes available, which in subsequent studies will be further validated and made available to NARS partners in diverse regions.

In sweetpotato, estimates for genetic gains across years for the arid Pacific Coast (APC), irrigated environments, humid tropics of the Amazon Basin (HTA), and semi-arid savanna of Southern Africa suggest that genetic progress can be sustained over the years to come. Within APC and HTA zones, genetic gains for storage root yield over the last two decades leading up to 2014 ranged from 0.18 to 0.34 t/ha per year and 0.36–0.58 t/ha per year for 90- and 120-day harvests, respectively.

Enabling partners in developing countries to enhance and speed up the delivery of improved varieties benefitting smallholders

ABS potato breeding schemes are already being deployed in Vietnam and Kenya, and with additional support would be progressively expanded to all NARS partners interested in reducing breeding cycles.

Trainings on HIDAP were conducted in Vietnam, Rwanda, and CIP–Kenya to enable breeders from NARS partners to manage the data flow of their potato breeding programs. In addition, research done on participatory breeding in Ethiopia concluded that farmer panels must me trained and standardized before evaluation to fully exploit their contribution, as otherwise they might select genotypes carrying undesirable traits that can curb their wide adoption.

The 2017 version of CIP's interactive catalogue, "Advanced Potato Clones and Varieties," was published online in nine languages. It includes more than 450 advanced clones and 70 varieties with images and information on main attributes, molecular data, pollen viability, and nutritional contents.

Final remarks

USAID's investment in the potato/sweetpotato breeding project has delivered value from several perspectives:

- Progress was achieved, in several cases with final outputs. In others, the intermediate outputs achieved, upon validation steps subject to funding availability, would become outputs to be taken by NARS partners to be transformed into towards outcomes.
- Substantial breeding progress was achieved. If additional funding is made available, potato hybrid varieties at the diploid level could be developed and transferred to NARS partners, in addition to the traditional potato tetraploid advanced clones and varieties bred by CIP.
- The progress mentioned here, together with the ABS already in place at CIP and the progress in molecular breeding achieved at CIP and elsewhere, would accelerate the delivery of benefits to the myriad of smallholders around the world who depend on potato and/or sweetpotato as a source of nutrition security, income, and wellbeing.

1. PROJECT GOALS

The International Potato Center (CIP) dedicates much of its scientific research to global food and nutrition security and to enhancing smallholder's ability to adapt to climate change through the development of more resilient potato and sweetpotato varieties. Potato and sweetpotato are important food crops for addressing issues of global concern, including hunger, poverty, public health, and threats to the environment. CIP's breeding programs develop and disseminate improved potato and sweetpotato populations and build capacity locally for the selection of varieties that will enhance productivity; reduce farmers' dependence on external inputs; and help to improve incomes and nutrition in target regions, cropping systems, and value chains. The use of innovative breeding approaches targeting population improvement and variety selection will effectively ensure higher yields and yield stability, and harness diversity toward dynamic sets of specific objective traits in new potato and sweetpotato varieties. The specific project financed by the USAID contribution between October 2015 and September 2017 supported the overall potato and sweetpotato breeding program to deliver on its goals and purpose.

1.1 PROJECT PURPOSE

In the medium term, smallholder farmers in Asia, Africa, and Latin America will have access to new stable and high-yielding potato and sweetpotato varieties that are resilient to disease and climate change, enabling these farmers to improve their capacity to manage constraints affecting sustainability and household economy. The impacts of new varieties, when accompanied by functioning seed systems, successful crop management, and competitive value chains, can reduce poverty and malnutrition and enhance the resilience of food security and farming and food systems. CIP follows a comprehensive scheme for breeding, comprising variety development and population improvement (Gallais 2003). Variety development aims to select the best clones and maximize the use of genetic variation. Population improvement aims to select the best parents to generate new genetic variation relevant for end-users around an improved population mean. Variety development is relatively straightforward and done in cooperation with national agricultural research systems (NARS). Population improvement is complex. It has to be carried out for a given agro-ecological zone or set of traits—for example, potato for subtropical lowlands or tropical highlands, and/or orange-fleshed sweetpotato (OFSP) with short growing season requirements. CIP's global potato and sweetpotato crop improvement programs emphasize genetic improvement and dissemination of populations. Variety selection and releases from improved populations are carried out in cooperation with partners in target countries.

1.2 OVERVIEW OF BREEDING OBJECTIVES AND OUTPUTS AT CIP TO BE SUPPORTED BY THE PROJECT

1.2.1 Breeding objectives

CIP's potato and sweetpotato breeding programs contribute to the center's overall goals through three main objectives:

1. Develop nutritious and biodiverse potato and sweetpotato populations with recognized added value and high variety ability.
2. Develop resilient potato and sweetpotato varieties by strengthening regional and local networks.

3. Improve selection accuracy and intensity, and shorten the breeding cycle in order to accelerate genetic gains.

1.2.2 Breeding outputs

CIP's breeding programs work toward five broad outputs. Each output consists of deliverables, activities, and milestones contributing to CIP's strategic and corporate plan. Outputs include:

1. Dynamic and nutrient-dense breeding populations available as sources of early-maturing, high, and stable-yielding varieties with resistance to biotic and abiotic stresses and quality traits.
2. Alignment of research with farmers' and end-users' preferences through participatory varietal selection (PVS) from initial selection stages.
3. Accelerated breeding methods (ABMs) and tools to help breeders select genotypes and parental lines in fewer years than with traditional clone-breeding schemes.
4. Improved and shared breeding databases and knowledge management, including databases of accurate phenotypic and breeding values of selected breeding lines and specific protocols and catalogues to support the orientation of breeding products and facilitate decision-making and outcomes from breeding research.
5. New capacities for applying knowledge, tools, and modern breeding approaches developed for more efficient progress in variety-oriented breeding programs of NARS.

2. FINAL REPORT

2.1 SUMMARY OF ACHIEVEMENTS BY OUTPUT—*POTATO*

2.2.1 Output 1: Dynamic and nutrient-dense breeding populations available as sources of early-maturing, high, and stable-yielding varieties with resistance to biotic and abiotic stresses and quality traits

Deliverable 1: Advanced cycle of recurrent selection of main breeding populations developed and true seed (TS) families generated for variety selection in target countries.

Development of a new cycle of selection in population B groups targeting tropical highland and mid-elevation agro-ecologies

Milestone achieved: Foreign varieties and breeding lines with LB resistance, table and processing quality, and short growing period identified for enriching population B by Q3 2016 (2017).

CIP aims to introduce new sources of resistance to late blight (LB) into its breeding programs. Seven varieties with high levels of LB resistance have been identified in five countries (Table 1).

TABLE 1. LB-RESISTANT VARIETIES FOR INTRODUCTION INTO CIP'S LB BREEDING PROGRAMS

#	Country	Institution	Varieties
1	Chile	INIA	Patagonia (INIA)
2	Mexico	INIFAP	Adelita, Milagros
3	Netherlands	HZPC	Innovator
4	United States	USDA	Defender
5	India	CPRI	Kufri Himsona, Kufri Sadabahar

NOTE: INIA = Instituto de Investigaciones Agropecuarias; INIFAP = Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias; HZPC = an agricultural business concern in the Netherlands; USDA = U.S. Department of Agriculture; CPRI = Central Potato Research Institute.

In 2016, import procedures were initiated, with risk studies for the importation of germplasm between SENASA (Peru) and the institutions that own the varieties in Chile, Mexico, the United States, India, and the Netherlands.

To minimize the risk of dispersion and introduction of regulated pests through the entry of plants and plant products into Peru, the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organization, the International Plant Protection Convention, and the General Secretariat of the Andean Community[1] establish that National Plant Protection Organizations (SENASA–Peru) can implement phytosanitary measures based on the respective risk analysis. This is a technical tool to identify and apply phytosanitary measures justified and necessary to maintain an adequate level of phytosanitary protection for the country and international trade.

SENASA-Peru has sent letters to all countries to initiate these studies; however, only INIA–Chile and CPRI India have responded; no responses have been received from HZPC, INIFAP, and USDA. These studies take 1–2 years, depending on responses from owners of the varieties.

After a risk analysis is conducted, all necessary import procedures will be pursued to have these varieties for use as controls and to incorporate other programs' advances into CIP's breeding efforts.

Milestone achieved: Thirty TS families from best progenitors combining LB resistance, heat tolerance, cooking quality, and mid-maturity generated and disseminated to NARS and regional hubs for use in accelerated breeding schemes (ABS).

Since 2004, CIP has sought to improve the heat tolerance of its LB-resistant population B3 by developing the new "LBHT" population in order to obtain potato clones with the following traits: high levels of resistance to LB, high tuber yield under high temperatures (>20°C at night), heat tolerance, low glycoalkaloid content, early maturity (90 days), and adapted to tropical mid-elevation environments.

Breeding clones were evaluated and selected under the following conditions in Peru: (1) San Ramón, a warm rainforest environment at 800 masl, 11°08'S and 75°20'W, where day and night temperatures averaged 28°C and 22°C, for heat tolerance; (2) Oxapampa, at 1,950 masl, for LB resistance, where optimal environmental conditions result in high endemic disease pressure; and (3) Majes, at 1,294 masl, 16°28'S and 72°06'W, for drought tolerance. Extreme resistance (ER) to potato virus X (PVX) and PVY were evaluated under greenhouse conditions in La Molina. Tests for glycoalkaloid content was determined in tubers harvested under high temperature conditions in San Ramón.

[1] "Decreto Legislativo No 1059. Ley General de Sanidad Agraria (2008)" and the "Decreto Supremo No. 018. Reglamento de la Ley General de Sanidad Agraria (2008).

The selected clones with resistance to LB (better than 'Kory', a resistant variety control), heat tolerance, extreme resistance to PVX and/or PVY, and drought tolerance were evaluated to determine their parental value for commercial yield of tubers under high temperature conditions in San Ramón, La Molina, and Majes.

We identified 18 clones with high parental value (Table 2) and which were intercrossed (Table 3) in the cross-breeding greenhouses in Huancayo from February 2016 to January 2017. We used as male parents 4 clones that had viable pollen; 14 clones were used as females.

TABLE 2. LBHT-RESISTANT CLONES WITH GOOD BREEDING VALUE FOR MARKETABLE TUBER YIELD UNDER HIGH TEMPERATURES

#	Clone	Drought Tolerance	ER PVX	ER PVY
1	CIP398098.570		ER	
2	CIP398192.213	Tolerant	ER	
3	CIP398192.553		ER	
4	CIP398201.510		ER	ER
5	CIP398208.704		ER	
6	CIP302531.43		ER	
7	CIP302534.17			ER
8	CIP304079.10		ER	ER
9	CIP304081.44			ER
10	CIP398098.203	Tolerant		ER
11	CIP398190.200			
12	CIP398208.505		ER	ER
13	CIP398208.670		ER	ER
14	CIP302551.26		ER	ER
15	CIP398208.219	Tolerant	ER	ER
16	CIP398208.620		ER	
17	CIP398203.5		ER	
18	CIP398208.33	Tolerant	ER	

ER: EXTREME RESISTANCE

TABLE 3. CROSSING PLAN FOR LBHT CLONES WITH GOOD PARENTAL VALUE FOR MARKETABLE TUBER YIELD UNDER HIGH TEMPERATURES

Female	Male			
	398208.22	398208.62	398203.5	398208.33
398098.57	X	X	X	X
398192.213	X	X	X	X
398192.553	X	X	X	X
398201.51	X	X	X	X
398208.704	X	X	X	X
302531.43	X	X	X	X
302534.17	X	X	X	X
304079.1	X	X	X	X
304081.44	X	X	X	X

398098.203	X	X	X	X
398190.2	X	X	X	X
398208.505	X	X	X	X
398208.67	X	X	X	X
302551.26	X	X	X	X

Fifty-six family progenies were generated with 166,880 TS (Table 4) and are being distributed to regions and NARS to identify varieties using ABS.

TABLE 4. INVENTORY OF PROGENIES AND TS

#	Female	Male	Number of TS
1	302531.43	398208.219	4,500
2	302531.43	398208.62	4,000
3	302531.43	398203.5	3,000
4	302531.43	398208.33	6,000
5	302534.17	398208.219	800
6	302534.17	398208.62	300
7	302534.17	398203.5	500
8	302534.17	398208.33	1,800
9	302551.26	398208.219	4,000
10	302551.26	398208.62	1,500
11	302551.26	398203.5	1,000
12	302551.26	398208.33	4,000
13	304079.1	398208.219	4,500
14	304079.1	398208.62	4,500
15	304079.1	398203.5	5,000
16	304079.1	398208.33	5,000
17	304081.44	398208.219	3,500
18	304081.44	398208.62	2,000
19	304081.44	398203.5	1,700
20	304081.44	398208.33	5,000
21	398098.203	398208.219	1,700
22	398098.203	398208.62	1,800
23	398098.203	398203.5	400
24	398098.203	398208.33	2,500
25	398098.57	398208.219	2,000
26	398098.57	398208.62	3,500
27	398098.57	398203.5	4,800
28	398098.57	398208.33	5,500
29	398190.2	398208.219	2,500
30	398190.2	398208.62	2,000
31	398190.2	398203.5	3,000
32	398190.2	398208.33	4,000
33	398192.213	398208.219	4,500

#	Female	Male	Number of TS
34	398192.213	398208.62	2,500
35	398192.213	398203.5	2,500
36	398192.213	398208.33	4,500
37	398192.553	398208.219	2,800
38	398192.553	398208.62	2,000
39	398192.553	398203.5	1,500
40	398192.553	398208.33	3,500
41	398201.51	398208.219	3,000
42	398201.51	398208.62	2,000
43	398201.51	398203.5	4,000
44	398201.51	398208.33	9,800
45	398208.505	398208.219	3,500
46	398208.505	398208.62	2,500
47	398208.505	398203.5	2,500
48	398208.505	398208.33	5,500
49	398208.67	398208.219	1,200
50	398208.67	398208.62	780
51	398208.67	398203.5	1,800
52	398208.67	398208.33	3,800
53	398208.704	398208.219	1,500
54	398208.704	398208.62	1,200
55	398208.704	398203.5	1,200
56	398208.704	398208.33	2,500

Development of a new cycle of selection in population LTVR for sub-tropical lowland and temperate ecologies

Milestone achieved: PLRV resistance from Andigena source introduced to LTVR by Q1 2017.

Though potato leafroll virus (PLRV) is a biotic constraint that causes potato yield losses and tuber seed degeneration worldwide, it is much more prevalent in tropical regions where high populations of the aphid vector are commonly found. A novel major gene controlling high resistance to PLRV (named Rl_{adg}) was identified previously at CIP in three accessions of *S. tuberosum* Andigenum Group and also in Andigena-derived LB-resistant breeding lines from CIP's B3 population. The gene was introduced by crossing these resistance sources to advanced breeding lines from the Lowland Tropics Virus Resistance population (LTVR) with ER to PVY. Thus, we generated a TS pre-breeding population segregating for this major gene. Tuber families developed from 24 TS hybrid families were field-evaluated for agronomic traits and yield components under warm conditions in the lowland subtropics of the Peruvian coast. Out of 2,400 plants, 350 genotypes were selected based on breeders' scores on tuber size, shape, number, and uniformity and depth of tuber eyes. Screening for ER to PVY under greenhouse conditions yielded 147 ER genotypes. We analyzed the multiplex polymerase chain reaction (PCR) marker previously developed at CIP that allows simultaneous detection of markers associated with Rl_{adg} and Ry_{adg} controlling high levels of PLRV resistance and ER to PVY, respectively, in the selected group. We identified 54 genotypes positive for both markers.

Upon cloning to obtain enough seed tubers, the 54 genotypes underwent intentional field exposure to PLRV-infested aphids under warm conditions in the lowland subtropics of Peru. Low levels of PLRV infection prevented validation of PLRV resistance in the selected group, although environmental conditions allowed selection for heat tolerance. We identified 9 clones that showed at most 30% of tuber heat defects (second growth, knobby tubers, sprouted tubers); total tuber weight ranged from 239 to 800 g/plant with 1–3 marketable tubers/plant. Upon PLRV resistance validation, these clones will undergo in-vitro cleaning until healthy enough to be distributed as parental clones for high levels of PLRV resistance to breeders in CIP's regional programs who are pursuing agile potatoes adapted to the tropical lowland areas of target countries in Asia. In addition, enough TS from 24 hybrid progenies segregating for the novel major gene Rl_{adg} for PLRV resistance in a genetic background of heat tolerance and ER to PVY are available for quick distribution.

Milestone partially achieved: At least 40 TS families generated and disseminated to NARS and CIP regional hubs for use in ABS by Q2 2017.

Selected clones from CIP' tetraploid biofortified population (cycle I) were intercrossed in order to generate a second cycle of recurrent selection with higher yield, varying for earliness, tolerance to abiotic stress, combined resistances to LB and PVY, nutrition quality with higher iron (Fe) and zinc (Zn) concentration, and higher dry matter content. More than 140 different crosses with more than 82,000 seeds were generated. In the same way, advanced clones from CIP's LTVR-resistant population were intercrossed to generate a sixth cycle to improve earliness with shorter growing period, high yield, and better tolerance to heat and drought without losing disease resistance.

Twenty-six TS families (200 seeds each) from a total of 5,400 seeds (Table 5) from crosses of the biofortified population were dispatched to Yunnan Province, China, in August 2017. This was done for selection and evaluation under ABS with the support of protocols and data management tools in HIDAP, a CIP database to unify best practices for data collection, data quality, and data analysis for clonally propagated crops. In the following months, 40 TS families of 200 seeds each will be dispatched to Bangladesh, Nepal, and Bhutan.

TABLE 5. TS FAMILIES DISPATCHED TO CHINA

CIP Family Number	Pedigree		Seeds
	Female CIP Number	Male CIP Number	
CIP316556	CIP312637.139	CIP312527.026	200
CIP316557	CIP312725.088	CIP312527.026	200
CIP316558	CIP312725.088	CIP312721.083	200
CIP316559	CIP312725.100	CIP312527.026	200
CIP316561	CIP312747.056	CIP312527.026	200
CIP316562	CIP312747.056	CIP312721.083	200
CIP316563	CIP312747.073	CIP312527.026	200
CIP316565	CIP312751.021	CIP312527.026	200
CIP316566	CIP312842.155	CIP312527.026	200
CIP316567	CIP312527.033	CIP312527.026	200
CIP316568	CIP312527.033	CIP312721.083	200
CIP316570	CIP312562.735	CIP312527.026	200
CIP316571	CIP312562.735	CIP312721.083	200

CIP Family Number	Pedigree		Seeds
	Female CIP Number	Male CIP Number	
CIP316574	CIP312562.735	CIP312527.024	200
CIP316575	CIP312586.061	CIP312527.026	200
CIP316577	CIP312637.004	CIP312527.026	200
CIP316578	CIP312637.004	CIP312721.083	200
CIP316580	CIP312637.063	CIP312527.026	200
CIP316582	CIP312637.098	CIP312527.026	200
CIP316583	CIP312637.098	CIP312721.083	200
CIP316584	CIP312637.098	CIP312725.024	200
CIP316585	CIP312637.107	CIP312527.026	200
CIP316586	CIP312637.107	CIP312721.083	200
CIP316587	CIP312637.107	CIP312725.024	200
CIP316588	CIP312637.118	CIP312527.026	200
CIP316591	CIP312725.068	CIP312527.026	200
CIP316594	CIP312725.069	CIP312527.026	200

Deliverable 2: First recurrent potato hybrid selection pool for heterosis exploitation developed.

Recurrent potato hybrid selection pool for combining LB and virus resistance, heat and drought tolerance, and early bulking from populations LTVR and B3 (1st reciprocal recurrent selection cycle)

Milestone achieved: At least 20 TS families generated from LTVR x B3 selected parents disseminated to NARS and CIP regional hubs for use in ABS by Q2 2016.

Selected parents from CIP's LTVR and LBHT populations were intercrossed using line by tester mating design, in direct and reciprocal crosses: LBHT x LTVR and LTVR x LBHT. This was done to exploit heterosis for yield in hybrid families varying for earliness, tolerance to abiotic stress, and combined resistances to LB and virus. A total of 108 families and more than 87,000 seeds were generated. Standard heterosis of the interpopulation hybrid families was determined using the variety 'Unica' as control in a series of experiments in multiple locations in Peru. The assessment showed high heterosis of the progenies up to 53% for number of tubers per plant and up to 37% for tuber weight (yield of 'Unica' was 0.5 kg/plant, progenies were 0.7 kg/plant). Most of the progenies were higher in dry matter content compared with the control, reaching up to 17% heterosis increments (dry matter content of 'Unica' was 15.3%, progenies mean was 17.9%).

A group of 20 TS families, mostly heterotic for yield from LBHT x LTVR and LTVR x LBHT crosses, were identified. Some 300 seeds of each family—6,000 seeds in total (Table 6)—were dispatched to Ethiopia in August 2017. From there, sets of tuber families will be redistributed to Ethiopia, Kenya, Rwanda, and other countries in sub-Saharan Africa (SSA) to practice variety selection under ABS and supported by respective protocols and data management tools in HIDAP.

TABLE 6. 20 TS HETEROTIC FAMILIES DISPATCHED TO ETHIOPIA

#	CIP Number	Female	Male	Population
1	CIP312887	CIP398098.119	CIP302476.108	LBHT x LTVR
2	CIP313165	CIP398098.57	CIP302476.108	LBHT x LTVR
3	CIP312908	CIP398201.51	CIP304350.118	LBHT x LTVR
4	CIP312909	CIP398203.244	CIP304350.118	LBHT x LTVR
5	CIP313171	CIP302534.17	CIP302476.108	LBHT x LTVR
6	CIP312913	CIP398208.62	CIP304350.118	LBHT x LTVR
7	CIP312915	CIP398208.704	CIP304350.118	LBHT x LTVR
8	CIP313169	CIP398208.33	CIP302476.108	LBHT x LTVR
9	CIP312924	CIP398203.244	CIP304372.7	LBHT x LTVR
10	CIP312927	CIP398208.505	CIP304372.7	LBHT x LTVR
11	CIP312064	CIP388615.22	CIP398208.620	LTVR x LBHT
12	CIP312070	CIP392797.22	CIP398098.119	LTVR x LBHT
13	CIP312079	CIP392820.1	CIP398208.620	LTVR x LBHT
14	CIP312083	CIP392820.1	CIP398098.203	LTVR x LBHT
15	CIP312088	CIP396311.1	CIP398208.620	LTVR x LBHT
16	CIP312092	CIP396311.1	CIP398098.203	LTVR x LBHT
17	CIP312171	CIP304350.118	CIP398208.620	LTVR x LBHT
18	CIP312174	CIP304350.118	CIP398098.203	LTVR x LBHT
19	CIP312359	CIP302476.108	CIP398208.620	LTVR x LBHT
20	CIP312360	CIP302476.108	CIP398208.670	LTVR x LBHT

Milestone achieved: At least 10 heterotic hybrid clones with resistance to LB and virus, early bulking, drought, and heat tolerance (widely adapted clones) selected for cleaning to HS2 by Q1 2017.

As previously reported in 2016, the first recurrent potato hybrid selection pool for heterosis exploitation developed from interpopulation hybridization seeks to capitalize on CIP's advanced and genetically divergent populations B with resistance to LB and LTVR. The aim was to achieve additional genetic gains and harness heterosis by an overarching scheme of reciprocal recurrent selection. Selected parents from CIP's LTVR population and group LBHT from population B with LB resistance and heat tolerance were intercrossed in order to exploit heterosis for yield in hybrid families varying for earliness, tolerance to abiotic stress, and combined resistances to LB and virus.

In addition to the genetic variability study and heterosis measurement as already reported, clonal selection was practiced to identify heterotic clones with high yield and resistance to diseases (LB and virus). Multi-environment trials for evaluation and selection were carried out for 3 years in divergent environments of Peru, imposing biotic and abiotic stresses:

- The warm, humid climate at San Ramón experiment station, at 800 masl, in the central jungle of Junin, with average day and night temperatures of 28°C and 22°C
- The highland temperate, rainy climate at Huancayo experimental station, at 3,200 masl, with average day and night temperatures of 18°C and 5°C

- The desert, arid lowland coast of Santa Rita, at 1,300 masl
- The warm, humid, mid-central jungle Oxapampa, at 1,900 masl (used to evaluate and screen for resistance to LB due the natural high pressure for this disease).

All these locations provide representative evaluation sites of tropical lowland and highland potato target environments, and permit the identification of stable genotypes with high chance of good performance in other latitudes.

More than 14,000 seedlings were grown from April to September 2012, in the greenhouse at La Molina, Peru, to obtain tuber families. The TS families were sown in trays and individual genotypes were transplanted to 4-in. pots. At harvest, two sets of tuber families were recovered prior to the individual genotypes being labeled with bar codes. The first set was maintained in quarantined greenhouse conditions. The second set was planted in the field in Huancayo in January 2014, to obtain sufficient number and adequate size of tuber seeds for field evaluations. A first clonal selection by yield and tuber appearance was practiced in this location. More than 2,000 clones were selected in the first clonal generation: 1,300 from crosses LTVR-LBHT and 578 from reciprocal crosses (LBHT-LTVR). A group of 100 clones remain selected (77 from crosses LBHT-LTVR and 23 from LTVR-LBHT) after three clonal generation and evaluation-selection through divergent biotic and abiotic stressful environments. All these clones show high to moderate resistance to LB, early maturing with a growing period of 80–90 days, and a yield range of 15–50 t/ha under warm heat conditions. Meanwhile, control varieties 'Desiree' and 'Unica' yielded 12–14 t/ha and 24–27 t/ha, respectively. Figures 1 and 2 show the yield performance of the top clones of both populations under warm conditions in San Ramón in 2015 and 2016. These clones will be introduced in vitro at the beginning of 2018, and available for distribution for variety selection by NARS by the end of 2018.

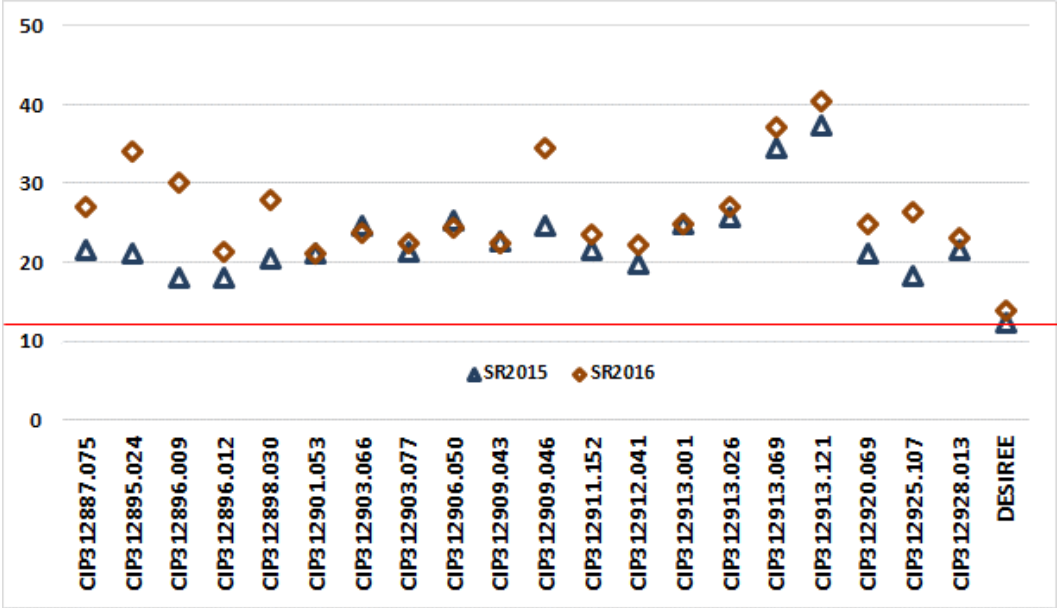

Figure 1. Marketable yield (t/ha) of top 20 clones from LBHT-LTVR population under warm conditions (SR2015: San Ramón 2015, SR2016: San Ramón 2016).

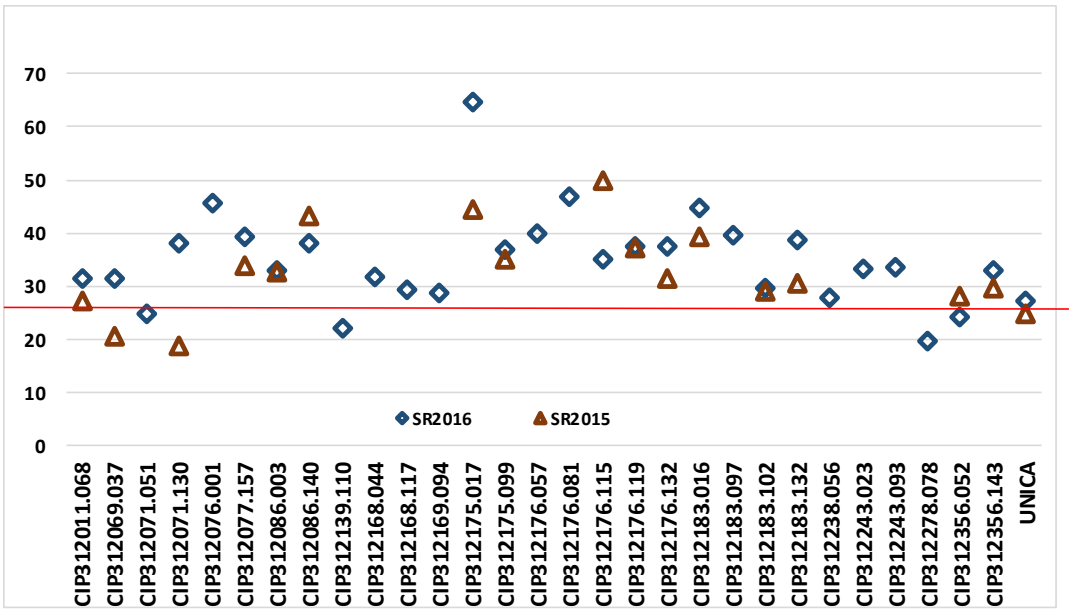

Figure 2. Yield (t/ha) of top 28 clones from LTVR-LBHT population under warm conditions (SR2015: San Ramón 2015, SR2016: San Ramón 2016).

Deliverable 3: Populations comprising new sources of needed traits: micronutrient density, stress tolerance, and preference traits characterized and promising clones and progenitors identified.

Consolidation of biofortified populations with high nutrient content and health-promoting traits

Milestone achieved: At least five advanced tetraploid clones with 35 ppm Fe, 35 ppm Zn, and high vitamin C adapted to tropical highland and mid-elevation ecologies selected for international distribution by Q2 2017.

Potato biofortification breeding at CIP has resulted in new generations of biofortified potatoes with up to 45 mg/kg dry weight (DW) Fe and 37 mg/kg DW Zn. Depending on location, mineral concentrations increased by recurrent selection at the diploid level and strategic interploid crossing with top tetraploid parental lines, leading to higher yielding, disease-resistant populations of biofortified potatoes.

Advanced disease-resistant (LB and/or virus) and heat-tolerant clones as female tetraploid parents, together with high density Fe and Zn clones from the biofortification program capable of producing high frequency of 2n pollen as male diploid parents, were used to perform interploid (4x–2x) crosses. More than 12,000 seedlings from more than 200 families were generated. Ploidy level was determined and tetraploid genotypes were identified. Screening for yield, resistance to LB and PVY, and micronutrient concentration was performed. Field trials in five locations of Peru—La Molina, Huancayo, Paucartambo, Cusco, and Oxapampa (for LB screening)—were carried out for yield and micronutrient evaluation. Genotypes with high Fe and Zn values over 30 ppm and high yield and resistance to LB and PVY were

identified. A group of 142 tetraploid clones with high yield, LB and/or virus resistance, and high concentration of Fe and Zn (over 30 ppm) were introduced for in-vitro conservation and dispatch to NARs.

Table 7 shows the summary of clones combining high and medium concentration of Fe and Zn and resistances to PVY and LB. Remarkably, 85 clones show high ranges (27–46 ppm) of Fe through the different sites of evaluation, and medium to high levels of Zn concentration (20–30 ppm); 44 of these are resistant to LB and 19 are resistant both to LB and PVY. Fifty-six clones show intermediate levels of Fe (range of 24–30 ppm) from which 31 are resistant to LB and 17 are resistant both to LB and PVY. The average of Fe and Zn concentrations of check varieties are 19 mg/kg DW and 14 mg/kg DW, respectively.

Two to three healthy plants coming from quarantined greenhouse-grown tubers of the 142 clones were delivered to the genebank for in-vitro introduction by February 2017. Testing for phytosanitary status is still in process. In-vitro disease-free plants should be available for distributions by Q1 of 2018.

TABLE 7. NUMBER OF SELECTED CLONES FOR IN-VITRO INTRODUCTION, COMBINING HIGH LEVELS OF IRON AND RESISTANCE TO LB AND PVY

Genetic Resistance	Iron Level	
	High	Medium
	85	56
LB	44	31
PVY	22	6
LB + PVY	19	17

NOTE: High Iron levels = >27–46 ppm; Intermediate (Medium) Iron levels = 24–30 ppm

Base population for abiotic stress tolerance assembled and characterized to inform breeding strategies

Milestone achieved: At least five dihaploid tuber families from elite bred lines available for drought tolerance assessment.

Haploid techniques are normally applied to improve cultivar breeding in crop plants. In potato, this is normally achieved by use of related *Solanum* species with haploid induction capacity when crossed with cultivated tetraploid potato. These dihaploids (2n = 2x) provide genetic material for targeted polyploid hybrid breeding that maximizes heterotic combinations for the traits of interest. They also present simpler genotypes for understanding complex traits. Drought tolerance is a complex trait, and different genotypes have different adaptation mechanisms. Our objective was to develop dihaploid families from different elite clonal backgrounds that would maximize different combinations of mechanisms for drought tolerance as a first step toward genetic studies and hybrid breeding for drought tolerance, once inbred lines can be derived.

We selected five elite clones based on their drought tolerance index (DTI) from a prior multi-environment evaluation of elite clones for drought tolerance (Table 8). These elite clones also have resistance to PVX and PVY. Three inducer lines from *Solanum phureja* (IVP-35, IVP-101, and PL-4) were used in the haploidization process as males. The first crossing plan (2015–2016) resulted in 237 dihaploid genotypes, and the second crossing plan (2016–2017) resulted in 225 dihaploid genotypes (Table 8). Ploidy assessment was based on phenotypic markers, chloroplast counting, and flow cytometry.

Tubers from the dihaploid progeny are being multiplied to obtain enough tubers for genetic studies on morphological and physiological components of drought tolerance in 2018, and to test the validity of these genotypes in future breeding for drought tolerance.

TABLE 8. DIHAPLOID FAMILIES SHOWING THE PARENTAL CLONE, OTHER IMPORTANT TRAITS IN THE PARENTAL CLONES, THE HAPLOID INDUCER LINE USED IN THE CROSS, AND THE NUMBER OF DIHAPLOID INDIVIDUALS

CIP Family	Female CIP Number	Female Breeder Code	Important Traits	Male Breeder Code	Dihaploids 2015-2016	Dihaploids 2016-2017
CIP315028	CIP300056.33	LR00.014	PVX and PVY ER	PL-4	28	28
CIP315029	CIP300056.33	LR00.014	PVX and PVY ER	IVP-35	16	31
CIP315044	CIP397073.16	WA.104	PVX ER	PL-4	8	4
CIP315045	CIP397073.16	WA.104	PVX ER	IVP-35	4	13
CIP315038	CIP390637.1	93.003	PVX and PVY ER	PL-4	35	23
CIP315039	CIP390637.1	93.003	PVX and PVY ER	IVP-35	6	20
CIP315040	CIP391931.1	458	PVX resistant	PL-4	4	6
CIP315047	CIP397077.16	WA.077	PVX and PVY ER	PL-4	84	14
CIP315048	CIP397077.16	WA.077	PVX and PVY ER	IVP-35	49	57
CIP315049	CIP397077.16	WA.077	PVX and PVY ER	IVP-101	3	28
Total					**237**	**225**

2.2.2 Output 2: Breeding research aligned with farmers' and end users' preferences.

Deliverable 1: Strengthen regional breeding hubs.

Implementing ABS at early selection stages with NARS in CIP's regional hub

Milestone achieved: Workshops on breeding and selection scheme conducted in African and Asian countries by Q3 2017.

In 2016, Manuel Gastelo, a potato breeder from CIP's headquarters in Lima, Peru (CIP–Lima), spent 3 months in SSA supporting several local breeding programs in the region. Three training events were held on the use of HIDAP, a data management platform that can manage all aspects of a potato breeding program, including information from potato selection in ABS to researchers of the National Potato Program of Ethiopia and Rwanda and CIP researchers in CIP–Kenya. Also in Rwanda, potato researchers were trained to formulate the objectives and strategies of a potato breeding program.

In Rwanda on November 21st, Rwanda Agriculture Board researchers were trained in objectives and strategy for Rwanda's potato breeding program and management of the HIDAP database to the board's

potato team as well as researchers from other crops. Nineteen people (5 women, 14 men) attended (Table 9). The presentation was very welcome, and many asked how they could implement the program. Many researchers were familiar with Data Collector software provided by CIP before HIDAP was available. They mentioned that HIDAP was easier and friendlier to use. HIDAP was installed on their computers.

TABLE 9. PARTICIPANTS IN TRAINING IN RWANDA (NOV. 2016)

#	Name	Position	Email	Gender
1	Senkesha Ottizo	Research fellow potato	senkesha@yahoo.fr	Male
2	Ndayisaba Celestin	Research technician	pierre.celestin.ndayisaba@rab.gov.rw	Male
4	Gshebrick N. Engles	Researcher	egashalenkans@yahoo.fr	Male
5	Abandibakobwa Charlotte	Research technician	acharloty@gmail.com	Female
6	Kamenge J. Bosco	Researcher	sanobosco@gmail.com	Male
7	Uwizeye Christine	Researcher	christine.Uwizeye@gmail.com	Female
8	Nyiramigisha Philomene	Researcher	philoscultive@yahoo.fr	Female
9	Niyomugaba Francois Regis	Researcher	niyofraregis@gmail.com	Male
10	Ndayambaje Alexis	Researcher	andayambaje15@yahoo.com	Male
11	Munyabarame Denis	Research technician	munybden13@gmail.com	Male
12	Gafishi Martin	Researcher	gafishi.martin@yahoo.com	Male
13	Nyawika Pierr	Researcher	nkanikapierre@yahoo.com	Male
14	Nayigiziki Jonathan	Lab technician	jonathannayigiziki@gmail.com	Male
15	Niyomugaba Alphonse	Researcher	niyalpha05@gmail.com	Male
16	Karantowa Damoscene	Researcher	kjdstrongk@gmail.com	Male
17	Umwiszrwa Aline	Researcher	umwizerwaaline@gmail.com	Male
18	Mumyeshyaka Lambert	Researcher	mulambe03@gmail.com	Female
19	Sebahire Marcellin	Researcher	mariselini1985@gmail.com	Male

In Kenya on October 14th, HIDAP training was delivered to five researchers from the CIP–SSA region: Daniel Mbiri, James Mugo, Benson Kisinga, Mercy Kitavi, and Bruce Ochieng. Lists of clones, families, field books, and analysis of variance with its own report in Word were prepared. HIDAP software was installed on their computers.

In Ethiopia on November 8th, HIDAP was presented at the Holeta Experimental Station for seven researchers (one woman, six men) from the Ethiopian Institute of Agricultural Research's National Potato Program (Table 10). HIDAP software was installed on their computers.

TABLE 10. PARTICIPANTS IN TRAINING IN ETHIOPIA (NOV. 2016)

Name	Email	Profession	Gender
Atsede Solomon	atsede123@yahoo.com	Breeder/agronomist	Male
Abebe Chindi	abechindii@yahoo.com	Breeder/agronomist	Male
Egata Shunka	shunka2007@gmail.com	Agronomist and breeder	Male
Girma Gashu	gashugirma21@gmail.com	Biotechnology	Female
Kasaye Negash	Kasaye2006@gmail.com	Agronomist/breeder	Male
Gebremedhin W/Giorgis	gebregiorgis2003@yahoo.com	Breeder/agronomist	Male
Melaku Demissie			Male

Training in Vietnam. To improve the collection and quality of data, a small and preliminary training workshop on database management was carried out in Dalat (Feb. 16, 2017) and Hanoi (Feb. 21, 2017). Participating were 12 technicians of the Potato Vegetable and Flower Research Center in Dalat and 12 from the Field Crops Research Institute of Hanoi (Table 11). Participants were trained on the use of HIDAP. Data management; generation of genotype lists and field books, statistical analysis, and reports; bar coding; and use of CIPCROSS software to manage crosses were the main topics.

TABLE 11. PARTICIPANTS IN THE DALAT AND HANOI TRAININGS

#	Name	Cell Phone No.	Email
1	Nguyễn Thị Nhung	0983 718 656	Nguyenthinhungccc@gmail.com
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4	Nguyễn Thị Thu Hương	0976 828 014	nguyenthithuhuongccc@gmail.com
5	Ngô Thị Huệ	0916 341 278	ngohueccc78@gmail.com
6	Giang Thị Lan Hương	0916 813 658	gianghuong220607@gmail.com
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11	Nguyễn Đạt Thoại	0915 151 786	nguyendatthoai@gmail.com
12	Dao Huy Chien*	0919 959 080	c.daohuyvn@gmail.com

*CIP–Hanoi.

Milestone partially achieved: Data from clonal evaluation in Bangladesh, Vietnam, Uzbekistan, and Ethiopia collected and accessible in HIDAP by Q1 2017.

Regardless of successful training events and enthusiastic participation of NARS researchers, data collection from the regional trials remains challenging. So far, 11 trials conducted in Rwanda between 2013 and 2016 involving the B population are available in HIDAP and under revision for being included in the potato database.

The Lima breeding team is in contact with the NARS researchers and supporting their efforts in integrating the clonal evaluation data from the other breeding materials such as the diploid biofortified clones under evaluation in Rwanda, LBHT x LTVR heterotic clones in Ethiopia and Kenya.

More emphasis needs to be put in the continued support of the use of the HIDAP by the NARS partners starting from the design of the experiments to ensure the accessibility of the data.

Participatory selection in ABS with NARS and CIP regional hubs.

Milestone achieved: Breeders' and farmers' selections and preference profiles documented by clonal generation by Q2 2017.

Adoption of varieties may increase if farmers' and consumers' preferences are known and incorporated into the potato breeding program. PVS was implemented by CIP in collaboration with the Adet Agricultural Research and Experimental Station in Ethiopia to document the breeders' and farmers'

(women and men) selections and preference profiles. An experiment with 16 advanced clones was established in a randomized complete block design and two repetitions. The PVS methodology comprised three key stages: (1) evaluating potato at the time of flowering; (2) evaluating potato at the time of harvest, which also includes standard yield evaluation and organoleptic evaluation; and (3) postharvest evaluation at 45 days and 90 days to assess how well clones behave under storage conditions. Results show that men and women farmers preferred different traits (Fig. 3). The data also illustrate that although men and women are interested in marketable traits, women have additional requirements, particularly related to processing, that men do not consider. Both men and women agreed on non-selection criteria such as small tuber size and attacks by pests and diseases. Men mentioned that they sometimes keep their potato longer in the field before they harvest. If potato clones are already attacked by insects in the first harvest, it shows that the losses would only increase the longer the potato stayed underground. This element was therefore grounds for non-selection for some varieties in the scientists-managed plots at the station during farmer evaluation.

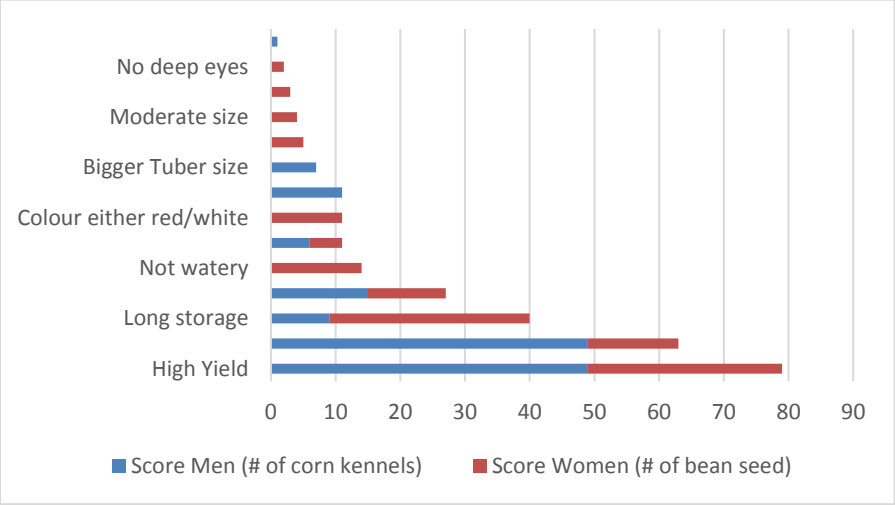


Figure 3. Men and women free listed selection criteria at harvest time.

The top six clones selected by breeders and farmers at harvest time are shown in Figure 4. Gender-mainstreamed PVS activities in Ethiopia showed that farmer perspectives need to be integrated in order to ensure that released clones meet their needs. Men and women had different preferences in their selection of potato clones. This study shows that, as above, men and women are interested in marketable traits, but women had additional requirements than did men.

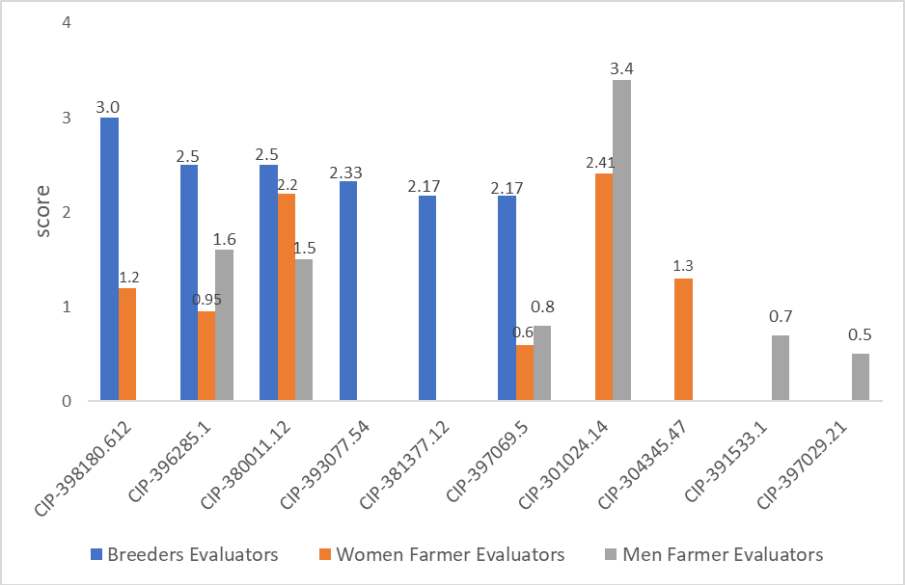


Figure 4. Top six selected clones by breeders and men and women farmers at harvest time.

Evidence from this evaluation suggests the need for trained farmer panels during evaluation. For example, the number one clone at harvest time selected by both men and women (CIP-301024.14) was rejected by breeders because more than three tubers were cracked, which showed a possible lack of tolerance to dry spells. Trained men and women farmer panels will be able to spot potential issues with different clones, especially related to pest and disease susceptibility and drought tolerance, while also ensuring that selected clones also met other farmer-based criteria. Clonal selection based on organoleptic test, yield, disease resistance, and famers' and breeders' preference at harvest time are presented in Table 12.

TABLE 12. CLONAL SELECTION BASED ON ORGANOLEPTIC TEST, YIELD, DISEASE RESISTANCE, AND FARMERS' AND BREEDERS' PREFERENCE AT HARVEST TIME

						Organoleptic properties					Choice of clones at Harvest		
Clones	Days to 50% emergence	Days to 50% flowering	Yield indicators above 3	Marketable tubers	Disease and quality	Appearance	Test at harvest	Taste 45 days post-harvest	Texture at harvest	Texture 45 days post-harvest	Breeders	Women farmers	Men Farmers
CIP-398180.612		✓	✓	✓	✓						✓	✓	
CIP-393227.66	✓	✓	✓	✓		✓		✓	✓	✓			
CIP-380011.12			✓	✓	✓		✓	✓	✓	✓	✓	✓	✓
CIP-301024.14			✓	✓	✓				✓	✓		✓	✓
Ater Abeba	✓	✓			✓	✓	✓		✓	✓			
CIP-396285.1		✓					✓	✓	✓	✓	✓	✓	✓
CIP-395077.12								✓	✓	✓			
CIP-397069.5	✓					✓		✓			✓	✓	✓
CIP-393077.54	✓										✓		
CIP-381379.12		✓				✓					✓		
CIP-397014.2	✓					✓							
CIP-397029.21						✓							✓
CIP-391533.1													
CIP-304371.67													✓
CIP-304345.47												✓	
CIP-302499.30													

Milestone achieved: At least 1 NARS from Asia successfully complete the first clonal cycle of ABS.

Results of the research on potato breeding in Vietnam, 2016–2017

Research results on potato breeding by a CIP consultant in Vietnam in 2016–2017 are discussed below. The results are the cumulative outcomes of the potato-breeding research that began in Vietnam in 2009 and supported by CIP.

From 2007 to 2014, three cycles of recurrent selection were performed in Vietnam. In the fourth cycle of the recurrent selection, the breeding works were performed in Dalat highland (1,500 masl) in the Southern Central Highlands, far from Hanoi (1,600 km), from August 8 to December 1, 2016, using 36 clones selected from third cycles of the recurrent selection as parents.

The breeding materials generated in Vietnam and clones introduced from CIP were evaluated and selected using ABS in the lowland of the Red River Delta (RRD, 5 masl) in two experiments to identify adaptable genotypes for possible release and for using as parental clones in the recurrent selection.

The objectives of this potato breeding program were to (1) identify adaptable clones from germplasm materials introduced from CIP clones to the growing conditions in RRD; (2) generate and identify new genotypes adaptable to the growing conditions in RRD; and (3) identify potential parental clones for breeding works.

A set of 105 potato clones bred in Mochau highland (in the third cycle of recurrent selection) and 'Solara' (a local check) were evaluated in Chuong My, Hanoi, Vietnam, in the 2016–17 winter crop season, planted on November 16, 2016, and harvested on February 27, 2017. The experiment was conducted in random planting without replication and plot size of 2 m^2 in single-row bed of 0.8 m x 2.5 m and 10 plants/clone. Fertilization, irrigation, insecticide, and fungicide were applied (see Table 13).

The clones were evaluated under field conditions in the winter crop season of 2016–17. The unexpectedly warm winter conditions, with high heat stress and severe infections by mites and thrips, lowered tuber yields of potato clones. Tuber number/plant ranged from 2.9 (VR01-5-2-1412) to 10.5 (VR08-5-5-144), with an average of 6.5. Tuber yield ranged from 5.6 t/ha (398192.41-2) to 35.0 t/ha (VR24-5-10-149), with an average of 11.2 t/ha. Control variety 'Solara' tuber yield only reached 11.0 t/ha. The average tuber weight ranged from 19.1 g (398192.41-2) to 46.8 g (VR 01-5-2-147), with an average of 34.6 g. Marketable yield ranged from 38.5% (VR08-5-5-145) to 89.5% (VR24-5-10-7), with an average of 72.8%. Seventeen clones were selected.

Conclusions and recommendations

In the evaluation of potato germplasm in the RRD in Vietnam during crop season 2016–17, warm winter conditions were encountered which depressed potato productivity. Potato clones faced not only heat stress but also serious infection from mites and thrips. Therefore, the productivity of potato clones was very poor. However, clones with higher tuber yields could be considered as promising clones with resistance to mites and thrips.

Among 17 clones selected from 105 clones evaluated, only 3 had significantly higher tuber yields than 'Solara'. These were VR24-5-10-149, VR01-5-2-147, and VR08-5-5-144. These clones need to be further evaluated in the farmers' fields for advancement towards registration trials (Table 13).

TABLE 13. YIELDS AND COMPONENTS OF YIELDS OF THE CLONES SELECTED FROM A SET OF 105 CLONES AND LOCAL CHECK 'SOLARA' EVALUATED IN WINTER 2016–17, PLANTED ON NOV. 16, 2016, AND HARVESTED ON FEB. 27, 2017, CHUONG MY, HANOI, VIETNAM

Clones	Tuber/ Plant	Tuber Weight/ Plant (g)	Yield (t/ha)	Average Tuber Weight (g)	Large Tuber Number (%)	Marketable Yield (%)	Small Tube Number (%)	Non-marketable Yield (%)
VR24-5-10-149	15.3	700	35	45.7	60.9	85.7	39.1	14.3
VR01-5-2-147	9.4	440	22	46.8	36.2	81.8	63.8	18.2
VR08-5-5-144	10.5	250	12.5	23.8	34.9	60	65.1	40
LB44-1-4-5	6.8	241.4	12.1	35.3	56.1	79.3	43.9	14.1
VR01-8-3-142	5.2	233.3	11.7	45.2	48.4	78.6	51.6	21.4
VR01-5-2-143	7.9	227.1	11.4	29	38.4	65.8	61.6	13.3
SOLARA	6.4	220	11	34.4	62.5	72.7	37.5	27.3
LB44-1-4-2	5.9	200	10	34	56.6	77.8	43.4	22.2
VR01-8-3-147	5	195.8	9.8	37.8	49.6	88.8	50.4	6.3
VR24-5-10-7	3.8	190	9.5	50	78.9	89.5	21.1	10.5
VR08-5-5-141	4.5	175	8.8	38.9	55.6	71.4	44.4	28.6
VR01-5-2-13	3.9	155.6	7.8	40	48.6	78.6	51.4	21.4
398192.41-9	5.5	150	7.5	27.3	52.7	73.3	47.3	26.7
VR24-5-10-4	4.9	150	7.5	30.6	46.9	73.3	53.1	26.7
C88-1	6.4	144.4	7.2	22.4	39.7	61.5	60.3	38.5
VR08-5-5-145	7.1	130	6.5	18.3	15.5	38.5	84.5	61.5
VR01-5-2-1412	2.9	128.6	6.4	45	50	77.8	50	0
398192.41-2	5.9	112.5	5.6	19.1	42.6	55.6	57.4	44.4
Average	6.5	224.7	11.2	34.6	48.6	72.8	51.4	24.2

2.2.3 Output 3. Accelerated breeding methods (ABMs) and tools to help breeders select genotypes and parental lines in fewer years than with traditional clone-breeding schemes.

Deliverable 1: Strategies for selection and trait transfer in and among gene pools implemented.

Development and validation of molecular marker-assisted selection (MAS) for major disease resistances

CIP applied MAS and genomic selection (GS) approaches that accelerate breeding by increasing selection accuracy and incorporate new traits into potato.

Milestone achieved: Multiplex PCR marker to assist selection for two potato virus resistances (PVY and PLRV) validated in respective source-based test populations by Q3 2016.

A novel gene for resistance to PLRV from sources of *S. tuberosum* Andigenum Group was introduced into elite clones with ER to PVY, and 340 hybrid clones were selected for agronomical attributes from 24 segregating progenies. CIP had developed a tool that allows amplification of two PCR products, one the "RYSC3" molecular marker indicative of ER to PVY controlled by Ryadg, and "RGASC850," a sequence characterized amplified region marker linked to a novel gene for resistance to PLRV (Rl_{adg}). It was analyzed in this selected sample to validate the efficiency of the multiplex PCR marker to assist selection for both virus resistances (Fig. 5). A total of 68 hybrid clones were positive for the two markers (i.e., both markers were present), 115 were positive for the marker for PLRV resistance and 57 for that for ER to PVY; 100 lacked the two markers. The efficiency of the marker for ER to PVY was 88% as determined by comparing phenotypic responses from artificial inoculation versus marker presence. That for PLRV resistance was still not determined since low levels of PLRV infection of clones and controls after three exposure seasons did not provide reliable phenotypic responses to estimate the marker efficiency. A new trial under greenhouse conditions for more effective control of PLRV infection was established by the time of this final report. Once this multiplex PCR marker is validated, its usefulness on selecting breeding individuals would become mainstream in CIP's potato disease resistance efforts.

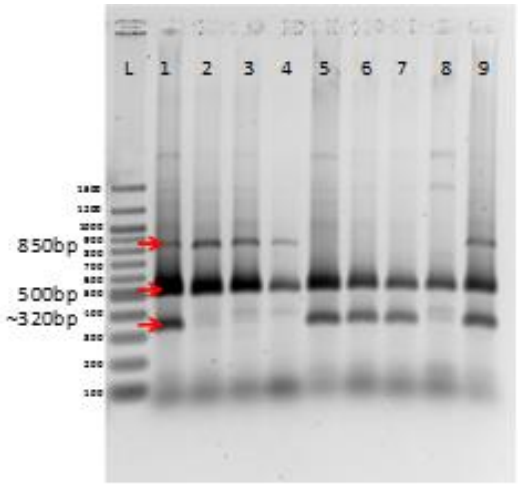


Figure 5. Amplification of a multiplex PCR marker for assisted selection of PVY and PLRV resistance. Restriction fragments were size separated by agarose gel electrophoresis on 1.5% agarose and visualized by GelRed nucleic acid gel staining (Biotium). Resistance gene markers "RGASC850" (850 bp) and "RYSC3" (320 bp) amplified by multiplex PCR marker using a mix of equal amounts of DNA from the PLRV highly resistant Andigena source LOP-868 and the PVY ER var. 'Costanera' (lane 1, resistance gene markers and a non-specific PCR product of 500 bp are indicated by arrows). Multiplex PCR marker amplified in the PLRV highly resistant Andigena sources LOP-868, HUA-332 and OCH-7643 (lanes 2–4), the var. 'Costanera' (lane 5), two CIP-bred PVY ER breeding lines, CIP 391180.6 and CIP 392820.1 (lanes 6 and 7), a PVY and PLRV susceptible breeding line (CIP 397073.16) (lane 8), and a hybrid genotype obtained from crossing CIP 391180.6 with HUA-332 (lane 9). Molecular weight marker 100 bp ladder, New England BioLabs (lane L).

A multiplex PCR marker that allows simultaneous selection for PVY and PLRV resistance is in its final validation step. This multiplex PCR marker, once

released, will support breeders interested in introducing and incorporating a novel major gene for high levels of PLRV resistance from Andigenum Group in their programs by requesting pre-bred parental clones and TS progenies from CIP developed from original sources under a background of heat tolerance and ER to PVY.

Milestone achieved: A marker to assist selection for LB resistance developed, tested, and validated (Q2 2017).

The Solanaceae Coordinated Agricultural Project marker SolCAP_SNP_c2-56148 tightly linked to LB resistance was converted to a high-resolution melting marker. A total of 396 breeding lines from CIP's potato breeding program belonging to the LB resistance (A, B1, B3), LTVR, and bacterial wilt resistance (BW) populations, combinations between B3 and LTVR, and the B3-HT population were analyzed for the marker genotype. Among the 396 breeding lines genotyped, the most frequent SNP c2_56418 marker genotypes detected by high-resolution melting were AAAB and AAAA. Only a few breeding lines presented duplex allele dosage for the resistance allele (B), and no triplex or quadruplex individuals for this single nucleotide polymorphism (SNP) allele were detected. The marker genotype AAAA was more frequent among the potato genotypes in the breeding populations A, B1, and LTVR, whereas the marker genotype AAAB was more frequent in the B3 population that has been specifically bred for quantitative LB resistance (Table 15). In the newer populations that are in their earlier cycles of selection (combinations between B3 and LTVR, and the B3-HT population) and in the BW population, the frequencies of both marker genotypes are nearly equal (Table 15).

TABLE 15. FREQUENCY OF THE SNP C2_56418 MARKER GENOTYPES AAAA AND AAAB (OR AABB) IN CIP POTATO BREEDING POPULATIONS

Population	A	B1	B3	B3-HT	B3-LTVR	BW	LTVR	LTVR-B3
(n)	-13	-22	-100	-37	-13	-15	-168	-12
AAAB	23%	14%	58%	51%	46%	47%	31%	50%
AAAA	77%	86%	42%	49%	54%	53%	69%	50%

A subset of 215 breeding lines, mostly from the B3 and LTVR populations, were phenotyped for LB resistance in the field in Peru under high endemic disease pressure. The resistance in the field was evaluated using alpha lattice design, with three replications and 10 hills per replication per repetition and including susceptible and resistant control varieties. The disease levels were evaluated weekly until the susceptible controls were 100% infected and the percent leaf area affected values were used to calculate the area under the disease progress curve (AUDPC) values for each genotype. The disease levels were evaluated weekly until the susceptible controls were 100% infected and the percent leaf area affected values were used to calculate the AUDPC values for each genotype. The AUDPC values were converted to sAUDPC values that are easier to compare among locations and years. The conditions in the field were favorable for disease development, judging by the reactions of the control varieties. The most susceptible control variety, 'Tomasa', reached 100% infection at the fifth evaluation and the remaining susceptible controls were fully infected by the seventh evaluation. The resistant control variety LBr40 remained uninfected throughout the experiment. The AUDPC values ranged from 0 to 3,043. For calculating the sAUDPC, a susceptible cultivar 'Desiree' with AUDPC 2376 was assigned the value of sAUDPC 8. The distribution of the sAUDPC values was skewed toward low values, especially in the populations B3 and B1, which target LB resistance as the main trait (Fig. 6a).

The presence of the contrasting marker genotypes AAAA versus AAAB (or AABB) was as follows: LTVR, 72 versus 26; B3, 39 versus 54; and B1, 14 versus 2. In both B3 and LTVR populations, the potato genotypes that contained the B allele were on average more resistant than those that contained only the A allele (Fig. 6b). In the B3 population, there were 35 genotypes with sAUDPC value 0 and all contained the marker allele B. The same was true in the LTVR population only for 1 potato genotype.

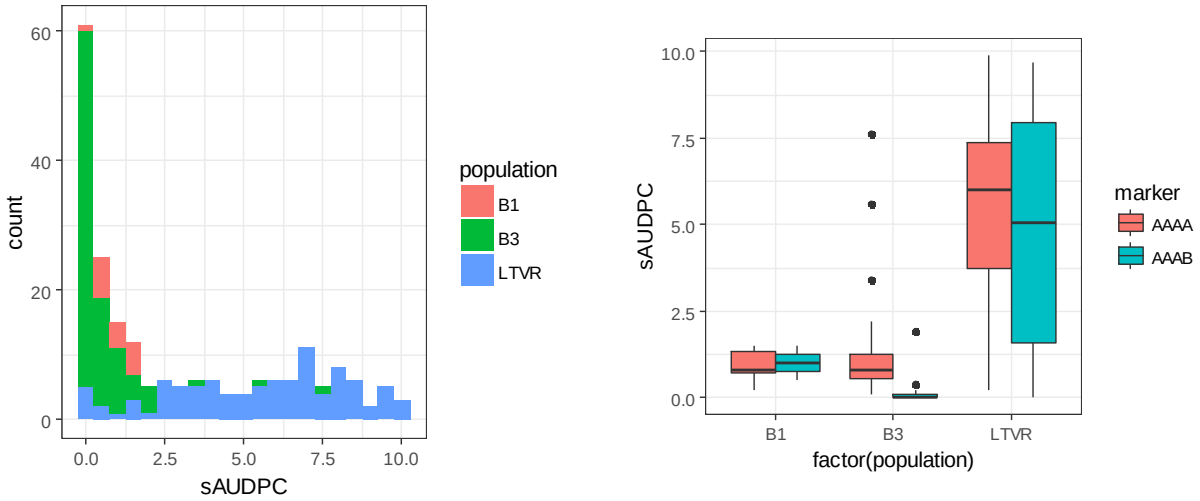


Figure 6a. Histogram of the distribution of LB resistance levels in the breeding populations B1, B3, and LTVR in the field trial at 2014. LB resistance was measured on the susceptibility scale (sAUDPC), where increasing values indicate decreasing level of resistance. Figure 6b. Boxplot on the marker SNP c2-56418 genotype (AAAA vs. AAAB) effect on the LB resistance level in the breeding populations B1, B3, and LTVR. LB resistance was measured on the susceptibility scale (sAUDPC), where increasing values indicate decreasing level of resistance.

On the basis of the marker genotype and resistance phenotype, 11 breeding lines from B3 and LTVR populations were chosen as parents, and crosses were made to study the marker genotype and resistance phenotype segregation in the progenies. The crosses were made in 2015, and the TS from the crosses were planted in the greenhouse in seedling trays and finally transferred in pots for minituber production. Leaf samples were collected from all individuals for DNA extraction following the protocol described above. Minitubers were harvested and planted in the field for seed multiplication in 2017. Finally, the basic seed was planted in the field for resistance phenotype evaluation following the same protocol as detailed above for LB resistance evaluation.

The SNP marker c2_56418 is linked to ER for LB resistance in the B3 population but not in the LTVR and B1 populations. The marker allele for resistance is most frequently present in simplex form and is present in almost 60% of the breeding lines in the B3 population. This marker can be used in forward selection of highly resistant individuals in B3 population intracrosses. Final validation in crosses among different breeding populations is pending the phenotypic evaluation taking place late 2017.

Milestone achieved: A set of diverse, complementary genes for building durable resistance to LB from wild sources identified by Q3 2018.

CIP tetraploid breeding population B3 has been bred for quantitative LB resistance. We have shown that the resistance in this population is largely based on a quantitative trait locus (QTL) dPI09c in chromosome 9 (Li et al. 2012; Lindqvist-Kreuze et al. 2014). The resistance conferred by this QTL is expected to be more durable because it is quantitative and confers broad spectrum resistance. Since 2013, in collaboration with Huazhong Agricultural University, China, and with substantial co-funding by China NSF, we have fine-

mapped this resistance to a 186-kb region utilizing a bi-parental population B3C1HP developed at CIP. Owing to the large effect (explaining up to 70%) of the QTL for resistance phenotype we hypothesized that an R gene could explain the resistance. Therefore, we conducted a dRenSeq (Van Weymers et al. 2016) analysis to enrich the genomic DNA for NB-LRR type R genes utilizing the resistant parent 301071.3, susceptible parent 703308, and bulks consisting of 27 resistant and 27 susceptible progenies. DNA sequence reads were mapped, at high stringencies, against 9 functional LB NB-LRR genes: Rpi-blb1, Rpi-blb2, R1, R2, R3a, R3b, R8, R9a, and Rpi-vnt1.1. Mapping results demonstrated that the reads of the resistant parent and resistant bulk generated full coverage of R8, while only partial coverage was achieved using reads from the susceptible parent or the susceptible bulk. These results strongly suggest that R8 could be a main contributor toward the function of dPI09c for LB resistance. To confirm the presence of a complete and intact R8 in the B3C1HP population, R8-specific primers were utilized to amplify a 7-kb fragment that encompasses functional R8 (including coding and regulatory sequences). Our analysis confirmed that the resistant parent and the resistant progenies contained the R8 gene and no sequence variation was identified. R8 originates from the wild species *Solanum demissum* (Vossen et al. 2016) and has been considered as a valuable resistance source because the virulence to this gene is rarely found in the populations of *P. infestans.*

As shown in the previous section, the resistance conferred by chromosome 9, and most likely based on the R8 gene, is the main source of resistance in the CIP B3 potato breeding population. To expand the genetic basis of LB resistance, other wild sources have been utilized in pre-breeding. They have already been introduced to the tetraploid materials with the help of bridge crosses with diploid landraces of Phureja and Stenotonum groups and subsequent introgression by unilateral sexual polyploidization to 4x advanced breeding lines. The resistance sources include species from the Series Piurana and Tuberosa. The resistance in these species is hypothesized to be unique, since especially the Piurana clade species are taxonomically distinct from the cultivated potatoes. Earlier work had demonstrated that the LB resistance from *S. paucissectum* (Series Piurana) is conferred by a major QTL in chromosome11 (Villamon et al. 2005), suggesting that it would be a welcome diversifying source of resistance. With co-funding from the European Union (Project G2PSOL), we are currently investigating the genomic location of the resistance provided by three more species, *S. chiquidenum* (Series Piurana) and *S. cajamarquense* (Series Tuberosa), as well as *S. paucissectum.* The resistant genotypes from these species that had been used as resistance donor in the pre-breeding program described above were crossed with self-compatible hybrid derived from *S. tuberosum* Phureja Group and Sli-bearing *S. chacoense*. TS (range = 522–4,579 seeds/progeny) of the progenies were germinated and multiplied by stem cuttings. Finally, 150 genotypes of each of the three families will be planted in the field to evaluate LB resistance. Leaf samples from original parents and the 150 progeny genotypes from each family were collected and stored at -20°C for subsequent DNA extraction. Phenotypic data on LB resistance and SNP marker data generated by genotyping by sequencing from these families will be used for genetic mapping of major genes or QTL associated with LB resistance derived from these novel sources.

LB resistance gene R8 originating from Mexican species *S. demissum* was discovered in the CIP LB-resistant population B3. Resistances from other wild South American sources from Series Piurana and Tuberosa have been introduced in the advanced tetraploid breeding lines. The genetic characterization of these new sources is under investigation.

Identification of trait-specific markers for nutritional content and abiotic stresses for pre-screening of progenies and identification of parental lines

Milestone achieved: Candidate genes for iron and zinc content identified by Q3 2016.

CIP's breeding efforts are oriented toward current demands for disease-resistant and highly nutritious potatoes, and its biofortification efforts are focused on the development of diploid and tetraploid potatoes with high micronutrient contents. Genetic variability for Fe and Zn content and good tuber quality for fresh and processing consumption of diploid-cultivated potatoes of Stenotomum and Phureja groups have been exploited across three cycles of recurrent selection, followed by 4x-2x crosses performed to transferred genetic gains into 4x potatoes. Marker-assisted breeding is a tool that may enhance selection accuracy and expedite selection cycles. Two positional candidate SNP markers for Fe and Zn contents were identified by genome-wide association studies (GWAS) in 2x potato landraces: one associated with both minerals is located at 1.0 Mbp of a gene encoding a ZIP metal transporter protein on chromosome 8, and the other associated with Zn content at 8.0 Mbp from another ZIP metal transporter on chromosome 1. This latter showed stability as proved by its detection in two locations of the Peruvian highlands. Further work, including finding the tag sequences from which these genotyping by sequencing-derived SNP were identified and aligning them to the potato reference genome, will contribute long sequences for developing SNP-based Kompetitive Allele Specific PCR genotyping assays for MAS. Validation of these markers may provide a molecular tool for early screening of 2x derived biofortified potatoes. Furthermore, their application in conjunction with other genomic approaches may support conventional breeding for high nutritional potatoes.

Milestone achieved: Candidate genes for early tuberization and bulking under warm and long photoperiod identified by Q2 2017.

Tuber initiation and bulking are key traits in determining potato yield and adaptation. We evaluated several relevant variables related to these traits under long photoperiod and warm conditions in a panel of 171 4x breeding lines, and conducted a GWAS for identifying trait-associated SNP markers using 4738 SNP obtained from genotyping the population with the SolCAP Infinium SNP array (8303 SNP). We found three interesting SolCAP SNP markers highly associated with tuber initiation and bulking whose allelic dosage displayed an additive effect on these traits. The Solcap_snp_c2_56256 located at `12.4 cM on chromosome 4; Solcap_snp_c2_25926 at 46 cM on chromosome 6, and Solcap_snp_c1_2280 at 39.5 cM on chromosome 11 showed their minor frequency allele (the allele with less frequency in the population) to increase tuber bulking at 90 days after planting (DAP), tuber bulking at 75 DAP, and tuber initiation, respectively (Table 16, Fig. 7). SNP markers Solcap_snp_c2_56256 and Solcap_snp_c1_2280 are located within genes annotated as Pheophorbide A oxygenase (PaO) and pullulanase, respectively. PaO plays a key role in the regulation of the biochemistry of chlorophyll catabolism during senescence. It is located in the inner envelope of senescing chloroplast and is responsible for the loss of pigment color. Since chlorophyll turns into a threat to the plant when it is released from its apoprotein environment during uncontrolled senescence, PaO is a key enzyme in chlorophyll catabolism and hence it is a senescence-specific regulated protein (Pruzinska et al. 2003). On the other hand, pullulanase is a glucan hydrolase (starch debranching enzyme) whose function has been implicated in starch synthesis and degradation (Dinges et al. 2003). The role of PaO and pullulanase in potato plant senescence, tuberization, and starch accumulation has not yet been studied. We observed that genotypes with the least frequent allele, denoted as "B" in Table 16, in duplex (AABB) and greater state (ABBB and BBBB) simultaneously at the three associated SolCAP SNPs, display greater tuber initiation (as indicated by tuber induction values), bulking, and consequently number of marketable tubers. The opposite was true for the allele at the highest frequency denoted as "A" (Table

16). Further studies and the development of a genotype dosage assay such as high-resolution melting for applying these markers to assist in early identification of tuberization and bulking of potatoes under long day-length are required. Recombinant individuals have been identified in this survey (Table 17), and associated SNP markers in combination are not able to unambiguously classify all breeding lines analyzed (n = 171) into early, intermediate, or late-bulking maturity. Therefore, we suggest that these markers be used for pre-screening, to select and discard extreme genotypes at early stages before another approach such as genomic selection (GS) is applied. Otherwise, they can be tracked in the course of GS.

With support from the United States Agency for International Development (USAID), CIP's potato breeding program is applying novel MAS-based genomic tools to open new ways for accelerating and enhancing selection of early bulking potatoes under long day-length conditions. Three SNP markers positively associated with early tuberization and bulking were identified by GWAS and were located within genes annotated as senescence-specifically regulated and starch synthesis and degradation proteins. SNPs showed additive allele effects on early tuber initiation and bulking, and thus require development of a genotype dosage assay for testing and validation.

TABLE 16. MEANS PER DOSAGE STATE OF SNP MARKERS ASSOCIATED WITH TUBER INDUCTION AND BULKING (AVERAGE OF 171 ADVANCED BREEDING CLONES)

SolCAP SNP	solcap_snp_c1_2280	solcap_snp_c2_56256	solcap_snp_c2_25926
Annotation	Pullulanase	Pheophorbide A oxygenase	Nucleic acid binding protein
Chromosome	Chr 11	Chr 4	Chr 6
Allelic dosage stage / Trait	Tuber induction[1]	Bulking 90 DAP[2]	Bulking 75 DAP[2]
AAAA	4.5 d	19	10 c
AAAB	4.7 cd	24 b	20 c
AABB	5.3 b	30 ab	27 b
ABBB	5.3 bc	33 a	28 b
BBBB	7.3 a	35 a	39 a

NOTE: Values with the same letter are not statistically different, $p<0.05$.

[1] Scale 1 (no induction), 9 (strong induction). Percentage of well-developed tubers at 75 or 90 DAP.

TABLE 17. GENOTYPIC CONFIGURATION FOR THREE TUBERIZATION AND BULKING-ASSOCIATED SOLCAP SNP MARKERS AND THEIR EFFECT ON THE PERFORMANCE OF VARIETIES AND CIP'S 4X BREEDING CLONES TESTED UNDER LONG DAYS AND WARM CONDITIONS

Description	Clone name/ Number of clones	solcap_snp_c1_2280	solcap_snp_c2_56256	solcap_snp_c2_25926	Tuber Induction[1]	Bulking[2] (75 DAP)	Bulking[2] (90 DAP)	Number of marketable tubers	Number of marketable tubers
Long day-length adapted controls	Atlantic	AABB	AABB	ABBB	6	49	58	19	18
	Desiree	AABB	ABBB	AABB	7	41	41	20	19
	DTO-33	BBBB	AABB	AABB	8	32	38	21	26
	Spunta	AABB	AABB	ABBB	8	46	52	15	21
CIP advanced breeding clones	Mean of 29 clones	ABBB/AABB/BBBB	ABBB/AABB/BBBB	ABBB/AABB/BBBB	**5.4**	**37**	**40**	**19**	**24**
	Mean of 7 clones (recombinants?)	ABBB/AABB/BBBB	ABBB/AABB/BBBB	ABBB/AABB/BBBB	**5.4**	**12**	**19**	**5**	**11**
Short day-length adapted controls	Tomasa	AAAB	AAAB	AAAB	4	0	4	0	1
	Yungay	AAAB	AAAB	AAAB	5	20	31	8	23
CIP advanced breeding clones	Mean of 13 clones	AAAA/AAAB/ AABB	AAAA/AAAB	AAAA/AAAB	**4.5**	**14**	**21**	**5**	**10**

[1] Scale 1 (no induction), 9 (strong induction).

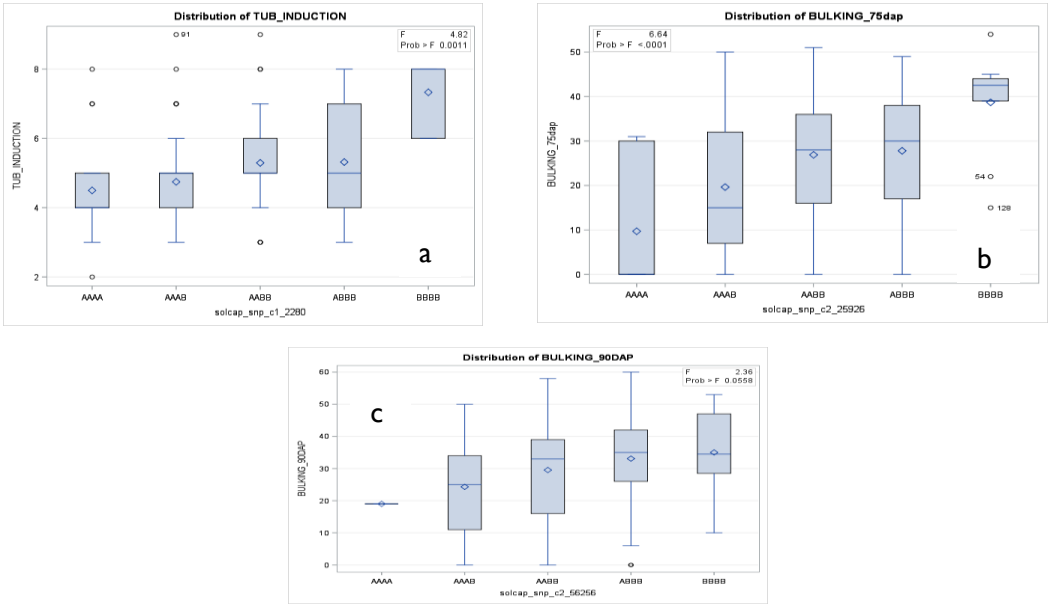


Figure 7. Boxplot of the distribution of breeding lines based on their genotype for SNP markers associated with tuber induction and bulking at 75 and 90 DAP.

Diploid pre-breeding for capturing new diversity and developing complementary inbred lines

Milestone achieved: The S-locus inhibitor gene confirmed as a source for overcoming reproductive barriers in new interspecific crosses by Q3 2016.

We proposed that the S-locus inhibitor (*Sli*) gene from wild *S. chacoense* species that is able to change self-incompatible (SI) status to self-compatibility (SC) may overcome pre-zygotic interspecific reproductive barriers. Following the rule of unilateral compatibility by which SI x SC crosses are incompatible while SC x SI crosses are compatible, we proposed that pollen of SI wild species from the Piurana and Tuberosa

clade might be accepted on pistils of 2x cultivars that have been turned into compatible hybrids by introgression of the *Sli* allele via hybridization. However, this hypothesis was not successfully proved, as very few seeds/berry (average of 4 seeds/berry) or no seeds at all were obtained as had occurred in original crosses of wild *S. piura*e and *S. chiquidenum* genotypes with SI 2x landraces. Most crosses required mentor pollen intervention to prevent premature fruit drop. Two crosses were the most successful, one between a *S. piura*e genotype with a SC 2x hybrid that produced 34 seeds in one berry, and the other with a *S. chiquidenum* genotype that produced on average 35 seeds/berry in 8 berries harvested. In both cases mentor pollen was required to prevent early fruit drop. These crosses were completely incompatible (no seed produced) when they were crossed to 2x SI landraces (i.e., without *Sli*), even with intervention of mentor pollen. Nevertheless, these results look promising because these cross combinations normally require pollination of a much larger number of flowers (a range of 20–60 flowers) to obtain a berry. Upon learning more about the mechanism of *Sli*, and in light of these results, we have modified the approach to testing whether *Sli* might overcome interspecific reproductive barriers by using *Sli*-bearing donors as male parents. This approach is supported by previous studies that have shown *Sli* to be a sporophytic pollen side mutation; hence it could prevent pollen rejection by evading pistil S-RNase cytotoxicity. This new approach will be tested in 2017–2018.

Milestone partially achieved: Inheritance of Self Compatibility in 2x landraces determined by Q1 2017.

Breeding gains in tetraploid potato are limited by low recombination, a long generation cycle, polyploidy, and inbreeding depression. Diploid inbred line potato breeding is a new strategic approach for the development of genetically uniform TS varieties, self-fertile, and highly desirable TS-propagated lines. This approach seeks to fully exploit the advantages that breeding potato at the 2x level offers with regard to tetraploid (4x) breeding. The use of closely related diploid species and dihaploid potatoes to develop inbred lines is hampered by gametophytic SI controlled by multi-allelic S genes. The *Sli* identified previously in certain accessions of *S. chacoense* that alters SI plants to SC ones (Hosaka and Hanneman 1998) have been widely used in the development of diploid inbred lines, although with the imminent dragging of unfavorable alleles from these wild SC sources (Phumichai et al. 2006). Previous studies at CIP identified five SC sources in the cultivated *S. tuberosum* Phureja and Stenotomum groups of germplasm. With USAID funding, we studied whether SC was genetically transmitted to progenies generated from selfing these sources. We found progenies segregating for SC and SI from selfing SC CIP705468, CIP703320, and CIP701165. Lack of flowering in almost half of each progeny was most likely due to the unfavorable season as well as pollen sterility in some plants, reduced the population size of progenies to 55, 61, and 41 individuals that, respectively, displayed SC to SI ratios of 2.4 SC: 1 SI, 0.5 SC: 1 SI, and 1.7 SC: 1 SI (Table 18).

SC plants due to pistil side mutations that compromised S-RNase or HT factors segregate only SC plants. The same is true for gain of function mutations of the SLF gene—the pollen determinant of S-specificity (McClure et al. 2011). On the other hand, *Sli* locus is a sporophytic pollen side mutation independent from the gametophytic SI system. Therefore, a SC plant with a *Sli* mutation in heterozygocity produces 3 SC: 1 SI plants regardless of its haplotype condition in the SLF locus (i.e., SxSx, SxSy, SySy). Hence, the presence of SI plants in the progenies of SC 2x landraces in our study suggests a pollen side *Sli*-like mutation since this is the most plausible scenario that yields SI progenies. Unfortunately, small population sizes yielded different segregation ratios across progenies, and none of them fit the expected Mendelian ratio of 3:1. Further studies that include performing reciprocal crosses between SI and SC plants within a segregating progeny, provided that both siblings have the same S haplotypes, will allow us to prove if the defect

corresponds to a *Sli*-like mutation. Here we expect SC x SI to be incompatible whereas SI x SC should be compatible. If SC in 2x landraces is a pollen-side mutation independent of the gametophytic SI system, these will provide a more desirable SC source for developing 2x inbred lines than the wild species donor *S. chacoense*.

Genetic studies to elucidate the nature of SC in three SC landraces from group Stenotomum suggested a novel pollen-side gain of function factor or *Sli* gene variant as responsible for SC. These SC cultivars will provide a more desirable SC source for developing 2x inbred lines than the wild species donor *S. chacoense*.

TABLE 18. SEGREGATION FOR S AND SI OF THREE SC 2X CULTIVARS OF *S. TUBEROSUM* STENOTOMUM GROUP

CIP Number	Family Code	Pedigree	Pop. Type	No. of Not Flowering Plants	No. of Plants with Sterile Pollen	No. of SI Plants	No. of SC Plants	Total SI + SC	% of SC*
CIP515017	BSEL2	705468 (x)	S_1	91	13	16	39	55	71
CIP 515018	BSEL3	703320 (x)	S_1	96	14	42	19	61	31
CIP 515019	BSEL4	701165 (x)	S_1	62	28	15	26	41	63

*Percentage of well-developed tubers at 75 or 90 DAP. Number of marketable tubers in 6 hill plots.

Milestone achieved: Promising dihaploids derived from 4x breeding lines with earliness, heat tolerance, and virus resistance identified by Q1 2017.

The first step in a potato breeding program at the diploid level is the production of dihaploids (2n=2x=24). Dihaploids extracted from 4x potatoes are produced for better understanding of the genetics of complex agronomic and quality traits, full use of current genomics tools for mapping, and as a route for developing 2x inbred lines and capturing genetic diversity from 2x wild species in diploid inbred line potato breeding. We set up a crossing plan for haploidization of 36 4x elite breeding lines with variation for yield, quality traits, resistances to biotic constraints, heat tolerance, and long photoperiod adaptation from CIP's breeding program and two long photoperiod adapted foreign varieties—'Desiree' (HZPC, UK) and 'Atlantic' (USDA, USA). Three haploid inducers were used as pollen parents (IVP-35, IVP-101, and PL-4). A total of 21,274 botanical seeds were produced from 32 breeding lines and the two foreign varieties. A total of 4,596 seeds (22%) were visually regarded as putative dihaploids since they lacked the embryo seed marker spot indicative of hybrid seed inherited from the haploid inducer. A unique identification (CIP number) was assigned to each seed progeny and registered in CIP-Cross database. We identified 1,599 dihaploids upon spotless seed planting, and ploidy confirmation through seedling nodal band assessment (homologous to embryo spot), cell guard chloroplast counting, and flow cytometry. Tubers were recovered at harvest from 1,044 dihaploids, but only 885 dihaploids could be planted in 2017 because 155 genotypes had "blind eyes" and never sprouted. We evaluated plant vigor, earliness, and growth habit during plant growing and characterized tubers for shape, size, skin color, and breeders' opinion at harvest. A total of 833 dihaploids tuberized from 31 advanced breeding lines and varieties, recovering 1–106 tubers/genotype from a total of three plants per genotype. We identified 167 dihaploid that showed appealing tubers of more than 2.5 cm. Worth highlighting is the production of 211 dihaploid genotypes from the parental breeding line C93.154 (CIP 392820.1). The line has several attributes such as ER to PVY, resistance to PLRV and LB, high fertile pollen production, and combining ability for yield and tuber quality traits, as well as being progenitor of several breeding clones selected for adaptation to long days and tolerance to heat and

drought. This 2x segregating population is suitable for applying modern genetic and genomic tools for discovery and mapping of genes for biotic and abiotic stresses, earliness, and tuber quality and yield. On the other hand, the collection of 167 selected dihaploids will represent a 2x genetic resource for the development of 2x inbred stocks for TS hybrid potato breeding following current procedures for inhibiting SI and tuber shape and size grading of the recipient inbred line. This collection will be screened for heat tolerance under greenhouse conditions during spring–summer 2017–18 at CIP's station in La Molina (Lima, Peru). Table 19 shows morphological characterization and agronomical attributes of 21 top selected dihaploids, photographed in Figure 8.

TABLE 19. TOP 21 SELECTED DIHAPLOIDS BASED ON TUBER APPEALING AND NUMBER OF TUBER GREATER THAN 2.5 CM

Item	Dihaploid Breeder Code	Female CIP No.	Female Breeder Code	Haploid Inducer Male Parent	Vigor	Earli-ness	Growth Habit Type	Tuber Shape	Tuber Skin Color	Tuber Skin Color Intensity	No. of Tubers >25 Mm	Total No. of Tubers
1	**35-DT1.001**	CIP300056.33	LR00.014	IVP-35	5	7	Tuberosum	6	2	2	33	44
2	**PL-HT10.191**	CIP392820.1	C93.154	PL-4	7	3	Tuberosum	6	2	1	33	65
3	**PL-HT1.019**	CIP300048.12	LR00.006	PL-4	5	3	Tuberosum	4	2	2	31	51
4	**PL-DT8.103**	CIP397077.16	WA.077	PL-4	5	3	Tuberosum	4	2	2	25	43
5	**PL-HT11.004**	CIP392822.3	LR-93.073	PL-4	5	3	Tuberosum	7	2	2	25	38
6	**PL-HT2.030**	CIP300072.1	LR00.022	PL-4	7	3	Tuberosum	6	2	1	25	30
7	**35-HT6.001**	CIP392820.1	C93.154	IVP-35	5	5	Tuberosum	4	1	3	24	65
8	**PL-HT11.005**	CIP392822.3	LR-93.073	PL-4	5	3	Tuberosum	7	2	2	22	34
9	**PL-HT18.002**	CIP398190.89	398190.89	PL-4	-	-	-	6	2	1	22	27
10	**101-HT3.004**	CIP388615.22	C91.640	IVP-101	5	5	Tuberosum	2	2	1	21	29
11	**35-HT1.006**	CIP300048.12	LR00.006	IVP-35	7	7	Tuberosum	4	2	1	21	36
12	**PL-DT4.016**	CIP390637.1	93.003	PL-4	3	5	Tuberosum	4	2	2	21	41
13	**PL-HT10.022**	CIP392820.1	C93.154	PL-4	3	5	Tuberosum	4	2	1	21	30
14	**PL-HT2.017**	CIP300072.1	LR00.022	PL-4	5	5	Tuberosum	4	2	1	21	41
15	**35-DT5.021**	CIP390637.1	93.003	IVP-35	5	5	Tuberosum	4	1	2	20	42
16	**35-DT5.005**	CIP390637.1	93.003	IVP-35	5	5	Tuberosum	4	2	1	20	47
17	**35-DT8.022**	CIP397077.16	WA.077	IVP-35	5	7	Tuberosum	5	2	2	20	73
18	**35-HT1.005**	CIP300048.12	LR00.006	IVP-35	7	7	Tuberosum	4	2	1	20	37
19	**PL-DT8.105**	CIP397077.16	WA.077	PL-4	5	5	Tuberosum	7	2	1	20	30
20	**PL-HT15.009**	CIP397039.53	C97.182	PL-4	5	3	Tuberosum	6	2	1	20	25
21	**PL-HT2.012**	CIP300072.1	LR00.022	PL-4	5	3	Tuberosum	4	2	2	20	40

NOTE: Vigor: 1 (the least), 9 (the most); Earliness: 1 (very late), 9 (very early); Growth habit type: tuberosum, intermediate, or Andigena type; Tuber skin color and intensity following CIP descriptors.

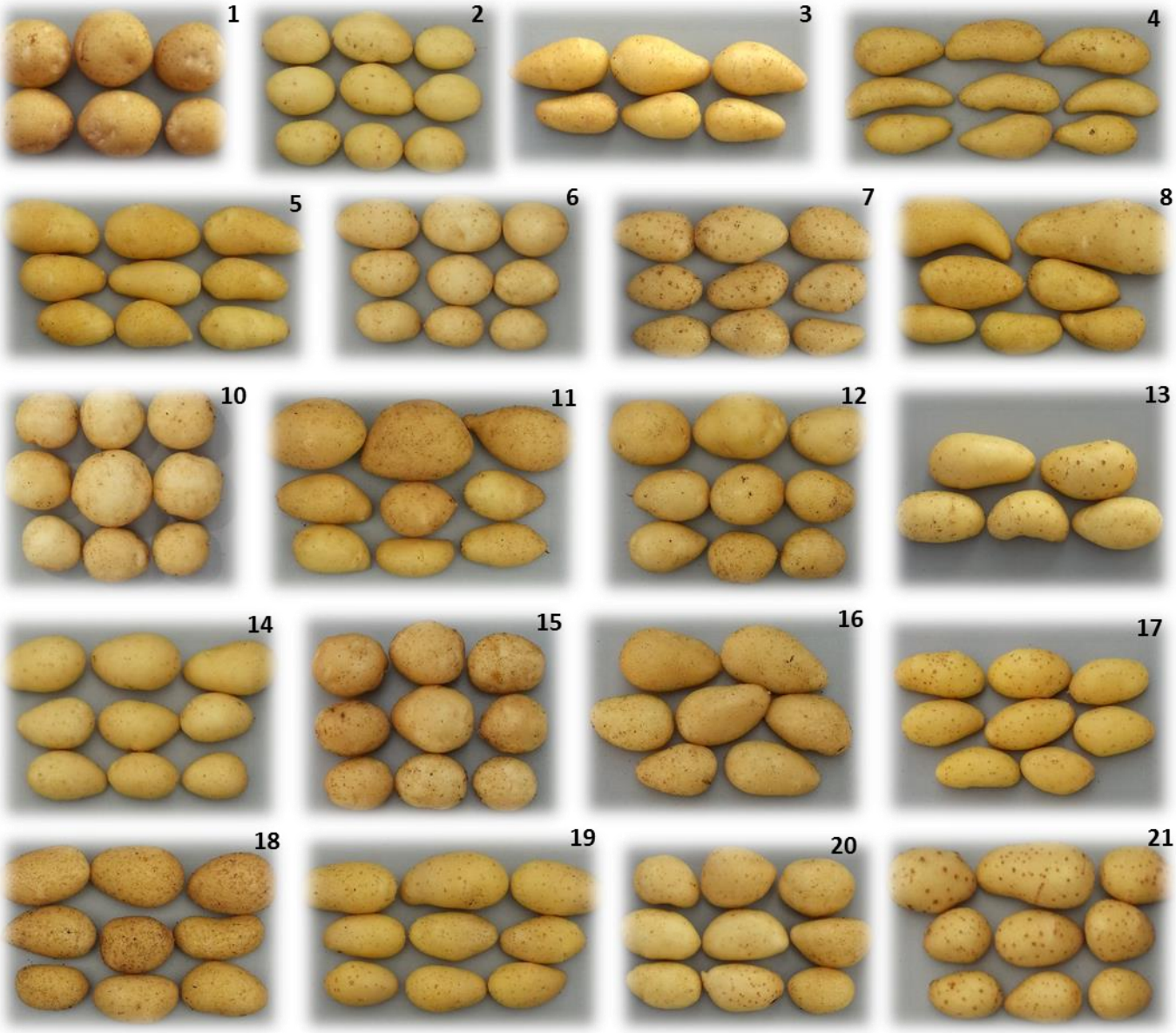


Figure 8. Top 20 dihaploids selected based on number of tuber >25 cm (see Table 19 for dihaploid genotype identification).

Milestone partially achieved: *Sli* introgressed into promising dihaploid material for inbreeding initiation by Q3 2017.

This deliverable was not achieved because top dihaploids in which *Sli* had to be introgressed were selected at the time this report was prepared.

Application of genomic selection to accelerate genetic gains for complex traits

Milestone achieved: GS models for tuberization and tuber bulking under warm and long photoperiods validated in potato hybrids selected in target countries by Q3 2017.

Application of molecular genetics in crops aims at using information at the DNA level to achieve faster genetic gains than in conventional breeding based on phenotypic data only. GS is a model-based molecular approach that estimates marker effects across the whole genome using a prediction model developed in a training breeding population that have been genotyped and phenotyped for complex traits of interest. Prediction models are used to produce genomic-estimated breeding values (GEBV) from which candidate breeding lines can be selected by genotyping before phenotypic evaluation. Feasibility for applying GS for traits associated with early tuberization and bulking was previously performed at CIP in a "training population" (TP) of 177 advanced breeding clones by means of cross-validation (CV) prediction. Predicting ability for traits associated with tuberization and bulking was performed by comparing CV predictions with actual phenotypic observations in several testing sets created at random from the TP. We obtained acceptable prediction accuracies ($r^2 \geq 0.3$) for bulking ratio at 75 and 90 DAP, stolon length, and marketable tuber number at 75 DAP. In this project, 24 TS progenies were generated from crosses among 16 breeding lines selected based on GEBV estimated for these traits in the TP. Progenies were dispatched to our partners at the Root Crop Research and Development Center (RCRDC) in Hanoi, Vietnam, where 191 hybrid clones were selected for agronomical attributes. The selected panel that would represent the GS "validation population" (VP) was phenotyped for stolon number and length as well as number of tiny, small, medium-, and large-sized tubers under field conditions. Data on tuber size were used to calculate bulking ratio as the percentage of the number of well-developed tubers (medium and large tubers) over the total number of tubers (tiny + small + medium + large) as well as number and total weight of marketable tubers using medium- and large-sized tubers. Leaf samples for DNA extraction were taken from each clone and sent to CIP–Lima for DNA extraction; unfortunately, samples arrived rotten as they were not properly dried. Therefore, genotyping of this validation set was not possible. Genotypic data are required to calculate the GEBVs for each trait in the 191 selected hybrid clones using models developed in the TP. These GEBVs are required to calculate predicted breeding values for being validated by comparison with actual phenotypic data. Accuracy of GEBVs in the VP is expected to be maximized since the TP is representative of the selected 191 candidates. However, since selections were performed in another environment—though representing also the lowland subtropics under which the TP was evaluated—QTL interactions with the environment may hamper accurate estimation of marker effects and therefore decrease GEBV accuracies. These hypotheses need validation. Table 20 and Figure 9 illustrate some selections and phenotypic data from 21 hybrid clones from validation set.

TABLE 20. TOP 21 HYBRID CLONES FROM GS VALIDATION POPULATION EVALUATED AT THE RCRDC

CIP No.	Breeder Code	Female	Male	Tuber Shape	Skin Color	Tuber Flesh Color	Eye Depth	Bulking Ratio (%)	No. of Marketable Tubers/Plant	Marketable Tuber Weight/ Plant (g/plt)	Mean Weight of Marketable Tubers (g)
CIP314969.012	VIET23-15.12	C91.640	C95.276	Oval	Pale yellow	Light yellow	Shallow	73	4	286	75
CIP314957.016	VIET11-15.16	C95.416	C95.276	Oval	Intermediate pink	Light yellow	Medium	50	3	270	84
CIP314959.068	VIET13-15.68	C97.182	LR00.022	Oval	Pale yellow	White	Shallow	68	3	240	74
CIP314957.008	VIET11-15.8	C95.416	C95.276	Oval	Pale yellow	Cream yellow	Shallow	55	2	238	108
CIP314960.069	VIET14-15.69	LR00.022	C95.276	Oval	Deep cream	Yellow	Medium	87	3	230	69
CIP314966.007	VIET20-15.7	95.118	C95.276	Oval	Pale yellow	Yellow	Shallow	47	4	223	64
CIP314957.001	VIET11-15.1	C95.416	C95.276	Oval	Pale yellow	Yellow	Shallow	59	4	215	59
CIP314965.051	VIET19-15.51	Maria Bonita-INIA	C95.276	Oval	Pale yellow	Yellow	Medium	50	2	214	97
CIP314959.047	VIET13-15.47	C97.182	LR00.022	Oval	Pale yellow	Cream yellow	Medium	43	4	210	53
CIP314969.079	VIET23-15.79	C91.640	C95.276	Flat round	Pale yellow	White	Medium	77	2	206	103
CIP314964.032	VIET18-15.32	Maria Bonita-INIA	C93.154	Oval	Deep cream	Yellow	Shallow	50	3	205	63
CIP314960.053	VIET14-15.53	LR00.022	C95.276	Oval	Pale yellow	Yellow	Shallow	45	3	200	80
CIP314968.073	VIET22-15.73	C91.640	C93.154	Oval	Pale yellow	Yellow	Medium	80	2	176	73
CIP314970.015	VIET24-15.15	LR-93.073	C93.154	Flat round	Pale yellow	Yellow	Medium	40	3	164	61
CIP314958.069	VIET12-15.69	C97.182	C93.154	Oval	Intermediate cream	Yellow	Medium	43	2	160	89
CIP314960.013	VIET14-15.13	LR00.022	C95.276	Oval	Pale yellow	Light yellow	Shallow	40	2	156	64
CIP314970.019	VIET24-15.19	LR-93.073	C93.154	Round	Pale yellow	White	Shallow	81	2	151	62
CIP314965.024	VIET19-15.24	Maria Bonita-INIA	C95.276	Oval	Intermediate cream	Yellow	Medium	62	2	136	85
CIP314960.056	VIET14-15.56	LR00.022	C95.276	Oval	Pale yellow	Yellow	Shallow	38	2	120	67
CIP314960.011	VIET14-15.11	LR00.022	C95.276	Oval	Pale yellow	Yellow	Shallow	67	2	107	64
CIP314960.050	VIET14-15.50	LR00.022	C95.276	Oval	Pale pink	Cream yellow	Medium	57	2	104	61

Figure 9. Top nine hybrid clones from GS validation population evaluated at the RCRDC in Hanoi, Vietnam.

Milestone partially achieved: GS models for nutrient content validated in 2x landrace-derived hybrids from 3rd cycle of recurrent selection by Q3 2017.

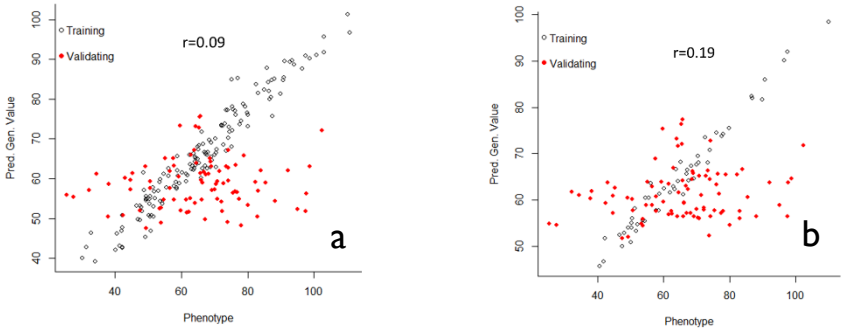
CIP has undertaken biofortification as a breeding objective to enhance micronutrient content of potato tubers at the diploid level. We previously studied the potential of applying GS to enhance genetic gains for micronutrient content in a panel of 150 2x landraces from *S. tuberosum* Groups Phureja and Stenotomum. By means of CV, we obtained predictive correlations of 0.7, 0.3, and 0.5 for vitamin C, Fe, and Zn, respectively—sufficiently high enough to merit implementation of GS. In this project, we aimed at validating GS models for Zn, Fe, and vitamin C developed in the panel of 150 2x landraces (i.e., TP) in 89 derived 2x hybrids, designated as "M3" population, generated upon three cycles of recurrent selection performed in CIP's biofortification diploid pre-breeding program. This M3 population was regarded as the VP and was grown under a randomized complete block design during the rainy season of 2016–2017 in Huancayo (Junin, Peru, 3,200 masl). This location in the Peruvian highlands was also used previously to grow the TP from which phenotypic data on Fe, Zn, and vitamin C were obtained. Available soil Fe and Zn were of 22 and 25 ppm, respectively. Fertilizers containing Zn were applied in combination with standard N-P-K to optimize the soil Zn level and achieve a soil Zn content similar to the suitable content of 38 ppm encountered in the soil analysis performed in the field where the TP was previously grown in

2014. It is known that Zn concentrations in potatoes increase with Zn fertilizers (Kromann et al. 2017). Tuber samples recovered at harvest were analyzed for vitamin C content by the spectrophotometric method of Egoville et al. (1988), whereas for Fe and Zn content, freeze-dried tuber samples were sent to CSIRO laboratory in Australia to be analyzed by inductively coupled plasma–optical emission spectrophotometry. By the time of this report we were still awaiting Fe and Zn content analysis results. However, we evaluated the accuracy obtained for vitamin C content by estimating marker effects in the TP of 150 2x landraces and predicting the breeding values in the 89 M3 2x hybrids (VP). Surprisingly, we found a prediction accuracy significantly lower (r = 0.09) than that obtained (r = 0.7, see above) when a tenfold CV was previously conducted in the TP. The semiparametric reproducing kernel Hilbert spaces that yielded the best predictive correlation compared with Bayesian Lasso and Bayesian Ridge Regression for vitamin C using testing sets from the TP was also used for GS validation here. Since vitamin C content in tubers has a high heritability (Pavek and Corsini 2004), other reasons may account for this decrease in prediction accuracy. Potential reasons for this decrease may include (1) allele frequency changes across cycles; (2) differences in the number and effect sizes of QTL between the TP and VP; (3) epistasis (QTL x genetic background); (4) QTL X E effects; (5) insufficient size of the training population; and (6) genetic distances between TP and VP.

Since the reproducing kernel Hilbert spaces model achieves accuracy by using relationships between individuals, we analyzed the genetic distance between TP and VP by pedigree and by exploring simple sequence repeat (SSR) marker distance analysis performed previously at CIP in 425 2x landraces. It has been shown that the prediction accuracy decreases when the genetic relatedness between individuals in the training population and those in the prediction set decreases (Würschum et al. 2013). We found that only 14 2x landraces were used as base population to develop the 89 M3 2x hybrids, and only 5 are present in the TP. We looked in SSR marker distance analysis performed previously in 2x landraces, for closely related genotypes to the 14 parental landraces that were in turn present in our TP. We found 47 2x landraces closely related to the 14 parental landraces used to generate the VP. Hence, we used 52 landraces (47 related to the VP and 5 parents from VP base population) for running the analysis again. Even though the TP was smaller (N = 52 vs. N = 150), we obtained a better accuracy (r = 0.19 vs. r = 0.09; Fig. 10), indicating that genetic distance between TP and VP was one of the reasons accounting for the decrease in prediction accuracy. This result highlights the importance that genotypes from the population in which prediction will be done be represented in the TP. Other aforementioned reasons, such as the size of the TP, allele frequency changes across cycles, and QTL x E, may also account for decrease in the prediction accuracy in this study. QTL x E because TP and VP were grown in different seasons even though in the same location. If changes in allele frequencies across selection cycles affected prediction accuracy, though it was not shown here, recalibration of GS should be considered.

GS was applied as a MAS-based genomic tool to predict GEBV for micronutrient content in 2x potato biofortification. Prediction accuracy was significantly low after three cycles of recurrent selection. It decreased from r = 0.7 in the TP to 0.19 in the VP for vitamin C. Genetic distance between TP and VP was found as one of the factors accounting for prediction decrease after three cycles of recurrent selection. We also suggested other factors, such as changes in allele frequencies and QTL x E.

Figure 10. Correlation between TP and VP for vitamin C content after three cycles of recurrent selection in



CIP's diploid biofortification pre-breeding program. "a" shows prediction values versus phenotypic outcomes when using a TP of 150 2x landraces that are not as closely related to the VP as it was to the TP of 54 landraces used to predict breeding values of VP in "b" (see text).

Capturing and tracking novel genes for wild resistance sources through nested association mating design (NAM)

Milestone achieved: NAM population developed from crop wild relative-derived 2x hybrids resistant to LB by Q2 2017.

Crosses between 6 wild potato species that were previously identified as LB resistant (*Solanum circaeifolium* var. *capsicibaccatum*, *S. commersonii*, *S. megistacrolobum, S. microdontum*, *S. sogarandinum*, and *S. tarijense*) and 4 selected SC BSLI hybrids (BSLI-3.122, BSLI-8.72, BSLI-1.35, and BSLI-5.5) were used to develop a NAM design to study novel sources of LB resistance. BSLI hybrids were used as male parents to pollinate 72 LB-resistant genotypes from 7 different accessions from the aforementioned species, and 129 hybrid families with a range of 50 to 3,460 seeds/progeny were generated (Table 21). The NAM populations developed provide a community resource for collaborative projects that can be used to generate recombinant inbred lines through single-seed descent with selfing to the F5 or F6 generation, for mapping and gene discovery and for pyramiding genes.

TABLE 21. SEEDS GENERATED FROM CROSSES BETWEEN WILD POTATO LB-RESISTANT GENOTYPES AND 4 SC BLSI HYBRIDS

Species	Accession	Genotype	Number of Seeds				
			BSLI-3.122	BSLI-8.72	BSLI-1.35	BSLI-5.5	Total
S. microdontum	760534	760534.204	1,400	2,700	375	835	5,310
		760534.205	2,305	3,460	700	1,180	7,645
		760534.208	500	270	0	430	1,200
		760534.211	620	770	110	595	2,095
		760534.212	115	60	0	0	175
		760534.214	575	490	0	335	1,400
		760534.216	790	260	0	470	1,520
		760534.221	355	255	0	40	650
		760534.222	335	150	0	170	655
		760534.224	260	380	0	0	640
		760534.23	390	165	105	221	881
		760534.232	145	490	0	0	635
		760534.236	750	960	0	0	1,710
		760534.237	90	730	0	200	1,020
		760534.238	1,435	440	0	0	1,875
		760534.239	0	300	0	0	300
		760534.24	705	1,450	130	12	2,297
		760534.242	990	780	40	390	2,200
		760534.243	3,183	2,225	0	1,085	6,493
		760534.245	415	215	0	0	630
		760534.247	0	160	0	0	160
S. megistacrolobum	760535	760535.201	275	112	0	80	467
		760535.216	347	225	0	200	772
		760535.217	624	305	0	70	999
		760535.218	200	40	0	0	240
		760535.221	63	86	0	8	157
		760535.224	33	10	0	123	166
		760535.225	277	79	7	187	550
		760535.228	350	79	100	18	547
		760535.229	25	40	0	0	65
		760535.23	335	71	0	10	416
		760535.24	83	0	0	40	123
S. tarijense	761007	761007.203	1,655	891	150	70	2,766
		761007.207	2,920	660	50	9	3,639
		761007.209	1,254	1,425	0	253	2,932
		761007.211	3,390	680	75	155	4,300
		761007.212	815	2,025	0	130	2,970
		761007.218	1,435	2,045	50	618	4,148
		761007.219	1,708	619	0	280	2,607
		761007.22	1,195	1,290	30	130	2,645
		761007.226	965	765	0	180	1,910
		761007.238	1,920	1,342	0	190	3,452
		761007.241	1,490	1,570	0	435	3,495
		761007.244	2,205	1,148	105	115	3,573

A NAM population composed of 129 diploid families of botanical seed, which recombines new sources of LB resistance and SC, was developed and is available for generating recombinant inbred lines for mapping, gene discovery, pyramiding genes, and as a community resource for collaborative projects.

2.2.4 Output 4: Improved and shared breeding databases and knowledge management, including databases of accurate phenotypic and breeding values of selected breeding lines and specific protocols and catalogues to support the orientation of breeding products and facilitate decision-making and outcomes from breeding research.

Deliverable 1: Standardized methodologies and protocols to assess adaptive traits and communicate intrinsic qualities and add value to breeding materials developed and available in a global trial data management system (GTDMS).

Develop and validate standard methodologies for trait assessment

Milestone achieved: Protocols for data collection under ABS adapted for potato by Q3 2017.

The approach conventional breeding programs have followed for decades starts with population development and ends with selection of the "best" individuals in the genetic variation/breeding population and variety release. This conventional breeding scheme takes 7–8 years. Given our findings, and the pressure to make a difference in farmers' lives faster, such a scheme is no longer adequate. ABS, on the other hand, requires 1 year for crossing and multiplication of planting material. In the second year, all genotypes developed from seeds that have been cloned in year 1 are planted concurrently in two or four distinct environments as observational trials in small plots (3–5 plants per row) without replication. After evaluating the trials, the best selections are entered into a series of preliminary and advanced yield trials across environments (multilocational trials) as before in the conventional breeding scheme to be evaluated principally for yield components; nutritional content (Fe, Zn, and vitamin C); processing quality; and resistance to viruses (PVY and PVX) and LB in years 3 and 4. In year 4, these are conducted concurrently with on-farm trials using PVS–Mother and Baby trial design. Moreover, traits are aggregated into an index. This enables varieties to be released in year 5.

Implementation and data collection under ABS in potato breeding requires capacity to apply protocols for the selection of clones from the seedling trial through the observational trials and on to the preliminary and advanced yield trials, each accompanied by field books and structures for data collection, analysis, and reporting. Protocols for 13 potato evaluation trials were updated in CIP's GTDMS (see https://research.cip.cgiar.org/gtdms/) in the course of transferring them to the new HIDAP. A key advance in 2017 has been the development of data management structures to connect breeding material lists of families and clones with the institutional pedigree and corporate database at CIP. This connectivity permits verification and maintenance of the identity of clones across the different selection stages and to follow their pedigrees. The HIDAP network enables researchers to share field books with colleagues, regional breeding programs, and/or partners. To use this network, it is necessary to register and create a login account. Once logged in, the user can share, download, and receive field books of different selection stages in a user-friendly interface. A download count helps to keep track of users and uses of this tool.

Milestone achieved: Protocol for assessing drought tolerance available by Q2 2016 and salinity in 2017.

Precise and accurate phenotyping of component traits is a prerequisite for proper genetic dissection of complex phenomena such as drought tolerance. CIP's guidelines for standard evaluation of advanced potato clones, "Assessing Potato Clones for Drought Tolerance under Field Conditions," have been written to establish standard procedures for conducting and documenting (1) drought tolerance phenotyping and (2)

standard evaluation trials (https://research.cip.cgiar.org/potatoknowledge/abioticstress.php). The extensive drought tolerance phenotyping protocol aims to aid in the design of field trials to characterize the performance of potato genotypes under drought stress. Such trials are used for research focused on improving the understanding of the relationship of agro-morphological and physiological plant and tuber traits with tolerance and susceptibility and, in turn, unravelling the genetic basis of this variation for more efficient breeding. For this protocol 36 morphological and physiological traits and means for assessing those at up to four stages of plant development have been defined in CIP's trait dictionary. Of these, 19 morphological and physiological traits are measured twice before drought initiation and twice after drought initiation; 17 are evaluated at harvest. For analysis purposes, 31 additional traits are described to be calculated using evaluated traits.

Related protocol for practical evaluation of potato clones for drought tolerance is derived from the phenotyping protocol based on accumulated knowledge of the most informative subset of traits, in light of phenotyping a diversity panel in several locations of Peru, China, and Ethiopia. Traits that show enough variation to enable discrimination of potato genotypes' response to drought stress were identified in a panel of 394 advanced breeding clones from CIP's breeding program. Clones were distributed using an augmented design subject to two water restriction treatments. In the first treatment water was withheld 45 DAP (terminal drought); in the second watering was reestablished at 75 DAP (recovery). A total of 23 traits were assessed among agronomical and physiological ones, highlighting a good correlation of stem diameter and chlorophyll content index (Chlspad) correlated with canopy reflectance normalized difference vegetation index (CR_NDVI) ($r \geq 0.6$) in Peru; a high correlation of plant canopy with Chlspad and stem diameter in China ($r \geq 0.43$). On the other hand, drought tolerance index (DTI), which is estimated as a function of total tuber fresh weight (FW), showed a great difference between both terminal drought and recovery treatments. This was confirmed by results in Ethiopia, where only harvest traits were evaluated.

For further analysis of potato genotype's response to drought identification, lineal discrimination analysis was performed to identify groups with different responses than individuals under non-stress, using a previous selection of informative traits designated by the Gini index decrease in a random forest model (Fig. 11). We found ChlSPAD, CR_NDVI, stem diameter, and DTI as the most relevant traits that can be used in turn to efficiently discriminate potato genotype's response to drought and therefore be relevant as variables to address in breeding for adaptation to drought environments.

On the other hand, trials from 2014 and 2015 focused on salinity tolerance were used to establish discriminating treatments (salinity levels and monitoring methods) and trait measurements to systematize procedures for assessing salinity tolerance of potato genotypes under screenhouse conditions. A set of 22 traits is proposed with different measurement levels. Analysis results showed plant height, Chlspad, and harvest index as the most indicative traits for salinity tolerance to be included in CIP's trait dictionary. For salinity tolerance assessment, 15 traits were proposed and used as part of CIP's drought tolerance protocol. Furthermore, a protocol describing the correct management of a salt experiment in screenhouse condition has been written. It is available as module 8.3 at abiotic stress protocols webpage (link described above).

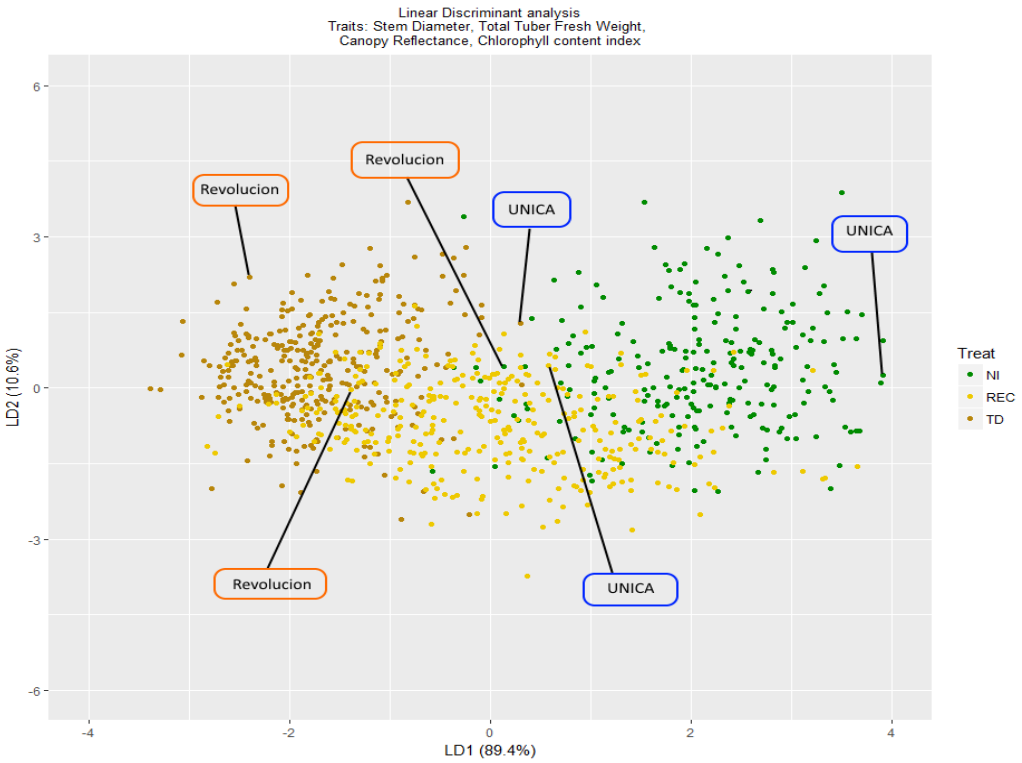


Figure 11. Lineal discriminant analysis—results example. Note the differentiation of treatment groups: Ni = normal irrigation, REC = recovery, TD = terminal drought. It can be seen how a drought-tolerant potato variety ('Unica') shows a tendency to the NI treatment group even when it is under drought stress. A drought-susceptible potato variety ('Revolution') tends to behave as TD despite its being under non-stress. Many different scenarios were tested, in which the best possible prediction model for the differentiation of clone behaviors by treatment was the main concern.

Milestone achieved: Fast protocol for glycoalkaloid estimation developed by Q2 2017.

Glycoalkaloids are naturally occurring, secondary plant metabolites that are found in a number of foods including potatoes, tomatoes, and eggplants. They serve as natural defenses against insects and other pests; but in high doses they can produce various toxic effects in humans. The toxicity may be due to adverse effects on the central nervous system and disruption of cell membranes, which adversely affects the digestive system and general body metabolism. Contents of glycoalkaloids in potato are generally expressed as total glycoalkaloids (TGA), calculated as the sum of α-chaconine and α-solanine, which form about 95% of the glycoalkaloids present in potatoes.

Glycoalkaloid levels can vary greatly in different potato cultivars. They may be influenced postharvest by environmental factors such as light, mechanical injury, and storage. They are also influenced by stress such as heat and drought during production. This raises concern for maintaining the quality of potato harvests under climate change and suggests increased attention may be needed to glycoalkaloid concentrations of potato varieties bred for or grown in warm environments.

The generally accepted safe upper limit for TGA in potato tubers is 20 mg/100 g of FW. However, owing to the large and often unpredictable variation in levels of TGA, which can arise from differences in variety, locality, season, cultural practices, and stress factors, and in view of the more recently discovered synergism between α-chaconine and α-solanine in inducing toxic effects, it has been suggested that the limit should be reduced to 6–7 mg/100 g FW.

Given the importance of potato glycoalkaloids, it is critical that every potato breeding program includes their evaluation. However, the evaluation of thousands of samples produced as a part of a breeding program by conventional high-performance liquid chromatography method would be very expensive and time consuming. Therefore, a need exists for rapid, sensitive, and selective analytical techniques.

Near-infrared reflectance spectroscopy (NIRS) is a common technique for routine analysis because it allows fast and non-destructive analysis of samples. Different compounds present in food samples can be detected and quantified. NIRS has already been applied for the quantitation of the three main alkaloids caffeine, theobromine, and theophylline in roasted coffee and in green tea leaves.

The aim of the present study was to examine the potential of NIRS as a rapid method to estimate TGA in freeze-dried potato samples. Potato samples came from an experiment where 20 clones were tested in San Ramón and Majes (Peru) in three replications under heat and drought conditions. Samples were analyzed by photo-spectrometry.

NIRS calibration equations developed for TGA showed medium-high coefficients of determination for the calibration (0.81), with a medium coefficient of determination for cross validation (0.62); this needs improvement. It was expected that the NIRS technique works much better for potato glycoalkaloids. However, it will be possible to improve calibrations performance by increasing the number of samples and locations used. Research at CIP has been done to develop an ultra-performance liquid chromatography (UPLC) method to analyze individual glycoalkaloids in potato tubers as better reference methods for NIRS calibration development. The UPLC method will be fully implemented by Q4 2017. Results of UPLC reference method will be used for NIRS calibration development for fast (individual) glycoalkaloid screening in potato-breeding material at early stage.

Milestone partially achieved: Protocol for assessing sensorial traits in potato developed and available in GTDMS by Q1 2017. (co-funded by CRP-RTB and USAID)

The aim of this study is to explore diversity for flavor-related compounds in raw, freshly harvested, boiled, and 4°C stored boiled potatoes in a panel of landrace, bred, and wild species genotypes. The study also aims to identify associated metabolites that could be included as flavor traits in potato breeding programs. A panel of 32 advanced potato breeding clones, 15 diploid landraces from Groups Phureja and Stenotomum, 15 diploid biofortified bred clones, and 10 wild genotypes from species indicated as ancestors of cultivated potato were grown in a field trial during 2016 in the Peruvian highlands of Huancavelica at Mullaca (Tayacaja, Huancavelica, at 4,100 masl) using local agronomic practices. Some 15–30 uniform-sized and visually healthy tubers of each genotype were selected at harvest from each of two replications. These formed the sample used for sensory evaluation, dry matter determination, amylose and amylopectin content, and metabolite analysis. Raw and boiled tubers (after harvest and storage at 4°C) were frozen in liquid nitrogen and freeze-dried. Freeze-dried samples were milled and 6-g samples dispatched to our partner, Dr. Paul Fraser from RHUL, for metabolite analysis. Metabolite analysis is in progress.

Sensory evaluation was performed with boiled tubers at harvest and after 4 months' storage. Flavor criteria were determined based on known varieties and evaluations provided by a taste panel of 15 members. The selection of the evaluation panel is described below.

Selection of the evaluation panel

Students at the National Agricultural University in La Molina (Lima, Peru) and CIP staff were invited for sensory analysis; 50 students and 8 CIP staff were selected. Candidates completed a preselection form, indicating availability, general health, and eating habits.

1. **Evaluation of pre-candidates**. Fifty-eight pre-candidates were evaluated for their ability to discriminate sweet and salty flavors at different concentrations using a triangle test. We identified 35 candidates who could differentiate these two flavors.

2. **Evaluation of candidates**. The 35 candidates were evaluated for their ability to discriminate five basic flavors: sweet, salty, bitter, acid, and umami in different concentrations. Eighteen panelists were finally identified.

3. **Training of panelists**. The 18 panelists were trained in four sessions in quantitative descriptive analysis for the recognition of flavors, textures (firmness, creaminess, and granularity), and other characteristics (sweetness, bitter, residual flavor, etc.), and on the use of an unstructured scales of 0–10 cm, where 0 = lowest and 10 = highest intensity. Very well-known potato varieties were used for the training: 'Yungay', 'Perricholi', 'Huayro', 'Canchan', and 'Papa Amarilla'. The top 15 panelists were considered for the evaluation sessions and the other 3 remained as replacements if any evaluator was missing.

4. **Samples preparation**. Five to six tubers/genotype (30–60 g) were first washed and scrubbed to remove all soil particles. They were then placed in stainless-steel saucepans and covered with sufficient distilled water to cover them all. Unpeeled tubers were cooked covered to prevent excessive moisture loss. The water was replenished when required to ensure that tubers stayed fully immersed. After tubers were cooked they were removed from the saucepan, peeled manually, and placed on a glass container covered with aluminum foil and kept at 60°C in the stove before they were served to the panel. Each tuber was cut in half and coded using three random numbers and presented in the order defined by the incomplete block design.

5. **Samples evaluation**. Individual cabins with green artificial light were used for each panelist to avoid preferences for color during the evaluation. Samples were delivered on white melamine plates. Care was taken to ensure uniformity of each sample (volume served and serving temperature) and of each replication of the different samples. Each panelist received evaluation forms and water at room temperature, which served as palate cleansers among sample evaluations. Ten samples were evaluated by 15 panelists, and 4 different samples were evaluated by each panelist in each session.

The survey included the following criteria: potato flavor intensity, sweetness, savoriness, sourness, bitterness, and mealiness assessed on an unstructured intensity scale from 1 (the lowest) to 10 (the highest). Results on fresh-boiled potatoes showed savoriness, potato flavor intensity, and bitterness as criteria that can discriminate germplasm groups, explaining together 76% of the variation. Overall, wild potatoes were scored high for bitterness (7.2) and low for flavor intensity (2.9) and savoriness (1.3). Diploid landraces and their biofortified-bred derivatives were scored low for bitterness (1.3) and highest for savoriness (5.3) and flavor intensity (5.7). On the other hand, advanced breeding clones were scored the lowest for bitterness (0.9), though medium for flavor intensity (2.7) and savoriness (2.2). Analysis of boiled potatoes after storage at 4°C did not include wild potatoes due to lack of sufficient tubers. Mealiness, savoriness, flavor intensity, and sweetness discriminated advanced breeding clones from diploid landraces and their derivatives biofortified-bred clones, explaining together 95% of the variation. Overall,

diploid landraces and their derivative-bred clones scored highest for sweetness (3.5), flavor intensity (6.3), and savoriness (5.7). It is worth highlighting that after storage, sweetness increased slightly while sourness perception decreased in diploid landraces and their derivative-bred clones. Texture criteria (i.e., mealiness vs. creaminess) was hard to perceive by the panel in fresh-boiled potatoes, whereas this became more noticeable in boiled potatoes after storage.

We expect that results from metabolite analysis may reveal associations between flavor attributes and secondary metabolite evaluation

Test and standardize remote sensory tools for population screening for abiotic and biotic stress

Milestone achieved: Feasibility of applying proximal sensing tools in early-generation selection and clonal selection for abiotic stress tolerance determined and validated by Q2 2017.

The study of the genetic variability for drought tolerance was performed in the arid zone of Peru (Majes, Arequipa) under field conditions. Two treatments, normal irrigation and deficit irrigation, were used under a strip plot design with three replications and 30 plants per replication. The regression of mid-parent values on progeny performance and slope "b" of the regression as estimation of narrow-sense heritability and correlation coefficients were calculated in a population of 27 progenies from 16 progenitors. Rapid remote sensing methods (CR_NDVI and chlorophyll concentration) were tested for ability to assist with identification of best families with tolerance to abiotic stress.

Normalized difference vegetation index (NDVI) showed high negative correlation (-0.66) with harvest index-FW, but high correlation (0.74) with plant wilting and moderately high (0.50) with plant vigor under drought conditions. The families with a lower canopy temperature under drought stress use more of the available soil water to avoid excessive dehydration. A significant negative correlation (r = -0.5) was found between canopy temperature and tuber yield (t/ha) in families representing CIP's advance breeding populations. The results suggest that remote-sensing techniques provide means for saving resources and accelerating genetic gains by the early identification of best families with tolerance to abiotic stress in a breeding program. However, the phenotypic and genotypic assessment of the panel of 300 advanced clones (analysis in process) will give more insights and validate the use of remote sensing in breeding.

Establishing methodologies to assess morphological, biochemical, or physiological traits as components of abiotic stress tolerance

Milestone not achieved: Root system architecture (RSA) assessed and validated as a component of drought tolerance by Q2 2016.

RSA refers to the configuration of roots in space and how the root axes are deployed within the growing environment. This spatial distribution is important as it determines a plant's ability to utilize the usually unevenly distributed resources in the growing environment. RSA is genetically controlled but responds to environmental conditions. The genetics is poorly understood in most crops due to the difficulty associated with root evaluation. Our objective was to identify QTL associated with different component traits of RSA as a first step to understanding the genetic control of RSA in potato.

We evaluated 64 genotypes from the mapping population used to anchor (give chromosome positions) the potato reference genome commonly known as "DMDD." The population is a diploid backcross progeny between a doubled monoploid (DM) sequenced for the reference genome and a heterozygous

diploid accession (D) as the recurrent parent. The genotypes included 61 DMDD progeny plus the three pedigree parents, DM, D, and DM/D (Table 22). Lateral sprouts were obtained from treated tubers for homogenous lateral sprout production. Given the complexity of evaluating roots under soil media (i.e., destructive sampling and difficulty in recovering most fine roots), the evaluation was done in hydroponic conditions under water. Sprouts were placed at the borders of a 30 x 40 cm tray and distilled water was poured over until it touched the bottom of each sprout (Fig. 12). The experiment was continued for 14 days. Root traits such as root break, root volume, root surface area, root projected area, number of roots, and root depth were evaluated at 3, 6, 9, and 14 days after shoots were placed in the trays. Fresh and dry weights of shoots and roots were also evaluated at harvest. Root volume, root surface area, and root projected area were evaluated by image analysis using RootGraph and ImageJ software. Analysis of QTL was done using MapQTL software.

TABLE 22. THE 64 GENOTYPES USED IN THE LATERAL SPROUTS ROOT PHENOTYPING EXPERIMENT

CIP Number	Breeder Code	Parent by Species	Population Group
CIP305156.17	DMD-7.17	PHU x GON	DM cross
CIP308328.1	DMDD-1.1	(PHU x GON) x GON	DM Back cross
CIP308328.104	DMDD-1.104	(PHU x GON) x GON	DM Back cross
CIP308328.116	DMDD-1.116	(PHU x GON) x GON	DM Back cross
CIP308328.125	DMDD-1.125	(PHU x GON) x GON	DM Back cross
CIP308328.128	DMDD-1.128	(PHU x GON) x GON	DM Back cross
CIP308328.130	DMDD-1.130	(PHU x GON) x GON	DM Back cross
CIP308328.133	DMDD-1.133	(PHU x GON) x GON	DM Back cross
CIP308328.134	DMDD-1.134	(PHU x GON) x GON	DM Back cross
CIP308328.140	DMDD-1.140	(PHU x GON) x GON	DM Back cross
CIP308328.144	DMDD-1.144	(PHU x GON) x GON	DM Back cross
CIP308328.145	DMDD-1.145	(PHU x GON) x GON	DM Back cross
CIP308328.146	DMDD-1.146	(PHU x GON) x GON	DM Back cross
CIP308328.148	DMDD-1.148	(PHU x GON) x GON	DM Back cross
CIP308328.16	DMDD-1.16	(PHU x GON) x GON	DM Back cross
CIP308328.161	DMDD-1.161	(PHU x GON) x GON	DM Back cross
CIP308328.163	DMDD-1.163	(PHU x GON) x GON	DM Back cross
CIP308328.17	DMDD-1.17	(PHU x GON) x GON	DM Back cross
CIP308328.173	DMDD-1.173	(PHU x GON) x GON	DM Back cross
CIP308328.18	DMDD-1.18	(PHU x GON) x GON	DM Back cross
CIP308328.181	DMDD-1.181	(PHU x GON) x GON	DM Back cross
CIP308328.182	DMDD-1.182	(PHU x GON) x GON	DM Back cross
CIP308328.2	DMDD-1.2	(PHU x GON) x GON	DM Back cross
CIP308328.22	DMDD-1.22	(PHU x GON) x GON	DM Back cross
CIP308328.23	DMDD-1.23	(PHU x GON) x GON	DM Back cross
CIP308328.24	DMDD-1.24	(PHU x GON) x GON	DM Back cross
CIP308328.25	DMDD-1.25	(PHU x GON) x GON	DM Back cross
CIP308328.26	DMDD-1.26	(PHU x GON) x GON	DM Back cross
CIP308328.27	DMDD-1.27	(PHU x GON) x GON	DM Back cross
CIP308328.28	DMDD-1.28	(PHU x GON) x GON	DM Back cross
CIP308328.30	DMDD-1.30	(PHU x GON) x GON	DM Back cross
CIP308328.32	DMDD-1.32	(PHU x GON) x GON	DM Back cross
CIP308328.33	DMDD-1.33	(PHU x GON) x GON	DM Back cross
CIP308328.36	DMDD-1.36	(PHU x GON) x GON	DM Back cross

CIP Number	Breeder Code	Parent by Species	Population Group
CIP308328.37	DMDD-1.37	(PHU x GON) x GON	DM Back cross
CIP308328.38	DMDD-1.38	(PHU x GON) x GON	DM Back cross
CIP308328.40	DMDD-1.40	(PHU x GON) x GON	DM Back cross
CIP308328.42	DMDD-1.42	(PHU x GON) x GON	DM Back cross
CIP308328.43	DMDD-1.43	(PHU x GON) x GON	DM Back cross
CIP308328.44	DMDD-1.44	(PHU x GON) x GON	DM Back cross
CIP308328.48	DMDD-1.48	(PHU x GON) x GON	DM Back cross
CIP308328.49	DMDD-1.49	(PHU x GON) x GON	DM Back cross
CIP308328.5	DMDD-1.5	(PHU x GON) x GON	DM Back cross
CIP308328.51	DMDD-1.51	(PHU x GON) x GON	DM Back cross
CIP308328.52	DMDD-1.52	(PHU x GON) x GON	DM Back cross
CIP308328.54	DMDD-1.54	(PHU x GON) x GON	DM Back cross
CIP308328.55	DMDD-1.55	(PHU x GON) x GON	DM Back cross
CIP308328.58	DMDD-1.58	(PHU x GON) x GON	DM Back cross
CIP308328.59	DMDD-1.59	(PHU x GON) x GON	DM Back cross
CIP308328.6	DMDD-1.6	(PHU x GON) x GON	DM Back cross
CIP308328.60	DMDD-1.60	(PHU x GON) x GON	DM Back cross
CIP308328.63	DMDD-1.63	(PHU x GON) x GON	DM Back cross
CIP308328.65	DMDD-1.65	(PHU x GON) x GON	DM Back cross
CIP308328.67	DMDD-1.67	(PHU x GON) x GON	DM Back cross
CIP308328.68	DMDD-1.68	(PHU x GON) x GON	DM Back cross
CIP308328.7	DMDD-1.7	(PHU x GON) x GON	DM Back cross
CIP308328.71	DMDD-1.71	(PHU x GON) x GON	DM Back cross
CIP308328.72	DMDD-1.72	(PHU x GON) x GON	DM Back cross
CIP308328.79	DMDD-1.79	(PHU x GON) x GON	DM Back cross
CIP308328.9	DMDD-1.9	(PHU x GON) x GON	DM Back cross
CIP308328.97	DMDD-1.97	(PHU x GON) x GON	DM Back cross
CIP308328.99	DMDD-1.99	(PHU x GON) x GON	DM Back cross
CIP703825	OCH 5648	-	-
CIP801092	-	-	-

The phenotypic root traits were significantly different among genotypes. However, no significant QTL was observed for any traits, with logarithm of odds scores below 2.5 (data not shown). This is significantly lower than logarithm of odds of 3.0 normally used to declare significant QTL in most studies. Among the reasons for this is the size of the population used for mapping. Given the heterozygous nature of the recurrent parent, a larger population size than 61 would be necessary to identify significant QTL. However, a trade-off was considered here, given the volume of work involved in measuring the root traits. Also, given the plasticity of root traits based on the growing environment, we could not validate root surface area as a component trait for drought tolerance using the current data since the experiment was carried out under hydroponic conditions as a trade-off for destructive sampling and losing fine roots under soil conditions. Our efforts show that genotypes differ for different RSA traits. It remains to be examined if these differences translate into differences in drought tolerance under field conditions, and future experiments under drought in the field will help answer this question. However, the formidable challenge of phenotyping roots in soil conditions remains and hindered the achievement of this milestone. Among the lessons learned is investment in non-destructive root phenotyping methods under field conditions,

like ground-penetrating radar, would enhance better understanding of RSA as a very important trait in adaptation to abiotic stresses.

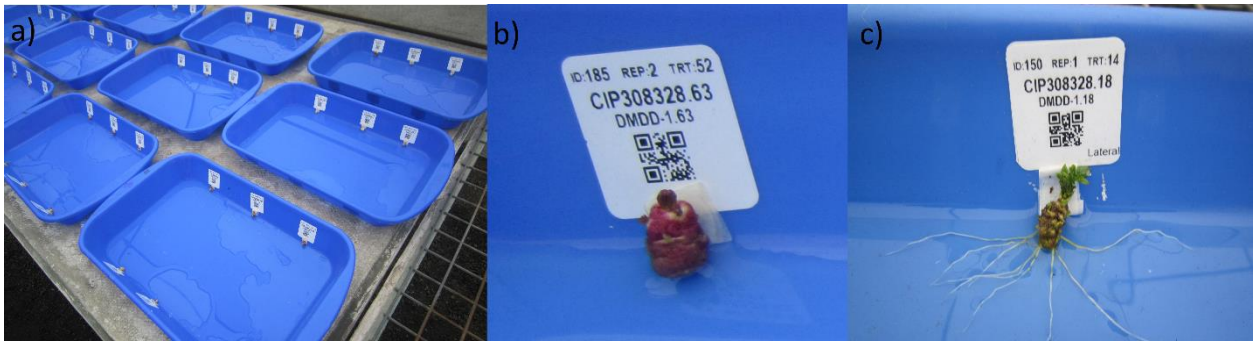


Figure 12. a) Sprouts were placed along the borders of the trays; b) water was filled until it touched the base of the sprout; and c) grown roots of a sprout.

NOTE: Trait abbreviations: RVOL, root volume; RSFA, root surface area; RPA, root projected area; RDPTH, root depth; RN, number of roots; RFW, root fresh weight; RDW, root dry weight; SFW, shoot fresh weight; SDW, shoot dry weight, BFW, biomass fresh weight; BDW, biomass dry weight, and RBREAK, root break.

Milestone achieved: Fast and inexpensive screening methods based on NIRS to estimate metabolites associated with stress response developed by Q3 2016.

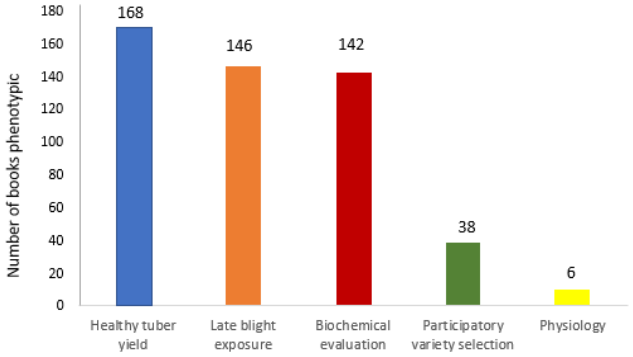
Metabolite profiling can help to decipher molecular and biochemical mechanisms underlying genetic variation for complex traits. We coupled metabolite profiling to explore variation among potato genotypes with the development of inexpensive assays, such as NIRS, to monitor metabolites associated with tolerance to drought. This adds value to the discovery pipeline and should enable breeding of future varieties suitable for sustainable production. Potato leaf samples coming from a greenhouse experiment in Huancayo, Peru, with five advanced clones (LTVR392797.22, LTVR 394611.112, LTVR 390637.1, LTVR 397077.16, and BW 395448.1) that were grown under water restriction in greenhouse conditions were double scanned by NIRS XDS with sample cup refilling. The two NIRS scans per sample were checked visually and then averaged. Samples were sent to London University for metabolite profiling.

NIRS calibration development needs around 100 samples with a wide range of trait concentrations in order to correlate spectral NIRS information with chemical reference values for the same samples. Initial NIRS calibrations within a feasibility study can be developed with 40–50 samples, but a large range of reference results is needed. These critical criteria in available datasets were given for 13 out of 22 metabolites identified: aspartic acid, citric acid, fructose, GABA, glucose, glyceric acid, inositol, maleic acid, malic acid, phosphate, proline, pyroglutamic acid, and sucrose_iso1_8TMS. NIRS calibration equations for 9 metabolites (citric acid, fructose, glucose, GABA, inositol, phosphate, proline, pyroglutamic acid, and sucrose_iso1_8TMS) showed high coefficients of determination for the calibration curve (0.86–0.97) and medium to high coefficients of determination in CV (0.77–0.91). The standard errors of calibration and the standard errors in CV were low for all 9 metabolites. The NIRS calibration equations for 2 metabolites (glyceric acid and malic acid) showed medium coefficients of determination in calibration curves, 0.73 and 0.84, and consequently lower coefficient of determination in CV (0.58 and 0.55). The NIRS calibration equations for the remaining 2 metabolites (aspartic acid and maleic acid) showed low coefficients of determination in calibration curves and consequently low coefficient of determination in CV. This is likely due to low concentrations and limited variation for these metabolites in the reference data sample set.

Deliverable 2: Database of accurate phenotypic values for main constraints, quality, and adaptation traits available for promising materials.

Milestone achieved: Genotypic and phenotypic information incorporated into database of breeding populations, clones, and progenitors by Q1 2017.

The data generated in the CIP potato breeding program is stored in a ***"Global Roots & Tubers Base***" utilizing the free BioMart software https://research.cip.cgiar.org/gtdms/biomart/. The data have been structured for storage of phenotypic, genotypic, pedigree, geographical, and environmental data. Through the metadata and the search function using filters, the user can retrieve data from the experiments conducted by CIP scientists or NARS partners using CIP materials. The availability of the data is managed in conjunction with the Dataverse following CGIAR open access guidelines. Currently, the database holds data from 500 experiments in 11 different countries and 71 localities conducted from 2002 to 2015. The phenotypic data include important potato traits such as healthy tuber yield, LB resistance, and vitamin and mineral composition. The genotypic data consist of approximately 3,000 SNP marker genotypes on a set of 103 B3 population breeding lines using the SolCAP Infinium Potato SNP platform. Stakeholder preference data from PVS trials are also included in the database: biochemical evaluation (142 books), physiology (6 books), LB exposure (146 books), healthy tuber yield (168 books), and PVS (38 books) (Fig. 13).



FiFigure13. Number of field books registered in *Global Roots&Tubers Base* by experimental trial.

CIP's potato base is linked with the CIP breeding tools such as HIDAP data processing interface and CIP cross software. This allows for retrieval and selection of clones from the database, utilizing their CIP number as a unique identifier of each breeding line. This process minimizes the errors generated by typing and streamlines the whole process of planning for field trials and crossing blocks.

The methods of data generation and processing that are utilized in plant breeding have radically changed in recent years. With the advancement of new high-throughput technologies, data have grown in terms of quantity and complexity; better data management and storage facilities are needed. CIP's potato base provides a simple data management solution to connect different types of data and make the data accessible for the scientific community.

Milestone achieved: NIRS and XRF data for micro- and macronutrients integrated and available with corresponding field trial data by Q1 2017.

At the beginning of 2016, CIP defined specific needs and gaps in data management systems in which a collaboration with IBM could be established. CIP started a consultancy with IBM's Corporate Citizenship and Corporate Affairs to help in one of the gaps: to consolidate a global laboratory information

management system using the quality and nutrition laboratory at CIP as an entry point. In September 2017, IBM presented the Technology Road Map for the quality and nutrition laboratory. Content included:

1. **General considerations**
   - CIP considers generating a platform for the quality and nutrition laboratory. Each NIRS analysis, including the needed sample preparation, costs $15. Nutritional data generated by NIRS analysis are connected to different field trials and are required to do data quality check and to have both types of data: raw (spectral information) and processed (estimated nutritional values using CIP-developed NIRS calibration equations for each nutritional trait). Many databases exist at CIP. There should be an institutional effort to integrate all types of datasets, having non-isolated platforms between them.
2. **Objective**
   - To define a road map to implement a solution to consolidate the information management system of the quality and nutrition laboratory, including NIRS.
3. **Needs**
   - Store all the information in a unique repository
   - Work with CIP's four NIRS labs worldwide (Peru, Ghana, Mozambique, and Uganda)
   - Back-up security.
4. **Organization goals**
   - Open access data
   - Build analytics with the information of lab files
   - Store lab files in a secure environment
   - Optimize the way to manage the NIRS information between labs
   - Support the existing applications and systems.
5. **Addressing the technology gap**

   Current status:
   - Silos of information, many historical archives and in four countries
   - Do not take advantage of "raw" data for estimation new traits
   - There is no repository that supports open data for the laboratory results.

   Recommendations:
   - Store the raw data in a single centralized repository that integrates the data
   - Display raw data (spectra files) in a single, open consolidated data structure
   - Evaluate the Hadoop platform as the central repository for open access.

   Future status:
   - Integrated information, shared data

- Open data base to analyze "raw" data (spectral information)
- Large structured and unstructured data repository that will serve as containers for open data.

6. **To-do recommendations:**
   - Open Hadoop platform cluster environment
   - Define structure and loading form
   - Import historical files from the four laboratories
   - Integrate with open data system
   - Internal connectivity to reporting and predictive modeling applications.

Figure 14 illustrates the road map to implement a solution to consolidate the information management system of the quality and nutrition laboratory.

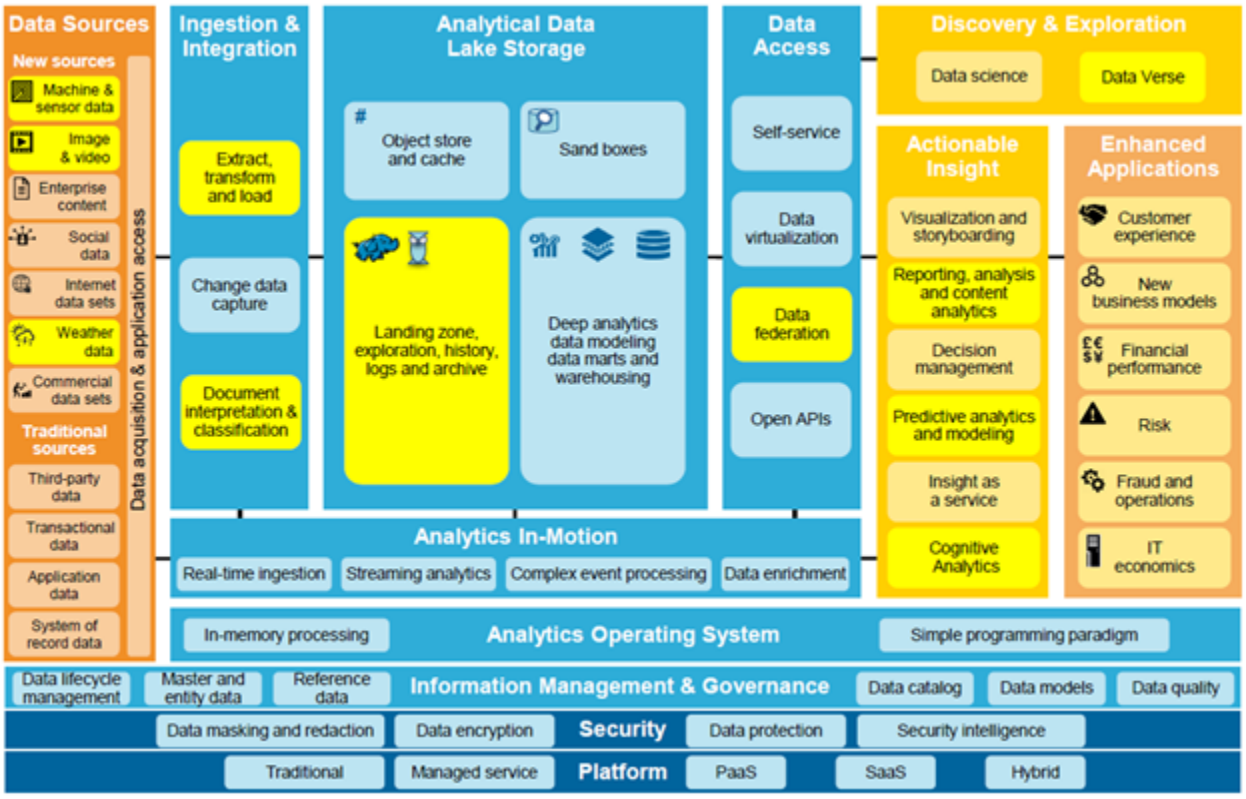


Figure 14. Analytics reference architecture: Recommendations that can be implemented at CIP (in yellow).

Milestone achieved: Biannually updated online clonal and variety catalogues by Q3 2017.

CIP's interactive 2017 catalogue, "Advanced Potato Clones and Varieties," has been published online in nine languages: English, Spanish, French, Chinese, Korean, Russian, German, Portuguese, and Hindi. The catalogue includes 459 advanced clones and 70 varieties with images and information on main attributes, molecular data, pollen viability, and nutritional contents.

The new additions to the earlier catalogue format include 20 new elite potato clones, a user-oriented search tool, a facility for updating contents with new information, print-out capacity for "breeders' choice" clones, and

full documentation in two new languages, Hindi and Portuguese. The source code of each component in the catalogue can be easily adapted to other clonal crops using a very simple user-interface administrator panel.

a. At least 600 families (PGH15, about 8000 clones) evaluated at least at 3 locations by ABS by June 2016 and selection 40 parents and families respectively (selected fraction has on average at least 14 t/ha after 100 days, root dry matter 26%, 50 ppm b-carotene on fwb).
b. Recombination of 40 elite families (repeat of best crosses) and true seed dissemination to NARS, ARIs, universities in SWA and SEA (mid to long term target: <100 day sweetpotato in SWA&SEA) by December 2016.
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The software was developed in hypertext preprocessor, HTML, and Cascading Style Sheets, to be compatible with a wide range of mobile devices and desktop computers. User-friendly access to descriptive information and images of CIP elite potato clones makes the catalogue a valuable tool for communicating and documenting intellectual assets. The new catalogue was published online on October 30, 2017 (https://research.cip.cgiar.org/potatoknowledge/catalogues.php).

2.3 SUMMARY OF ACHIEVEMENTS BY OUTPUT—*SWEETPOTATO*

2.3.1 Output 1: Dynamic and nutrient-dense breeding populations developed as sources of early-maturing, high, and stable-yielding varieties with resistance to biotic and abiotic stresses and quality traits

Deliverable 1: Global OFSP breeding population for wide adaptation and earliness.

Milestone achieved:

a. At least 600 families (PGH15, about 8000 clones) evaluated at least at 3 locations by ABS by June 2016 and selection 40 parents and families respectively (selected fraction has on average at least 14 t/ha after 100 days, root dry matter 26%, 50 ppm b-carotene on fwb) – milestone achieved: 661 families evaluated / 587 families selected for statistical analysis.
b. Recombination of 40 elite families (repeat of best crosses) and true seed dissemination to NARS, ARIs, universities in SWA and SEA (mid to long term target: <100 day sweetpotato in SWA&SEA) by December 2016. – milestone partially achieved: 12 elite families were selected due to the possibility of higher selection intensities.
c. Recombination of 2 x 20 parents in partial diallel cross resulting in true seed for 2x 120 partially inbreed families by December 2016 – milestone achieved: 2 x 24 parents were selected and recombined.

Population development for the global sweetpotato breeding program has complete hybrid breeding schemes in which OFSP populations are developed to select for widely adapted and early-maturing OFSP varieties. Our program is actually the first within the CGIAR Research Program on Roots, Tubers and Bananas (RTB) that fully implemented hybrid breeding schemes. The global hybrid population was developed in the context of this project by controlled crossings between different gene pools (PJ07 x PZ08, PJ07 x

PZ06, and PJ05 x PZ08). In total, 990 families were developed with a number of seeds from 2 to 250; 661 cross-combinations comprising 8,193 genotypes were evaluated. After discarding all cross-combinations with missing or zero storage root yields in at least one environment, 587 cross-combinations comprising 7,345 genotypes were analyzed together with 57 PJ parents and 41 PZ parents. The population was evaluated in two locations: Satipo (humid tropics of the Amazon Basin [HTA]) and Ica (temperate arid Pacific Coast [APC]) for 2 years. We lost data from Ica 2015 due to a stolen data pocket device. Thus final data from three environments were available to (1) select elite crossings (disseminated by TS shipments to regions) and (2) selected PJ and PZ parents on basis of "offspring–parent" analysis for the next breeding cycle. For the details of the selection process see Appendix 1.

The global H0 population shows variety ability for harvest after 90–100 days. A key trait for variety ability is the number of commercial storage roots/plant (values >1.5 indicate variety ability). The breeding program at CIP is targeting the 12-weeks OFSP. Table 23 shows elite cross-combinations for which so far about 4,000 TS have been produced. TS from these cross-combinations have been shipped to India and Indonesia; TS shipments to Bangladesh, Nepal, the Philippines, and Vietnam are planned for 2018.

TABLE 23. H0 GLOBAL ELITE OFSP HYBRID CROSS-COMBINATIONS FOR 100-DAY GROWING PERIODS

Cross-combination PJ x PZ	No. of Commercial Roots/Plant	Storage Root Yield (t/ha)	Foliage Yield (t/ha)	Root Dry Matter (%)
Low dry matter elite OFSP cross-combinations				
PJ05.124-PZ08.038	3.5	27.4	30	25.7
PJ05.120-PZ08.011	3.31	31	16.1	25.8
PJ05.130-PZ08.038	2.83	27.9	25.2	26.2
PJ05.213-PZ08.038	2.78	24.3	40.3	27.4
PJ05.213-PZ08.090	2.7	25.7	42.3	27.3
PJ07.265-PZ08.011	2.68	20.1	30.7	28
High dry matter elite OFSP cross-combinations				
PJ07.061-PZ08.038	2.67	27.8	31.3	29.8
PJ07.061-PZ06.085	2.62	19.5	30	28.7
PJ07.690-PZ06.304	2.17	16.3	29.8	29.7
PJ07.079-PZ06.304	2.17	20.5	40.4	28.3
PJ05.213-PZ08.153	2.15	25.8	23.8	32.3
PJ05.064-PZ08.153	2	17.4	14.7	31.6

Parents for the next global breeding population have been selected based on offspring–parent analysis (Table 24); 12 PJ and 12 PZ parents are undergoing intra-gene pool crossings and general combining ability (GCA). Owing to project funding it was possible to establish a sustainable TS pipeline for OFSP variety selection to all partners across regions, especially those that are not directly supported by projects in Africa.

TABLE 24. SELECTED PARENTS FOR INTRA-GENE POOL RECOMBINATION AND GCA AND OFFSPRING ESTIMATES IN GLOBAL HO HYBRID POPULATION FOR OBSERVED TRAITS

Parent	GCA Com. Roots/ Plant	GCA Root Yield (t/ha)	GCA Foliage Yield (t/ha)	GCA Dry Matter (%)	Offspring Mean			Offspring	N
					Com. Roots/ Plant	Root Yield (t/ha)	Foliage Yield (t/ha)	Mean Dry Matter	Offsprings
Selected PJ parents for intra-gene pool crossing									
PJ07.245	1.07	6.3	4.1	1.4	3.31	26.4	33	28.8	2
PJ05.124	0.59	5.2	5.5	-1.7	2.83	25.3	34.4	25.6	5
PJ05.210	0.55	4.4	-2.4	0	2.79	24.4	26.5	27.4	11
PJ07.305	0.42	0.9	2.5	-0.4	2.66	21	31.4	26.9	3
PJ07.265	0.41	-0.5	3.3	0.3	2.65	19.6	32.2	27.7	4
PJ05.180	0.37	1.4	-0.7	-0.6	2.61	21.5	28.2	26.8	11
PJ05.212	0.31	7.3	2.4	1	2.55	27.4	31.3	28.4	12
PJ05.217	0.25	0.5	-1.3	1.1	2.49	20.6	27.6	28.5	15
PJ05.171	0.22	4.5	-0.9	0.4	2.46	24.5	28	27.8	1
PJ05.213	0.21	4.6	4.7	1.1	2.45	24.6	33.6	28.5	10
PJ05.120	0.21	2.8	-3.3	-0.1	2.45	22.8	25.6	27.3	14
PJ07.304	0.2	3.5	6.8	0.1	2.44	23.5	35.7	27.5	9
Selected PZ parents for intra-gene pool crossing									
PZ06.349	0.58	0.3	5.3	0.5	2.78	20.4	33.8	28	14
PZ08.017	0.53	6.6	-1.3	1.3	2.73	26.6	27.2	28.8	3
PZ08.011	0.35	2.9	-4.2	0.5	2.55	23	24.3	28	14
PZ08.137	0.33	2.8	-1.7	1.8	2.53	22.9	26.8	29.4	3
PZ08.038	0.33	3	-2.4	0.2	2.53	23.1	26.1	27.8	20
PZ08.008	0.3	2	-2.8	-0.2	2.5	22	25.7	27.3	22
PZ08.048	0.28	2.3	-1.2	1.1	2.48	22.4	27.3	28.7	5
PZ08.086	0.26	0.2	1.8	-0.9	2.46	20.3	30.3	26.6	5
PZ08.090	0.14	1.8	0.5	0.4	2.34	21.8	29	27.9	20
PZ08.066	0.14	0.6	1.2	-0.8	2.34	20.6	29.7	26.8	12
PZ08.053	0.14	1.7	-2.2	0.7	2.34	21.8	26.3	28.3	24
PZ08.153	0.12	1.9	-1	0.5	2.32	22	27.5	28	29

Deliverable 2: Pre-breeding population for sweet potato virus disease (SPVD).

Milestone achieved:

a. At least 500 true seeds from crosses of CIP-105086.1 / Arne x (CIP-110025.1, CIP-110025.2, CIP-110025.3, CIP-110025.4, CIP-110025.5, CIP-110025.7, CIP-110025.8, CIP-110025.10, CIP-110025.11, CIP-110019.16, CIP-110019.17, and CIP-110019.21) and test for self-compatibility in 110025 and 110019 family completed by June 2016 – milestone achieved.

b. Field and screen house evaluations of 500 clones completed by December 2016 – milestone achieved with 426 evaluated clones.

c. At least one OFSP SPVD resistant pre-breeding population reaching variety ability (average yield 6 to 10 t/ha, 50 ppm b-carotene, resistance to SPCSV) in 50 clones by mid-2017 – milestone achieved with 61 clones above target levels.

Sweetpotato has serious quarantine and production constraints due to viruses; however, the crop has a pronounced resistance to nearly all viruses, except sweet potato chlorotic stunt virus (SPCSV). This virus is breaking the natural defense mechanism of sweetpotato to viruses and in co-infection with other viruses—mainly sweetpotato feathery mottle virus (SPFMV)—is forming the so-called SPVD. The SPVD resistance breeding is complex due to recessive inheritance (SPCSV and SPFMV), dosage effects, the long time to finally decide about "true" resistance in field testing, and the limited test capacity (N = 500) for SPVD resistance under controlled conditions. CIP is working with different approaches to enhance SPVD resistance. One of these approaches is pre-breeding (classical sense of pre-breeding: weak material with the desired attribute and no variety ability is improved in agronomic performance).

The pre-breeding population developed comprises 79 families (N = 436 clones) and can be divided into two groups:

1. Clones tracing back to grandparents with repeatedly confirmed SPVD resistance: CIP-107729.9 (VJ08.330) and/or CIP-189151.34 (PJ05.064). Through selfing offspring CIP-110025 was developed from CIP-107729.9 (VJ08.330) and by crossing CIP-107729.9 (VJ08.330) x CIP-189151.34 (PJ05.064) the offspring CIP-110019 was developed for a total of 17 families (N = 120 clones).

2. Other clones tracing back to clones with strong indications of SPVD resistance: in total 62 families with 316 clones.

Family 110025 are all SC and, for some clones (N=2) from family 110019, SC was observed.

The pre-breeding population is also called SPVD-resistant-3 population (see previous annual report). Three crossing steps and generations, respectively, were made since the finding of the SPCSV resistance in CIP-107729.9 (VJ08.330) and CIP-189151.34 (PJ05.064). The agronomical performance in SPVD-resistant-3 population is higher as expected (Table 25), but the evaluation so far is based on only one environment (San Ramón, Peru).

TABLE 25. MEAN, MINIMUM, AND MAXIMUM IN 436 SPVD RESISTANCE PRE-BREED CLONES FOR OBSERVED TRAITS

		Population	Genotypic	
Variable	**N**	**Mean**	**Minimum**	**Maximum**
Com. roots N/plant	426	1.6	0	5.8
Foliage yield (t/ha)	436	6	0	43.8
Root yield (t/ha)	426	9.2	0	35.4
Beta-carotene (ppm FW)	417	56	0	14.4
Root form*	436	2.5	0	3

* Scale from 0 to 5, with 0 = very bad and 5 = very good.

In group 1 SPVD-resistant-3 population we selected 22 clones. The average performance of these clones was 2.5 commercial storage roots/plant, storage root yield of 32.3 t/ha, and root beta-carotene content of 76.2 ppm on fresh matter basis. In group 2 we selected 61 clones, whose average performance was 2.3 commercial storage roots/plant, storage root yield of 29.5 t/ha, and root beta-carotene content of 77.9 ppm on fresh matter basis. Eight selected clones in group 2 are tracing back to crosses of CIP110025 clones with PZ06.085 (variety 'Arne', adapted to the APC with good SPVD field resistance and low virus titers in controlled SPVD testing). Selected pre-breeding clones are listed in Appendix 2. The entire pre-breeding population was germinated in vitro, which allows rapid incorporation into CIP's genebank and dissemination of pre-bred clones to the regions. With these results 1 OFSP SPVD-resistant pre-breeding population has reached variety ability (>1.5 commercial storage roots per plant). In future projects, we are planning to

test at least 30 pre-bred clones (22 of group 1 and 8 of group 2) in areas with high SPVD prevalence such as Uganda.

2.3.2 Output 2: Farmers' and end-users' preferences integrated into varietal development and selection approaches

Deliverable 1: Varieties as well as farmers' and end-users' priorities and preferences documented and integrated into varietal breeding.

Milestone achieved:

a. At least CTCRI and ILETRI in SWA and SEA multiply 500 families from previous seed introductions (June 2015) with the target of at least 3000 clones by June 2016 – milestone achieved for ILETRI but not for CTCRI due to delays in quarantine in India.
b. ABS selection with at least two locations (one location is managed and selected by farmers) and documentation of breeder selections and farmer selections and clones entering PYT / AYT trials available by April 2017 – milestone achieved for ILETRI but not for CTCRI due to delays in quarantine in India.

TS from the global hybrid breeding population, including elite crossings, has been sent to Indonesia and India.

Indonesia. TS from 650 families (10,494 seeds) was sent to the Indonesian Legumes and Tuber Crops Research Institute (ILETRI), TS from 158 families (3,603 seeds) was sent to Padjadjaran University, and 164 families (7,584 seeds) were used by CIP in Indonesia. The material was evaluated in observational trials in early breeding stages at both ILETRI and Padjadjaran University, which evaluated at three locations (Bandung, Sumedang, and Karawang) in an ABS. ILETRI evaluated at one location in farmers' fields (Wringin Songo/ Malang). CIP evaluated at two locations, Bogor (250 masl) and Lembang (1,250 masl), and harvested at 90 days. In total 324 OFSP clones for short crop duration were selected for preliminary yield trials in Indonesia; so far, the trials were carried out at Bogor (250 masl) at 90 days harvest. The preliminary trials were harvested on September 5, 2017. The average of yield across 324 accessions was 7.6 t/ha and higher than that of local check varieties—most clones exhibited intermediate to dark orange-fleshed color. (NB: In Indonesia, there is a high demand of early-maturing OFSP with abundant upper biomass production to be used for foliage for animal feed/goats and milking cows, for example).

India. TS from 737 families was sent to the Central Tuber Crops Research Institute of India (CTCRI); the material is under multiplication. Sweetpotato is an important food crop in India in the eastern states of Odisha, West Bengal, Eastern Uttar Pradesh, and Bihar. It contributes largely to food and nutritional security to the resource poor population. Odisha is the largest consumer of sweetpotato in India (80 g per person per day). The Indian Council of Agricultural Research–CTCRI, which has the mandate to conserve and maintain genetic material of tuber crops, contributes largely to crop improvement in India and promotes tuber crops. After delays of more than half a year due to quarantine issues, CTCRI received 737 TS families to be used for its evaluation and breeding program. In 2017, these families have been germinated and are undergoing multiplication. Farmer preferences in Odisha are high dry matter and starchy OFSP with short crop duration. CTCRI in the state of Kerala and CIP in the state of Odisha have so far selected 100 sweetpotato varieties; however, high dry matter OFSP with short crop duration is still a bottleneck in the breeding and variety selection pipeline.

2.3.3 Output 3: ABMs and tools to help breeders to select genotypes and parental lines in fewer years than with traditional clone-breeding schemes

Deliverable 1: ABM developed by improved strategies for selection and trait transfer in early-breeding stages after applying field surface/nearest neighbor models.

Milestone achieved:

a. Field surface / nearest neighbor models implemented at two breeding platforms to achieve higher precision in hybrid population trials and other trials with very large number of genotypes by August 2016 – milestone achieved

Field heterogeneity in large trials comprising more than 100 clones (often thousands of clones) in a major block is a critical issue. Options that we already have implemented to adjust for soil heterogeneity are (1) the alpha lattice designs, or methods for spatial analysis using splines. For this second option we can use the R package to fit generalized additive models, or the more specific package SpATS, a package for spatial analysis of field trials with splines among others. Additionally, we have tested commercial software: ASReml and AGROBASE (www.agronomix.com). With ASReml it is possible to fit models for AR1 x AR1-correlated errors and fit polynomial models or smoothing splines for error trends in the field. Whereas with AGROBASE, it is possible to fit the more traditional nearest neighbor adjustment. (Unfortunately, AGROBASE provides only adjusted means across major blocks and not adjusted values for each major block.) For all methods it is essential to have a row and column mapping from the field to the data field book. We have implemented functions, freely available by the R package st4gi (https://github.com/reyzaguirre/st4gi), to create field books with row and column mapping for several statistical designs (i.e., completely randomized designs, randomized complete block design, alpha lattice, augmented block design, split plot design, strip split plot design, two-way factorials, and the Westcott design. It also creates randomizations with row and column mapping in non-replicated trials (often carried out in early breeding stages). As a standard design in non-replicated trials, the Westcott design has been implemented in HIDAP (https://research.cip.cgiar.org/gtdms/hidap/), which is a free software developed by CIP to support breeders. The Westcott design is used since the beginning of 2017 on all CIP sweetpotato breeding platforms (CIP–Lima, CIP–Mozambique, CIP–Uganda, and CIP–Ghana).

Deliverable 2: ABM developed by using diversity arrays technology (DArT) markers.

Milestones achieved:

a. Validation population VZ08 comprising at least 500 clones phenotyped for SPCSV and SPFM and statistical analysis completed by October 2016 – milestone achieved with 482 clones.
b. DNA conservation of VZ08 population with at least 500 clones and in storage by November 2016 – milestone achieved with 482 clones.
c. Validation population VZ08 available through phenotypic SPCSV and SPCSV data and DNA for validation of potential molecular markers by December 2016 – milestone achieved with 482 clones.

d. At least the currently available AFLP and SSR markers for SPCSV and SPFMV validated by June 2017 – milestone not achieved because the activity has now to be out-sourced.

MAS for SPVD resistance would be of high value in applied sweetpotato breeding, provided that (1) genetic markers could predict this resistance with R^2 >0.4 and (2) costs and capacity allow one to screen at least 5,000 samples per season. CIP–Lima has identified six interesting amplified fragment length polymorphisms (AFLP) band associations (E44M34.533, E33M48.460, E36M34.400, E33M48.343, E39M32.440, and E39M34.156), seven SSR markers associated with susceptibility to SPCSV (IBS204-172, IBS169-162, Ib-286-125, IbJ559-262, IbJ559-269, IbJ116a-229, and IBS149-225), and five DArTs marker associations with SPCSV (758044, 7563062, 7572542, 753123, and 7574925). It is critical to have enough SPVD-resistant clones (more than 10) and "true" values for resistance and susceptibility, in our case population VJ08 was indicating marker associations. A VP with "truly" SPVD resistant clones, in our case population VZ08, also is critical.

In total 482 genotypes were screened for SPCSV and SPFMV under controlled conditions in a screenhouse (San Ramón, Peru), including population VZ08 with 455 clones. Using a double antibody sandwich-enzyme linked immune-sorbent assay (ELISA) and triple antibody sandwich-ELISA 444 VZ08 clones were evaluated, virus titer estimates, and SPVD symptoms were recorded with two plant replications and two repeated measurements (inflections by grafting on infected material). Five response groups due to SPCSV and SPFMV infection with strains from East Africa were distinguished: (1) very susceptible to SPVD (N = 23), (2) susceptible to SPVD (N = 377), (3) slightly tolerant to SPVD (N = 17), (4) tolerant to SPVD (N=26), and (5) resistant to SPVD (N = 1).

Phenotypic SPCSV and SPCSV data and DNA are available for validation of potential molecular markers. The phenotypic data are complete but not yet uploaded into a database. With respect to genotypic data the DNA of resistant, susceptible groups and their parents from the VJ08 and the VZ08 population are stored. The DNA of 452 clones of the population VZ08 will be used in future projects at CIP and made available to all advance research institutions on request by September 2018. About 75% of the DNA samples from population VZ08 has already been sent to and an institute in Australia for DArTs genotyping in Augusts 2017. Results were obtained by the end of September; the data are being analyzed.

The milestone "At least the currently available AFLP and SSR markers for SPCSV and SPFMV validated by June 2017" has been delayed because the laboratory services were not able to cope with our orders in September. Genotyping with SSR markers is programed to start in the second week of October and will be followed by AFLP markers.

2.3.4 Output 4: New capacities for applying knowledge, tools, and modern breeding approaches developed for more efficient progress in variety-oriented breeding programs of NARS

Deliverable 1: Regional breeding hubs strengthened. Workshops

Milestone achieved:

a. At least 20 breeders from CIP-HQ, Peru, Brazil, Panama, Bangladesh, India, and Indonesia learned about advance in sweetpotato breeding by December 2016 – milestone achieved.

b. At least 4 sweetpotato breeders from SWA and SEA (Bangladesh, India, Indonesia) trained in SEA at the annual sweetpotato breeders meeting Asia – milestone achieved.

From 31 May to 3 June 2016, the 3nd Annual Meeting of the Asia Sweetpotato Breeders Network was held in Malang, Indonesia; participants from seven Asian countries attended.

From 30 to 31 May 2017, a workshop on heterosis and hybrid breeding in potato and sweetpotato was held with 28 participants (10 participants by web-ex from Africa); 10 presentations were given. Invited speakers were Shelly Jansky from the University of Wisconsin ("The development of diploid inbred lines in potato – progress and challenges") and Jochen Reif from IPK/Germany ("Heterotic groups the single most important element in implementing hybrid breeding – maize and wheat as model crops"). After the workshop, J. Reif visited CIP's hybrid population trials in Canete, San Ramón, and Satipo. We have established a collaboration on heterosis and hybrid breeding between CIP and IPK. The plan is to submit a joint project proposal to BMZ/Germany at the end of 2017 or early 2018. An executive summary of the workshop is provided in Appendix 3.

2.3.5 Output 5: Improved and shared breeding databases and knowledge management, including trait-specific protocols and catalogues to support the orientation of breeding products and facilitate decision-making and outcomes from breeding research

Deliverable 1: Database for early bulking (the <100-day sweetpotato).

Milestone achieved:

a. Documentation about early bulking genotypes in SWA and SEA available by June 2016 – milestone achieved.
b. Heritability estimates for early bulking and outline the strategy to breed for 90 day sweetpotato determined by April 2016 – milestone achieved.

Early bulking. Information for 49 early-bulking released/launched varieties were uploaded in a database on the Sweetpotato Knowledge Portal. Early maturing (time from planting to harvest of 2.5–3.5 months) is a sweetpotato attribute found across all sweetpotato production zones: APC (1 variety), HTA (2 varieties), humid tropics of South Asia (7 varieties), Indian subcontinent (8 varieties), temperate West Pacific (7 varieties), West Africa (2 varieties), East Africa (9 varieties), and semi-arid Southern Africa (SASA) (12 varieties). The database has reporting gaps for Southeast Asia, but recent information claims that selection for 90-day OFSP clones for variety release was very promising in Indonesia. Certainly, the attribute 90 days to harvest is relatively easy to incorporate into OFSP breeding populations (though to date not emphasized by project funding). Early-maturing OFSP varieties for the humid tropics are considered to be a funding opportunity with low risk of failure.

New early-bulking breeding clones from CIP's global program. These will be disseminated in the future, mainly by TS through **elite crossing**s (see Deliverable 1). Clones in registration and virus cleaning are divided into two groups. **(1) Moist and sweet** comprises 31 clones (Z06.077, PZ06.085 (Arne), PH09.718, PZ08.127, PZ08.018, PH09.5176, PJ07.609, PJ07.057, PJ07.586, PJ07.522, PZ08.008, PJ05.212, PH09.2294, PZ08.048, PH09.2582, PH09.893, PH09.3534, PJ07.660, PJ07.119, PZ08.017, PH09.5313, PZ08.174, PJ07.096, PH09.1609, PH09.4137, PZ08.011, PJ07.305, PH09.1687, PH09.3314, PJ07.508, and PJ07.678. **(2) Dry and starchy** comprises 22 clones **(**PJ05.236, PJ05.052, PJ05.312, PZ08.053, PH09.3323, PZ06.698, PH09.753, PJ07.588, PJ07.602, PH09.2616, PJ07.084, PJ07.544, PJ07.028, PJ05.324, PZ08.153, PJ07.147, PJ07.061, PJ07.064, PZ06.050, PH09.4543, PJ07.079, and PH09.1699). The material requires a further selection step (15 clones to be selected) due to high costs of virus cleaning (expected costs of $1,000 per clone).

Deliverable 2: Database for non-sweet sweetpotato.

Milestone achieved:

a.Zero amylase activity or non-sweet after cooking sweetpotato tested (zero / low amylase activity identified) and documented by January 2017 – milestone achieved.

Non-sweetpotato. Information for 21 non-sweet sweetpotato clones have been uploaded in a database on the Sweetpotato Knowledge Portal. The information contains CIP-code, accession name/variety name/breeding code, aroma, and taste. The quality of information and material in the database for non-sweet sweetpotato are low compared with that in the early-bulking database. CIP–Lima has larger numbers of clones considered as low in sugar after cooking, but we had to realize that there are issues with our screening methods for sweetness after cooking and the determination of beta-amylase activity after cooking. The sweetpotato breeding group at CIP–Lima decided that all clones have to be evaluated for taste and aroma in order to decide about non-sweet after cooking.

Deliverable 3: Database for genetic gains by modified demonstration trials.

Milestone achieved:

a. Genetic gain estimates for OFSP in humid tropics determined on basis of modified demonstration trials (comprising about 10 OFSP and 10 varieties from the past in Peru) available and documented by September 2017 – milestone achieved.

b. Genetic gain estimates for OFSP in semi-arid tropics/subtropics under terminal drought conditions (no irrigation after 80 days) determined on basis of modified demonstration trials (comprising about 10 OFSP and 10 varieties from the past in Peru) available and documented by September 2017 – milestone achieved.

Estimates for genetic gains across years are important for predicting yield developments for the decades and for demonstrating value of investments into breeding. Genetic gain studies became available for three climatic zones: APC (irrigated environments), HTA, and SASA (Table 26). Within the climatic zones of APC and HTA, genetic gains over the last two decades leading up to 2014 were for storage root yield at 90 days after harvest in the range of 0.18–0.34 t/ha/year and at 120 days after harvest (0.36–0.58 t/ha/year). We observed genetic gains for 90 days after harvest, and sweetpotato can indeed be transformed into a crop for short crop duration. In addition to root yield, the crop produces substantial amounts of animal feed through upper biomass production. It is suggested that, in addition to current use as fodder and silage, the pelletizing of upper biomass production be investigated. For SASA the total storage root yield increase estimates range 0.29–0.30 t/ha/year, with a foliage yield increase in the range of 0.08–0.10 t/ha/year (estimates based on variety releases from 2000 and 2016). Genetic gains are estimated from modified demonstration trials in which old and new varieties are evaluated together (continuous variety release trials are the basis of genetic gain estimates in the United States and Europe, but in the developing world release trials are only conducted irregularly). The data from APC, HTA, and SASA have been stored in a database and will be put into open access in September 2018. Population improvement has so far not been intensively applied in sweetpotato breeding, except at CIP–Lima (CIP–Uganda, however, just started with systematic population improvement). So one can assume that breeding progress for sweetpotato will show stability in the mid- to long term in this century.

TABLE 26. ANNUAL GENETIC GAINS BY REGIONS ESTIMATED ON BASIS OF VARIETY RELEASES ACROSS TWO DECADES

Agro-ecological Zone	Storage Root Yield (t/ha/year)		Foliage Yield (t/ha/year)	
	90 days after harvest	120 days after harvest	90 days after harvest	120 days after harvest
APA	0.18	0.36	n.p.	n.p.
HTA*	0.34	0.58	n.p.	n.p.
SASA	n.a	0.29–0.30	n.a	0.08–0.10

* Assume to be transferable to other humid topical zones with high rainfall.
n.a. = not available; n.p. = so far not predicted.

APPENDIX 1

Statistics for the Global H0 Hybrid Population (100-day OFSP)

Field design. A total of 661 cross-combinations comprising 8,193 genotypes were evaluated. All cross-combinations with 3 or fewer genotypes and all cross-combinations without data across three environments (Satipo 2015, Satipo 2016, and Ica 2016) were excluded from the statistical analysis. **Harvest was conducted at 100 DAP at all three environments**. In total 587 families comprising 7,345 H0 global hybrid genotypes were analyzed together with 57 PJ parents and 41 PZ parents (2 parents, Santo Amaro and SR01.018, were treated as PZ clones due to SSR marker data). A set of 64 cross-combinations comprised reciprocal families (2 families per cross-combination using the same PJ and PZ parent as male and female parent). Thus this study comprised 57 PJ parents combined with 41 PZ parents, resulting in 523 parental combinations with additional 64 combinations available as reciprocals. Previous studies indicate no reciprocal effects in sweetpotato, except for the trait beta-carotene content. Trials in each environment were not replicated.

Outlier elimination. The model used was "y = g + l + err," where g is genotype, l is location, and err is plot error. All global H0 genotype observations with residuals ± 4 were set to missing values.

First analysis step. The lsmeans cross-combination by location were calculated. The model used was "y = c + l + cxl + err," where c is cross-combination, l is location, cxl is cross-combination by location interaction, and err is the error. Population mean and cross-combination (minimum/maximum) for the global H0 population were calculated from lsmeans cross-combination by location data (Table 27).

TABLE 27. HO GLOBAL HYBRID POPULATION MEAN AND CROSS-COMBINATION MINIMUM AND MAXIMUM FOR OBSERVED TRAITS ACROSS ENVIRONMENTS (SATIPO 2015, SATIPO 2016, ICA 2016)

Variable	N	Population	Cross-Combination	
		Mean	Minimum	Maximum
Root yield (t/ha)	523	19.9	9.7	39.2
Com. roots (N/plant)	523	2.18	0.78	4.11
Fol. yield (t/ha)	523	28.4	12.2	67.4
Dry matter root (%)	523	27.6	19.3	34.8

Second analysis step. Variance components were estimated for the global H0 population using data classified by cross-combinations, genotypes within cross-combinations, and environments. The model used was "y = e + c + g(c) + cxe + err," where e is environment, c is family, g(c) is genotype within cross-combination, cxe is cross-combination by environment interactions, and err is the error. Note that the error includes gxe(c) in unreplicated trials. Variance component estimates and their 95% confidence limits are presented in Table 28.

The σ_E^2 estimate for storage root (383.7 t²/ha²) and foliage yield (194.7 t²/ha²) was large in the global H0 hybrid population (Table 27). The locations used (Ica and Satipo) belong to different agro-ecological zones (APC and HTA). In addition, the population was harvested 100 DAP in all environments; usual harvest is 120 DAP. The σ_C^2 was significant (different from zero) for all traits. The $\sigma_C^2 : \sigma_{G(c)}^2$ ratios for storage root yield, number of storage roots per plant, and foliage yield were 1 : 3.16, 1 : 2.83, and 1 : 12.57. This

provides an estimation of the magnitude of variation both among and within crosses. The large variation within crosses appears to be a characteristic for hexaploid sweetpotato (large segregations within crosses) and have been observed in other populations such as those developed for the Sweetpotato Action for Security and Health in Africa (SASHA) project.

TABLE 28. VARIANCE COMPONENT ESTIMATIONS σ_E^2, σ_C^2, $\sigma_{G(C)}^2$, σ_{CxE}^2, AND σ_ε^2 WITH 95% CONFIDENCE LIMITS IN GLOBAL H0 HYBRID POPULATION (524 CROSS-COMBINATIONS) FOR OBSERVED TRAITS EVALUATED AT THREE ENVIRONMENTS (SATIPO 2015, SATIPO 2016, AND ICA 2016)

Variable	Para-meter	σ_E^2	σ_C^2	$\sigma_{G(C)}^2$	σ_{CxE}^2	σ_ε^2
Root yield t^2/ha^2	Estimate	383.7	3.7	11.7	26.6	114.8
	CL limits	72.1-2231.9	1.3-6.4	9.6-13.8	23.3-30.3	111.9-117.8
Com. roots $N^2/plant^2$	Estimate	3.48	0.06	0.17	0.26	1.66
	CL limits	0.65-20.26	0.03-0.09	0.14-0.20	0.22-0.30	1.62-1.70
Fol. yield t^2/ha^2	Estimate	194.7	1.4	17.6	95.4	217.9
	CL limits	36.4-1133.2	0-8.0	13.7-21.6	85.6-105.7	212.3-223.6
DM root	Estimate	3.8	<0.0001	2.72	2.72	16.74
2%	CL limits	0.42-33.41	0-0.56	2.06-3.26	2.06-13.26	15.9-17.36

NOTE: σ_E^2, σ_C^2, $\sigma_{G(C)}^2$, σ_{CxE}^2, and σ_ε^2 are variance components due to environment, cross-combination, genotype within cross-combination, cross-combination by environment interactions, and the plot error, respectively. The error includes in unreplicated trials the genotype by environment interactions within cross-combination.

Third analysis step. The lsmeans cross-combination by location data were used to selected best cross-combinations. Selection was divided into two sub-steps. First, all cross-combinations were discarded with fewer than 1.2, 2, and 1.5 number of commercial storage roots/plant at environments Ica 2016, Satipo 2015, and Satipo 2016, respectively. Two selected sets of cross-combinations were formed: (1) for low dry matter OFSP by discarding all cross-combinations with fewer than 2.2 commercial storage roots/plant and more than 28% dry matter, and (2) for high dry matter OFSP by discarding all cross-combinations with fewer than 1.7 commercial storage roots/plant and less than 28% dry matter. The top 30 cross-combinations for low dry matter OFSP sorted by number of storage roots/plant in descending order are listed in Table 29. The top 20 cross-combinations for high dry matter OFSP sorted by number of storage roots/plant in descending order are listed in Table 30.

The cross-combinations in Tables 29 and 30 were used as the source for elite cross-combinations for low and high dry matter OFSP for 100 DAP, which CIP is providing to regions as TS shipments.

TABLE 29. H0 GLOBAL OFSP HYBRID CROSS-COMBINATIONS FOR 100-DAY GROWING PERIODS WITH MORE THAN 2.2 COMMERCIAL ROOTS/PLANT AND FEWER THAN 28% DRY MATTER ACROSS EXPERIMENTAL SITES AND THEIR STORAGE ROOT AND FOLIAGE YIELDS

Rank	Cross-combination PJ x PZ	No. of Commercial Root/Plant	Storage Root Yield (t/ha)	Foliage Yield (t/ha)	Root Dry Matter (%)
1	PJ07.265-PZ06.349	4.11	24.1	28.4	25.8
2	**PJ05.124-PZ08.038***	3.5	27.4	30	25.7
3	PJ05.180-PZ08.018	3.38	29.2	27.6	23.3
4	PJ07.239-PZ06.353	3.33	24.5	43.7	25.4
5	**PJ05.120-PZ08.011***	3.31	31	16.1	25.8

Rank	Cross-combination PJ x PZ	No. of Commercial Root/Plant	Storage Root Yield (t/ha)	Foliage Yield (t/ha)	Root Dry Matter (%)
6	PJ07.544-PZ08.008	3.08	26.6	20.8	26
7	PJ05.324-PZ08.086	3	23.6	27.3	25.1
8	PJ05.124-PZ08.008	2.99	23.5	36.4	24
9	**PJ05.130-PZ08.038***	2.83	27.9	25.2	26.2
10	PJ05.216-PZ08.053	2.78	28.8	26	27.1
11	**PJ05.213-PZ08.038***	2.78	24.3	40.3	27.4
12	PJ07.336-PZ06.349	2.76	21	40.6	27.8
13	PJ07.152-PZ08.170	2.75	28.8	26.8	23.2
14	PJ07.602-PZ08.011	2.74	25.2	24.9	27.8
15	PJ07.298-PZ08.018	2.74	24.8	27.9	26
16	PJ05.180-PZ08.008	2.73	25.5	28.3	25.4
17	**PJ05.213-PZ08.090***	2.7	25.7	42.3	27.3
18	**PJ07.265-PZ08.011***	2.68	20.1	30.7	28
19	PJ05.213-PZ08.008	2.66	26.6	35.3	26.8
20	PJ07.298-PZ08.011	2.63	23.5	30.2	25.5
21	PJ07.522-PZ08.170	2.6	27.2	27	26.1
22	PJ07.262-PZ06.196	2.59	15.8	41.9	25.7
23	PJ07.298-PZ08.153	2.54	24	29.7	27.5
24	PJ05.180-PZ08.090	2.52	22.5	36.6	27.1
25	PJ07.061-PZ08.127	2.49	21.8	28.5	26.3
26	PJ05.257-PZ08.066	2.48	17.8	32.7	26.9
27	PJ07.032-PZ08.066	2.46	24.7	29.3	26.1
28	PJ07.336-PZ06.353	2.38	20.7	47.1	27.4
29	PJ07.064-PZ08.031	2.38	20.2	28.3	27.6
30	PJ07.069-PZ06.115	2.34	20.8	32.6	25.5

NOTE: Selected elite cross-combinations shown in bold.

* Selected elite cross-combinations used additional information about GCA of parents.

TABLE 30. H0 GLOBAL OFSP HYBRID CROSS-COMBINATIONS FOR 100-DAY GROWING PERIODS WITH MORE THAN 2.2 COMMERCIAL ROOTS/PLANT AND MORE THAN 28% DRY MATTER ACROSS EXPERIMENTAL SITES AND THEIR STORAGE ROOT AND FOLIAGE YIELDS

Rank	Cross-combination PJ x PZ	No. of Commercial Root/Plant	Storage Root Yield (t/ha)	Foliage Yield (t/ha)	Root Dry Matter (%)
1	PJ07.037-PZ06.349	2.71	22.7	34.2	28.7
2	**PJ07.061-PZ08.038***	2.67	27.8	31.3	29.8
3	**PJ07.061-PZ06.085***	2.62	19.5	30	28.7
4	PJ07.691-PZ06.196	2.59	21.7	47.3	29.7
5	PJ07.061-PZ08.170	2.47	25.4	26	28.8
6	PJ05.219-PZ08.090	2.43	17	21.3	30.5
7	PJ07.023-PZ06.029	2.3	21.7	24.6	28.4
8	PJ05.216-PZ08.011	2.26	22.9	23.7	31.1
9	PJ07.037-PZ06.304	2.21	20.9	22.5	28.5
10	PJ07.336-PZ06.196	2.19	13.4	27.9	30
11	**PJ07.690-PZ06.304***	2.17	16.3	29.8	29.7
12	**PJ07.079-PZ06.304***	2.17	20.5	40.4	28.3

Rank	Cross-combination PJ x PZ	No. of Commercial Root/Plant	Storage Root Yield (t/ha)	Foliage Yield (t/ha)	Root Dry Matter (%)
13	PJ07.262-PZ06.042	2.15	16.4	37.8	30
14	**PJ05.213-PZ08.153***	2.15	25.8	23.8	32.3
15	PJ05.219-PZ08.127	2.14	19.2	21.9	29
16	PJ05.324-PZ08.008	2.1	20.8	36.4	28.4
17	PJ07.032-PZ06.304	2.02	16.4	33.5	29.5
18	**PJ05.064-PZ08.153***	2	17.4	14.7	31.6
19	PJ05.216-PZ08.018	1.98	17.6	35.6	28.4
20	PJ07.310-PZ08.011	1.73	21.6	24.5	30.8

NOTE: Selected elite cross-combinations shown in bold.

*Selected elite cross-combinations used additional information about GCA of parents.

Fourth analysis step. An offspring–parent analysis was conducted using lsmeans cross-combination by location data in order to estimate GCA and SCA. This was divided into two sub-steps. In the first, the hybrid offspring performance was partitioned into the variance components due to GCA of parental groups PJ and PZ, respectively, and due to SCA (PJ x PZ interaction). In the second sub-step, GCA of individual parents (average cross performance of parents) was determined for individual PJ and PZ parents.

First analysis sub-step. Variance components were estimated for the global H0 OFSP population using lsmean cross-combination by location data which were classified by parent PJ, parent PZ, and location. The model used was "PJ + PZ + E + PJ x PZ + PJ x E + PZ x E." Variance component estimates and corresponding 95% confidence limits were determined (Table 31).

TABLE 31. VARIANCE COMPONENT ESTIMATIONS FOR GCA FOR PJ ($\sigma^2_{GCA\ PJ}$) AND PZ ($\sigma^2_{GCA\ PZ}$) AND SCA ($SCA\ \sigma^2_{PJxPZ}$) IN H0 GLOBAL OFFSPRINGS (523 CROSS-COMBINATIONS) FOR OBSERVED TRAITS ACROSS ENVIRONMENTS (ICA 2016, SATIPO 2015, SATIPO 2016)

Variable	Parameter	$\sigma^2_{GCA\ PJ}$	$\sigma^2_{GCA\ PZ}$	σ^2_{SCA}	$\sigma^2_{GCAxloc\ PJ}$	$\sigma^2_{GCAxloc\ PZ}$	σ^2_{err}
Root yield t^2/ha^2	Estimate	2.6	2.1	0.2	12.9	7.5	28.1
	CL limits	0 - 7.2	4.6 - 12.0	0 - 1.8	8.9 - 18.8	4.6 - 12.0	25.6 - 30.6
Com. roots $N^2/plant^2$	Estimate	0.028	0.024	0.02	0.082	0.091	0.4
	CL limits	0 - 0.07	0 - 0.07	0 - 0.05	0.051 - 0.13	0.052 - 0.16	0.37 - 0.45
Fol. yield t^2/ha^2	Estimate	0	1.1	6.3	53.4	12	69.9
	CL limits	0 - 6.4	0 - 6.4	2.0 - 11.1	39.6 - 72.0	7.1 - 19.1	63.7 - 76.9
DM root	Estimate	0.3	0.32	0	1.12	0.06	6.1
2%	CL limits	0-1.10	0.02-0.78	0-0.45	0-2.18	0- 0.46	5.41-6.77

The H0 global offspring performance was observed to be determined to a larger proportion by GCA and to a lower proportion by SCA, especially for root yield-related traits. The SCA : GCA_{PJ} : GCA_{PZ} variance component ratios are for storage root yield 1 : 13 : 10.5 and for number of commercial storage root 1 : 1.4 : 1.2. A very small SCA variance component was observed for root dry matter content.

Second analysis sub-step. Average cross performance of parents ~~were~~was estimated using lsmean cross-combination by location data classified by parent PJ, parent PZ, and location. The model used was "PJ + PZ + E + PJ x PZ + PJ x En + PZ x E" (Parent PJ and "aren't PZ" fixed all remaining effects random). The

average cross performance of parents minus the overall mean of the lsmean cross-combination by location data was used to calculated the GCA effect for each parent. The GCA for number of commercial root per plant, storage root yield, foliage yield, and root dry matter together average cross performance of parent and the frequency of cross-combinations are provided in Table 32 for PJ parents and in Table 33 for PZ parents.

The GCA for PJ and PZ parents was used to select parents for the next generation cycle for low and high dry matter OFSP for 100 days harvest. For the PJ gene pool, all parents were discarded with GCA values of (1) less than 0.2 number commercial storage roots per plant, (2) less than 0.4 t/ha storage root yield, (3) less than -6.0 t/ha foliage yield, and (4) less than -1% storage root dry matter. For the PZ gene pool, all parents were discarded with GCA values of (1) less than 0.1 number commercial storage roots per plant, (2) less than 0.3 t/ha storage root yield, (3) less than -6.0 t/ha foliage yield, and (4) less than -1.8% storage root dry matter.

TABLE 32. GCA IN PJ PARENTS (N = 56) FOR COMMERCIAL ROOTS/PLANT, STORAGE ROOT YIELD, FOLIAGE YIELD, AND ROOT DRY MATTER WITH GCA ESTIMATES FOR NUMBER OF COMMERCIAL ROOTS/PLANT LARGER THAN ZERO SORTED IN DESCENDING ORDER, TOGETHER WITH AVERAGE CROSS PERFORMANCE OF PARENT EVALUATED ACROSS EXPERIMENTAL SITES (ICA 2016, SATIPO 2015, SATIPO 2017)

Parent	GCA Com. Roots/ Plant	GCA Root Yield (t/ha)	GCA Foliage Yield (t/ha)	GCA Dry Matter (%)	Offspring Mean				N Offsprings
					Com. Roots/ Plant	Root Yield (t/ha)	Foliage Yield (t/ha)	Offspring Mean Dry Matter	
PJ07.245	1.07	6.3	4.1	1.4	3.31	26.4	33	28.8	2
PJ05.130	0.81	7.8	0	-1.1	3.05	27.9	28.9	26.2	2
PJ05.052	0.74	9.5	-3	-2	2.98	29.5	25.9	25.4	1
PJ05.124	0.59	5.2	5.5	-1.7	2.83	25.3	34.4	25.6	5
PJ05.210	0.55	4.4	-2.4	0	2.79	24.4	26.5	27.4	11
PJ07.305	0.42	0.9	2.5	-0.4	2.66	21	31.4	26.9	3
PJ07.265	0.41	-0.5	3.3	0.3	2.65	19.6	32.2	27.7	4
PJ07.586	0.4	0.3	-3.7	-1.6	2.64	20.4	25.2	25.8	1
PJ05.180	0.37	1.4	-0.7	-0.6	2.61	21.5	28.2	26.8	11
PJ07.158	0.34	2.9	-6.2	-2.8	2.58	23	22.7	24.6	9
PJ05.212	0.31	7.3	2.4	1	2.55	27.4	31.3	28.4	12
PJ07.691	0.29	-0.2	18.7	1.9	2.53	19.8	47.6	29.3	3
PJ05.217	0.25	0.5	-1.3	1.1	2.49	20.6	27.6	28.5	15
PJ05.233	0.24	3.5	-3.2	-2.7	2.48	23.6	25.7	24.7	6
PJ05.171	0.22	4.5	-0.9	0.4	2.46	24.5	28	27.8	1
PJ05.213	0.21	4.6	4.7	1.1	2.45	24.6	33.6	28.5	10
PJ05.120	0.21	2.8	-3.3	-0.1	2.45	22.8	25.6	27.3	14
PJ07.304	0.2	3.5	6.8	0.1	2.44	23.5	35.7	27.5	9
PJ07.544	0.11	3.5	-3.5	-0.8	2.35	23.6	25.4	26.6	16
PJ07.079	0.09	-0.6	4	1.5	2.33	19.4	32.9	28.8	4
PJ07.602	0.07	0.5	0.4	-0.4	2.31	20.5	29.3	27	9
PJ07.690	0.06	-1.7	1.3	0.8	2.3	18.3	30.2	28.2	3
PJ07.560	0.06	-7	12.7	0.1	2.3	13	41.6	27.5	4
PJ07.037	0.06	0.7	6.7	1.2	2.3	20.8	35.6	28.6	5
PJ05.219	0.05	0.1	-3.7	2.9	2.29	20.1	25.2	30.3	12
PJ07.522	0.02	1.8	-5.1	-0.2	2.26	21.9	23.8	27.2	26
PJ07.625	-0.02	-0.3	-1.4	0.5	2.22	19.8	27.5	27.9	19

Parent	GCA Com. Roots/ Plant	GCA Root Yield (t/ha)	GCA Foliage Yield (t/ha)	GCA Dry Matter (%)	Offspring Mean			Offspring Mean Dry Matter	N Offsprings
					Com. Roots/ Plant	Root Yield (t/ha)	Foliage Yield (t/ha)		
PJ05.236	-0.03	0.6	-3.5	-0.9	2.21	20.7	25.4	26.5	12
PJ07.336	-0.04	-3	10.8	0.8	2.2	17.1	39.7	28.2	5
PJ07.061	-0.05	-1.2	0.2	0.7	2.19	18.8	29.1	28.1	24
PJ07.069	-0.05	0.9	-3	-1.6	2.19	21	25.9	25.7	11
PJ05.303	-0.05	-0.2	-2.9	-0.8	2.19	19.8	26	26.6	8
PJ05.216	-0.08	1.8	-4.2	0.5	2.16	21.9	24.7	27.9	11
PJ07.023	-0.11	-0.4	-2.1	-0.7	2.13	19.6	26.8	26.7	17
PJ05.324	-0.13	-2.9	-1.8	-0.1	2.11	17.1	27.1	27.3	9
PJ05.257	-0.14	-2.9	2.1	1.8	2.1	17.2	31	29.2	9
PJ05.255	-0.21	-2.1	-3.6	1.6	2.03	18	25.3	29	15
PJ07.002	-0.21	0.3	-0.5	0.9	2.03	20.3	28.4	28.3	5
PJ07.298	-0.22	-0.4	-0.2	0	2.02	19.7	28.7	27.3	25
PJ07.609	-0.24	0.8	-3.4	.	2	20.9	25.5	.	2
PJ05.064	-0.24	-2.7	-14.2	4.2	2	17.4	14.7	31.6	1
PJ05.304	-0.27	-1.9	-1.4	-0.3	1.97	18.2	27.5	27.1	13
PJ07.505	-0.27	-2.3	-1.6	0.3	1.97	17.7	27.3	27.6	3
PJ07.064	-0.27	-2.3	-4.3	0.6	1.97	17.7	24.6	28	19
PJ07.239	-0.28	-2	3.5	1.1	1.96	18.1	32.4	28.4	9
PJ07.325	-0.31	-3.3	10.3	-2.2	1.93	16.7	39.2	25.2	3
PJ05.248	-0.31	-1.9	-6.2	0.3	1.93	18.1	22.7	27.7	12
PJ07.262	-0.32	-3.7	1.4	-0.2	1.92	16.4	30.3	27.2	6
PJ07.032	-0.35	-1.8	5.9	0.4	1.89	18.2	34.8	27.8	25
PJ07.133	-0.35	-2.9	-8	-1.8	1.89	17.2	20.9	25.6	1
PJ07.024	-0.37	-4	0.2	0.1	1.87	16.1	29.2	27.5	12
PJ07.678	-0.38	-0.8	-2.3	0	1.86	19.2	26.6	27.4	8
PJ07.015	-0.41	-7	0.2	-2.3	1.83	13.1	29.1	25.1	3
PJ07.152	-0.43	-1.1	1.7	-1.5	1.81	19	30.6	25.9	6
PJ07.086	-0.62	-4.6	0.5	-1	1.62	15.4	29.4	26.4	3
PJ07.310	-0.65	-5	-0.2	0.9	1.59	15	28.7	28.2	27
PJ07.663	-0.74	-5.4	-8.3	-0.9	1.5	14.6	20.6	26.5	2

NOTE: Selected parents for the next breeding cycle shown in bold.

TABLE 33. GCA IN PZ PARENTS (N=41) FOR COMMERCIAL ROOTS/PLANT, STORAGE ROOT YIELD, FOLIAGE YIELD, AND ROOT DRY MATTER WITH GCA ESTIMATES FOR NUMBER OF COMMERCIAL ROOTS/PLANT LARGER THAN ZERO SORTED IN DESCENDING ORDER, TOGETHER AVERAGE CROSS PERFORMANCE OF PARENT EVALUATED ACROSS EXPERIMENTAL SITES (ICA 2016, SATIPO 2015, SATIPO 2017)

Parent	GCA Com. Roots/ Plant	GCA Root Yield (t/ha)	GCA Foliage Yield (t/ha)	GCA Dry Matter (%)	Offspring Mean			Offspring Mean Dry Matter	N Offsprings
					Com. Roots/ Plant	Root Yield (t/ha)	Foliage Yield (t/ha)		
PZ06.307	0.84	6.3	15.4	-2.8	3.04	26.4	43.9	24.8	1
PZ06.349	0.58	0.3	5.3	0.5	2.78	20.4	33.8	28	14
PZ08.017	0.53	6.6	-1.3	1.3	2.73	26.6	27.2	28.8	3
PZ06.077	0.47	3.6	-9.1	.	2.67	23.6	19.4	.	1
PZ08.011	0.35	2.9	-4.2	0.5	2.55	23	24.3	28	14

Parent	GCA Com. Roots/ Plant	GCA Root Yield (t/ha)	GCA Foliage Yield (t/ha)	GCA Dry Matter (%)	Offspring Mean				
					Com. Roots/ Plant	Root Yield (t/ha)	Foliage Yield (t/ha)	Offspring Mean Dry Matter	N Offsprings
PZ08.137	0.33	2.8	-1.7	1.8	2.53	22.9	26.8	29.4	3
PZ08.038	0.33	3	-2.4	0.2	2.53	23.1	26.1	27.8	20
PZ08.008	0.3	2	-2.8	-0.2	2.5	22	25.7	27.3	22
PZ08.048	0.28	2.3	-1.2	1.1	2.48	22.4	27.3	28.7	5
PZ08.086	0.26	0.2	1.8	-0.9	2.46	20.3	30.3	26.6	5
PZ06.235	0.2	3.7	-4.7	-1.7	2.4	23.8	23.8	25.9	9
PZ08.090	0.14	1.8	0.5	0.4	2.34	21.8	29	27.9	20
PZ08.066	0.14	0.6	1.2	-0.8	2.34	20.6	29.7	26.8	12
PZ08.053	0.14	1.7	-2.2	0.7	2.34	21.8	26.3	28.3	24
PZ06.353	0.14	0.3	5.2	-1.6	2.34	20.3	33.8	25.9	18
PZ08.153	0.12	1.9	-1	0.5	2.32	22	27.5	28	29
PZ08.018	0.09	1.2	-3	0	2.29	21.3	25.5	27.5	19
PZ06.672	0.05	0.2	-2	-1.5	2.25	20.2	26.5	26	3
PZ08.170	0.05	0.7	-2.3	-0.1	2.25	20.8	26.2	27.4	20
PZ08.127	0.03	1.8	-5.4	-0.7	2.23	21.9	23.1	26.8	21
PZ06.029	0	1	-5	0	2.2	21.1	23.5	27.5	12
PZ06.085	0	0	1.4	-1	2.2	20.1	29.9	26.6	12
PZ08.094	-0.02	-0.1	-3.5	0.9	2.18	19.9	25	28.4	5
PZ08.052	-0.06	0.1	-1.7	0.7	2.14	20.2	26.8	28.2	13
PZ08.031	-0.07	-1.4	-1.5	0	2.13	18.7	27	27.5	25
PZ06.698	-0.14	0.1	0.4	1	2.06	20.1	28.9	28.5	15
PZ06.196	-0.16	-3.2	6.5	2.1	2.04	16.9	35	29.6	15
PZ06.709	-0.22	-4.1	-3.8	1.9	1.98	15.9	24.7	29.5	2
PZ06.023	-0.22	-3.3	-2.1	-0.6	1.98	16.8	26.4	26.9	11
PZ06.117	-0.26	-2.2	-0.4	0.1	1.93	17.9	28.2	27.6	16
PZ08.171	-0.29	-1.1	3	0.9	1.91	19	31.5	28.5	12
PZ06.042	-0.31	-3.9	6	0.4	1.89	16.1	34.5	27.9	25
PZ06.050	-0.31	-2.7	0.2	-0.9	1.89	17.4	28.7	26.6	10
PZ06.120	-0.32	-1.4	1.5	-0.1	1.88	18.7	30	27.5	6
PZ06.048	-0.34	-3	-1.1	.	1.86	17	27.4	.	5
PZ06.359	-0.37	-2.4	2.1	-1	1.83	17.7	30.6	26.5	13
PZ06.115	-0.43	-3.7	-0.9	-0.3	1.77	16.4	27.6	27.2	14
PZ06.114	-0.46	-3.1	0.1	-0.4	1.74	17	28.6	27.1	18
PZ06.124	-0.51	-3	6.1	0.4	1.69	17.1	34.6	28	4
PZ06.072	-0.6	-3.9	5.6	-0.5	1.6	16.1	34.1	27	10

NOTE: Selected parents for the next breeding cycle shown in bold.

APPENDIX 2

SPVD pre-breeding

TABLE 34. SELECTED PRE-BREEDING CLONES FROM SPVD PRE-BRED POPULATION 3

Clone	Female Parent CIP Code	Male Parent CIP Code	Female Parent Breeding Code	Male Parent Breeding Code	Parental Group	Com. Roots (N/Plant)	Root Yield (t/ha)	Root Form	BC (ppm)
CIP112168.3	CIP107729.9	CIP110025.7	VJ08.330	VJ11.050	i	2.0	22.5	3	1.2
CIP112169.5	CIP107729.9	CIP110025.4	VJ08.330	VJ11.047	i	2.2	25.8	3	0.2
CIP112169.7	CIP107729.9	CIP110025.4	VJ08.330	VJ11.047	i	1.6	22.6	3	13.2
CIP112170.1	CIP107729.9	CIP110025.1	VJ08.330	VJ11.042	i	2.3	24.7	3	105.0
CIP112170.3	CIP107729.9	CIP110025.1	VJ08.330	VJ11.042	i	1.3	14.4	3	49.2
CIP112170.4	CIP107729.9	CIP110025.1	VJ08.330	VJ11.042	i	1.8	33.0	3	143.7
CIP112170.5	CIP107729.9	CIP110025.1	VJ08.330	VJ11.042	i	2.7	39.3	3	143.7
CIP112170.7	CIP107729.9	CIP110025.1	VJ08.330	VJ11.042	i	1.5	25.6	3	143.7
CIP112170.8	CIP107729.9	CIP110025.1	VJ08.330	VJ11.042	i	3.0	55.8	3	143.7
CIP112184.8	CIP189151.34	CIP189151.34	PJ05.064	PJ05.064	i	2.0	16.1	3	16.5
CIP112184.12	CIP189151.34	CIP189151.34	PJ05.064	PJ05.064	i	1.7	11.0	3	105.0
CIP112185.8	CIP189151.34	CIP107734.5	PJ05.064	VJ08.390	i	3.4	36.2	3	1.5
CIP112185.11	CIP189151.34	CIP107734.5	PJ05.064	VJ08.390	i	3.6	42.9	3	49.2
CIP112185.16	CIP189151.34	CIP107734.5	PJ05.064	VJ08.390	i	4.8	79.7	3	54.6
CIP112186.1	CIP189151.34	CIP107729.9	PJ05.064	VJ08.330	i	3.0	29.3	3	0.0
CIP112186.2	CIP189151.34	CIP107729.9	PJ05.064	VJ08.330	i	4.7	40.1	3	143.7
CIP112186.3	CIP189151.34	CIP107729.9	PJ05.064	VJ08.330	i	2.0	33.2	3	49.2
CIP112186.4	CIP189151.34	CIP107729.9	PJ05.064	VJ08.330	i	2.3	39.0	3	105.0
CIP112186.9	CIP189151.34	CIP107729.9	PJ05.064	VJ08.330	i	1.7	9.5	3	105.0
CIP112186.10	CIP189151.34	CIP107729.9	PJ05.064	VJ08.330	i	3.0	42.3	3	16.5
CIP112186.12	CIP189151.34	CIP107729.9	PJ05.064	VJ08.330	i	2.0	23.7	3	143.7
CIP112186.15	CIP189151.34	CIP107729.9	PJ05.064	VJ08.330	i	2.7	44.3	3	143.7
CIP112145.4	CIP110025.7	CIP110025.3	VJ11.050	VJ11.046	ii	1.5	21.1	3	0.0
CIP112146.4	CIP110025.7	CIP110025.7	VJ11.050	VJ11.050	ii	1.8	34.5	3	0.0
CIP112146.7	CIP110025.7	CIP110025.7	VJ11.050	VJ11.050	ii	2.0	18.5	3	105.0
CIP112146.16	CIP110025.7	CIP110025.7	VJ11.050	VJ11.050	ii	1.5	12.4	3	143.7
CIP112146.18	CIP110025.7	CIP110025.7	VJ11.050	VJ11.050	ii	1.2	18.2	3	16.5
CIP112147.17	CIP110025.7	CIP110025.4	VJ11.050	VJ11.047	ii	1.3	28.6	3	0.0
CIP112149.4	CIP110025.7	CIP107734.5	VJ11.050	VJ08.390	ii	1.8	48.0	3	1.5
CIP112150.7	CIP110025.7	CIP107729.9	VJ11.050	VJ08.330	ii	1.5	39.3	3	1.2
CIP112150.12	CIP110025.7	CIP107729.9	VJ11.050	VJ08.330	ii	1.3	26.1	3	0.0
CIP112152.9	CIP110025.4	CIP110025.7	VJ11.047	VJ11.050	ii	2.3	50.3	3	1.2
CIP112156.4	CIP110025.4	CIP107729.9	VJ11.047	VJ08.330	ii	1.8	21.7	3	143.7

Clone	Female Parent CIP Code	Male Parent CIP Code	Female Parent Breeding Code	Male Parent Breeding Code	Parental Group	Com. Roots (N/Plant)	Root Yield (t/ha)	Root Form	BC (ppm)
CIP112158.3	CIP110025.1	CIP110025.7	VJ11.042	VJ11.050	ii	1.6	21.1	3	13.8
CIP112160.1	CIP110025.1	CIP107734.5	VJ11.042	VJ08.390	ii	1.3	25.9	3	13.2
CIP112176.4	CIP107701.5	CIP110025.3	VJ08.476	VJ11.046	ii	2.0	26.5	3	105.0
CIP112177.1	CIP107701.5	CIP110025.4	VJ08.476	VJ11.047	ii	2.3	20.0	3	105.0
CIP112177.10	CIP107701.5	CIP110025.4	VJ08.476	VJ11.047	ii	2.8	48.3	3	1.2
CIP112177.15	CIP107701.5	CIP110025.4	VJ08.476	VJ11.047	ii	2.0	21.0	3	105.0
CIP112177.16	CIP107701.5	CIP110025.4	VJ08.476	VJ11.047	ii	3.0	22.4	3	143.7
CIP112177.17	CIP107701.5	CIP110025.4	VJ08.476	VJ11.047	ii	1.5	19.9	3	105.0
CIP112208.2	CIP110019.17	CIP107729.9	VJ11.029	VJ08.330	ii	1.6	17.6	3	143.7
CIP112210.1	CIP110019.17	CIP110025.4	VJ11.029	VJ11.047	ii	2.5	30.4	3	105.0
CIP112210.7	CIP110019.17	CIP110025.4	VJ11.029	VJ11.047	ii	2.0	21.0	3	1.2
CIP112210.10	CIP110019.17	CIP110025.4	VJ11.029	VJ11.047	ii	1.2	16.2	3	105.0
CIP112212.10	CIP110019.17	CIP110025.3	VJ11.029	VJ11.046	ii	4.5	64.4	3	0.0
CIP112215.7	CIP110019.16	CIP110019.16	VJ11.028	VJ11.028	ii	2.3	22.9	3	143.7
CIP112215.10	CIP110019.16	CIP110019.16	VJ11.028	VJ11.028	ii	1.6	12.5	3	39.6
CIP112218.9	CIP110019.16	CIP110025.4	VJ11.028	VJ11.047	ii	2.3	22.2	3	105.0
CIP112219.12	CIP110019.16	CIP107729.9	VJ11.028	VJ08.330	ii	2.3	26.7	3	105.0
CIP112223.5	CIP110019.21	CIP107734.5	VJ11.034	VJ08.390	ii	1.7	27.9	3	1.2
CIP112223.6	CIP110019.21	CIP107734.5	VJ11.034	VJ08.390	ii	2.0	22.5	3	105.0
CIP112225.1	CIP110019.21	CIP110019.21	VJ11.034	VJ11.034	ii	2.3	24.7	3	105.0
CIP112226.2	CIP110019.19	CIP110019.19	VJ11.031	VJ11.031	ii	1.6	18.4	3	123.9
CIP112240.1	CIP110019.20	CIP107734.5	VJ11.033	VJ08.390	ii	2.4	34.7	3	0.0
CIP112240.5	CIP110019.20	CIP107734.5	VJ11.033	VJ08.390	ii	1.4	19.1	3	0.0
CIP112242.3	CIP110019.20	CIP110025.3	VJ11.033	VJ11.046	ii	1.2	8.7	3	1.2
CIP112244.11	CIP110019.20	CIP110025.7	VJ11.033	VJ11.050	ii	2.0	21.7	3	6.9
CIP112245.2	CIP110001.1	CIP107729.9	VJ11.001	VJ08.330	ii	2.3	25.6	3	105.0
CIP112245.3	CIP110001.1	CIP107729.9	VJ11.001	VJ08.330	ii	2.4	30.9	3	72.3
CIP112246.8	CIP110001.1	CIP110025.7	VJ11.001	VJ11.050	ii	2.0	20.9	3	143.7
CIP112248.7	CIP110001.1	CIP110025.4	VJ11.001	VJ11.047	ii	1.5	18.4	3	143.7
CIP112249.3	CIP110001.1	CIP110025.1	VJ11.001	VJ11.042	ii	1.5	15.7	3	0.0
CIP112250.2	CIP110001.1	CIP107734.5	VJ11.001	VJ08.390	ii	2.5	23.2	3	10.4
CIP112259.5	CIP105086.1	CIP110025.4	PZ06.085	VJ11.047	ii	3.2	36.9	3	143.7
CIP112259.8	CIP105086.1	CIP110025.4	PZ06.085	VJ11.047	ii	2.2	30.8	3	143.7
CIP112260.2	CIP105086.1	CIP110025.1	PZ06.085	VJ11.042	ii	1.8	28.4	3	110.3
CIP112260.5	CIP105086.1	CIP110025.1	PZ06.085	VJ11.042	ii	2.9	41.6	3	143.7
CIP112261.7	CIP105086.1	CIP110025.3	PZ06.085	VJ11.046	ii	4.0	70.7	3	123.9
CIP112261.9	CIP105086.1	CIP110025.3	PZ06.085	VJ11.046	ii	2.2	39.6	3	143.7
CIP112261.12	CIP105086.1	CIP110025.3	PZ06.085	VJ11.046	ii	2.5	33.0	3	143.7

Clone	Female Parent CIP Code	Male Parent CIP Code	Female Parent Breeding Code	Male Parent Breeding Code	Parental Group	Com. Roots (N/Plant)	Root Yield (t/ha)	Root Form	BC (ppm)
CIP112261.14	CIP105086.1	CIP110025.3	PZ06.085	VJ11.046	ii	2.8	32.3	3	105.0
CIP112263.2	CIP105086.1	CIP107729.9	PZ06.085	VJ08.330	ii	3.0	66.7	3	143.7
CIP112263.3	CIP105086.1	CIP107729.9	PZ06.085	VJ08.330	ii	3.2	34.3	3	105.0
CIP112263.7	CIP105086.1	CIP107729.9	PZ06.085	VJ08.330	ii	2.3	30.3	3	110.3
CIP112263.10	CIP105086.1	CIP107729.9	PZ06.085	VJ08.330	ii	3.8	50.2	3	105.0
CIP112264.9	CIP105086.1	CIP105086.1	PZ06.085	PZ06.085	ii	2.8	31.6	3	143.7
CIP112265.1	CIP105086.1	CIP107734.5	PZ06.085	VJ08.390	ii	5.7	49.3	3	143.7
CIP112265.8	CIP105086.1	CIP107734.5	PZ06.085	VJ08.390	ii	3.0	34.7	3	143.7
CIP112265.13	CIP105086.1	CIP107734.5	PZ06.085	VJ08.390	ii	4.0	45.4	3	123.9
CIP112278.1	CIP110019.4	CIP107734.5	VJ11.036	VJ08.390	ii	1.2	15.0	3	105.0
CIP112278.2	CIP110019.4	CIP107734.5	VJ11.036	VJ08.390	ii	2.3	25.4	3	1.2
CIP112161.1	CIP107734.5	CIP110025.3	VJ08.390	VJ11.046	ii	3.8	33.9	3	0.0

Note: BC = Beta-carotene root content in ppm on fresh matter basis.

APPENDIX 3

Heterosis and Hybrid Breeding in Potato and Sweetpotato
Workshop May 30-31 – CIP Lima Peru

Executive summary

This workshop was organized to discuss within the CIP breeding community and external experts the current state of exploiting heterosis and hybrid breeding schemes to develop superior cultivars both in sweetpotato and potato more efficiently.

Unlike previous attempts on TS 4x (tetraploid) potato, at the moment there is enough experimental evidence, interest of private companies, and global academic critical mass to warrant revamping CIP's research on the topic. The explicit objective is to develop superior varieties in terms of productivity, agronomic traits, and, particularly, yield stability—the latter being a key trait for smallholders benefited from CIP work. Though research questions remain, CIP has conducted enough basic breeding research to sustain both sweetpotato and potato hybrid breeding programs targeting hybrid breeding populations in sweetpotato and TS 2x (diploid) hybrid varieties in potato.

This brief document summarizes discussions held, defines current research gaps, and suggests a road map strategy to achieve the overall objective of making available to smallholders potato and sweetpotato hybrid varieties by hybrid breeding technology in the midterm.

1. General

Participants: Two experts, Dr. Shelly Jansky, professor at the University of Wisconsin-Madison (US) and Dr. Jochen Reif, IPK Gatersleben, Germany, participated as invited speakers. CIP potato and sweetpotato breeders from Lima and SSA, plus Marc Ghislain, leader of CIP's strategic program 4, served as speakers and/or participants.

Goals of the workshop: To discuss the exploitation of heterosis in potato and sweetpotato with emphasis on 2x (diploid) TS potato hybrid breeding, performance of 2x potatoes and hybrids derived from homozygous inbred lines, potential pathways for potato and sweetpotato to systematically exploit heterosis, and to identify potential fund-raising pathways.

Note: Several private companies are investing in 2x TS potato hybrid breeding (i.e., Solynta, KWS, and PepsiCo). It appears that at least Solynta has TS hybrid material in the breeding pipeline for variety release testing.

2. Summary of All Presentations

The single most important element in hybrid breeding—Heterotic groups

Why develop hybrid varieties? To (1) achieve yield increase, (2) ease stack simple inherited traits such as disease resistance, and (3) obtain elevated yield stability. Achieving yield stability and resilience to climate change is much more effective via hybrid breeding than increasing current breeding efforts. The backbone of a successful hybrid breeding program is developing so-called heterotic groups and economic, feasible hybridization systems. When breeding parents from different heterotic groups are crossed, their siblings

express hybrid performance above their parent's. Hybrid varieties are more productive than either parent, display a higher environmental stability than regular varieties, and sustain a higher genetic gain, thus benefitting the smallholders CIP serves.

Sweetpotato

CIP started in 2004 to separate gene pools. In 2008 its research began to exploit heterosis in hybrid breeding populations with the goal of achieving better breeding populations and enhanced multi-trait genetic gains in population improvement by combining inbreeding and outbreeding into the breeding scheme. PJ x PZ hybrid breeding populations exhibit on average a storage root yield advantage of 20%, yet many crosses exhibit storage root yield heterosis increments over 40%. High GCA was detected in PJ x PZ hybrid combinations. On the basis of these encouraging results, three hybrid populations were developed at CIP-Lima within the SASHA2 project, targeting (1) OFSP for wide adaptation and earliness (90 days), (2) non-sweet OFSP, and (3) high-Fe OFSP.

A similar effort is underway in Uganda within SASHA2. Its objectives are to increase root yield and SPVD resistance, the current most significant breeding issue with extremely low frequencies of SPVD resistance in breeding populations under high SPVD pressure (0.1–0.2%).

An additional, significant advantage of developing a sweetpotato hybrid breeding program will be rapid hybrid seed dissemination to NARS partners. This advantage is already used by CIP's global program by repeating the best cross-combinations and TS shipment to NARS partners.

Diploid potato, self-compatibility, and hybrid performance

The consensus of potato breeders is that the benefits of working with inbred lines or partially inbred lines to target true F1 hybrid potato are too substantial to be ignored any longer. Further, the large German breeding company KWS sold all 4x breeding material after a larger gene pool of 2x potatoes were developed from 4x potatoes. Work at the University of Wisconsin has shown, based on a model population of two homozygous 2x potato lines, that it is possible to develop a large number of diploid advanced breeding lines 100% SC using a SI inhibitor *Sli* from *S. chacoense*. Through breeding and genetic selection, improved 2x populations have been created which in best cases deliver tuber yield heterosis of 300–400% over homozygous parents and even out-yielding 4x check varieties 'Superior' and 'Atlantic' by 58% and 16%, respectively. There were intensive discussions about the need of using completely or partially homozygous lines to develop 2x seed hybrid varieties. In maize, the development of hybrid varieties started with inbreed but still heterozygous parents.

Tetraploid potato gene pools at CIP and heterosis increments

Two advanced populations for resistance to LB adapted to the Andean highlands are available. Populations B3C1 and B1C4. B3C1 carry several sources of LB resistance (*S. demissum, S. tuberosum* Tuberosum*, S. tuberosum* Andigena*)*, whereas B1C4 is entirely derived from *S. tuberosum* Andigena and exhibits high dry matter content, 120 days from planting to harvest and high levels of resistance to LB. Using a limited, small sample size from interpopulation crosses, heterosis increments for tuber yield up to 150% have been observed. In addition, significant heterotic effects for tuber yield have been observed when crossing two other CIP breeding populations, namely LTVR (adapted to lowland tropics, early maturity, and multiple virus resistance) and B3 (adapted to highland tropics and LB resistant).

True diploid hybrid potato breeding at CIP

True 2x hybrid potato at CIP aims at uniform, high-yielding TS hybrids from complementary inbred breeding parents. The TS hybrids at CIP have yet to establish a breeding pipeline. Diploid breeding has

several advantages over 4x breeding in potato: (1) easier trait combination, (2) easier phenotypic assessment, (3) efficient removal of unfavorable alleles, (4) compatibility with 2x wild species (genetic diversity and desirable traits), and (5) genomic tools developed in other 2x crops can be used. CIP has also started to use the dominant *Sli* allele from *Solanum chacoense* to make SC inbreds, though additional SC sources have also been observed. CIP has developed three 2x gene pools to create inbred parents with different breeding objectives: (1) dihaploid germplasm from advanced/elite 4x lines from CIP populations, (2) 2x biofortified hybrids from 2x selected cultivars of groups Phureja and Stenototmum, and (3) crop wild relative-derived germplasm with novel genes for resistance to biotic and abiotic stress.

Hybrid breeding and heterosis exploitation in other RTB crops and sugarcane

Across RTB crops breeders observed outstanding clones and offsprings surpassing mid-parent performance. In cassava breeding at IITA this had led to considerable investments into genome-wide prediction. However, no studies on heterotic pattern have been made and no gene pools have been separated for systematic heterosis exploitation. In cassava breeding at CIAT, a larger number of inbred lines have been generated. However, no studies on heterotic pattern have been made and no gene pools for systematic heterosis exploitation or hybrid breeding have been formed either. In banana breeding at IITA (East African highland bananas), there are very impressive observations to which extend 3x clones can surpass 2x and 4x parents, but no systematical resynthesize of 3x clones with 2x and 4x gene pools have been conducted. In sugarcane breeding the observation of outstanding families has led to intensive prediction of parental values based on offspring performance by mixed model statistic. But despite the huge economic relevance of sugarcane for sucrose and ethanol production, no heterotic pattern has been studied and no gene pools for systematic heterosis exploitation and elevated offspring performance have been formed.

3. Research gaps and pathways

Potato research gaps and pathways over the short term (2–3 years):

- Discussions with institutions (Michigan State University and University of Wisconsin-Madison, and IPK, Germany) and private companies with expertise in hybrid breeding: KWS (Germany), HZPC (The Netherlands).
- Use of documented pedigree data for 2x farmer varieties (potato catalogue) and diploids derived from tetraploids (about 1,100 2x lines).
- Evaluation of the entire 2x potato germplasm at CIP in multilocation trials to identify 150 2x potato lines with superior performance.
- Prove new SC sources other than *S. chacoense*.
- Fast-track approach using Atzimba, R-218.6, Serrana, and LT-7 (parental basis of two superior TPS varieties)—diploidization and use of *Sli* system.

Potato research gaps and pathways over the medium term (3–5 years):

- F1 2x derived from 3-way crosses followed by 2-stage selection in segregating generation for self-crossing -> target 150 S1 or S2 best performing inbreds.
- Evaluation of 700 hybrids (at least eight locations) developed based on 150 S1 (S2) best performing inbreds and applying genome-wide prediction to establish a full diallel cross prediction matrix.

- Establish heterotic groups by simulated annealing algorithm using full diallel cross prediction matrix and select 30 parental lines in each gene pool, narrowing down to just 15 parents per pool after selection.
- Reproduce best hybrids considering tuber yield attributes and TP seed attributes for entering into variety release.
- **Diploid hybrid breeding program fully established in year 6.**

Sweetpotato research gaps and technical pathway over the short term:

- Implementation of a heterosis exploiting breeding scheme at the breeding platform in Uganda with moderate emphasis on yield and strong emphasis on SPVD resistance (model breeding scheme at CIP–Lima for non-sweet sweetpotato).
- Identification of a set of testers in populations PJ and PZ and increase fivefold selection intensity on parental material.

4. Follow-ups and fund-raising strategy to fill in gaps identified

- Use GIZ proposal call in October 2017 to support midterm potato research gaps/pathways in partnership with IPK.
- RTB breeding platform breeders alignment for GIZ proposal call in 2018/19 to submit a joint proposal on heterosis across centers (CIP, IITA, CIAT). A side session at ISTRC meeting in Cali in 2018 will focus on heterosis exploitation in RTB crops and to join with nextgen breeders to use genome-wide predictions to establish heterotic groups based on hybrid/hybrid-offspring performance data from multi-environmental trials.
- Develop a concept note for BMGF on developing RTB hybrid crops.
- Link RTB effort on heterosis exploitation and hybrid breeding to FFAR Effort to Accelerate Crops of the Future.
- Sweetpotato will target SASHA phase 3 to exploit heterosis with strong emphasis on SPVD resistance in Uganda. Depending on expertise and skills available on breeding platforms in Mozambique and Ghana, will make first steps into heterosis exploitation.
- Sweetpotato global will target RTB-FOOD WP5 for non-sweet sweetpotato heterosis exploitation breeding (already observed to be successful despite very high selection intensities) and USAID for wide adaptation and earliness by sweetpotato heterosis exploitation.

APPENDIX 4

Publications (1st year, Oct. 2015–Sept. 2016)

Andrade MI., A. Naico, J. Ricardo, R. Eyzaguirre, GS. Makunde, and WJ Grüneberg. 2016. Genotype x environment interaction and selection for drought adaptation in sweetpotato (*Ipomoea batatas* [L.] Lam.) in Mozambique. *Euphytica* 209: 261–280.

Andrade MI., A. Alvaro, J. Menomussanga, GS. Makunde, J. Ricardo, WJ Grüneberg, R. Eyzaguirre, J. Low, and R. Ortiz. 2016. 'Alisha', 'Anamaria', 'Bie', 'Bita', 'Caelan', 'Ivone', 'Lawrence', 'Margarete', and 'Victoria' Sweetpotato. *HortScience* 51(5): 597–600.

Mwanga, ROM., G. Kyalo, GN. Ssemakula, C. Niringiye, B. Yada, MA. Otema, J. Namakula, A. Alajo, B. Kigozi, RNM. Makumbi, A. Ball, WJ. Grüneberg, JW. Low, and GC. Yencho. 2016. 'NASPOT 12 O' and 'NASPOT 13 O' Sweetpotato. *HortScience* 51(3): 291–295.

Publications (2nd year, Oct. 2016–Sept. 2017)

Alvaro A., MI. Andrade, GS. Makunde, F. Dango, O. Idowou, and W. Grüneberg. 2017. Yield, nutrition quality, and stability of orange-fleshed sweetpotato cultivars successively later harvesting periods in Mozambique. *Open Agriculture* 2: 464–468. (published by De Gruyter Open)

Andrade MI., J. Ricardo, A. Naico, GS. Makunde, J. Low, R. Ortiz, and WJ. Grüneberg. 2017. Release of orange-fleshed sweetpotato (*Ipomoea batatas* [L.] Lam.) cultivars in Mozambique through an accelerated breeding scheme. *Journal of Agricultural Science* 155: 919–929.

Andrade MI., GS. Makunde, J. Ricardo, J. Menomussanga, A. Alvaro, and WJ. Grüneberg. 2017. Survival of sweetpotato (*Ipomoea batatas* [L.] Lam) vines in cultivars subjected to long dry spells after the growing season in Mozambique. *Open Agriculture* 2: 58–63. (published by De Gruyter Open)

Gastelo Benavides, Manuel, Luis Diaz, Gabriela Burgos, Thomas Zum Felde, Merideth Bonierbale 2017 Heritability for Yield and Glycoalkaloid Content in Potato Breeding under Warm Environments. Open Agriculture. 2017; 2: 561–570. https://doi.org/10.1515/opag-2017-0059

Gastelo Benavides, Manuel, Luis Diaz, Gabriela Burgos, Thomas Zum Felde, Merideth Bonierbale 2017 Heritability for Yield and Glycoalkaloid Content in Potato Breeding under Warm Environments. Open Agriculture. 2017; 2: 561–570. https://doi.org/10.1515/opag-2017-0059 In press

Makunde GS., MI. Andrade, J. Ricardo, A. Alvaro, J. Menomussanga, and WJ. Grüneberg. 2017. Adaptation to mid-season drought in a sweetpotato (*Ipomoea batatas* [L.] Lam) germplasm collection grown in Mozambique. *Open Agriculture* 2: 133–138. (published by De Gruyter Open)

Mwanga ROM., MI. Andrade, EE. Carey, JW. Low, GC. Yencho, and WJ. Grüneberg. 2017. Sweetpotato (*Ipomoea batatas L.*). *In* H. Campos and PDS. Caligari (eds.) *Genetic Improvement of Tropical Crops*. Springer International Publishing AG.

Hirut B, Shimelis H, Fentahun M, Bonierbale M, Gastelo M, Asfaw A (2017) Combining ability of highland tropic adapted potato for tuber yield and yield components under drought. PLoS ONE 12(7): e0181541. https://doi. org/10.1371/journal.pone.0181541