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Final Report BMZ Project Funding

General Information

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Closing date	At the latest 5 months subsequent to termination of the project
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1. Basic Data

Name of IARC	International Potato Center (CIP)
Project title	Accelerating the Development of Early-Maturing-Agile Potato for Food Security through a Trait Observation and Discovery Network
Funding type, GIZ Project number and Contract number	**Project number**: 14.1432.5-001.00 **Contract number**: 81180345
Reporting period	**Final report**: January 2015–December 2018, including a no-cost extension during 2018
Project coordinator and project scientists	**Project coordinator**: Hannele Lindqvist-Kreuze, Av. La Molina 1895, Apartado 1558, La Molina, Lima, Peru. Phone number: +511 349 6017 (3065). Email: h.lindqvist-kreuze@cgiar.org **Principal staff members**: • Merideth Bonierbale, Hannele Lindqvist-Kreuze, Elisa Salas, Elisa Mihovilovich, Junhong Qin, Hirut Getinet, Dorcus Gemenet, Awais Kahn, Xie Kaiyn, Greg Forbes. Coordination of final report: Phillip Kear, Thiago Mendes
Project partners, including national agricultural research systems (NARS)	Max Planck Institute for Molecular Plant Physiology (MPI-MP): Karin Köhl Yunnan Academy of Agricultural Sciences (YAAS): Xianping Li Gansu Agricultural University (GAU): Zhang Junlian Heilongjiang Academy of Agricultural Sciences (HAAS): Sheng Wanmin Ethiopian Institute of Agricultural Research (EIAR): Gebremedhin Woldegiorgis Amhara Regional Agricultural Research Institute (ARARI): Alemu Worku

2. Final Report

State of Project Implementation

Output 1: Broad genetic diversity of advanced-bred lines genotyped and phenotyped in key environments

1.1 Extend and adapt phenotyping protocols for key adaptive traits. (*Ongoing)*

CIP updated phenotyping protocols available in its Global Trial Data Management System (GTDMS) http://cipotato.org/resources/databases/ for conducting large, multi-environment trials and developed procedures to enable field books to be uploaded and process them for open access available in DataVerse.

- CIP improved its tuber bulking and late blight (LB) resistance evaluation protocols in Data Collector software to account for larger numbers of pairwise comparisons using statistical testing than was possible in earlier versions.
- CIP updated its *Participatory Varietal Selection of Potato Clones Using the Mother & Baby Trial Design A Gender Responsive Trainer's Guide* with analytical software and graphical capacity to support the electronic field book. It was uploaded to the GTDMS portal (https://research.cip.cgiar.org/potatoknowledge/pvs.html).

- Improvements were made to CIP's protocol and data collection tool for assessing potato clones for drought tolerance under field conditions. Twenty-six morphological or physiological traits and means for assessing them at five stages of plant development have been defined in CIP's trait dictionary. Fourteen morphological and physiological traits are measured twice before and twice after drought initiation; another 12 traits are evaluated at harvest.
- CIP developed the fieldbooks interface with which individual researchers can upload data generated using the Data Collector software into the CIP database for safe data storage. The field books are searchable by username and other filters.

MPI-MP worked to identify phenotypic markers for use in drought-tolerance breeding. They used automatic phenotyping tools, including laser and infra-red thermometry systems, to identify morphological or developmental features of potato that are associated with drought tolerance and could be used to simplify selection. (See detailed report in Annex 3.)

- MPI-MP characterized two populations of genetic material available in Germany under field and greenhouse conditions. They subjected potato populations to three different drought-stress patterns (early, late, and repeated) and calculated a drought-tolerance index as the deviation of the relative starch yield from the experimental median of the relative starch yield of all genotypes.
- The relationship between features (e.g., maximum plant height) or derived growth curve parameters (e.g., growth rate leaf area) observed on optimally watered and drought-stressed plants and drought tolerance was studied. Leaf angle measured pre-dawn was proposed as a possible marker for drought tolerance, with validation in different genetic backgrounds still pending. The canopy temperature depression calculated as the difference between canopy surface temperature and the air temperature was tested as a putative drought-tolerance marker.
- To handle the large amount of data generated with automatic phenotyping, staff and the project's PhD student developed a workflow system. The system handled the exchange of data between the storage database, the joining of metadata and environmental data, quality control, filtering algorithms, and statistical analysis in SAS.

The objective of the automatic phenotyping trials of MPI-MP was to identify simple markers that can readily be applied in target environments, which requires field validation and training of local staff. The planned testing of marker assessment by simple methods in field trials in Ethiopia and their dissemination among Ethiopian breeders had to be cancelled as a consequence of severe delays in G. Mulugeta Aneley's PhD. Owing to a late start, illness, and technical problems, data are still being analyzed. Candidate drought-tolerance markers were identified, but validation in the target environment will have to be done in a subsequent project. Data analysis, validation experiments, and PhD supervision will continue at MPI-MP through 2019; completion of the thesis is expected for 2019 with institutional funds. The final data evaluation will most likely not be finished at the end of the PhD work. Karin Köhl has started to conduct additional data analysis and will continue this work to write a manuscript for a peer-reviewed journal in 2019.

1.2 Phenotype the genetically diverse potato panel for 7 traits. *(Completed)*

CIP provided more than 360 advanced potato clones from its breeding program comprising the project's "trait observation network" (TON) panel and standardized protocols for phenotyping the clones for the projects key traits. Partners introduced the germplasm and produced seed for trials, prioritizing trait evaluations according to their needs. For example, GAU in semi-arid North China concentrated on drought tolerance and crop duration. LB and virus resistances were emphasized by YAAS in the humid subtropical highlands of China.

EIAR in Ethiopia's mid-elevation tropics evaluated panel clones for drought tolerance and LB resistance.

- Seed production involved exchange between programs, with YAAS supplying minitubers to other institutions in China.
- Approximate plans and schedule for conducting the phenotyping trials were developed at the start-up workshop in Germany.
- CIP project members maintained contact with national program researchers throughout the project to address queries regarding protocols and data collection. They visited each program during at least one cropping season.
- Field evaluations for all traits planned in the start-up workshop have been completed. The field books have been processed and will be made open access in CIP's database for phenotypic data. Datasets used in upcoming scientific publications will also be made available in Dataverse.

The application of standardized evaluation protocols requires intentional exposure to the targeted stress and the use of local and introduced standard clones as controls to assess severity. All partners said that they appreciated the rigor of the protocols, which are prerequisite for accurate genetic and genomic analysis; however, few applied the recommended procedures. CIP project staff accommodated the different trial designs and data sets through data collection and collaborative data analysis to enable the multilocation assessment required for stability analysis, marker-trait association, and prediction models that make up work package 3. CIP staff in Peru, China, and Kenya will use institutional funds to follow up with all project partners to understand and address limitations to applying standard evaluation procedures following the project period. (The details of the phenotyping trials are given in Annex 1.)

1.3 Genotype the genetically diverse potato panel by GBS. *(Completed)*

In total, 380 tetraploid potato genotypes were subjected to genotyping by sequencing (GBS) to identify single nucleotide polymorphism (SNP) markers for trait-association studies and to develop the genomic-estimated breeding values (GEBV) of the potato genotypes comprising the TON panel when genotypic and phenotypic data are analyzed together. DNA extraction was done at CIP and genotyping was outsourced to Cornel University Institute for Biotechnology Genomic Diversity facility (www.biotech.cornell.edu/brc/genomic-diversity-facility). (Details regarding the marker identification are shown in Annex 1.)

1.4 Marker-trait associations: Associate genotype and phenotype data in GWAS. *(Completed)*

A bioinformatics workflow was setup to identify SNP markers with tetraploid allele dosage from the raw sequence reads generated by the GBS genotyping. Marker trait associations were modeled for all the traits separately, and with four different marker-effect models utilizing software specifically developed for polyploids. Large-effect quantitative trait loci (QTL) for LB and virus resistance were identified in genomic regions known to harbor resistance loci to these same diseases. Small-effect QTL were also identified for tuber bulking and drought tolerance. The number of association studies and QTL identified are summarized in the table below. (Further analysis details are given in Annex 1.)

Trait	No. of Tests	Total No. of QTL in Chromosomes
Bulking-based maturity	41	7 (chr0, chr1, chr3, chr5, chr9, chr11, chr12)

LB resistance	4	3 (chr0, chr5, chr9)
Virus resistance	5	7 (chr3, chr6, chr7, chr11)
Drought tolerance	6	3 (chr5, chr7, chr12)

Output 2: New tools and capacities to evaluate traits and link genotypes with phenotypes available and used by NARS of China and Ethiopia

2.1. Convene a workshop on modern genomics for crop improvement and phenotyping for NARS researchers. *(Completed)*

To bring researchers up to speed in the assessment of new traits and improve the efficiency of variety development, it is necessary to build capacity in the use and integrated analysis of modern phenotyping and genotyping tools and technologies that can help increase experimental accuracy and traits heritabilities and reduce the time needed to make decisions that lead to genetic gain. CIP and MPI-MP co-organized a workshop at MPI in Potsdam-Golm from November 15 –19, 2015. The workshop represented the start-up of the project and was critical to establishing a common understanding of the project's objectives and expectations. Five female and nine male participants from China and Ethiopia registered for the event. Five female and four male researchers conducted the teaching.

The workshop consisted of expert lectures, demos, and hands-on exercises that integrated computer, greenhouse, and laboratory work. The participants received hands-on training in the collection of phenotyping data, data management, sampling from biological experiments, genetic linkage analysis, and the design of field experiments. Plans for conducting the phenotyping trials were discussed and agreed. A full report of the workshop was presented in the project's first annual report.

2.2. Develop tutorials for the use of the online database hosting data from Output 3. *(Completed)*

User-friendly tutorials focusing on the explanation of protocols and online data collection, analysis, and visualization tools and database were made available at CIP's GTDMS (https://research.cip.cgiar.org/confluence/display/GDET4RT/Home).

Training materials to support the use of morphological markers identified by drought-tolerance phenotyping with laser and infra-red scanners at MPI-MP can only be developed once potential markers are validated, which is pending for 2019. The thesis proposal of Gedif Mulugeta includes the development of a tutorial for testing the method in breeding programs. This is expected to be completed during his PhD project.

Output 3: Next generation selection systems for directing and scaling out genetic gain defined with network of NARS and end-users

3.1 Compile stakeholder and expert knowledge and observations on local (subregional) potato productivity; crop rotation; and pest, soil, and water risk and management practices. *(Completed)*

A cropping calendar for potato was made to provide information on agro-ecological, geo-physical, and temporal characteristics and planting patterns of potato cropping, per country and agro-ecological zone, with a focus on Asia. It is modeled after the Food and Agriculture Organization's (FAO) cropping calendar that currently covers more than 100 crops located in 43 African countries. A template for data collection was designed for each Asian country and sent to potato specialists from CIP or partner organizations to collect expert information on potato-cropping systems and environments. By overlaying information on potato production and yield at subnational level and the farming systems' map, this tool can help in identifying

and targeting intervention regions. The information can also be analyzed quantitatively to query the database to see how much potato is grown per country-period combination or compare yields for the different cropping periods. The calendar has been taken as part of the institutional set of tools at CIP, and its development continues with other funding sources.

The Climate and Soil Similarity Tool (https://research.cip.cgiar.org/gtdms/similaritytool.html) connects statistically analogous locations in Peru, China, and Ethiopia using World Climate and soil data from FAO. It can be visualized as a map. Several important improvements were made by adding high-resolution data on different soil variables, World Wildlife Fund ecoregions, and several variables of climate data. The tool now has the possibility to connect with CIP's BioMart database to extract the localities of the field trials + agronomic data of interest. The new architecture allows the inclusion of other mathematical models to identify similar regions based on climate and soil. It can be used to predict varietal performance or develop recommendation domains for promising technologies or management practices. Connecting the phenotypic data with the Climate and Soil Similarity Tool further facilitates the understanding of genotype-by-environment interaction patterns.

3.2 Conduct gender-integrated, multistakeholder participatory varietal selection, and consumer preference studies in Ethiopia. *(Completed)*

A gender-mainstreamed participatory varietal selection (PVS) method was used to evaluate potato genotypes at Adet Agricultural Research Center experimental station in Amhara, Ethiopia. Fifteen potato genotypes new to the farmers were evaluated together with a widely grown farmers' variety. Thirty-two farmers (12 females, 20 males) participated in listing the important features they employ in choosing varieties to grow and market. The goals were to document the farmers' trait preferences and to identify potato genotypes with potential for variety release. Seven researchers attended as facilitators. Female and male farmers had different trait preferences, which also differed from those of the breeders. The results confirm that the perspectives of both sexes need to be integrated into breeding programs to ensure that new technologies do not disadvantage either one. For example, out of five important genotypes, the male and female farmers' preferences matched for only the top two selected by the breeders. And although both men and women were interested in productivity and market traits, women had additional requirements, particularly relating to processing.

3.3 Establish and compare predictive models for GEBV for their predictive accuracy in potato based on Output 1. *(Completed)*

Genomic selection offers the ability to select parents within a shorter interval and increase selection intensity by predicting the performance of untested genotypes. This study used the phenotypic and genotypic data reported in Output 1. CIP researchers developed a pipeline for calling SNPs considering marker dosage and applied it to identifying the GS models that best predict traits related to tuber yield, resistance to LB and viruses, and bulking-based maturity in tetraploid potato. They used the univariate genomic best linear unbiased predictor (G-BLUP) method to estimate predictive ability of three models that partition genetic effects into additive and non-additive types. Since not all genotypes were evaluated in all locations, they selected the location with the lowest missing data per trait for model training. A pseudo-diploidized model gave the best prediction abilities across traits, showing an outstanding advantage for resistance to potato virus Y (PVY). Predictive abilities were generally higher for LB and PVY resistance than for potato leafroll virus (PLRV) resistance, bulking rate, and tuber weight. (The strength and advantages of the three models and other considerations like number of markers and size of the training population are detailed in Annex 2.)

3.4 Apply multitrait selection index using data generated in work package 1. *(Partially completed)*

The predictive ability—and thus the progress that can be made in improving multiple traits by genomic selection under multivariate models—depends on the genetic and residual correlations among the traits considered and genetic correlations across environments. CIP used phenotypic data for 144 TON panel genotypes evaluated for four traits (of which one was assessed in 2 years) in three locations in Peru to develop a multitrait selection index.

They based the index on sum of the ranks following analysis of the correlations among BLUPs of individual traits from mixed models applied to each of the five traits: LB resistance in Oxapampa 2014; PVY and PLRV resistance in Lima 2016; and total tuber weight in Ica 2016 and 2017. They then used the sum of ranks index for multiple traits in genomic prediction under the pseudo-diploidized model developed in Activity 3.3, and compared its predictive ability to those of the individual traits. (The approach and results are detailed in Annex 2.)

The multitrait index had the least predictive ability with an average of 0.15 compared with 0.7 for PVY (highest among the individual traits) and 0.2 for total tuber weight in 2016 (lowest among traits). This is attributed to low genetic correlation among the traits and lack of positive genetic correlation among the environments in which they were measured. The results of this study illustrate the importance of the definition of target populations of environments (TPEs) for the success of multivariate predictive models. This activity anticipated the use of site characteristics to be documented under Activity 3.1 to define TPEs and the assignment of weights to the traits required for each of them. However, the tools developed in Activity 3.1 have not yet been applied to defining TPEs among those used by the project. Nor have weights been assigned to the traits required for each TPE. CIP will continue to refine site characterization data toward the definition of TPEs for potato variety development. It is committed to incorporating environmental data and national program priorities as well as gender preferences into product profiles. These profiles will complement emerging genomic data toward the development and application of multitrait selection indices in collaboration with the Excellence in Breeding Platform and the Gender in Breeding Initiative of the CGIAR. CIP and partners will seek additional collaborative projects for testing and implementation of new prediction and selection methods that utilize the phenotypic and genotypic data and models developed in the project.

3.5 Develop and apply performance prediction tools to support variety recommendation. *(Ongoing)*

CIP used GEBV in a univariate approach to carry out prediction for 29 traits grouped into bulking rate traits, disease traits, and tuber weight traits, using the phenotypic and genotypic data from Output 1. Genomic prediction was applied to predict the performance of the untested genotypes per location using the genotypes with phenotypic data for each trait in each location as a training set. Prediction used the pseudo-diploidized model based on the distribution of cross-validation iterations carried out in Activity 3.3. Predictive abilities reported are for cross-validation and prediction of missing genotypes within each respective trial. (Detailed results are presented in Annex 2.) Predictive abilities varied across trait type and environment, with the size of the training set (ranging from 58 to 334 genotypes) seeming to play the largest role in prediction accuracy. For the bulking traits (ATMW = average tuber marketable weight and AYP = average yield per plant) in three locations, prediction ability ranged from 0.25 to 0.53. Disease traits had the highest predictive ability, ranging from 0.57 to 0.73. Prediction accuracy varied with size of the training population (TP), but some differences were found even when TPs were not significantly different. This may be a reflection of the accuracy of phenotypic data, which can be influenced by disease pressure or other factors that could compromise the resistance data collected. Traits in the total tuber weight category had the lowest predictive ability across several locations. Predictive ability ranged from 0.12

to 0.56 for the traits and locations concerned. Yield is a more complex trait and environment is expected to play a more important role in affecting prediction accuracy. Therefore, proper definition of target environments in terms of genetic correlations and multivariate prediction across environment is expected to improve prediction accuracy for this type of trait.

The success of prediction based on the univariate analysis was tested for the case of bulking traits by calculating the selection differential (i.e., the difference in trait values between the mean of the training set and the mean of the 5% selected fraction based on GEBV). ATMW increased in the selected fraction by about 19.4 and 21.0 g in Holetta and Lima, AYP by 0.26 and 0.07 kg, respectively, in Kunming and Lima, and WMT increased by 0.28 and 1.2 kg in Kunming and Lima, respectively. There was no negative selection differential, indicating that progress can be made using GEBV as long as the factors affecting prediction accuracy are taken into account. Future multivariate analysis may further improve predictive ability and selection using genomic selection models combined with breeders' definitions of target populations of environments. This simulated selection exercise illustrates how GEBV can be used to select the best bet set of clones in a breeding program, but the elaboration of multivariate models along with definition of target populations of environments are pending before selections can be made to support the recommendation of best bet clones across environments. (See the detailed report in Annex 2.)

IDO Contribution

IDO 1 refers to improved productivity in roots, tubers and bananas (RTB) cropping systems. IDO 2 refers to increased and stable access to food commodities by rural and urban poor.

Direct beneficiaries (next-users) of the project's outputs are potato breeders in the key potato producing regions of China and Ethiopia and surrounding countries as well as local potato research institutions that will improve their breeding methods, materials, and efficiency to evaluate and release potato varieties. The ultimate beneficiaries (end-users) of this project's outputs and outcomes will be potato farmers in poor rural regions who will benefit by having access to resilient potato varieties.

CIP and the CGIAR Research Program on Roots, Tubers and Bananas (CRP–RTB) regularly conduct ex post and ex ante studies of potato area and yield in intervention countries and regions. They participate in systems-oriented CRPs' monitors combined cropping outputs and development and sustainability indicators. The project targeted 300,000 ha of cereal-based systems in China and nine other Asian countries with new next-generation potato-breeding capacities, methods, and materials from international collaboration. The introduction of agile potato varieties can still be expected to increase potato production by 12% and contribute to an estimated 8% increase in the total crop output of 205,000 poor households in China and 260,000 poor households in nine other Asian countries. Increases in the area productivity in at least six sub-Saharan Africa countries will be realized with 5% increments in potato productivity. Economic and environmental benefits are expected from productivity gains and less frequent seed replacement and pesticide use where these are currently significant requirements for potato production, once resilient potato varieties are adopted,

More than five NARS organizations (project partners) have accessed the tools and technologies from CIP and stand to benefit from research in drought-tolerance markers identified by MPI-MP or CIP in collaboration with partners. Smallholder farmers in Ethiopia (22 women, 23 men) took part in PVS trials that started to involve development partners in matching the demand of smallholders with the supply of new potato varieties. The project's strengthened breeding and networking capacities will require continued scientific exchange and communication as well as upstream and downstream links with academic institutions and development projects to realize impact.

New candidate varieties identified as resilient, productive, and adapted to local conditions of the selected environments of China and Ethiopia reached the stages of performance trials and will be incorporated into each national program's variety development scheme from seed production through farmers' evaluation. The success of NARS programs will be enhanced as a result of the large amount of new, improved genetic diversity introduced and used in population and variety development and capacities of the research teams.

Research Outputs

Output 1. Broad genetic diversity of advanced-bred lines genotyped and phenotyped in key environments. **Overall rating: 2.4**

Indicators	Rating and Comments
Phenotyping protocols refined and standardized for key traits by month 24	**Rating: 1** Improvements were made to four protocols as well as to data management pipelines, user interfaces, and structures enabling open access.
The diversity panel multiplied for seed tuber production in China and Ethiopia	**Rating: 2** Minituber and seed production is the greatest bottleneck in clonal evaluation and variety development. The project's strong advantage was the achievement of germplasm distribution prior to start-up using CIP's and partners' institutional funds, so that seed production could largely be accomplished in year 1. Strong programs (YAAS) with excellent facilities for tuber production contributed resources to producing starting material of the panel for other programs in China. Ethiopian partners struggled to produce seed sufficiently; thus their trials were conducted without the full complement of the panel.
Diversity panel phenotyped for 7 traits across sites by month 36	**Rating: 2** Nearly all trials planned were completed and data compiled. However, the standard protocols recommended to ensure comparability and optimize the utility of results in genetic analysis were generally not applied by the NARS partners.
GBS data of the diversity panel ready by month 24	**Rating: 1** This was generated without incident and available ahead of schedule.
Low-tech shoot phenotyping parameter identified from high-tech laser scanning data by month 24	**Rating: 2** Strong hypotheses were developed from successful experiments and the application of automatic phenotyping.
Field validation of new parameter on multiple sites by month 30	**Rating: 6** Validation script has not yet been done due to delays in the PhD research following a late start and illness. Thus extension of new methods to Ethiopian breeders will not be achieved during the project as planned.
Data evaluation pipeline defined and flow charts and commented evaluation scripts published online by month 24	**Rating: 3** Data analysis scripts will be made available in the scientific publications to follow the project period.

Output 2. New tools and capacities to evaluate traits and link genotypes with phenotypes available and used by NARS of China and Ethiopia. **Overall rating: 2**

Indicators	Rating and Comments
10 NARS researchers with updated capacity in current breeding approaches (trait evaluation, marker-assisted selection, data management and evaluation) during project year 1	**Rating: 1** More than 10 national researchers attended the workshop, which was of high quality and well-rated by participants.
Training of young researcher from target countries in automatic phenotyping and modern data management, leading to PhD thesis on validation of phenotypic parameter derived from automatic phenotyping in agro-environments	**Rating: 3** Training was strong in terms of practical research, workshop, and conference attendance as well as instruction on research and writing skills. But the PhD was not completed during the project life time due to a late start and illness. It will continue with institutional funds at MPI-MP.

Output 3. Next generation selection systems for directing and scaling out genetic gain defined with network of NARS and end-users. **Overall rating: 3.6**

Indicators	Rating and Comments
Key informant survey results of baseline data for risk assessment and sustainable productivity gains available by month 24	**Rating: 2** The cropping calendar was developed, but this was not as diligently applied to site characterization and documentation of conditions and constraints in the projects target environments as would have been ideal. Our intention was for additional surveys to be conducted, but these were not performed as they were not specifically budgeted in the project.
Seed for participatory trials available by month 12	**Rating: 2** This was done successfully by the Ethiopian partner. However, the clones were not from the TON panel as we had hoped they would be, due to the time required for identifying locally adapted material for use in farmer assessment.
Detailed report on multistakeholder PVS and consumer preference studies with gender integration	**Rating: 2** Report on PVS was provided on time, but no specific survey of consumer preferences was performed.
Site characterization data and interrelation with phenotypic data documented by month 30	**Rating: 4** Although data collection template (i.e., cropping calendar) was developed and applied for Asian countries and the climate similarity tool was improved to address Peru, China, and Ethiopia, these tools were not specifically applied to characterizing the experimental sites or the breeding targets addressed by the project.
Locally important traits ranked and weights assigned for breeding by end of year 3	**Rating: 5** The choice of trait phenotyping experiments made by each partner reflected local priorities, but the exercise of converting these to weighting factors that could be used in multitrait selection models was not achieved.
PhD thesis on predictive	**Rating: 5**

models for GEBV for potato ready by the end of the project (with complementary funds)	An additional PhD was not achieved since complementary funds were not identified. However, CIP project members and supporting statisticians used genotypic and phenotypic data of the project to develop and test prediction models and multitrait selection indices. In addition, the project coordinator HLK planned and is currently conducting a sabbatical study in the UK on bioinformatics to advance methods for performance prediction with GBS. An MSc thesis was completed on drought tolerance assessment of the diversity panel in Ethiopia.
Stability analyses of LB resistance published by month 3	**Rating: 3** The analysis has been conducted and a scientific paper is in preparation based on the work reported in Annex 1.
Cross-locational meta-analysis of virus resistance published by month 36	**Rating: 3** The analysis has been conducted and a scientific paper is under preparation based on the work reported in Annex 1.

Achievement of the Purpose

Purpose: Increase the capacity of NARS in the project's target regions to identify new trait diversity and/or superior potato genotypes and use new methods to release resilient potatoes to end-users in a reduced time frame. **Overall rating: 1.7**

Indicators	Rating and Comments
At least 3 resilient candidate varieties ready for regional trials of new variety registration in each country	**Rating: 2** With present procedures for potato variety assessment, at least 4 years are required to identify most promising materials and produce sufficient seed for regional variety testing, even when accelerated release schemes are applied. Each NARS partner has identified 7–40 promising clones that are being advanced in their selection schemes. This project was dedicated to accelerating procedures through prediction models that could enable successful recommendation of elite clones for direct variety testing in target environments. Its objectives were diversity enhancement, capacity building, and research toward new efficient breeding methods that may allow faster progress in variety identification.
30 new parental lines selected for crossing program of NARS	**Rating: 1** Achieved by mid-project; progenies already are developed and under selection by the partners.
Greater resistance and higher yields in reduced cropping season	**Rating: 2** Advantages of selected TON panel clones over predominant varieties were reported by each program.

Achievement of the Goal

Goal: To increase food security and income through sustainable intensification of cropping systems and value chains by increasing the availability and access to early-maturing, robust, and low-input potato varieties. **Rating: 2**

Indicators	Comments
At least 5 NARS organizations engage with CIP in an improved germplasm distribution and selection system	All partners and additional programs received TON panel clones and protocols. The 5 NARS partners built new capacities in evaluation and selection and are participating in research, analysis, and publication of results. Additional interaction is needed to agree on evaluation methods for each trait since standard protocols were rarely applied by NARS; to provide training on genome-wide association studies (GWAS) and prediction models; and to test and compare these under field conditions before we can say that next-generation breeding is in practice.

Gender Equity Aspects

Rating: 2

The team was prepared to stress the importance of a gender-integrated approach when engaging scientists, students, laborers, and end-users. This would help to ensure that the project reaches a gender-balanced participant and user pool, in an effort to promote gender integration and equity in decision-making, access to training opportunities, and ultimately, to benefit from the project's results. Specific project activities targeted 50% of women's participation. This was achieved for the participants of the Phenomics and Genomics workshop, in the engagement of researchers at all levels from leadership to technical, and in the PVS trial conducted in Ethiopia.

Activities of Output 3 intended to guide breeding decisions and strategies, included the activity to "compile stakeholder and expert knowledge and observations on local (subregional) potato productivity, crop rotation and pest, soil and water risk and management practices." This was realized through the development of a cropping calendar (Activity 3.1); in hindsight, however, it could have been an excellent opportunity to collect information on gender roles in potato production and enrich the description of target environments and market segments. Ideally, this activity might have been conducted in collaboration among breeders and social and other biophysical scientists.

3. Major Research Findings

This project constituted CIP's largest and most concerted international distribution of elite-bred potato materials to strategic locations and partner programs. Genomic data were generated and applied to improve understanding of the genetic structure and genomic constitution of elite breeding materials and trait sources. New experience and capacities were developed for genomic-assisted breeding under a new collaborative modality.

Main highlights from trait analysis include the identification of genotypes with stable LB resistance across all test locations, with resistance to PVY, PLRV, or PVS, and with early-bulking-based maturity. Main highlights from genomic analysis include the development of a set of SNP markers for CIP germplasm that take tetraploid allele dosage into account. Some of these markers will be selected for a mid-density molecular marker assay that is being developed in collaboration with the Excellence in Breeding platform. The marker set enabled the estimation of linkage disequilibrium and population structure in CIP-bred germplasm, which will support additional future marker-trait association studies and molecular breeding approaches. Specific markers associated with important traits were identified (see Annex 1). Main highlights from statistical approaches include the use of mixed models and incorporation of the row-column design into phenotypic data analysis. These enabled more accurate assessment of the performance of the genotypes in the different trials and thus improved the

precision of the marker trait associations and prediction models developed.

GEBV were incorporated into selection indices using univariate and multivariate models that enabled the prediction of performance of panel genotypes in and across environments. The most important factors contributing to accurate genomics-assisted prediction of individual and combined trait levels were illustrated for the case of simple and more complex traits addressed in potato breeding (see Annex 2).

Each partner program identified outstanding candidate varieties and/or trait sources for immediate incorporation into their breeding programs with the following types of highlights:

YAAS evaluated 336 panel clones for LB resistance and bulking-based maturity, and more than 200 clones for drought tolerance and virus resistance. It reported on the 5–10 top clones for each trait, prioritizing earliness in the maturity trial. Two clones were identified for variety (DUS) testing in Yunnan: CIP398180 and CIP397036.7. They selected 48 LB-resistant clones as parents for breeding and obtained 9,430 seeds from 10 crosses in 2017 and 21,266 seeds from 114 crosses in 2018. A crossing plan was implemented with 80 locally selected, top-performing TON panel clones in Yunnan during 2018.

Among the selections used in crosses to enrich their breeding populations, **HAAS** partners found CIP 393371.157 to be outstanding for its generation of seedlings with uniformly long, oval shape, yellow flesh, and yellow-skin tubers with shallow eyes. Attention to the project's evaluation protocols gave more precise results that are of great value to the breeding program. Some aspects of the drought phenotyping protocol were found inconvenient and in need of improvement. These involve the measurement of canopy cover, which is complicated by senescence, and the measurement of stolons when these emerge from the soil and become stems. Better attention may be needed to hilling of the crop in such cases. A minimum number or weight of tubers should be specified as requirement for determining starch or dry matter content, as the method proposed is not reliable on very small samples attainable from stressed or poorly adapted clones.

GAU managed to evaluate 330 panel clones for drought tolerance and maturity applying the project's standard protocols over two seasons. The many measurements required for drought tolerance phenotyping made costs for travel to field sites very high, and they are thus very interested in establishing efficient high throughput field phenotyping methods. While access to the diversity panel was highly appreciated, they expressed interest in contributing to basic research on drought-tolerance mechanisms. GAU carried out a more basic research project on drought tolerance of potato simultaneously with this one, for which the TON panel clones were used. Synthetic analysis across projects would be extremely valuable.

One trial at **EIAR** intended to assess drought tolerance had to be considered for yield and quality only due to inability to exclude rain from the drought treatments. Five early-maturing clones (CIP397036.7, CIP396285.1, CIP304405.42, CIP304371.58, and CIP304351.109) gave total tuber yields equal or higher than 'Belete', which yielded 50 t/ha. Likewise, 22 mid-maturing and 14 late-maturing clones yielded more than 50 t/ha. Production of sufficient seed for all of the planned trials was not possible due to the large number of clones in the panel. Sufficient seed could only be produced from 125 panel clones. When 75 were selected for LB resistance, only 57 of these could be advanced and preliminary yield trials due to insufficient quantitates of seed. Nineteen clones were selected for resistance to LB and will be advanced to farmer participatory trials. Among these CIP-304366.46, CIP-393077.159, CIP-393371.157, CIP-393077.54, and CIP-398190.605 yielded better than the standard variety.

4. Assessment of Research Findings

CIP's global mandate to develop and share breeding materials, practices, capacities, and information to improve the lives of poor male and female potato farmers and consumers has been strengthened by the collaborative mode of the project.

Five NARS programs contributed to the evaluation of an immense wealth of elite-bred potato germplasm, accessing standardized protocols and best practices for trait assessment that will help ensure favorable results in their potato breeding and research programs. Their capacities were further enhanced through collaboration on data analysis and training on genomic approaches and tools. As a result of the project, the partners are more prepared to take advantage of emerging technologies and tools that will help accelerate and deliver genetic gains. CIP is more aware of abilities and gaps that influence the transfer of technology required for impact. Resilient candidate potato varieties are in national variety development pipelines, and elite sources of needed traits have been incorporated into NARS potato populations for sustained improvement that will proceed with increased speed and precision. The German partner brought the potential of automatic phenotyping to bear on potato improvement and provided expert knowledge and experience to male and female researchers of China, Ethiopia, and Peru to help them and their downstream partners address climate change. Women played major roles in proposal development and research, training, and communication for project implementation and assessment on the part of MPI, CIP, and the NARs.

PVS coordinated by ARARI in Ethiopia, as well as first steps in variety testing in each project country, provided links with extension programs. Because of increased awareness and rigor regarding evaluation and selection, these and development projects in Ethiopia, Peru, and China will have earlier access to adapted and resilient potato varieties for extension to farmers and release authorities. Demo trials can be established with locally adapted, disease- and stress-resistant varieties identified in the project to illustrate economic and ecological advantages of these characteristics to policymakers, farmers, and students. Policymakers' awareness of national program researchers' needs for knowledge and facilities may translate into sound investments in scientific research and training in agriculture and, particularly, for women.

It is regrettable, however, that more varieties are always released than adopted by farmers. Thus the involvement of men and women farmers in seeing, assessing, and selecting the latest material from breeding programs is positive for breeders and farmers alike. Breeders gain perspective on the market segment they are addressing by which to add critical features to their selection criteria, and access relevant, lower input production environments before variety release. This helps to ensure stability and robust performance of new varieties under the variable conditions of end-users. Where allowed by national policy, PVS is an economical means to assess genotype-by-environment interaction and provide seed of new candidate varieties to poor farmers otherwise unlikely to access them after release.

5. Knowledge Sharing and Partnerships for Impact

5.1 Research institutes (IARC, NARS)

Seven NARS programs received most of the TON panel clones for trait evaluation and use in breeding, research, and variety development. The five partner programs were given access to protocols and the projects phenotypic and genotypic data, data analysis, and management pipelines. These partner programs also participated in the project workshop and hosted expert visits from CIP's trait specialists and project coordinator. Frequent contact was maintained by Skype and by several opportunities for CIP staff to visit partners, or for partners to meet and discuss progress in their respective national potato-breeding meetings.

The project achieved a fair gender balance in terms of technical staff involved in research and training. Recruitment of the PhD student originally identified a female candidate who was accepted for studies in Germany, although she later cancelled her plans and recruitment had to begin again. The German partner, also serving as PhD supervisor, and three of the six originally committed, internationally recruited CIP project scientists (including the project coordinator) were women. Five female and nine male participants from China and Ethiopia registered for the project workshop; teaching was done by five female and four male researchers. The Ethiopian PhD student is male. A female Chinese research associate supported the project from CIP–China and is now conducting her PhD at the Chinese Academy of Agricultural Sciences, which was one of the institutions outside of the project receiving the diversity panel. However, the main contact and principal scientist at each of the participating national programs is male. Outreach to potential beneficiaries through PVS in Ethiopia sought to include women farmers; one-third of the participants were women.

Follow-up is needed with each partner program. Tutorials should be adapted to accommodate the facilities and limitations that prohibited partner programs from applying CIP's standardized phenotyping protocols. Accurate phenotyping and characterization of stress conditions in test environments are needed to enhance understanding of complex traits like drought tolerance and crop duration, and are required for reliable genomic and genetic analysis and the development of prediction models.

Putative morphological markers for drought tolerance identified through automatic phenotyping by the German partner have a strong theoretical basis. These have been partially validated under experimental conditions but remain to be tested in target environments and extended to national program breeders. Delays in the PhD research necessitated a transfer of funds from the planned trials in Ethiopia, which should be conducted, and research and analysis are completed in 2019.

As a complement to the phenomics and genomics workshop held in year 1, the national program partners are anxious to begin to apply genomic and morphological markers and prediction models for next-generation breeding. This should be facilitated by a workshop in which final project results based on analyses that were conducted in the last year of the project can be detailed and discussed. When the PhD student returns to Ethiopia, he should present his thesis results to his own and additional national program institutions in order to share the knowledge gained. Project scientists are also committed to presenting results in scientific conference and literature.

The CRP–RTB and CIP are committed to next-generation breeding, including through the RTB clusters on this subject and by leading the RTB breeders' community of practice and the CGIAR Gender in Breeding Initiative.

5.2 Development partners like extension and training institutions, farmers, agribusiness, and policymakers

The majority of the project's investments in research and training targeted national program breeders, with farmers, consumers, and businesses being the ultimate beneficiaries. PVS coordinated by ARARI in Ethiopia provided the only direct link with extension programs. However, CIP's standardized protocol and tutorial on PVS, which was improved during the project, are accessible to and have been used by several development projects running in parallel with this current one. It enables training of trainers through practical exercises directed to rural populations and makes special efforts to integrate gender into the variety assessment and uptake process.

And though specific efforts were not made to reach policymakers or connect with development partners, there may be benefit to interacting with a larger range of institutions to raise

awareness of the importance of resilience traits in crop varieties, and even of new research methods such as genomics. Increased awareness by development project members and policymakers of the advantages of resilient or climate-proof varieties would contribute to their extension and use in production. Demo trials can be established with locally adapted, disease- and stress-resistant varieties identified in the project to illustrate economic and ecological advantages of these characteristics to policymakers, farmers, and students. Policymakers' awareness of national program researchers' needs for knowledge and facilities may translate into sound investments in scientific research and training in agriculture and, particularly, in plant breeding.

Broad communication of the project's outputs through avenues like donor fact sheets, newspapers, and press will be valuable for informing the public on new technologies. This will contribute to sustainable agricultural production, in turn influencing consumers' and farmers' choice of resilient seed and safe food and contributing to a clean environment.

The typical period of 15 or more years for new varieties to be released to farmers delays the impact of research on their livelihoods. Involvement of downstream partners in product development, combined with efficient breeding methods, should help create demand and result in better fitting varieties in reduced timeframes.

As several TON panel clones have been identified for variety testing in each partner program, these should be advanced to demo trials and PVS that expose female and male farmers as well as consumers to the new diversity. Information collected on trait preferences of women and men will be incorporated into breeders' selection schemes; the involvement of women in scaling-up of seed quantities should be emphasized. National program scientists should join development partners in demonstrating and communicating the positive contributions of resilient varieties to sustainable production, incomes, and food security.

6. Training

Individual young researchers from target countries were trained via thesis research on trait assessment, automatic phenotyping, and modern data management and through related capacity-building events.

Mr. Gedif Mulugeta from Ethiopia conducted PhD research in Germany on the identification and validation of phenotypic parameters derived from automatic phenotyping in agro-environments. His thesis is entitled, "Identification of phenotypic markers for the prediction of drought tolerance in potato *Solanum tuberosum* L.)," and he was supervised by Prof. Dr. Michael Lenhard, Potsdam University, and Dr. Karin Koehl, MPI-MP. His thesis research involved hands-on training in all aspects of expertise needed to conduct drought-tolerance trials, set up laser and infrared scanner phenotyping system, and carry out statistical analysis, including the development of a workflow for data integration and analysis. He attended conferences, workshops, and academic meeting presentations. Instruction was provided on abstract writing and poster presentation techniques. Mr. Mulugeta participated in a 1-day course on Good Scientific Practice at MPI, Plant Phenotyping Forum at Tartu, Estonia, and an image analysis workshop at Wageningen University Research Center. He was part of a scientific team of two women and one man (50% of the PhD students at MPI-MP are female). His university supervisor is male, his group leader female. The PAC consisted of two men and one woman. The supervisor of the IMPRS PhD school is female. *Completion is anticipated for 2019.*

ARARI reported that Zerihun Kebede submitted his thesis to the School of Plant Sciences, School of Graduate Studies, Haramaya University, in partial fulfillment of the requirements

for a MSc in agriculture (plant breeding). He completed and defended his thesis in 2018.

7. Lessons Learned

The research project was well planned, starting with proposal development and the logframe. The start-up workshop provided an opportunity for all partners to meet and become familiar with the project plan, in addition to its objective of building capacity and phenotypic and genotypic assessment for crop improvement.

For CIP major lessons learned concern the need for more careful attention to budgeting for travel and time for interaction among project partners. While the start-up workshop was successful for orientation and technical training, additional face-to-face meetings should have been planned and carried out to communicate the importance of, and understand the limitations to, the application of standard phenotyping protocols by each partner. Major aspects of CIP's collaboration were confirmation of the TON diversity panel and its provision to the partners, communication and back-stopping on standard phenotyping protocols, improvement of these and data management pipelines, provision of genotyping data, GBS pipeline development, analysis of trait variation by phenotyping and GWAS, and development of genomic and phenotypic selection models for single and multiple traits. CIP co-organized the workshop on Phenomic and Genomics for Crop Improvement. It provided two instructors and training materials in the form of software for phenotyping and experimental design, data collection and analysis, and data sets for the hands-on exercise on genetic mapping.

It was disappointing that despite clarity of protocols and support for their use, NARS partners used their own established designs and methods for trait assessment. This made comparative analysis challenging. Accurate phenotyping is required for reliable marker-trait associations. It is possible that the use of diverse and nonstandardized procedures for trait evaluation across the partner programs may result in weak or even false associations and prediction models. On the other hand, the provision of a large panel of elite germplasm representing the breadth of CIP's long-term breeding efforts is sure to enable phenotypic selection of trait donors for improvement of NARS breeding populations and resilient varieties for incorporation into the targeted cropping systems and environments. Successful identification of useful parents and clones by each partner program was due to the inclusion of elite materials expected from long-term collaboration to be adapted to the target environments, and bred for the traits prioritized in the project, and existing capacity of the NARS to grow out and select potato genotypes.

CIP collaborated on PVS in Ethiopia by providing time and expertise of a gender expert and an agronomist—both women. Data from PVS in Ethiopia were collected under the PVS module of CIP's GTDMS and contribute to a repository of information on preference traits of female and male farmers and consumers for new potato varieties. It was not possible to use newly introduced panel clones in the PVS trials. The large number of materials involved necessitated considerable investment in seed production and selection before a small number of the most promising candidate varieties could be identified.

8. Outlook Future Research and Development Pathway

Introduction of a large panel of advanced-bred potato clones from CIP to partners in China and Ethiopia, coordinated trait evaluation trials, and the development of new tools and approaches to assist breeding and enhance capacity of project partners to use the new trait sources and methods were proposed to help speed up the selection and release of early-maturing and disease-resistant potato varieties. As all partners were familiar with evaluation

for LB resistance, application of the standardized protocol required only minor interventions to ensure comparability of results across locations. However, in the case of drought tolerance, CIP's protocol proved to be too complicated for most program to apply due to recommendation for the measurement of many parameters. For this reason some programs conducted it only once during the project, or did not follow the standard protocol. Drought-tolerance phenotyping for potato is in its infancy. The complex procedures were intended to help define which component traits—physiological, morphological, or biochemical—contributed to drought tolerance and good performance under which type of drought scenarios and in which genetic backgrounds. In fact, the multilocation trials conducted in the project aimed at understanding drought tolerance by phenotyping, and a precise and efficient protocol for characterization was to be developed with the results. Instead, partners became bogged down with the measurements assigned. Moreover, they may not have understood that the purpose (like that of the German partner) was to improve understanding of this complex trait and, eventually, identify a subset of parameters associated with drought tolerance that would comprise an evaluation protocol for use in future trials and breeding programs. Such research objectives are difficult to address with large amounts of germplasm and across programs with varying capacities and conditions.

Likewise, the determination of crop duration across environments that influence phenology is complex, but better understanding from the integration of genetic, physiological, and environmental perspectives would inform breeding and germplasm exchange and variety recommendation schemes. The bulking-based maturity trial is intensive in seed requirements and data collection as three harvests are performed and tubers are classified by size to indicate plant development. A lecture and practical training in potato development, phenology, and maturity were given by CIP at YAAS, which contributed to better understanding of the protocol for its reliable application. However, not all programs benefited from this training, and few may have appreciated the purpose of the bulking-based maturity assessment, as related to adaptation and thus crop duration under a given set of conditions.

CIP's virus-resistance evaluation protocol is also complex, in that it requires three seasons of assessment with exposure to virus pressure (PVY or PLRV) as well as carryover of seed and assessment of both infection and impact on yield/degeneration. CIP's program in Peru and YAAS's in Yunnan applied the virus-resistance assessment protocol, which will enable validation of previous assessments and increased the precision of phenotypic data for the diversity panel.

Utility of the project results such as prediction models will require relevant and accurate descriptions of target populations of environments. The cropping calendar established the means for collecting data for environmental classification and was used to support actors in the potato sector in making decisions. However, to embrace the diversity of potato-cropping systems in Asia and, therefore improve targeting of development interventions, it is important that the next phase collect data according to smaller areas within the current farming systems. The development of this tool continues at CIP using other funding sources. Once in an advanced stage, the maps with potato-related cropping information per country and agro-ecological zone will be made available online on the FAO's calendar webpage. Synthetic analysis of environmental and systems data began in the context of the prediction models to support the development of recommendation domains for varieties or next-generation selection tools. The collaborative stability analysis CIP and YAAS conducted for LB resistance comes close to this objective and should be carried out for the remainder of the project's traits with the partners.

Prediction models remain to be validated in and across environments. The advantages, costs, and benefits of phenotypic versus genomic selection models should be communicated

with NARS. Promising clones selected by either approach still need to be tested with development partners in the interest of release and adoption. Short-term funding opportunities make continuity along impact pathways very challenging. Though this project may not qualify for it, the new opportunity for second-phase projects offered by GIZ is a welcome contribution to the need for longer term collaboration that is required to realize goals such as networks of NARS applying and benefiting from next-generation breeding approaches.

9. Summary

The project sought to increase the capacity of NARS in and beyond the project's target regions to identify new trait sources and methods to select and release early-maturing, resilient potato varieties to end-users in a shorter time. Automatic phenotyping, GWAS, genomic selection, and PVS were variously adapted and applied by five NARS programs in Ethiopia and China, CIP, and MPI–MP (which also hosted a PhD student from Ethiopia) in a highly coordinated network of inter-linked activities. These partnership arrangements were designed to facilitate an inter-connected series of research trials spanning the biophysical environments that challenge sustainable potato and systems productivity gains.

The three project outputs were achieved with the indicator-based rating fully in line with the expectations. CIP managed the project's finances and each annual report met donor expectations. For the project period, the project provided approximately one-fifth the operational resources available to the Genomics and Crop Improvement Program that is executed at CIP. The foundation of the project was a genetically diverse potato panel (TON panel), which assimilates 40 years of CIP's and partners' research in germplasm enhancement. The project built on CIP's long-term linkages with NARS in Africa and Asia to realize the project's three major outputs: (1) Panel of diverse, elite potato lines genotyped and phenotype for key traits; (2) New tools and capacities to evaluate traits and link genotypes with phenotypes available and used by NARS in China and Ethiopia; and (3) Next generation selection systems for directing and scaling out genetic gain defined with network of NARS and end-users. Phenotyping and genotyping of the TON panel was largely successful, with genotyping and necessary analytical pipelines being developed ahead of or on time (although NARS faced limitations in producing sufficient seed for inclusion of the entire panel in their field trials). Germplasm distribution was rapid and efficient, beginning in fact before the project was approved. But the unprecedented number of genotypes in the panel stretched the capacity of most partners for seed production. In some cases, the quality of phenotypic data suffered due to lack of experience or ability to carry out the recommended intentional exposure trials with limited interference from natural causes such as rainfall on a drought trial or uncontrolled disease. Remarkably, one partner (YAAS) with excellent facilities for potato research produced and provided seed tubers of the majority of the panel clones to other partners in China, enabling a rapid start on pivotal phenotyping activities. An additional challenge came from the interdependent nature of the types of data to be collected and analyzed on their own account and in combination with other types. The phenotypic and genotypic data generated in this project constitute a significant amount of extremely valuable information on the performance of CIP breeding products across different agroecologies generating much value in relation to the monetary investment. The application of the genomic selection developed in Output 3 was successful when applied for predicting trait values of missing genotypes in the project's field experiments. Yet it could not be applied to developing recommendations for genetic material across locations due to insufficient data on the characteristics of test and target environments. Related to this, the ranking and weighting of locally important traits that required the development of multitrait selection indices could not be achieved. In hindsight, along with greater interaction on the use of standard phenotyping

protocols and training on data analysis, the proposal should have contemplated more than one face-to-face meeting/workshop to maximize understanding of complex objectives and accomplish group tasks. CIP researchers also learned of the risk of insufficiently budgeting for all commitments, since their plan to include a second PhD student in the project with complementary funds could not be fulfilled as such funds could not be raised.

10. Publications, Papers, Reports, and Other Media

Already published conference presentations:

- Oral presentation by Hannele Lindqvist-Kreuze at the APA 2016–10th Triennial Conference (Ethiopia, ~50 attendance).
- Oral presentation by Karin Köhl and co-authors: Integrated Plant and Algal Phenomics Meeting (Prague, August 2018, ~100 attendance); EAPR/EUCARPIA Joint Meeting (Rostock, December 2018, ~100 attendance).
- Poster by Gedif Mulugeta Aneley (Integrated Plant and Algal Phenomics Meeting (Prague, August 20).
- Oral presentation by Xianping Li, Junhong Qin, Wei Jiang et al. Evaluation of potato resources introduced from International Potato Center in Yunnan. Proceedings of the China potato congress, Harbin map publishing house. 2018: 174–177 (in Chinese, ~100 attendance).
- Oral presentation by Hannele Lindqvist-Kreuze: Seminar at the Earlham Institute, UK, 2019 (~30 attendance).
- PhD advisory committee 1st report (submitted June 7, 2017)
- PhD advisory committee 2nd report (final version submitted May 11, 2018)

Planned publications:

Articles/journals:

- Aneley, G., Haas, M., and Köhl, K. Prediction of drought tolerance in potato from shoot phenotyping. To be submitted 2019/20 to *Functional Plant Biology.*
- Lindqvist-Kreuze, H., et al. Stability of late blight resistance and virus resistance and the identification of QTL in CIP breeding materials evaluated in Peru, China and Ethiopia. To be submitted 2019/20.

Conference presentation:

Oral presentation by Lindqvist-Kreuze, H. MPMI XVIII Congress, July 14–18, 2019, Glasgow, Scotland. “New potato germplasm for resistance breeding and variety release in China.”

PhD thesis: Aneley, G.

Other media:

Submission of manual marker assessment method to public repository after acceptance of manuscript.

Submission of original data to FAIR data repository.

Genome-Wide Association Mapping in a Tetraploid Potato Diversity Panel from the International Potato Center (CIP)

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Introduction

Although potato genetic resources comprise a polyploid series, most commercially cultivated potato varieties are tetraploid (2n=4x=48). The genome of modern potato varieties consists mostly of the *Solanum tuberosum* Group *tuberosum* with variable levels of introgressions from wild species and cultivated landrace groups. Tetraploid potato is a highly heterozygous, outcrossing autopolyploid, which complicates genetic analysis. Therefore, most early genetic mapping studies utilized bi-parental populations at the simpler, diploid level. (2n=2x=24) However, this approach did not permit the assessment of large gene pools or multi-allelic interactions that influence traits in polyploids. Significant progress has recently been made in the development of algorithms and software for genotype calling, linkage and QTL analysis in polyploid species. SNP arrays have been developed for potato: SolCAP (Hamilton et al., 2011) and the 20K SolSTW array (Vos et al., 2015). These were developed using North American and European potato germplasm, respectively, and are not necessarily the best options for genotyping CIP germplasm that contains more introgressions from the native South American genepool. According to our previous experience, less than 50% of the SNP on the 8K SolCAP array were informative in a test sample of CIP germplasm (Lindqvist-Kreuze et al., 2014). Genotyping by sequencing (GBS) has been applied to tetraploid potato (Uitdewilligen et al., 2013, Sverrisdottir et al., 2017); and variant calling from short read sequencing data considering allele dosage is now possible using several different tools, such as GATK, Freebayes, SAMtools to name a few (Clevenger et al., 2015). Together, these advances make genomic analysis of tetraploid potato more informative and applicable to evolutionary and breeding studies.

The genes affecting many agronomic traits in potato remain unknown, and the accurate molecular dissection of these in CIP-held and CIP-bred potato germplasm is our goal. Previous studies have identified genetic markers for several traits in bi-parental populations, but these are rarely transferrable to different germplasm sources and populations. To discover markers that are robust across a wider germplasm pool, or in a specific one, it is advisable to use association mapping (GWAS) and there are numerous examples of successful identification of trait linked QTL in tetraploid potato using this method (Sharma et al., 2019, Rosyara et al., 2016, Lindqvist-Kreuze et al., 2014 among others). The identification of QTL in autopolyploids is facilitated by a new tool called GWASpoly that considers allele dosage effects (Rosyara et al., 2016).

Here, we report the genotyping, estimation of linkage disequilibrium and population structure of a “diversity panel” comprised of 380 tetraploid genotypes from CIP’s breeding populations with GBS. Our objective was to identify QTL for bulking based maturity, late blight resistance, drought tolerance and virus resistance via GWAS.
A large number of traits were evaluated in a coordinated series of international field trials, and here we focus on selected trials and traits as an example. The data recorded in each experiment is available in CIP Biomart; and future plans include inviting students from NARS organizations to work on the data to learn about data management and analysis toward full exploitation of the project’s phenotypic and genotypic data sets.

Materials and methods

Germplasm

The germplasm included in this study consisted of the advanced tetraploid clones from seven different breeding populations of CIP as well as a group of old varieties with variable origins (Table 1.). The population A for late blight resistance was developed between 1980 and 1990. This population underwent three recombination cycles and approximately 300 resistant clones contained in 10 family groups from overall cycles were selected. Sources of late blight resistance were improved materials with *S. demissum*-derived resistance from breeding programs around the world, native Andean cultivars *S. tuberosum* groups *andigena*, *phureja* and *stenotomun*, wild species *S. acaule* and *S. bulbocastanum.* The population B3 genotypes were derived from the A population with emphasis on increasing frequencies and levels of horizontal resistance to late blight. The B1 population is derived from *S. tuberosum* group *andigena*. The LTVR population is characterized mainly for its resistance to the most important virus diseases (PVY, PVX and PLRV), earliness in short days, and adaptation to warm environments. B3-HT population combines late blight resistance from the B3 population and heat tolerance from North American and European bred varieties, and the LTVR population. B3-LTVR population contains hybrid genotypes originating from crosses between B3 and LTVR populations. Pre-Bred population has genotypes that have LB resistance introduced from wild *Solanum* species into the tetraploid background of B3 or LTVR. Varieties group consists of a group of potato varieties: Desiree, Atlantic, Spunta, Granola, Yungay, Tomasa Condemayta, DTO-33, Kufri Yoti. CIP numbers and the pedigrees of the 380 genotypes are given in the Supplementary Table S1.

Table 1. Summary of the TON panel genotypes originating from CIP breeding populations.

Breeding population	Genotypes evaluated	main traits
A	13	late blight resistance
B1	11	late blight resistance
B3	100	late blight resistance
B3-HT	37	late blight resistance, heat tolerance
B3-LTVR	25	late blight resistance, heat tolerance, virus resistance
LTVR	186	virus resistance, heat tolerance
PREBRED	2	late blight resistance
VARIETY	6	varied
Grand Total	380	

Field trials

A trait observation diversity panel consisting of 380 tetraploid potato clones from the breeding populations of the International Potato Center (CIP) were evaluated for bulking/ crop duration- and drought tolerance related traits, virus resistance and late blight resistance in a series of field trials in Peru, China and Ethiopia following the standard CIP protocols (Bonierbale, 2007). The experiment sites were located in variable agroecological areas in the sub-tropics, tropics and the temperate areas of Ethiopia, China and Peru (Table 2). The number of genotypes evaluated varied across locations, and different experimental designs were used (Table 3.). The data was collected using the field book system of the CIP Datacollector, which during the project was upgraded to HiDAP (https://research.cip.cgiar.org/gtdms/hidap/).

To evaluate bulking based maturity, the tubers were harvested sequentially with three harvest dates in each trial. As the trials took place in different environments with variable latitudes and temperature regimes, the harvesting dates varied in days after planting, but

were always three. At harvest, the tubers were divided into marketable and non-marketable classes, counted and weighed. From these number the variables WMT (weight of marketable tubers/plant (kg)), ATMW (Average marketable tuber weight (g)) and AYP (average yield/plant (kg)) were calculated for each harvest time and location.

Late blight resistance was evaluated under endemic disease pressure. The disease level in the plots was recorded at 7-day intervals until the susceptible controls were fully infected and these values were used to calculate the area under the disease progress curve (AUDPC) and relative AUDPC (rAUDPC).

Drought tolerance was evaluated under different treatments depending on the experiment. In Ica (Peru), the genotypes were first divided into early and late maturing groups. Three treatments were applied: normal irrigation, terminal drought and recovery. The drought trials in China only had the drought treatment, while in Ethiopia there were two treatments: normal irrigation and drought.

Virus resistance was evaluated by exposing the test genotypes to viruliferous aphids carrying PLRV and PVY, and planting virus infected potatoes as infector rows among the test plants. The infected tubers from first year were used as seed to plant the second years experiment and tubers from the second year were used as seed to plant the third years experiment. Between 14-20 plants in Lima, and six plants in Kunming of each genotype were collected from the third year's experiment for serological testing using ELISA test.

Table 2. Location of the trial sites and their main agroecological zone.

Country/Location		Agro-ecologies
Peru	Lima, La Molina 12.0820° S, 76.9282° W	Lowland sub-tropics
	Ica, Ica 14.0755° S, 75.7342° W	
	Pasco, Oxapampa 10.5853° S, 75.4053° W	Highland tropics
China	Yunnan, Kunming 24.8801° N, 102.8329° E Yunnan, Dehong 24.4334° N, 98.5849° E	Mixed agriculture systems, lowland & highland
	Heilongjian, Harbin 45.8038° N, 126.5350° E	Temperate (long day)
	Gansu, Lanzhou 36.0611° N, 103.8343° E	Dry land
Ethiopia	Amhara, Koga 11.2643° N, 37.4921° E Oromia, Holeta 9.0633° N, 38.4902° E	Mid, highland

Table 3. Description of the field trials that were undertaken under the GIZ funded project. The corresponding field books are available in CIP Biomart database.

Main trait	location, country, year	number of genotypes evaluated	Trial design
Bulking based maturity	Holeta, Ethiopia 2016	159	Augmented
	Gansu, China, 2016	330	RCBD
	Kunming, China, 2016	317	RCBD
	Heilongjiang, China, 2016	256	RCBD
	La Molina, Peru, 2016	89	RCBD

Late blight resistance	Kunming, China, 2016	336	RCBD
	Kunming, China, 2015	306	RCBD
	Holeta, Ethiopia 2016	60	RCBD
	Holeta, Ethiopia 2015	128	Augmented
Drought tolerance	Ica, Peru, 2016	269	Augmented
	Ica, Peru, 2017	258	Augmented
	Gansu, China, 2016	324	Augmented
	Heilongjian, China, 2016	316	Augmented
	Koga, Ethiopia, 2016	113	Augmented
Virus resistance	La Molina, Peru, 2016-2018	341	RCBD
	Dehong, China, 2016-2018	261	RCBD

Statistical analysis of phenotypic data

The best linear unbiased predictor (BLUP) and best linear unbiased estimator (BLUE) and values as well as ranked predictors for bulking based maturity and drought related traits were calculated using ASREML package. The earliness to tuberize was assessed from the average marketable tuber weight (ATMW (g)) variable from the mixed model for ranked BLUP predictions, averaging over the 3 harvest times as well as for each of the harvest times (t1, t2, t3) separately. Drought tolerance was only analysed from the Ica 2017 trial and considering the ranked BLUP prediction for the tuber yield/plot (kg). The earliness type and the different drought treatments were considered as factors.

The late blight resistance BLUEs based on the rAUDPC values were estimated with R lm4 package. The virus resistance levels were estimated based on the serological tests of the virus titres (PVY, PLRV and PVS) of 6-9 individual plants/genotype. The individual plants were considered infected with the virus when the virus titre was higher than that in the background (negative control). The proportion of infected plants per genotype three seasons after initial exposure was used as an estimate of the level of resistance.

Genotyping and variant calling

In total 380 potato clones were genotyped. Library construction and genotyping by GBS was outsourced to Cornell University. The DNA was digested with EcoT221 restriction enzyme and the libraries were 48x multiplexed for sequencing. The variants were called using GATK HaplotypeCaller option (Poplin et al., 2017), disabling the duplicate read filter (this is recommended for GBS data) and joint genotyping using the -ERC GVCF mode. From the vcf files different sets of SNP markers were filtered depending on the downstream analysis requirements. The filtering was done using bcftools for SNP sets for GWAS and LD estimation, while for population sub-structuring the filtering was done directly using SNPrelate package (Figure 1).

For population sub-structuring analysis, the biallelic SNP from the GATK pipeline were LD pruned, using the threshold of 0.16 and missing rate of 0.1. The population sub-structuring was analysed using the SNPrelate package, thus not considering the allele dosage information.

The measure of polymorphic information content (PIC) for each SNP was calculated according to Botstein *et al.* (1980).

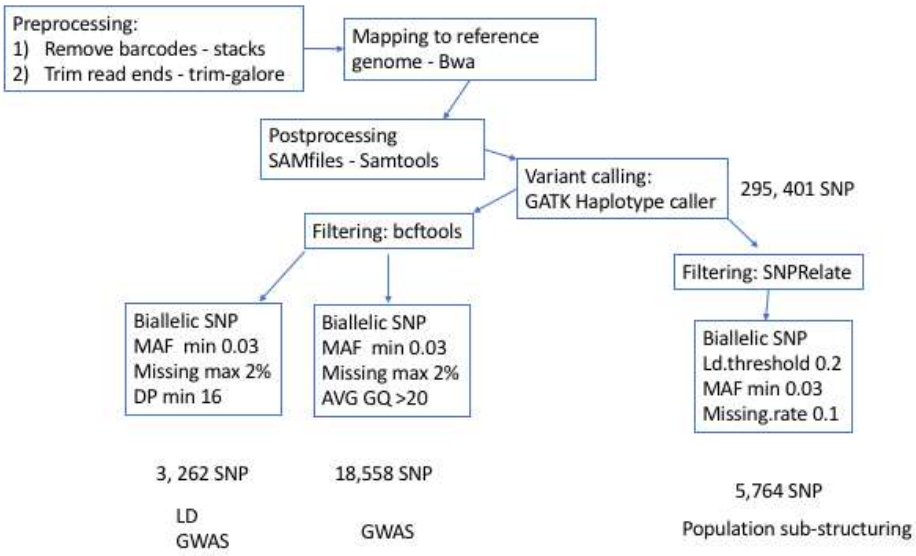

Figure 1. Workflow of the bioinformatics analysis starting from the raw sequencing reads (fastq files) and ending with the three SNP sets generated for the analysis of LD, GWAS and population sub-structuring.

Linkage disequilibrium

SNP markers (3262 high confidence SNP) were coded for the dosage of the alternative allele (0-4) and Pearson correlation coefficient (r^2) was calculated between marker-pairs. LD was calculated based on marker pairs located within the whole chromosomal region for all 12 chromosomes. Extent of LD decay was estimated by implementing Quantile regression (R package 'quantreg'; Koenker 2017) on the 90th percentile as recommended by Vos et al. (2017). From the fitted regression two estimators were obtained: 1) for LD $_{1/10,\ 90}$, where r^2 equals 0.1 on the 90th percentile and 2) for LD $_{1/2max,\ 90}$, where r^2 equals 0.5 on the 90th percentile.

GWAS

Marker trait associations were modelled for all the traits separately and using four different marker-effect models (general, additive, 1-dom and 2-dom) available in the GWASpoly package (Rosyara et al., 2016). Two different statistical models were used depending on the composition of the genotypes included in the study: 1) including the information on individual relatedness (K) and population structure (Q); 2) using only K-matrix. The eigenvectors of the first four principal components from the SNP relate package analysis were used to define the population sub-structuring (Q). Bonferroni correction (with genome-wide $\alpha = 0.05$) was used for establishing a p-value detection threshold for statistical significance. Missing genotypes were estimated with the population node.

Results and discussion

Population sub-structuring

There were in total 295,401 biallelic SNP after the GATK variant calling. After excluding monomorphic SNP and applying a 3% minor allele frequency and 10% missing rate there were 34,479 SNP. Filtering for LD threshold of 0.2 using the 500Kb sliding window, 5,764 markers were selected (Figure 1). The majority of the markers have minor allele frequency below 10% (Figure 2A). Polymorphic information content of the markers ranged from 2 to 50%, with the mean of 19% (Figure 2B). In the principal component analysis based on this set of markers, population B1 was clearly separated from the rest of the germplasm (Figure 3). The genetic background of the B1 population is *S. tuberosum* group *andigena*, while the rest are mostly group tuberosum type. B3-HT shares alleles with the B3 population, as expected since these clones are hybrids with B3 clones in their pedigrees. B3 and LTVR population clones are also mostly separated with a few exceptions of clones that may have been incorrectly assigned to the population groups. B3-LTVR, which is a hybrid between the two populations and this can be clearly seen in the PCA plot as well. Not surprisingly, Population A is intermingled within population B3 since the ancestors of the B3 clones were bred from selected clones of the A population.

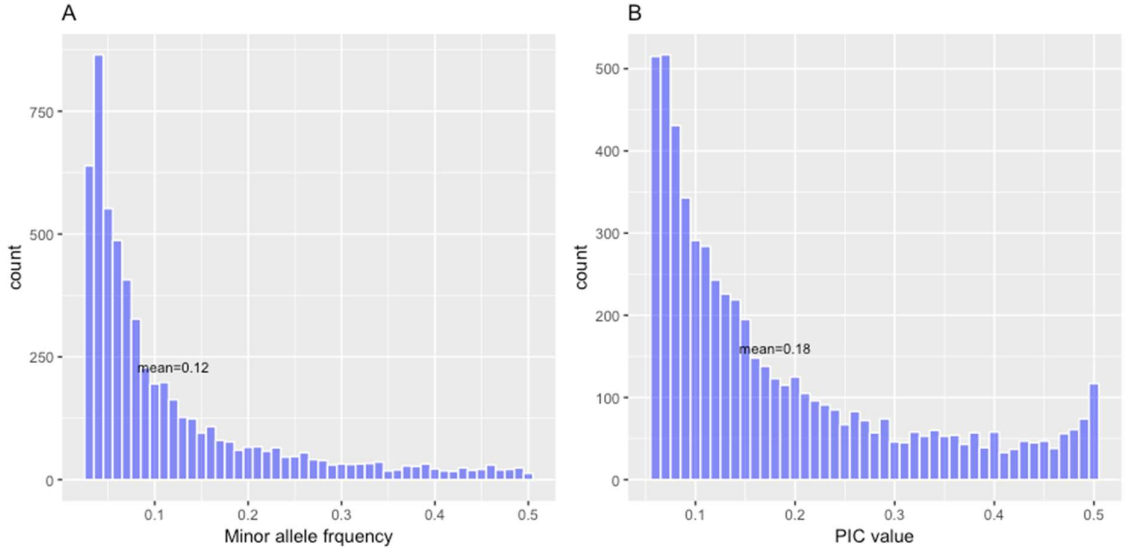

Figure 2. Minor allele frequency (MAF) (A) and polymorphic information content (PIC) (B) distribution of 5,764 SNP in 380 tetraploid potato genotypes called without the information on heterozygous allele dosage.

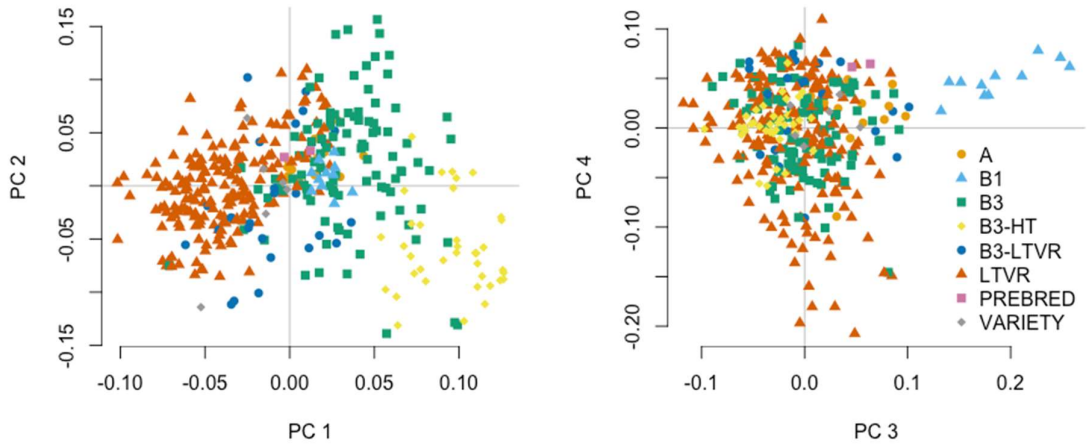

Figure 3. Population sub-structuring based on 5,764 LD pruned polymorphic bi-allelic SNP with minor allele frequency >0.03 and missing rate <10%.

SNP sets for LD estimation and GWAS

To obtain a set of biallelic SNP markers with reasonably accurate allele dosage for tetraploid genotypes only the SNP with the minimum site depth of 16 reads in each sample was utilized. In addition, minor allele frequency cut-off was 3% and maximum number of missing genotypes was 9 for each marker. The stringent filtering reduced the SNP number to 3,262 (Figure 1). The distribution of the minor allele frequencies of the SNP (Figure 3A) is similar to that of the SolSTW in the European germplasm described by Vos et al., (2015). Polymorphic information content (PIC) among the 3,262 markers ranged from 6% to 50% with the average of 22.4% (Figure 4B). This is somewhat lower than those reported by Sharma et al (2018) and Stitch et al., (2013) for the SNP from the SolCAP potato array. The SNP set of 3,262 markers was used to estimate the LD and in the GWAS analysis to identify trait linked QTL.

To increase the number of markers for the GWAS, another biallelic marker set was generated using the same MAF and missingness filters, and the average genotype call quality (GQ) above 20 (Figure 1). This means that the average accuracy of all genotypes called for that SNP has to be above the threshold of 0.01(thus there is the chance of 1% that the genotype call is not correct).

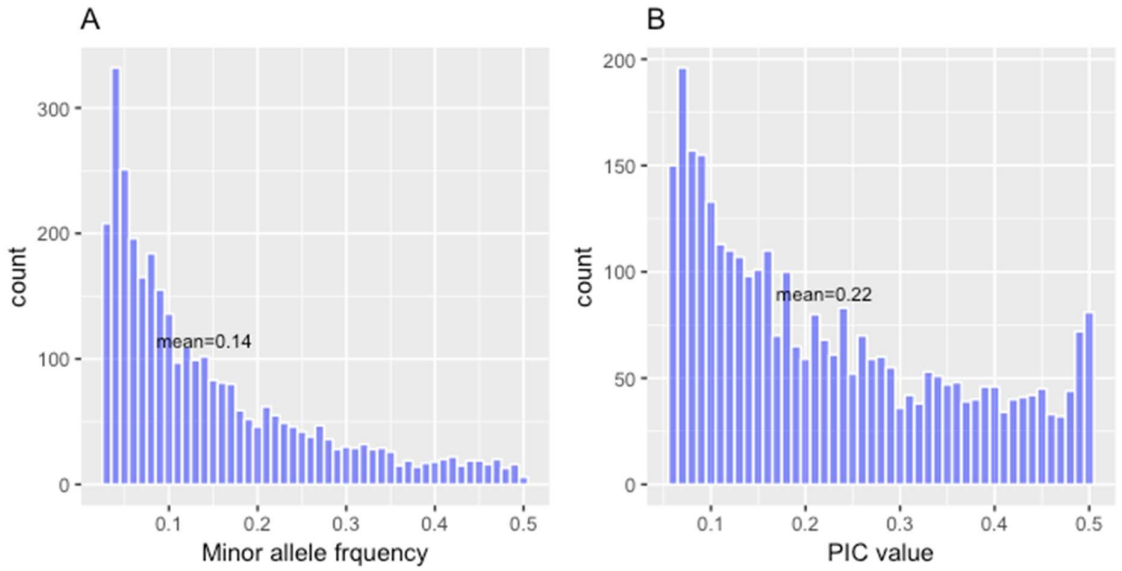

Figure 4. Distribution of the 3,262 SNP markers with tetraploid allele dosage information based on minor allele frequency (A) and the polymorphic information content (PIC) (B).

Linkage disequilibrium

LD decay was estimated using the set of 3,262 SNP markers described above. Spline was fitted on the 90th percentile of the r^2 and the distance between the pairs of the markers on the short distance (Figure 5A) and long distance (Figure 5B) over all chromosomes. From the fitted spline, the intersection of the significance threshold r^2=0.1, different estimates are obtained for the short distance vs long distance LD decay. On the short distance the threshold is reached at 2Mb, while on the long distance it is reached at 5.5Mb. Considering the short distance LD-decay estimate of $r^2_{1/2max,\ 90}$ that was suggested as the most consistent estimator for LD decay in potato by Vos et al (2017), we obtain the value of 0.55 Mb. This value is like that in the Vos (2017) data for the recent European potato varieties (0.6 Mb) and a bit lower compared to the study of Sharma et al., (2018) where the value was 0.91 Mb. The average r^2 for the short distance in our dataset was 0.091, which is a bit lower than that (0.19-0.22) reported for the European varieties (Vos et al., 2017), indicating that there are probably more founder haplotypes in the CIP diversity panel as compared to the European pool of varieties.

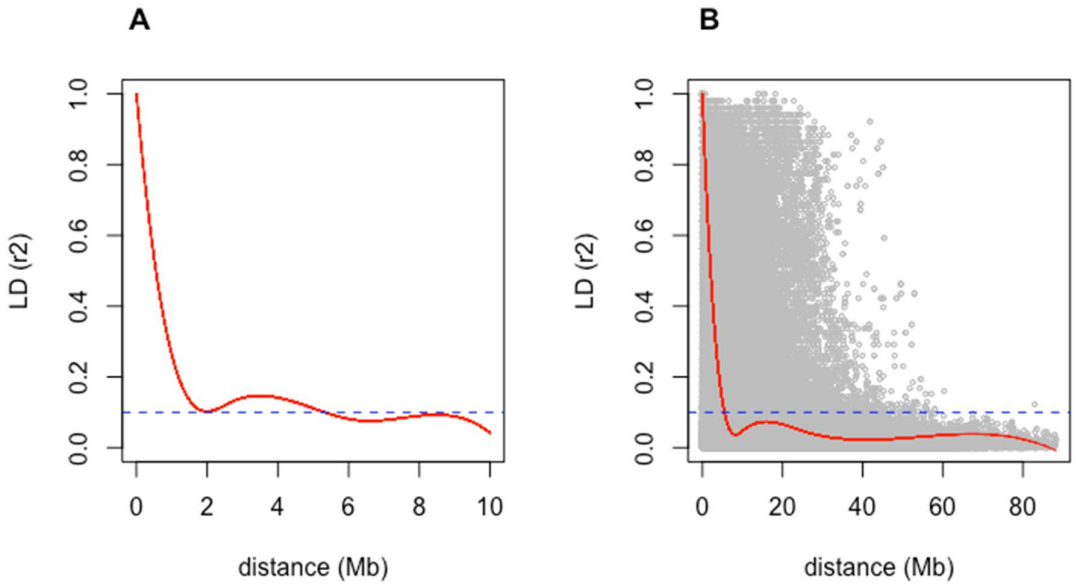

Figure 5. Linkage disequilibrium (LD) estimated in the TON panel based on Pearson correlation coefficient (r^2) plotted against the physical map distance (Mb) between pairs of SNPs in each of the 12 chromosomes. The red line depicts the trend line of the nonlinear quantile regression of r^2 (90th percentile) on short distance (A) and on long distance (B). LD decay threshold (r^2 = 0.1) is indicated by a dashed blue line.

Phenotypes

Bulking based maturity

The most important factor determining the yield of the potato crop is the tuber bulking rate. Bulking rate is the slope of the linear curve described by the increase in tuber weight with time. Information on bulking rate is valuable for assessing performance and adaptation of genotypes particularly in areas with short growing seasons. In these experiments, TON panel clones were evaluated for yield components, such as number and weight of tubers in different size classes in yield trials with three harvest dates: early, intermediate, and late (Table 4). The histograms of the distribution of the ranked BLUB predictions of the average marketable tuber weight (ATMW) (g) of the genotypes in each environment are shown in Figure 6. Based on the ranked BLUP predictions averaging over the three harvest times, ten genotypes showing an early-bulking pattern in each of the four different locations were identified. The average tuber weight of these ten genotypes ranged from 126.79 to 208.65 g across the harvest times (Table 5). The marketable tubers of these outstanding genotypes were smaller at the first harvest in all experiments but still over 90 g, indicating that they had reached a marketable size at the early harvest stage, and can be considered early bulking genotypes in their respective environments.

Genotypes shared among all four trials were analysed for the AMTW ranked BLUP variable in principal component analysis to identify trends among the different locations. Overall genotypes had more similar marketable tuber weight in Lima, Kunming and Heilongjiang as compared to Holeta. From this comparison four genotypes, CIP304369.22 (LTVR population), CIP392633.64 (B3 population), CIP395448.1 (LTVR population) and CIP398208.670 (B3-HT population) emerge as universally early bulking genotypes (Figure 7).

Table 4. The harvest dates expressed as days after planting in the different field trials.

location, country, year	number of genotypes evaluated	Harvest dates (days after planting)
Holeta, Ethiopia 2016	159	80, 100, 120
Kunming, China, 2016	317	100, 121, 141
Heilongjiang, China, 2016	256	80, 100, 120
Lima, Peru, 2016	89	90, 120, 140

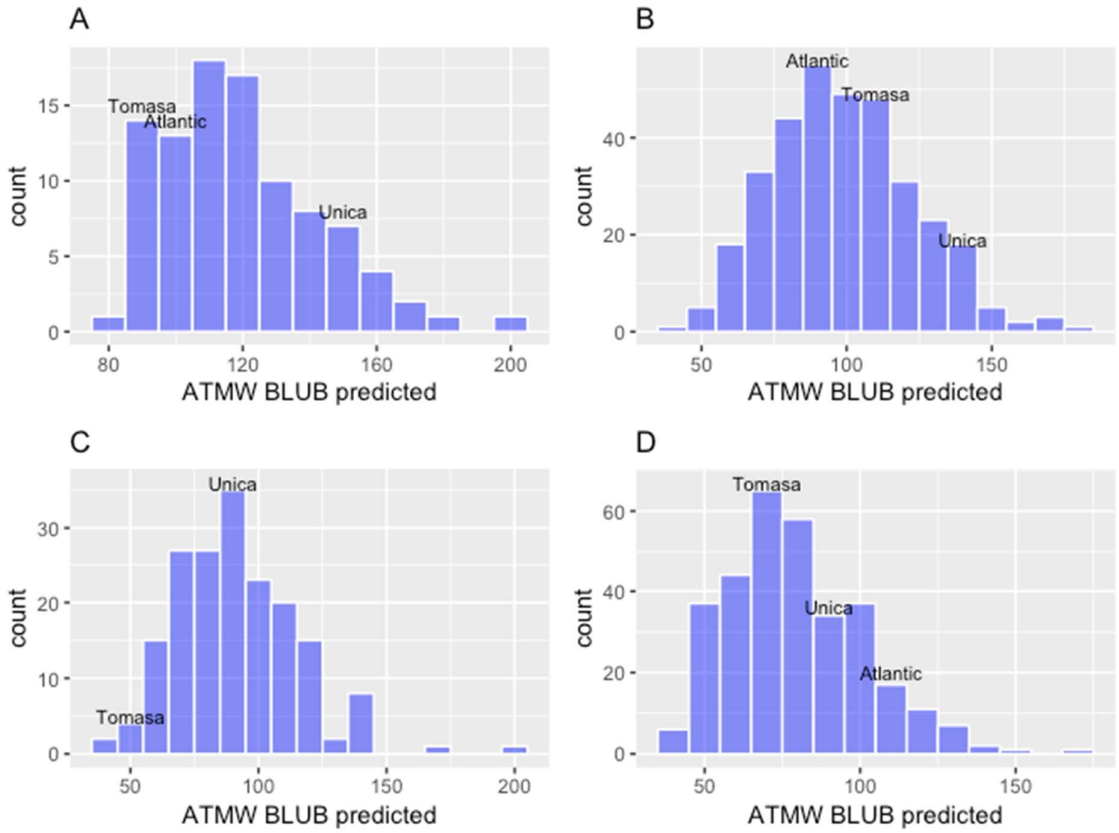

Figure 6. Histogram of the distribution of the ranked BLUB predictions of the average marketable tuber weight (ATMW) (g) of the TON panel clones across the three harvest times in Lima (A), Kunming (B), Holeta (C) and Heilongjiang (D).

Table 5. The average weight of marketable tuber (ATMW) (g) averaged over the three harvest times and at the three different harvest times of ten early-bulking genotypes identified in each of the four trials.

Trial site	Genotype	Ranked BLUP predictions	pred.HT1	pred.HT2	pred.HT3
Lima	CIP309028.32	208.65	179.25	198.27	217.56
	CIP392633.64	187.47	162.22	178.68	194.84
	CIP309064.76	180.32	157.03	172.51	187.77
	CIP309024.1	179.74	153.72	168.65	182.78
	CIP393371.157	167.10	146.79	160.21	173.29
	CIP309074.123	162.53	143.27	156.24	168.52
	CIP398208.620	162.20	145.81	159.18	172.58
	CIP398180.612	160.16	136.51	148.91	159.56
	CIP389746.2	159.35	140.63	153.62	165.54
	CIP304351.109	158.09	142.57	155.93	168.95
Kunming	CIP393073.179	195.90	133.94	205.38	215.26
	CIP393371.58	178.98	105.47	191.17	218.79
	Yunshu_No.401	177.31	129.20	190.62	195.34
	CIP398098.231	174.33	129.29	185.18	187.21
	CIP398180.144	166.21	101.29	175.32	198.29
	CIP387164.4	164.89	130.85	172.74	166.73
	CIP398180.253	155.09	117.23	165.77	167.76
	CIP398180.289	154.06	113.06	161.06	165.10
	CIP397036.7	153.23	124.81	160.12	152.60
	CIP398208.670	150.98	103.00	159.44	171.63
Holeta	CIP396268.9	196.13	154.45	198.87	233.23
	CIP304351.5	168.45	131.30	171.48	201.11
	CIP396269.14	145.03	111.81	148.15	174.39
	CIP391046.14	144.96	111.74	148.04	174.14
	CIP396272.2	144.06	110.98	147.18	173.06
	CIP391065.81	140.59	108.11	143.82	169.35
	CIP302499.24	138.29	106.21	141.19	166.34
	CIP391207.2	136.41	104.61	139.61	164.41
	CIP391002.6	135.43	103.81	138.38	163.00
	CIP304383.41	126.79	96.56	129.91	153.01
Heilongjiang	Kexin_No.23	174.52	117.21	161.33	222.98
	CIP396180.22	154.89	110.45	143.07	201.63
	CIP397036.7	144.45	104.50	136.05	179.64
	CIP394223.19	142.88	102.34	132.17	187.82
	CIP394579.36	136.84	102.96	128.68	172.46
	CIP390478.9	135.74	98.83	129.93	162.43
	Zhongshu_No.18	130.28	96.48	119.94	169.70
	CIP398098.65	129.48	92.60	121.65	165.92
	Zaodabai	129.46	98.32	121.06	167.68
	CIP395186.6	128.18	92.90	119.93	166.47

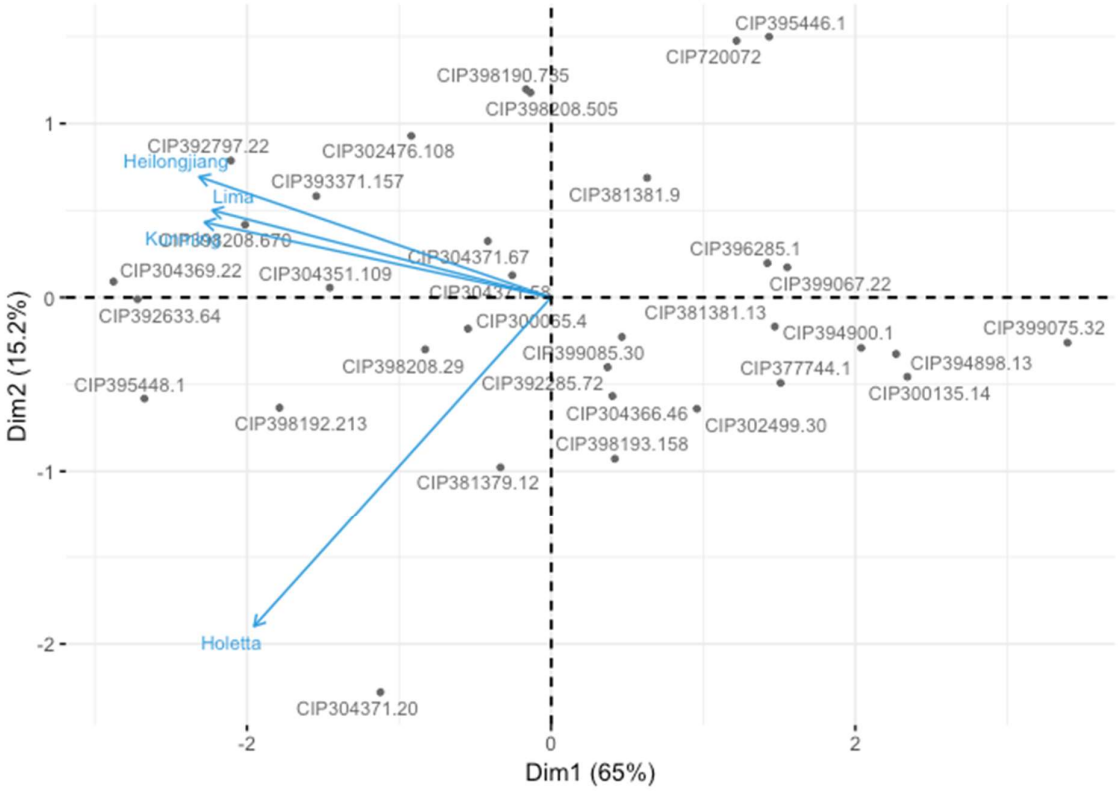

Figure 7. Principal component bi-plot of the genotypes shared in the trials in Heilonjiang, Kunming, Lima and Holeta based on the predicted BLUB AMWT (g) averaged over the three harvest time's.

Late blight resistance

Late blight resistance was evaluated in field trials with high endemic disease pressure. From the weekly observations of the disease incidence in the plots, the AUDPC was calculated and the estimated means (BLUEs) were transformed to the relative AUDPC (rAUDPC) to facilitate the comparisons among the different locations. There was a high frequency of genotypes with rAUDPC values comparable to the resistant control genotype which in Peru is released as a variety called Chucmarina and Ethiopia as Belete (Figure 8). Notably in China most of the genotypes tested were more resistant than the local variety C-88 that has been popular because of its good late blight resistance. To visualize similarities among the environments, the 58 shared genotypes rAUDPC values were analysed by PCA, and genotypes with similar level of resistance were identified. Genotypes CIP393227.66, CIP396043.226, CIP395077.12, CIP392634.52, CIP396023.109, CIP392634.49 and CIP395169.17, all from the B3 population are among the most resistant in all four environments (Figure 9).

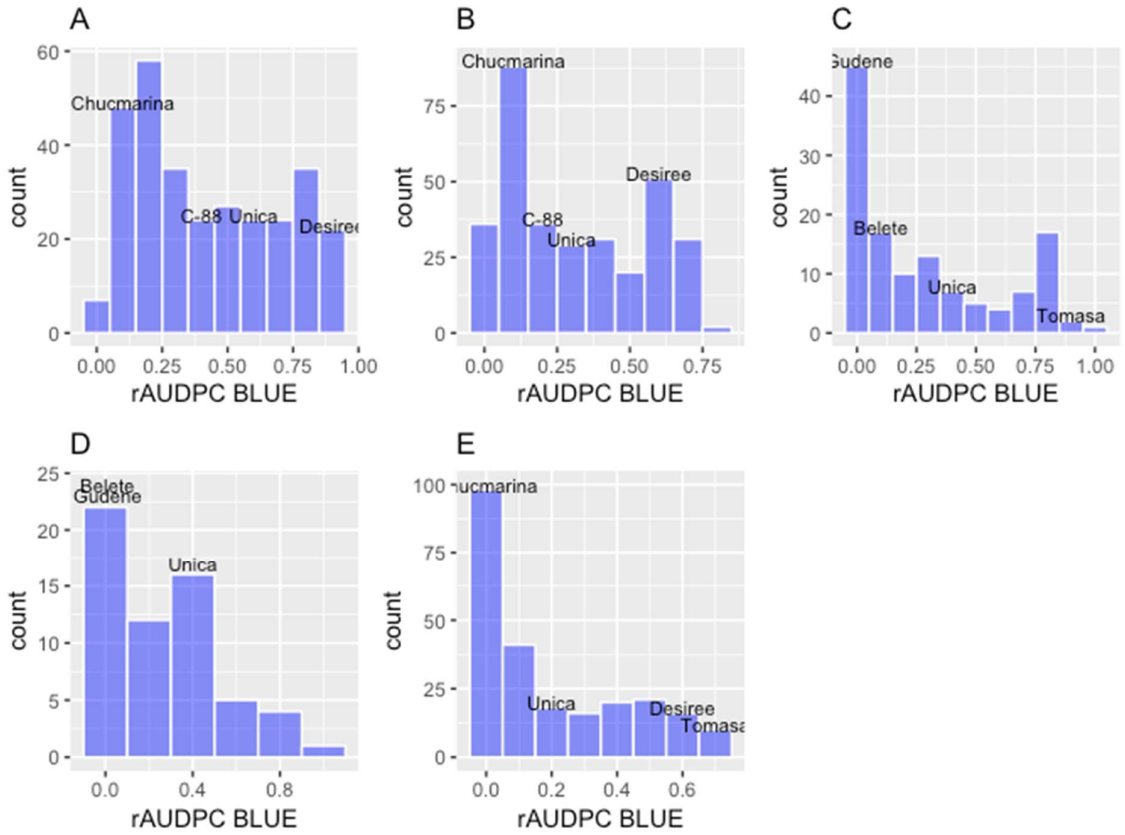

Figure 8. Histograms of rAUDPC values in Kunming, China in 2015(A) and in 2016 (B), Holeta, Ethiopia in 2016 (C) and 2017 (D), and Oxapampa, Peru at 2014 (E). The control genotypes are indicated in the plots based on their rAUDPC value in each trial.

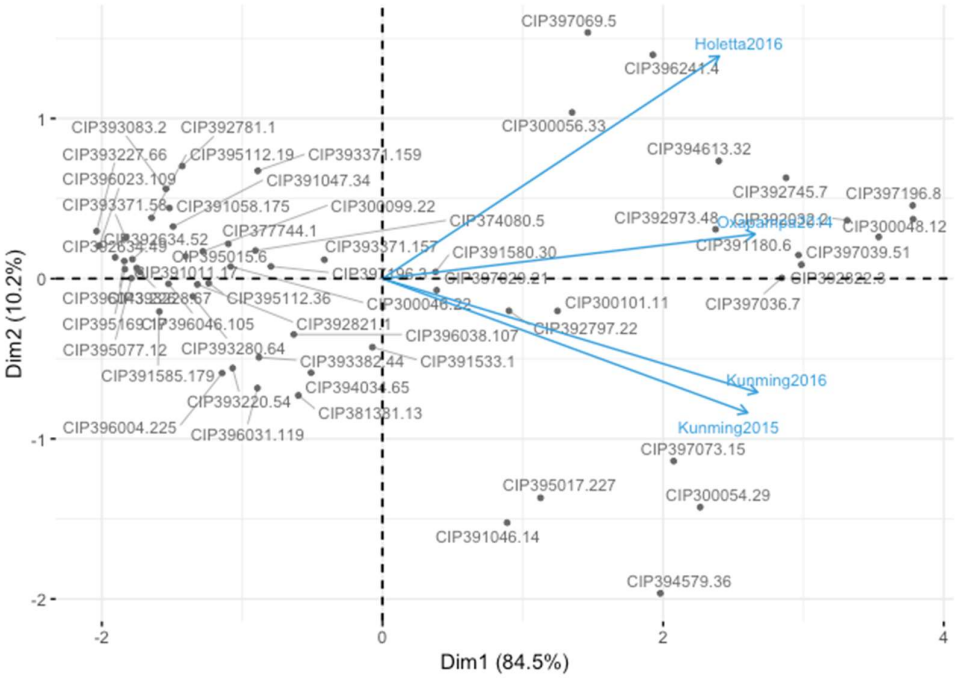

Figure 9. Principal component analysis of the shared genotypes evaluated in the trials of Kunming 2015 and 2016, Oxapampa 2014 and Holeta 2016.

Drought tolerance

Drought tolerance was evaluated in a trial in Ica, Peru in 2016 and 2017 under three treatments: normal irrigation, recovery and terminal drought, and various traits were recorded. Here, we focus on the predicted BLUB of the fresh tuber yield/plant as a proxy to estimate the level of drought tolerance among the genotypes tested. As shown in Figure 10, the yield distributions under the three treatments look very different in the two experiments suggesting a significant genotype x environment interaction. This is confirmed by the PCA, where the treatments within each year are more similar to each other than the same treatments across the years (Figure 11). Although the highest yielding genotypes in both years are of the early maturing type, the maturity type does not seem to have a significant effect on the yield overall. Ten most tolerant genotypes identified based on the PCA belong to both maturity types (Table 6).

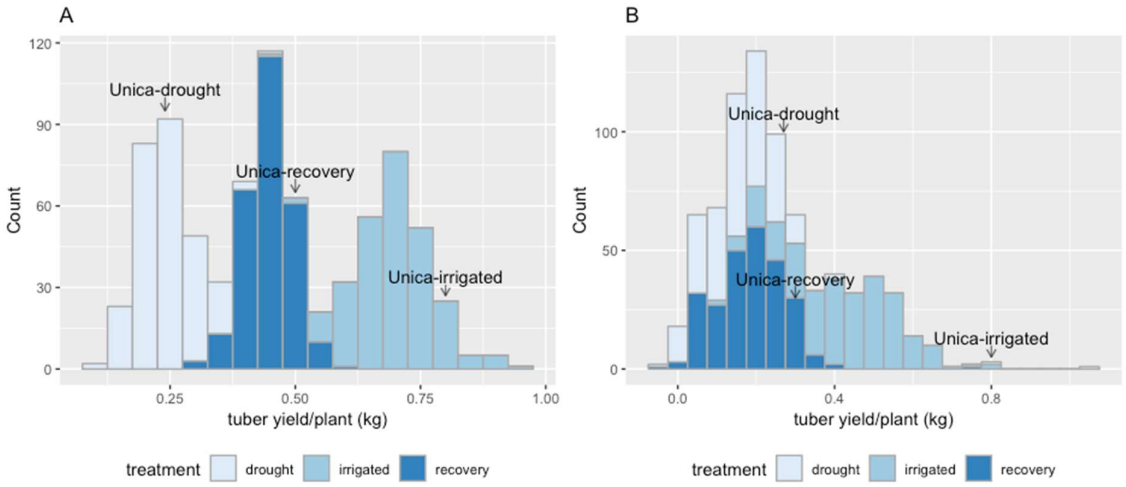

Figure 10. Histograms illustrating the tuber yield/plant (kg) of the potato genotypes under normal irrigation, recovery and drought treatment in Ica in 2016 (A) and in 2017 (B). The yield of a reference genotype Unica is shown above the corresponding category at each treatment.

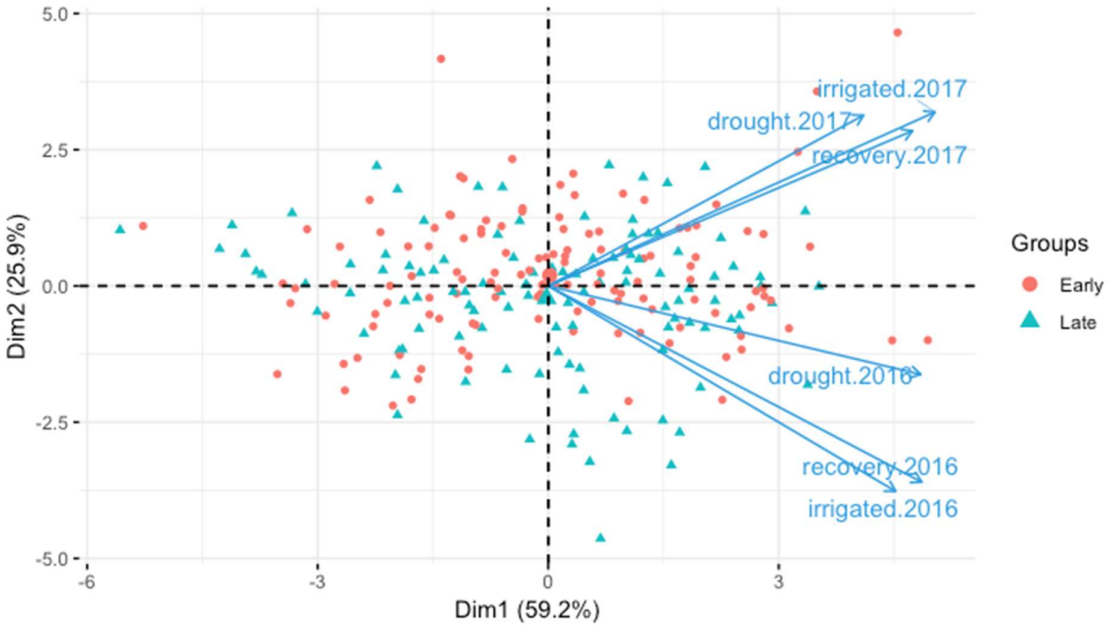

Figure 11. Principal component analysis (PCA) on the three different treatments: irrigated, recovery and drought, of the genotypes that were shared in Ica 2016 and 2017 trials. The genotypes have been coloured based on their maturity type (early <100 days, or late>100 days).

Table 6. Tuber yield/plant (kg) of the 10 genotypes least affected by drought treatments in the experiments in Ica, Peru 2016 and 2017 identified based on the principal component analysis.

	2017			2016			
	irrigated	recovery	drought	irrigated	recovery	drought	Maturity type
CIP389746.2	0.658	0.352	0.216	0.791	0.515	0.340	Late
CIP393371.159	0.541	0.303	0.244	0.773	0.507	0.350	Late
CIP397014.2	0.574	0.290	0.275	0.812	0.518	0.289	Early
CIP304371.58	0.566	0.300	0.257	0.794	0.511	0.304	Early
CIP397079.6	0.586	0.324	0.217	0.776	0.503	0.317	Early
CIP309024.1	0.585	0.281	0.240	0.740	0.491	0.365	Late
CIP381379.12	0.606	0.297	0.248	0.762	0.496	0.323	Late
CIP397029.21	0.573	0.313	0.229	0.781	0.504	0.307	Early
CIP302499.30	0.526	0.289	0.226	0.766	0.502	0.346	Early
CIP393371.157	0.531	0.250	0.219	0.757	0.500	0.360	Late

Virus resistance

A large proportion of the genotypes evaluated were resistant to PVY and PLRV in Lima (Figure 12A) as well as in Yunnan (Figure 12B). Most of the genotypes with high levels of PVY and PLRV resistance come from the LTVR population and there is a large number of genotypes that show high resistance to both viruses (Figure 13). The reactions of the

genotypes to both viruses are highly correlated between the environments suggesting that the resistances are functional in both environments. The top 20 genotypes that are highly resistant to PVY and PLRV in both environments can be seen in figure 12 in the two squares at bottom left. Those genotypes are also listed in Table 7. As expected these are mostly from the LTVR population, but there are two genotypes from the B3 population and three from the LTVR-B3 population. The resistant check Costanera is among these most resistant genotypes. Interestingly, 75 clones were also reported as resistant to PVS in China although PVS is not among the breeding objectives of CIP. This is an interesting preliminary finding that merits more research.

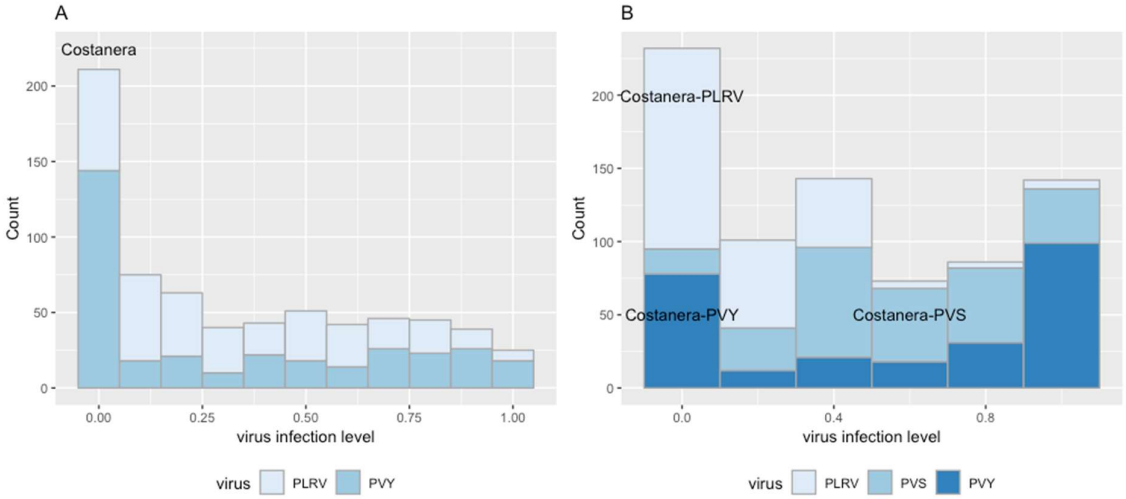

Figure 12. Histograms illustrating the infection levels of PLRV and PVY in the potato genotypes in Lima (A) and of PLRV, PVS and PVY in China (B). The infection level of the control genotype Costanera is indicated in each trial.

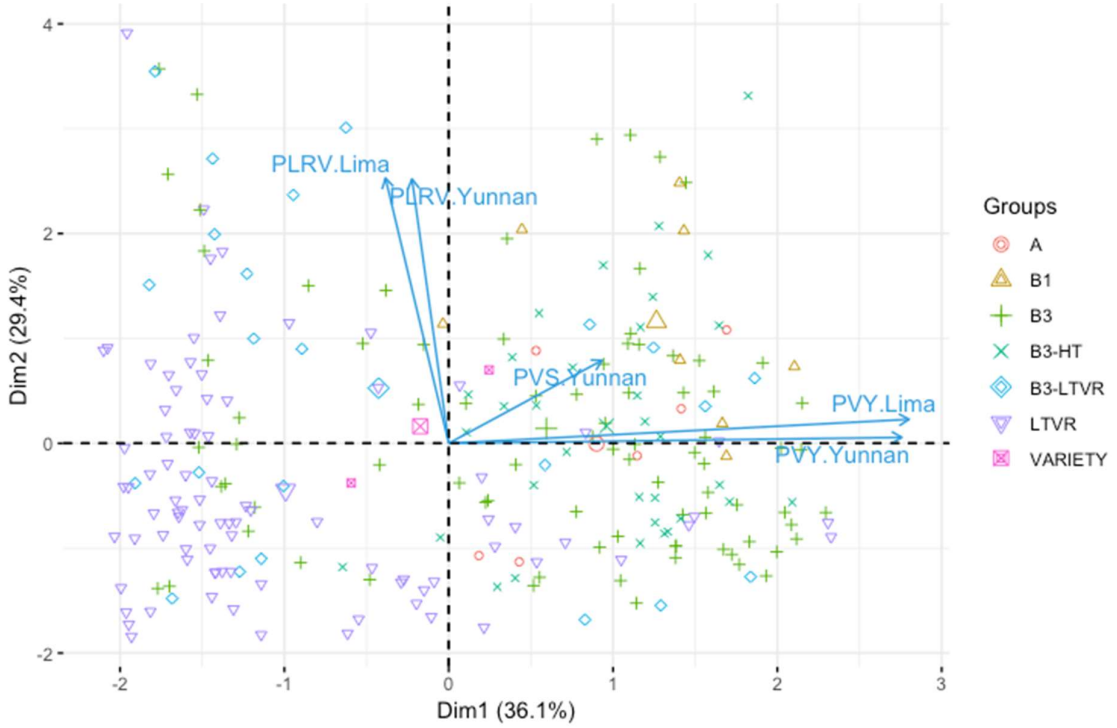

Figure 13. Principal component analysis (PCA) on the virus resistance of the genotypes that were shared in Lima and Yunnan trials. The genotypes are coded based on the breeding population that they belong to.

Table 7. Twenty genotypes with the highest levels of PVY and PLRV resistance, expressed as % of infected plants in Lima and Yunnan.

	Lima		Yunnan			
	PVY	PLRV	PVY	PLRV	PVS	population
CIP395432.51	0	0.2	0	0	0	LTVR
CIP397100.9	0	0.1	0	0	0	LTVR
CIP390478.9	0	0.05	0	0	0	LTVR
CIP388615.22	0	0	0	0	0	LTVR
CIP304387.92	0	0.05	0	0	0.17	LTVR
CIP391046.14	0	0.12	0	0	0.25	B3
CIP396009.240	0	0.1	0	0	0.33	B3
CIP301023.15	0	0.05	0	0	0.33	B3-LTVR
CIP390637.1	0	0.2	0	0	0.5	LTVR
CIP304405.47	0	0.05	0.17	0.17	0.17	LTVR
CIP300066.11	0.05	0	0	0	0.5	LTVR
CIP391207.2	0	0.05	0	0	0.67	LTVR

CIP397029.21	0	0.05	0	0	0.67	LTVR
CIP303381.30	0.1	0.1	0	0	0.5	LTVR
CIP391533.1	0.05	0.05	0	0	0.67	LTVR
CIP392740.4	0.05	0	0.17	0	0.33	LTVR
CIP301044.36	0	0	0	0	0.83	B3-LTVR
CIP304387.39	0	0	0.5	0	0	LTVR
CIP379706.27 (Costanera)	0	0	0.17	0	0.67	LTVR
CIP301041.26	0	0	0	0	1	B3-LTVR

Genome-Wide Association Analysis

QTL for bulking based maturity

Marginally significant marker-trait associations were identified for the average yield/plant (AYP) in Lima and Kunming, for the average weight of marketable tuber (AWMT) in Lima and Holeta, and weight of marketable tubers (WMT) in Kunming and Lima (Table 8). Some of these markers are in regions previously identified in potato as tuber traits related (tuber yield, number of tubers per plant) (Rak et al., 2017), while two of the chromosome 9 markers detected in the Kunming dataset are in the region linked to late blight resistance (Lindqvist-Kreuze et al., 2014). The trait associated markers were unique among the different trials, which is not surprising, since the yield and tuber development are highly influenced by environmental conditions. Furthermore, these traits are known to be controlled by several genes with minor effects, and hence will require a larger set of markers to enable more reliable QTL identification. In the Kunming trial, late blight infection appeared at the end of the growing season and apparently had a significant effect on the tuber weight.

Table 8. Significant marker trait associations for bulking based maturity related traits.

Marker	Ref	Alt	Trait	Model	Score	Effect
ST4.03ch00_36073482	A	G	AYP_BLUPpred_KUN_H3	additive	11.32	-0.21
				general	11.32	NA
				1-dom	11.32	-0.21
			WMT_BLUPpred_HT_KUN_H3	additive	9.37	-0.19
				general	9.37	NA
				1-dom	9.37	-0.19
ST4.03ch01_44934786	A	G	ATMW_BLUPpred_HT_LIM17_H1	additive	5.64	-11.18
				2-dom	5.31	-28.35
			ATMW_BLUPpred_HT_LIM17_H2	additive	5.69	-13.17
				2-dom	5.32	-33.3
			ATMW_BLUPpred_HT_LIM17_H3	additive	5.72	-15.27
				2-dom	5.37	-38.67
			ATMW_BLUPpred_ranked_LIM17	additive	5.69	-13.21
				2-dom	5.33	-33.44
ST4.03ch01_71274982	C	T	ATMW_BLUE_HT_HOLE_H1	general	5.38	NA

ST4.03ch03_5413372	A	T	AYP_BLUPpred_KUN_H3	1-dom	5.22	-0.14
			WMT_BLUPpred_HT_KUN_H3	1-dom	5.53	-0.15
ST4.03ch03_61269153	C	T	AYP_BLUPpred_HT_LIM17_H1,	2-dom	5.29	-0.18
			AYP_BLUPpred_HT_LIM17_H2	2-dom	5.29	-0.18
			AYP_BLUPpred_HT_LIM17_H3	2-dom	5.29	-0.18
ST4.03ch05_29452181	G	T	ATMW_BLUE_HT_LIM17_H1	1-dom	5.54	46.83
			ATMW_BLUE_HT_LIM17_H2	1-dom	5.54	46.83
			ATMW_BLUE_HT_LIM17_H3	1-dom	5.54	46.83
ST4.03ch09_48586503	A	C	WMT_BLUPpred_HT_LIM17_H1	2-dom	5.36	-0.15
ST4.03ch09_59967523	A	T	AYP_BLUPpred_KUN_H3	additive	5.51	0.11
				1-dom	6.21	0.14
ST4.03ch09_60067335	A	G	AYP_BLUPpred_KUN_H3	additive	9.6	0.18
				general	8.84	NA
				1-dom	9.69	0.19
			WMT_BLUPpred_HT_KUN_H3	additive	7.39	0.16
				general	7	NA
				1-dom	7.87	0.17
ST4.03ch11_42597235	C	T	AYP_BLUPpred_HT_LIM17_H1	general	5.47	NA
			AYP_BLUPpred_HT_LIM17_H2	general	5.47	NA
			AYP_BLUPpred_HT_LIM17_H3	general	5.47	NA
ST4.03ch12_44213394	C	T	AYP_BLUPpred_HT_LIM17_H1	general	5.5	NA
			AYP_BLUPpred_HT_LIM17_H2	general	5.5	NA
			AYP_BLUPpred_HT_LIM17_H3	general	5.5	NA
			WMT_BLUPpred_HT_LIM17_H1	general	5.81	NA

QTL for late blight resistance

Significant marker-trait associations for late blight resistance were identified in all field trials and using different models (Table 9, Figure 14). Three of the markers map in chromosome 9 in the same region previously found associated with late blight resistance in Peru (Lindqvist-Kreuze et al., 2014, Li et al., 2010). The results here confirm that the same genomic region is effective regardless of the environment. This result suggests that the late blight resistance breeding strategy at CIP has been successful in selecting for alleles that confer broad spectrum late blight resistance, since the *Phytophthora infestans* populations found in Peru, China and Ethiopia are different. We have recently shown that the R8 gene originating from *Solanum demissum* co-locates in this QTL (Jiang Rui et al., 2018).

Table 9. Markers tagging QTL for late blight resistance.

Marker	Ref	Alt	Trait	Model	Threshold	Score	Effect
ST4.03ch00_36073482	A	G	Oxa2014	additive	5.47	10.33	0.19
				general	5.39	10.33	NA
			Yun2015	additive	5.47	26.75	0.3

							2
				general	5.43	26.75	NA
			Yun2016	additive	5.47	25.81	0.27
				general	5.44	25.81	NA
ST4.03ch05_9022783	C	T	Yun2015	general	5.43	5.84	NA
ST4.03ch09_58779951	A	G	Oxa2014	additive	5.47	5.88	0.11
				general	5.39	6.21	NA
			Yun2015	general	5.43	6.07	NA
ST4.03ch09_59299540	A	C	Oxa2014	additive	5.47	6.02	0.09
			Oxa2014	general	5.39	5.89	NA
ST4.03ch09_59967523	A	T	Oxa2014	additive	5.47	6.24	-0.11
				general	5.39	5.89	NA
			Yun2015	additive	5.47	11.38	-0.16
				general	5.43	12.24	NA
			Yun2016	additive	5.47	14.17	-0.16
				general	5.44	13.98	NA
ST4.03ch09_60067335	A	G	Oxa2014	additive	5.47	12.09	-0.19
				general	5.39	12.97	NA
			Yun2015	additive	5.47	24.2	-0.29
				general	5.43	25.92	NA
			Yun2016	additive	5.47	24.4	-0.25
				general	5.44	24.85	NA
ST4.03ch09_61106174	C	T	Yun2015	general	5.43	7.82	NA
			Yun2016	general	5.44	7.77	NA

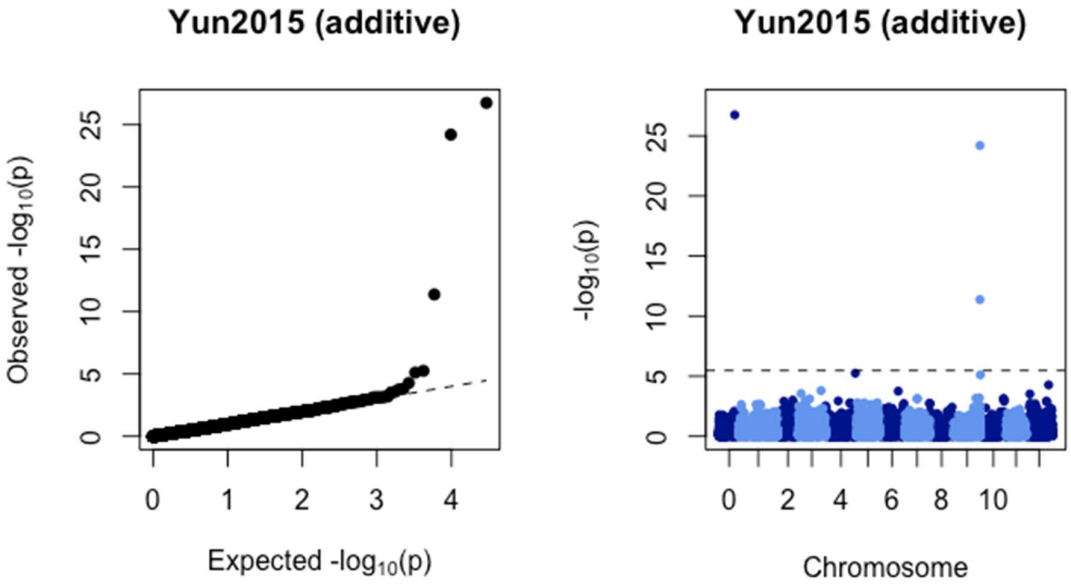

Figure 14. Example of a quantile plot (left) indicating deviation from normality and Manhattan plot (right) depicting the significant QTL for LB resistance after GWASpoly analysis using additive model.

QTL for drought tolerance

Several morpho- physiological processes are influencing the tuber yield under drought, and these are controlled by many genes with relatively small effects (Anithakumari et al., 2012). In this study 81 markers tagging all chromosomes were found significant either in the drought treatment 2017 or recovery treatment in the same experiment. Most of these associations are marginally significant, therefore only those with the highest significance level in chromosomes 5, 7 and 12 are listed in Table 10. The general GWAS model identified most of the significant associations (Figure 15). The shape of the quantile plot indicates that more markers are needed to cover the genome for more precise QTL identification for the drought traits. These associations are preliminary and require further validation steps.

Table 10. Markers associated with drought tolerance using 18,558 SNP and the general model with the threshold of 5.42.

Marker	Ref	Alt	Trait	Score
ST4.03ch05_13265796	A	G	drought.2017	20.15
ST4.03ch05_18425413	A	T	drought.2017	20.42
ST4.03ch05_33095381	C	T	drought.2017	19.38
ST4.03ch05_33848950	G	T	drought.2017	19.55
ST4.03ch05_33860722	A	C	drought.2017	18.93
ST4.03ch05_33979154	G	T	drought.2017	19.53
ST4.03ch05_37422939	A	G	drought.2017	8.28
ST4.03ch07_32240570	C	T	drought.2017	6.64

ST4.03ch07_32240574	C	T	drought.2017	6.64
ST4.03ch07_45308907	C	T	drought.2017	19.01
ST4.03ch12_15539343	A	G	drought.2017	19.39
ST4.03ch12_19070339	C	T	drought.2017	9.47
ST4.03ch12_35967269	C	T	drought.2017	19.52
ST4.03ch12_42901527	A	G	drought.2017	18.75
ST4.03ch12_49456205	C	T	drought.2017	6.32
ST4.03ch12_50342965	C	T	drought.2017	20.76

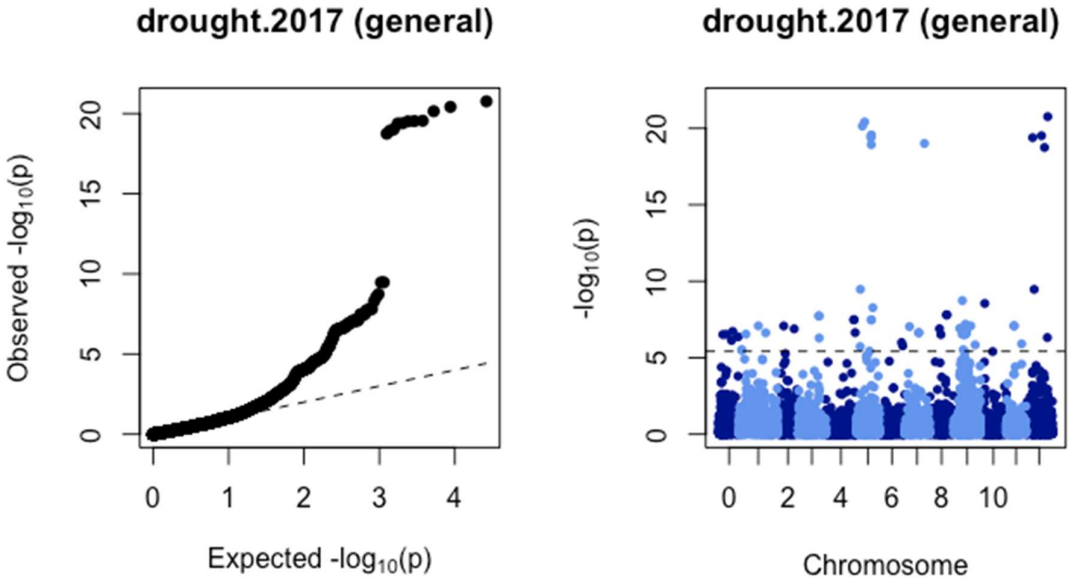

Figure 15. Example of a quantile plot (left) indicating deviation from normality and Manhattan plot (right) depicting the significant QTL for drought tolerance after GWASpoly analysis using the general model.

QTL for virus resistance

A QTL for PVY resistance, in both Lima and Yunnan trials was found in chromosome 11 in the same region where the resistance gene Ry_{adg} has previously been mapped (Hamalainen et al., 1997) (Table 11, Figure 16). Ry_{adg} confers extreme resistance to all PVY strains, and this is clearly demonstrated by the histogram of the infection severity (Figure 11) as a large proportion of genotypes tested were resistant to PVY. The strength and predominance of this QTL (Figure 17) as well as the type of data collected (qualitative) most likely contributed to the lack of identification of minor factors or QTL that may contribute to partial or field resistance to PVY.

The PLRV resistance in the CIP LTVR population is understood to have come from European and North American germplasm. PLRV resistance in these sources is generally attributed to a major QTL in chromosome 11 and two minor QTL in chromosomes 6 and 5 (Marczewski et al., 2001). Here the QTL in chromosome 6 was identified, while the QTL in Chromosome 11 stays below the statistical significance level (Figure 18). Another source of PLRV resistance has been identified from the andigena germplasm which has been heavily

used in breeding at CIP. The corresponding RI_{adg} gene confers high levels of PLRV resistance and has been mapped in chromosome 5 (Velasquez et al., 2007), but GWAS did not identify QTL for PLRV in this region. The lack of QTL for this resistance in the TON panel confirms a previous survey that found RI_{adg} to be rare in CIP-bred as well as andigena landrace germplasm. Nevertheless, the relatively low number of SNP used in the GWAS assay does not favour the detection of small effect QTL or rare haplotypes that contribute to them. According to the recent estimate by Vos et al (2017) up to 40,000 markers are needed for the GWAS to cover the entire tetraploid genome if 10 founder haplotypes are assumed. To test this, we ran the GWAS with the entire unfiltered biallelic SNP set containing 24,1478 markers for the Lima PLRV data. From this data QTL were identified in chromosomes 4, 6 and 11 (Table 12). The markers in chromosome 11 are located close to the resistance gene hotspot that was reported for the PLRV resistance QTL by Marczewski et al (2001).

Table 11. Markers associated with PVY resistance using the 18K SNP set. The significance thresholds for the additive model 5.47 and 5.45 for the general model.

Marker	Ref	Alt	Trait	Model	Score	Effect
ST4.03ch03_30142264	C	T	PVY.Yunnan	additive	6.17	-0.15
ST4.03ch06_683421	A	G	PVY.Lima	additive	12.85	0.21
			PVY.Lima	general	15.01	NA
			PVY.Yunnan	additive	13.63	0.31
ST4.03ch06_55496024	G	T	PLRV.Lima	additive	15.56	-0.23
				general	15.44	NA
ST4.03ch06_55496153	A	G	PLRV.Lima	additive	13.57	0.24
				general	12.8	NA
ST4.03ch07_7941692	G	T	PLRV.Lima	additive	5.71	0.14
ST4.03ch07_21073029	C	T	PVY.Lima	additive	11.64	-0.26
			PVY.Yunnan	additive	11.66	-0.4
ST4.03ch11_149549	A	T	PVY.Yunnan	additive	6.03	-0.15
ST4.03ch11_1310453	A	T	PVY.Lima	additive	22.05	0.27
				general	28.13	NA
			PVY.Yunnan	additive	27.87	0.45
			PVY.Lima	additive	22.05	0.27
				general	28.13	NA
			PVY.Yunnan	additive	27.87	0.45
ST4.03ch11_2116439	A	G	PVY.Lima	additive	17.79	-0.34
				general	18.32	NA
			PVY.Yunnan	additive	24.44	-0.56
ST4.03ch11_2116475	A	T	PVY.Lima	additive	17.79	0.34
				general	18.32	NA
			PVY.Yunnan	additive	24.44	0.56
ST4.03ch11_2635239	A	G	PVY.Lima	additive	9.76	0.17
				general	12.99	NA
			PVY.Yunnan	additive	14.86	0.33

ST4.03ch11_2943577	A	G	PVY.Lima	additive	13.74	0.2
				general	14	NA
			PVY.Yunnan	additive	17.06	0.31
ST4.03ch11_2943595	C	T	PVY.Lima	additive	12.86	-0.2
				general	13.64	NA
ST4.03ch11_3249012	C	T	PVY.Lima	additive	10.83	-0.26
				general	10.66	NA
ST4.03ch11_3268566	C	T	PVY.Lima	additive	12.02	-0.26
ST4.03ch11_3268579	A	G	PVY.Lima	additive	11.99	0.25
ST4.03ch11_3268590	A	C	PVY.Lima	additive	12.02	-0.26
ST4.03ch11_3270456	A	G	PVY.Lima	additive	8.35	-0.13
				general	11.58	NA
ST4.03ch11_3281377	A	T	PVY.Lima	general	5.54	NA
ST4.03ch11_3281404	A	G	PVY.Lima	additive	12.27	0.26
				general	12.65	NA
ST4.03ch11_3306950	A	G	PVY.Lima	additive	11.07	0.25
				general	11.97	NA
ST4.03ch11_3306991	G	T	PVY.Lima	additive	11.07	-0.25
				general	11.97	NA
ST4.03ch11_3756922	A	G	PVY.Lima	additive	8.57	-0.19
				general	10.44	NA
ST4.03ch11_3807202	C	T	PVY.Lima	additive	11.34	0.24
				general	12.66	NA
ST4.03ch11_3954286	A	T	PVY.Lima	additive	9.97	0.2
				general	12.77	NA
ST4.03ch11_4561679	A	T	PVY.Lima	additive	7.41	-0.18
				general	7.31	NA
ST4.03ch11_4667044	A	G	PVY.Lima	additive	11.52	0.26
				general	12.67	NA
ST4.03ch11_5208548	A	G	PVY.Lima	additive	9.37	0.21
				general	9.48	NA
ST4.03ch11_5208601	A	C	PVY.Lima	additive	9.65	-0.21
				general	10.42	NA
ST4.03ch11_5208610	C	G	PVY.Lima	additive	10.95	-0.23
				general	11.23	NA
ST4.03ch11_5294929	G	T	PVY.Lima	additive	11.14	0.28
				general	10.57	NA
ST4.03ch11_5294940	A	C	PVY.Lima	additive	10.56	0.27
				general	9.97	NA
ST4.03ch11_5294964	A	G	PVY.Lima	additive	10.56	-0.27
				general	9.97	NA
ST4.03ch11_5376333	A	T	PVY.Lima	additive	8.76	0.17

				general	8.55	NA
ST4.03ch11_5468329	A	T	PVY.Lima	additive	11.34	-0.28
				general	10.57	NA
ST4.03ch11_5535999	C	G	PVY.Lima	additive	6.2	-0.2
				general	5.58	NA

Table 12. Markers tagging QTL for PLRV resistance in Lima using the additive model and unfiltered 24,1478 markers. The significance threshold was 6.17.

Marker	Ref	Alt	Score	Effect
ch04_8429456	C	T	7.46	-0.14
ch04_8429555	C	T	15.39	-0.11
ch06_55496024	G	T	14.75	-0.23
ch06_55496153	A	G	13.69	0.24
ch11_5344490	A	G	8.1	-0.1
ch11_5407524	A	C	9.55	-0.07
ch11_5407540	A	G	8.19	-0.08
ch11_5707246	G	T	19.24	0.08
ch12_3278804	G	T	6.96	-0.08
ch12_3278859	A	G	6.41	0.06

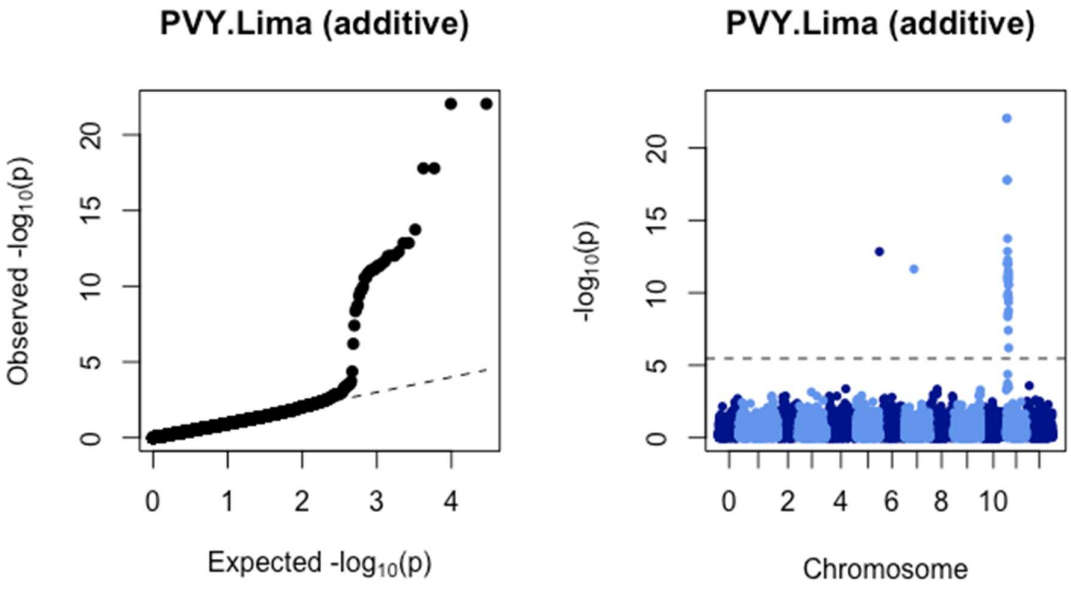

Figure 16. Quantile plot (left) indicating deviation from normality and Manhattan plot (right) depicting the significant QTL for PVY resistance after GWASpoly analysis using the additive model.

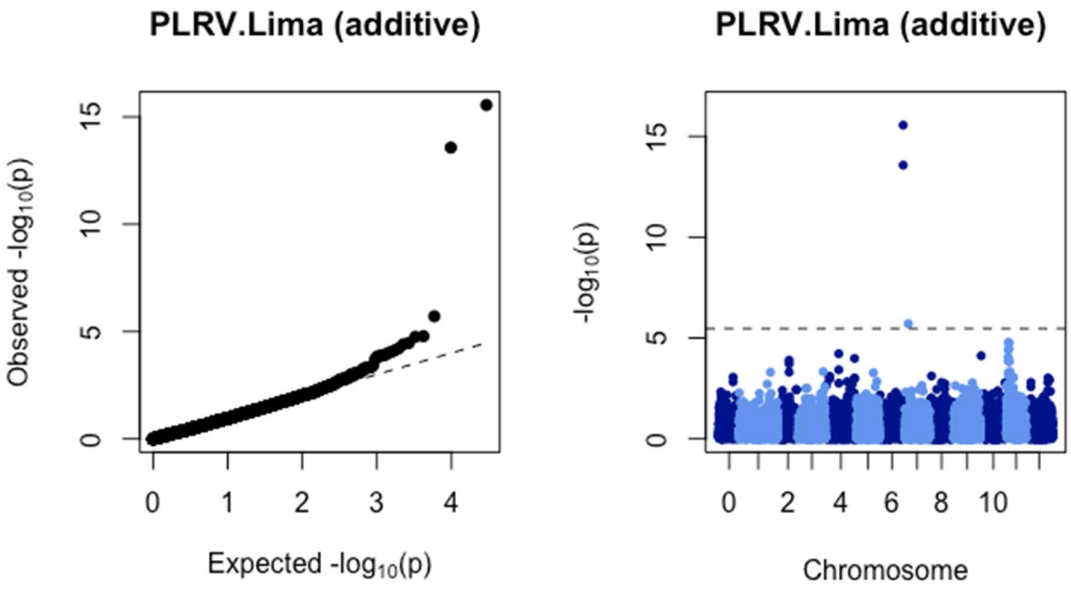

Figure 17. Quantile plot (left) indicating deviation from normality and Manhattan plot (right) depicting the significant QTL for PLRV resistance after GWASpoly analysis using the additive model.

Conclusions

This report describes the identification of SNP markers using genotyping by sequencing (GBS) in a set of diverse germplasm from the breeding populations of the International Potato Center (CIP) and their use in describing the population structure, the extend of LD and identification of QTL for various important traits. In total 380 advanced clones, pre-breeding lines and varieties were genotyped. Significant population sub-structuring was identified, particularly due to the population B1 that was derived from *S. tuberosum* group *andigena* germplasm. The remaining populations partially overlapped with each other, but the structure was still visible along the four principal components.

The LD decay discovered was modest, and comparable to that found in the European potato germplasm. Estimates based on the average r^2 of the markers along the short distance suggest that high diversity is retained in the germplasm and that tens of thousands of markers would be needed for sufficient coverage of the entire tetraploid genome. The 48x plex multiplexing of the samples during the sequencing enabled the identification of approximately 14K SNP with reasonable quality that effectively tagged several important traits. More stringent filtering for minimum read depth in all samples yielded few SNP (3,462), but even with this reduced set several marker-trait associations were found.

Phenotyping of a large set of germplasm in variable locations is challenging and the data collection and storage, as well as statistical analysis bring along further challenges. We have

successfully collected and stored the field data of most of the experiments and this will serve as an excellent platform for future trait-marker identification in many more traits. Improved statistical analysis were applied in the datasets incorporating mixed models and row-column design when applicable. This is likely to improve the precision of phenotypic estimates and facilitate the QTL identification.

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Table S1. Population denominations, accession names and pedigrees of the potato genotypes evaluated in this study.

population	Name	accession_name	parent_female	parent_male
A	CIP384866.5	Amarilis-INIA	376724.1=(85LB70.5)	BULK PRECOZ
A	CIP381379.12	A LB Group III	378356.895	PRECOZ BULK
A	CIP381381.9	Rukinzo	378493.915	PRECOZ BULK
A	CIP381381.13	IDIAP 92	378493.915	PRECOZ BULK
A	CIP381403.16	A LB Group IV	378507.833	BULK
A	CIP381178.14	A LB Group I	378943.565	PHY BULK
A	CIP384321.3	A LB Group V	380479.15	BULK 3
A	CIP391691.96	INIA 309, SERRANITA	381381.9	LB-CUZ.1
A	CIP387224.11	A LB Group IX	382121.25	676008=(I-1039)
A	CIP374080.5	Perricholi	801013=(MEX 72 =I-1058)	700764=(Casa Blanca EE-2010)
A	CIP380011.12	A LB Group VI	GRETA	SEEDLINGS 79 BULK
A	CIP380496.6	Chagllina-INIA	INDIA-1058 B	XY BULK
A	CIP377744.1	Kori-INIA	M-1266-14 MEX	374035.1
B1	CIP399053.15		395230.1	395322.11
B1	CIP399067.22		395257.2	395271.6
B1	CIP399075.32		395266.2=(B1C4046.2)	395282.3=(B1C4062.3)
B1	CIP399075.7	INIA 312, Puca Lliclla	395266.2=(B1C4046.2)	395282.3=(B1C4062.3)
B1	CIP399078.11		395266.3=(B1C4046.3)	395260.8=(B1C4040.8)
B1	CIP399048.24	B1C5	395272.2	395257.6
B1	CIP399079.22		395274.1	395257.6
B1	CIP399085.17		395296.2=(B1C4076.2)	395256.1=(B1C4036.1)
B1	CIP399085.30	INIA 317, Altiplano	395296.2=(B1C4076.2)	395256.1=(B1C4036.1)
B1	CIP399083.4		395296.2=(B1C4076.2)	395247.1=(B1C4027.1)
B1	CIP399085.23	INIA 311, Pallay Poncho	395296.2=(B1C4076.2)	395256.1=(B1C4036.1)
B3	CIP389746.2		381379.9	386614.16=(XY.16)
B3	CIP393220.54		381400.22	387170.9
B3	CIP387164.4	LBr-40	382171.1	575049=(CEW-69-1)

B3	CIP391046.14		386209.1	387338.3
B3	CIP391047.34		386209.1	387338.3
B3	CIP393228.67		386209.1	387170.9
B3	CIP391002.6		386209.1	386206.4
B3	CIP393227.66		386209.1	381400.22
B3	CIP391583.25		386209.15	387170.9
B3	CIP392617.54		387002.11	387170.9
B3	CIP393248.55		387002.11	386614.16=(XY.16)
B3	CIP393242.50		387002.11	381400.22
B3	CIP391580.30		387002.2	387214.9
B3	CIP393079.4		387004.13	390357.4
B3	CIP393079.24		387004.13	390357.4
B3	CIP391004.18		387004.4	386206.4
B3	CIP393284.39		387015.12	387170.9
B3	CIP393073.179		387015.13	389746.2
B3	CIP393073.197		387015.13	389746.2
B3	CIP393280.82		387015.3	386316.14=(XY.14)
B3	CIP393280.64		387015.3	386316.14=(XY.14)
B3	CIP393280.57		387015.3	386316.14=(XY.14)
B3	CIP391011.17		387041.12	386206.4
B3	CIP391585.179		387132.2	387170.9
B3	CIP391585.5		387132.2	387170.9
B3	CIP392633.64		387132.2	387334.5
B3	CIP392634.49		387136.14	387170.9
B3	CIP392634.52		387136.14	387170.9
B3	CIP392637.10		387143.22	387170.9
B3	CIP392637.27	B3C1	387143.22	387170.9
B3	CIP392639.34		387143.22	387334.5
B3	CIP393339.242		387164.4	SANI IMILLA
B3	CIP393371.		387170.16	389746.2

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B3	CIP393371.58	INIA 310;Chucmarina	387170.16	389746.2
B3	CIP393371.164		387170.16	389746.2
B3	CIP393371.159		387170.16	389746.2
B3	CIP391058.175		387170.16	387338.3
B3	CIP393349.68		387170.6	387338.3
B3	CIP392650.12	B3C1	387181.7	387170.9
B3	CIP393382.44		387205.5	387338.3
B3	CIP393385.47		387231.7	387170.9
B3	CIP393385.39		387231.7	387170.9
B3	CIP393399.7	Nova	387303.71	387338.3
B3	CIP393075.54		387315.27	389746.2
B3	CIP393083.2		387315.27	390357.4
B3	CIP393084.31		387326.27	390357.4
B3	CIP392657.171		387341.1	387170.9
B3	CIP392657.8		387341.1	387170.9
B3	CIP393077.159		387348.2	389746.2
B3	CIP391065.81		387348.2	387338.3
B3	CIP393077.54		387348.2	389746.2
B3	CIP393085.5		387348.2	390357.4
B3	CIP391065.69		387348.2	387338.3
B3	CIP396008.104		391002.15	393382.64
B3	CIP396004.263		391002.6	393382.64
B3	CIP396004.225		391002.6	393382.64
B3	CIP396004.337		391002.6	393382.64
B3	CIP396012.266		391004.1	393280.58
B3	CIP396009.240		391004.4	393280.58
B3	CIP396009.258		391004.4	393280.58
B3	CIP395037.107		391004.4	391679.12
B3	CIP396018.241		391046.14	393280.58

B3	CIP396023.109		391047.34	393280.57
B3	CIP396244.12		391580.3	392633.1
B3	CIP395077.12		391586.109	393053.6
B3	CIP395109.29		391589.26	393079.4
B3	CIP395109.34		391589.26	393079.4
B3	CIP395112.19		391686.15	393079.4
B3	CIP395112.32		391686.15	393079.4
B3	CIP395112.6		391686.15	393079.4
B3	CIP395112.36		391686.15	393079.4
B3	CIP395111.13		391686.5	393079.4
B3	CIP396027.205		392633.23	393382.64
B3	CIP396026.101		392633.4	393280.64
B3	CIP396026.103		392633.4	393280.64
B3	CIP395084.9		392633.6	393053.6
B3	CIP396031.119		392633.64	393382.64
B3	CIP396031.108		392633.64	393382.64
B3	CIP396241.4		392634.52	392626.9
B3	CIP396033.102		392639.53	393382.64
B3	CIP395169.17		392652.8	391679.12
B3	CIP396034.268		393042.5	393280.64
B3	CIP396034.103		393042.5	393280.64
B3	CIP395123.6		393046.7	393079.4
B3	CIP396036.201		393077.51	393382.64
B3	CIP396038.101		393077.54	393280.64
B3	CIP396037.215		393077.54	393382.64
B3	CIP396038.107		393077.54	393280.64
B3	CIP396038.105		393077.54	393280.64
B3	CIP395015.6		393083.2	391679.12
B3	CIP395017.14		393085.13	392639.8
B3	CIP395017.		393085.13	392639.8

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B3	CIP395017.242		393085.13	392639.8
B3	CIP395017.227		393085.13	392639.8
B3	CIP395011.2		393085.5	392639.8
B3	CIP395096.2		393085.5	393053.6
B3	CIP396240.2		393371.58	391679.12
B3	CIP396240.23		393371.58	391679.12
B3	CIP396043.226		393401.55	393280.57
B3	CIP396046.105		TXY.4	393280.64
B3-HT	CIP398180.612		392657.171	392633.64
B3-HT	CIP398180.289		392657.171	392633.64
B3-HT	CIP398180.292		392657.171	392633.64
B3-HT	CIP398180.253		392657.171	392633.64
B3-HT	CIP398180.144		392657.171	392633.64
B3-HT	CIP398193.650		393077.54	392633.64
B3-HT	CIP398192.213		393077.54	392633.54
B3-HT	CIP398190.735		393077.54	392639.2
B3-HT	CIP398190.112		393077.54	392639.2
B3-HT	CIP398192.41		393077.54	392633.54
B3-HT	CIP398192.592		393077.54	392633.54
B3-HT	CIP398190.571		393077.54	392639.2
B3-HT	CIP398190.615		393077.54	392639.2
B3-HT	CIP398190.404		393077.54	392639.2
B3-HT	CIP398190.530		393077.54	392639.2
B3-HT	CIP398193.553		393077.54	392633.64
B3-HT	CIP398193.158		393077.54	392633.64
B3-HT	CIP398190.605		393077.54	392639.2
B3-HT	CIP398192.553		393077.54	392633.54
B3-HT	CIP398190.200		393077.54	392639.2
B3-HT	CIP398190.523		393077.54	392639.2

B3-HT	CIP398201.510		393242.5	392633.64
B3-HT	CIP398203.509		393280.82	392633.64
B3-HT	CIP398098.65		393371.58	392639.31
B3-HT	CIP398208.58		393371.58	392633.64
B3-HT	CIP398208.33		393371.58	392633.64
B3-HT	CIP398098.205		393371.58	392639.31
B3-HT	CIP398208.219		393371.58	392633.64
B3-HT	CIP398208.670		393371.58	392633.64
B3-HT	CIP398098.231		393371.58	392639.31
B3-HT	CIP398098.203		393371.58	392639.31
B3-HT	CIP398098.570		393371.58	392639.31
B3-HT	CIP398208.704		393371.58	392633.64
B3-HT	CIP398098.119		393371.58	392639.31
B3-HT	CIP398208.29		393371.58	392633.64
B3-HT	CIP398208.505		393371.58	392633.64
B3-HT	CIP398208.620		393371.58	392633.64
B3-LTVR	CIP301056.54		385205.5	393613.2=(TXY.2)
B3-LTVR	CIP301037.85		387205.5	702853=(LOP-868)
B3-LTVR	CIP301045.74		387205.5	391207.2=(LR93.050)
B3-LTVR	CIP301024.14		388615.22=(C91.640)	387170.9
B3-LTVR	CIP301024.95		388615.22=(C91.640)	387170.9
B3-LTVR	CIP301026.23		389746.2	BOGNA
B3-LTVR	CIP301041.26		389746.2	LOP-886
B3-LTVR	CIP301055.53		389746.2	393617.1=(TXY.11)
B3-LTVR	CIP301023.15		391180.6=(C90.266)	387170.9
B3-LTVR	CIP301044.36		392025.7=(LR93.221)	LOP-886
B3-LTVR	CIP396063.1		392633.1	TXY.12
B3-LTVR	CIP396063.16		392633.1	TXY.12
B3-LTVR	CIP396180.22		392633.6	393615.6=(TXY.6)
B3-	CIP396268.		392639.34	393613.2=(TXY.2)

LTVR	9			
B3-LTVR	CIP396272.18		392639.34	TXY.12
B3-LTVR	CIP396268.1		392639.34	393613.2=(TXY.2)
B3-LTVR	CIP396272.21		392639.34	TXY.12
B3-LTVR	CIP396272.12		392639.34	TXY.12
B3-LTVR	CIP396272.2		392639.34	TXY.12
B3-LTVR	CIP396272.37		392639.34	TXY.12
B3-LTVR	CIP396273.48		393220.54	TXY.12
B3-LTVR	CIP396269.16		393371.58	393613.2=(TXY.2)
B3-LTVR	CIP396269.14		393371.58	393613.2=(TXY.2)
B3-LTVR	CIP301029.18		C97.255	C95.397
B3-LTVR	CIP301040.63		UNICA	702853=(LOP-868)
LTVR	CIP394899.5		28.68	C90.205
LTVR	CIP394898.13		28.68	BWH-87.344R
LTVR	CIP385558.2		32) 2	NT 91.002
LTVR	CIP394901.2		34.73	393617.1=(TXY.11)
LTVR	CIP394900.1		34.73	BWH-87.344R
LTVR	CIP392285.72		36.14	382157.3
LTVR	CIP379706.27	Costanera	377257.1=(LT-1)	PVX + PVY BULK
LTVR	CIP388676.1	Maria Bonita-INIA	378015.18	PVY-BK
LTVR	CIP385561.124		38) 8	ML 91.007
LTVR	CIP391180.6		385305.1=(XY.9)	378017.2=(LT-7)
LTVR	CIP388972.22		386316.1=(XY.20)	377964.5
LTVR	CIP397079.6		386768.10=(MARIA TAMBEÃ‘A)	392820.1=(C93.154)
LTVR	CIP397079.26		386768.10=(MARIA TAMBEÃ‘A)	392820.1=(C93.154)
LTVR	CIP392797.22	UNICA	387521.3	APHRODITE
LTVR	CIP303381.30		388611.22=(C91.612)	676008=(I-1039)
LTVR	CIP395434.1		388611.22=(C91.612)	N93.067
LTVR	CIP394600.52		388611.22=(C91.612)	388972.22=(C89.315)
LTVR	CIP395192.1		388611.22=(C91.612)	C92.044

LTVR	CIP395195.7		388611.22=(C91.612)	C92.167
LTVR	CIP397044.25		388611.22=(C91.612)	391180.6=(C90.266)
LTVR	CIP395193.6		388611.22=(C91.612)	C92.030
LTVR	CIP303381.106		388611.22=(C91.612)	676008=(I-1039)
LTVR	CIP397197.9		388615.22=(C91.640)	388972.22
LTVR	CIP304345.102		388615.22=(C91.640)	676008=(I-1039)
LTVR	CIP395432.51		388615.22=(C91.640)	C92.030
LTVR	CIP397039.53		388615.22=(C91.640)	388972.22=(C89.315)
LTVR	CIP395436.8		388615.22=(C91.640)	388615.22=(C91.640)
LTVR	CIP397039.51		388615.22=(C91.640)	388972.22=(C89.315)
LTVR	CIP392759.1		388676.1=(Y84.027)	PENTLAND CROWN
LTVR	CIP397006.18		389468.3=(92.119)	88.052
LTVR	CIP397067.2		390663.8=(C91.628)	392820.1=(C93.154)
LTVR	CIP300101.11		390674.33=(95.303)	387170.9
LTVR	CIP397065.2		391180.6=(C90.266)	392820.1=(C93.154)
LTVR	CIP397065.28		391180.6=(C90.266)	392820.1=(C93.154)
LTVR	CIP399101.1		391213. 1	388972.22
LTVR	CIP300066.11		391382.18=(95.108)	392820.1=(C93.154)
LTVR	CIP300065.4		391382.18=(95.108)	387170.9
LTVR	CIP397098.12		391533.1=(LR93.060)	391207.2=(LR93.050)
LTVR	CIP397012.20		391846.5=(LR93.309)	88.052
LTVR	CIP397012.22		391846.5=(LR93.309)	88.052
LTVR	CIP397078.12		391846.5=(LR93.309)	392820.1=(C93.154)
LTVR	CIP393617.1		391896.15=(DXY.15)	DXY.33
LTVR	CIP393613.2		391896.15=(DXY.15)	391894.7=(DXY.7)
LTVR	CIP396311.1		391925.2	C92.030
LTVR	CIP397036.7		392011.1=(LR93.160)	392745.7=(92.187)
LTVR	CIP397077.16		392025.7=(LR93.221)	392820.1=(C93.154)
LTVR	CIP397014.2		392739.4=(92.062)	88.108
LTVR	CIP397060.		392739.4=(92.062)	392820.1=(C93.154)

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LTVR	CIP397196.8		392797.22	388611.22=(C91.612)
LTVR	CIP397196.3		392797.22	388611.22=(C91.612)
LTVR	CIP397069.11		392797.22=(C92.140)	392820.1=(C93.154)
LTVR	CIP397069.5		392797.22=(C92.140)	392820.1=(C93.154)
LTVR	CIP304347.6		392820.1=(C93.154)	676008=(I-1039)
LTVR	CIP397099.4		392822.3=(LR93.073)	391207.2=(LR93.050)
LTVR	CIP397099.6		392822.3=(LR93.073)	391207.2=(LR93.050)
LTVR	CIP397073.15		392823.4=(LR93.120)	392820.1=(C93.154)
LTVR	CIP397073.7		392823.4=(LR93.120)	392820.1=(C93.154)
LTVR	CIP397100.9		392823.4=(LR93.120)	391207.2=(LR93.050)
LTVR	CIP397073.16		392823.4=(LR93.120)	392820.1=(C93.154)
LTVR	CIP304366.46		392823.4=(LR93.120)	676008=(I-1039)
LTVR	CIP397035.26		392823.4=(LR93.120)	92.187
LTVR	CIP300048.12		392973.48=(95.048)	392820.1=(C93.154)
LTVR	CIP300046.22		392973.48=(95.048)	393613.2=(TXY.2)
LTVR	CIP300099.22		393533.2=(95.302)	392820.1=(C93.154)
LTVR	CIP300063.9		393536.13=(95.103)	392820.1=(C93.154)
LTVR	CIP300063.4		393536.13=(95.103)	392820.1=(C93.154)
LTVR	CIP396285.1		393617.1=(TXY.11)	104.12 LB
LTVR	CIP395448.1		393617.1=(TXY.11)	BWH-87.344R
LTVR	CIP385499.11	E86.011	65-ZA-5	377964.5
LTVR	CIP391919.3		69.4 (1043) BW	-
LTVR	CIP392780.1	BASADRE	703364=(SEDAFIN)	YY.3
LTVR	CIP389468.3		720087=(SERRANA)	388216.1=(YY.5)
LTVR	CIP390478.9	Tacna	720087=(SERRANA)	386287.1=(XY.4)
LTVR	CIP390663.8		720087=(SERRANA)	386316.14=(XY.14)
LTVR	CIP388611.22	REICHE	720091=(MEX-32)	385305.1=(XY.9)
LTVR	CIP394904.20		720118.1=(37-35A)	C90.205
LTVR	CIP302498.70		720139=(YAGANA-INIA)	391180.6=(C90.266)

LTVR	CIP302499.30		720139=(YAGANA-INIA)	392820.1=(C93.154)
LTVR	CIP394611.112		780280=(PW-88-6203)	676008=(I-1039)
LTVR	CIP304383.41		800824=(RED PONTIAC)	92.187
LTVR	CIP304383.80		800824=(RED PONTIAC)	92.187
LTVR	CIP391724.1		800959=(GRANOLA)	386316.1=(XY.20)
LTVR	CIP391207.2		800959=(GRANOLA)	385305.1=(XY.9)
LTVR	CIP392739.4		86001	386614.16=(XY.16)
LTVR	CIP392740.4		87055	386614.16=(XY.16)
LTVR	CIP397054.3		87059	392820.1=(C93.154)
LTVR	CIP397055.2		88052	392820.1=(C93.154)
LTVR	CIP392745.7		88078	386316.1=(XY.20)
LTVR	CIP397029.21		92.118	92.187
LTVR	CIP397016.7		92.119	88.108
LTVR	CIP397030.31		93.003	92.187
LTVR	CIP300054.29		95.059	392820.1=(C93.154)
LTVR	CIP300056.33		95.071	387170.9
LTVR	CIP300055.32		95.071	393613.2=(TXY.2)
LTVR	CIP300072.1		95.139	392820.1=(C93.154)
LTVR	CIP300137.31		95.187	387170.9
LTVR	CIP300093.14		95.206	392820.1=(C93.154)
LTVR	CIP388615.22		B-71-240.2	386614.16=(XY.16)
LTVR	CIP392781.1	Primavera	B71-74-49.12	385280.1=(XY.13)
LTVR	CIP394034.65		B79.638.1	676008=(I-1039)
LTVR	CIP394034.7		B79.638.1	676008=(I-1039)
LTVR	CIP394881.8		B84-606.5	386287.1=(XY.4)
LTVR	CIP393536.13		BEROLINA	386287.1=(XY.4)
LTVR	CIP394895.7		BWH-87.230R	C90.205
LTVR	CIP391930.1		BWH-87.338	SELF
LTVR	CIP395438.1		BWH-87.344R	393617.1=(TXY.11)
LTVR	CIP395445.		BWH-87.415	391894.7=(DXY.7)

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LTVR	CIP394906.6		BWH-87.420	C90.205
LTVR	CIP395446.1		BWH-87.446R	393613.2=(TXY.2)
LTVR	CIP395186.6		C91.902	C92.032
LTVR	CIP395197.5		C91.921	BK-RKN-3
LTVR	CIP398014.2		C91.923	N93.107
LTVR	CIP395194.9		C93.059	C93.030
LTVR	CIP304350.78		CHIEFTAIN	392820.1=(C93.154)
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LTVR	CIP393615.6		DXY.33	391896.15=(DXY.15)
LTVR	CIP395196.4		ES-92.005	BK-RKN-1
LTVR	CIP391533.1		G-7445	385280.1=(XY.13)
LTVR	CIP394579.36		KONDOR	393615.6=(TXY.6)
LTVR	CIP392973.48		KRASA	385280.1=(XY.13)
LTVR	CIP392025.7		LINEA 21	386614.16=(XY.16)
LTVR	CIP392032.2		LOTOS	385280.1=(XY.13)
LTVR	CIP392822.3		MARIELA	YY.1
LTVR	CIP302428.20		MARIELA	392745.7=(92.187)
LTVR	CIP391382.18		MARIELA	386287.1=(XY.4)
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LTVR	CIP304371.67		MONALISA	92.187
LTVR	CIP392820.1		MONALISA	388216.1=(YY.5)

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LTVR	CIP393708.31		PW-31	391895.10=(DXY.10)
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LTVR	CIP304387.92		REINHORT	92.187
LTVR	CIP304387.17		REINHORT	92.187
LTVR	CIP304394.56		SHEPODY	391207.2=(LR93.050)
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LTVR	CIP394638.3		TXY.8	TITIA
LTVR	CIP396287.5		TXY.8	387170.9
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LTVR	CIP304405.42		WA.018	676008=(I-1039)
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LTVR	CIP394223.19		XY.13	C-282LM87B
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LTVR	CIP304349.4	0	CHIEFTAIN	92.187
LTVR	CIP304351.15	0	CHIEFTAIN	676008=(I-1039)
LTVR	CIP304351.	0	CHIEFTAIN	676008=(I-1039)

	9			
LTVR	CIP309003.11	0	388611.22	304387.17
LTVR	CIP309017.101	0	395438.1	801088
LTVR	CIP309024.1	0	397036.7	392820.1
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LTVR	CIP309028.32	0	397036.7	801152
LTVR	CIP309062.106	0	303381.106	302499.24
LTVR	CIP309064.42	0	303381.30	392797.22
LTVR	CIP309064.76	0	303381.30	392797.22
LTVR	CIP309074.123	0	304330.34	392745.7
LTVR	CIP309078.56	0	304330.34	304356.32
LTVR	CIP309088.120	0	304347.6	302499.24
LTVR	CIP309093.50	0	304349.25	392820.1
LTVR	CIP309103.85	0	304349.8	801152
LTVR	CIP309128.87	0	304368.46	304356.32
LTVR	CIP309129.11	0	304368.46	304371.19
LTVR	CIP309131.16	0	304387.31	392820.1
LTVR	CIP309137.95	0	800258	396311.1
LTVR	CIP380389.1	Canchan-INIA	BL-1.2	MURILLO III-80
LTVR	CIP720043	Revolucion	NARANJA	(KATAHDIN x MANTARO)
LTVR	CIP720088	Achirana-INTA	MPI 61.375/23	B 25.65=(Atleet x Huinkul MAG)
PREBRED	CIP694474.16		4x-84.1	2x-5.26
PREBRED	CIP694474.33		4x-84.1	2x-5.26
VARIETY	CIP720072	Tomasa Condemayta	(B 606.37 X KATAHDIN)	(RENACIMIENTO x YANA IMILLA)
VARIETY	CIP800258	KUFRI JYOTI	3069D (4)	2814A (1)
VARIETY	CIP800827	Atlantic	800823=(WAUSEON)	B-5141.6
VARIETY	CIP800923	Spunta	BEA	USDA X 96.56
VARIETY	CIP800048	Desiree	URGENTA	DEPESCHE
VARIETY	CIP800174	DTO-33	WISC 639	W5295.7

Development of genomic selection in a panel of advanced clones of tetraploid potato: Models and estimated progress.

Dorcus Gemenet

International Potato Center

3.3. Establish and compare predictive models for genome estimated breeding values for their predictive accuracy in potato based on output

3.4. Apply multi trait selection index using data generated in WP1

Phenotyping under recurrent selection has been the main approach for variety development in plant breeding, with substantial success. However, in potato this process takes a long time, for example, it takes a year to develop tubers from botanical seed obtained from crossing nurseries. This is followed by at least two years of field evaluation for qualitative traits, with evaluation for most quantitative traits in replicated multi-environment trials beginning in around year four (**Endelman et al 2018**). The estimation of parental value based on genetic designs can add years to the selection cycle.

The use of markers for selection offers potential to reduce the breeding cycle as selection can be done at an early stage. However, identifying quantitative trait loci (QTL) via QTL mapping and genome-wide association studies (GWAS) has had little practical application in plant breeding since identifying the causal genes underlying QTL which may be needed to make their application practical is costly (**Xu and McCouch 2008**). Genomic selection which offers the ability to select parents within a shorter interval and increase selection intensity by predicting untested genotypes is emerging as the approach of choice to circumvent the limitations associated with phenotypic selection and QTL mapping for marker-assisted selection (**Meuwissen et al 2001**). This approach uses genome-wide marker data to predict the performance of untested genotypes and estimate their breeding values (genomic estimated breeding values, GEBV), based on a genotyped and phenotyped training population. The Predictive ability of genomic selection, i.e., the correlation between phenotypic best linear unbiased estimators (BLUPs) and GEBV, is influenced by several factors. These include trait architecture, the size of the training population, the relationship between the training and validation populations, heritability of the trait, the level of linkage disequilibrium (LD), marker density, environmental variances and covariance among traits (**Covarrubias-Pazaran et al 2018**).

This study used the phenotypic and genotypic (genotyping-by-sequencing (GBS) single nucleotide polymorphism (SNP) data reported for the panel of 380 potato clones in Output 1. We used the univariate genomic best linear unbiased predictor (G-BLUP) method to estimate predictive ability of models that partition genetic effects into additive and non-additive types.

Activity 3.3. Establish and compare models for genome estimated breeding values for their predictive accuracy in potato based on Output 1.*:*

Methods

The AGHmatrix package (Amadeu et al. 2016) was used to develop kinship G-matrices partitioning genetic variation based on three models: (i) Only additive effects, according to **VanRaden (2008; Add_4x)**, (ii) additive plus non-additive effects, according to **Slater et al (2016; Add+Non_4x**) and (iii) pseudo-diploidized additive effects according to **VanRaden (2008; Add_2x)**. During kinship matrix development, the full model (Add+Non_4x) could only differentiate genotypes when minor allelic frequency (MAF) was set to 40%. Adjusting the data set for MAF of 40% reduced the number of markers to 176 SNPs. We first used this marker set to develop all three matrices. We used G-BLUP to compare the predictive ability of the three models using the kinship matrices as variance-covariance matrices to fit the compressed linear mixed model (**Zhang et al 2010**) and estimate genomic best linear unbiased predictors (G-BLUPs). The package GAPIT (**Lipka et al 2012**) was used in the G-BLUP prediction. Cross-validation was done by setting 25% of the population to missing phenotypes to be used as a validation set. We used 1000 iterations to estimate the predictive ability of the three models for bulking traits (ATMW, AYP and WMT), late blight resistance (LB), virus resistance (PVY, PLRV and PVS), and total tuber weight per plant (TTWPL). Since not all genotypes were evaluated in all locations, we selected the locations with the lowest missing data per trait for model training.

Results

The summary of predictive ability based on the iterations is shown in **Figure 1**. In most cases, the pseudo-diploidized model (Add_2x) performed better than the other two models, Add_4x and Add+Non_4x, that considered dosage. We attributed this to the genotyping platform. Read depth is important in determining genotyping quality. Most commercially available service providers aim for low depth and many genotypes, and since most of these methods have originally been developed for diploids, calling dosage data for polyploids reduces genotype quality. **Gemenet et al (unpublished)** compared hexaploid sweetpotato data from DArTseq, which is the same platform as the one used in the current study, and data from a genotyping platform specifically optimized for hexaploid sweetpotato named GBSpoly (**Wadl et al. 2018**) using the same models. They obtained similar results in that a pseudo-diploidized additive model using data from DArTseq performed as well as using additive effects with dosage (Add_6x) from a high read depth genotyping platform, especially for traits with a simpler genetic architecture. However, they found that this was only true if using a low read depth platform. With a high read depth platform like GBSpoly, pseudo-diploidization significantly reduced predictive ability.

The full model (Add+Non_4x) and the additive model with dosage (Add_4x) performed equally in this study with lower predictive ability than the pseudo-diploidized model (Add-2x). In the sweetpotato study, however, while this was true for simple traits, non-additive effects were important for more complex traits like yield. **Endelman et al (2018)** showed that not considering nonadditive effects in potato reduced prediction accuracy by about 0.13 on average. They registered predictive ability ranging from 0.06 to 0.63 for specific gravity, yield and fry color, and using data from the SolCAP potato SNP array. We attribute the contrast in the current result to the lower genotype quality data based on our genotyping platform.

Given our data, we considered the pseudo-diploidized (Add_2x) model as the best model to carry out genomic prediction. While in the first step we could use only 176 markers to be able to partition the genetic effects into additive and non-additive types, this partitioning was not necessary once we decided to use the pseudo-diploidized model for our prediction. The reduction of MAF to 10% enabled

the increase of marker number in the relationship matrix to 1710 SNPs. Upon comparing with the initial matrix results, this matrix with more markers increased predictive ability for one of the tuber weight traits from 0.32 to 0.40, i.e. a 0.08 increase in predictive ability. This minimal increase in predictive ability was not surprising given that other studies have reported a plateau in the maximum number of markers required to achieve the best predictive ability, and addition of more markers after this number did not result in significant increase in predictive ability. For instance, **Covarrubias-Pazaran et al (2018)** using three biparental populations of the American cranberry showed that addition of markers after 500 markers only resulted in a 0.01 increase in predictive ability. We used this matrix of 1710 SNP markers for the predictions for all traits in the following objectives.

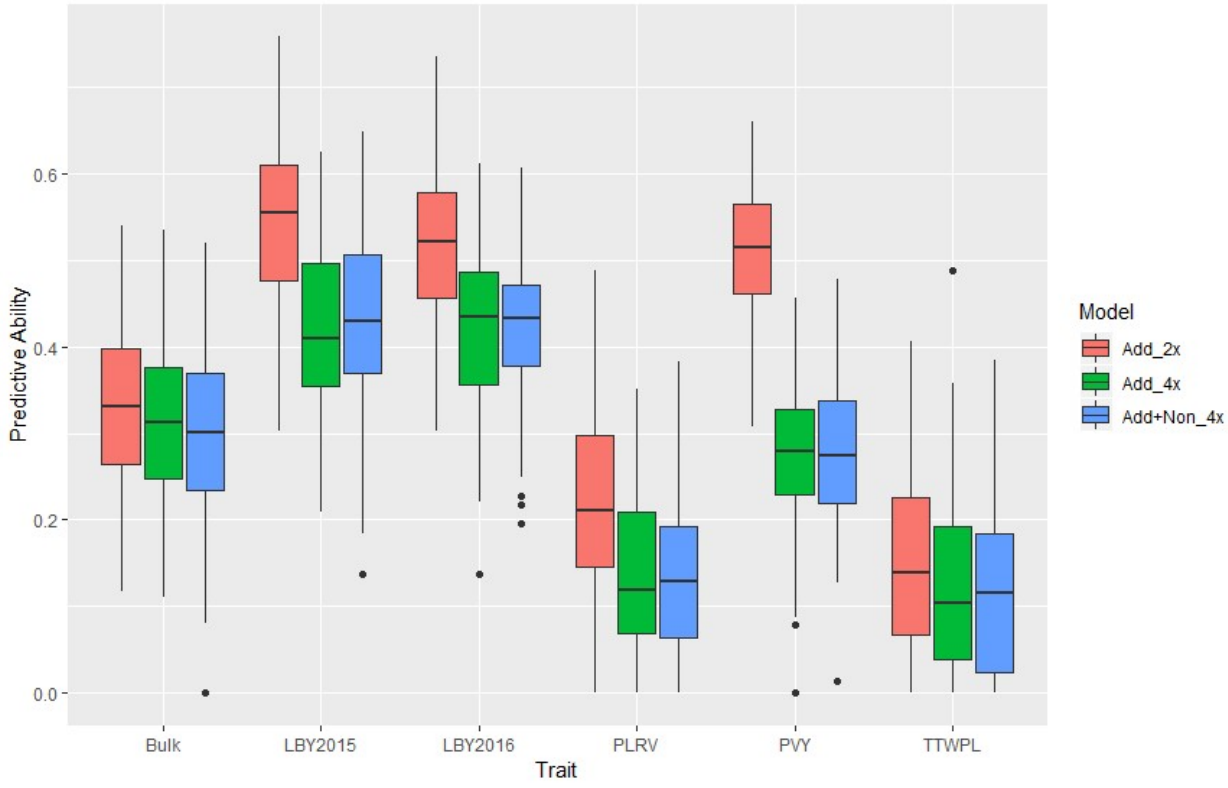

Figure 1. Model comparison showing predictive ability distribution from cross-validation iterations comparing pseudo-diploidized (Add_2x), tetraploid dosage additive (Add_4x) and tetraploid full model (Add+Non_4x). Traits include bulking (Bulk), late blight in Kunming(Yunnan) 2015 and 2016, potato leaf roll virus (PLRV), potato virus Y (PVY) and total tuber weight per plant (TTWPL).

Activity 3.4. Apply multi-trait selection index using data generated in WP1.

Breeders normally have to select for more than one trait in a given population or clone. The amount of progress made in the breeding program depends on the number of traits to be selected for and the genetic correlations among the traits (**Thompson and Meyer 1986**). Multi-trait analysis can also be applied in genomic selection, with the predictive ability of the multivariate models depending on the genetic and residual correlations among the traits considered **(Covarrubias-Pazaran et al 2018).**

Phenotypic data was collected on the TON panel for several traits in multiple locations. However, not all genotypes and not all traits could be evaluated in all the locations/seasons. For this objective, we used data from Peru where a maximum number of genotypes were evaluated for several traits per location. Late blight resistance was measured in Oxapampa in 2014, PVY and PLRV were measured in Lima during 2018 and total tuber weight was measured in a drought resistance trial in Ica during 2016 and 2017. We used the total tuber weight trait data averaged across all drought treatments per season. We used 144 genotypes for which data was available for all five traits as the training population.

We used a two-step multi-trait analysis since the phenotypic data was collected in different locations and seasons. First, mixed models were applied to each experiment in each location and season separately assuming heterogeneity of variances and covariances among the environments as described in Objective 1. The BLUPs resulting from this were then used in multi-trait analysis. We used an index based on sum of ranks according to **Mulamba and Mock (1978).** To rank the genotypes, we first analyzed the correlations among BLUPs of individual traits as shown in **Figure 2**.

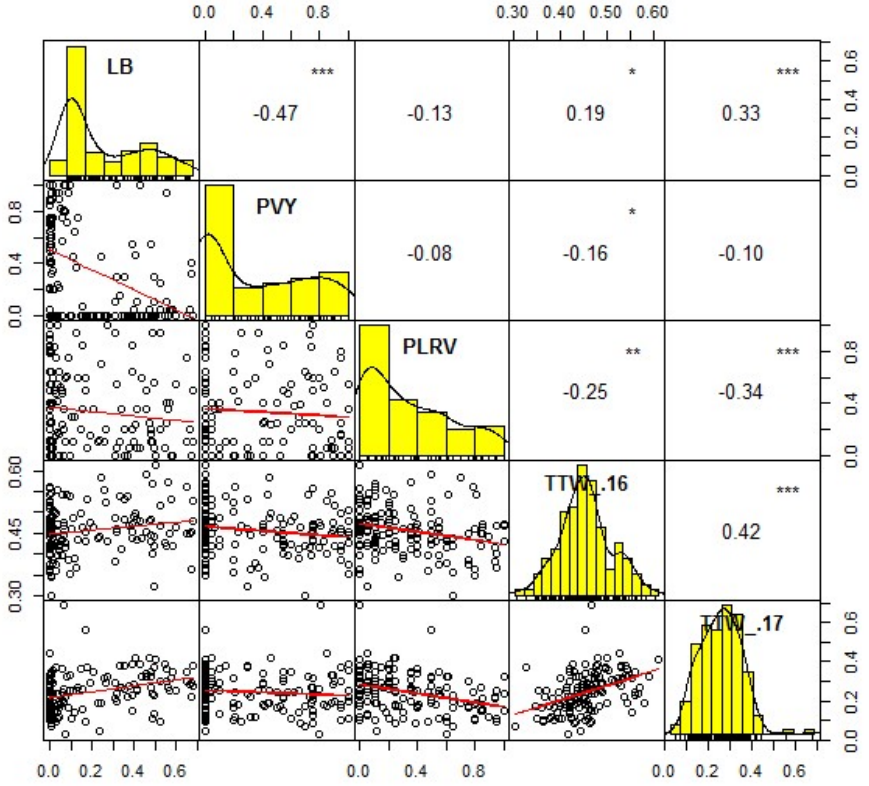

Figure 2. Genotypic correlations among trait BLUPs measured in 144 genotypes of a 380-genotype panel of advanced clones of potato. Traits: LB=late blight, PVY=potato virus Y, PLRV=potato leaf roll virus, TTW_.16 and .17= total tuber weight per plant in 2016 and 2017. ***=p<0.001, **=p<0.01, *=p<0.05.

We observed that LB scores were positively associated with tuber weight traits, whereas PVY and PLRV were negatively associated with tuber traits. Ranking for LB and TTW was therefore done in a descending order while that for PVY and PLRV was done in an ascending order. The sum of ranks was

obtained from these rankings and used in genomic prediction using the selected pseudo-diploidized model. We then compared the predictive ability from the sum of ranks index to that of the individual traits. Results are summarized in **Figure 3**.

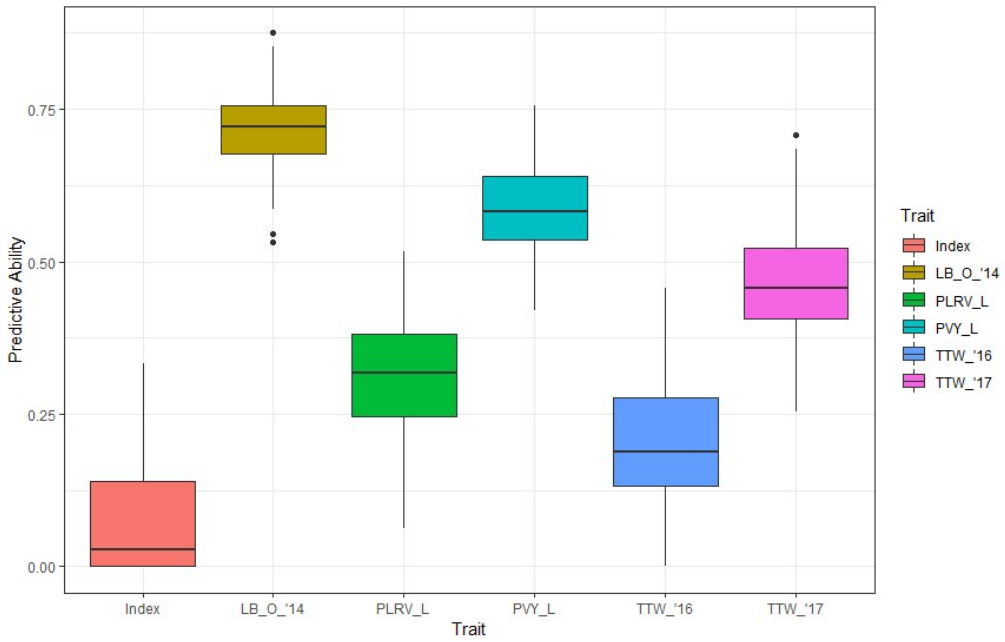

Figure 3. Box plots for predictive ability of a multi-trait index based on sum of ranks for five traits and those of individual traits. Trait abbreviations: LB_O_'14=late blight in Oxapampa in 2014, PLRV_L=potato leaf roll virus in Lima, PVY_L=potato virus Y in Lima, TTW_'16 and '17= total tuber weight per plant in Ica 2016 and 2017.

Our results show that the index based on sum of ranks (left-most boxplot) had the least predictive ability with an average of 0.15. We attribute our results to the low genetic correlation among traits measured as indicated by genotypic correlations. Multivariate analyses with mixed models to estimate the actual genetic correlations between pairs of traits will support this premise further. In our data, except for the tuber weight traits which were measured in one location for two seasons, all the other traits were measured in separate locations. The low genotypic correlations observed can therefore also be

attributed to the lack of positive genetic correlation among environments where different traits were expressed and measured. **Covarrubias-Pazaran et al (2018)** also reported no advantage of a multivariate prediction model when the genetic correlation of traits across environments was not significant. The results of this study illustrate the importance of the definition of target populations of environments for the success of multivariate predictive models.

Predictive abilities based on individual traits were higher for late blight and PVY resistance than for PLRV or tuber weight. This is not surprising, given the genetic architecture and heritability known for each. The lower predictivity of TTW in 2016 versus 2017 is likely a factor of heterogeneity of the former years' trial when conditions for field assessment of drought tolerance were just being established.

Activity 3.5. Develop and apply performance prediction tools to support variety recommendation. *Status: **ongoing**.*

We used a univariate (within-experiment) approach to carry out prediction for 29 traits grouped into bulking rate traits (**Figure 4**), disease traits (**Figure 5**) and tuber weigh traits (**Figure 6**), using the pseudo-diploidized model developed in Activity 3.3. The 29 traits included similar traits measured in the TON panel across locations and/or seasons as described in Output 1. Training sets were composed of the genotypes with phenotypic data in each respective trial. The predictive abilities reported are from cross-validation and prediction of the trait values for missing genotypes within each respective trial.

Bulking traits

We found predictive abilities ranging from 0.25 to 0.53 for the bulking traits. The predictive ability was the highest for the traits in Yunnan (Kunming) mainly because this location had a training set of 318 genotypes while Lima and Holeta had 90 and 160 genotypes in the training sets, respectively. It is shown from genomic selection studies that the size of a training population contributes significantly to prediction accuracy **(Nakaya and Isobe 2012**). Although we see the effect of training population size, the effect of the environments can also be seen to affect predictive ability. Even though Holeta had almost twice the number of genotypes in the training set as Lima, Lima still had 0.07 better prediction ability than Holeta. The data shows that given a large enough training population, genomic prediction can be applied to select for early bulking traits. However, genetic correlations across environments need to be assessed in order to plan such a breeding scheme if targeting multiple environments.

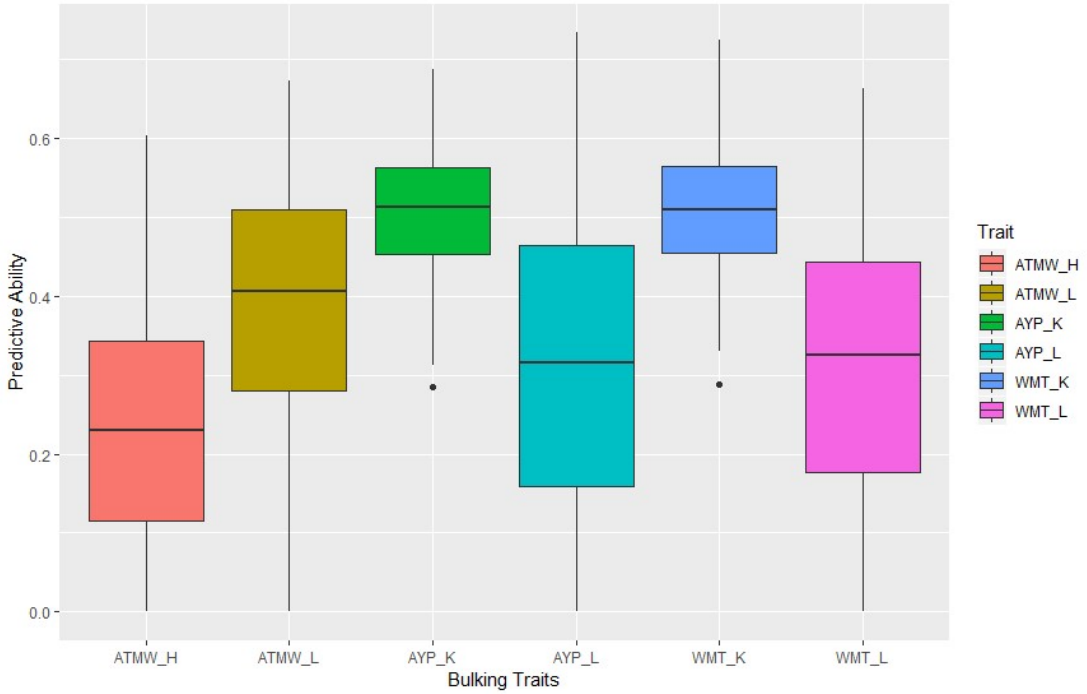

Figure 4. Prediction ability for bulking traits using the pseudo-diploidized model based on the distribution of cross-validation iterations. Traits: ATMW=average tuber marketable weight AYP = average yield per plant. _H=Holeta, _L=Lima, _K=Yunnan(Kunming).

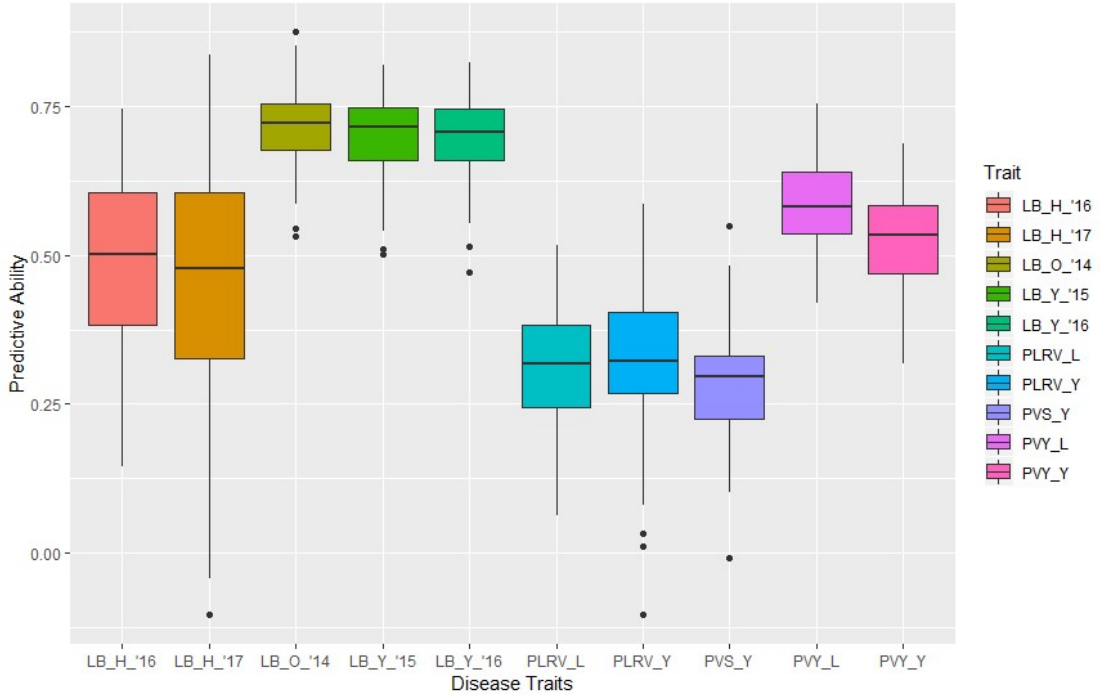

Figure 5. Prediction ability for disease traits using the pseudo-diploidized model based on the distribution of cross-validation iterations. Traits: LB=late blight, PLRV = potato leaf roll virus, PVS=potato virus S, PVY=potato virus Y. _H = Holeta, _O=Oxapampa, _Y=Yunnan(Kunming), _L=Lima. _'14=2014, _'15=2015, _'16=2016 and _'17=2017.

Disease traits

Disease traits had the highest predictive ability ranging from 0.57 to 0.73, indicating the effect of genetic architecture and heritability of the traits on prediction accuracy. Late blight resistance had similar predictive ability in Oxapampa 2014, Yunnan (Kunming) 2015 and 2016, with the lowest predictive ability being registered in Holeta. The training population in Holeta for late blight was 58 in 2016 and 125 in 2017 while those in Oxapampa and Yunnan (Kunming) were 214 and 309 respectively. PVY, PLRV and PVS had similar predictive abilities indicating that good success is expected to be made for these traits using genomic selection.

Total tuber weight

This category of traits had the lowest predictive ability across several locations. Predictive ability ranged from 0.12 to 0.56. We did not observe a variation in prediction accuracy due to drought tolerance treatments normal irrigation (NI), recovery (REC) and terminal drought (TD) in either location where this was measured i.e. Koga (ARA), Ethiopia and Ica (ICA), Peru, although we examined variations in prediction due to environment (locations or seasons). Predictive ability in Koga for NI and TD was 0.12, Lima 2016 was 0.23 for NI, REC and TD, whereas in Lima 2017, the accuracy was 0.56 for NI, REC, and TD. Predictive ability of Gansu (GNZ) and Heilongjiang (HLJ) was comparable to that of Lima 2016, at 0.22. Although Lima 2017 had the highest predictive ability at 0.56 for NI, REC and TD, using the adjustment across all three treatments resulted in a prediction accuracy of 0.38. Yield is a more complex trait and environment is expected to play a more important role in affecting prediction accuracy. Therefore, proper definition of target environments in terms of genetic correlations and multivariate prediction across environment is expected to improve prediction accuracy for this type of trait.

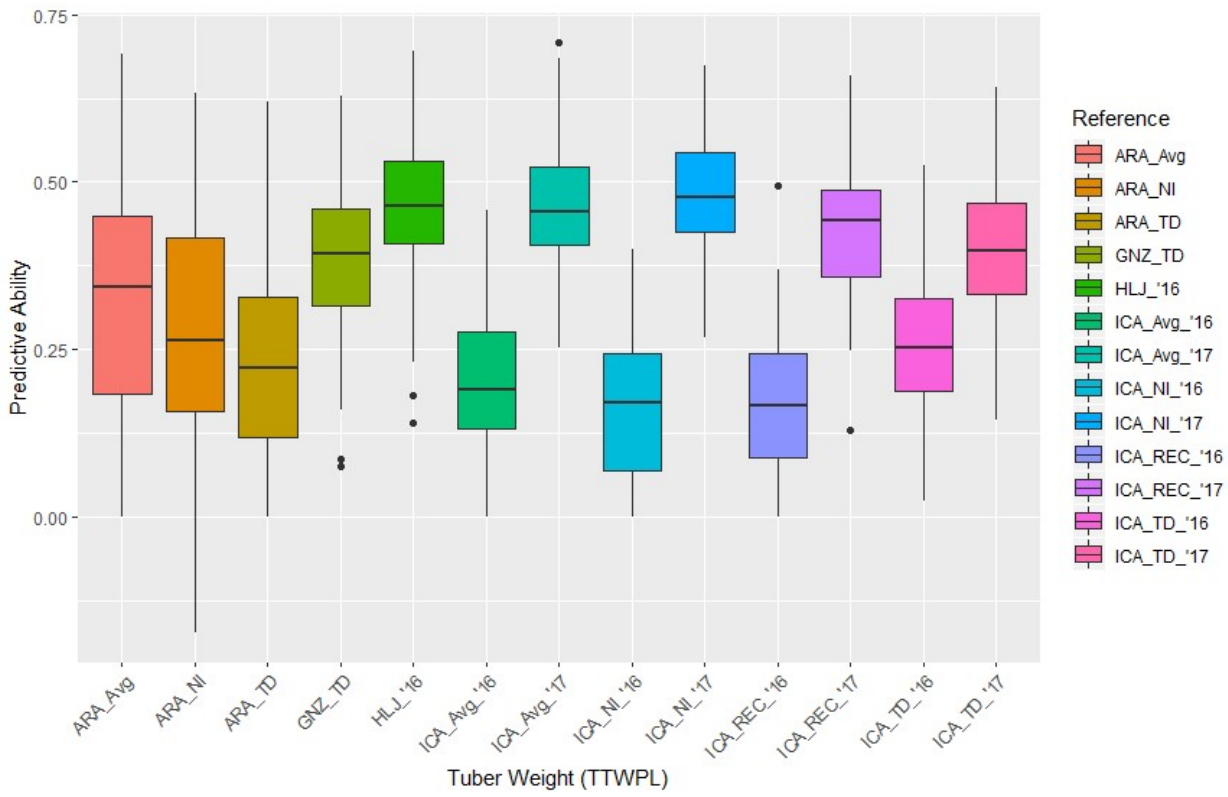

Figure 6. Prediction ability for total tuber weight per plant (TTWPL) using the pseudo-diploidized model based on the distribution of cross-validation iterations. ARA=Koga, GNZ=Gansu, HLJ=Heilongjiang, ICA=Ica. Avg=Average, NI=normal irrigation, REC=recovery, TD=terminal drought. _'16=2016, _'17=2017.

Selection differential between BLUPs and GEBV

In the current study, not all 380 genotypes could be evaluated in all locations or seasons, although all were genotyped. Genomic prediction came in handy in this case as we could predict the performance of the untested genotypes per location using the number of tested genotypes per trait as listed in **Table 1**. We used bulking traits in the current study to demonstrate the selection differentiation i.e. the difference between the mean of the base population (the training set in this case) and the mean of the selected fraction based on genomic estimated breeding value (GEBV) at 5% fraction as shown in Table 2. ATMW increased in the selected fraction by about 19.4 and 21.0g in Holeta and Lima respectively. AYP by 0.26 and 0.07 kg respectively, in Yunnan (Kunming) and Lima while WMT increased by 0.28 and 1.2 kg in Yunnan (Kunming) and Lima respectively. There was no negative selection differential indicating that progress can be made using GEBV as long as the factors affecting prediction accuracy are taken into account. Future multivariate analysis may further improve predictive ability and selection using genomic selection models combined with breeders' definitions of target populations of environments.

Table 1. Best Linear Unbiased Predictor means, training population size (TP) and mean predictive ability (PA) for the traits measured in the TON population across several locations and seasons.

Group	Trait	Mean	TP	PA
Bulking traits	ATMW_H	65.8	159	0.25
	ATMW_L	124.1	89	0.41
	AYP_K	0.72	317	0.53
	AYP_L	0.42	89	0.32
	WMT_K	0.66	317	0.53
	WMT_L	0.32	89	0.33
Disease traits	LB_O2014	0.21	214	0.72
	LB_Y2015	0.43	293	0.73
	LB_Y2016	0.32	309	0.73
	LB_H2016	0.29	125	0.62
	LB_H2017	0.27	58	0.79
	PVY_L	0.35	334	0.6
	PLRV_L	0.35	334	0.6
	PVY_Y	0.57	253	0.57
	PLRV_Y	0.16	253	0.57
	PVS_Y	0.58	253	0.57
Total tuber weight	ICA_Avg_2016	0.46	269	0.15
	ICA_NI_2016	0.69	269	0.23
	ICA_REC_2016	0.45	269	0.23
	ICA_TD_2016	0.24	269	0.23
	ICA_NI_2017	0.42	256	0.56
	ICA_REC_2017	0.19	256	0.56
	ICA_TD_2017	0.16	256	0.56
	ICA_Avg_2017	0.26	256	0.38
	GNZ_TD	0.20	307	0.22
	HLJ_2016	0.5	300	0.22
	ARA_NI	0.24	96	0.12
	ARA_TD	0.55	96	0.12
	ARA_Avg	0.40	96	0.22

H=Holeta, L=Lima, Y=Yunnan(Dehong), K=Yunnan(Kunming), ICA=Ica, GNZ=Gansu, ARA=Koga

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Accelerating the Development of Early-Maturing-Agile Potato for Food Security through a Trait Observation and Discovery Network

Final Technical Report: Max Planck Institute of Molecular Plant Physiology (MPI-MP)

PI Karin Köhl

3. Major Research Findings

Highlight important achievements: technologies and products developed and new or improved research methodologies. What has been done to overcome limitations or unexpected problems? Detailed research reports should be added in the form of annexes.

Output 1 Activity 1.1. Extend and adapt phenotyping protocols for key adaptive traits

WP 7 Development of low-tech methods for marker quantification (ongoing)

Characterization of yield and drought tolerance in potato populations

The basis for the identification and validation of phenotypic markers for drought tolerance in potato were two potato populations, which were characterized for yield potential and drought tolerance in container (pot, bigbag) and field trials (Design see supplemental material **Table 1** and **Table 2**). The first population (A) contained 60 lines from a cross between one sensitive and two tolerant potato cultivars, the three parent cultivars and a check cultivar. Their drought tolerance was determined in 13 trials in 2014 to 2016 by the VALDIS TROST consortium (Haas et al. submitted 2018). The second population (B) contained 14 genotypes from population A and seven German cultivars, for which drought tolerance had been characterized previously (http://dx.doi.org/10.1071/FP15013). This population was subjected to three different drought stress patterns (early, late, and repeated) in container and field trials at the MPI-MP in 2017 and 2018 (see supplemental material **Figure 1**). The response variable for the drought stress experiments was starch yield, which was calculated from the mass and the starch content of harvested tubers. Analysis of variance (ANOVA) showed a significant effect of the drought

stress treatment on starch yield in all experiment. Significant effects of genotype and genotype × treatment on starch yield in both populations and both test systems (container, field) indicated that both populations contained genetic variance for drought tolerance and were thus suitable for marker identification. The drought tolerance index DRYM (see **Figure 1**) was calculated as the deviation of the relative starch yield from the experimental median of the relative starch yield of all genotypes (DRYM) (http://dx.doi.org/10.1071/FP15013), or of the parent genotypes (DRYMp) (Haas et al submitted 2018).

Identification of marker candidates by laser-scanner measurements

Analysis of laser-scanner data

Two PlantEye infrared laser-scanner systems (Phenospex; NL) mounted on an automobile Fieldscan platform were used to phenotype shoot development in four bigbag trials that were performed in the screenhouse of the MPI-MP between 2015 and 2018 (see supplemental material **Figure 2**). The laser-scanner system and the infrared-thermometry system (see 'Identification of marker candidates by infrared thermometry') yielded large data volumes in the range of several Terabyte. The handling of these vast amounts required the setup of a script-based data analysis workflow that handled the exchange of the data between the storage database, the joining with metadata and environmental data, quality control and data analysis (see **Figure 3**). This workflow was programmed in SAS.

For each plant, the laser scanner yielded eight surface images per day (see supplemental material **Figure 4** A), which were used to calculate the features plant height (PH, **Figure 4** B, C) 3d surface area (A3D), projected surface area (A2D, **Figure 4** D), leaf angle (LA), leaf inclination (LI) and plant volume (PV). These features were significantly affected by genotype, treatment and time (ANCOVA Analysis). The time effect resulted from the processes leaf movement, shoot growth and shoot lodging. Leaf movement results in a diurnal oscillation of plant height (**Figure 4** B), surface area (not shown) and leaf angle (**Figure 5** C). Growth results in a saturation curve, when the daily median of height or surface area were plotted against the time after planting (**Figure 4** C, D). At a genotype- and treatment-dependent age, plant height decreased because of shoot lodging and intermingling of neighboring plants (see **Figure 2**). We included quality control and filtering algorithms in the evaluation workflow (see **Figure 3**) to identify valid observations. Subsequently, growth curves for plant height and leaf area were parameterized by calculating mean and maximum parameter values, initial linear growth rate by linear regression and polynomial regression, and the parameters maximum (max), growth rate k

and time of half-maximum (tm) from logistic regression curves (**Figure 4** E). Those parameters associated with maximum height and leaf area were most significantly affected by genotype, treatment and their interaction in both populations A (2015, 2016) and B (2017). Growth rate k and tm were less consistently affected by treatment and genotype × treatment interaction.

The diurnal leaf movement was studied by analyzing the effect of treatment, genotype, plant age and time of the day on leaf angle. The leaf angle is defined as the angle between the leaf plane and the horizontal (see **Figure 5** A). Low values indicate almost horizontal leaves, high values erect leaves. A general analysis of variance based on the daily median of the leaf angle and its daily variance yielded weak treatment effects, which were inconsistent between years. The correlation between mean or standard deviation of a genotype's leaf angle and its drought tolerance index was not significant. The leaf angle changed significantly with the diurnal cycle (see **Figure 5** B, C). A closer analysis of effect of the time of day on the leaf angle showed a significant treatment effect on the location of minimum and maximum values of the leaf angle in the diurnal cycle. In both, 2015 and 2016, optimally watered plant changed from an erect leaf position during the night to a low angle during the day (**Figure 5** D, F). Stressed plants maintained an erect leaf position throughout the diurnal cycle, with the highest percentage of plants with leaves in the maximum position at the end of the day. The largest treatment effect on leaf angle was therefore found in the afternoon (interval 4PM and 5DK in **Figure 5** D, F). Additionally, plant age affects the effect of the diurnal cycle on leaf angle ((**Figure 5** E). Water supply, time of day and plant age thus have to be taken into account when determining the optimal conditions for leaf angle measurements.

Relationship between laser-scanner derived parameters and drought tolerance

After quality control and descriptive data analysis, we studied the relationship between descriptive statistics of features (e.g. maximum plant height) or derived growth curve parameters (e.g. growth rate of leaf area) observed on optimally watered and drought-stressed plants and drought tolerance to elucidate, which features may yield phenotypic markers. We started by using the drought tolerance index DRYM calculated from the starch yield data of the same experiment, in which the phenotypic measurements were done. To find out, whether these results can be generalized, we analyzed the relationship between of phenotypic data from a single experiment and the DRYMp values based on yield data from several experiments in the same test environment (bigbag in screenhouse) or different test system (pot in screenhouse, soil on field).

We tested the relationship between features or derived parameters and drought tolerance measured in the same experiment by ridge regression on single values of features (e.g. plant height) and by Pearson and Spearman (rank) correlation analysis and multiple regression analysis on descriptive statistics (mean, range, maximum) and regression parameters from linear and polynomial regression of features on plant age. The ridge-regression based approach was abandoned as it did not yield reproducible or interpretable results. Pearson correlation analysis (see **Table 3**) revealed a negative correlation between the maximum, the initial slope and the linear term of the polynomial regression of plant height on plant age in both populations and under optimal as well as reduced water supply. Slow initial internode growth and reduced final plant height thus seem to be associated with increased drought tolerance. In contrast, there was a positive relationship between the initial slope of the linear regression of leaf area on age, suggesting that drought tolerant genotypes develop a closed leaf canopy more rapidly than sensitive genotypes.

Subsequently, we tested the relationship between the parameters from the logistic regression of plant height and area 2d on plant age (see above) and drought tolerance estimated from multiple experiments by Spearman regression analysis (see **Table 4**). For population A, drought tolerance was estimated from bigbag, pot and field experiments. The most significant and reproducible correlations with drought tolerance were found for the estimated maximum of plant height under stress and control conditions, which correlated negatively with drought tolerance, and the maximum leaf area under stress conditions, which correlated significantly positively. Likewise, we found a positive correlation between half-maximum time (tm) and initial slope of leaf area development under stress and drought tolerance in bigbag experiments, suggesting an association between rapid canopy development under stress and drought tolerance. Comparing the correlations for DRYMp values from different test systems revealed that the closest correlations were obtained when phenotyping and tolerance determination were performed in the same test system. This means that the system has to be validated in the target environment.

The analysis of the leaf angle data in population A had shown a strong diurnal effect, an interaction between diurnal effect and treatment as well as an age effect. Spearman correlation analysis of mean leaf angles in different time intervals revealed a significant positive correlation between leaf angle during the early night and drought tolerance index DRYMp from bigbag trials (**Table 5**). However, a more detailed analysis (**Table 6**) revealed a strong effect of age and water supply on this correlation. In contrast, the negative correlation between leaf angle in the

intervals 1LN and 2DW were less affected by plant age. Leaf angles measured pre-dawn may thus yield a suitable marker for drought tolerance.

The ongoing analysis of the data for population B will show whether the results can be used in genetically different population.

Identification of marker candidates by infrared thermometry

In 2017 and 2018, canopy surface temperature was monitored continuously by 16 infrared thermosensors ((IR120, Campbell) that were mounted on the Fieldscan platform. The temperature data were linked to the plant metadata from the PlantEye dataset based on the time stamp (see **Figure 3**). In addition, the data were joined with the micrometeorological parameter (air and soil temperature, soil and air humidity, light intensity, wind speed) from the screenhouse weather station. From these data, we calculated the canopy temperature depression (CTD) as the difference between canopy surface temperature and the air temperature. CTD has been suggested as a marker for drought tolerance in various crops (10.3389/fphys.2012.00429), as it is linked to transpiration rate and thus photosynthesis. CTD is known to be affected by water status, but also by micrometeorological conditions, the diurnal cycle and the developmental stage of the plant.

The analysis of the 2017 data had revealed a significant correlation between CTD measured in drought-stressed plants and the drought tolerance determined in the 2017 bigbag experiment. During the analysis of the 2018 data, we detected an error in the analysis workflow that failed to take the different time systems of the two phenotyping systems (UTC and CEST) into account. A reanalysis of the 2017 thermography data is thus paramount before any further statements can be made.

Practicable methods for marker assessment in the target environment

There are two general approaches to employ phenotypic markers for drought tolerance breeding in the target environment: development of simple markers from automatic phenotyping and introduction of automatic phenotyping to the target environment. This project initially followed the first approach and aimed to use automatic phenotyping to identify markers that could be quantified with simple techniques. One example could be leaf temperature measurements by cheap handheld infra-red thermometers, used by breeders or farmers in participatory breeding programs. The fast readout allows on-site tagging of plants with low leaf temperatures as potentially drought tolerant genotypes, thus removing the need for extensive

data management. The critical aspect is the method validation with respect to the diurnal cycle, plant development and meteorological conditions, which still needs to be done in the target environment.

The second approach, introduction of automatic phenotyping to the target environment, became more feasible in the last years, as the prices for both drones as carrier devices and sensors decreased substantially. Presently, the bottleneck is the data evaluation, as it requires sophisticated image analysis and data management technologies and high performance computers. The growing number of publications on analysis techniques (https://doi.org/10.1186/s13007-015-0072-8) will alleviate the first obstacle. The second problem could be addressed by making high performance computers accessible to scientists in the target environments through international collaborations or funding schemes.

WP 8 Dissemination *of methods in Ethiopia (cancelled)*

The testing of marker assessment by simple methods in field trials in Ethiopia and their dissemination among Ethiopian breeders had to be cancelled as a consequence of severe delays in the PhD work of G. Mulugeta Aneley's PhD (see below; 6.2 Capacity building).

WP9 Final data evaluation and manuscript writing

The final data evaluation will most likely not be finished at the end of the PhD work. Karin Köhl has started do additional data analysis. She will continue this work to write a manuscript for a peer-reviewed journal in 2019.

4. Assessment of Research Findings

The use of phenotypic markers by breeders in the target environment requires validation in field trials in Ethiopia.

5. Knowledge Sharing

Describe what has been done and what still needs to be done to ensure that the research findings (products and research methodologies) will be used and/or further developed by the various users groups.

Name (and, if possible, quantify by gender-differentiated figures) recipients to whom the research findings have already been transferred. Specify which partnership arrangements were created for participatory research and achieving impact on the ground.

1.1. Research institutes (IARC, NARS)
1.2. Development partners like extension and training institutions, farmers, agribusiness, policy makers.

6. Training

6.1. Training on the technical level

Training of breeders and field staff in the target environment was to be done as part of the field experiment in Ethiopia, which had to be cancelled (see 6.2. Problems).

6.2 Capacity development on the academic level

Workshop at the Max Planck-Institute of Molecular Plant Physiology

A 'Workshop on modern genomics for crop improvement and phenotyping' was organized by Hannele Lindqvist-Kreuze and Karin Köhl in December 2015. Five female and nine male participants from China and Ethiopia registered for the event. Teaching was done by five female and four male researchers.

Achievements by PhD student Gedif Mulugeta Aneley

- Participation in two drought tolerance trials with potato breeding lines and cultivars (population A) in 2016. Conduction of four drought tolerance trials with potato breeding lines and cultivars in the screenhouse and field in 2017 and 2018, assisted by the plant cultivation staff and Manuela Haas (2017).
- Assisted setup and operation of the laser scanner phenotyping system in the screenhouse trial 2017. Independent setup and operation of laser scanner system in screenhouse trial 2018.
- Evaluation of yield and drought tolerance data from trials 2017 and 2018 trials.
- Setup of an infrared thermometry system in the screenhouse trial in 2017, monitoring of measurements in 2017 and 2018.
- Establishment of a data evaluation workflow for laser scanner and thermometry data: data retrieval from database system, quality control, join with metadata (genotype and treatment data, meteorological information).

- Data analysis to identify markers for the prediction of drought tolerance: analysis of variance, correlation analysis, multiple regression analysis
- Preparation and presentation of two progress seminars (institute-wide seminar for PhD students and postdocs) 2017 and 2018. Tuition on presentation techniques.
- Preparation of two reports for the PhD advisory committee (PAC). (see 10.1). Meeting with the PAC on 4.8.17, 5.10.18 and 30.11.2018.
- Tuition on scientific writing. Attendance on scientific writing course in December 2018.
- Preparation of contribution (abstract, poster) to Integrated Plant and Algal Phenomics Meeting (Prag, 26 – 29th August). Tuition on abstract writing and poster presentation techniques.
- Attendance on conferences and workshops: Phenotyping summer school Wageningen (July 2016), Plant Phenotyping Workshop (November 2017), Integrated Plant and Algal Phenomics Meeting (Prag, August 2018)
- Attendance to weekly progress seminar and institute's seminar (external speakers) obligatory for the third year of the PhD.
- G. Mulugeta Aneley will have to fulfill his teaching obligations as a PhD student at the University of Potsdam by working as a teaching assistant in a practical course in 2019 before submitting his thesis.

Problems

The preparation of the progress report and thus the decision of the PAC regarding the extension of the time allowed for the PhD was delayed by four months to the 30.11.2018. In consequence, the field trial in Ethiopia was cancelled, as there are reasons to belief that G. Mulugeta Aneley will not manage to do both, conduct the trial in Ethiopia and write his thesis. As he has considerable difficulties to summarize his results in writing and to link his results to published work, he will have to concentrate on writing the thesis in the last months of his PhD. The PAC advised a four months extension of the PhD period to 31.05.2019. These five months will be funded by the Karin Köhl's institute funds. If he submits during this time, he will receive additional three months extension to write a manuscript for a peer-reviewed journal and prepare a method description for breeders. If he does not submit, the grant will end on 31.05.2019 and

he would have to finish the writing of the thesis in Ethiopia. He will get supervision during writing until 31.05.2020.

Gender-aspect

The PhD student is male. 50 % of the PhD students at the MPI-MP are female. The PhD student was part of a scientific team of two women and one man. His university supervisor is male, his group leader female. The PAC consisted of two men and one women. The supervisor of the IMPRS PhD school is female.

7. Lessons Learned

The main problem of the project resulted from the delayed start of the PhD project, which began 13 months after the start of the main project. This was due to the delays in signing the contracts, long waiting times for VISA and the cancellation of the first PhD student shortly before she was to start in Golm. The situation was aggravated by a serious illness of the second PhD student. Thus, half of the project was over before the main work of the PhD student started. The PhD project was therefore under constant time pressure. The situation was made worse by the student's lower degree of training with respect to self-organization, project presentation and reporting compared to a typical PhD student at the institute. This required more time for the training. The main conclusion for the future is that a PhD project with an extensive training aspect must not begin later than six months after the start of a project.

As the student's professional and personal life depends on the successful submission and defense of the thesis, the final months of the project now had to concentrate on this goal at the expense of producing outputs for the technical aims and the dissemination of the results. He will receive funds for additional five months from the MPI to cover the expense for the final months of his official PhD time.

8. Outlook Future Research and Development Pathway

It is highly likely that the data evaluation will be incomplete by the end of the PhD period. If the student manages to increase his efficiency and submit his thesis within the time allowed by the MPI, the Karin Köhl will invest additional money from her institute budget to grant the student money for additional time to finalize the evaluation, produce a manuscript and engage in dissemination activities. Otherwise, Karin Köhl will continue the data evaluation and write a manuscript on the results. The phenotyping method should be validated in the target

environments in Africa. This requires a new project in collaboration with the CIP and its partners in Africa.

9. Summary

Aim of the project was the development of phenotypic markers for drought-tolerance selection in breeding. The basis for the identification and validation of phenotypic markers were two potato populations, which were characterized for yield potential and drought tolerance in container (pot, bigbag) and field trials. Laser-scanners and infrared sensors mounted on an automobile scanning device were used to phenotype shoots continuously. A script-based evaluation workflow was established to analyze the vast amount of data. As a result of severe delays due to late start, illness and technical problems, data analysis is still ongoing. Preliminary results indicate that maximum plant height and leaf area, pre-dawn leaf angle and potentially canopy temperature depression may be suitable drought tolerance markers in potato. Marker validation in the target environment has to be done in a subsequent project.

10. Publication, papers, reports and other Media

10.1 Peer-reviewed articles in periodicals (give DOI number)

none

10.2 Conference presentations and other documents:

Talk by Karin Köhl and coauthors: Integrated Plant and Algal Phenomics Meeting (Prag, August 2018, ~100 attendance); EAPR/EUCARPIA Joint Meeting (Rostock, December 2018, ~ 100 attendance)

Poster by Gedif Mulugeta Aneley (Integrated Plant and Algal Phenomics Meeting (Prag, August 2018, ~100 attendance)

10.3 Thesis

none

10.4 (Hand-) Books (hardcover/paperbacks)

none

10.5 Other media (like websites, video-clips etc.)

PhD advisory committee 1st report (submitted 6.7.2017)

PhD advisory committee 2nd report (final version submitted 5.11 2018)

*List and categorize here all relevant documents, which are still under review or are **planned to be published** later on:*

10.1 Articles / journals

Aneley, G; Haas, M; Köhl,K; Prediction of drought tolerance in potato from shoot phenotyping. To be submitted 2019/20 to Functional Plant Biology

10.2 Conference presentations and other documents

Talk by Karin Köhl and coauthors: GRC – Applied Bioinformatics for Crops (Gatersleben, March 2019);

Presentation Keystone meeting on Climate Change and Plant Resilience (Hannover; may 2019)

Presentation Botanikertag 2019 (Rostock, September 2019)

10.3 Thesis

Gedif Mulugeta Aneley, Identification and validation of phenotypic markers for the prediction of drought tolerance in *Solanum tuberosum. To be submitted in May 2019*

10.4 (Hand-) Books (hardcover/paperbacks)

none

10.5 Other media (like websites, video-clips etc.)

Submission of manual marker assessment method to public repository after acceptance of manuscript.

Submission of original data to FAIR data repository.

Supplemental material

***Table 1. Design of VALDIS TROST experiments on population A**. Experiments, in which automatic phenotyping was performed, are highlighted in blue. Culture = experiment reference Id in the MPI database limsdb2(http://dx.doi.org/10.1186/1746-4811-4-11). T= number of treatment levels: 1 optimal, 2 optimal and drought stress treatment, 3 optimal (50% field capacity), reduced irrigation (30% field capacity) and drought stress. n = number of replicate plots or pots per treatment. pl = number of plants per replicate. Number of lines without parent lines (3 lines). Start date = date of planting into final pot size or field. End date = date of shoot destruction. Further information on the locations see http://dx.doi.org/10.1071/FP15013.*

Trialtype	Trial-Id	Culture	Location	T	repl	pl	Number of lines	Start date	End date
bigbag	P2	67199	Golm FGH	2	3	1	227	16.04.2014	17.07.2014
pot	P3	68015	JKI Shelter	2	1	2	195	15.05.2014	01.08.2014
bigbag	P4	72247	Golm FGH	2	2	3	60	09.04.2015	19.07.2015
pot	P5	72292	JKI Shelter	2	4	2	60	12.05.2015	10.08.2015
bigbag	P6	76240	Golm FGH	2	5	1	60	14.04.2016	17.07.2016
pot	P7	76354	JKI Shelter	2	4	2	60	09.05.2016	11.08.2016
field	F1	67516	Golm Field	2	1	5	197	22.04.2014	28.08.2014
field	F2	67518	Groß Lüsewitz	2	1	2	191	28.04.2014	27.08.2014
field	F3	72275	Golm Field	2	3	5	60	22.04.2015	17.08.2015
field	F4	72396	Groß Lüsewitz	2	2	6	60	28.04.2015	04.09.2015
field	F5	72482	Dethlingen	3	2	16	60	20.04.2015	31.08.2015
field	F6	76219	Golm Field	2	3	8	60	21.04.2016	09.08.2016
field	F7	76529	Groß Lüsewitz	2	2	6	60	02.05.2016	10.08.2016
field	F8	76528	Dethlingen	3	2	16	60	19.04.2016	01.09.2016

1

***Table 2. Design of experiments on population B.** Experiments, in which automatic phenotyping was performed, are highlighted in blue. Culture = experiment reference Id in the MPI database limsdb2(http://dx.doi.org/10.1186/1746-4811-4-11). T= number of treatment levels. n = number of replicate plots or pots per treatment. pl = number of plants per replicate. Number of lines without parent lines (3 lines). Start date = date of planting into final pot size or field. End date = date of shoot destruction.*

Trialtype	Trial-Id	Culture	Location	T	repl	pl	Number of lines	Start date	End date
bigbag	P2017	81251	Golm FGH	4	7	1	21	11.4.2017	21.7.2017
bigbag	P2018	85178	Golm FGH	4	7	1	20	17.4.2018	09.7.2018
field	F2017	81256	Golm Field	4	2	5	21	24.4.2017	14.8.2017
field	F2018	85442	Golm Field	4	2	5	21	02.5.2018	02.8.2018

Table 3. Correlation between phenotypic features and drought tolerance. *Pearson correlation coefficient between estimated parameters for the laser-scanner derived features plant height, leaf area 2d and leaf angle measured on control (c) and stress (s) plants in bigbag experiments 2017 (population B) or 2016 and 2015 (population A) and the drought tolerance index DRYM estimated from the same experiment. Range = difference between minimum and maximum, slope = average slope of a linear regression of the daily mean against plant age for the initial growth period, maximum = average maximum of the genotype, poly linear = linear regression coefficient of a polynomial regression of the parameter on plant age, poly quad = quadratic regression coefficient of the polynomial regression. Values printed in bold are significant (α = 0,05)*

	2017 c	2017 s	2016 c	2016 s	2015 c	2015 s
Range						
Plant height	0,04	-0,03	-0,09	0,04	0,09	-0,12
Leaf area 2D	0,07	0,03	0,09	-0,14	**0,25**	0,11
Leaf angle	0,05	-0,12	-0,15	-0,14	-0,16	-0,04
Slope						
Plant height	**-0,46**	-0,41	-0,16	0,16	-0,12	0,07
Leaf area 2D	0,14	0,03	0,16	0,21	**0,38**	**0,49**
Leaf angle	-0,32	-0,09	-0,05	-0,03	-0,22	**-0,27**
Maximum						
Plant height	**-0,45**	**-0,52**	0,08	**-0,26**	-0,23	-0,21
Leaf area 2D	**-0,48**	**-0,6**	-0,08	-0,06	0,22	0,19
Leaf angle	**0,52**	0,25	-0,15	-0,05	0,08	-0,08
Poly linear						
Plant height	-0,46	-0,45	-0,08	-0,24	-0,23	-0,18
Leaf area 2D	0,22	0,2	-0,23	-0,36	**0,24**	0,16
Poly quad						
Plant height	0,44	0,42	-0,02	0,2	0,22	0,17
Leaf area 2D	-0,16	-0,06	0,21	0,32	**-0,2**	-0,11

***Table 4. Correlation between parameter estimates and drought tolerance in test systems bigbag, field and pot**. Spearman correlation coefficient for correlation between parameters estimated from logistic regression of features area 2d and plant height estimated in experiment 2015 or 2016 under stress or control conditions in bigbag experiments and drought tolerance index DRYMp estimated from bigbag, field and pot experiments of project VALDIS Trost (see table 1).*

		Phenotype stress			Phenotype control		
Feature	Para-meter	bigbag	field	pot	bigbag	field	pot
Area 2d, 2015	tm	0,28	-0,03	0,25	-0,01	-0,13	-0,06
Area 2d, 2016	tm	0,54	0,02	0,34	0,20	-0,04	0,13
Height, 2015	tm	0,09	0,08	0,14	0,19	0,09	0,21
Height, 2016	tm	0,02	-0,19	-0,08	-0,21	-0,31	-0,13
Area 2d, 2015	max	0,24	0,25	0,09	0,02	0,10	0,00
Area 2d, 2016	max	0,34	0,20	0,10	-0,16	-0,04	-0,06
Height, 2015	max	-0,24	-0,06	0,03	-0,07	-0,03	0,17
Height, 2016	max	-0,32	-0,14	-0,02	-0,39	-0,12	-0,21
Area 2d, 2015	k	-0,37	-0,02	-0,08	-0,08	0,15	0,20
Area 2d, 2016	k	-0,02	-0,02	0,09	-0,08	-0,07	0,13
Height, 2015	k	0,27	0,09	-0,02	-0,20	0,18	-0,13
Height, 2016	k	0,34	-0,01	0,19	0,28	0,19	0,25

Table 5. Correlation between leaf angle and drought tolerance of genotypes of population A. *Spearman correlation coefficient for the correlation between the drought tolerance index DRYMp (measured in bigbag experiments) and the mean leaf angle in different time intervals of the day in the screenhouse experiments 2015 and 2016. Time intervals 1LN (0:30 – 4:30 CEST), 2DW (4:30 – 8:30), 3AM (8:30 – 12:30), 4PM (12:30 – 16:30), 5DK (16:30 – 20:30), 6EN (20:30 – 0:30). Sunrise approx. 5:15 CEST, Sunset 21:00 (CEST).*

Class (time)	Treatment	2015	2016
1LN	C	-0,198	0,058
1LN	S	-0,061	0,188
2DW	C	-0,218	-0,016
2DW	S	0,022	0,044
3AM	C	-0,122	-0,200
3AM	S	0,174	-0,286
4PM	C	-0,150	-0,139
4PM	S	0,226	-0,069
5DK	C	0,157	0,053
5DK	S	0,492	-0,031
6EN	C	0,152	0,297
6EN	S	0,364	0,259

Table 6 Spearman correlation coefficient *for correlation between mean leaf angle in time interval (class(time) and age interval (class(age)) in experiment 2015 and 2016 and drought tolerance (mdrymp), starch yield under stress (msy_norm_str) and control (msy_norm_ctrl) conditions in bigbag trials. Definition of time intervals see Table 5, age intervals see Figure 5.*

			2015			2016		
Class (time)	Class (age)	Treatment	mdrymp	msy_norm_str	msy_norm_ctrl	mdrymp	msy_norm_str	msy_norm_ctrl
1LN	U30	C	-0,191	-0,233	-0,072	-0,107	-0,174	-0,064
1LN	U30	S	-0,153	-0,191	-0,088	-0,049	-0,162	-0,167
1LN	U45	C	-0,243	-0,316	-0,260	-0,152	-0,273	-0,256
1LN	U45	S	-0,090	-0,219	-0,301	-0,039	-0,174	-0,269
1LN	U65	C	-0,157	-0,150	-0,145	0,150	0,158	0,057
1LN	**U65**	**S**	-0,421	-0,429	-0,051	-0,348	-0,384	-0,145
2DW	U30	C	-0,185	-0,200	-0,021	-0,246	-0,246	-0,013
2DW	U30	S	-0,121	-0,156	0,020	-0,131	-0,175	-0,056
2DW	U45	C	-0,300	-0,366	-0,250	-0,007	-0,150	-0,233
2DW	U45	S	-0,193	-0,256	-0,173	0,099	-0,019	-0,115
2DW	U65	C	-0,307	-0,225	-0,091	0,068	0,083	0,058
2DW	**U65**	**S**	-0,456	-0,442	-0,032	-0,356	-0,388	-0,147
3AM	U30	C	0,126	0,124	0,003	-0,194	-0,195	-0,039
3AM	U30	S	0,124	0,161	0,110	-0,145	-0,153	-0,074
3AM	U45	C	0,014	-0,040	-0,184	-0,141	-0,280	-0,322
3AM	U45	S	0,001	0,024	0,019	-0,089	-0,178	-0,138
3AM	U65	C	0,150	0,123	-0,026	-0,059	-0,024	-0,019
3AM	**U65**	**S**	-0,295	-0,253	0,057	-0,292	-0,323	-0,096
4PM	U30	C	0,084	0,019	-0,075	-0,078	-0,090	-0,110
4PM	U30	S	-0,172	-0,122	0,055	-0,108	-0,073	0,089
4PM	U45	C	0,013	-0,075	-0,231	-0,102	-0,164	-0,160
4PM	U45	S	-0,107	-0,045	0,041	-0,059	-0,066	-0,054
4PM	U65	C	0,018	0,066	0,151	-0,011	0,093	0,083
4PM	U65	S	-0,088	-0,073	0,148	-0,161	-0,076	0,200
5DK	U30	C	0,057	-0,020	-0,097	-0,027	-0,036	0,003
5DK	U30	S	-0,029	-0,074	-0,016	0,027	-0,020	0,021
5DK	U45	C	0,053	0,000	-0,092	-0,256	-0,245	-0,116
5DK	U45	S	-0,233	-0,180	0,067	-0,195	-0,291	-0,217
5DK	U65	C	-0,221	-0,204	-0,062	-0,060	0,018	-0,008
5DK	U65	S	-0,085	-0,077	0,142	-0,237	-0,207	0,078
6EN	U30	C	0,053	-0,032	-0,063	-0,010	-0,091	-0,163
6EN	U30	S	-0,009	-0,030	-0,037	0,213	0,039	-0,271
6EN	U45	C	-0,039	-0,126	-0,173	-0,200	-0,249	-0,177
6EN	U45	S	-0,113	-0,181	-0,187	-0,225	-0,374	-0,345
6EN	U65	C	-0,089	-0,080	0,050	-0,034	0,060	0,057
6EN	**U65**	**S**	-0,310	-0,331	-0,019	-0,253	-0,291	-0,077

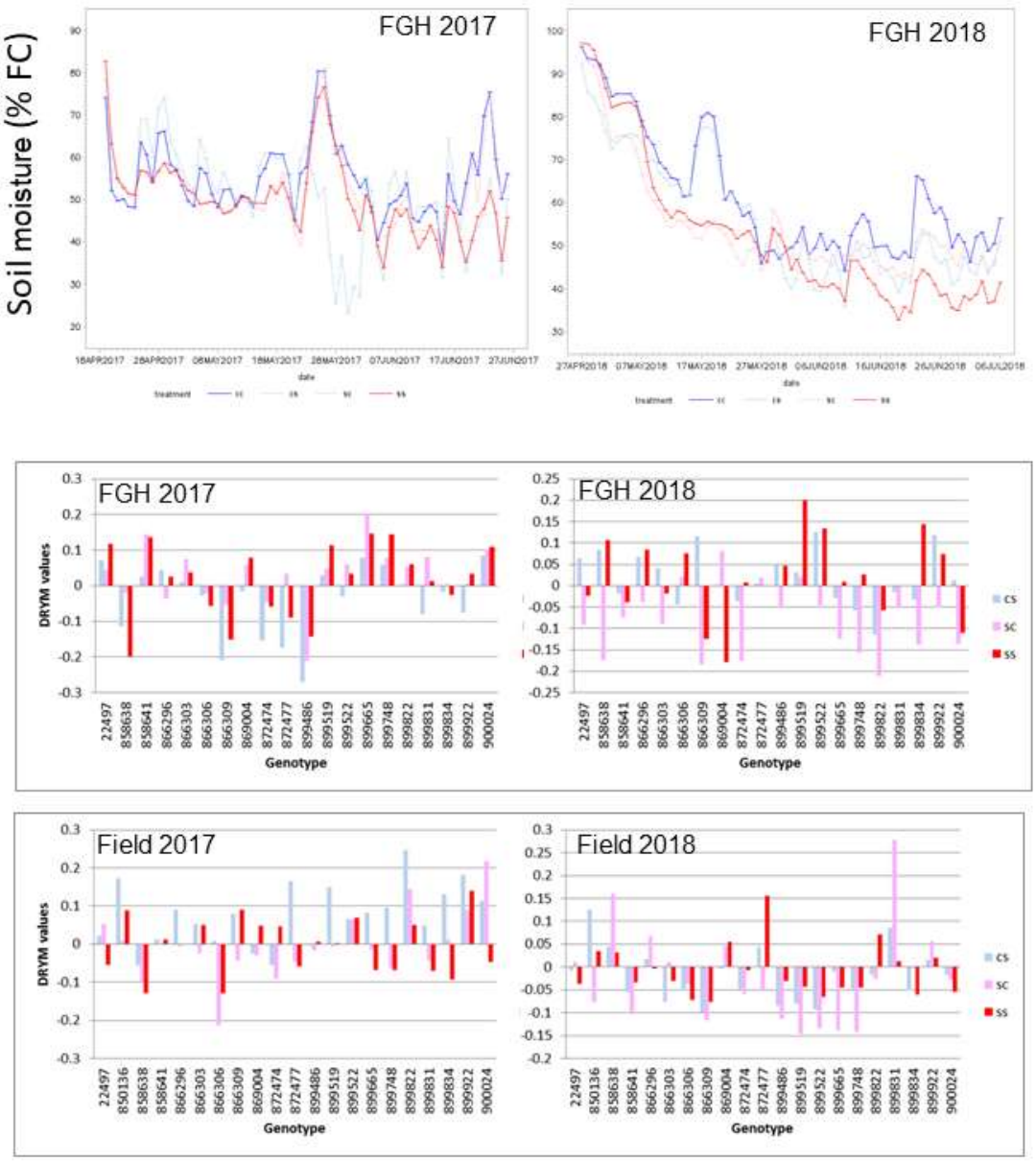

Figure 1 Drought stress experiments and drought tolerance of population B*. Upper panel: soil moisture content in % of field capacity against date for the treatments control (cc), early stress (sc), late stress (cs) and repeated stress (ss) in 2017 (left) and 2018 (right). Middle panel: Mean drought tolerance index for genotypes from population B, calculated from starch yield data obtained in treatments cs, sc and ss relative to treatment cc in screenhouse experiment 2017 (left) and 2018 (right). Lower panel: Mean drought tolerance index for genotypes from population B, calculated from starch yield data obtained in treatments cs, sc and ss relative to treatment cc in field experiments 2017 (left) and 2018 (right).*

Figure 2. Automatic phenotyping on potato genotypes subjected to drought stress in a screenhouse experiment at the MPI-MP, *Potsdam-Golm. A and B: optimally watered (left) and drought stressed plants (right) of population A before (A) and after (B) onset of shoot lodging. C: optimally watered (left) and drought stressed (right) plants of population B with phenotyping device (2017). D Fieldscan with thermosensors (bracket) and PlantEye laserscanner (2018).*

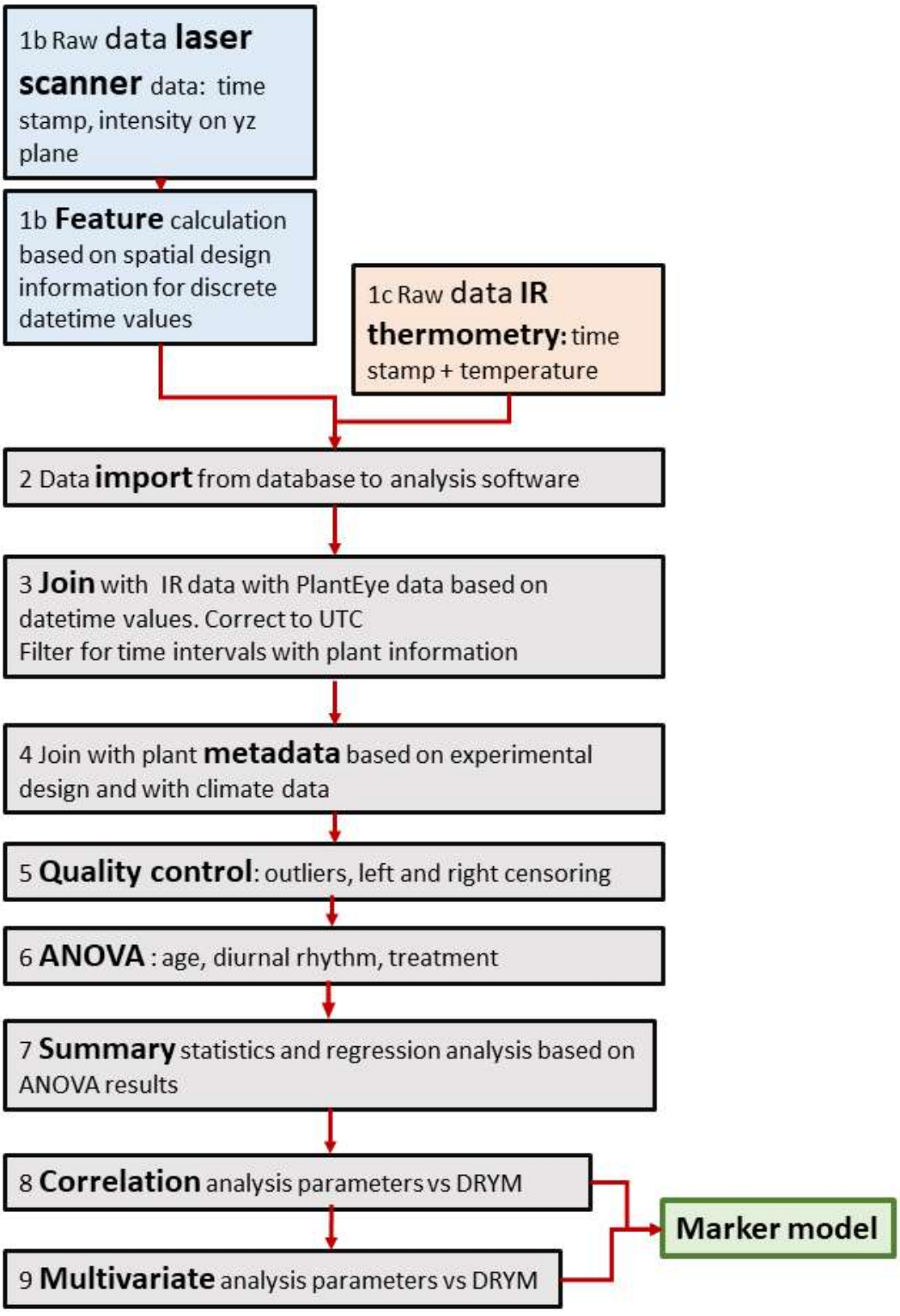

Figure 3. Data analysis workflow *for laser-scanner and infrared-thermometry data.*

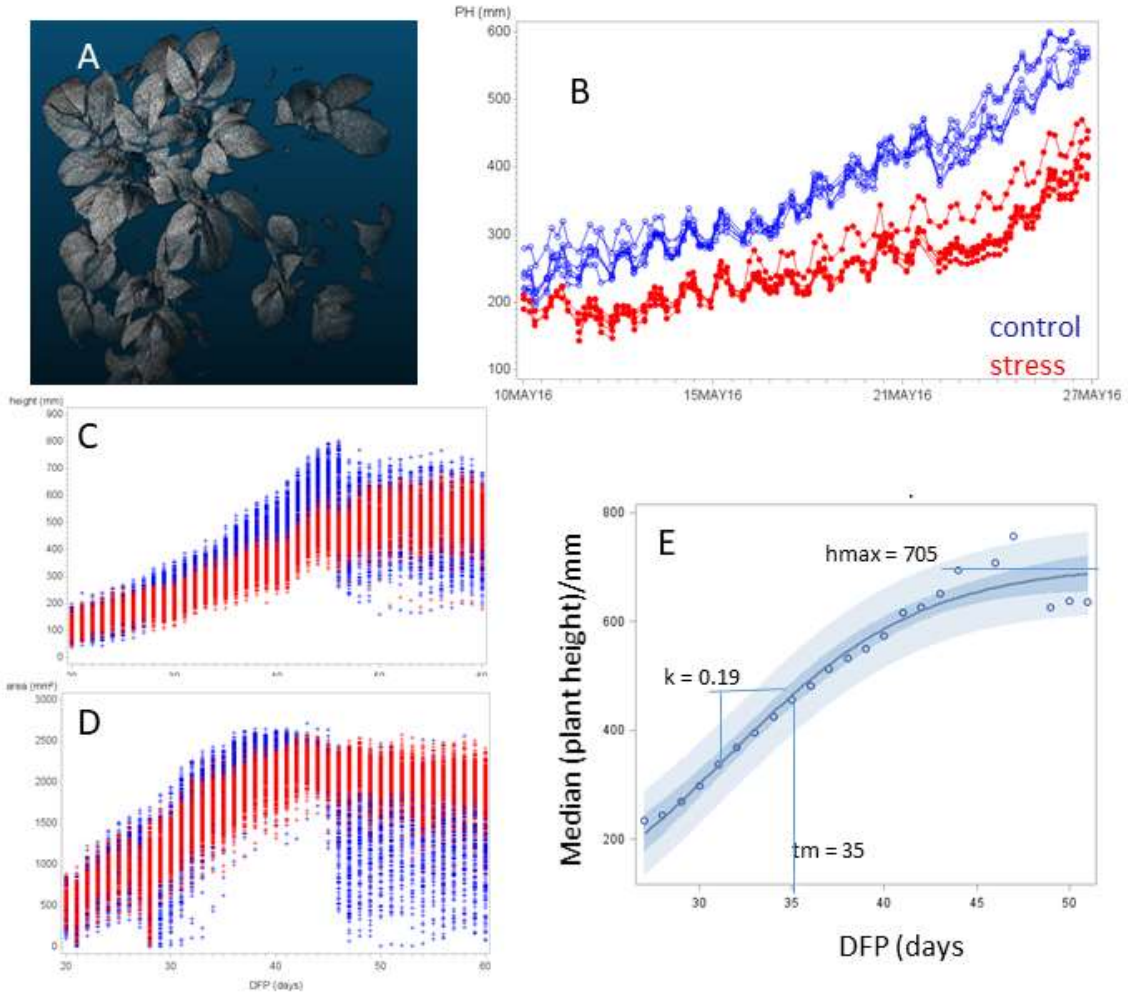

Figure 4 . Illustration of raw data and data evaluation of PlantEye laser scanner data. *A Scanner image of plant A-1:4 2017 B Plant height of 6 independent control and stress plants of genotype 22497 against measurement time, C, D daily median of plant height (C) and leaf area 2d (D)against plant age, 2016. Notice decrease of values in control plants when age > 46 days. E Example of logistic regression on daily median of plant height with confidence interval and estimated regression parameters*

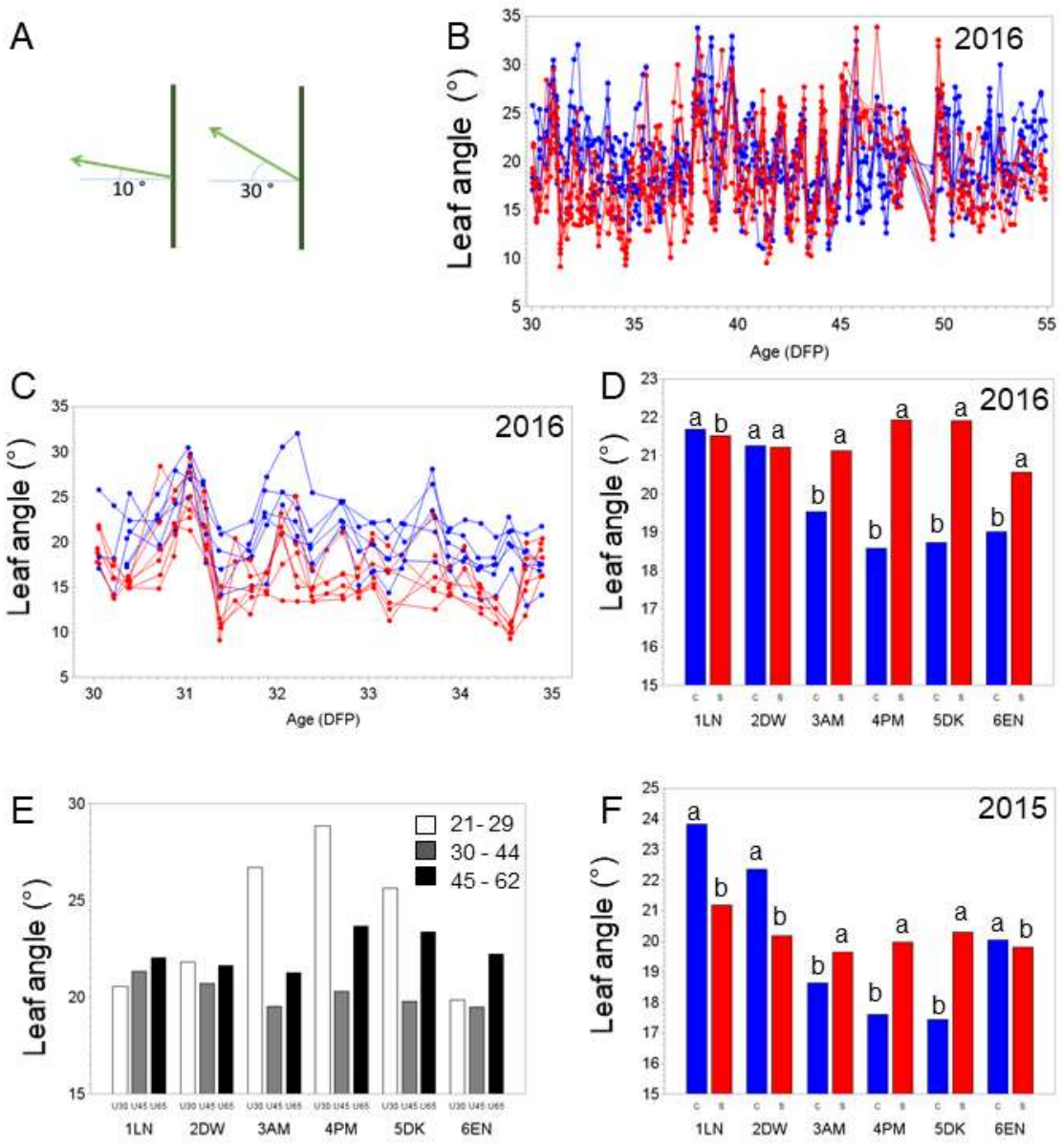

Figure 5. Effect of treatment, diurnal cycle and age on leaf angle. *A Illustration of low (10 °C) and high (30 °C) leaf angle. Leaf light green, stem dark green, horizontal blue line. B Leaf angle of cultivar 22497 depicted against plant age for control (blue) and stressed (red) plants; experiment 2016. C Zoom in on day 30 to 35 of B. D Effect of treatment and 4 h time intervals of the diurnal cycle on mean leaf angle of all cultivars under control (blue) and stress treatment in 2016. Different letters indicate significant differences between treatment means (REGWQ-Test, alpha = 0.05). 1LN = late night, 2DW dawn, 3AM morning, 4PM afternoon, 5DK dusk, 6EN early night. E Effect of plant age class (days after planting) on mean leaf angle in different time intervals of the day in stress treated plants in experiment 2016. F Same as figure D for experiment 2015*