Exploring the potential and limitations of genotyping-by-sequencing for SNP discovery and genotyping in tetraploid potato

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Exploring the potential and limitations of genotyping-by-sequencing for SNP
discovery and genotyping in tetraploid potato

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Abstract

Genotyping-by-sequencing (GBS) potentially offers a cost-effective alternative for SNP discovery and genotyping. Here, we report the exploration of GBS in tetraploid potato. Both *ApeKI* and *PstI/MspI* enzymes were used for library preparation on eight diverse potato genotypes. *ApeKI* yielded more markers than *PstI/MspI* but provided a lower read coverage per marker, resulting in more missing data and limiting effective genotyping to the tetraploid mode. We then assessed the accuracy of these SNPs by comparison with SolCAP data (5,824 data points in diploid mode and 3,243 data points in tetraploid mode) and found the match rates between genotype calls was 90.4% and 81.3%, respectively. Imputation of missing data did not prove very accurate due to incomplete haplotype discovery, suggesting caution in setting the allowance for missing data. To further assess the quality of GBS-derived data, a genome-wide association analysis was performed for flower color on 318 clones (with *ApeKI*). A strong association signal on chromosome 2 was obtained with the most significant SNP located in the middle of the dihydroflavonol 4-reductase (DFR) gene. We conclude that an appropriate choice of enzyme for GBS library preparation makes it possible to obtain high-quality SNPs in potato and will be helpful for marker-assisted genomics.

Keywords: genotyping-by-sequencing, potato, complexity reduction, genome-wide association mapping, marker-assisted genomics.

41 Introduction

42

43 The use of molecular markers derived from next-generation sequencing (NGS) has
44 begun to revolutionise the way plant genetic research is being conducted. Such
45 developments have been slower in potato than other species due to the complex nature
46 of its autotetraploid genome (Mehra et al. 2015; Hardigan et al. 2016). The most popular
47 NGS platform to this day is the Infinium 8303 Potato Array developed in 2012 by Felcher
48 et al. More recently, a subset of 4,179 SNPs from this array was included in the
49 development of a larger array (SolSTW) which comprises 19,747 SNPs (Vos et al.
50 2015). These arrays have been used for different purposes, such as fingerprinting (Bali
51 et al. 2016), pedigree analysis (Endelman et al. 2017), linkage mapping (Hackett et al.
52 2014; Manrique-Carpintero et al. 2015; Massa et al. 2015; Muktar et al. 2015; Obidiegwu
53 et al. 2015; Bourke et al. 2016; Endelman and Jansky 2016; Rak et al. 2017), diversity
54 analysis (Kolech et al. 2016) and linkage disequilibrium analysis (Vos et al. 2017).
55 However not all of these SNP markers can actually be used successfully in a given
56 experiment as they may not be polymorphic or may present a low minor allele frequency
57 (MAF) in a given population. For instance, a study based on the Infinium 8303 array
58 found 6,373 usable SNPs in a diploid calling mode and 3,763 usable SNPs in a
59 tetraploid calling mode within a diversity panel comprising 250 clones (Hirsch et al.
60 2013). Another work conducted on a collection of 350 tetraploid cultivated potato
61 varieties found 4,239 usable SNP markers, in tetraploid mode, after removing SNP
62 markers that mapped to multiple locations and SNP markers having more than 20%
63 missing data as well as SNP markers whose minor allele frequency was less than 5%

(Esnault et al. 2016). Similarly, the number of SNP markers available in various data sets from a panel of 537 varieties using the SolSTW array varied from 5,282 to 14,530 (Vos et al. 2015, 2017).

Another drawback of such highly parallel SNP genotyping platforms is that they typically carry a high cost per sample for first-time or small-scale users. In contrast, approaches that enable the simultaneous discovery and genotyping over the entire genome are becoming an appealing alternative. For instance, an optimised RAD-seq approach was reported in potato (Jiang et al. 2016). Similarly, genotyping-by-sequencing (GBS) approaches offer a highly cost-effective alternative for simultaneous SNP discovery and genotyping (Elshire et al. 2011; Poland et al. 2012; Sonah et al. 2013). Moreover, GBS provides other advantages such as reduced sample handling, few PCR & purification steps, no DNA size fractionation, efficient barcoding system and ease of scalability. The first work labelled as GBS in potato was conducted by Uitdewilligen et al. (2013). The targeted-GBS method used in this work was atypical in that complexity reduction was achieved through in-solution hybridisation targeting only 807 genes. This resulted in a very large number of biallelic SNPs (105,812) enjoying deep read coverage and accurate genotype assignment but all these SNPs resided within only 807 genic regions. Recently, Sverrisdóttir et al. (2017) reported a more typical GBS approach where complexity reduction was achieved strictly through enzymatic digestion (*ApeKI*). However, rather than calling actual genotypes at each SNP locus, they simply estimated variant allele frequencies at each SNP. No assessment of the accuracy of genotype calls made from GBS data was provided.

In the present investigation, we explore the use of GBS with standard complexity reduction achieved through enzymatic digestion (*ApeKI* or *PstI/MspI*) in potato and

88 devise a python script to call genotypes in diploid or tetraploid mode. We assess the
89 accuracy of these GBS-derived SNPs by comparison with SolCAP data and explore the
90 use of imputation for filling gaps in GBS data. Finally, we demonstrate the quality of
91 GBS-derived datasets by performing an association mapping for flower color using GBS
92 data among a collection of over 300 potato clones representing the diversity of an
93 Eastern Canadian breeding program.

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Material and Methods

Plant Material

A set of eight diverse potato genotypes (Set A) of different origins was used for restriction enzyme tests and optimization of SNP-calling parameters. The set included three cultivars from eastern Canada (Conestoga, Envol and Roselys), two cultivars from the United States (Eva and Russet Burbank) as well as three cultivars from Europe (Argos, Divina and Hindenburg). A collection of 375 clones (Set B) representing the extent of diversity present in a public potato breeding program in the province of Quebec (Canada) was used to conduct phylogenetic analyses and genome-wide association mapping (GWAM). This collection comprised advanced breeding clones, landraces, registered varieties and ten accessions of wild potato species (*S. phureja*, *S. cardiophyllum*, *S. sparsipilum* and *S. stenotomum*). A subset of 52 genotypes from this collection (Set B2) was selected for comparison of GBS data with the Infinium 8303 Potato Array. Among these, 49 are registered varieties and three are advanced breeding lines.

DNA Extraction, Library Preparation and Sequencing

DNA was extracted from 50 mg of fresh young leaves using the DNeasy 96 Plant kit (Qiagen, cat. no. 69181) following the manufacturer's protocol. DNA was quantified using a Thermo Scientific Nanodrop 8000 spectrophotometer (Wilmington, DE). DNA concentrations were normalized to 10 ng/μl and subsequently used for library preparation. For optimization of the method (Set A), eight potato samples were

sequenced as part of a 48-plex GBS library. The GBS libraries were prepared with enzymes that have been used for GBS library preparation in several species: *ApeKI* as per Elshire et al. (2011) and *PstI/MspI* as per Poland et al. (2012) with minor modifications described in Sonah et al. (2013). For Set B (375 clones), three 96-plex and one 87-plex *ApeKI* libraries were prepared according to the GBS protocol described by Elshire et al. (2011). For all libraries, single-end sequencing was performed on an Illumina HiSeq2000 (at the McGill University-Génome Québec Innovation Center in Montreal, QC, Canada).

Processing of Illumina Raw Sequence Read Data and SNP Calling

The IGST-GBS pipeline described by Sonah et al. (2013) was used for data processing of Illumina 100-bp reads and alignment on version 4.03 of the genome (Sharma et al. 2013). Two dosage models were used in this work. A diploid model with three marker classes for each SNP (AA, AB and BB) was used for all analyses. The minimum number of reads for genotype calls to be kept was 11. Four levels of tolerance for missing data were compared for set A (≤ 50 , ≤ 25 , ≤ 12.5 or 0%), while for Sets B and B2, markers with more than 20% missing were excluded from the dataset using vcfTools version 0.1.7 and 0.1.13 (Danecek et al. 2011).

For the genotypic data derived from Set A with the use of *ApeKI* or *PstI/MspI*, a tetraploid model with five marker classes (AAAA, AAAB, AABB, ABBB and BBBB) was also used. Here, genotype calls were conducted using the default parameters of FreeBayes v.0.9.14 (Garrison and Gabor 2012). In this case, the ploidy level was set to four and a minimum of three reads supporting an alternate allele was required to call a

polymorphism. An in-house Python script was then used to filter out markers based on read depth. Eleven reads per site per genotype were used to call homozygotes (Simko 2004) while 53 reads were deemed sufficient to distinguish between the three types of heterozygotes (AAAB, AABB, ABBB). Two filters were thus compared for genotype calling: a relaxed filter requiring 11 reads per clone to call all genotypic classes and a stringent filter requiring 11 reads to call homozygotes (AAAA & BBBB) and 53 reads to distinguish the heterozygotes (AAAB, AABB & ABBB). Marker datasets were then submitted to further filtration using the vcfilter command of vcflib (<https://github.com/ekg/vcflib>) to assess the impact of the proportion of missing data tolerated per marker on marker number and depth. Again, four levels of missing data were compared in the tetraploid model: ≤ 50 , ≤ 25 , ≤ 12.5 or 0%. Finally, markers with a minor allele count inferior to 2 were excluded from the dataset.

Comparison of GBS and Infinium 8303 Potato Array SNP calls

Genotype calls reported by Hirsch et al. (2013) were used as a reference to assess the accuracy of GBS SNP calls obtained for shared clones in this work (Set B2). Three measurements of genotype accuracy were conducted: GBS-derived diploid genotype calls, GBS-derived tetraploid genotype calls, and imputed diploid genotype calls. These imputed genotype calls were obtained using Beagle v.4.0 (Browning and Browning 2007) to impute missing data at three different levels: $\leq 20\%$, $\leq 50\%$ and $\leq 80\%$ missing data.

Genome-wide association mapping

SNP data obtained in diploid mode were used to study the population structure of set B. First, indels and SNPs with more than two alternate alleles, more than 20% missing data or a minor allele frequency inferior to 5% were removed from the datasets. The remaining markers were used to characterize population structure using a Bayesian clustering approach implemented in FastStructure algorithm (Raj et al. 2014). An association mapping analysis for flower pigmentation, assessed in a field trial in 2014 as white vs any color, was performed using the Genomic Association and Prediction Integrated Tool (GAPIT version 2) (Tang et al. 2016). As GBS had failed for one of the clones while phenotypic information was missing for 46 clones, these clones and the ten wild accessions were removed from the dataset. Thus, a total of 318 clones were used to perform association mapping. A mixed linear model (Q + K model) taking into account both population structure (Q matrix) and relative kinship (K matrix) was used. The Q covariate and the kinship matrix calculated were used to determine relatedness among individuals and eliminate false positives. The q -values for assessing the significance of marker-trait associations were calculated based on their corresponding p -values (Story and Tibshirani 2003). The q -value is a measure of significance in terms of the false discovery rate similar to the p -value that relates to the false positive rate. This approach limits the number of false positive results, while offering a more liberal criterion than the Bonferroni correction factor. A q -value of 0.1 was used as a significant association threshold (Lee et al. 2002; Benjamini et al. 2005).

Results

Comparison of *ApeKI* and *PstI/MspI* enzyme combinations

The first series of analyses was conducted to compare GBS datasets generated using a 5-base (*ApeKI*) and a 6-base/4-base (*PstI/MspI*) restriction enzyme combination as well as the diploid and tetraploid calling modes. Eight diverse potato genotypes (Set A) were used to produce both *ApeKI* and *MspI/PstI* GBS libraries. Sequencing of the GBS libraries on an Illumina HiSeq2000 yielded approximately 19.1 million reads and 19.6 million reads in total for the 8 potato genotypes with *ApeKI* and *PstI/MspI* restriction-enzyme combinations, respectively (Table 1). Sorting of raw reads was performed using the barcode information associated with each read. The number of sorted raw sequence reads ranged from 1.6 million reads (Hindenburg, *ApeKI*) to 3.0 million reads (Russet Burbank, *PstI/MspI*). On average, 72.1 and 75.4% of the reads were successfully mapped to the potato reference genome v.4.03.

In the diploid genotyping mode, using the IGST-GBS pipeline, a total of 30,605 polymorphisms (SNPs and indels) were identified from Set A using the *ApeKI* library after removing markers having more than 12.5% missing data and a minor allele frequency below 10%. Indels represented 3.2% of the total, while markers with more than two alleles accounted for a very small proportion (0.3%) of polymorphisms (Table 2). Similarly, the *PstI/MspI* library yielded a total of 13,584 polymorphisms with indels representing 4.1% of the total and markers with more than two alleles accounting for 0.8% of polymorphisms. In the tetraploid genotyping mode, using the relaxed filter, a

total of 22,946 polymorphisms (SNPs, indels, multi-nucleotide polymorphisms and complex events) were identified from Set A within the *ApeKI* library after removing markers with more than 12.5% missing data or a minor allele count below 2. Indels represented 3.9% of the total, while multi-nucleotide polymorphisms and complex events represented 3.8% of the total. Markers with more than two alleles accounted for 3.6% of polymorphisms. The *PstI/MspI* library yielded a total of 12,047 polymorphisms with indels representing 4.8% of the total while multi-nucleotide polymorphisms and complex events represented 4.1% of the total. Three- and 4-allele markers accounted for 3.8% of polymorphisms.

The impact of the tolerance towards missing data on the total number of markers and mean read depth was then examined. For Set A, in diploid mode, the smallest number of markers (15,615) was obtained using the *PstI/MspI* enzyme combination without missing data (Fig. 1a). Under these conditions mean read depth per scored genotype was 112.5 (Fig. 2a). When tolerating up to 50% missing data, the number of SNP markers rose by 59% (to 24,817 markers), while mean read depth per scored genotype decreased only by 18% (91.7). Compared to *PstI/MspI*, when keeping only markers without missing data, *ApeKI* yielded 27,263 markers with a mean read depth of 33.3 (Fig. 1a and 2a). When tolerating up to 50% missing data, the *ApeKI* restriction enzyme yielded three times more markers (74,308) than *PstI/MspI* with a read depth per scored genotype 3.5 times lower (26.0).

A similar trend was observed in tetraploid mode with both relaxed and stringent filtration. Under the basic filter condition (minimum of 11 reads required to call genotypes), the

greatest number of markers (40,199) was obtained using the restriction enzyme *ApeKI* while tolerating up to 50% missing data per marker (Fig. 1b). A mean depth of 24.9 per scored genotype was recorded under these conditions (Fig. 2b). When keeping only markers with no missing data, the total number of markers decreased to 15,336, while mean marker depth increased moderately to 30.6. The *PstI/MspI* enzyme combination yielded 2.7 times fewer markers than *ApeKI* (14,815) with four times higher depth coverage (100.2) when tolerating up to 50% missing data. When keeping only markers with no missing data, the number of markers only decreased slightly (10,324) while mean marker depth increased moderately (118.7).

The impact of enzyme choice and tolerance to missing data was more striking under the stringent filter condition where 53 reads were needed to discriminate the three heterozygous genotype classes or 11 required to call a homozygote). Here, the greatest number of markers (9,148) was obtained using the restriction enzyme *PstI/MspI* while tolerating up to 50% missing data per marker (Fig. 1c). A mean depth of 144.1 reads per scored genotype was recorded under these conditions. When keeping only markers with no missing data, the total number of markers dropped by one third (6,024 markers), while mean marker depth increased slightly (169.5) (Fig. 2c). The *ApeKI* enzyme yielded only 1,621 markers with a depth of coverage of 62.0 when tolerating up to 50% missing data (Fig. 1c). When keeping only markers with no missing data, the number of markers was reduced to only 199 while mean marker depth increased to 98.5.

Genotyping a collection of 375 clones

A second series of analyses was conducted using *ApeKI* and the diploid calling mode on a collection of 375 clones (Set B) representing the extent of diversity present in a public potato breeding program in the province of Quebec (Canada). The purpose of the analysis was to compare SNP calls made using GBS and the Infinium 8303 Potato Array and then to conduct phylogenetic as well as association analyses. For Set B, sequencing of the four *ApeKI* GBS libraries on an Illumina HiSeq2000 yielded approximately 670.3 million reads. GBS failed to yield a sufficient number of reads (<100k reads) for one clone; it was removed from the ensuing analyses. The number of sorted raw sequence reads ranged from 0.37 million reads to 2.55 million reads with a mean of 1.79 million reads. On average, 73.2% of the reads were successfully mapped to the potato reference genome.

The IGST-GBS pipeline first yielded a total of 1,514,110 markers in diploid mode. To obtain a high-quality dataset, genotype calls supported by less than 11 reads, indels and markers with more than 20% missing data were excluded. After applying these filters, a total of 42,786 SNPs remained. As this collection of clones included ten wild relatives of potato, we then examined the impact of filtration against rare alleles on the dataset. Eliminating markers with a minor allele frequency below 1% or 5% yielded 22,545 and 15,424 SNPs, respectively. The distribution of these SNPs was similar for all chromosomes and is illustrated in supplemental figure 1. It proved to be highest in gene-rich terminal regions and lowest in highly repetitive centromeric and pericentromeric regions of chromosomes. The number of SNPs varied from one chromosome to another, but was only moderately correlated with the physical length of the chromosome

($r^2=0.15$). Chromosome 10 yielded the smallest number of SNPs (767) while chromosome 1 yielded the largest number of SNPs (1,978) (Table 3). The frequency of SNPs on the twelve potato chromosomes averaged 21.1 SNPs/Mb.

Figure 3 shows the distribution of genotypic classes within the catalogue of SNPs called among Set B. Among the called genotypes, the vast majority were homozygous for the reference allele (44.8%) or heterozygous (40.5%), whereas 5.9% were homozygous for the alternate allele and 8.8% were missing.

Comparison of GBS and Infinium 8303 Potato Array SNP calls

To establish the accuracy of GBS-derived genotype calls, a comparison with data obtained using the SolCAP 8303 SNP Infinium array was conducted. From the initial collection (374 clones), a subset of fifty-two genotypes (Set B2) was selected for this purpose. Diploid genotype calls for these clones were made based on a minimum of 11 reads/genotype call while filtering out markers with more than 20% missing data and keeping all informative markers (i.e. with at least one alternate allele). Such conditions yielded a total of 45,153 markers of which 126 were in common with the Infinium 8K array. After removing 728 missing data in the GBS or 8K array data, the actual number of data points available for comparison was 5,824. Amongst these, the match rate between genotype calls was 90.4%. For homozygotes, the match rate was 98.2%, while it dropped to 84.6% for heterozygotes. Most of the 560 discordant genotype calls (83.6%) occurred when GBS did not detect an alternate allele (Table 4).

To assess if this degree of marker coverage could adequately capture the underlying haplotypes and make it possible to accurately impute missing data, we performed imputation of missing information on the GBS-derived set of SNPs. Imputed genotypes (formerly missing data) at the 126 SNP loci common between the three GBS-derived datasets and the 8K array data were then compared. As shown in Table 4, there was a slight decrease in the accuracy of these imputed data compared with GBS-called SNPs, with an accuracy of 84.2% over all SNPs. Thus, globally imputation of GBS data in diploid mode proved challenging in this polyploid crop.

In addition, a comparison between data obtained through GBS or the SolCAP 8303 SNP Infinium array was conducted for genotypes called in tetraploid mode. Again, a subset of fifty-two genotypes from the 374-clone collection (Set B2) was selected for this purpose. Tetraploid genotype calls for the 52 clones were made based on the relaxed filter requiring 11 reads per clone to call all genotypic classes while filtering out markers with more than 20% missing data and keeping all informative markers (i.e. with at least one alternate allele). Such conditions yielded a total of 16,884 GBS-derived markers of which 72 were in common with the 3,763 SNPs called in tetraploid mode using the Infinium 8K array. After removing 480 missing data in the GBS or 8K array data and 21 data where a third allele was detected with GBS, the actual number of data points available for comparison was 3,243. Amongst these, the match rate between genotype calls was 81.3%. Most of the 606 discordant genotype calls (91.4%), occurred when the two genotyping tools differed slightly in their assessment of allele dosage, for example monoplex vs duplex or duplex vs triplex (data not shown).

Genome-wide association mapping in potato

The genetic structure of the 374 clones of Set B was explored using diploid data after removing genotype calls supported by fewer than 11 reads, markers with >20% missing data and with a MAF <5%. In total 15,424 SNPs were available to perform this analysis. fastStructure analysis determined the optimal value of K to be 5 and was used to generate bar plots to visualize proportions of admixture (Fig. 4). Clones with pigmented skin were clustered in subgroup 1, all of the wild species and genetic stocks were found in subgroup 2, whereas most clones known to belong to a specific market class (yellow, round table white and French fry processing, chip processing) were found in subgroups 3, 4 and 5, respectively.

Then, to demonstrate the utility of dense SNP coverage as achieved here using GBS, we performed GWAM on a subset of Set B composed of 318 potato lines for which flower color had been determined. A total of 17,392 SNPs were selected for GWAM after removing genotype calls supported by less than 11 reads and excluding SNPs with >20% missing data and with a MAF <5%. To reduce false positive signals, population structure (covariate Q) and estimates of relatedness among individuals (covariate K) were incorporated as covariates in the MLM. In this case, the most likely value for K was found to be 4, as wild accessions had been removed when creating this subset. This model was tested on the flower colour trait and a quantile-quantile plot was produced to assess the extent to which the analysis produced more significant results than expected by chance (supplemental figure 2). A single highly significant region of marker-trait association was seen on chromosome 2, with the peak SNP showing a *p*-value as low

as 2.00×10^{-8} (Fig. 5, Supplemental Table 1). In total, 5 significantly associated SNPs spanned a region of approximately 69 kb (between 40.29 and 40.35 Mb on St02), encompassing eight genes, among which the dihydroflavonol 4-reductase locus (40293862 to 40297510) (Supplemental Table 2). Two additional, but much weaker, associations were detected on chromosomes 5 and 8.

Discussion

Choice of GBS protocol

Genotyping-by-sequencing is a versatile complexity reduction approach because it makes it possible to focus a given sequencing effort on a fraction of the genome in such a way that a higher read depth can be achieved in these regions. The required depth of coverage will depend on the level of genotyping precision required. In the case of tetraploid potato, a simplified genotypic classification wherein only three classes are called (“diploid mode”; AA, AB, BB) can be achieved with as few as 11 reads at each SNP locus. This number represents the number of reads of a single type (allele) that is sufficient to confidently call a homozygote (Simko 2004). In contrast, a full characterization into all five possible genotypic classes (“tetraploid mode”; AAAA, AAAB, AABB, ABBB and BBBB) requires a much greater depth of coverage. In this work, we estimated that 53 reads were sufficient to distinguish between the five expected genotypic classes based on a Chi-square distribution. This is similar to the conclusions drawn by Uitdewilligen et al. (2013), who concluded that read depths of ~60-80X could be used as a lower boundary for reliable assessment of allele copy number of sequence

variants in autotetraploids. It stands to reason that different degrees of complexity reduction may be needed to achieve these different levels of read depth.

Here, two restriction enzymes were compared, a five-base cutter (*ApeKI*) and an enzyme pair comprising a six-base (*PstI*) and a four-base cutter (*MspI*). It was expected that the five-base cutter would yield more markers than the six-base cutter at the expense of a lower read depth per scored marker. In diploid calling mode, *ApeKI* yielded three times more markers than *PstI/MspI* (74,308 vs 24,817) when tolerating up to 50% missing data. Under these conditions, the mean read depth per scored marker was 3.5 times lower using *ApeKI* instead of *PstI/MspI* (26.0 vs 91.7), but was still high relative to the minimum number of reads required to confidently distinguish between homozygotes and all types of heterozygotes (11 reads). Under the most demanding conditions, aiming to determine allele dosage, the *PstI/MspI* protocol yielded 5.6 times more markers than *ApeKI* (9,148 vs 1,621) when tolerating up to 50% missing data. On average, 2.3 times more reads per scored genotype (144.1 vs 62.0) were obtained under these conditions. Thus, under conditions used in this study, *PstI/MspI* should be preferred over *ApeKI* to call genotypes in tetraploid mode, while the *ApeKI* protocol maximizes the number of SNP loci that can be called in diploid mode.

GBS genotype calls are accurate

Assessing the quality of GBS genotype calls is of paramount importance for the adoption of such an approach in a new species. In this work, to assess the accuracy of our genotype calls, we compared genotypes called for the same potato clones at loci in common with the Infinium 8303 Potato Array SNP (Hirsch et al. 2013). This comprised a set of 126 SNPs and 52 genotypes that were common to both studies. In diploid mode,

after removing 728 missing data in the GBS or 8K array data, the actual number of data points available for comparison was 5,824. In the absence of imputation of missing data, the match rate between genotype calls was 90.4%. As data imputation has been demonstrated to provide highly accurate genotype calls in diploid soybean, (Torkamaneh and Belzile 2015) we wanted to explore if it could be used successfully in potato as well. This turned out not to be the case, as the proportion of concordant genotype calls fell to 84.1%. This is likely due to an insufficient marker coverage leading to incomplete capture of haplotype diversity. Although a relatively large number of markers was available to perform missing data imputation (45k), haplotype diversity in a tetraploid species such as potato tends to be quite high (Uitdewilligen et al. 2013). Accuracy of missing data imputation has been shown to decline markedly when marker coverage is insufficient (Torkamaneh and Belzile 2015). This result is lower than those reported by Uitdewilligen et al. (2013): 97.9% of the homozygous and 99.9% of the heterozygous GBS calls having been found concordant with the results of KASP genotyping. The authors argued that their method of bait-hybridisation enrichment was of great importance for genotyping accuracy because it avoided complex multicopy and repetitive elements of the genome, thus circumventing potential difficulties in distinguishing allelic variants from paralogous sequences. This could indeed explain the lower match rate reported here. In polyploid species, SNP detection is confounded by a highly similar homeologous sequence where a polymorphism between subgenomes must be differentiated from a SNP (Clevenger and Ozias-Akins 2015). Almost half of the *S. tuberosum* genome (48.8%) is composed of repetitive elements and such complex elements are associated with >95% of genes (Mehra et al. 2015). Moreover, extensive copy number variation has been documented in potato, impacting 219.8 Mb (30.2%) of

its genome with nearly 30% of genes subject to at least partial duplication or deletion (Hardigan et al. 2016). Nonetheless, these results are difficult to compare directly with ours as this work focused on a limited set of genomic regions (803) and cannot provide whole-genome coverage comparable to the one achieved above.

Aside from the complex nature of the potato genome, other causes may account for the 9.6% discordant genotype calls between GBS and 8K array in diploid mode and 18.7% discordant genotype calls in tetraploid mode. Some of these (83.6%, in diploid mode, 19.6% in tetraploid mode) occurred when GBS did not detect an alternate allele, a situation referred to as allele dropout. Such events may occur if the SNP underlying the alternate allele created or abolished an *ApeKI*, *MspI* or *PstI* recognition site and could not be captured during the library preparation process. Other polymorphisms on raw reads corresponding to a missing allele could lead to similar results. In addition, a GBS haplotype corresponding to an alternate allele could map to several locations on the genome and be eliminated in the GBS pipeline.

Lastly, the Infinium 8303 SNP array data generates variable cluster positioning and quality for each SNP. Therefore, a custom cluster file was generated for each SNP (Hirsch et al. 2013). In contrast GBS IGST pipeline treats all markers uniformly after removing all low-quality reads during the read cleaning step (Sonah et al. 2013). This sole fact could account for a sizable proportion of discrepancies between the two approaches. Nevertheless, the high match rate of genotype calls support is a proof of the high quality of GBS in potato.

Genome-wide association mapping is possible in potato

441 The success of GWAM depends on achieving an adequate marker coverage within a set
442 of germplasm. The most recent and comprehensive study on this subject, conducted
443 using the 20K SolSTW array which comprises 14,530 SNPs, concluded that such
444 coverage should be adequate to detect sufficient QTLs (Vos et al. 2017). However it is
445 important to underline that only a fraction of the SNP loci tested by an array are
446 polymorphic within a population and show a minor allele frequency high enough to
447 perform GWAM. For instance, in the same work, the number of available SNPs within
448 genotypes released after 2004 (n=255) was only 5,282 and 7,926 following filtering at
449 MAF > 10% and MAF > 1%, respectively.

450 In this work, the number of markers used to perform GWAM (17,392 SNPs) was higher
451 than what the SolSTS array could provide in a perfect situation where all SNPs would be
452 polymorphic. The analysis of the population structure allowed to group together clones
453 sharing similarities in terms of domestication (wild species and genetic stock vs
454 cultivated clones) or in terms of market classes (yellow, round table white and French fry
455 processing, chip processing), consistent with a previous study conducted using the
456 Infinium 8303 Potato Array SNP (Hirsch et al. 2013).

457 Using this dataset, we found a single strong association signal on chromosome 2 and
458 two other significant signals on chromosomes 5 and 8. Interestingly, our most significant
459 SNP on chromosome 2 at position 40,294,266 ($p = 2.00 \times 10^{-8}$) was in the middle of the
460 dihydroflavonol 4-reductase (DFR) gene and the second one at position 40,301,913 ($p =$
461 1.07×10^{-7}) was located only 4.4 kb away. The DFR gene underlies the R locus, a key
462 enzyme in anthocyanin synthesis (Zhang et al. 2009). This concurs with a QTL reported
463 to explain over 40% of the variation for flower color and believed to correspond to the R
464 locus (McCord et al. 2011).

Intriguingly, a recent study where a GWAM analysis for flower colour was conducted among a collection of 809 accessions did not identify markers associated to the DFR gene on chromosome 2 (Berdugo-Cely et al. 2017). The p values reported were slightly high, as only three markers associated with flower primary color were below 0.001. This could have been caused by an insufficient marker coverage to account for linkage disequilibrium on chromosome 2, since the Infinium 8303 potato SNP array yielded only 5,968 useful markers, almost three time less than here. In addition, the mode of scoring for flower colour could account for the differences between both studies, as their coding for primary flower colour was on a scale from 1 to 8 while a binary code (white or any colour) was used here. Globally, our ability to identify SNP markers associated to flower color through GWAM is another demonstration of the usefulness of GBS in potato.

In this work, we showed that GBS can be successfully used for various genetic analyses in autotetraploid potato. Depending on the aim of the study, different library preparation protocols are available that yield different numbers of markers and depth of coverage, one being achieved at the cost of the other. The resulting catalogues of SNPs are found to be of good quality and were used successfully to perform useful genetic analyses such as phylogenetic trees and GWAM.

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Draft

Table 1 Summary of sequenced raw and processed reads in eight potato genotypes obtained on an Illumina HiSeq2000

	Raw (Millions)		Processed (Millions)		Processed (%)	
	<i>ApeKI</i>	<i>PstI/MspI</i>	<i>ApeKI</i>	<i>PstI/MspI</i>	<i>ApeKI</i>	<i>PstI/MspI</i>
Argos	2.1	2.9	1.5	2.2	71.2	74.0
Conestoga	2.4	2.5	1.7	1.8	71.5	74.1
Divina	2.9	2.4	2.1	1.8	71.6	76.0
Envol	2.7	2.3	2.0	1.7	72.0	74.7
Eva	2.4	2.2	1.7	1.7	72.0	75.1
Hindenburg	1.6	1.9	1.2	1.4	72.7	75.2
Roselys	1.9	2.4	1.3	1.8	71.7	75.8
Russet Burbank	3.0	3.0	2.2	2.4	73.9	78.5
Average	2.5	2.5	1.7	1.9	72.1	75.4

Table 2 Summary of markers among eight potato genotypes with two restriction enzyme combinations and two calling modes for markers sustained by less than 12.5% missing data and having a minor allele frequency lower than 10% (diploid) or a minor allele count (tetraploid) lower than three

	IGST		Freebayes	
	Diploid mode		Tetraploid mode	
	<i>ApeKI</i>	<i>PstI/Mspl</i>	<i>ApeKI</i>	<i>PstI/Mspl</i>
Total number of markers	30,605	13,584	22,946	12,047
Number and	29,625	13,027	21,181	10,970
% of SNPs	96.8%	95.9%	92.3%	91.1%
Number and	980	557	904	584
% indels	3.2%	4.1%	3.9%	4.8%
Number and	/	/	861	493
% of MNPs & complex events	/	/	3.8%	4.1%

Table 3 Number of SNPs detected per chromosome by GBS among a collection of 374 clones in diploid mode after removing genotype calls supported by less than 11 reads, indels, markers with more than 20% missing data or with a minor allele frequency inferior to 5%

CHR	Length (Mb)	Nb of SNPs
1	82.6	1978
2	45.3	1539
3	57.2	1697
4	66.3	1361
5	49.5	1169
6	55.1	1133
7	52.9	1244
8	52.4	1079
9	57.3	1288
10	56.0	767
11	42.5	1061
12	57.4	1108
Total	674.4	15,424

Table 4 Comparison of GBS and SolCAP 8K array SNP calls in diploid mode with and without imputation

Genotype call		Data	
solCAP	GBS	Original	Imputed
0/0	0/0	2237	2430
0/1	0/1	2823	2902
1/1	1/1	204	31
1/1	0/0	45	240
0/1	0/0	417	644
1/1	0/1	6	8
0/0	0/1	50	57
0/1	1/1	41	38
0/0	1/1	1	20
Total concordant calls		5264	5363
Total discordant calls		560	1007
Total nb. of datapoints		5824	6370

Fig. 1 Number of markers according to percentage missing data in a) diploid mode, b) tetraploid mode under relaxed filter conditions and c) tetraploid mode under stringent filter conditions. Relaxed filter requires 11 reads to call all genotypic classes while stringent filter requires 11 reads to call homozygotes (AAAA & BBBB) and 53 reads to distinguish the heterozygotes (AAAB, AABB & ABBB)

Fig. 2 Depth of coverage per scored genotype in a) diploid mode, b) tetraploid mode under relaxed filter conditions and c) tetraploid mode under stringent filter conditions according to percentage missing data. Relaxed filter requires 11 reads to call all genotypic classes while stringent filter requires 11 reads to call homozygotes (AAAA & BBBB) and 53 reads to distinguish the heterozygotes (AAAB, AABB & ABBB)

Fig. 3 Distribution of genotypes calls within the 374 clones collection amongst 15,424 SNPs with less than 20% missing data and a minor allele frequency greater than 5%. A represents the reference allele and B represents the alternate allele

Fig. 4 Graphical display of population substructure for 374 lines based on 15,424 SNP markers with diploid genotype calls and $K = 5$ subpopulations

Fig. 5 Genome-wide association scan for flower pigmentation. The $-\log_{10}(p)$ values from the genome-wide scan are plotted against the single nucleotide polymorphism (SNP) positions on the physical map of each chromosome. The horizontal line shows the threshold corresponding to a false discovery rate < 0.10

101x76mm (300 x 300 DPI)

101x76mm (300 x 300 DPI)