Article

Kompetitive Allele Specific PCR (KASP) Markers for Potato: An Effective Tool for an Increased Genetic Gain

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Abstract: *Phytophthora infestans* (Mont.) de Bary causing potato late blight (LB) and potato virus Y (PVY), are serious constraints to cultivated potatoes causing important yield reduction, and phenotyping for resistance is challenging. Breeding operations for vegetatively propagated crops can lead to genotype mislabeling that in turn reduces the genetic gain. Low-density and low-cost molecular marker assessment for phenotype prediction and quality control stands as a viable option for breeding programs. We report here the development of Kompetitive Allele Specific PCR (KASP) markers for LB and PVY resistance, and for quality control assessment, and their routine use in different breeding populations. Two KASP markers for LB resistance and two for PVY Ryadg were validated with an estimated assay power ranging between 0.65 and 0.88. Developed QC KASP markers demonstrated the power of tetraploid calls in discriminating the breeding material, including full sibs and half sibs. The routine implementation of developed markers in a breeding program will allow a better allocation of resources and a precise characterization of the breeding material, leading to an increased genetic gain.

Keywords: Late blight, PVY, quality control, KASP

1. Introduction

Vegetatively propagated, cultivated potato (*Solanum tuberosum* L.) is an autotetraploid crop (2n = 4x = 48), leading to high levels of genetic heterogeneity and tedious genomic studies and breeding [1]. Phenotypic evaluations of several disease related traits are challenging, costly, and depend heavily on the environmental conditions in the test sites.

Tetraploid potato recurrent selection, as applied in CIP breeding programs, consists of creating variability from parents with known breeding values and selecting for traits of interest (including disease resistance, yield, tuber quality traits and environmental adaptation) through various selection stages [2]. Hence, heterozygous individuals are multiplied and tested through several stages in different environmental conditions based on the breeding stage, with the aim of identifying parents for the next recurrent selection cycle, advanced clones for material sharing and promising clones for variety release. Clones evaluated in advanced and multi-environment trials are therefore expected to be no different from those in early observational trials. Tubers from field trials are generally not used as seeds to prevent viral diseases' spread; therefore, tuber multiplication is done parallelly to the field

trials, and tuber seed of the breeding population are maintained in controlled environments. However, this process may increase the odds of mislabeled genotypes and sampling errors throughout the breeding cycle and across environments in the same breeding stage. Such blunders lead to a waste of time and resources, and a reduction of achieved genetic gains because of a low accuracy of selection along the breeding cycle. Therefore, quality control (QC) analysis is essential to ensure the traceability and the identity of the clones at key stages during the breeding process (e.g., identity control, hybrid purity), during conservation in the germplasm bank and when sharing breeding materials. QC analysis refers to the process of identifying the defects or errors in the quality of breeding lines, germplasm accession, variety, or any other product throughout the breeding pipeline [3].

The oomycete *Phytophthora infestans* (Mont.) de Bary causing potato late blight (LB) and potato virus Y (PVY), a potyvirus, are of the most important pathogens of potatoes worldwide and if not controlled can damage the entire plants and cause yield reduction of up to 80% [4–6]. The occurrence of these two diseases is increased by several factors, including climate variations, non-adequate crop management, the strain and pressure of the pathogen, the use of non-resistant varieties [7–9]. Resistance to late blight and PVY is controlled by several genes and the development of molecular markers [10,11] had allowed an acceleration of breeding cycles and a reduction of operational costs. The use of molecular markers for selecting resistant clones gains more interest from the breeders and producers. This if of upmost importance for smallholder farmers and the environment given the lack of information, the stringent crop management requirements (e.g., clean seeds, crop rotation), and the cost and unsustainability nature of the chemical control [12–14].

Ryadg, *Rysto*, and *Rychc* were identified as PVY strain nonspecific resistance (R) genes derived from *S. tuberosum* subsp. *andigena, stoloniferum*, and *chacoense* respectively. These genes confer extreme resistance phenotype to all PVY strains. Several markers linked with various Ry genes and allele-dosage assays have been developed and validated for identifying multiplex Ry progenitors and breeding line with resistance to PVY. The sequence-characterized amplified region marker RYSC3 was developed for *Ryadg* on chromosome 11 [15]. Further linked **a**mplified **f**ragment **l**ength **p**olymorphism (AFLP) markers (M5, M6, M17, M33, M35 and M45) and one RFLP marker (GP259) were identified in different segregating populations [16]. RYSC3, M6 and M45 DNA markers flanking the *Ryadg* gene were multiplexed and validated on the International Potato Center (CIP) germplasm and breeding stocks [17]. YES3-3A and YES3-3B for *Rysto* were mapped on chromosome 12 [18], and sequence-tagged site markers have been developed and validated on cultivars and breeding clones and segregating populations [19]. The introgression of Ry genes into potato germplasm allowed the identification and selection of potato clones with extreme resistance against PVY, reducing therefore the cost and accuracy of screening for resistant material [20–22].

LB resistance in potato is controlled by several R genes originating from wild potato species, e.g., *S. demissum*, *S. stoloniferum*, *S. albornozii*, *S. trifidum*, and the cultivated *S. tuberosum* groups Andigena and Phureja [23–25]. Research efforts in identifying several R genes were reviewed in [26] and several molecular markers were developed to characterize the breeding material. While single R gene resistance can be rapidly overcome by an evolution of

the pathogen, the presence of several R genes could possibly favor potato quantitative late blight resistance [27]. The development of molecular markers has recently enabled stacking such resistance genes into elite backgrounds and pre-breeding lines in tetraploid potato [28,29], and to accelerate their introgression into breeding lines.

The development of molecular markers for applied potato breeding is evolving rapidly [30] with the availability of a potato reference genome [31–34] and the development of recent sequencing technologies. Thus, the cost-efficiency ratio for the same number of datapoint, the laboratory workload, the flexibility, data quality and bioinformatics required to characterize the breeding material [35] are the key factors to consider while planning on a long-term and effective use of low-density markers.

Kompetitive Allele Specific polymerase chain reaction (KASP) is a simplified fluorescence-based methodology for single nucleotide polymorphism (SNP) genotyping assay where the DNA sample is amplified with thermal cycler using allele specific primers [36,37, www.biosearchtech.com]. KASP markers enable bi-allelic scoring of single nucleotide polymorphisms (SNP) and indels at a specific locus and offer extremely high levels of robustness and precision at a relatively low cost. Whether the SNP are identified from an association analysis, resistance gene enrichment sequencing, or a simple selection from SNP markers, KASP markers can be developed provided the flanking bases/sequences are available (Figure 1). The process and bioinformatics needed to develop KASP markers and analyze the output data is less demanding as compared with the previously developed trait markers for potato [11,20]. KASP markers were developed from previous efforts on PVY, *Ryadg* [17] and *Rysto* [19], late blight [38,39].
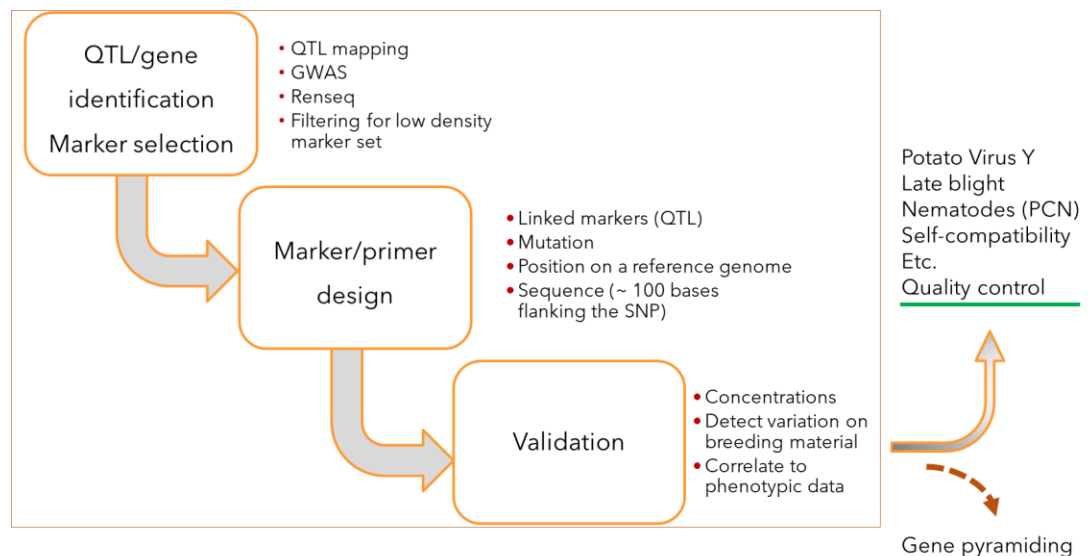

Figure 1. Workflow for KASP marker development and validation from association analysis or SNP marker selection. QTL, quantitative trait loci; GWAS, genome wide association study; Concentration, two different DNA concentrations (standard and diluted), results from the two concentrations allow to determine the stability of the assay and therefore its sensitivity to DNA concentration; SNP, single nucleotide polymorphism; PCN, potato cyst nematodes

Developed KASP markers are now routinely used in CIP breeding programs for characterizing the breeding material with respect to these important diseases for potato cultivation. Due to their discriminatory ability, SNP markers are ideal for developing low-cost QC KASP marker sets. Discrimination of breeding material is possible with a relatively small set of appropriately selected SNP markers. Our objective is to report the development and use of KASP markers for LB and *Ryadg* resistance, and QC analysis for CIP breeding programs.

2. Materials and Methods

2.1. Trait KASP marker development

SNP markers in the *Ryadg* M6 resistance locus on chromosome 11, and in chromosome 9 late blight resistance locus were converted into KASP markers. The M6 markers' allele sequences representing resistant and susceptible alleles previously described by [17] were aligned for SNP identification to develop markers linked to *Ryadg* gene. In total 9 SNP or indels were selected for KASP marker design, and the amplification results were compared with the high-resolution melting (HRM) assay using probe M6P2 [17] on a quality-evaluation set of 72 tetraploid potato genotypes from the CIP breeding program. The late blight markers were selected from previous association studies using genotyping by sequencing (GBS) [38] or solcap [39] markers and the KASP results were compared with the original marker scores using a set of 73 and 78 tetraploid potato clones from CIP breeding program, respectively. Validated SNP markers in chromosome 9 late blight resistance locus, and in the *Ryadg* M6 resistance locus on chromosome 11 were converted into KASP markers by Intertek/Agritech® (https://www.intertek.com/agriculture/agritech/).

2.2. Assay verification and routine analysis

Assay verification was conducted using a set of 83 advanced tetraploid clones (S_1) from CIP breeding program with known resistance to PVY [40] and late blight [41] (Supplementary Table 1). Assay verification for the PVY markers snpST0052 and snpST00073 was conducted by comparing the 78 available PVY resistance phenotypes previously recorded in the breeding program by enzyme-linked immunosorbent assay (ELISA) tests with their corresponding genotypes. Clones were classified in five categories: extremely resistant, resistant, hypersensitive, susceptible, highly susceptible. For the verification calculations, the three first phenotypic classes were considered as resistant to PVY and the two last ones as susceptible. There were three possible categories for the markers snpST0052 and snpST0073: AA, AG, and GG, where A and G are the dominant favorable alleles linked to the resistance phenotype for snpST0052 and snpST0073, respectively.

An assay verification was similarly conducted for the LB markers snpST0020 and snpST0023 by comparing resistance phenotypes recorded in Peru and their respective genotypes. LB phenotypic data were available for all S_1 samples. Clones were classified in seven categories: Rpi-gene, highly resistant, resistant, moderately resistant, moderately susceptible, susceptible, highly susceptible. For the verification calculations, the three first categories were considered as resistant to late blight and the remaining ones as

susceptible. There were three possible categories for the tested markers, i.e., snpST0020: CC, CA, and AA and snpST0023: TT, TG, and GG, where C and T are the favorable alleles linked to the resistance phenotype for snpST0020 andsnpST0023, respectively.

The number of resistant and susceptible potato clones in S_1 were counted in each marker category. The false positive (α), and false negative (β) rates as well as the assay power (s) were determined as follows: α=FP/(FP+TN); β=FN/(FN+TP) and s=1-β, where FP=false positive, FN=false negative, TN=true negative, TP=true positive.

Further, a sample of 59 clones from the late blight heat tolerant (LBHT) [42] and the LBHT×LTVR (LTVR= lowland tropics virus resistance) [2] breeding populations (S_2) was tested with validated KASP makers for resistance to late blight and PVY (Table 1). The genotypic data of the 59 clones were compared to previously recorded LB phenotypic data from field trials in Oxapampa (high late blight pressure environment) between 2012 and 2018.

Table 1. List of KASP markers validated for late blight R8-QTL and PVY *Ryadg* resistance, the allele variants, and their position in the DM_v6.1 potato genome are indicated. Alleles in bold denote the favorable allele that confers resistance.

SNP ID	Intertek id	Gene /QTL	Reference sequence	Chromo-some	Position
S9_6126 1167	snpST00020	R8-QTL	tgaaggtattcattttctgatgaaa tgcattcctg-gactttcttcatc[a/**c**]tgttt-gggcgctctttgtacag-gacatgcatt-	9	6738007 6
sol-cap_snp _c2_564 18	snpST00023	R8-QTL	gctgttttctccccccca ggatactgtcgcaatggttgtt-gcaacggcaataactatgagtgc-tatag[t/**g**]tgctgttaattatggg gagcaataaatatttgtaatgg-caaagatgtaat	9	6637926 7
M6F1R4 _711	snpST00052	*Ryadg*	aaacatgagctactcggggtcac-cacttaacaaagattttagttaga-taa[g/**a**]gacagaatacaaaatc cactaactatcacatttatcac-ggatgggttcca	11	2499608
M6F1R4 _817	snpST00073	*Ryadg*	acgtgctaactagttagggat-tcaaattcaa-gattgtattaaacccg-gat[a/**g**]tacatatacagggaa-gctttaac-cacacatgcaaggttcaga-tatcca	11	2499502

2.3. QC marker selection pipeline

The 8,303 SNP solcap Infinium array [43] was used to genotype 206 clones from two CIP B3 [44] and LTVR [2] breeding populations. The marker selection from the 3285 markers with no missing data is summarized in Figure 2. Markers with a minor allele frequency (MAF) < 0.2 in both populations separately based were removed, and only the 1523 markers with a in both populations were considered for further steps.

Low sequencing depth in next generation sequencing methods can lead to an overestimation of homozygous calls, and therefore to an underestimation of heterozygosity, besides the genotyping errors and several missing data that in turn could lead to biases in subsequent population genetics analyses [3,45–47]. The markers were hence reduced to their diploid form (AAAA = AA, BBBB = BB, heterozygous (AAAB, AABB and ABBB = AB) for further analyses, to reduce heterozygous dosage bias. A further MAF filtering was perform at diploid level, removing 19 markers with MAF <0.2. Physical positions and the allele variants (DM v03) of the 1504 remaining markers were retrieved from the solcap database (http://solanaceae.plantbiology.msu.edu/). The linkage disequilibrium (LD) between markers on each separate chromosome and for each population separately was computed using the R package "genetics" [48]. Finally, two markers on each chromosome having the lowest median LD (either in both populations or in one population) and laying in different linkage blocks were selected for KASP conversion (Intertek/Agritech®). The 21 validated QC markers is available at the Excellence in Breeding webpage (https://excellenceinbreeding.org/module3/kasp).
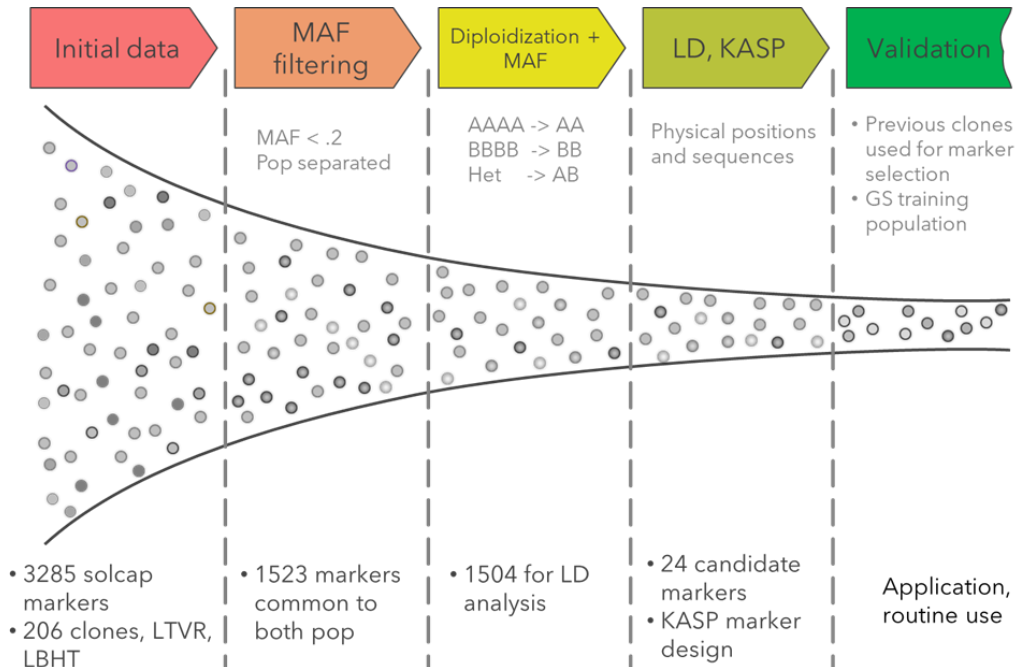

Figure 2. Selection pipeline of quality control markers. 3285 initial solcap markers had no missing data. GS, genomic selection; KASP, Kompetitive allele specific PCR; LBHT, late blight heat tolerance population; LD, linkage disequilibrium; LTVR, lowland tropics virus resistant population; MAF= minor allele frequency.

2.4. Routine analysis with QC KASP markers

2.4.1. Study design

A QC marker assessment was completed on a subset of the LBHT population (second cycle recurrent selection). Samples from field trials were taken at two different stages of the breeding cycle. The preliminary trial was constituted of about 2500 clones, selected from the greenhouse observational evaluation of the first clonal generation, and evaluated in Oxapampa in 2019. The intermediate trials with 500 clones selected from Oxapampa were planted in 2020 in 3 locations: Huanuco, Huancayo and Oxapampa with two replications per location. A safeguard copy of all clones being evaluated in the fields is maintained at La Molina research station by the breeder; these genotypes are therefore considered as the reference for any breeding material identity verification.

The 114 clones sampled from Huancayo intermediate trial (Hyo) were randomly selected from the 500 tested clones. Samples in Hyo (for a total of 224 samples) were taken from the first replication (r1, one single sample of 96 different clones) and the second replication (r2, 77 of the 96 r1 samples). Additionally, three samples (a, b, and c) of 17 clones were taken in 17 random plots of the first replication in Hyo to assess potential within plot variations. The 34 clones sampled from Oxapampa preliminary trial were randomly selected from the 114 Hyo-sampled clones. As reference for the 114 sampled clones in field experiments, each of the 114 clones was sampled from La Molina safeguard in 2020 (Table 2).

Table 2. Counts of genotypes and samples used for QC assessment. r1 and r2 denote the first and the second replication of Huancayo intermediate trial, respectively.

	La Molina	Huancayo	Oxapampa	Total
Total samples (number of clones)	114 (114)	224 (114)	38 (38)	376 (114)
r1 samples		96		
r2 samples		77		
r1 within plot samples		17		

Samples were collected from young leaves and desiccated in silica gel before storage. Hence, the 376 samples were prepared at once for 21 QC KASP marker assessment. The DNA extraction and marker analysis were performed by Intertek/Agritech®.

2.4.2. QC data analysis

Marker snpST00174 was removed from the following analysis because having a high number of missing genotypes (over 75%). Tetraploid allele dosage calling was performed from raw intensity data using the R [49] package fitTetra [50]. To evaluate the discriminatory effectiveness of developed markers on the breeding material, dendrograms using diploid and tetraploid genotypes were generated from the 114 reference samples with a neighbor joining clustering [51]. Samples with more than 50% missing data (10 out of 20 markers) and markers with more than 30% missing data were filtered out for the QC analysis. For each clone, the genetic distance between test samples and the greenhouse reference samples was calculated using package ape [51].

Samples were considered different when their genetic difference from the reference was higher than the arbitrary 10% (~2 markers).

3. Results

3.1. Trait markers for late blight and PVY resistance

3.1.1. Selected markers and KASP assay verification

Two KASP markers for PVY resistance, i.e., snpST0052 and snpST0073, had 100% concordance with the M6P2 HRM assay (Table 3). Interestingly, the marker snpST0050 that is diagnostic for the same SNP as the M6P2 had a relatively lower concordance. The genotypic analysis of 78 clones in S_1 with the PVY resistance markers snpST0052 and snpST0073 classified 62 clones as resistant and 16 clones as susceptible to PVY. The very low type I error and relatively high assay power (s=0.83), based on the count of clones of S_1 with the three different marker genotypes and their PVY resistance phenotypes (Table 4), suggests that the PVY markers snpST0052 and snpST0073 are excellent markers for MAS in CIP potato breeding program.

Eight of the 11 tested markers for late blight resistance had over 90% concordance between the original marker score and the KASP marker score (Table 3). The two best markers, snpST0020 and snpST0023, were selected as markers for routine genotyping. Marker snpST0020 appears to be much better suited for the used gene pool , as compared to snpST0023. The assay efficiency for snpST0023 based on the number of potato clones of with three different marker genotypes and their LB resistance phenotypes (Table 4) point to the poor performance of this marker in the tested material.

Table 3. Quality evaluation of the single nucleotide polymorphism (SNP) markers converted into Kompetitive allele specific PCR (KASP) system. Markers selected for breeding are indicated with an asterisk. SNP ID is the identification in Intertek potato KASP marker system.

SNP ID	Marker name	Location†	Concordance‡
Late blight resistance			
snpST0010	GBS_S9_58779951	Chr9: 58779951	93%
snpST0012	GBS_S9_59226671	Chr9: 59226671	0%
snpST0013	GBS_S9_59301204	Chr9: 59301204	92%
snpST0014	GBS_S9_59997331	Chr9: 59997331	95%
snpST0015	GBS_S9_60067335	Chr9: 60067335	95%
snpST0016	GBS_S9_60225630	Chr9: 60225630	71%
snpST0017	GBS_S9_60510506	Chr9: 60510506	90%
snpST0018	GBS_S9_61095507	Chr9: 61095507	93%
snpST0019	GBS_S9_61205320	Chr9: 61205320	0%
snpST0020*	GBS_S9_61261167	Chr9: 61261167	99%
snpST0023*	solcap_snp_c2_56418	Chr9: 60182930	97%
PVY resistance			
snpST0049	M6F1R4_672	Chr:11: 1708290	0%
snpST0050	M6F1R4_679	Chr:11: 1708297	50%
snpST0051	M6F1R4_691	Chr:11: 1708309	45%
snpST0052*	M6F1R4_711	Chr11: 1708329	100%
snpST0053	M6F1R4_732	Chr11: 1708350	74%
snpST0054	M6F1R4_739	Chr11: 1708357	55%
snpST0071	M6F1R4_797	Chr11: 1708415	99%
snpST0072	M6F1R4_798	Chr11: 1708416	0%
snpST0073*	M6F1R4_817	Chr11: 1708435	100%

† Position on the Solanum tuberosum group Phureja DM1-3 PGSC v4.03 pseudomolecules

‡ % of concordance between original marker and KASP marker

Table 4. Assay verification of the PVY *Ryadg* (snpST00052 and snpST00073) and late blight (snpST00020 and snpST00023) KASP markers tested respectively on three samples of CIP breeding program.

Marker ID	Trait	Number of clones	α	β	s
snpST00052	PVY	78	0.0	0.16	0.83
snpST00073	PVY	78	0.0	0.16	0.83
snpST00020	Late blight	73	0.02	0.13	0.88
snpST00023	Late blight	77	0.22	0.35	0.65

α, β and s are the false positive and negative rates, and the assay power, respectively.

3.2. Markers tested on two different breeding populations

We found in both LBHT and LBHT×LTVR S$_2$ samples resistant clones to LB and PVY (Figure 3). In both populations, 23 clones had the late blight resistance genotype with snpST0020 and snpST0023. Six of the 7 genotypes with only snpST0023 resistance allele were from the LBHT×LTVR population. Likewise, 15 clones out of 59 showed resistance with both *Ryadg* markers. The poor correspondence between LB marker assessment and the phenotypes of S2 samples from the LBHT×LTVR population (Figure 4) may be due to a recombination between the markers and the resistance gene, or biased phenotypic evaluation in the greenhouse.

These two LB markers have a physical distance of approximately 1Mb in the potato DM_v6.1 reference genome, while the two *Ryadg* markers are separated by 106 bases along the chromosome 11 (Table 1). The LB markers are tightly linked in the CIP B3 population as they are always found together in the resistant genotypes (in S1 and S2). In the LTVR population, however, there is a recombination between the markers, and only snpST0020 associates significantly with late blight resistance in this population. Screenhouse experiments are labor demanding and costly and field evaluations rely heavily on erratic environmental conditions in the testing sites [52,53]. These molecular markers will allow a precise and cost-effective characterization of breeding material for PVY and late blight, as compared to phenotypic evaluations. However, the genetic distance between the used markers and LB and PVY genes defines the accuracy of marker assisted selection. The suitability of LB markers for each breeding population must be assessed, mostly when there is a recombination between the two resistance alleles.
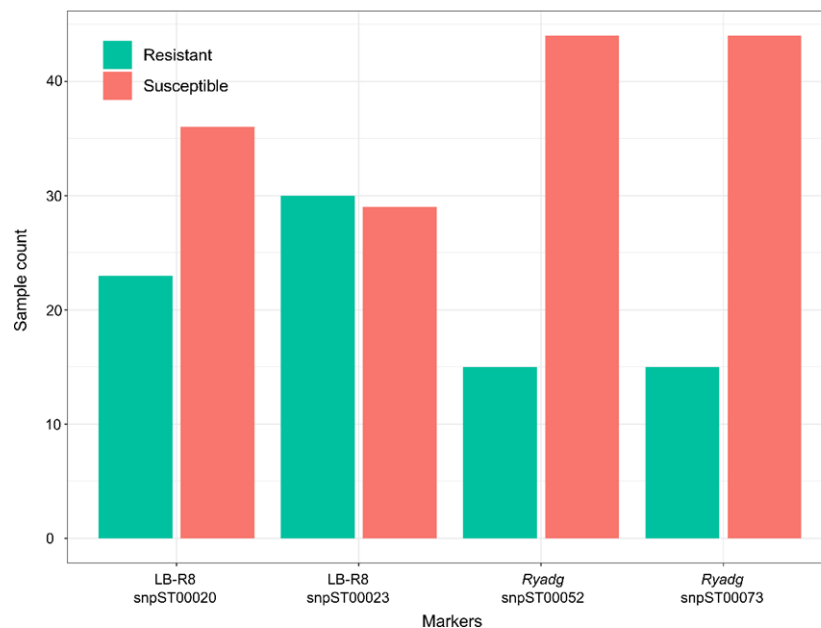

Figure 3. Count of resistant and susceptible samples (n=58) in the LBHT population with the late blight and *Ryadg* KASP markers.
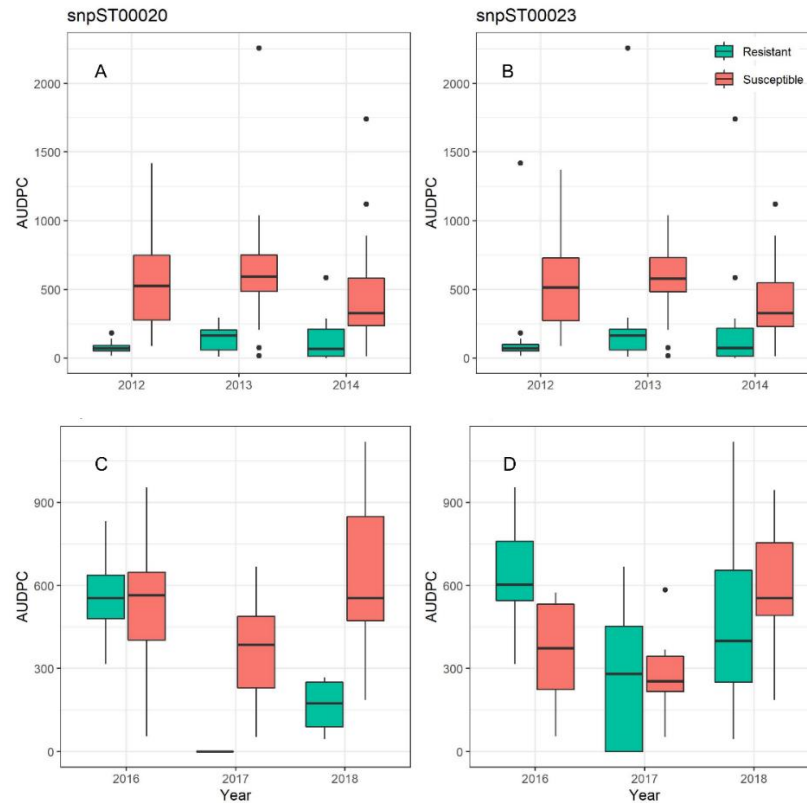
Figure 4. Boxplots of phenotypic evaluation for late blight resistance of 59 clones of the LBHT (A and B, 43 clones) and the LBHT×LTVR (C and D, 16 clones) breeding populations, separated by their snpST0020 and snpST0023 genotypes. Phenotypic data were recorded from field and screenhouse experiments for the LBHT and LBHT×LTVR, respectively (see materials and methods).

3.3. Tetraploid calls enhance QC marker efficiency

The phylogenic tree constructed using the diploid calls could not separate every two clones, whereas the tetraploid-based tree clearly differentiated all genotypes, including full- and half-sibs (Figure 5). Including the tetraploid allele dosage information for each marker-genotype combination increased the levels of heterozygosity. Three levels of heterozygosity could therefore be obtained (simplex, duplex, and triplex), allowing the separation of one-level heterozygous samples at diploid level. Further, half and full sibs did not cluster together (Figure 5), pointing towards a good discrimination ability of used markers, despite the relatively low number of used markers and the fact that one marker had been filtered out due to missing data. Such markers can therefore be used for identity analysis and are expected to serve efficiently fingerprinting purposes. Although breeding at polyploid level is complex [54,55], 4 alleles at each locus appears beneficial for QC analysis since few discriminatory markers are needed to efficiently separate the material. Lower amount of data is generated, and fewer markers should contribute to relatively lower QC genotyping cost (marker design and routine use) and data-point per genotype (computation), as compared to diploid breeding material [56–59].
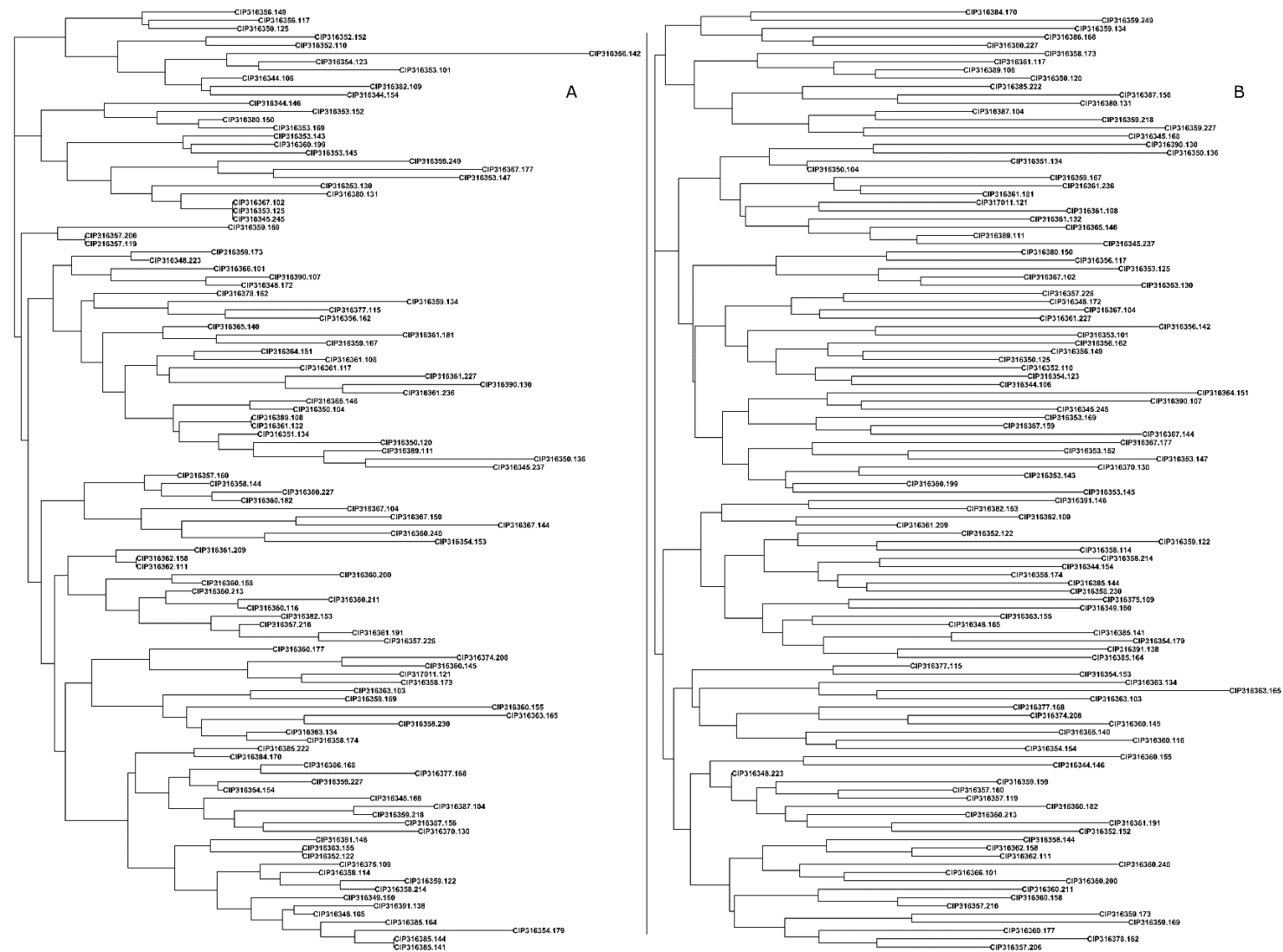
Figure 5. Neighbor joining tree constructed with 114 clones from la Molina greenhouse and 20 QC KASP markers with diploid (A) and tetraploid (B) calls. Elements before the dot sign represent the family identification.

3.4. Discrepancy in breeding material genetic identity revealed by QC markers

Considering the clones from the greenhouse as the reference genotypes, we found dissimilarities with clones from the preliminary and the intermediate trials as well as differences within the Huancayo intermediate trial (Figure 6). Out of the 38 clones sampled in Oxapampa, 7 (18.4%) were differed from the reference, against 25 of 224 samples (11.2%) in Huancayo. Within Huancayo trial, seven sampled clones from the first replication were different from their greenhouse respective reference sample. There were an intraplot plot variation in 2 of the 17 plots where three samples were taken. Additionally, we found 2 dissimilarities with the 77 clones sampled in both replications of Huancayo intermediate trail.

Mislabeled genotypes seem to occur with more frequency in breeding stages involving large number of clones in evaluation. Although mislabeled genotypes are common in vegetatively propagated crops, and QC screening of all tested clones would increase operational costs, testing a subsample of the breeding material, if not all, at each stage of the breeding program and in the seed multiplication sites may be worthwhile for maximizing breeding outputs. The higher rate of mislabeled genotypes in the preliminary trial, as compared with the intermediate trial, could occur at any step between the seed multiplication, the field operations, the material sampling, and the QC evaluation in the lab. Finding the problematic step or steps is an essential task for a consistent selection and evaluation of the breeding material throughout the breeding cycles. Accurate clonal identity has important implications in breeding progress as mislabeled clones can significantly affect the expected gains from breeding. Thus, the development of this QC marker set and its proper implementation in the routine breeding program is crucial and would be an effective strategy to reduce mislabeling and achieve the targeted genetic gain. The threshold between the number of genotypes to test and the number of markers should be defined for each breeding program, considering the genotyping costs and the available implementation budget.
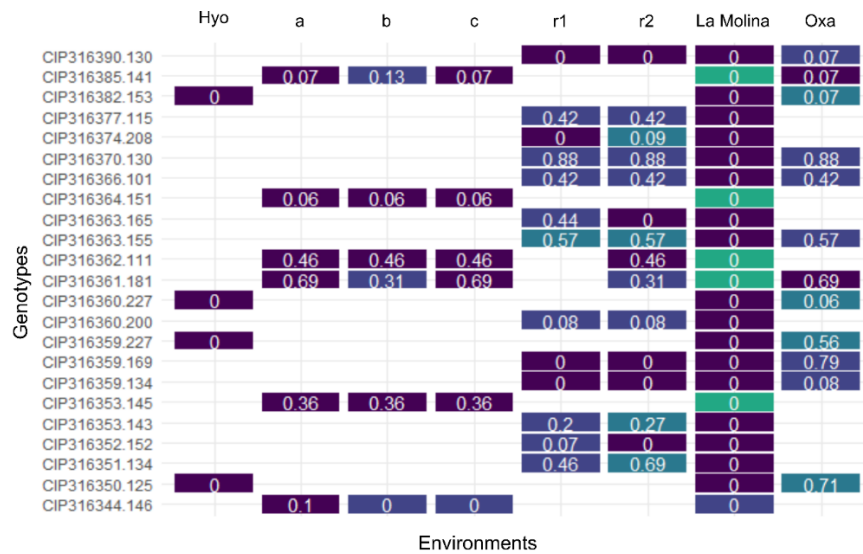
Figure 6. Proportion of dissimilarity between samples of clones taken in Hyo and Oxapampa and their corresponding reference genotype grown in the greenhouse at La Molina. Out of 114 samlples, only those with at least one difference from La Molina samples were represented. Different colors in the same row denote a somewhat genetic distance (see materials and methods) between samples of the same clone. Environments are the locations or any specification within Huacayo location where samples were taken. Hyo, Huancayo intermediate trial, samples were taken from the first replication alone; a, b and c represent the three samples taken within random plots in the first replication of Hyo; r1 and r2 denote the two different replications in Hyo; Oxa, Oxapampa preliminary trial.

4. General remarks and conclusions

SNP markers for late blight and PVY resistance, two important traits in potato, were successfully converted into KASP markers, that are amenable for high throughput system. The dosage level for each marker can be computed and selection of susceptible or simplex parents can be avoided to generate progenies with resistance to LB or PVY. More sources of resistance and markers for PVY and LB should be identified and introgressed into the breeding material, with the purpose of stacking several resistance genes with molecular assisted selection. Identified resistance SNPs can be further incorporated in any targeted sequencing platform for a more accurate genomic prediction.

The set of KASP markers tested and validated for quality control in the LBHT population may be suitable for the LTVR population. There is a need for defining a QC analysis pipeline for routine use throughout the breeding cycle. Thousands of clones are tested in the early generations of the breeding program, and a QC analysis may be a very costly activity. QC analysis only on a subset of a whole population will not inform about the non-tested clones. A mislabeled untested genotype can still be selected and therefore may show poor performance when the true tuber seed (from multiplication plots) are used for following field experiments. Although testing a subset of tested genotypes would prove more efficient than not testing at all, a routine application of QC markers should be defined to test as many genotypes as possible. Waiting for further analysis to test the QC markers' ability to assess parental purity in hybrids, a QC assessment of parental plants in the crossing blocks could be systematically envisaged, complemented by precocious crosses.

Routine QC genotyping at all stages could be possible through a genotyping budget increase or a reduction genotyping costs. Including QC markers into a targeted sequencing platform or selecting QC markers from such platform and converting them into KASP markers could also reduce the long-term QC genotyping costs. When genotyping for genomic prediction at early stages, the reference QC marker data would be generated from the targeted sequencing work and QC test in next stages could be implemented with KASP markers. The correspondence between these two marker sets must be maximal to

allow a fair comparison. In any case, KASP QC analysis will be a key molecular tool if applied at all stages of the breeding process and can contribute to a more accurate selection of breeding products, thus to an increased genetic gain.

The KASP marker assessment for potato in CIP breeding is outsourced, thus requires only minimal work for sample preparation. The turnaround time from sending the samples to Intertek/Agritech® and receiving the genotyping results is approximately 14 days, which is more than sufficient to allow a decision based on the marker genotype before the next planting or crossing season. Developed markers present therefore a great potential and their implementation will be beneficial to the breeding program.

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