2018 FINAL REPORT

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

31 October 2018

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October 2017–September 2018

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ACRONYMS

ABMs	Accelerated breeding methods
ABS	Accelerated breeding scheme
APC	Arid Pacific coast
AUDPC	Area under the disease progress curve
BW	Bacterial wilt
CIP	International Potato Center
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
GTDMS	Global trial data management system
GWAS	Genome-wide association studies
HIDAP	Highly Interactive Data Analysis Platform
HTA	Humid tropics of the Amazon Basin
LB	Late blight
LBHT	LB-resistant, Heat Tolerant potato population
LTVR	Lowland tropic virus-resistant potato population
NARS	National agriculture research system
NIRS	Near-infrared reflectance spectroscopy
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
RSA	Root system architecture

RTB	CGIAR Research Program on Roots, Tubers and Bananas
SASA	Semi-arid savanna of Southern Africa
SC	Self-compatibility
SCA	Specific combining ability
SI	Self-incompatible
Sli	S-locus inhibitor
SNP	Single-nucleotide polymorphism
SSA	Sub-Saharan Africa
SSR	Simple sequence repeat
TGA	Total glycoalkaloids
TS	True seed
UPLC	Ultra performance liquid chromatography
USAID	United States Agency for International Development
Zn	Zinc

EXECUTIVE SUMMARY

This report covers the period Oct. 2017–Sept. 2018 of project "Advancing Achievements in Breeding for Early, Resilient, and Nutritious Potato" 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 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 and their families.

Report organization

The "Advancing Achievements in Breeding for Early, Resilient, and Nutritious Potato" is a technical project that advances targeted and complex potato breeding programs. 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 previous report submitted to USAID. This wealth of data helps to sustain the science behind breeding research and its potential for delivery and impact on end-users.

Section 2 summarizes the achievements of potato breeding programs by outputs. The section is organized by individual project output, and the set of deliverables and milestones is reported for specific outputs. The report concludes with a supporting appendix.

Most salient results, and their relevance, are as follows:

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

<u>Result</u>

20 advanced clones from inter-population crosses (LBHT x LTVR) exhibiting substantial heterosis for tuber yield have been selected in Peru.

<u>Relevance</u>

Reported results represent valuable progress within the context of CIP's potato breeding program transitioning from a program only delivering traditional, 4X varieties, into one developing hybrid 2X varieties and 4x traditional varieties.

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

<u>Result</u>

Three high yielding (>40 t/ha), late blight resistant (better than resistant variety Belete) and high cooking quality have been identified for Ethiopian highlands growing conditions, and in addition advanced clones adapted to heat stress condition in mid-altitude and low land areas have been selected in Ethiopia.

Relevance

Breeding efforts deployed during the timeframe reported represented additional sources of resistance to the main disease affecting potato – Late Blight - and climate resilience for Ethiopian smallholders.

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

Result

Several elite clones have been introduced to CIP's Tissue Culture labs in Nairobi, Kenya, with the intend of becoming disease clean and so suitable to be distributed to interested SSA countries. Furthermore, the cleaning process for Rwandan local varieties, biofortified clones selected in Ethiopia, and 5 LTVR x LBHT clones selected in Kenya, started in anticipation to their distribution to interested SSA countries

Relevance

The process of cleaning is an essential step before distributing elite, advanced clones to interested countries, in order to preserve the genetic potential of these clones and to realize their contribution to smallholder's production efforts.

Final remarks

Within the period herein reported, CIP's potato breeding program has delivered the genetic progress committed to USAID across several foci of work. On one side, the program kept developing novel sources of resistance to main diseases such as late blight, enhanced climate resilience to withstand increasing climate unpredictability, and also nutrient enriched (both Fe and Zn) elite advanced clones. On the other, the program was able to achieve progress towards its transformation into becoming, in the medium term, a breeding program able to deliver not only traditional, 4X varieties, but also 2X potato hybrid varieties, and so better suited to fulfill the varied and increasing expectations of the multiple African and Asian countries where smallholder producers rely on potato as a source of income, food, nutrition and wellbeing for they and their families.

1. PROJECT GOALS

The International Potato Center (CIP) focuses 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 varieties. Potato is an important food crop 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 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 2017 and September 2018 supported the overall potato 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 varieties more 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, improve well-being 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. 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. CIP's global potato crop improvement program emphasizes 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 breeding program contribute to the center's overall goals through three main objectives:

1. Develop nutritious and biodiverse potato populations with recognized added value and high variety ability.
2. Develop resilient potato 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 developed as sources of early maturing, high and stable yielding varieties with resistance to biotic and abiotic stresses and quality traits.
2. Breeding research aligned with farmers and end users' preferences.
3. Accelerated breeding methods and tools to help breeders select potato genotypes and parental lines available.
4. Improved and shared breeding databases available and knowledge management, including trait specific protocols and catalogs to support the orientation of breeding products and facilitate decision making.
5. Iron biovailability of biofortified potatoes in humans determined.

2. FINAL REPORT

2.1 SUMMARY OF ACHIEVEMENTS BY OUTPUT

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 targeting tropical highland and mid-elevation agro-ecologies

Milestone achieved: Phenotypic Stability for tuber yield and Late Blight resistance in advanced clones from B3C3

Means of verification: Technical report

Group B3C3 comes from the cross between elite clones of B3C2, started in 2011, planting 30,000 genotypes in greenhouses in La Molina, and at harvest 21,685 clones were selected for their good agronomic characters. Then, in 2011-2012, they were planted in the field in Huancayo, Peru and at harvest 3,005 clones were selected for yield and good agronomic characters such as skin color, flesh color, tuber shape and eyes depth, plant vigor, uniformity of tubers.

In 2012-2013 these clones were planted in Oxapampa, Peru in order to evaluate their late blight (LB) resistance, tuber yield, selecting 507 at harvest. These clones continued to be evaluated and selected in Oxapampa and Huancayo for LB resistance and yield respectively during 2013 to 2015, 80 clones were selected with high level of LB resistance, high yield, some of them good aptitude for French fries and/or chips, resistance to virus PVX and/or PVX, heat tolerance, precocity (90 days) and low glycoalkaloid content. Out of such selection process, the best 30 clones were selected for this study.

During 2016 until 2018, the phenotypic stability for late blight resistance and tuber yield in 30 advanced clones belonging to population B group B3, third cycle 3 - B3C3 was studied (Table 1). Eight experiments were performed; Three in Oxapampa -OXA (2,000 masl) to study the phenotypic stability of resistance to late blight and five in La Molina-LAM 2016 (150 masl), Huancayo – HYO, 2016 and 2017 (3,200 masl) and San Ramon – SRA, 2016 and 2017 (800 masl) for the tuber yield. A randomized complete blocks statistical design was used, with three repetitions of 10 plants each.

Information on late blight resistance was taken, through the percentage of leaf area damaged by this disease, for 6 weeks at 7-day intervals. With the information obtained, the area under the curve of progress of the disease, AUDPC and the scale of susceptibility to late blight (SAUDPC), with values from 1 to 9 were calculated. At harvest for tuber yield, we taken the number of plants harvested, the number and weight of commercial and, non-commercial tubers, then the commercial (MTY) and total yield (TTY) per Hectare was calculated.

For the analysis of phenotypic stability, the AMMI (additive main effects and multiplicative interaction) methodology was used. We used for late blight resistance the values of AUDPC and the Scale of susceptibility to late blight, and for tuber yield we used commercial and total yield per hectare.

Analysis of variance for total and commercial tuber yield and resistance to late blight (AUDPC and SAUDPC), shows statistically significant differences (α =0.01) for environments, clones and the interaction of clones x environments, the CVs were within the normal range. Principal components (PC) contributed significantly in the explain of the interaction clones x environment (Table 2).

From the biplot of the AMMI phenotypic stability analysis for resistance to late blight, measured through the AUDPC, shown in Figure 1, the principal components PC1 and PC2 explain 60.6% and 39.4% of the interaction of clones x environments respectively. 24 of the 30 clones under study are phenotypically stable in their resistance to late blight with average values of AUDPC from 78.33 to 535.69, and 6 clones do not show stability in their resistance. These results indicate that stable clones maintain their resistance in comparison to non-stable clones that vary in their resistance over time.

In the biplot for the commercial tuber yield (MTY), the principal components PC1 and PC2 explain 48.5% and 34.5% of the interaction clones x environments respectively. (Figure 3), showing that the clones CIP308427.194, CIP308436.173, CIP308436.245, CIP308441.201, CIP308452.167, CIP308482.163, CIP308486.187, CIP308486.355, CIP308490.407, CIP308493.22, CIP308510.80, CIP308513.318, CIP308518.201 and CIP308518.7 are phenotypically stable with average tuber yields in the range of 22.57 to 28.32 th^{-1}. The clones CIP308480.287, CIP308497.212, CIP308498.191, CIP308498.280, CIP308513.404, CIP308517.91 and CIP308519.433, were also phenotypically stable but with average tuber yields less than 20 th^{-1}. The clones CIP308478.59, CIP3078487.157 and CIP308487.197 are phenotypically unstable and are adapted under HYO conditions with an average yield of 22.43 to 40.68 th^{-1}, clones CIP308474.153, CIP308479.56 and CIP308480.292 with 25.89, 26.06 y 23.80 th^{-1}, are also unstable but they are adapted to SRA. (Table 2)

TABLE 1. CLONES B3C3 WITH RESISTANCE TO LATE BLIGHT IN STUDIO FOR PHENOTYPIC STABILITY FOR RESISTANCE TO LATE BLIGHT AND TUBER YIELD

#	Clone	Female	Male	Skin Color	Flesh Color	Tuber Shape	Eyes Deep	TTY th^{-1}	MTY th^{-1}	AUDC	SAUDPC
1	CIP308427.194	CIP395017.229	CIP395011.2	pink	cream	oblong	superficial	32.57	28.32	192.50	1.34
2	CIP308436.173	CIP395111.13	CIP395011.2	cream	cream	elliptical	superficial	30.68	28.30	220.28	1.53
3	CIP308436.245	CIP395111.13	CIP395011.2	cream	cream	elliptical	superficial	31.12	26.37	293.06	2.16
4	CIP308441.201	CIP395114.5	CIP396240.2	cream	cream	elliptical	superficial	28.82	24.93	405.28	3.07
5	CIP308452.167	CIP396026.101	CIP395011.2	cream	cream	elliptical	superficial	25.66	24.04	117.50	0.94
6	CIP308474.153	CIP395037.107	CIP395096.7	Red/Cream	cream	oval	Superficial	28.33	25.89	464.17	4.46
7	CIP308478.123	CIP395096.2	CIP396264.14	cream/pink	cream	oval	superficial	25.71	23.73	355.56	2.68
8	CIP308478.59	CIP395096.2	CIP396264.14	cream	cream	oblong	superficial	43.50	40.68	315.28	2.17
9	CIP308479.56	CIP395096.5	CIP395017.242	cream	cream	rounded	superficial	29.01	26.06	453.33	3.42
10	CIP308480.287	CIP395109.29	CIP395017.242	cream	cream	elliptical	superficial	20.02	17.73	78.33	0.50
11	CIP308480.292	CIP395109.29	CIP395017.242	cream	cream	elliptical	superficial	26.00	23.80	320.56	2.53
12	CIP308482.163	CIP395109.34	CIP396038.107	pink	cream	Oblong	superficial	29.80	27.13	330.28	2.52
13	CIP308486.187	CIP395112.32	CIP396012.288	Cream	Cream	Round	Superficial	27.40	24.39	319.72	2.37
14	CIP308486.355	CIP395112.32	CIP396012.288	Purple	Cream	Round	Superficial	31.14	27.62	510.56	3.91
15	CIP308487.157	CIP395112.32	CIP396264.14	Red	Cream	Oval	Superficial	24.72	22.43	211.39	1.47
16	CIP308487.197	CIP395112.32	CIP396264.14	red	cream	oblong	superficial	29.74	26.79	387.78	2.80
17	CIP308490.332	CIP395112.36	CIP396263.8	cream/pink	cream	oblong	superficial	22.04	19.57	376.11	2.47
18	CIP308490.407	CIP395112.36	CIP396263.8	cream/pink	cream	oval	superficial	30.12	27.04	633.06	4.51
19	CIP308493.22	CIP395117.3	CIP395096.3	cream	cream	oval	superficial	27.90	22.57	366.11	2.48
20	CIP308497.212	CIP396004.225	CIP396041.102	Red	yellow	oval	superficial	24.37	20.50	428.06	2.59
21	CIP308498.191	CIP396004.263	CIP395017.229	Cream	yellow	oval	superficial	17.41	15.48	338.61	3.57
22	CIP308498.280	CIP396004.263	CIP395017.229	Cream	Cream	Oval	Superficial	20.71	18.46	366.11	2.63
23	CIP308505.377	CIP396009.239	CIP396004.337	cream/pink	cream	oblong	superficial	22.40	20.39	535.69	5.80
24	CIP308510.80	CIP396031.118	CIP395077.12	Pink	yellow	oval	superficial	27.56	24.15	256.39	1.82
25	CIP308513.318	CIP396033.102	CIP395152.16	Purple	Cream	Oval	Superficial	29.13	26.95	224.44	2.53
26	CIP308513.404	CIP396033.102	CIP395152.16	purple	cream	oblong	superficial	19.24	17.59	336.94	2.39
27	CIP308517.91	CIP396034.103	CIP396038.107	Red	cream	Oblong	superficial	22.64	19.94	70.83	0.50
28	CIP308518.201	CIP396034.103	CIP396041.102	Pink	cream	oblong	superficial	30.25	25.55	71.39	0.46
29	CIP308518.7	CIP396034.103	CIP396041.102	Red	cream	oblong	superficial	26.70	23.74	151.11	1.07
30	CIP308519.433	CIP396046.105	CIP396017.227	cream/pink	cream	oblong	superficial	21.38	19.71	411.94	2.88
31	Kory - INIA	Control								587.19	3.76
32	Amarilis - INIA	Control								1076.26	5.69
33	Yungay	Control								1095.49	6.00

TABLE 2.- ANALYSIS OF VARIANCE AMMI FOR RESISTANCE TO LATE BLIGHT (AUDPC Y SAUDPC) AND TOTAL AND MARKETABLE TUBER YIELD OVER ENVIRONMENTS 2016-2018

Source of variation	df	Mean Square	Mean Square	df	Mean Squar	Mean Square
		TTY	MTY		AUDPC	SAUDPC
Environment	4	12989.50**	11688.20**	2	10671116.00**	618.70**
Replicactions/Environment	10	64.10	54.40	6	13681.00**	1.07**
Clones	29	390.20**	335.60**	32	483011.00**	18.78**
clones x environment	116	223.80**	211.40**	64	126878.00**	10.30**
PC1	32	379.07**	372.04**	33	149154.40**	16.60**
PC2	30	190.36**	282.13**	31	103163.50**	3.61**
PC3	28	87.39**	85.58**			
PC4	26	33.75**	67.60**			
Pooled error	290	35.70	34.00	192	3163.00	0.22
CV (%)		25.41	24.30		15.09	17.25

SAUDP = Scale of susceptibility to late blight

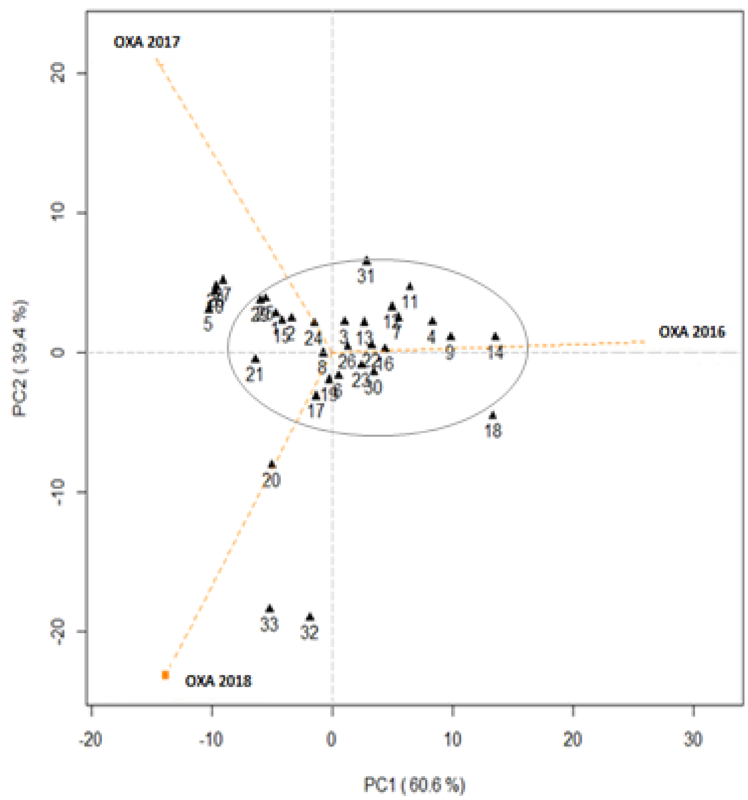


Figure 1.- Biplot from AMMI ANALYSIS for Late Blight resistance showing first and second principal components (PC1 and PC2) for three locations and 30 genotypes 2016-2018

For total tuber yield (TTY), the biplot for principal components PC1 and PC2 explain 50.4% and 32.5% of the interaction clones x environments. 18 clones showed phenotypic stability for this character with tuber yields from 25.71 to 31.14 th^{-1} (Table 3, Figure 2).

13 clones combine stability for commercial and total tuber yield and late blight resistance, these clones can be used to initiate registration processes or, once their merit as parents determined, as parental in crossing plans by NARS (Table 3).

TABLE 3.- PHENOTYPIC STABILITY AMMI FOR COMMERCIAL AND TOTAL TUBER YIELD AND RESISTANCE TO LATE BLIGHT (AUDPC)

#	Clone	TTY	MTY	Resistance to LB - AUDPC
1	CIP308427.194	Unstable	Stable	Stable
2	CIP308436.173	Stable	Stable	Stable
3	CIP308436.245	Stable	Stable	Stable
4	CIP308441.201	Stable	Stable	Stable
5	CIP308452.167	Stable	Stable	Unstable
6	CIP308474.153	Unstable	Unstable	Stable
7	CIP308478.123	Unstable	Unstable	Unstable
8	CIP308478.59	Unstable	Unstable	Stable
9	CIP308479.56	Unstable	Unstable	Stable
10	CIP308480.287	Stable	Stable	Stable
11	CIP308480.292	Unstable	Unstable	Stable
12	CIP308482.163	Stable	Stable	Stable
13	CIP308486.187	Unstable	Stable	Stable
14	CIP308486.355	Stable	Stable	Stable
15	CIP308487.157	Unstable	Unstable	Stable
16	CIP308487.197	Unstable	Unstable	Stable
17	CIP308490.332	Stable	Unstable	Stable
18	CIP308490.407	Stable	Stable	Unstable
19	CIP308493.22	Stable	Stable	Stable
20	CIP308497.212	Stable	Stable	Unstable
21	CIP308498.191	Stable	Stable	Stable
22	CIP308498.280	Stable	Stable	Stable
23	CIP308505.377	Unstable	Unstable	Stable
24	CIP308510.80	Unstable	Stable	Stable
25	CIP308513.318	Stable	Stable	Stable
26	CIP308513.404	Stable	Stable	Stable
27	CIP308517.91	Stable	Stable	Unstable
28	CIP308518.201	Unstable	Stable	Unstable
29	CIP308518.7	Stable	Stable	Stable
30	CIP308519.433	Stable	Stable	Stable

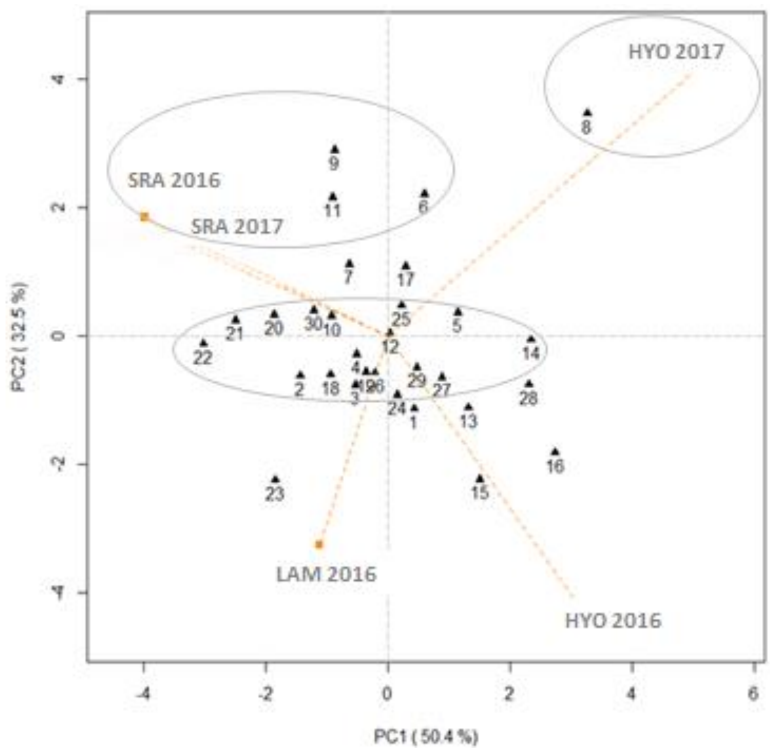


Figure 2.- Biplot from AMMI ANALYSIS for total tuber yield showing first and second principal components (PC1 and PC2) at five locations and 30 genotypes 2016-2018

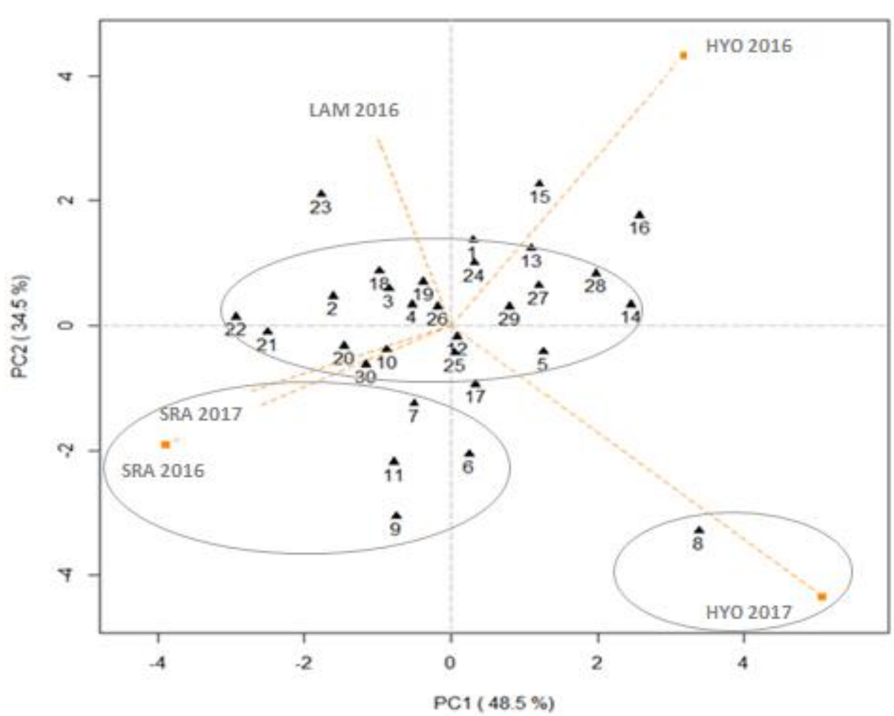


Figure 3.- Biplot from AMMI ANALYSIS for Commercial tuber yield showing first and second principal components (PC1 and PC2) at five locations and 30 genotypes 2016-2018

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

Milestone achieved: 20 advanced clones from inter-population crosses (LBHT x LTVR) selected in Peru and available for introduction in In vitro clean for international distribution.

Means of verification: Technical report

Population LBHTxLTVR comes from inter-crosses between advanced clones from LBHT and LTVR populations. In 2013, Forty five true seed families (TS) with 250 seed each, were planted in Greenhouse at La Molina (Peru) to obtain tuber families (TF), the TS families were sown in trays and individual genotypes were transplanted to 4" pots. At harvest, two sets of TF with 80 genotypes were recovered, previously the individual genotypes labeled with bar codes. The first set of TF is maintained in quarantine greenhouse conditions, and the second set of TF 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, 528 clones were selected in the first clonal generation.

From 2015 to 2018, these clones were tested in divergent biotic and abiotic stressful environments: Huancayo for yield, San Ramon a warm environment at 800 masl for heat tolerance and Oxapampa for late blight resistance, Oxapampa at 1,950 masl is a place with very good conditions for high pressure of late bligh.

Twenty eight advanced clones selected after four clonal generation (Tables 4, 5). All these clones show resistance to late blight with AUDPC values from 112 to 1210 (Figure 4), meanwhile variety Yungay, susceptible control with 1518, early maturing with a growing period of 90-110 days, yield from 16 to 35 th^{-1} under warm heat conditions, meanwhile control varieties Desiree (heat tolerant) to Amarilis (non-heat tolerant) yielded 12.5 and 4.27 th^{-1} respectively. (Figure 5)

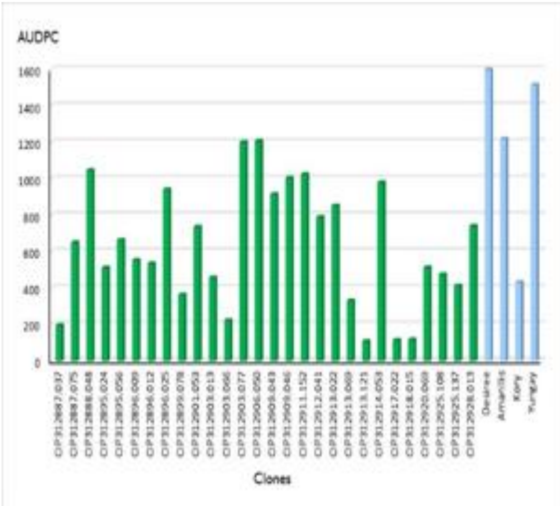
These clones will be introduced in vitro, for virus cleansing (HS2 health status) and will be ready for international distribution to the regions and NARS

TABLE 4.- NUMBER OF SELECTED CLONES FROM LBHT X LTVR POPULATION THROUGH THE YEARS

True seed Family	Female	Male	Population	Number of clones selected				
				HYO 2013-2014	HYO 2014-2015	San Ramon 2015	San Ramon 2016	San Ramon and Oxapampa 2017-2018
CIP312886	398017.53	302476.108	LBHTxLTVR	10	4	2	1	0
CIP312887	398098.119	302476.108	LBHTxLTVR	10	5	5	3	2
CIP312888	398098.203	302476.108	LBHTxLTVR	20	12	2	2	1
CIP312889	398180.144	302476.108	LBHTxLTVR	9	5	2	2	0
CIP312890	398180.292	302476.108	LBHTxLTVR	12	5	2	1	0
CIP312891	398192.213	302476.108	LBHTxLTVR	8	0	0	0	0
CIP312892	398192.553	302476.108	LBHTxLTVR	6	2	1	0	0
CIP312893	398201.51	302476.108	LBHTxLTVR	4	1	0	0	0
CIP312894	398203.244	302476.108	LBHTxLTVR	22	10	4	2	0
CIP312895	398208.219	302476.108	LBHTxLTVR	11	4	3	3	2
CIP312896	398208.29	302476.108	LBHTxLTVR	16	12	6	6	3
CIP312897	398208.505	302476.108	LBHTxLTVR	6	2	1	0	0
CIP312898	398208.62	302476.108	LBHTxLTVR	13	5	4	3	0
CIP312899	398208.67	302476.108	LBHTxLTVR	14	5	3	2	1
CIP312900	398208.704	302476.108	LBHTxLTVR	10	4	3	2	0
CIP312901	398017.53	304350.118	LBHTxLTVR	2	2	2	1	1
CIP312902	398098.119	304350.118	LBHTxLTVR	4	2	1	1	0
CIP312903	398098.203	304350.118	LBHTxLTVR	11	7	6	6	3
CIP312904	398180.144	304350.118	LBHTxLTVR	3	0	0	0	0
CIP312905	398180.292	304350.118	LBHTxLTVR	15	3	2	1	0
CIP312906	398192.213	304350.118	LBHTxLTVR	11	4	4	3	1
CIP312907	398192.553	304350.118	LBHTxLTVR	8	0	0	0	0
CIP312908	398201.51	304350.118	LBHTxLTVR	15	9	5	3	0
CIP312909	398203.244	304350.118	LBHTxLTVR	5	2	2	2	2
CIP312910	398208.219	304350.118	LBHTxLTVR	8	1	1	1	0
CIP312911	398208.29	304350.118	LBHTxLTVR	16	6	2	2	1
CIP312912	398208.505	304350.118	LBHTxLTVR	8	4	4	3	1
CIP312913	398208.62	304350.118	LBHTxLTVR	20	7	8	6	3
CIP312914	398208.67	304350.118	LBHTxLTVR	10	5	3	2	1
CIP312915	398208.704	304350.118	LBHTxLTVR	8	4	3	1	0
CIP312916	398017.53	304372.7	LBHTxLTVR	18	12	1	1	0
CIP312917	398098.119	304372.7	LBHTxLTVR	25	15	7	3	1
CIP312918	398098.203	304372.7	LBHTxLTVR	13	7	5	2	1
CIP312919	398180.144	304372.7	LBHTxLTVR	12	4	0	0	0
CIP312920	398180.292	304372.7	LBHTxLTVR	19	8	2	2	1
CIP312921	398192.213	304372.7	LBHTxLTVR	8	3	0	0	0
CIP312922	398192.553	304372.7	LBHTxLTVR	20	8	1	0	0
CIP312923	398201.51	304372.7	LBHTxLTVR	13	5	3	1	0
CIP312924	398203.244	304372.7	LBHTxLTVR	6	3	0	0	0
CIP312925	398208.219	304372.7	LBHTxLTVR	15	9	5	4	2
CIP312926	398208.29	304372.7	LBHTxLTVR	15	12	4	1	0
CIP312927	398208.505	304372.7	LBHTxLTVR	8	5	2	2	0
CIP312928	398208.62	304372.7	LBHTxLTVR	11	6	5	2	1
CIP312929	398208.67	304372.7	LBHTxLTVR	13	5	1	0	0
CIP312930	398208.704	304372.7	LBHTxLTVR	17	6	3	0	0
TOTAL SELECTED CLONES				**528**	**240**	**120**	**77**	**28**

TABLE 5.- ADVANCED CLONES SELECTED IN LBHT X LTVR POPULATION

								Marketable tuber yield th^{-1} San Ramon			AUDPC Oxapampa				
#	Clone	Female	Male	Skin color	Flesh color	Tuber shape	Eyes deep	2015	2016	2017	2015-2016	2016-2017	2017-2018	Scale LB	Glycoalkaloids mg/100 g. Fresh Weight
1	CIP312887.037	CIP398098.119	CIP302476.108	cream	yellow	oval	superficial	18.07	18.39	20.15	543.00	0.00	53.00	0	
2	CIP312887.075	CIP398098.119	CIP302476.108	red	cream	elliptical	superficial	15.56	27.11	21.70	620.00	837.50	502.00	2	7.41
3	CIP312888.048	CIP398098.203	CIP302476.108	red	cream	elliptical	superficial	15.45	25.25	26.32	952.00	1220.83	974.00	4	4.27
4	CIP312895.024	CIP398208.219	CIP302476.108	cream	cream	oblong	superficial	21.04	34.06	15.58	419.00	0.00	1125.00	4	
5	CIP312895.056	CIP398208.219	CIP302476.108	cream	cream	oblong	superficial	20.34	25.82	19.78	587.00	950.83	461.00	2	12.89
6	CIP312896.009	CIP398208.29	CIP302476.108	cream	cream	elliptical	superficial	18.04	30.21	19.46	462.00	870.83	338.00	1	25.23
7	CIP312896.012	CIP398208.29	CIP302476.108	cream	cream	elliptical	superficial	18.00	21.34	19.88	224.00	847.50	543.00	2	2.85
8	CIP312896.025	CIP398208.29	CIP302476.108	cream	cream	elliptical	superficial	19.70	20.29	19.85	770.00	1355.83	706.00	3	2.65
9	CIP312899.078	CIP398208.67	CIP302476.108	red	yellow	oval	superficial	21.78	20.66	19.19	832.00	0.00	269.00	1	19.73
10	CIP312901.053	CIP398017.53	CIP304350.118	cream/pink	cream	oval	superficial	21.04	21.17	12.94	669.00	638.75	910.00	3	
11	CIP312903.013	CIP398098.203	CIP304350.118	red	cream	oblong	superficial	20.19	23.10	18.94	1136.00	0.00	245.00	1	
12	CIP312903.066	CIP398098.203	CIP304350.118	cream	cream	oblong	superficial	24.52	23.74	23.21	573.00	0.00	105.00	0	
13	CIP312903.077	CIP398098.203	CIP304350.118	cream	cream	oval	superficial	21.41	22.43	15.41	953.00	1105.00	1552.00	6	
14	CIP312906.050	CIP398192.213	CIP304350.118	cream/pink	cream	elliptical	superficial	25.19	24.43	24.10	954.00	1568.75	1406.00	5	15.26
15	CIP312909.043	CIP398203.244	CIP304350.118	red	cream	oval	superficial	22.67	22.35	11.83	815.00	842.50	1097.00	4	
16	CIP312909.046	CIP398203.244	CIP304350.118	red	white	elliptical	superficial	24.52	34.40	26.89	733.00	1166.25	1120.00	4	
17	CIP312911.152	CIP398208.29	CIP304350.118	cream	cream	oval	superficial	21.59	23.43	16.69	729.00	1274.17	1079.00	4	
18	CIP312912.041	CIP398208.505	CIP304350.118	pink	white	oval	superficial	19.78	22.29	18.62	733.00	598.75	1044.00	4	
19	CIP312913.022	CIP398208.62	CIP304350.118	cream	cream	elliptical	superficial	14.45	28.88	24.27	574.00	1174.17	817.00	3	
20	CIP312913.069	CIP398208.62	CIP304350.118	pink	cream	oblong	superficial	34.59	37.06	26.74	754.00	65.00	181.00	1	
21	CIP312913.121	CIP398208.62	CIP304350.118	cream/pink	cream	oblong	superficial	37.26	40.31	25.68	278.00	0.00	58.00	0	
22	CIP312914.053	CIP398208.67	CIP304350.118	pink	cream	oblong	superficial	16.15	29.59	25.93	697.00	997.50	1254.00	5	
23	CIP312917.022	CIP398098.119	CIP304372.7	cream	cream	oblong	superficial	13.41	19.39	19.38	56.00	112.50	187.00	1	
24	CIP312918.015	CIP398098.203	CIP304372.7	cream	cream	elliptical	superficial	15.63	20.47	18.52	317.00	0.00	47.00	0	
25	CIP312920.069	CIP398180.292	CIP304372.7	cream	cream	elliptical	superficial	21.11	24.73	14.02	489.00	508.75	548.00	2	
26	CIP312925.108	CIP398208.219	CIP304372.7	cream	cream	elliptical	superficial	6.96	22.51	19.28	284.00	581.25	566.00	2	7.89
27	CIP312925.137	CIP398208.219	CIP304372.7	cream	cream	elliptical	superficial	16.15	23.55	19.56	205.00	570.00	473.00	2	6.34
28	CIP312928.013	CIP398208.62	CIP304372.7	cream/pink	cream	oblong	superficial	21.56	23.17	21.70	557.00	728.75	945.00	4	11.52
	Desiree							12.35	13.79	12.72				0	
	Amarillis							3.65	6.25	2.91	763.75	411.04	1680.00	6	
	Kory										532.00	369.58	402.50	1	
	Yungay										824.29	495.52	1610.00	6	



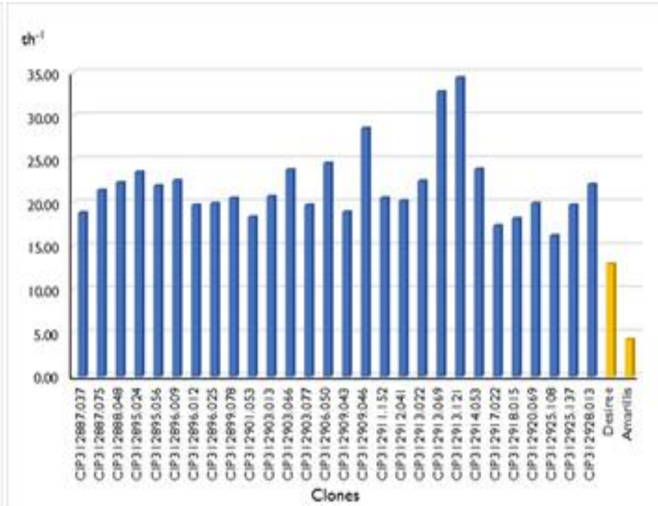


Figure 4.- Late blight resistance – AUDPC Average in Oxapampa 2016-2018

Figure 5.- Average marketable tuber yield under warm conditions in San Ramon 2015-2017

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

At least 20 advanced tetraploid clones with > 30 ppm Fe, > 25 ppm Zn, high vitamin C and resistant to LB, adapted tropical highland and mid elevation ecologies available for International distribution (C1) (2018 Q4)

Means of verification: Technical report

Potato biofortification breeding at CIP has resulted in new generations of biofortified potatoes with up to 32–45 mg/kg dry weight (DW) iron (Fe) and 22–37 mg/kg DW zinc (Zn). Mineral concentrations were increased by recurrent selection at the diploid level. Although some of these biofortified diploid materials are being promoted as varieties in the Andean zone of Peru, however, the narrow range of adaptation, low yields and lack of resistance to the most important diseases makes these diploid potatoes vulnerable to biotic and abiotic stresses and not competitive with commercial tetraploid varieties. An Inter-ploidy breeding strategy, of crosses 4x – 2x, based on unilateral sexual tetraploidization, introducing genetic gains at 2x level to the advanced tetraploid potatoes was initiated in 2012, developing new tetraploid cultivars with resistance to the most important diseases such as late blight and viruses and tolerance to abiotic stresses heat and drought, maintaining the nutritional quality with high levels of iron and zinc.

A population of approximately 12,000 genotypes from more than 200 families from crosses of advanced tetraploid disease-resistant progenitors with selected diploid clones with high micronutrient concentrations and high frequency of 2n pollen from cycle II, were generated in 2013. All genotypes were characterized for ploidy by counting chloroplasts in stomatal guard cells. Tetraploid, triploid, and a few diploid genotypes were identified. Micronutrient concentrations were evaluated in six field trials in different locations and years, augmented experimental design without replications was used and evaluations for late blight and virus resistance performed simultaneously, applying an accelerated breeding scheme

In 2017, 147 selected clones were introduced to *in-vitro*, from which 116 clones are disease-free and available for international distribution. Remarkably, 57 of these were cataloged https://research.cip.cgiar.org/cipcatlg_ac/Catalogue.php?language=1&name=English ranging from 16 to 43 ppm iron and 7 - 39 ppm zinc., high yield and resistant to LB and PVY (Table 6). Presently these were already dispatched to Ethiopia, Buthan Kenya, Rwanda and India for evaluation and variety selection ().

A new population of biofortified 4x clones adapted to lowlands and resilient will be available by 2022.

TABLE 6.- LIST OF POTATO BIOFORTIFIED CLONES

#	CIP number	Collecting number/Breeder code	Female Accesion Number	Male Accesion Number	Ploidy Level	PVY	LB	Dry matter (%)			Iron (mg/kg dw)			Zinc (mg/kg dw)			Yield (tn/ha)			International Distribution	Link Catalogue
								N LOC	Average DM	SD	N LOC	Average FeDW	SD	N LOC	Average ZnDW	SD	N LOC	Average TTYA	Sd TTYA		
1	CIP312507.311	BIOT-507.311	CIP391058.175	CIP306416.68	4X	R	MR	4	22.67	3.23	3	25.21	7.03	3	22.21	7.45	3	33.6	7.6	Nepal,Buthan,India,Kenia	CIP312507.311
2	CIP312507.312	BIOT-507.312	CIP391058.175	CIP306416.68	4X	R	MR	5	25.10	3.33	4	22.29	5.27	4	17.89	5.18	4	36.1	5.7	Nepal,Buthan,India,Kenia	CIP312507.312
3	CIP312527.026	BIOT-527.026	CIP391930.1	CIP306418.69	4X	S	MR	5	22.44	3.54	4	30.86	4.05	4	25.84	1.81	4	19.7	6.1	Nepal,Buthan,India,Kenia	CIP312527.026
4	CIP312535.032	BIOT-535.032	CIP392025.7	CIP306416.68	3X	---	MR	4	21.87	1.38	4	23.18	6.11	4	26.64	1.58	3	13.5	7.0	Nepal,Buthan,India,Kenia	CIP312535.032
5	CIP312595.053	BIOT-595.053	CIP393073.179	CIP306416.68	3X??	S	MR	5	21.12	1.79	4	29.48	6.72	4	22.59	5.08	4	22.3	16.4	Nepal,Buthan,India,Kenia	CIP312595.053
6	CIP312609.247	BIOT-609.247	CIP393083.2	CIP306416.68	4X	---	R	7	22.42	3.79	6	25.21	7.60	5	21.41	7.50	7	31.0	19.3	Nepal,Buthan,India,Kenia	CIP312609.247
7	CIP312609.252	BIOT-609.252	CIP393083.2	CIP306416.68	4X	---	MR	3	22.02	3.01	3	24.40	5.80	3	20.75	4.61	2	30.7	22.1	Nepal,Buthan,India,Kenia	CIP312609.252
8	CIP312621.069	BIOT-621.069	CIP393382.44	CIP306416.68	3x??	S	R	7	24.47	4.14	6	29.18	5.73	5	22.55	7.57	7	32.9	14.6	Nepal,Buthan,India,Kenia	CIP312621.069
9	CIP312621.097	BIOT-621.097	CIP393382.44	CIP306416.68	4X	S	MR	7	26.16	3.42	6	29.18	7.44	5	19.77	5.63	7	24.7	16.7	Nepal,Buthan,India,Kenia	CIP312621.097
10	CIP312633.155	BIOT-633.155	CIP393536.13	CIP306416.68	4X	R	MR	5	27.38	4.20	4	25.16	4.96	4	26.07	4.75	4	21.0	7.0	Nepal,Buthan,India,Kenia	CIP312633.155
11	CIP312637.020	BIOT-637.020	CIP393536.13	CIP306418.69	4X	---	MR	4	25.44	1.55	4	33.36	4.28	4	29.64	3.76	3	16.0	7.0	Nepal,Buthan,India,Kenia	CIP312637.020
12	CIP312637.069	BIOT-637.069	CIP393536.13	CIP306418.69	4X	R	MR	3	22.58	0.83	3	26.27	6.38	3	27.11	4.55	3	13.8	9.4	Nepal,Buthan,India,Kenia	CIP312637.069
13	CIP312637.132	BIOT-637.132	CIP393536.13	CIP306418.69	4X	R	MR	4	21.69	0.68	4	31.24	5.56	4	27.15	5.22	3	19.6	5.7	Nepal,Buthan,India,Kenia	CIP312637.132
14	CIP312682.005	BIOT-682.005	CIP394600.52	CIP306416.68	4X	R	MR	4	21.09	1.70	4	28.15	5.21	4	23.49	2.70	3	17.4	7.2	Nepal,Buthan,India,Kenia	CIP312682.005
15	CIP312682.011	BIOT-682.011	CIP394600.52	CIP306416.68	4X	S	MR	5	22.08	4.27	4	28.71	3.77	4	24.94	4.56	4	30.3	10.5	Nepal,Buthan,India,Kenia	CIP312682.011
16	CIP312682.042	BIOT-682.042	CIP394600.52	CIP306416.68	4X	---	MR	6	21.26	3.10	5	28.13	6.06	4	23.07	7.03	5	22.4	15.0	Nepal,Buthan,India,Kenia	CIP312682.042
17	CIP312686.019	BIOT-686.019	CIP394600.52	CIP306418.69	4X	S	R	7	24.80	3.91	6	27.26	2.93	5	17.40	3.02	7	24.7	12.8	Nepal,Buthan,India,Kenia	CIP312686.019
18	CIP312686.050	BIOT-686.050	CIP394600.52	CIP306418.69	4X	S	R	4	22.22	1.01	4	30.93	5.47	4	24.20	1.92	3	17.8	11.8	Nepal,Buthan,India,Kenia	CIP312686.050
19	CIP312718.005	BIOT-718.005	CIP395017.229	CIP306087.82	4X	R	R?	5	20.10	3.28	5	27.61	6.49	4	25.95	8.92	4	23.0	6.3	Nepal,Buthan,India,Kenia	CIP312718.005
20	CIP312721.004	BIOT-721.004	CIP395017.229	CIP306416.68	4X	---	R	5	22.44	3.69	4	28.97	5.43	4	20.88	7.04	4	27.9	2.4	Nepal,Buthan,India,Kenia	CIP312721.004
21	CIP312721.029	BIOT-721.029	CIP395017.229	CIP306416.68	4X	R	R	7	24.24	4.53	6	25.73	4.77	5	19.31	6.89	7	31.9	12.4	Nepal,Buthan,India,Kenia	CIP312721.029
22	CIP312721.038	BIOT-721.038	CIP395017.229	CIP306416.68	3X??	R	MR	7	21.85	2.10	6	25.72	3.99	5	18.95	6.90	7	23.0	8.9	Nepal,Buthan,India,Kenia	CIP312721.038
23	CIP312721.163	BIOT-721.163	CIP395017.229	CIP306416.68	4X	---	HR	7	24.71	4.43	6	25.80	3.48	5	18.14	8.05	7	23.1	11.8	Nepal,Buthan,India,Kenia	CIP312721.163
24	CIP312721.169	BIOT-721.169	CIP395017.229	CIP306416.68	3X??	---	MR	7	22.68	3.18	6	28.37	4.83	5	20.07	4.46	7	24.5	11.8	Nepal,Buthan,India,Kenia	CIP312721.169
25	CIP312721.212	BIOT-721.212	CIP395017.229	CIP306416.68	4X	---	MR	5	21.97	3.36	4	28.33	3.59	4	24.76	6.98	4	21.0	2.9	Nepal,Buthan,India,Kenia	CIP312721.212
26	CIP312721.245	BIOT-721.245	CIP395017.229	CIP306416.68	3X??	---	MR	5	23.23	5.21	4	32.01	6.20	4	27.02	9.37	4	27.5	8.0	Nepal,Buthan,India,Kenia	CIP312721.245
27	CIP312721.286	BIOT-721.286	CIP395017.229	CIP306416.68	4X	S	R	7	23.90	2.89	6	25.24	3.85	5	21.60	5.01	7	36.2	23.4	Nepal,Buthan,India,Kenia	CIP312721.286
28	CIP312725.001	BIOT-725.001	CIP395017.229	CIP306418.69	4X	R	HR	6	21.14	2.38	6	28.38	4.05	5	22.17	5.23	7	18.7	12.1	Nepal,Buthan,India,Kenia	CIP312725.001
29	CIP312725.024	BIOT-725.024	CIP395017.229	CIP306418.69	4X	S	R	7	22.43	3.86	6	29.70	6.34	5	26.04	8.41	7	25.7	11.4	Nepal,Buthan,India,Kenia	CIP312725.024

#	CIP number	Collecting number/Breeder code	Female Accesion Number	Male Accesion Number	Ploidy Level	PVY	LB	Dry matter (%)			Iron (mg/kg dw)			Zinc (mg/kg dw)			Yield (tn/ha)			International Distribution	Link Catalogue
								N LOC	Average DM	SD	N LOC	Average FeDW	SD	N LOC	Average ZnDW	SD	N LOC	Average TTYA	Sd TTYA		
30	CIP312725.036	BIOT-725.036	CIP395017.229	CIP306418.69	4X	R	R	7	22.28	3.15	6	26.29	6.02	5	22.95	7.58	7	17.5	10.0	Nepal,Buthan,India,Kenia	CIP312725.036
31	CIP312725.041	BIOT-725.041	CIP395017.229	CIP306418.69	4X	R	MR	5	22.60	2.54	4	30.18	7.94	4	22.84	3.05	4	17.2	6.0	Nepal,Buthan,India,Kenia	CIP312725.041
32	CIP312725.047	BIOT-725.047	CIP395017.229	CIP306418.69	4X	---	HR	4	25.16	4.02	3	33.24	2.80	3	27.17	7.95	3	17.6	15.6	Nepal,Buthan,India,Kenia	CIP312725.047
33	CIP312725.048	BIOT-725.048	CIP395017.229	CIP306418.69	4X	---	HR	3	22.93	2.31	3	28.53	1.08	3	25.76	5.26	2	17.1	18.1	Nepal,Buthan,India,Kenia	CIP312725.048
34	CIP312725.050	BIOT-725.050	CIP395017.229	CIP306418.69	4X	S	R	7	23.07	3.37	6	25.90	4.04	5	18.19	6.28	7	29.0	16.5	Nepal,Buthan,India,Kenia	CIP312725.050
35	CIP312725.052	BIOT-725.052	CIP395017.229	CIP306418.69	4X	R	R	4	24.17	0.96	4	31.06	7.91	4	24.42	3.87	3	15.1	5.8	Nepal,Buthan,India,Kenia	CIP312725.052
36	CIP312725.055	BIOT-725.055	CIP395017.229	CIP306418.69	4X	S	HR	6	24.67	3.92	5	26.91	5.86	2	29.99	6.84	7	28.8	15.0	Nepal,Buthan,India,Kenia	CIP312725.055
37	CIP312725.057	BIOT-725.057	CIP395017.229	CIP306418.69	4X	R	HR	7	20.71	3.59	6	26.96	5.53	5	20.39	5.66	7	32.5	14.0	Nepal,Buthan,India,Kenia	CIP312725.057
38	CIP312725.062	BIOT-725.062	CIP395017.229	CIP306418.69	4X	S	R	7	21.76	2.55	5	27.60	3.61	5	20.77	5.45	7	23.1	13.4	Nepal,Buthan,India,Kenia	CIP312725.062
39	CIP312725.067	BIOT-725.067	CIP395017.229	CIP306418.69	4X	---	MR	7	21.38	3.64	6	26.76	5.34	5	23.98	7.41	7	20.8	10.9	Nepal,Buthan,India,Kenia	CIP312725.067
40	CIP312725.110	BIOT-725.110	CIP395017.229	CIP306418.69	4X	S	MR	3	21.12	1.36	3	29.77	6.12	3	25.72	3.26	2	19.4	1.6	Nepal,Buthan,India,Kenia	CIP312725.110
41	CIP312725.128	BIOT-725.128	CIP395017.229	CIP306418.69	4X	S	MR	4	23.35	4.43	4	26.40	4.15	3	23.02	7.77	3	31.3	22.2	Nepal,Buthan,India,Kenia	CIP312725.128
42	CIP312731.004	BIOT-731.004	CIP395017.242	CIP306087.82	3x??	---	R	6	23.47	2.42	5	26.70	5.53	5	25.70	8.48	6	22.0	9.5	Nepal,Buthan,India,Kenia	CIP312731.004
43	CIP312735.051	BIOT-735.051	CIP395017.242	CIP306416.68	4X	R	MR	4	19.17	1.43	4	25.37	4.13	4	21.00	3.02	3	23.4	4.0	Nepal,Buthan,India,Kenia	CIP312735.051
44	CIP312735.062	BIOT-735.062	CIP395017.242	CIP306416.68	4X??	R	R	4	20.73	2.35	4	27.27	2.80	4	21.15	4.75	3	37.6	3.1	Nepal,Buthan,India,Kenia	CIP312735.062
45	CIP312735.077	BIOT-735.077	CIP395017.242	CIP306416.68	3X??	S	R	7	23.61	2.70	6	24.97	4.57	5	20.94	3.59	7	27.9	14.3	Nepal,Buthan,India,Kenia	CIP312735.077
46	CIP312735.100	BIOT-735.100	CIP395017.242	CIP306416.68	4X	R	HS	5	25.00	5.17	4	26.31	3.31	4	23.47	2.30	4	23.7	5.4	Nepal,Buthan,India,Kenia	hCIP312735.100
47	CIP312735.105	BIOT-735.105	CIP395017.242	CIP306416.68	4X	R	MR	5	25.98	3.89	4	29.51	8.33	4	23.35	3.95	4	22.0	4.6	Nepal,Buthan,India,Kenia	CIP312735.105
48	CIP312735.114	BIOT-735.114	CIP395017.242	CIP306416.68	4X	R	MR	4	21.69	2.03	4	30.01	5.30	4	25.09	6.73	3	13.8	2.1	Nepal,Buthan,India,Kenia	hCIP312735.114
49	CIP312735.253	BIOT-735.253	CIP395017.242	CIP306416.68	4X	R	MR	4	25.75	1.09	4	25.98	3.28	4	21.28	3.92	3	41.1	8.7	Nepal,Buthan,India,Kenia	CIP312735.253
50	CIP312747.056	BIOT-747.056	CIP395112.32	CIP306416.68	3X??	S	MR	6	24.26	3.40	6	30.58	8.08	4	26.08	9.52	7	19.1	10.6	Nepal,Buthan,India,Kenia	CIP312747.056
51	CIP312751.021	BIOT-751.021	CIP395112.32	CIP306418.69	4X	R	R	6	24.18	2.15	5	28.11	6.69	4	20.96	8.33	6	34.1	19.6	Nepal,Buthan,India,Kenia	CIP312751.021
52	CIP312751.025	BIOT-751.025	CIP395112.32	CIP306418.69	4X	R	R	6	26.75	3.44	5	23.50	5.80	4	19.93	7.68	6	26.1	16.5	Nepal,Buthan,India,Kenia	CIP312751.025
53	CIP312751.028	BIOT-751.028	CIP395112.32	CIP306418.69	4X	---	HR	4	25.75	3.61	3	25.58	7.34	3	22.81	3.01	3	28.6	23.3	Nepal,Buthan,India,Kenia	CIP312751.028
54	CIP312763.441	BIOT-763.441	CIP395443.103	CIP306416.68	4X	R	MR	4	24.02	5.17	3	25.65	3.31	3	24.84	6.81	3	28.7	12.1	Nepal,Buthan,India,Kenia	CIP312763.441
55	CIP312764.013	BIOT-764.013	CIP395443.103	CIP306418.1	4X	R	MR	4	22.51	3.80	4	22.69	5.78	4	25.74	5.52	3	45.0	1.5	Nepal,Buthan,India,Kenia	CIP312764.013
56	CIP312767.014	BIOT-767.014	CIP395443.103	CIP306418.69	4X	---	MR	7	24.30	3.55	6	27.62	3.11	5	22.83	8.42	7	30.5	20.8	Nepal,Buthan,India,Kenia	CIP312767.014
57	CIP312871.043	BIOT-871.043	CIP780278	CIP306418.69	4X	---	R	7	25.72	2.44	6	27.08	5.74	5	23.51	4.39	7	28.1	13.8	Nepal,Buthan,India,Kenia	CIP312871.043
									X			X			X			X			
									23.19			27.55			23.18			25.12			

TABLE 7.- DISTIBUTION OF POTATO BIOFORTIFIED CLONES

#	Country	Genebank Request	# of cultivars	Requester	Status	FAO PID	Institution	e-mail
1	Ethiopia	2018-131	57	Baye Berihun Getahun	Dispatched	00AU36	Ethiopian Institute of Agricultural Research, EIAR	Baye.bgetahun@gmail.com
2	Bhutan	2018-128	50	Yadunath Bajgai	Dispatched	00AU00	National Potato Program, ARDC	ybajgai@moaf.gov.bt
3	Kenya	2018-167	57	Thiago Mendez	Ready to dispatch	No NEED	CIP- Nairobi	t.mendes@cgiar.org
4	Rwanda	2018-169	57	Placidse Rukundo	Propagating		Rwanda Agriculture and Animal Resources Development Board	rukundoplacide@gmail.com
5	India	2018-179	57	Vinay Bhardwaj	Propagating		CPRI, Shimla.	vinaycpri@gmail.com

Milestone: Individual glycoalkaloids concentration (solanine and chaconine) determined by UPLC in 13 late blight resistant, advanced clones grown in 4 contrasting environments in 3 replications in Peru by early June 2018.

Means of verification: Technical report, scientific manuscript about the effect of environment on the individual glycoalkaloid concentration of LB resistant clones for target countries.

Effect of the growing location in the individual glycoalkaloid concentration of late blight resistant and heat tolerant clones

Burgos Gabriela, Munoa Lupita, Chacaltana Clara, Gastelo Manuel and Zum Felde Thomas

1. Introduction

Glycoalkaloids are secondary plant metabolites that serve as natural defenses against bacteria, fungi, viruses and insects (Friedman 2004). They can be toxic for humans when present in high concentrations and can impart a bitter taste to potatoes. However, when present in low concentrations, glycoalkaloids have also anti-carcinogenic properties and other beneficial effects (Friedman 2015).

Glycoalkaloid content in potato tubers should not exceed 20 mg / 100 g FW, because this level is risky for the human health (Ruprish et al., 2009). The toxicity of glycoalkaloids may be due to adverse effects such as anticholinesterase activity on the central nervous system and to disruption of cell membranes adversely affecting the digestive system and general body metabolism (Friedman et al., 2003). The toxicity of glycoalkaloids is associated with the synergistic interaction between two main components of glycoalkaloids: α-solanine and α-chaconine. Glycoalkaloid levels below 7mg/100 g FW are preferred in new potato varieties (Jacob van Dam 2002). Experiments with human taste panels revealed that potato varieties with glycoalkaloid levels exceeding 14 mg / 100 g FW tasted bitter (Friedman 2006). Those in excess of 22 mg / 100 g FW also induced mild to severe burning sensations in the mouths and throats of panel members. In order to increase the adoption of potato cultivars bred by CIP, low glykoalkaloids levels as needed, as otherwise they would be rejected by end user as a consequence of their bitter taste.

Although there are many glycoalkaloids, α-chaconine and α-solanine make up 95% of the total glycoalkaloids present in potato (Friedman and McDonald, 1997); α-solanine is found in greater concentrations than α-chaconine, and α-solanine has only half as much specific toxic activity as a α-chaconine (Lachman et al., 2001).

Glycoalkaloid levels vary greatly in different potato varieties and may be influenced by factors such as light, mechanical injury, and storage. They are also influenced by stress such as heat and drought during production (Andre et al., 2009). Hence, increased attention may be needed to glycoalkaloid concentrations of potato varieties bred for or grown in warm and or water limited environments.

The International Potato Center (CIP), has developed late blight resistant potato clones that are also heat tolerant (Gastelo et al., 2017). These clones show adaptation to high temperatures in warm environments, one of the major abiotic factors affecting the production of tubers in potato crop under climate change; and there is the need to monitor the glycoalkaloid concentration of these clones.

The main objective of this study was to evaluate the effect of environment on the concentration of individual glycoalkaloid concentration of 8 late blight resistant and heat tolerant clones and 2 released varieties.

2. Material and Methods

2.1. Plant material

Eight late blight-resistant and heat-tolerant CIP-bred clones and 2 released varieties were planted in 4 Peruvian environments: La Molina (winter and summer season), Lima; San Ramon, Junín and Majes, Arequipa. Every clone was planted in three replications in each environment.

The harvest of the potato clones grown in La Molina during winter and in San Ramon was carried out in October 2014, while the harvest of the potato clones grown in La Molina during summer and in Majes occurred in February and March 2015, respectively. The average temperature for the growing season was 17 ^{0}C for La Molina during winter (Max 25 – Min 13), 25 ^{0}C for San Ramon (Max 36 – Min 16), 21 ^{0}C for La Molina during summer (Max 30 – Min 16) and 19 ^{0}C for Majes (Max 35 – Min 8).

2.2. Sampling and sample preparation

Freeze dried and milled samples from peeled and unpeeled tubers of each clone in each replication in each location were prepared and stored at 70°C until analysis of total glycoalkaloids was performed according to Porras et al., 2014. Briefly, each sample (1 per plot replication) was composed of seven unblemished medium sized tubers that were collected at random from different plants of each accession. The tubers of each sample were washed thoroughly with tap water, rinsed with deionized, distilled water and patted dry with paper towels. Tubers were quartered longitudinally and 2 opposite quarters were sliced to obtain a sample of approximately 50 g. The sample was immediately frozen at –20°C, freeze-dried, milled with a 0.425-mm grid (40 mesh) and stored at -70°C until analysis were done.

2.3. Glycoalkaloid analysis

2.3.1 Chemical and reagents

Methanol, chloroform, acetic acid, petroleum ether, acetonitrile and potassium dihydrogen phosphate were purchased from Merck (Darmstadt, Germany) while ammonium hydroxide was purchased from Sigma–Aldrich (St. Louis, MO, USA). The solvents used for the extraction of glycoalkaloids were all of analytical quality grade while the solvents used for Individual glycoalkaloids analysis were of HPLC grade. Standards of α-solanine (98%) and α-chaconine (98%) were purchased from Extrasynthese (Genay Cedex, France). Distilled water was purified with a Nano pure water system (Thermo Scientific, Dubuque, Iowa, USA).

2.3.2. Extraction and concentration

Extraction of individual glycoalkaloids was performed following the method reported in Mondy and Ponnapalam (1983) with some modifications. Briefly 500 milligrams of freeze dried and milled sample was weighed, hydrated with 7mL of distilled water during 10 minutes and extracted with 60 mL of a Methanol: Chloroform (2/1) solution in an Ultra Turrax T25 D homogenizer (IKA, Staufen, Germany). The extract was concentrated using a rotary evaporator (IKA-Werke, Staufen Germany) at 65°C and a refrigerant

(HAAKE GH, Karlsruhe, Germany) at 16°C. The concentrated extract was transferred to a solution of 2% acetic acid, brought to 20ml and cleaned with petroleum ether. Four ml of the aqueous extract was placed in a tube and 1.2 ml of ammonium hydroxide was added. The mixture was flocculated in a dry block (TECHNE, ST15 OSA, UK) at 85°C during 10 minutes, refrigerated at 4°C during 30 minutes and centrifugated in a Optima L-90K Ultracentrifuge (BECKMAN COULTER, Brea, California, USA) at 27,000 rpm during 90 minutes. The pellet was recovered for analysis in the UPLC.

2.3.3. Determination

The pellet containing the glycoalkaloids was resuspended in 1ml of the mobile phase, sonicated during one minute using sonicator (Bransonic, Danbury, USA) and passed through a 0.20 um nylon filter (MILLIPORE, Carrigtwohill, Co., Cork, Ireland). Two ul of the resuspended glycoalkaloids were injected in the Acquity-H/UPLC Class system (WATERS, Milford, Massachusetts USA) and separated using ACQUITY UPLC BEH AMIDE column and isocratic elution composed of acetonitrile and 20 mM potassium dihydrogen phosphate buffer (80:20). Flow rate was set as 0.2 ml/min. The run time was 25 minutes. The detection of α-solanine and α-chaconine was performed with a UV visible photodiode array detector. The identification and quantification of α-solanine and α-chaconine was based on the comparison of the retention times and the visible absorption spectra obtained in the standards of these glycoalkaloids and the samples.

3. Results and Discussion

The chaconine, solanine and the sum of chaconine and solanine (considered as TGA) in unpeeled tubers of 8 late blight resistant and heat tolerant clones and 2 varieties as determined by UPLC and expressed in fresh weight (FW) basis are presented in table 1.

The TGA concentration ranged from 1.97 to 47.60 mg / 100 g, from 2.33 to 41.35 mg / 100 g, from 4.16 to 29.67 mg / 100 g and from 1.37 to 20.32 mg / 100 g DW in San Ramon, Majes, La Molina – Summer and La Molina -Winter, respectively.

ANOVA indicated that the solanine, chaconine and TGA concentration depends strongly on the interaction clone × location ($P < 0.001$). Valcarcel et al (2014) also found a significant effect on the glycoalkaloid concentration for the interaction between variety and site, implying that environmental effect seem to act differentially and could inducing high levels in genetically predisposed varieties.

The clones 302533.49, 393371.58 and 398098.570 presented the highest TGA concentration in San Ramon under high temperature conditions and the lowest TGA concentration in La Molina – Summer under appropriate temperature conditions. The clone 398180.292 also presented the highest concentration in San Ramon but the lowest concentration in Majes. The clone 398208.620 presented the highest concentration in San Ramon and La Molina – Summer.

The clones 398192.213, 398192.553 as well as the varieties Desiree and Unica showed the highest TGA concentration in La Molina – Summer under relatively high temperature conditions and the lowest TGA concentration in La Molina Winter. The clone 398208.620 presented the highest concentration in La Molina – Summer and Majes.

Table 1. Chaconine, solanine and TGA (sum of chaconine + solanine) concentrations in 8 late blight and heat tolerant clones and 2 varieties as determined by UPLC and expressed in mg /100 g, FW

Clone	San Ramon		Majes		La Molina - Summer		La Molina - Winter	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Chaconine								
CIP302533.49	29.06	2.51	24.62	1.93	17.98	1.48	12.01	0.94
CIP393371.58	11.34	1.56	3.85	0.59	3.00	0.59	1.56	0.28
CIP398098.570	5.09	1.50	2.73	0.40	2.75	0.35	1.50	0.43
CIP398180.292	9.45	2.34	1.63	0.60	6.23	0.97	5.84	0.52
CIP398192.213	2.08	0.47	3.53	0.33	5.29	0.16	0.86	0.24
CIP398192.553	3.83	0.51	2.81	0.10	9.98	0.85	1.79	0.32
CIP398208.219	3.43	0.63	5.31	0.76	4.93	0.94	2.27	0.62
CIP398208.620	10.31	1.44	3.83	0.51	9.03	1.16	3.70	0.86
Desiree	2.22	0.35	4.76	0.39	7.23	1.14	1.50	0.42
Unica	1.45	0.35	2.01	0.26	4.07	0.33	1.04	0.12
Solanine								
CIP302533.49	18.54	1.61	16.73	1.87	11.69	1.12	8.31	0.80
CIP393371.58	10.32	1.60	2.46	0.63	1.73	0.36	0.72	0.10
CIP398098.570	3.15	1.21	1.03	0.41	1.41	0.09	0.60	0.22
CIP398180.292	6.55	2.02	0.69	0.33	4.15	0.80	3.24	0.40
CIP398192.213	1.20	0.30	1.78	0.15	3.35	0.35	0.51	0.16
CIP398192.553	3.52	0.88	1.86	0.15	9.37	0.94	1.23	0.23
CIP398208.219	1.75	0.40	2.50	0.35	2.50	0.14	0.97	0.36
CIP398208.620	5.12	0.74	1.24	0.15	4.30	0.58	1.56	0.27
Desiree	0.83	0.22	1.47	0.08	2.30	0.56	0.63	0.10
Unica	0.52	0.21	0.75	0.07	1.57	0.28	0.41	0.11
TGA (chaconine + solanine)								
CIP302533.49	47.60	3.82	41.35	3.73	29.67	2.55	20.32	1.74
CIP393371.58	21.66	3.11	6.31	1.14	4.74	0.94	2.29	0.37
CIP398098.570	8.23	2.71	3.76	0.80	4.16	0.33	2.10	0.65
CIP398180.292	16.00	4.30	2.33	0.92	10.38	1.72	9.08	0.70
CIP398192.213	3.28	0.77	5.31	0.47	8.64	0.38	1.37	0.40
CIP398192.553	7.34	1.38	4.68	0.17	19.35	1.77	3.02	0.55
CIP398208.219	5.18	0.99	7.80	1.09	7.43	0.90	3.24	0.98
CIP398208.620	15.43	2.18	5.06	0.64	13.33	1.74	5.27	1.13
Desiree	3.05	0.53	6.23	0.46	9.53	1.69	2.13	0.52
Unica	1.97	0.55	2.77	0.31	5.64	0.59	1.45	0.17

Verma and Shukla (2015) and Ashraf et al (2018) indicated that the biosynthesis of secondary metabolites is correlated with heat stress in plants with high-temperature stress increasing the production of secondary metabolites. For all the clones evaluated in this study, the highest glycoalkaloid concentrations were presented in San Ramon or in La Molina during summer where the temperature ranged from 36 to

16 °C, and from 30 to 16 °C; respectively); and the lowest concentration was showed in La Molina during winter where the temperature ranged from 25 to 13 °C. These results can be explained by the fact that the optimum temperature for potato production goes from 17 to 23 °C; hence, higher temperature conditions as those presented in San Ramon and La Molina during summer, are considered as heat stress conditions for the potato plant and probably are the reason for the increased glycoalkaloid concentration in those locations.

With exception of the clone 398180.292, all the clones presented higher glycoalkaloid concentration in Majes than in La Molina during winter. Majes represented a drought stressed environment having a max and min temperature of 35 and 8, respectively under desert conditions and a restricted irrigation regime. Andre et al. (2009) also reported 2.5-fold increments of glycoalkaloid concentrations in Andean varieties grown under drought compared to well-watered conditions.

In the 4 locations, the clone 302533.49 showed a TGA concentration above the safe limit for human consumption (20 mg / 100 g, FW). The clone 393371.58 showed a non-safe TGA concentration (21.66 mg / 100 g, FW) when grown in San Ramon while the clone 398192.553 showed a TGA concentration very near to the safe level (19.35 mg / 100 g, FW) when grown in La Molina – Summer.

The clones 398098.570, 398192.213, 398208.219 as well as the varieties Desiree and Unica presented a TGA concentration below 10mg / 100 G, FW across the 4 locations.

4. Conclusions

The effect of locality in the TGA concentration depends on the clone. The TGA concentrations of all the late blight and heat tolerant clones and varieties evaluated in this study was higher under heat and drought stress conditions. The clones 398098.570, 398192.213, 398208.219 have a safe level of glycoalkaloids under heat stress conditions and are recommended for distribution. The clones 393371.58 and 398192.553 presented high levels of TGA under heat stress conditions and should be removed from the list of recommended late blight resistant and heat tolerant clones. The clone 302533.49 must be eliminated from the list of recommended late blight resistant and heat tolerant clones as it has unsafe levels of TGA concentration under stress and non-stress conditions.

5. References

André C.M., Oufir M., Schafleitener R., Guinar C., Oufir M., Alvarado C., Nomberto G., Hoffmann L., Hausman J.-F., Evers D., Larondelle Y. 2009. Modification of the Health-Promoting Value of Potato Tubers Field Grown under Drough Stress: Emphasis on Dietary Antioxidant and Glycoalkaloid Content in Five Native Andean Cultivars (Solanum tuberosum L.). J. Agric. Food. Chem. 57:599-609.

Ashraf M., Iqbal M., Rasheed R., Hussain I., Riaz M., Arif M. 2018. Plant Metabolites and Regulation Under Environmental Stress: An Overview. http://dx.doi.org/10.1016/B978-0-12-812689-9.00008-X, Pages 153-167

Friedman M (2006) Potato glycoalkaloids and metabolites: roles in the plant and in the diet. J Agric Food Chem 54, 8655–8681.

Friedman M (2015) Chemistry and anticarcinogenic mechanisms of glycoalkaloids produced by eggplants, potatoes, and tomatoes. Journal of Agricultural and Food Chem 63, 3323–3337.

Friedman M, Lee KR, Kim HJ, Lee IS, Kozukue N (2005) Anticarcinogenic effects of glycoalkaloids from potatoes against human cervical, liver, lymphoma, and stomach cancer cells. J Agric Food Chem 53, 6162–6169.

Friedman M, Roitman JN, Kozukue N (2003) Glycoalkaloid and calystegine contents of eight potato cultivars. J Agric Food Chem 51, 2164–2173.

Lachman J, Hamouz K, Musilová J, Hejtmánková K, Kotíková Z, Pazderu K, Domkárová J, Pivec V, Cimr J (2013) Effect of peeling and three cooking methods on the content of selected phytochemicals in potato tubers with various colour of flesh. Food Chem 138, 1189-1197.

Mondy N.I., Ponnapalam Y., Determination of total glycoalkaloids (TGA) in dehydrated potatoes. 1983. J. Food Sci., 48(2), 612-614.

Ruprich J, Rehurkova I, Boon PE, Svensson K, Moussavian S, Van der Voet H, Bosgra S, Van Klaveren JD, Busk L. 2009 Probabilistic modeling of exposure doses and implications for health risk characterization: glycoalkaloids from potatoes. Food Chem Toxicol 47:2899–2905

Valcarce J., Reilly K., Gaffney M., O'Brien N. (2014) Effect of Genotype and Environment on the Glycoalkaloid Content of Rare, Heritage, and Commercial Potato Varieties. Journal of Food Science. Vol. 79, Nr. 5, 2014

Verma, N., Shukla, S., 2015. Impact of various factors responsible for fluctuation in plant secondary metabolites. J. Appl. Res. Med. Aromatic Plants 2 (4), 105–113. http://dx.doi.org/10.1016/j.jarmap.2

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

Deliverable: Strengthening Regional Breeding Hubs

Milestone achieved: Germplasm selection and evaluation network established in selected priority countries.

Means of verification: Technical report

In Rwanda two scientists from its Resources Development Board (RAB) were trained on data collection using Field Book app[1] and on HIDAP[2]. Field Book is an application developed by Kansas State University that can be used by researchers to easily take notes on field research plots. It's now been successfully used by the researchers at RAB. The first season of 2018 all breeding data were collected using this app. HIDAP is a platform developed by CIP to support clonal crop breeders on trial planning, data collection, data quality and data analysis in clonal crop breeding. They have used this platform for trial planning and data analysis as ANOVA and AMMI purposes. The breeding team in Ethiopia will be trained on data collection and HIDAP in October 2018.

CIP has evaluated and released disease-resistant, high-yielding varieties across several countries in SSA. A robust set of potato clones has been selected (Table 8) with traits such as heat tolerance, water-use-efficiency, earliness, virus resistance, and late blight resistance, in combination with good processing qualities. Recently, 18 clones were dispatched to Eritrea, Burundi and Rwanda for variety development. The clones were introduced by mini tubers and/or *in vitro* plantlets between Oct/17 – Feb/18. In Rwanda, with the support of PSDAG[3] project and CIP, RAB will be able to release a new potato variety soon. The other countries are managing multiplication plots and trials on station with their own resources. CIP SSA team has provided technical backstopping to all of them.

[1] http://wheatgenetics.org/field-book

[2] https://research.cip.cgiar.org/gtdms/hidap/

[3] USAID|PRIVATE SECTOR DRIVEN AGRICULTURAL GROWTH PROJECT

TABLE 8. CIP ADVANCED CLONE BEING TESTED IN BURUNDI, RWANDA AND ERITREA.

#	Clones	Location	Population Group	Use	Skin color	LB	PVX	PVY	PLRV	BW	NOTE
1	CIP393077.159	Burundi	B3C1	Table and french fry	White-cream	R	R	S	HR	MS	
2	CIP393280.64	Burundi	B3C1	Chips and french fry	Red	R	R	S	R	MS	
3	CIP395112.6	Burundi	B3C2	Chips and french fry	White-cream	R					(High in Zn & Fe)
4	CIP391691.96	Burundi	A	Chips and french fry	Purple	R	R	S	HR		
5	CIP399072.21	Burundi	B1C5	Chips and french fry	Pink	R	R	R	R		Long dormancy
6	CIP388676.1	Rwanda	B3C1	Chips	White-cream	R	S	ER	S	S	Drought – heat tolerant
7	CIP392657.8	Rwanda	B3C1	Chips and french fry	White-cream	R	ER	R	HS	MR	
8	CIP393371.164	Rwanda	B3C1	Table and french fry	White-cream	R	ER	S	S	S	
9	CIP398190.89	Rwanda	LBHT-1	Table and french fry	White-cream	R	S	S			Heat tolerant
10	CIP398193.511	Rwanda	LBHT-1	Table and french fry	White-cream	R	ER	S			Heat tolerant
11	Shangi	Rwanda	Kenya Landrace	Table and french fry	White-cream	MR	R	R		S	
12	CIP392617.54	Rwanda, Burundi	B3C1	Table and french fry	White-cream	R	S	S	HS	S	
13	CIP392797.22	Rwanda, Burundi	LTVR	Chips and french fry	Red	MR	R	ER	R	MS	
14	CIP394611.112	Rwanda, Burundi	LTVR	Chips and french fry	Red	R	R	ER	MS	S	
15	CIP393371.157	Rwanda, Eritrea	B3C1	Table and french fry	White-cream	R	R	ER	S	S	
16	CIP398208.704	Rwanda, Eritrea	LBHT-1	Table	White-cream	R	ER	S			Heat tolerant, mid elevation
17	CIP393079.4	Rwanda, Burundi, Eritrea	B3C1	Chips	White-cream	R	R	S	R	HS	Good taste
18	CIP398190.200	Rwanda, Burundi, Eritrea	LBHT-1	Table	White-cream	R	S	S			Heat tolerant, mid elevation

Milestone: Clean potato seeds of clones under evaluation from population LBHTxLTVR, SSA local crosses, biofortified, TON panel, will be conserved and multiplied in screen houses for future experiments.

Means of verification: Technical report

Over 800 clones were multiplied in green house/field and have been conserved for future breeding trials. An inventory list of materials is available with a detailed pedigree, principal traits of interest, country of custody (Kenya, Ethiopia) and the number of tubers available to facilitate the trial planning for coming season. The inventory list of advanced clones has been updated throughout the year. Country, population group and number of clones multiplied and conserved for breeding activities are presented in Table 9. The TON panel has been recently shared with a national partner in Uganda for further evaluation under the RTBFoods project which is supported by the Bill and Melinda gates Foundation. The BW population will be shipped to Kenya for bacterial wilt assessment. This activity has a crucial importance to keep on projects on Breeding and variety development across sub Saharan Africa.

TABLE 9. COUNTRY, POPULATION GROUP AND NUMBER OF CLONES MULTIPLIED AND CONSERVED FOR BREEDING ACTIVITIES

Country	Population Group	Number of clones	Note
Ethiopia	LBHT-1	15	Field harvests, need virus cleaning
Ethiopia	LBHT-2	140	Number will further be reduced after down selection based on field data
Ethiopia	SAP2014	81	Number will further be reduced after down selection based on field data
Ethiopia	TON	185	176 clones sent to Uganda
Ethiopia	BW	850	272 clones prepared to send to Nairobi
Kenya	LTVR	7	Under virus cleaning at KEPHIS
Kenya	SAP2013	6	Under virus cleaning at KEPHIS
Kenya	SAP2014	120	Stored in cold room
Kenya	BW	450	Stored in cold room

Milestone: Assessment of at least 200 lines from LBHT x LTVR population in two locations under heat stress. Selection based on yield and yield related traits.

Means of verification: Technical report

Resilient potato clones that are high yielding under heat stress condition and adapted to mid-altitude and low land areas have been selected in Ethiopia. A total of 229 clones including: 219 new clones from LBHT x LTVR population, 5 advanced clones from TON panel, 4 released varieties in Ethiopia and Kenya, and "Eva" (variety from USA) were planted in augmented design, 5 plants per plot (0.75m x 1.5m) in two warm locations: Gode and Melkasa (Table 10), from February to June 2018 under irrigation. The minimum maximum and average temperature during the growing season for the two locations are presented in Figure 6 and 7. Tubers were harvested 110 days after planting.

TABLE 10. DESCRIPTION OF THE LOCATIONS USED FOR THE YIELD TRIAL, ETHIOPIA 2018.

Locations	Alt (masl)	Lat	Longitude	Agro-zone	T° Ave°C		Soil pH	CEC	Soil type
					Max	Min			
Gode	295	6°00'N	43°45'E	Arid	35.3	22.3	7.82	18.10	sandy clay loam
Melkasa	1550	8°24'N	39°21'E	Arid to semi-arid	28.4	14.0	6.71	27.78	Loam

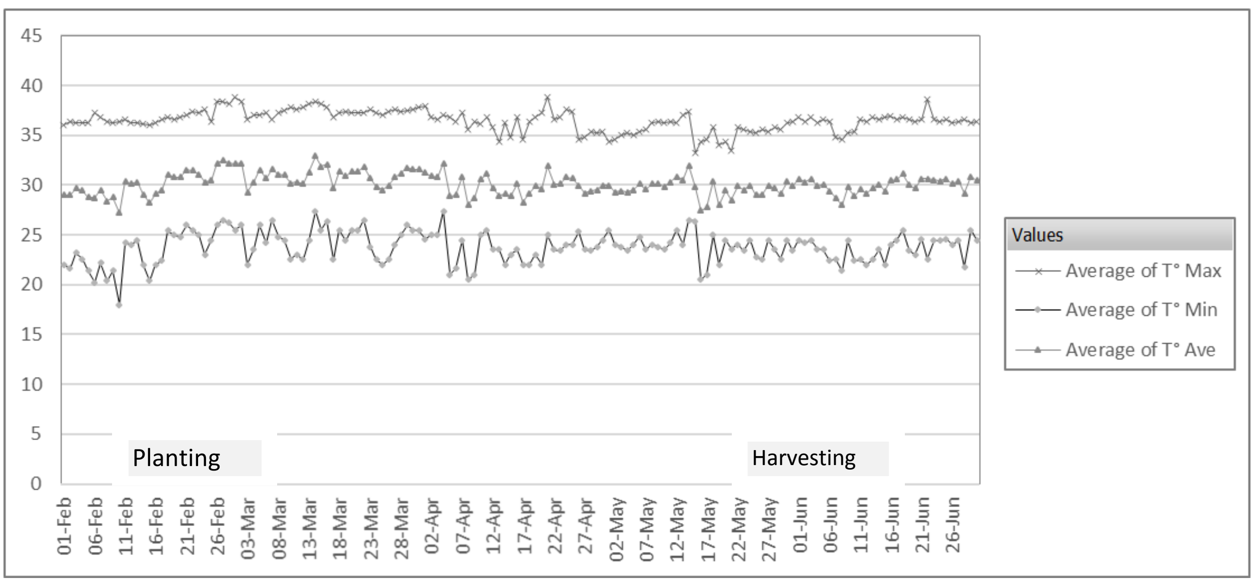


Figure 6. Temperature during growing season, Gode, Ethiopia 2018.

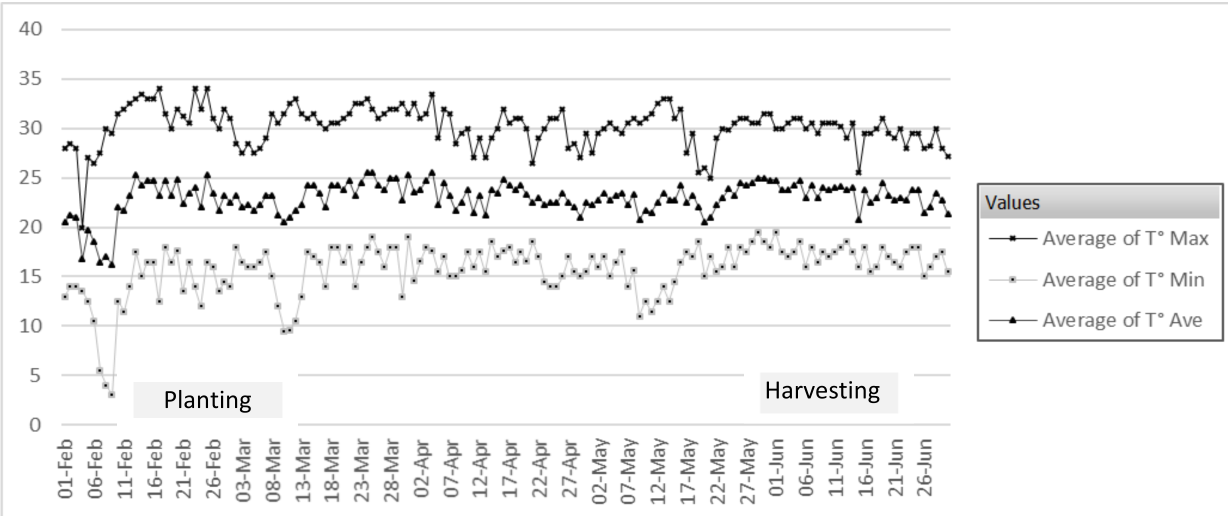


Figure71. Temperature during the growing season, Melkasa, Ethiopia 2018.

In Melkasa, the result displayed significant difference among clones. Forty-nine clones displayed above 10 t/ha yield advantage over the best check, CIP301056.54, and were free from tuber defects. Compared to Melkasa, the trial at Gode was exposed to extreme heat stress (Figure 16&7). As a result, the average yield in the experiment decreased from 25 to 1.2 t/ha. This could highlight the potential of the Gode site for severe heat stress screening. At Gode, the top 10% high performer, i.e. 25 clones in terms of tuber yield were selected for next season multilocation test. Figure 8 shows the potential of the selected clones against the main checks and discarded clones for yield. There was no correlation between the two sites for yield, indicating the experimental sites are distinct and selection was made separately for each location. However, eight common clones were selected for both locations.

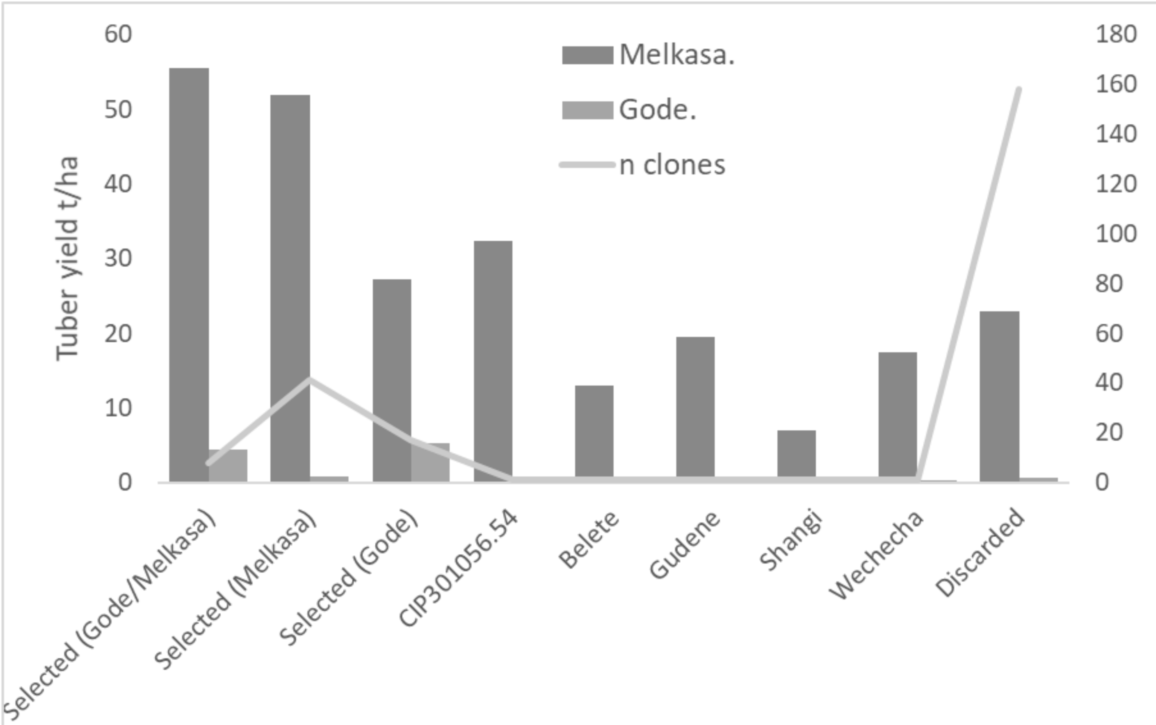
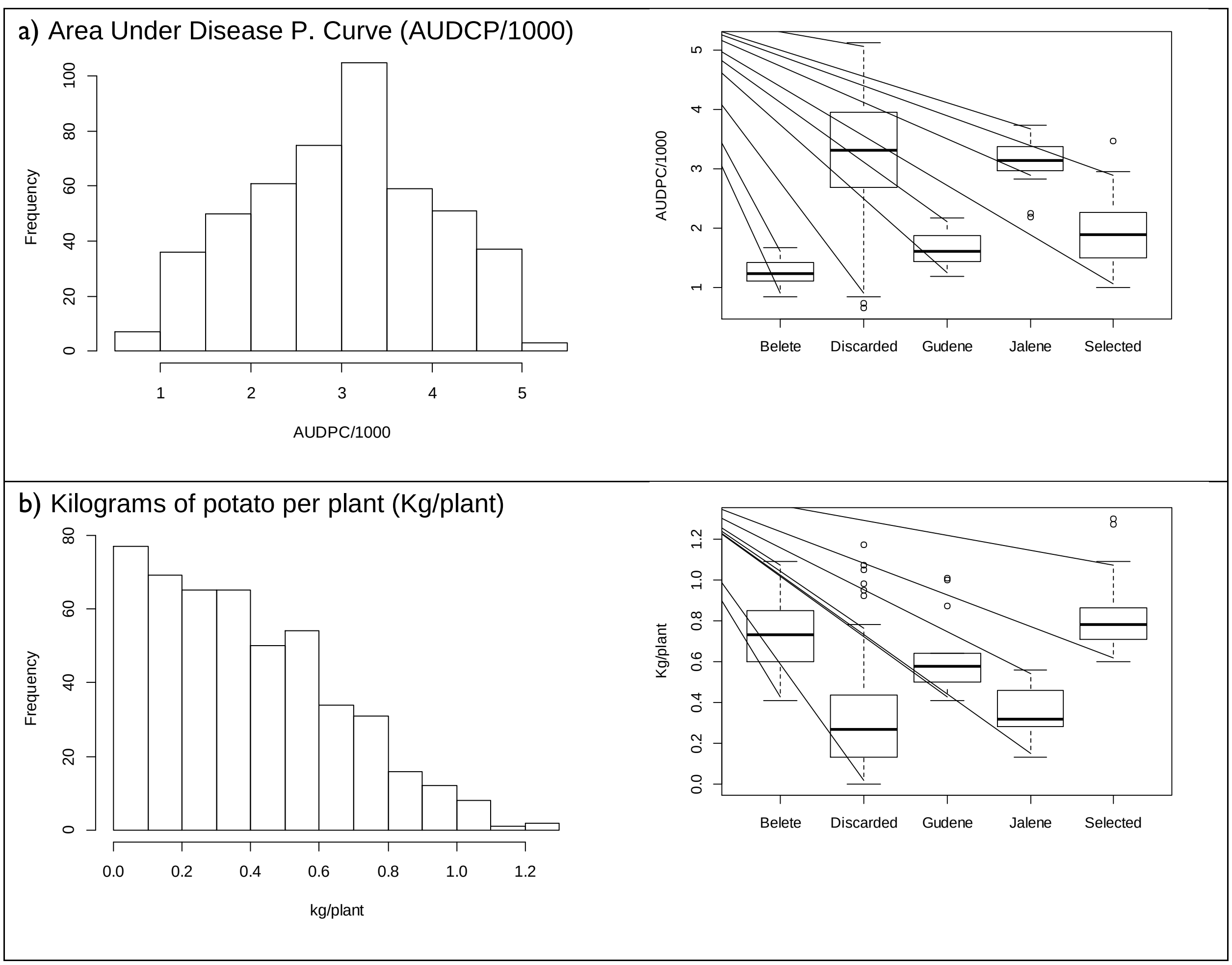


Figure *8*. Average yield of newly selected clones at Melkasa, Gode and at both locations versus checks and discarded clones, Ethiopia 2018.

Milestone: Assessment of at least 50 previously selected clones from LBHT x LTVR population and 80 clones from sub-Saharan Africa cross to develop high yielding clones under late blight disease pressure for traditionally potato growing highlands in Ethiopia.

Means of verification: Technical report

LBHTxLTVR: In the 2017 growing season, about 442 progenies derived from crossing of LBHT x LTVR populations were tested at Holetta Agricultural Research Center (HARC) for late blight resistance and yield under late blight pressure. About 63 clones that showed 46% and 63% yield and late blight resistance advantage, respectively over the entire population were selected. The selected 63 clones were planted in June 2018 with three check cultivars as a randomized complete blocks design, in two replications and 10 plants/plot for further selection. The three checks, Belete (late blight resistant), Gudene (late blight Moderately resistant) and Jalene (late blight susceptible), are widely grown high yielding cultivars in Ethiopia and included as a comparative control to identify the best clones with improved traits over the existing ones. The trials will be harvested on the third week of October 2018. Only clones yielding at least 10% above the best check and late blight tolerant will be selected. The clonal performance of 63 selected clones in the last season is presented on Figure 9 "results not reported". The selected groups showed a great performance with significant difference against the checks.



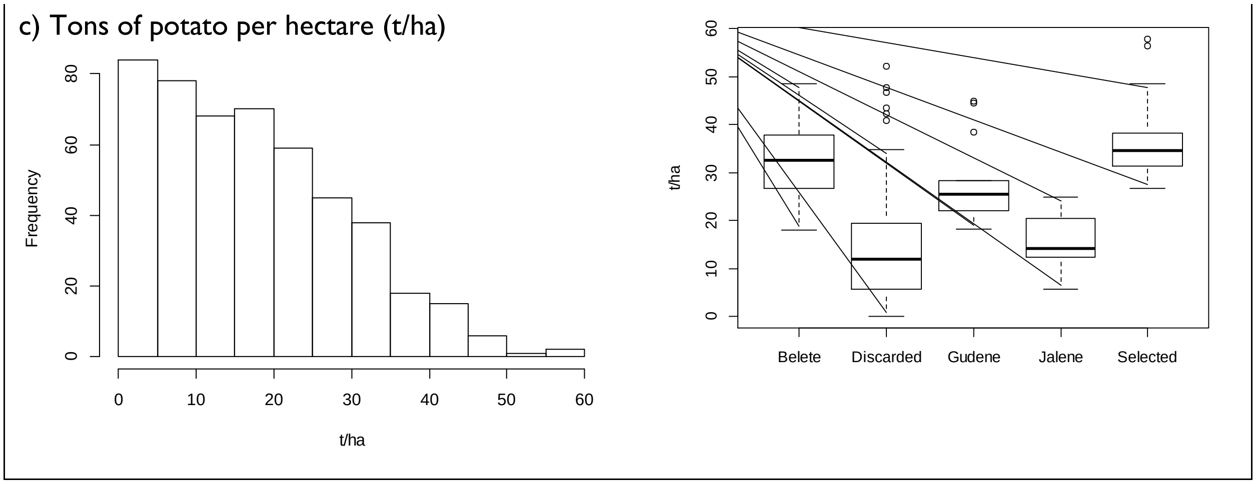


Figure 9. Yield and late blight resistant distribution from 442 clones from LBHT x LTVR population evaluated in 2017 growing season at Holetta, Ethiopia. a) Area Under Disease Progress Curve (AUDCP/1000) for late blight estimated based on a percentage of leaf area affected by late blight recorded seven times from the appearance of the first symptoms; b) Kilograms of potato per plant (Kg/plant): average yield per plant based on five plants per plot; c) Tons of potato per hectare (t/ha): estimated potato yield based on five plants per plot.

Sub-Saharan Africa cross: Since 2015, the sub-Saharan African population (SAP) was introduced to Ethiopia (Holetta and Adet) and the best clones have been selected with the objective of developing high yielding and late blight resistant clones for tropical highlands under rainfed condition. The crosses were made in Kenya in 2013 and 2014 using local cultivars and CIP advanced clones adapted to tropical highlands of East Africa. In 2017, 820 clones were planted in augmented design. No fungicide was sprayed during the growing season. Eighty-one clones were selected, representing about 10% of the tested clones that had AUDPC < 2300 or susceptibility scale ≤ 5 (where 9 is most susceptible and 1 is highly resistant), total yield per plant ≥ 0.45kg and marketable yield per plant ≥0.41 Kg, free from virus symptoms and external tube defects. The selection of the 10% best clone increased tuber yield two-fold, the marketable tuber yields more than four-fold and reduced the disease severity by half as compared to the original population planted in the 2017 growing season (Figure 10 and Figure 11) – "results not reported".

The selected 81 clones were planted in 2 replications using a randomized complete blocks design, with 5-10 plants/plot for further selection. Only clones yielding at least 10% above the checks and late blight tolerance will be selected. The trials will be harvested on the third week of October 2018. Its expected 20 highest yielding and late blight resistant clones will be used for parents and/or variety release after stability tasting for the traditional potato growing highlands.

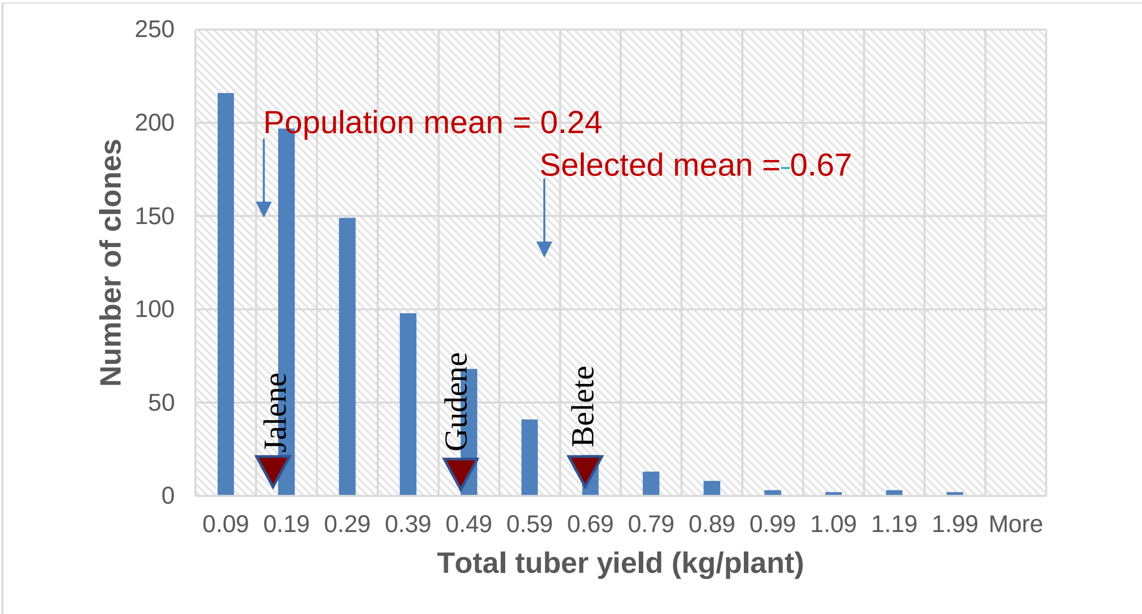


Figure 10. The Frequency distribution of clones derived from CIP x Kenyan local cultivar 2013/2014 crosses for tuber yield under late blight disease pressure in 2017 growing season at Holetta, Ethiopia.

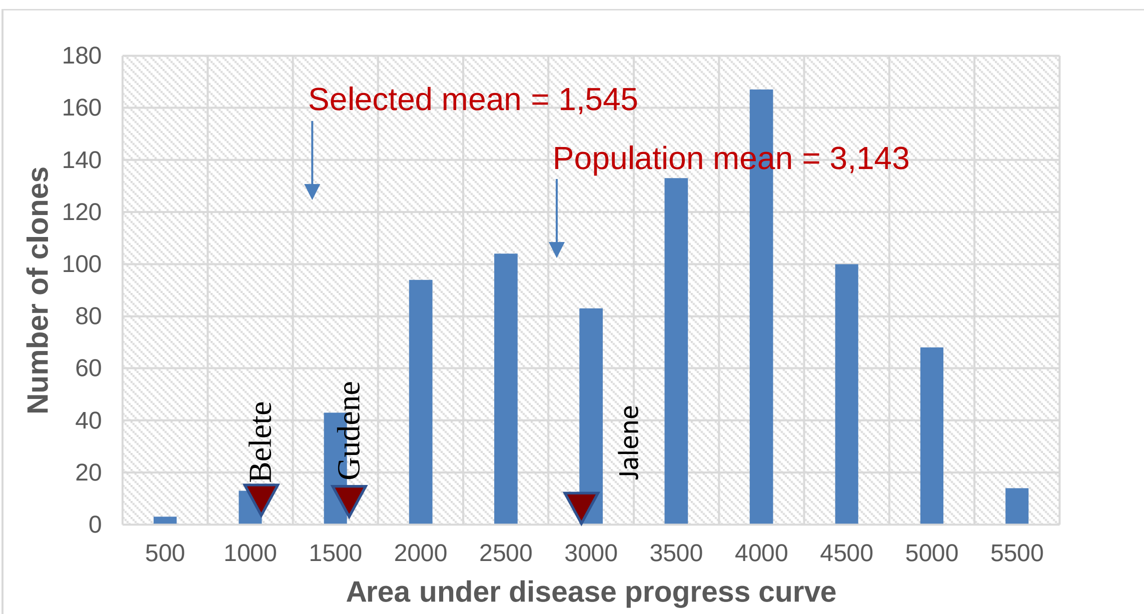


Figure 11. The Frequency distribution of clones derived from CIP x Kenyan local cultivar 2013/2014 crosses for late blight infection (AUDPC) in 2017 growing season at Holetta, Ethiopia.

Milestone: Assessment of at least 10 advanced clones from inter-population crosses (LBHT x LTVR) in Ethiopia and introduce them to Kenya for cleaning and further distribution.

Means of verification: Technical report

Clonal selection for yield and late blight resistance in LBHT x LTVR clones started in Ethiopia in 2015. From 300 initial clones, fifteen clones were selected in three years' field evaluation (2017 data was not reported) for late blight, yield and one-year pilot cooking quality tasting (Table 11). For further selection these fifteen clones and three local checks (Dagim, Gudene and Jalene) have been planted for evaluation in RCB design, 3 replications 40 plants/plot (2.25m x 3m) in two locations: Holetta and Adet. Participatory evaluation with male and female farmers is planned to be made 3 times: at flowering, harvest and organoleptic tasting 10 days after harvest. The first participatory evaluation was made at flowering. Fourteen women and 11 men farmers participated in the evaluation and selected their most preferred clones by gender (Table 11) based on their criteria presented in the Table 12. Both women and men identified their best three clones and among which, clones CIP312927.593 and CIP312927.610 were the most frequently selected one and common to both gender group. The female group uniquely selected CIP312922.528 as their second most preferred clones; the reason for this could probably attributed to women's sole selection criteria listed in the Table 12. The trials will be harvested in the second week of October 2018. It's expected three high yielding (>40 t/ha), late blight resistant variety (better than resistant variety Belete) with best cooking quality for traditional potato growing areas (altitude >1800m).

TABLE 11. YIELD, LATE BLIGHT DISEASE SEVERITY, PRELIMINARY ORGANOLEPTIC TESTING AND FARMERS EVALUATION AT FLOWING OF THE 15 SELECTIONS FROM LBHT X LTVR POPULATION, HARVESTED 90 DAYS AFTER EMERGENCE IN THE 2016-2018 GROWING SEASONS AT HOLETTA, ETHIOPIA

Clone	Total tuber yield[1] t ha^{-1}		Yield performance over Gudene (%)		AUDPC[2]		Average tuber weight[3]		Taste of[4] boiled tuber	Texture of boiled tuber	Frequency of selection at flowering stage by gender of farmers	
	2016	2017	2016	2017	2016	2017	2016	2017			Female	Male
CIP312923.634	41.8	50.2	159	192	56	1053	66.28	67.95	4.4	3.9	0	0
CIP312922.528	41.3	47.6	158	181	243	1238	67.13	71.5	3.6	2.2	15	1
CIP312921.603	23.1	33.8	88	129	363	2186	60.19	58.51	3.3	2.7	0	0
CIP312920.524	37.8	31.1	144	119	285	2376	33.09	34.65	4.2	2.9	0	0
CIP312920.515	40	35.6	153	136	641	2704	83.24	122.22	3.5	4	1	6
CIP312927.593	48	45.8	183	175	361	1673	114.14	93.94	3.9	3.5	19	16
CIP312921.525	26.2	34.7	100	132	434	1765	50.3	73.46	2.9	3.3	2	2
CIP312927.610	46.7	40.4	178	154	168	912	80.3	80.62	2.6	3	14	18
CIP312920.532	16	27.6	61	105	943	2251	35.18	60.52	3.3	1.8	0	1
CIP312921.543	28	27.1	107	103	148	1865	64.84	50.97	3.3	3	0	0
CIP312927.618	36.9	43.1	141	164	250	1257	75.32	90.5	3	3.2	10	9
CIP312920.563	22.2	24	85	92	111	2997	33.85	35.19	3.6	3	0	0
CIP312920.629	27.1	19.6	103	75	592	2796	47.28	37.69	3	3.3	0	0
CIP312921.651	30.7	24.9	117	95	173	2752	54.86	60.78	2.9	2.2	0	0
CIP312921.663	32.4	34.7	124	132	490	2163	92.91	96.99	2.5	3	2	0
Jalene	10.7	16.4	41	63	1889	3164	21.42	29.78	3.7	2.8	14	7
Belete	15.6	37.3	59	142	1073	1310	28.02	62.26	3.8	2.3	-	-
Gudene	26.2	26.2	100	100	289	1700	41.3	38.09	3.8	3.5	5	5
Dagim	-	-	-	-	-	-	-	-	-	-	1	0

[1]computed by converting the total weight of all the tubers harvested in a plot to t ha^{-1}
[2]Area under disease progress curve for late blight disease
[3]Obtained by dividing total tuber weight with number of tubers harvested per plot
[4]where 5 is excellent and 1 is poor taste and texture

TABLE 12 CHARACTERISTICS OF CLONES PREFERRED BY WOMEN AND MEN FARMERS AT FLOWERING AND HARVEST

At flowering					At Harvest				
Selection Criteria	Women		Men		Selection Criteria	Women		Men	
	frequency	rank	frequency	rank		frequency	rank	frequency	rank
Disease Resistance	53	1	8	4	High tuber Yield	61	1	53	1
Large number of stems	47	2	17	3	Tuber Colour (preferably white)	58	2	30	3
Thick and strong Stem	26	3	4	5	Medium sized tuber	40	3	34	2
Broad leaves	21	4	3	7	Free from crack	28	4	14	5
Abundant leaves that stays longer in the plant	20	5	-	-	Long Storability	20	5	-	-
Uniformly flowering	16	6	-	-	Easy to peel (shallow eye depth)	3	6	-	-
Deep Green foliage	16	7	4	5	Oval Shape	-	-	29	4
Medium plant height/no lodging	11	8	-	-	Shallow tuber eye depth	-	-	5	6
White flowers and that take long to drop	-	-	18	1					
Vigour	-	-	3	7					
Medium Maturing	-	-	18	1					

Milestone: Cleaning up at least 10 Rwanda local varieties, 30 2x biofortified potato introduced from Ethiopia and 5 LTVR x LBHT clones selected in Kenya for further distribution in SSA

Means of verification: Technical report

Out of 39 clones tested up to now, 19 have a clean copy at CIP SSA TC lab/KEPHIS (Kenya Plant health inspectorate Service) station. The cleaning process has started in early 2017 with total of 65 clones coming from different groups as Biofortified, LTVR x LBHT and Rwanda Landrace. To assess the incidence of virus in the plantlets, samples of 39 clones were tested using a serological technique (ELISA). This test can detect all common potato viruses: i) Potato leafroll virus (PLRV), ii) Severe mosaic (PVY, PVN), iii) Mild mosaic (PVX) and iv) PVS, PVA. The results showed the follow number of clones with virus: PLRV = 6, PVS = 9, PVX=8 and PVY = 6. All clones were free of PVA and PVM. The cleaning process is an ongoing activity for 2018 and it's expected to reach a higher number of clean copy of advanced clones.

TABLE 13. BREEDING POPULATION, NUMBER OF CLONES INTRODUCED AND CLEANED AT KEPHIS, KENYA 2018

Population	# clones introduced	# clones cleaned
Biofortified Ethiopia	40	14
Rwanda Landrace	10	3
LTVR	7	2

Milestone: Assessment of at least 40 clones from sub-Saharan Africa crosses in two locations for high yielding under late blight and virus disease pressure in Kenya.

Means of verification: Technical report

A group of 76 selected clones from sub-Saharan Africa crosses (SAP2013 and SAP2014) were evaluated for high yielding under late blight and virus disease pressure in two unrelated locations in Kenya. A set of 10 potential clones were identified yielding up to 62% above the best check (Kenya mpya) and showing smaller late blight score. These clones will be planted next season for further evaluation on Multi Location Trails.

The statistical analysis was performed throughout mixed models as described by the scripts in the Figure 12. It was adjusted a spatial model combining data from two locations. The boxplot of genotypic values divided in three groups (check, discarded and selected) for tuber yield per hectare (Yield t/ha) and late blight pressure (rAUDPC) from 76 advanced clones and local checks across two environments is presented in the Figure 13. For both traits (Yield t/ha and rAUDPC) the highest performance can be observed in the group of Selected.

```
# Model 1 - Basic Model with spatial components

#rAUDPC and Yield

spat1<-asreml(fixed=Yield~1,

        random=~ROWf+COLf+Variety,

        rcov=~ar1(COLf):ar1(ROWf), data=spatial,subset=Local==1)

summary(spat1)$varcomp

anova(spat1)

plot(variogram(spat1))


# Combining two Sites

met3<-asreml(fixed=Yield~Local,
```

```
        random=~at(Local):ROWf+at(Local):COLf+corgh(Local):Variety,
        rcov=~ar1(COLf):ar1(ROWf):at(Local),
        data=spatial)
summary(met3)$varcomp
plot(met3)
#BLUP
write.table(meanVariety_site$predictions,"Variety_Local_Yield.txt")
write.table(meanVariety$predictions,"Variety_Yield.txt")
```

Figure 12: Spatial analyses - Mixed model scripts

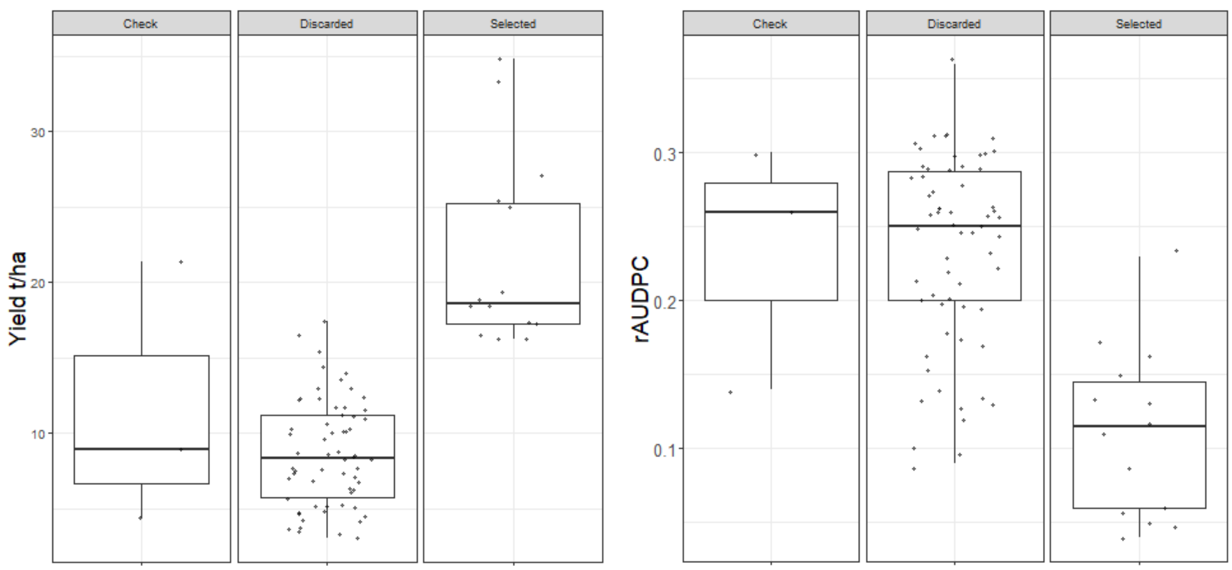


Figure 13: Boxplot of genotypic values divided in three categories (check, discarded and Selected) for tons of tuber per hectare (Yield t/ha) and late blight pressure (rAUDPC) from 76 advanced clones (Sub-Saharan Africa crosses) and local checks across two environments in Kenya, 2018.

On average the selected group yielded 11% above the best check (Kenya mpya). A visual complement for the selected group performance in the Figure 14 illustrates the distribution of genotypic values for tons of tuber per hectare (Yield t/ha) and late blight pressure (rAUDPC) from 76 advanced clones and local checks across two environments.

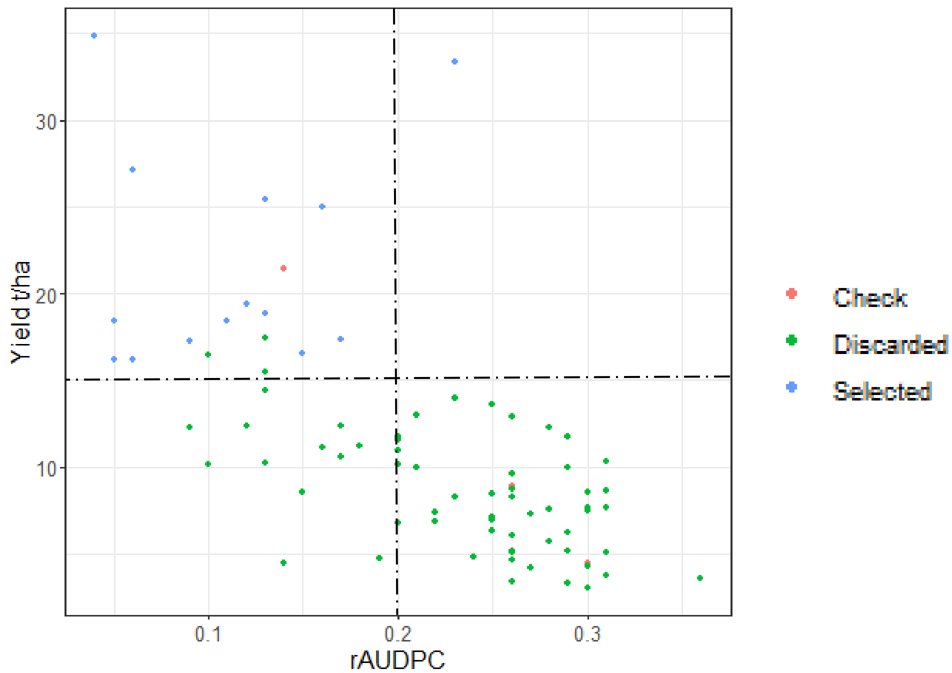


Figure 14: Distribution of genotypic values for tons of tuber per hectare (Yield t/ha) and late blight pressure (rAUDPC) from 76 advanced clones (Sub-Saharan Africa crosses) and local checks across two environments divided in groups of Checks, Discarded and Selected), Kenya, 2018.

Milestone: Five advanced clones introduced to SSA TC lab for cleaning and further distribution.

Means of verification: Technical report

Two populations of potato germplasm are being evaluated in the field: i) advanced inter-cross (LTVR x LBHT) population introduced as true potato seeds design from HQ in 2013, ii) SAP population from crosses made in Nairobi in 2013 and 2014. From LTVR x LBHT 11 clones trialed and 4 promising clones were identified at harvest. Two of them were introduced to NPT - Oct/2017. In SAP 2013, 34 clones were field selected by 2017 and 4 of best performing clones were introduced into National Potato Trial. Table 14 shows the clones introduced to TC lab at KEPHIS for virus cleaning in 2018.

Table 14. Clones introduced in the tissue culture laboratory at KEPHIS, Kenya 2018.

Clone	Female	Male	Population
CIP398180.292	CIP392657.171	CIP392633.64	LBHT_1
CIP398201.510	CIP393242.50	CIP392633.64	LBHT_1
CIP398190.89	CIP393077.54	CIP392639.2	LBHT_1
CIP398190.404	CIP393077.54	CIP392639.2	LBHT_1
CIP312010.759	CIP397069.11	CIP398098.119	LTVR x LBHT
CIP312084.731	CIP392820.1	CIP398208.219	LTVR x LBHT
CIP313002.4	CIP393371.58	CIP388615.22U	SAP13
CIP313010.15	CIP800048	Desiree	SAP13
CIP313009.84	Asante	CIP397073.7	SAP13

Milestone: Adaptability and stability of CIP clones for mid elevation in Kenya.

Means of verification: Technical report

To determine the patterns of adaptability and stability of potato clones for mid elevation in Kenya, 15 advanced materials were evaluated in five locations (Burn Forest, Cherangany, Kibirichia, Molo and Timau) for tuber yield per hectare (yield t/ha), tested in randomized complete block with four replications. The analyses were conducted and biplots generated by "GGEbiplot" software. Table 15 displays the parameter estimates from ANOVA of 15 clones of potato in multi-location trials.

TABLE 15. PARAMETER ESTIMATES FROM ANOVA OF 15 CLONES OF POTATO ASSESSED FOR TUBER YIELD PER HECTARE (T/HA) IN MULTI-LOCATION TRIALS, KENYA – 2017

Source	DF	MS
Genotype	14	41.77**
Environment	4	324.85**
G x E	56	20.97
LSD5%		5.92
CV%		23.00
Grand Mean		19.71
H = Vg/Vp		0.50

Three biplots were generated to explain: i) "which-won-where" patterns, ii) Interrelationship among environments and iii) Varieties mean yields and their stability

i) "which-won-where" patterns

The locations were divided into three groups and the best cultivars for each group reveled as follows: 1) Burn Forest: Mugaruro, 2) Cherangany: CIP392797.22, 3) Kibirichia, Molo and Timau: Tigone. It also revels the presence of a mega environment combining Kibirichia, Molo and Timau, which can be validated as a target region (Figure 15).

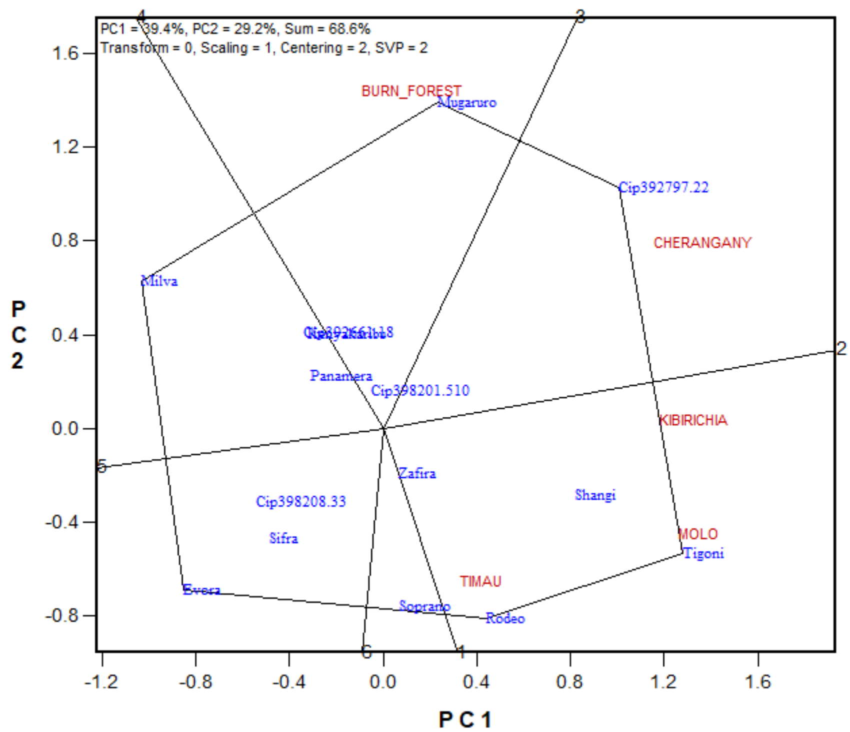


Figure 15. "Which-won-where" view of GGE biplot for tuber yield (t/ha) in Kenya across five locations.

ii) Interrelationship among environments

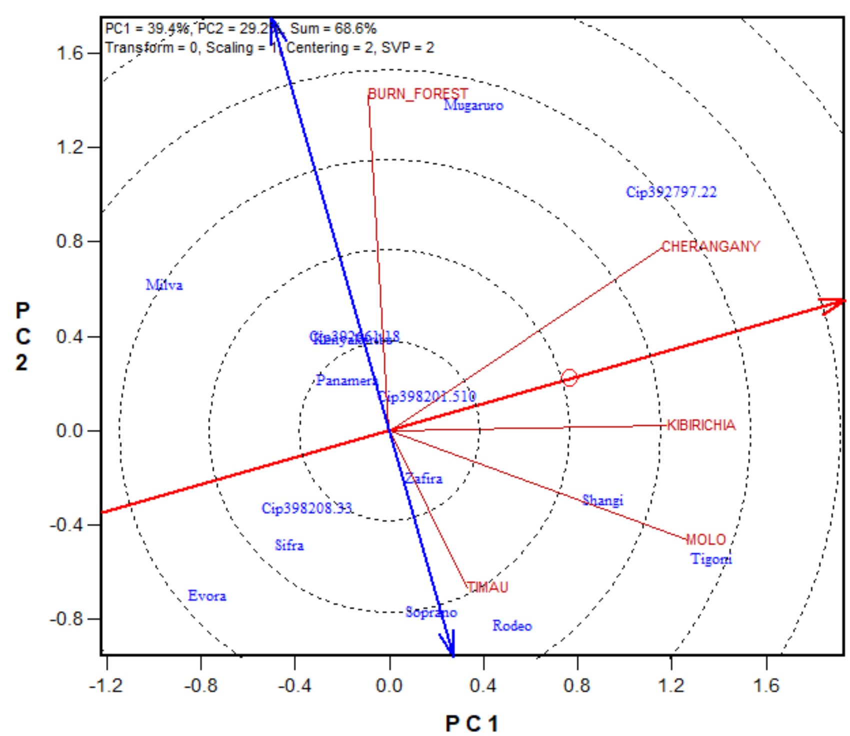


Figure 16. The relationship between five sites for tuber yield (t/ha) in Kenya.

Kibirichia, Molo and Timau presents vectors smaller than 90°, meaning that they are positively correlated and suggesting redundant environments for variety discrimination. Out of those three, Molo can be considered as the best location for clonal test, because it has the longest vector, which associates with a better ability to discriminate clones. Burn Forest has no relation with Kibirichia, Molo and Timau, i.e. the largest GxE will be between these two group. Molo, Cherangany and Burn Forest can be considered as the most discriminating locations and should be taken for next round of Multi Environment Trials (Figure 16).

iii) Varieties mean yields and their stability

Figure 17 displays the mean performance and stability of 15 clones for tuber yield (t/ha) across five environments in Kenya. The varieties are ranked along the red line, with the arrow pointing to a greater value. Thus, the best variety was CIP392797.22 follows by Tigone and Shangi. The blue line represents its midpoint and separates the varieties below and above the average.

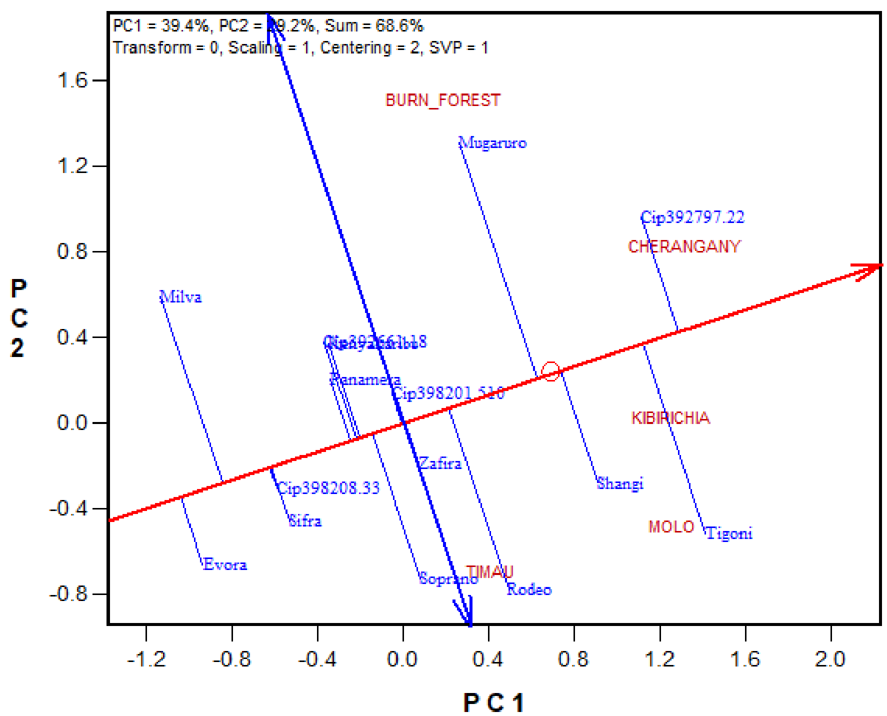


Figure 17. Mean performance and stability of 15 clones for tuber yield (t/ha) across five environments in Kenya.

The Figure 18 shows the predicted mean and instability parameter of 15 clones for tuber yield (t/ha) across five environments. The CIP398208.33 presented the highest stability associated with low yield, however CIP892797.22 showed good stability and high yield and should be released. Shangi, a local check, has proven to be a very competitive variety with similar stability of CIP892797.22.

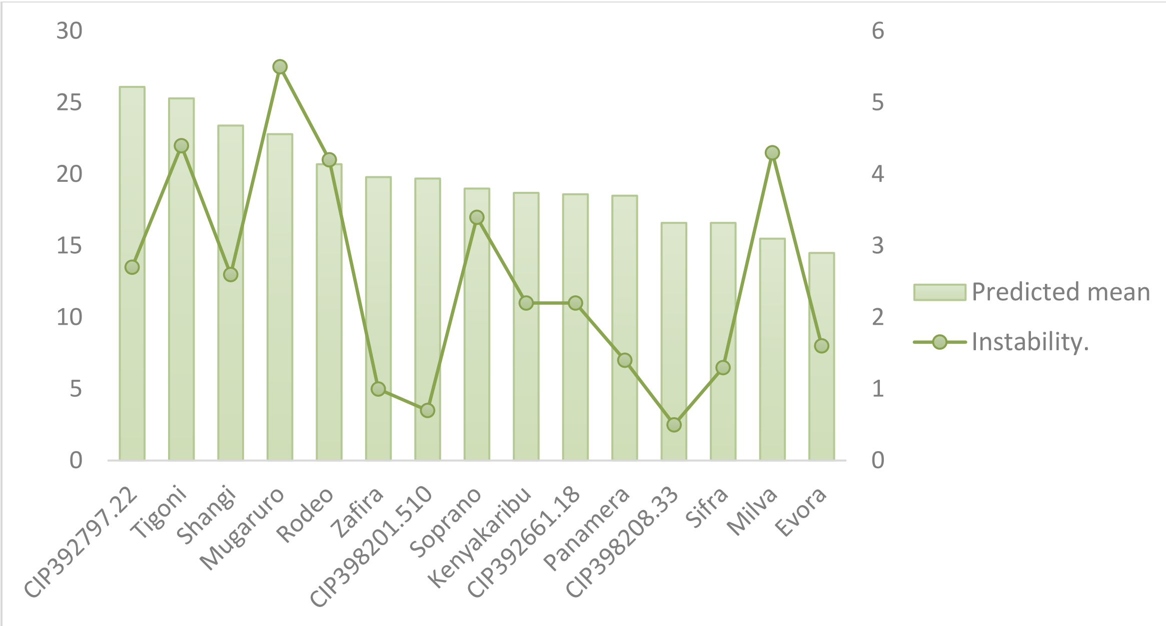


Figure 18. Predicted mean and instability parameter of 15 clones for tuber yield (t/ha) across five environments in Kenya.

Output 3: Improved and shared breeding databases available and knowledge management, including trait specific protocols and catalogs to support the orientation of breeding products and facilitate decision making.

Standardized methodologies and protocols to assess adaptive traits and communicate intrinsic qualities and add value to breeding materials developed and available in GTDMS

Milestone: Evaluate the level of Potato Leafroll Virus (PLRV) resistance in a set of advanced potato clones and publish the results in the online database CIP catalogue of standard evaluation trials (SET). M. Aponte

Means of verification: Database on website

Indicators: 70 advanced clones from SET7 with data of PLRV resistance (Database on Website)

https://research.cip.cgiar.org/cipcatlg_set/protected/views/Catalog/index.php

A panel of 70 elite genotypes from SET7 corresponding to 41 genotypes of the LTVR population and 29 genotypes of the LBHT-1 population and three checks (Perricholi = susceptible, Achirana-INTA = susceptible, DW84.1457 = resistant) were evaluated for PLRV resistance following the CIP protocol (https://youtu.be/K9mXF2A8AMI). To obtain tubers for the secondary infection trial the potato clones were planted in between rows of PLRV infected plants of cv. Flor Blanca and Perricholi and inoculated with virulent *Myzus persicae* aphids. Each genotype was evaluated in two replicates each consisting of 15 plants/genotype. From each inoculated genotype tubers were planted in the green house and subsequently infected again with viruliferous aphids. Seven days after the inoculation the aphids were killed by a systemic insecticide, and the PLRV infection was allowed to develop in the plants. Forty days later, DAS-ELISA was carried out on individual plants to assess the PLRV infection. Infection percentages were calculated as the number of plants that tested positive to DAS-ELISA in secondary infection cycles over the total number of plants tested per genotype and replication. The potato genotypes were assigned to one of the six reaction types ranging from highly susceptible to highly resistant depending on the infection percentages.

The PLRV infection was successful in both trials as shown by the reaction types of the checks: Perricholi was classified as highly susceptible, Achirana-INTA as moderately susceptible and DW84.1457 as resistant. While most of the test genotypes were classified as moderately resistant, there were 19 classified as highly resistant or resistant to PLRV, most of these originating from the LBHT-1 population (Figure 19). The data generate will be helpful for the breeders when selecting genotypes as progenitors for their crossing programs. The information regarding the reaction type was added in the in the online database CIP catalogue of standard evaluation trials (SET) that is open access to the general public. **https://research.cip.cgiar.org/cipcatlg_set/protected/views/Catalog/index.php**

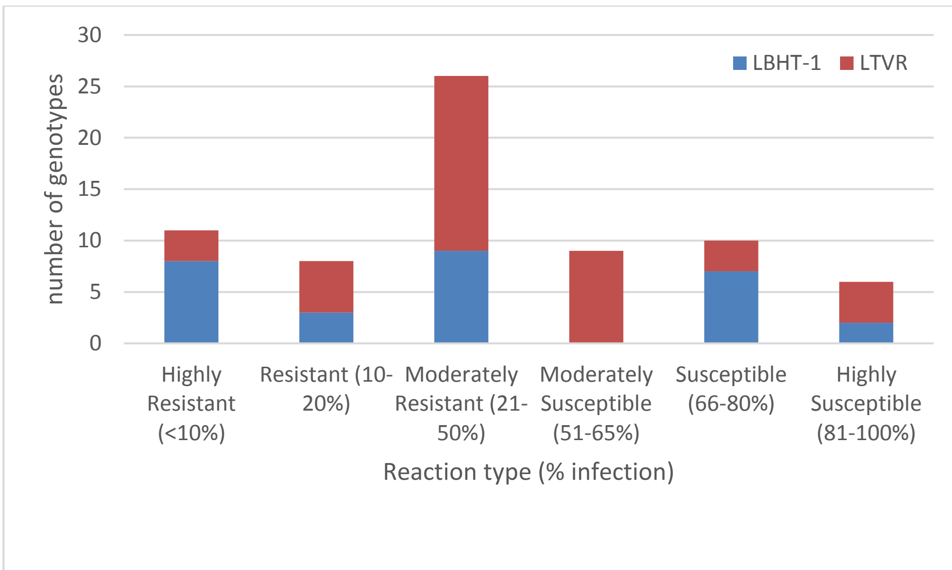


Figure 19. The level of PLRV resistance

APPENDIX

Publications (1st year, Oct. 2017–Sept. 2018)

Manuel A. Gastelo, Luis Diaz, Gabriela Burgos, et al. 2017 "Heritability for Yield and Glycoalkaloid Content in Potato Breeding under Warm Environments" *Open Agriculture*, 2.1 (2017): 561-570. doi:10.1515/opag-2017-0059.

De Haan, S.; Salas, E.; Fonseca, C.; Gastelo, M.; Amaya, N.; Bastos, C.; et al. 2017 Selección participativa de variedades de papa (SPV), usando el diseño mma y bebe: una guía para capacitadores con perspectiva de genereo,. Lima (Peru). Centro internacional de la Papa 82 pp.