SNP Markers Associated with Some Root, Stolon, and Tuber Traits in Tetraploid Potatoes (*Solanum tuberosum* L.) Grown Under Diverse Growing Systems

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Abstract

Numerous loci, environmental factors, and their interactions have an impact on the phenotypic diversity of several significant traits in plants. One approach put forth in recent years for genetic research and finding quantitative trait loci (QTLs) responsible for the specific trait is association mapping. The purpose of the current study was to pinpoint the genetic underpins of significant underground traits in potato. A panel of 192 diverse tetraploid potato genotypes from different countries were grown under different growing conditions (i.e., aeroponics and pot) to study root, stolon and tuber traits. Significant differences ($P \leq 0.01$) were found between the genotypes for all examined traits, and the heritability (H^2) of the traits ranged from 0.74 to 0.94. Genotyping was carried out using the SolCAP 25K array. 21,226 polymorphic SNPs were used for association mapping of underground traits. A *GWASpoly* R package was implemented for the marker-trait associations, and 78 genomic regions were found associated with the traits under investigation.. The history of potato breeding was reflected in LD patterns. The identified SNPs have their putative gene functions related to the root and stolon architecture and tuber growth (i.e., WRKY transcription factor, MAPK, the GTP cyclohydrolase 1 (i.e., *GTPCHI*), Glutathionyl-hydroquinone reductase, and pyrophosphate—fructose 6-phosphate 1-phosphotransferase subunit alpha (*PFPase*). The results of the present study provides a framework that could be

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helpful for future potato breeding programs to increase tuber production and reduce the challenges of feeding the world's population in the years to come.

Acronyms

AM	Association mapping
QTL	Quantitative trait loci
MAS	Marker-assisted selection
CRD	Completely randomized design
RD	Average root diameter
SFW	Stolon fresh weight
SDW	Stolon dry weight
TNT	Total number of tubers per plant
TS	Tuber shape
ATW	Average tuber weight
ATY	Average tuber yield
PCA	Principal component analysis
LD	Linkage disequilibrium
GWAS	Genome wide association study
SNP	Single nucleotide polymorphism
BLUP	Best linear un-biased prediction
NGS	Next generation sequencing

Introduction

Potato is a significant crop, as it ranks 4th in terms of production following maize, wheat, and rice and concomitantly holds 1st position among non-cereal crops. It has a lot of nutritional benefits, as it contains health promoting compounds such as carbohydrates, calcium, minerals, and antioxidants, all of which are considered essential for the human diet. Potato grows almost all over the world, and its consumption is high compared to many other crops due to its nutritious values and luscious taste. Moreover, it is also very popular and favorite among all ages of people. Due to its high consumption and the trend toward population growth, it has become necessary to breed new cultivars that are high-yielding and stress resistant in a wide range of environments.

In contrast to other major food crops, the underground portion of the potato (root, stolon, and tubers) is extremely important. An earlier study demonstrated that the root system is crucial for plant growth because it absorbs water, nutrients, and minerals while also protecting the plant from abiotic stress (Paez-Garcia et al. 2015). Root diameter trait has a significant impact on root development and functionality. The variation in root diameter trait affects root structure and the overall efficiency of the root system. Likewise, stolons have economic value. They are crucial for the storage of carbohydrates and are directly involved in the formation of tubers (final

product of the potato plant). Potato tuber traits such as shape, weight, the number of tubers per plant, and yield are considered important by farmers, fresh potato market, processing industry, and breeders as well. Results from earlier studies on the inheritance of these traits have been conflicting. Numerous loci on various chromosomes have been found to be involved in the variation of these traits in some studies, while others have suggested that they are monogenic traits (Taylor 1978; Okwuagwu 1982; Van Eck et al. 1994). Under field conditions, uncontrolled environmental factors can have a significant impact on these traits (Altman and Waisel 2012).

Using markers that are closely linked to the genes is one method of selecting the trait of interest. To find reliable markers, effective phenotyping protocols are needed (Trachsel et al. 2013). However, the lack of effective and precise phenotyping of large mapping populations has been a major impediment to the widespread application of genetic data related to underground traits in breeding programs (de Dorlodot et al. 2007; Chen et al. 2011). Traditional field evaluation methods can damage sensitive roots, stolons, and tubers. To overcome these issues, effective indoor growing platforms such as aeroponics and pot trials have emerged as feasible solutions (Chen et al. 2015). In phenotyping studies on underground traits, the entire root system is removed from pots or other containers filled with soil using destructive sampling, which provides an environment for crop growth that may mimic the field conditions (Chen et al. 2015).

In recent years, non-destructive techniques for phenotypic modeling of underground characteristics, such as aeroponics, have been developed (Chen et al. 2015). It improves accessibility and high-throughput phenotypic capacity of these essential underground traits in potato. High throughput imaging techniques can then be used to produce precise and accurate phenotyping results. When compared to other programs, "WinRHIZO" is the most flexible and reliable option for completing a variety of specialized tasks (Pierret et al. 2013).

The rapid development of genome-wide markers (SNPs) made possible by recent advances in next-generation sequencing (NGS) has allowed researchers to examine the relationship between genetic and phenotypic diversity at a level of resolution that was previously unattainable. An association mapping (AM) technique may help potato breeders to develop selection markers for underground traits. Therefore, the selection markers developed by AM can be used in marker assisted selection (MAS) and may boost tuber yield (Spindel et al. 2015). AM facilitates the identification of specific alleles associated with a trait (Oraguzie et al. 2007). AM has advantages over QTL mapping with biparental crosses because more variations can be observed in AM and the mapping resolution is typically better or higher (Gupta et al. 2005; Myles et al. 2009).

Using the NGS platform in AM studies for QTL identification, SNP variants of potatoes with biological variations of underground traits can be found (Bradshaw et al. 2008; Iwama 2008). Since these traits are polygenic, AM based on SNP markers is a potent tool for analyzing these complex traits and for discovering new QTLs and selection markers to select the genotype of interest with the desired traits, which ultimately supports MAS. Understanding the genetic underpinnings of these traits, and developing molecular markers for MAS can be accomplished by genotyping

different potatoes and comparing their underground traits' performance across various growing systems.

Very little or infrequent attention has been paid to the relationships between the underground traits of potatoes so far. The current study postulated that genotypic variation and measurable biological variation in the roots, stolons, and tubers exist in various potato genotypes, that can be utilized to identify the genomic regions associated with these traits. Therefore, the study aimed to investigate biologically significant variation in the root, stolon, and tuber traits under aeroponic and pot conditions for the discovery of genomic regions associated with these vital traits through association mapping.

Materials and Methods

Plant Material and Experimental Setup

A diverse panel of 192 tetraploid potato (*Solanum tuberosum* L.) genotypes was used in the experiment (detailed information about genotypes is available in Yousaf et al. (2021)). The panel was selected owing to the wide range of genotypes from Turkey and Germany and evaluated for the underground traits. To derive phenotypic data, the experiment was conducted under diverse growing conditions as (1) Aeroponic-2019, (2) Aeroponic-2020, and (3) Pot-2020.

Aeroponic

In the summer of 2019 and the winter of 2020, Aeroponic-2019 and Aeroponic-2020 experiments, respectively, were carried out in an aeroponic growing system in order to acquire the phenotyping data of underground traits. Sprouts were transplanted to aeroponic production beds with 20 cm spacing between rows and 10 cm in-row spacing, with a completely randomized design (CRD). Each genotype was planted in seven replicates. Complete information about aeroponic experimentation can be found in our previous study (Yousaf et al. 2021).

Pot

The pot experiment, Pot-2020, was carried out in the net house at the Faculty of Agricultural Sciences and Technologies (FAST), Nigde Omer Halisdemir University Nigde, Turkey (37°56′32 N, 34°37′25 E, 1229 m elevation). The phenotyping data of under-ground traits in the pot experiment were obtained by using specially made cylindrical pots (5 L) with dimensions of 22.5 cm top diameter, 16.5 cm bottom diameter, and 18 cm depth. Pots were filled with a 2:1 v/v mixture of peat and perlite before the tubers were planted. The experiment was conducted by following a completely randomized design (CRD) with three replications between June and

September 2020. Until harvest, plants received regular irrigation and two applications of N-P-K (18%-18%-18%) fertilizer.

Figure 1 showed minimum, maximum and mean temperature (°C) values during the experiment. During the growing season, standard potato production practices were used.

Phenotyping

In the present study, root traits such as average root diameter (RD); stolon traits including stolon diameter (SD), stolon fresh weight (SFW), and stolon dry weight (SDW); tuber traits such as total number of tubers per plant (TNT), tuber shape (TS), average tuber weight (ATW), and average tuber yield (ATY) were investigated. These traits were examined through five and three samples per genotype in aeroponic and pot experiments, respectively. Table 1 explains the phenotyping descriptors for the aforementioned traits. See Fig. 2 for phenotyping tuber shape.

Statistical Analysis

Using Statistix 8.1 software, a one-way analysis of variance (ANOVA) based on a completely randomized design was conducted on the data from Aeroponic-2019, Aeroponic-2020, and Pot-2020. Using the *lme4* package in the R, the best linear unbiased prediction (BLUP) was calculated (Bates et al. 2015). Through the *GWASpoly* R package, the marker-trait association was calculated using the BLUP values of all traits. The studied traits were correlated by Pearson correlations (r) using the SPSS software, and the *"psych"* package in R was used to analyze the correlation scatter matrix. Each trait's broad-sense heritability (H^2) was calculated using the equation ($H^2 = V_G/V_P$). Where;
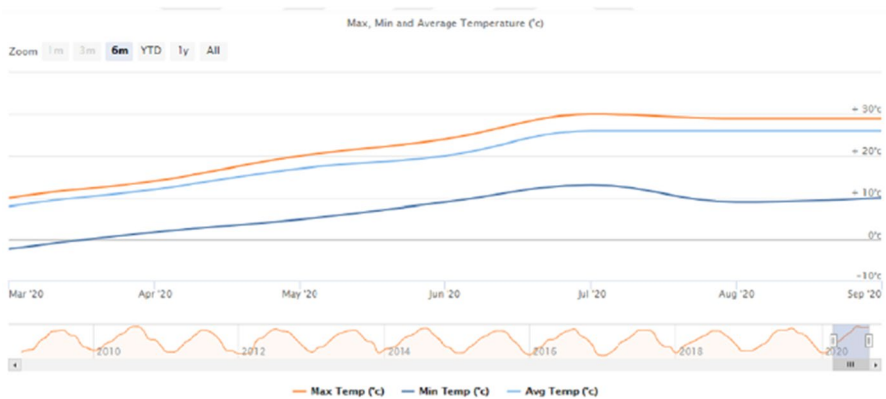
Fig. 1 During the growing season of 2020, the minimum, maximum, and average temperatures (°C) under pot experiment

Table 1 Phenotyping description of the studied traits

Trait	Phenotyping description
Average root diameter (RD)	The cumulative root diameter of the entire root system was measured using a scanner with the help of WinRHIZO software in millimeter (mm)
Stolon fresh weight (SFW)	The fresh weight of the entire stolon system was measured immediately at harvest by a calibrated precision scale
Stolon dry weight (SDW)	The stolons were oven dried at 70 °C until a constant mass was reached after 24 h and then the samples were weighed using a calibrated precision weighing balance
Total number of tubers per plant (TNT)	The total number of tubers per plant was counted and averaged
Tuber shape (TS)	The shape of tubers was scored according to the potato descriptors (Fig. 2) (Potato Guidelines by International Union for Protection of New Varieties of Plants in GENEVA). As per the descriptor, tuber shape was classified into six different classes i.e., Round-1, Short Oval-2, Oval-3, Long Oval-4, Long-5, Very Long-6
Average tuber weight (ATW)	The average tuber weight was calculated by the following formula: $\text{Average tuber weight} = \dfrac{\text{Total wt. of tubers/plant}}{\text{Total no. of tubers/ plant}}$
Average tuber yield (ATY)/genotype	The average tuber yield was calculated by using the following formula: $\text{Average tuber yield} = \dfrac{\text{Total wt. of tubers/genotype}}{\text{Total no. of replications/genotype}}$
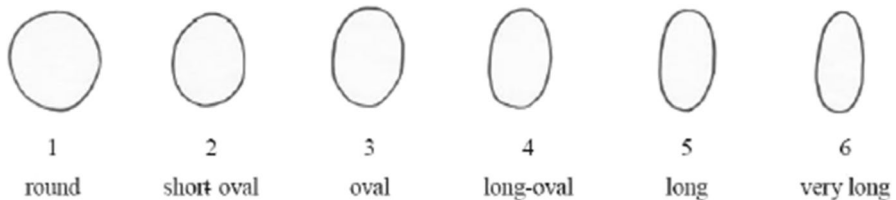
Fig. 2 Classes/descriptor of tuber shape

V_G and V_P are genotypic and phenotypic variance components, respectively (Bahmankar et al. 2014; Ogunniyan and Olakojo 2014).

Genotyping for SNP Markers

Genomic DNA was extracted according to standard protocol by using Gene JET Plant Genomic DNA Purification mini kit (Thermo Scientific). SNP genotyping was done by utilization of SolCAP 25 K potato genotyping array (Hamilton et al.

2011; Uitdewilligen et al. 2013). Further analysis to obtain 21,226 SNP markers were done according to the procedures outlined by Yousaf et al. (2021).

Analysis of the Population Structure, Principal Components (PCA) and Linkage Disequilibrium (LD) Assessment

STRUCTURE software was used to analyze the population structure (Pritchard et al. 2000). Principal component analysis (PCA) was performed by using *Prcomp* package R v. 3.6.3 as described by Yousaf et al. (2021).

Based on D′ and r^2, TASSEL was used to calculate the LD between marker pairs (Hill and Robertson 1968; Achenbach et al. 2008; Yousaf et al. 2021).

Association Mapping, Physical Map, and Gene Annotation

"Q + K" linear mixed model approach for autopolyploids was used for association mapping analyses in the *GWASpoly* R package (Rosyara et al. 2016). The equation for Q + K linear mixed model (Yu et al. 2006; Kang et al. 2008) is described as:

$$y = X\beta + ZS_T + ZQv + Zu + \varepsilon$$

where **y** is a vector of observed phenotypes, **β** is a vector of fixed effects with incidence matrix **X** to model environmental effects and effect of covariates, **v** is a vector of subpopulations effect, with incidence matrix **Q** for a population, and u is a vector of effects of polygenic, with covariance proportional to a kinship (or relationship) matrix, Var[u] = **g2K**. The incidence matrix **Z** maps genotypes to observations, and the SNP effect is represented by the vector **τ**, where the structure of the incidence matrix **S** and the dimension d depend on the genetic model, **ε** is a vector of residuals, Var[ε] = **I²ε.**

In the present study, additive gene action was taken into account. rrBLUP is necessary for *GWASpoly* R to function properly and accurately. Through the use of this package, Quantile–Quantile (q-q) plots were also created. Following Bonferroni correction method, a threshold of -log10(P) = 3.23 was used as illustrated by Yousaf et al. (2021). Manhattan plots were visualized utilizing the *GWASpoly* R package. Localization of SNP markers at particular regions on the chromosome were done through the R package "*LinkageMapView*". The gene annotation of SolCAP SNPs was revealed by the NCBI BLAST database (Hamilton et al. 2011).

Results and Discussion

Analysis of Phenotypic Traits

For all the studied traits, descriptive statistics and ANOVA revealed significant variation and highly significant (*P < 0.001*) differences (Table 2 and 3). The mean data

Table 2 ANOVA showing significant variation in measured traits

Trait SOV F value				
		Aeroponic-2019	Aeroponic-2020	Pot-2020
RD	Genotypes	6.42**	4.11**	3.86**
SFW	Genotypes	74.9**	7.52**	3.79**
SDW	Genotypes	49.1**	5.61**	1.57**
TNT	Genotypes	3.11**	4.34**	2.76**
TS	Genotypes	11.8**	7.41**	74.8**
ATW	Genotypes	5.93**	2.73**	3.22**
ATY	Genotypes	5.76**	2.54**	5.21**

SOV Source of variation

**means significant at $P < 0.01$

Table 3 Descriptive statistics of the studied traits

Traits	Aeroponic-2019 ± SE	Aeroponic-2020 ± SE	Pot-2019 ± SE	Mean ± SE	Min	Max
RD (mm)	0.95 ± 0.02	0.76 ± 0.01	0.89 ± 0.02	0.87 ± 0.01	0.51	1.33
SFW (g)	26.55 ± 2.57	8.53 ± 0.76	6.43 ± 0.49	14.09 ± 1.07	0.61	89.54
SDW (g)	1.63 ± 0.15	0.54 ± 0.04	0.49 ± 0.04	0.91 ± 0.07	0.03	6.06
TNT	2.01 ± 0.14	4.11 ± 0.18	5.96 ± 0.24	4.00 ± 0.13	1.00	13.67
TS	2.19 ± 0.12	3 ± 0.12	2.66 ± 0.09	2.61 ± 0.08	1.00	5.00
ATW (g)	8.85 ± 0.75	16 ± 0.79	21.32 ± 1.12	15.26 ± 0.53	2.62	36.64
ATY (g)	25.08 ± 2.24	62.44 ± 3.27	126.79 ± 7.88	71.00 ± 3.33	5.20	342.67

SE Standard error, *Min* Minimum, *Max* Maximum, *RD* Root diameter, *SFW* Stolon fresh weight, *SDW* Stolon dry weight, *TNT* Total number of tubers per plant, *TS* Tuber shape, *ATW* Average tuber weight, *ATY* Average tuber yield

of the traits (ARD, SFW, SDW, TNT, TS, ATW, and ATY) for the Aeroponic-2019, Aeroponic-2020, and Pot-2020 were 0.51 to 1.33 mm, 0.61 to 89.54 g, 0.03 to 6.06 g, 1.00 to 13.67, 1.00 to 5.00, 2.62 to 36.64 g, and 5.20 to 342.67 g, respectively. Boxplots were generated to display the mean data ranges (Fig. 3). Mean values were used to find the correlation among the traits. According to Bisognin et al. (2012), tuber shape is related to average tuber weight. Similarly, Khayatnezhad et al. (2011) discovered a highly significant correlation ($r = 0.99$) between average tuber weight and average tuber yield similar to our results like SFW and SDW ($r = 0.98$), ATY and ATW ($r = 0.69$), ATY and TNT ($r = 0.72$) showed a strong correlation (Fig. 4). The negative correlation of ATW, and ATY with SFW and SDW showed that more accumulation of assimilates in stolons increases stolon fresh weight and reduces average tubers yield (Lahlou and Ledent 2005). Midmore (1984) discovered that stolon length increased due to tuber initiation delay, which could be due to high temperatures (as observed in Aeroponic-2019), allowing more time and providing more assimilates for further stolon formation. This result corresponded to fewer tubers obtained in Aeroponic-2019 due to increased mobilization of assimilates
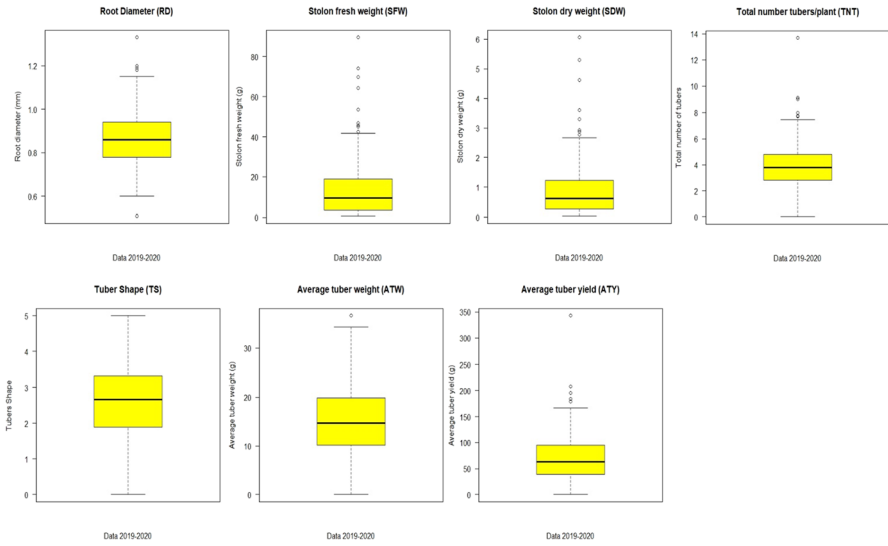
Fig. 3 Boxplots showing the means data range of studied traits

Fig. 4 Pearson correlation coefficients (*r*) (upper boxes), Pairwise scatter plot matrix (lower boxes), and histograms depicting data distribution (diagonal boxes) of studied traits of potato
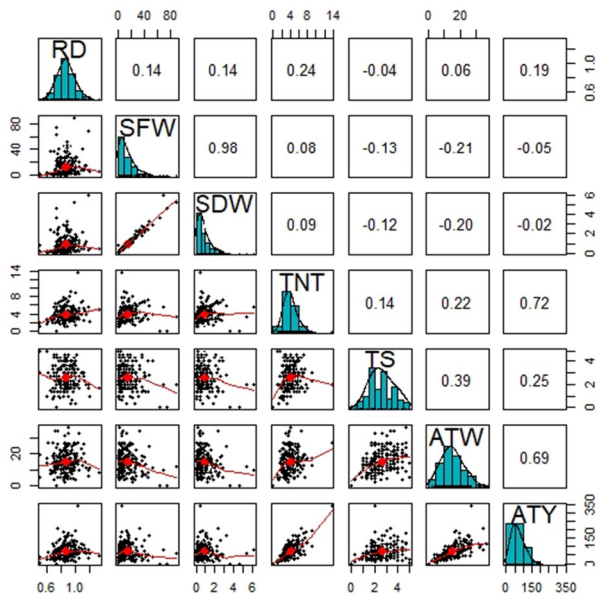
toward long and thick stolon formation with more stolon fresh weight. However, the Aeroponic-2020 and Pot-2020 experiments revealed more tubers with lower SFW and SDW (Table 3). Alongside, RD is more in Aeroponic-2019 as compared to Aeroponic-2020. The average temperature at the experiment of Aeroponic-2019 was relatively higher than Aeroponic-2020, which may influence these polygenic traits (Boguszewska-Mańkowska et al. 2020). Sattelmacher and Marschner (1990)

reported that potato plants reduced root size, root diameter, and root surface area if the temperature dropped to less than 20 °C. Again RD was higher in the pot experiment as compared to the aeroponic growing condition. Potato tuber yield is temperature sensitive, with a decreasing trend as the average temperature rises above 27 °C (Yandell et al. 1988). Lafta and Lorenzen (1995) and (DEMİREL et al. 2017) discovered that high temperatures resulted in the formation of fewer tubers. All tuber traits (TNT, ATW, and ATY) were higher at the Pot-2020 compared to the aeroponics. Better moisture retention and slower nutrient release under soil conditions may have contributed to the increase in tuber traits observed in the pot experiment. The longer growing season and multiple harvests in the aeroponics system, on the other hand, can attribute to an increase in the number of tubers per plant (Çalışkan et al. 2010). In our all experiments, single harvesting was done which may also contribute to the lower number of tubers in aeroponics especially.

A histogram showing the data distribution pattern and a visual representation of the Pearson correlation results are presented in Fig. 4. In our experiment, higher H^2 values illustrate the genetic contribution in the measured traits which implied that these were stable traits under the various growth conditions (Rodrigues and Pereira 2003; Phung et al. 2016; Liu et al. 2017) However, in the pot experiment the heritability ranges from 0.55 to 0.99 it means, the environment may also influence the biological variation among phenotypic traits (Table 4).

Population Structure and Linkage Disequilibrium (LD)

STRUCTURE analysis revealed that the genotypes were divided into four subpopulations or clusters, as indicated by the value of delta K = 4 (Yousaf et al. 2021). In the literature for GWAS, a variable number of "clusters/subpopulations" or "kinship groups" were identified, such as K = 6 (Rosyara et al. 2016), K = 3 (Vos et al. 2017), K = 10 (Sharma et al. 2018), K = 3 (Klaassen et al. 2019), and K = 4 (Zia et al. 2020). Our results revealed that studied population possesses genetic diversity as STRUCTURE outcome has been validated together with results of PCA analysis. These findings were also discussed in detail by Yousaf et al. (2021).

LD was performed on only polymorphic markers obtained after filtering steps carried out to curtail monomorphic markers. A total of 13,606 polymorphic SNP markers were obtained after filtering monomorphic 21,226 SNP markers. Results of LD showed that 49.98% of the markers in our population had a non-random association between different loci with average r^2 value of 0.29. Moreover, LD decay was 2.316 Mbps in a proposed marker data set. (Gupta et al. 2005; Stich et al. 2005) found that there is very less chance of recombination if the linkage is within 2 Mbps. Detailed information on genotypes' distribution into 4 clusters and admixed sub-population as well as LD analysis has been described in our previous study (Yousaf et al. 2021).

Table 4 Broad-sense heritability (H^2) of the traits for the year 2019 and 2020

Traits	Genotypic variance				Phenotypic variance				Heritability			
	Aero-ponic-2019	Aero-ponic-2020	Pot-2020	Mean	Aero-ponic-2019	Aero-ponic-2020	Pot-2020	Mean	Aero-ponic-2019	Aero-ponic-2020	Pot-2020	Mean
RD	0.13	0.05	0.12	0.10	0.15	0.06	0.15	0.12	0.86	0.79	0.78	0.81
SFW	3057.00	323.09	99.85	1159.98	3097.97	367.55	128.73	1198.08	0.99	0.88	0.78	0.88
SDW	11.09	1.21	0.95	4.42	11.32	1.44	1.71	4.83	0.98	0.84	0.55	0.79
TNT	9.41	14.56	18.14	14.04	12.70	18.11	25.64	18.82	0.74	0.80	0.71	0.75
TS	6.85	4.87	2.49	4.74	7.44	5.36	2.53	5.11	0.92	0.91	0.99	0.94
ATW	310.37	266.71	450.88	342.65	365.04	374.09	606.99	448.70	0.85	0.71	0.74	0.77
ATY	3163.24	4632.29	23,156.17	10,317.23	3736.85	6654.41	27,908.87	12,766.71	0.85	0.70	0.83	0.79

RD Root diameter, *SFW* Stolon fresh weight, *SDW* Stolon dry weight, *TNT* Total number of tubers per plant, *TS* Tuber shape, *ATW* Average tuber weight, *ATY* Average tuber yield

Association Mapping (AM)

Q-Q plots were drawn to examine the normal distribution of the data, by using a scale of -log10(P) between the observed and expected values. It further highlights the false positives, family structure, and population stratification. If there was a uniform distribution (1:1 straight line without tailing on either side between expected and observed -log10 (P) values), indicating no significant correlations or polymorphisms, the null hypothesis was accepted as true. The deviations from the straight line at the tail suggest that the null hypothesis was rejected with the presence of significant correlations or polymorphism. Utilizing BLUPs data of root, stolon, and tuber traits, association mapping was performed by integrating additive gene action in the *GWASpoly* R package and the results were reconfirmed with "potato genome assembly" and found to be the same. A total of 78 SNPs were found to be associated with root, stolon, and tuber traits. Among them, 8 were linked with a root trait (RD), 20 were associated with stolon traits such as SFW and SDW and 51 were related to tuber traits such as TNT, TS, ATW, and ATY, and 3 SNPs were found with pleiotropic effect means they were related to multiple traits. Furthermore, 34 SNPs (43%) were about PotVar, and 44 SNPs (56%) were SolCAP. PotVar SNPs were referred to by Uitdewilligen et al. (2013), while SolCAP SNPs were discovered by Hamilton et al. (2011).

Average Root Diameter (RD)

Root development and functionality are significantly influenced by root diameter. Root structure and the overall efficiency of the root system were affected by the variation in root diameter. On chromosomes 5, 6, and 11, eight significant SNP markers were identified to be associated with RD, whereas one SNP marker was discovered on an unmapped chromosome (Fig. 5 and 6). The identified SNPs were included in the genes of WRKY transcription factor, magnesium transporter, dihydropterin pyrophosphokinase-dihydropteroate synthase, UDP-glucose:protein transglucosylase, and mitogen-activated protein kinase kinase (MAPKK) (Supplementary Table 1S). According to Koch et al. (2020), magnesium transporters (*StMRS2*) play an important role in the regulation of root growth by re-mobilization and photoassimilate partitioning of Mg in potato, from source to sink. Abscisic acid was controlled by MAPKK, and this has an impact on plant roots' architecture (including cell division and root elongation) (Li et al. 2017).

Stolon Fresh Weight (SFW)

Carbohydrates are stored in a stolon which is necessary for the development of tubers, so they are crucial for tuber formation. Here we identified 9 significant SNP markers associated with the trait of interest on chromosome 4 (Fig. 1S). It has been
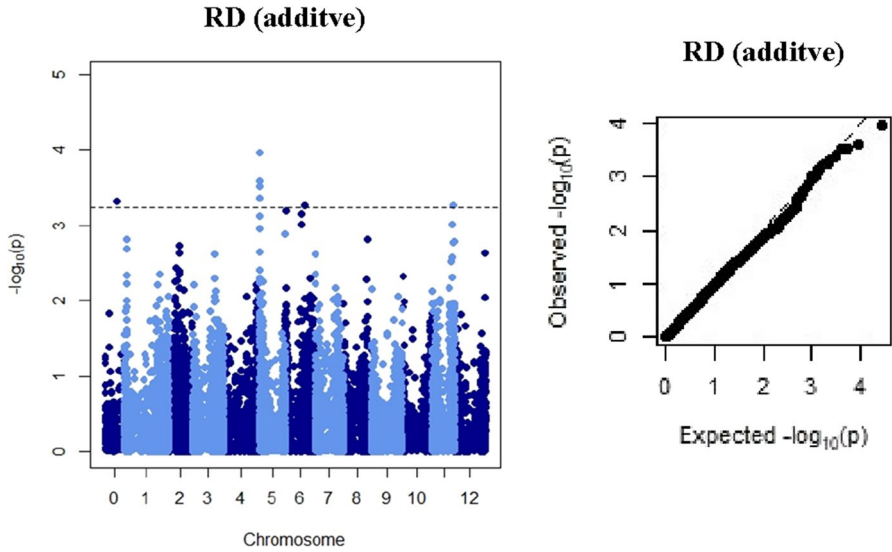
Fig. 5 Manhattan plot showing significant SNPs associated with root average diameter (left). Q-Q plot depicting the normal distribution of the data (right)
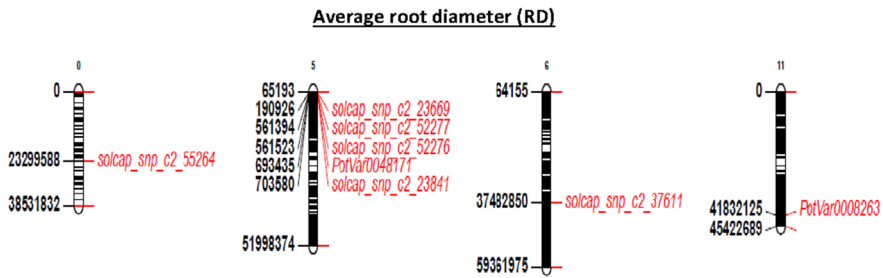
Fig. 6 Physical position of identified SNPs on the chromosomes

determined that chromosome 4 is crucial for stolon fresh weight. The majority of the SNPs on chromosome 4 are closely linked to each other (less than 2.31 Mbp) (Fig. 6S). Identified SNPs were in the genes of WRKY transcription factor, and Probable pectin methyltransferase *QUA2* (Table 1S). The research revealed that rice *OsQUA2* and the Arabidopsis mutant (*AtQUA2*) share a similar sequence, and a mutation in *OsQUA2* caused a decrease in HG esterification in the culture sieve element and root cell walls, which ultimately affects sucrose transport and the development of roots and other underground parts, respectively (LiangHuan et al. 2016; Xu et al. 2017). Other SNPs with putative functions, such as sucrose transporter 4, (*StSUT1*), and sucrose synthase genes (*Sus3* and *SuSy*), have been identified. These SNPs are involved in the transport and storage of sucrose in sink organs, such as potato tubers. (Table 1S). According to research by (Gallou et al. 2012), the WRKY transcription factor genes are involved in the mechanisms regulating

the establishment of arbuscular mycorrhizal fungi through the regulation of plant defense genes, which in turn contribute to the growth of underground parts (such as stolons) of potatoes.

Stolon Dry Weight (SDW)

On chromosomes 3 and 4, three SNP markers were discovered to be associated with SDW. On unmapped chromosomes, eight SNP markers were discovered (Fig. 2S. 4S and Table 1S). The narrow region of 0.07 Mbp (66.14 to 66.21 Mbp) on Chr 4 (having a pleiotropic SNP) was found to be associated with SFW and another stolon trait (not included in this article). These identified SNPs are included in some putative genes such as Protein TRANSPARENT TESTA GLABRA 1-like, Erg28, and Homeotic protein knotted-1 (Table 1S).

Total Number of Tubers per Plant (TNT)

Since TNT showed a non-normal distribution. − log10 transformation was implemented to normalize the data. Q-Q plots were produced using the normalized data (Fig. 4S). Three SNP markers were discovered on chromosomes 6 and 7 that were associated with the TNT (Table 1S and Fig. 5S). These identified SNPs were included in putative genes of NTGP4, GTP cyclohydrolase, and glucan endo-1,3-beta-glucosidase 12-like. The GTP cyclohydrolase 1 (i.e., *GTPCHI*) participates in the synthesis of the mineral folate, which is crucial for better growth and development. Using diploid potato species, Manrique-Carpintero et al. (2015) identified QTLs on chromosome 5 associated with TNT. Rak et al. (2017) used an auto-tetraploid family to conduct QTL mapping for the TNT trait and discovered that the trait is associated with chromosomes 4, 5, and 10. In our findings, we discovered an SNP associated with this trait on chromosomes 6 and 7, which contradicts the findings of Manrique-Carpintero et al. (2015) and Rak et al. (2017). Contradiction is quite normal because it is possible due to previous studies using diploid mapping populations and biparental QTL mapping technique.

Tuber Shape (TS)

TS is another very critical trait in the processing industry and breeding. Twenty-one SNPs were identified related to TS on the chromosomes of 1, 2, 8, 9, and 10. However, one SNP was found on the unmapped chromosome (Chr 0) (Fig. 5S). A physical map of the identified SNPs is presented in Fig. 7S. Eleven SNPs were present on Chr 10 within a narrow region of 0.56 Mbp (48.61 to 49.17 Mbp). The SNP named solcap_snp_c2_38942 found on Chr 2 is included in the gene of Glutathionyl-hydroquinone reductase (Table 1S) that might detoxify ROS species like H_2O_2 to H_2O and maintain cellular redox homeostasis during tuber development (Cheng et al. 2020). Several studies have been conducted to identify the genomic regions that control

the tuber shape trait. Similar to our findings, another genetic mapping study using heterozygous full-sib diploid potatoes (06H1) was conducted using a set of 8,303 single nucleotide polymorphism (SNP) markers. This study found significant QTLs on chromosomes 2 and 10 (Prashar et al. 2014). By using RFLP markers in a diploid potato population, some QTLs associated with tuber shape on chromosomes 2 and 10 were also discovered by Van Eck et al. (1994), and Endelman and Jansky (2016). In a diploid population of potatoes, two additional studies also identified the QTLs for tuber shape on chromosome 10 (Hara-Skrzypiec et al. 2018; Meijer et al. 2018). One more study was recently published in which they found significant SNPs related to TS on chromosomes 2, 4, and 10 by implementing association mapping coupled with SolCAP 20 K (Zia et al. 2020), that is in line with our findings as well as with Lindqvist-Kreuze et al. (2015). According to the prior literature, genomic regions discovered on chromosomes 2 and 10 were therefore regarded as potential QTLs related to TS.

Average Tuber Weight (ATW)

A non-normal distribution observed at ATW, therefore, a logarithmic transformation was executed for the data normalization. Twenty SNPs were found associated with the ATW on chromosomes 3, 4, 6, and 7 and a physical chromosomal map was drawn to visualize the location of identified SNPs (Fig. 9S, 10S). A region spanning 2.40 Mbp (69.62 to 72.02 Mbp) on Chr 4 carries eight SNPs positioned within a narrow distance of 0.74 Mbp (70.06 to 70.80 Mbp) linked to ATW. The identified SNPs are included in the gene of inositol-pentakisphosphate 2-kinase, which participates in the phytic acid biosynthetic process (Sun et al. 2007). Mature tubers contain phytic acid, which is a significant phosphorous mineral reservoir in tubers. In starchy crops like rice and potatoes, pyrophosphate—fructose 6-phosphate 1-phosphotransferase subunit alpha (*PFPase*) is involved in sucrose synthesis, glycolysis, and starch metabolism (Chen et al. 2020). In line with our findings, a previous study (Hara-Skrzypiec et al. 2018) found QTL for tuber weight on chromosomes 1, 4, 5, and 6 in a diploid potato mapping population consisting of 149 individuals. Similar to this, Rak et al. (2017) used the bi-parental family W9817 from the University of Wisconsin potato breeding program, which consists of 110 individuals obtained by a cross of chipping cultivar Liberator and Wisconsin breeding clone W4013-1, to draw a linkage map and identify 3 significant markers for average tuber weight on chromosome number 5. However, we could not identify any association with markers on chromosome 5 in our study. It is completely normal because it could be due to differences in the mapping populations used in the studies.

Average Tuber Yield (ATY)

A Manhattan plot revealed six significant SNP markers linked to ATY. These SNPs were spread out across chromosomes 5, 8, and 9 (Fig. 11S and Table 1S). Figure 12S shows a physical map based on the base pair distance between the SNP markers linked

to the investigated tuber trait. ATY was linked to a region on Chr 8 that spans 0.64 Mbp (47.37 to 48.01 Mbp). Additionally, the discovered SNP markers are included in genes such as F-box/kelch-repeat protein At3g23880-like, Carotenoid cleavage dioxygenase 4, DIAMINOPIMELATE EPIMERASE, and Isoamylase isoform 2. One SNP (PotVar0122020) included in a conserved gene of unknown function. Our findings are consistent with those of Manrique-Carpintero et al. (2015), who discovered that the tuber yield trait is linked to chromosome 5, as well as chromosomes 2 and 12. We discovered that tuber yield is linked to chromosomes 5, 8, and 9. Similarly, Rak et al. (2017) discovered QTLs on chromosomes 2, 5, and 11 during QTL mapping of the tuber yield trait using the auto-tetraploid family. Previous research, consistent with our findings, revealed that chromosome 5 was significantly important for ATY, indicating the presence of possible QTL controlling tuber characteristics such as tuber yield and can be used for future association mapping studies.

Conclusion

In our study, 78 SNP markers were identified related to some important underground traits in potato by implementing association mapping. Several discovered genomic regions were novel and can be potential selection markers for upcoming breeding programs. Among these 78 markers, 8, 9, 11, 3, 21, 20, and 6 markers were related to RD, SFW, SDW, TNT, TS, ATW, and ATY respectively. The average data from both aeroponic and pot experiments showed substantial phenotypic variation for the underground traits studied. Additionally, highly significant genotypic effects were found on the traits. While SFW was negatively correlated with SDW, ATY was positively correlated with ATW and TNT. Following validation, breeders can use the identified markers to expedite breeding programs and meet the deemed need for food due to population growth.

Declarations

Conflict of interes The authors declare no conflict of interest.

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