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Development of SSR Markers to Characterize Potato (*Solanum tuberosum* L.) Somaclones with Improved Starch Accumulation

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THIS STUDY aimed to establish functional SSR markers corresponding to variations in starch accumulation in potato somaclones. In addition to the original cultivar Lady Rosetta, fourteen callus-sourced somaclones were investigated for their starch and sugar contents as well as dry matter content. Of the selected somaclones, one represented clones with the same starch content of the original cultivar and another represented those of lower starch content. The remaining somaclones have higher starch content, compared with original cultivar. The combined results of two growing seasons unveiled significant positive correlation between dry matter content and starch content which was absent when replacing starch with soluble sugars. Insignificant correlations were recorded between sugar content and starch content. Based on the transferability of SSR markers between species, six SSR primers characterizing variation in starch accumulation in sweet potatoes were exploited to assess genetic diversity among the selected potato clones. The analysis generated distinct and reproducible banding patterns with 68 bands, of which 62 were polymorphic. The utilized primers reflected high resolving power appeared in PIC of 0.457 in average and amplification of 44 unique bands, distributed in all studied clones except one of those containing higher starch content, compared with the original cultivar. This fingerprint is a prerequisite for recruiting clones with distinguished starch accumulation potential, addressed in the present study, in breeding programs targeting the improvement of starch accumulation in potato tubers. On the other hand, the utilized primers failed to establish phylogenetic relationship corresponding to starch content of the addressed clones. This may be attributed to utilization of few primers that did not cover a considerable proportion of the addressed genome.

Keywords: Callus-sourced clones, Carbohydrate analysis, Genetic diversity, Phylogenetic tree, SSR-PCR, Starch accumulation.

Introduction

Potato (*Solanum tuberosum*) is the most fundamental nongraminaceous food crop worldwide. It is cultivated on 17.13 million hectares, yielding 376 million tonnes (FAO, 2021). In addition to carbohydrates, proteins, and dietary fibre, tubers are rich in minerals, including phosphorus, magnesium, potassium, zinc, and iron, as well as vitamins such as ascorbic acid, pyridoxine, thiamin, riboflavin, niacin, folate, and pantothenic acid (Devaux et al., 2021). These dietary facts put potatoes before the other food crops to produce energy, proteins, minerals, and vitamins per unit of time and land area (Zaheer & Akhtar, 2016). In addition to home culinary adoptions, tubers are utilized in many food industrial products (Zhang et al., 2017) that accrue an enormous bulk of peel directed to bioethanol manufacturing (Maroufpour et al., 2019).

Starch is the most abundant carbohydrate in

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potato tubers; it is an agriculturally important product with numerous food and non-food precious uses. It is the primary energy source for a great portion of the world's population. Starch offers the metabolic energy necessary for the performance of biological functions. In addition, starch has many industrial applications, including gelling, thickening, adhesion, coating, and encapsulation materials (Dupuis & Liu, 2019). Therefore, potato breeding programmes address starch content as a determinant quality criterion (Kumari et al., 2018). High starch and dry matter contents are prerequisites for high performance in processing attributes, particularly chip crispiness, and color (Das et al., 2021). Both are positively correlated (Baranowska, 2019) and based on cultural practices, climatic conditions, and the genetic makeup of the used potato clone (Islam et al., 2022).

Conventional potato breeding is labor-intensive due to the heterozygosity of the potato genomes, which is responsible for inbreeding depression and intraspecies incompatibilities. In addition, tetraploidy discourages the introduction of novel traits using conventional breeding (Diambra, 2011). Moreover, seeking genetic variation in taxonomically related wild species is exhausting and time-consuming (Hameed et al., 2018; Fouad et al., 2019). Added to these obstacles, genetic heterogeneity, the raw material of breeding programmes, is impaired by vegetative propagation (Bisognin, 2011), which is practised for potato farming in most areas of the world (Simmonds, 1997).

The term somaclonal variation was coined by Larkin and Scowcroft (Larkin & Scowcroft, 1981) to describe genetic variations arising in tissue cultures. These variations were explained later by structural and numerical chromosomal variations, point mutations, and epigenetic alterations, including hyper- and hypomethylation of DNA (Krishna et al., 2016). It is now well-documented that somaclonal variation is frequently detected in callus-sourced regenerated plants rather than those regenerated from preformed meristems (Zayova et al., 2010).

Somaclonal variation can add to the pool of genetic variation essential for breeding. It was successfully recruited to enhance genetic variation in potatoes for further selection of new lines with improved tolerance against cadmium (Ashrafzadeh & Leung, 2017), salinity (Zeid et al., 2022), drought (Albiski et al., 2012), early blight (Mirkarimi et al., 2013), and postharvest diseases (Soliman et al., 2019). Additionally, somaclonal variation produced potato lines with improved starch accumulation potential (Bayati et al., 2021; Adly et al., 2023).

Correctly identifying genetic variations is essential for germplasm certification and breeding programmes. Characterization of gene variation entails the development of cost- and time-efficient markers. Molecular markers are the favored tool to characterize genetic variation due to their insensitivity to environmental and developmental impacts (Ateş Sönmezoğlu & Terzi, 2018).

An arsenal of random molecular markers was developed to assess genetic diversity, including amplified fragment length polymorphism (AFLP), random-amplified polymorphism (RAPD), and inter-simple sequence repeat (ISSR). Although these markers do not require prior knowledge about the addressed genome sequence and detect high polymorphism levels, they suffer from several drawbacks, including the requirement of several time- and cost-demanding steps for AFLP (Vos et al., 1995), inconsistencies in data replication with RAPD (Perez et al., 1998), and the scarcity of polymorphism highlighted with some ISSR primer combinations (Nguyen & Wu, 2005). In addition, these markers are dominant and can be positioned away from genes, which was overcome using gene targeting markers, including start codon targeted polymorphism (SCoT). However, SCoT markers remain dominantly inherited.

Simple sequence repeats (SSRs) or microsatellites are DNA sequences with a tandem repeat motif of 1-6 bases in length. They have several attractive features, including high polymorphism, high reproducibility, multiple alleles per locus, genome-wide coverage, transferability between species, and low requirements for instrumentation and expertise (Bhattarai et al., 2021). In addition, functional SSR markers can be amplified using primers designed to target genomic regions hosting genes of interest (Samanta et al., 2015).

SSR markers were successfully employed in potatoes to identify cultivars (Moisan-Thiery et al., 2005) and varieties (Tiwari et al., 2018) and to recognize population structure and genetic diversity for late blight resistance (Bhardwaj et al., 2023), as well as to characterize variation in tuber

properties (Romano et al., 2018). Although there is no available literature for SSR markers related to variation in potato starch accumulation, (Nayak et al., 2022) highlighted such markers in rice. Similar markers were developed by Sa et al. (2023) in maize. In addition, (Zhang et al., 2016) developed functional SSR markers to characterize variation in expressed genes underlying variation in starch accumulation in sweet potatoes.

Developing SSR markers requires prior knowledge of the targeted genome, which is expensive and time-consuming. However, the transferability of SSR markers between species (Gupta et al., 1994; Adeyemo et al., 2020; Liu et al., 2021) may be exploited in de novo SSR primer selection. Therefore, the present investigation aimed to establish functional SSR markers corresponding to variations in starch accumulation in potato somaclones based on markers developed by Zhang et al. (2016) to characterize variations in starch accumulation abilities in sweet potatoes.

Materials and Methods

The plant material

In addition to the original potato cultivar Lady Rosetta, the present investigation used fourteen potato clones selected from callus-sourced clones regenerated as detailed in our previous publications (Adly et al., 2022, 2023). Virus-free tubers of potato (*Solanum tuberosum*) cv Lady Rosetta were kindly provided by the National Program for Potato Seed Production, ARC, Egypt. Fourteen clones were selected to lie in three categories concerning the starch accumulated in tubers. The first category included clone 1-9 with the same starch content as the original Lady Rosetta tubers. The second category was represented by clone B-17, characterized by an accumulation of significantly lower amounts of starch than Lady Rosetta tubers. In contrast, the last category hosted the remaining clones, characterized by their significantly higher starch accumulation potential, compared with Lady Rosetta tubers.

Potato cultivation

In the middle of February 2021, the tubers of each clone were planted in 100 X 100 X 25cm boxes filled with sand: peat moss: perlite: vermiculite: foam (40:40:5:10:5) in a greenhouse according to the instructions of the Egyptian Ministry of Agriculture for agricultural practices regarding cultivation, irrigation, fertilization, and pest and disease control. After four months, the G1 tubers produced by each clone were collected, counted, washed, and weighed. Tuber samples representing each clone were dried at 70°C until constant weight and utilized for dry matter percentage calculation and starch and sugar determination. At the same time, the remaining tubers were kept as seed tubers at 4°C.

As previously mentioned, the stored G1 tubers were cultivated in September 2021. The G2 tubers were harvested four months later, and samples were used to determine dry matter percentage and subsequent starch and sugar contents.

Carbohydrate analysis

Sugars

Sugars were extracted according to Islam et al. (2022), where the known weight of potato flesh corresponding to each clone was homogenized with 80% (v/v) ethanol and incubated at 80°C. After 30min, the homogenate was centrifuged at 5 000 × g for 10min. The supernatant was collected, and the pellets were subjected to two further extraction rounds. The residual tissues were kept for starch determination. On the other hand, the supernatant fractions were combined and evaporated to dryness in a rotatory evaporator, and the residue was dissolved in 3mL of distilled water for sugar determination. Based on the glucose standard curve, total soluble sugars were determined using the anthrone method (Morris, 1948).

Starch

Starch was determined in the sugar-free residual tissue prepared as described by Kumar et al. (2005). The tissue was oven dried at 70°C for 18 h, and then the dried tissues were homogenized in 60% (v/v) perchloric acid for starch hydrolysis. The liberated glucose was quantified utilizing the anthrone method (Morris, 1948).

DNA extraction and SSR-PCR

Genomic DNA was extracted from the two-month-old G2 potato tuber sprouts using the EZ-10 Spin Column Plant Genomic DNA Miniprep Kit (Bio Basic Canada Inc.) according to the manufacturer's protocol. The concentration and quality of extracted DNA were visually examined using stained 1% agarose gels and assessed using a NanoDrop2000 UV–Vis spectrophotometer (Thermo Fisher Scientific, USA).

Polymerase chain reactions (PCRs) were

conducted using a 20µL total reaction volume. This mixture comprised 2µL of template DNA containing 25ng, 4µL of 5X FIREPol Master Mix from Solis Biodyne, and 1µM concentration of each primer. To achieve the final 20µL volume, 13µL nuclease-free water (NF H_2O) was added. Sequences of SSR primers are presented in Table 1. PCR amplifications were performed in a Thermal Cycler (TECHNE, TC-312) under the following cycle profile: an initial denaturation of 5min at 94°C, followed by 35 cycles of 55 sec at 94°C, 45sec at an annealing temperature of 55°C, and 1min at 72°C, and a final elongation of 7min. PCR products were resolved on a 1.5% agarose gel containing ethidium bromide (0.5ug/mL). The amplified fragments were visualized using the Gel Documentation System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Band size was estimated using a 100-bp DNA ladder (Promega, Madison, WI 53711-5399, USA).

Analysis of SSR results

DNA banding patterns generated from the SSR-PCR were analyzed by the Gel Analyzer 3 programme. The bands were scored as (0) for absence or (1) for presence. Positive or negative unique bands were identified for each clone. For each primer, the polymorphism% was computed by dividing the number of polymorphic bands by the total number of scored bands. The polymorphic information content (PIC) of each marker was calculated using the equation $PIC = 1–\Sigma (P_i)^2$ (Nei, 1973), where P_i is the frequency of the ith allele of the addressed marker. The similarity between pairs of clones was calculated using Jaccard's coefficient (Nei & Li, 1979) and the NTSYS-PC programme. The unweighted pair-group method with arithmetic average (UPGMA) was used to generate clustering dendrograms (Rohlf, 1998) to illustrate genetic relationships among the clones.

Statistical analysis

The results of all clones are presented as the average of five replicates ± standard deviation (SD). Significant differences between different clones were tested using the least significant difference (LSD) posthoc test at the level of significance set at $P \leq 0.05$ using SPSS v. 14. The data of G1 and G2 were subjected to combined analysis after subjected to normality distribution test and homogeneity test. Correlations between starch content and dry matter content were calculated using Minitab v. 10.0 software based on Pearson correlation at P values < 0.05.

TABLE 1. SSR primer codes, sequences, and annealing temperatures according to Zhang et al. (2016)

Marker	Primer ID	Sequences 5'- 3'	Tm
SIP003	B04 F	TTCCATCTTCGCGTATCCAT	60.435
	B05 R	GGCCTCATCACTTCCACCTA	60.073
SIP004	B06 F	CCCAATCAAATCCCCTTCTT	60.124
	B07 R	CTATTGCTCCATTCCCACTGA	60.081
SIP025	B08 F	GGAAGGAACAGCAAAATCCA	60.051
	B09 R	TCGGCCACAATACAAGATCA	60.073
SIP031	B10 F	ATCAGGTGTGCTTTTGCTCC	60.263
	B11 R	CGGCTGAGGTTTGATCATTT	60.074
SIP137	C02 F	CCTCGCACAGCTGAAAAACT	60.571
	C03 R	TGCAACTGGCCGATACAATA	60.096
SIP150	C04 F	AGCTTTCCGCAATTTGTTTG	60.174
	C05 R	CCACCATTGGAGAGCCATAC	60.054
SIP132	B12 F	TGCCTGTTCAAATACAAGTCCA	60.008
	C01 R	CACTGAACCTGTCCAGAGCA	60.088
SIP151	C06 F	TGGTTCTTGGCCGTTCTTAG	60.241
	C07 R	CTTTTCTGCGGTATTGGCAT	60.096
SIP188	C08 F	CCCATGTCAGGCAGGTATTC	60.340
	C09 R	TCCATGTGAAGCAACCTGAA	60.240
SIP219	C10 F	CTTGGGAGCCCAGGATTACT	60.455
	C11 R	GTGGTATCAACGCGAGAGGT	60.142

Results

Starch content

The present investigation included three categories of potato callus-sourced clones, representing clones with equal, lower, and higher tuber starch content compared with tubers of the original cultivar Lady Rosetta. The results confirmed the significant differences among the aforementioned categories (Table 2). The first category included clone 1- 9 that accumulated approximately 741.2mg of starch per gram of dry weight, which is insignificantly different from the original cultivar. The second category, characterized by low starch content, hosted clone B-17, accumulates 683mg of starch per gram of dry weight. On the other hand, the starch content of the third category ranged from 788 to 821.7 mg for each gram of dry weight. However, the statistical analysis reflected no significant differences among clones in the third category, which included clones 1-7, Ros 119, 1-8, A-4, A-6, A-7, A-27, A-36, A-37, E-1, 2-2, and 2-6.

Soluble sugar content

The correlation analysis (Table 3) reflected an insignificant Pearson correlation between soluble sugar and starch content. Based on soluble sugar content, the addressed clones were distributed in four significantly different categories (Table 2). The first included clones 1-7, A-7, A-36, E-1, 2-2, and 2-6 with 1.4-1.5 mg soluble sugars/g dry weight, while the second category included the original cultivar Lady Rosetta and clones Ros 119, 1-8, 1-9, A-4 and A-27, which accumulated approximately 1.8 mg soluble sugars on a g dry weight basis. The third and fourth categories were represented by clones B-17 and A-37, which accumulated approximately 2 and 2.2mg/g dry weight, respectively.

TABLE 2. Dry matter, starch, and soluble sugar contents of the commercial cultivar Lady Rosetta and fourteen callus-sourced potato clones [Values are presented as the average of five replicates ± SD. Values with different letters are significantly different based on the LSD test at P< 0.05]

Potato clones	Dry Matter Content (% of Fresh Weight)	Total Starch Content (mg g^{-1} Dry Weight)	Total Soluble Sugars (mg g^{-1} Dry Weight)
Lady Rosetta	23.27 ± 0.54^{b}	737.8 ± 12.4^{b}	1.833 ± 0.031^{b}
Clone 1-7	23.67 ± 0.24^{bc}	799.3 ± 12.9^{d}	1.420 ± 0.026^{a}
Clone Ros 119	27.22 ± 0.12^{h}	821.7 ± 23.4^{d}	1.847 ± 0.055^{b}
Clone 1-8	25.26 ± 0.39^{ef}	799.1 ± 30.5^{d}	1.847 ± 0.071^{b}
Clone 1-9	23.31 ± 0.21^{b}	741.2 ± 14.4^{bc}	1.853 ± 0.038^{b}
Clone A-4	25.33 ± 0.81^{ef}	799.7 ± 28.8^{d}	1.850 ± 0.066^{b}
Clone A-6	24.19 ± 0.17^{cd}	788.7 ± 26.3^{cd}	1.480 ± 0.046^{a}
Clone A-7	25.65 ± 0.19^{fg}	808.5 ± 30.7^{d}	1.487 ± 0.059^{a}
Clone A-27	26.35 ± 0.63^{g}	816.7 ± 47.2^{d}	1.827 ± 0.107^{b}
Clone A-36	24.58 ± 0.59^{de}	795.5 ± 41.4^{d}	1.477 ± 0.075^{a}
Clone A-37	24.08 ± 0.81^{cd}	788.5 ± 26.5^{cd}	2.187 ± 0.075^{d}
Clone E-1	23.76 ± 0.25^{bc}	789.2 ± 28.5^{d}	1.410 ± 0.052^{a}
Clone 2-2	23.52 ± 0.46^{bc}	793.7 ± 23.0^{d}	1.440 ± 0.040^{a}
Clone 2-6	25.12 ± 0.20^{ef}	794.4 ± 39.4^{d}	1.463 ± 0.076^{a}
Clone B-17	22.43 ± 0.31^{a}	683.0 ± 17.4^{a}	1.987 ± 0.051^{c}

Dry matter content

The correlation analysis (Table 3) showed an insignificant Pearson correlation between dry matter content and soluble sugar content. Conversely, it highlighted a significant positive correlation between the dry matter and starch content. The highest and lowest values for dry matter content were recorded in clones Ros 119 and B-17 at 27.22 and 22.43%, respectively. Depending on dry matter content, the remaining clones were difficult to resolve into significantly different groups. However, the dry matter content of the original cultivar was 23.27%, which was insignificantly different from those recorded for clones E-1, 2-2, 1-9, and 1-7, and the remaining clones reflected significantly higher values.

SSR markers

Monitoring of the genetic diversity and estimation of the degree of polymorphism among the potato clones was conducted using six SSR primers (Table 4). The primers generated distinct and reproducible banding patterns (Supplementary Materials, Figure S1). 68 bands were generated, of which 62 were polymorphic (91.18%), while the remaining 6 were monomorphic (8.82%).

The total number of bands amplified by each primer ranged from 6 (primers SIP4 and SIP137) to 30 (primer SIP031) (Table 3). Each primer produced one monomorphic band. Consequently, the number of polymorphic amplicons ranged from 5 to 29, corresponding to 83.3 to 96.7% polymorphism. The average number of bands per primer was 11.3, distributed into 10.3 polymorphic bands and a monomorphic band.

The utilized primers amplified 44 unique bands (Supplementary Materials Table S1), with an average of 7.3 bands per primer, distributed in all clones except clone A-4. The number of unique bands per primer ranged from 2 generated by the primer SIP137 to 22 bands amplified by the primer SIP031. The unique bands generated by SIP137 were recognized in clones 1-8 and A-27, while the unique bands generated by primer SIP031 were recorded in clones 1-7, Ros 119, 1-8, A-6, A-7, A-37, E-1, 2-2, and 2-6. The number of unique bands per clone ranged from 1 in clones A-27 and A-37 to 8 in clone A-7. PIC, the probability for an individual to be polymorphic at a specific locus, ranged from 0.229 for SIP031 to 0.572 for SIP137, with an average of 0.457 per locus.

The phylogenetic tree constructed based on UPGMA analysis of SSR data was established for the studied potato clones (Fig. 1). It included five major clusters. The first cluster had clones 2-2, B-17, Ros 119, E-1, and 2-6, while the second cluster included clones 1-9, A-27, and A-6. The third cluster included Lady Rosetta and clones A-37, while the fourth cluster included the clones A-4, A-36, and 1-8. The rest of potato clones were clustered in the fifth cluster which included two clones (1-7, and A-7).

The binary scoring established by SSR analysis was utilized to calculate Jaccard's similarity matrices (Supplementary Materials Table S1). Based on six primers, SSR resolved all the potato clones. Among the resolved genotypes, similarity values ranged from 0.375 (estimated between clones 1-7 and Ros 119) to 0.786 (calculated between clones 2-2 and B-17).

Discussion

Starch is the most abundant carbohydrate in potato tuber, composed of amylose and amylopectin. The former is a linear, long α-glucan with few branches, incorporating approximately 99% α-(1,4) and 1% α-(1,6) linkages. Amylopectin is a heavily branched structure due to approximately 5% α-(1,6) linkages (Dupuis & Liu, 2019). Starch biosynthesis is localized in amyloplasts under the catalysis of granular-bound starch synthase (GBSS) and both starch branching enzymes (SBE) and starch synthases (SSs). GBSS is nearly restricted to the soluble phase and devoted to elongating the growing α−1,4 linkage. On the other hand, branched chains are constructed through reactions catalyzed by SBE and SSs (Nazarian-Firouzabadi & Visser, 2017).

TABLE 3. **Pearson correlation matrix for dry matter, starch, and soluble sugar contents**

	Dry matter content	Starch content
Starch content	0.693*	
Soluble sugars content	0.048	-0.177

* Correlation is significant at the 0.05 level.

TABLE 4. Band size range, total number of bands, distribution of bands (monomorphic, unique, and polymorphic), percent polymorphism, and polymorphism information content (PIC) for SSR markers

Marker	Size range	Total number of bands	Number. of mono-morphic bands	Number of unique bands	No. of polymorphic bands	% Poly-morphism	PIC values
SIP003	139-379	7	1	4	2	85.714	0.442
SIP004	103-139	6	1	4	1	83.333	0.422
SIP025	134- 755	12	1	8	3	91.667	0.526
SIP031	70 -381	30	1	22	7	96.667	0.229
SIP137	118- 556	6	1	2	3	83.333	0.572
SIP150	184- 1.154	7	1	4	2	85.714	0.552
Total		68	6	44	18	91.170	-
Average		11.3	1	7.3	3	87.738	0.457

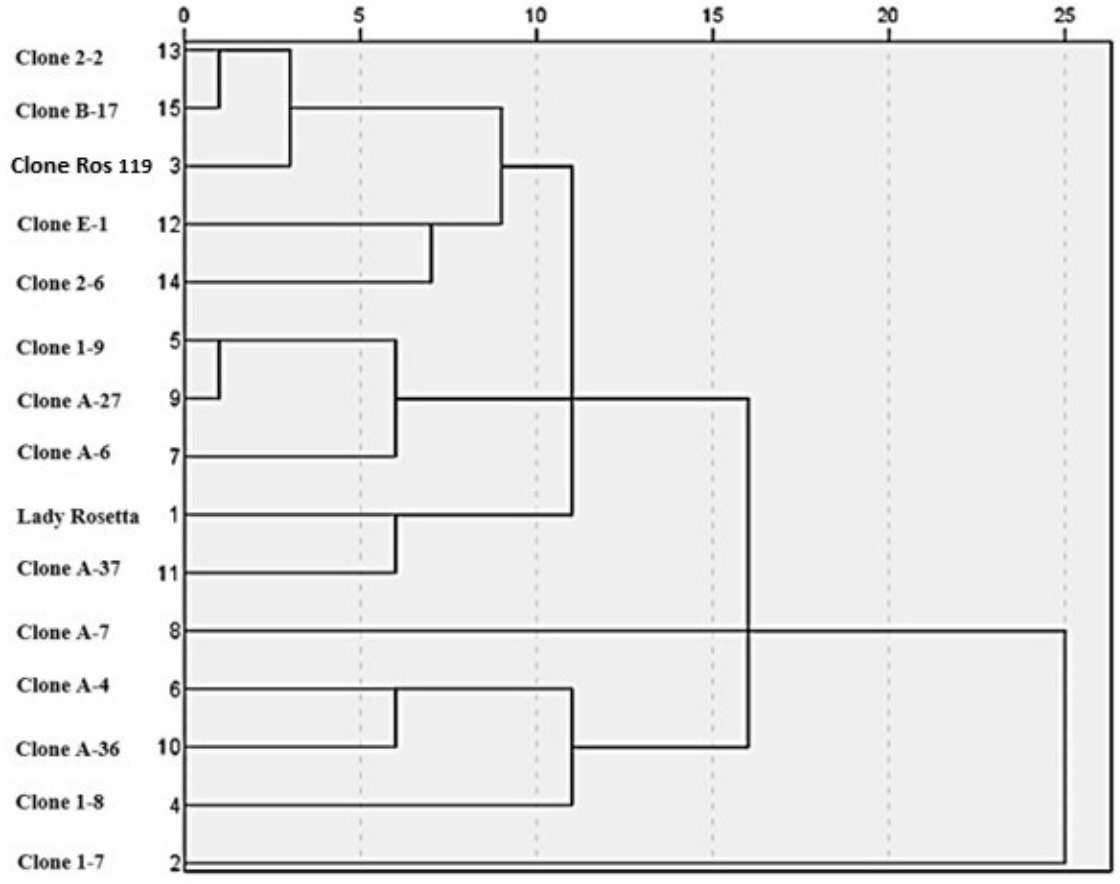

Fig. 1. Cluster analysis of 15 potato clones (the commercial cultivar Lady Rosetta and fourteen callus-sourced clones) generated from UPGMA analysis of the combined data produced by six SSR markers [The scale is % similarity]

ADP glucose pyrophosphorylase (AGPase) affords the glycosyl building blocks necessary for synthesizing amylose and amylopectin (Ferreira et al., 2010; Su et al., 2021). The glucosyl donor for starch synthesis in tubers is derived from sucrose, which is translocated through the phloem from the assimilating leaf tissues to the developing tuber. In tuber tissues, sucrose reaching the cytosol is converted to glucose 6-phosphate (G6P) and then imported into the amyloplast to be metabolized into ADP-Glc and finally starch (Van Harsselaar et al., 2017). ATP is also transported to tuber tissues

from photosynthetic tissues; it is imported into amyloplasts by a plastid adenylate translocator (NTT). Starch accumulation in potato tubers is highly sensitive to NTT activity (Tjaden et al., 1998).

The sugar and starch contents of plant storage organs are controlled by genetic factors and show genotype-dependent quantitative variations (Schreiber et al., 2014). In addition, the regulatory mechanisms affecting the activities of starch metabolic enzymes act at transcriptional and posttranslational levels (Van Harsselaar et al., 2017).

Compared with tubers of the original cultivar Lady Rosetta, the present investigation reflected variation in sugar and starch content in tubers of callus-sourced potato clones. These variations can be attributed to the somaclonal variation commonly observed in callus-sourced regenerated plants (Zayova et al., 2010). The somaclonal variation may alter the genetic scaffold underlying carbohydrate metabolism through point mutations and epigenetic alterations, including hyper and hypomethylation of DNA (Krishna et al., 2016). This resulted in alterations in the accumulation of starch (Thieme & Griess, 2005; Bayati et al., 2021; Adly et al., 2023) and soluble sugars (Nassar et al., 2011) in potato tubers.

Starch is the major component of potato tubers and is responsible for 15-20% of their fresh weight (Bertoft & Blennow, 2016; Tong et al., 2023). Consequently, our results highlighted the positive correlation between starch accumulation and dry matter content. Similar correlations were recorded by (Islam et al., 2022; Tong et al., 2023). In addition, dry matter content is genotype dependent (Scavo et al., 2023), which may explain variation in dry matter content by the genetic variations associated with somaclonal variation. Variations in potato somaclones' dry matter content were also observed by Wilson et al. (2010) and Bayati et al. (2021).

The present results reflect the ability of only six SSR primers (SIP003, 004, 025, 031, 137, and 150) to resolve fifteen potato clones, confirming SSR markers' high efficiency to reflect intraspecific diversity in potatoes. SSRs are excellent molecular markers for varietal identification, diversity analysis, and germplasm characterization due to their extensive genome coverage, high polymorphism, and co-dominant nature (Schönhals et al., 2016; Tiwari et al., 2018). Similar to our results, several research groups highlighted variations among potato genotypes using SSR markers (Anoumaa et al., 2017; Tiwari et al., 2018; Tillault & Yevtushenko, 2019; Bhardwaj et al., 2023). The previous authors calculated PIC values ranging from 0.477 to 0.93. On the other hand, the current results reflect PIC values ranging from 0.229 to 0.572. The genetic diversity parameters are based on the number of utilized primers, evaluated genotypes, and their genetic background (Anoumaa et al., 2017).

Regarding the tetraploid nature of potatoes, it is predicted to detect a maximum of four bands, corresponding to four alleles per SSR locus in each individual. However, the multilocus nature of the utilized markers can explain the detection of more than four bands in some clones using certain primers (Zhang et al., 2016). Another explanation for the extra bands is based on events related to somaclonal variation, including point mutations that may create new binding sites for the applied primer or numerical chromosome variations (Krishna et al., 2016). However, the increase in chromosome number only leads to increased alleles if combined with variations in the number of tandem repeats comprising the microsatellite.

The results of the present investigation reflected the ability of SSRs to generate a unique banding pattern for each clone, which is crucial for molecular marker-assisted breeding (Hasan et al., 2021). This fingerprint is a prerequisite for recruiting some clones with distinguished starch accumulation potential, addressed in the present study, in breeding programmes targeting the improvement of starch accumulation in potato tubers. The ability of SSR to characterize improved starch accumulation was also recorded in rice (Bao et al., 2006), maize (Zhang et al., 2008), and sweet potato (Zhang et al., 2016; Diaz et al., 2022).

Conclusion

SSR is a potent molecular marker able to resolve and characterize the genetic diversity underlying variation in starch content in potato clones using a few primers. However, constructing a proper phylogenetic relationship requires many primers to cover a considerable proportion of the addressed genome.

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Authors' contributions: Conceptualization: El-Sayed T. Abd El-Salam, Ahmed S. Fouad, Hayam S. Abdelkader, Mahasen A. Mohamed and Mohammad E. EL-Denary; methodology and software: Walaa M.R.M. Adly, Hayam S. Abdelkader and Ahmed S. Fouad; writing—original draft preparation: Walaa M.R.M. Adly; writing—review and editing: Ahmed S. Fouad, Hayam S. Abdelkader.

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استنباط علامات جينية من النوع "تكرارات السلسلة القصيرة" لتوصيف درنات البطاطس المستنبتة من خلايا نسيجية مختلفة ذات محتوى عالى من النشا

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تهدف هذه الدراسة الى انشاء المؤشرات التكرارية البسيطة (SSR) الوظيفية التى تتوافق مع الاختلافات في تراكم النشا في مستنسخات (someclones) البطاطس. بالإضافة إلى الصنف الأصلي ليدي روزيتا، تم فحص أربعة عشر مستنسخات (someclones) من مصدر الكالس لمعرفة محتويات النشا والسكر بالإضافة إلى محتوى المادة الجافة. من بين مستنسخات (someclones) المختارة، كان أحدها يمثل مستنسخات (someclones) التي تحتوي على نفس محتوى النشا للصنف الأصلي والآخر يمثل تلك التي تحتوي على محتوى أقل من النشا. تحتوي مستنسخات (someclones) المتبقية على نسبة نشا أعلى مقارنة بالصنف الأصلي. كشفت النتائج المجمعة للموسمين الزراعيين عن وجود علاقة إيجابية معنوية بين محتوى المادة الجافة ومحتوى النشا والتي كانت غائبة عند استبدال النشا بالسكريات القابلة للذوبان. تم تسجيل ارتباطات غير معنوية بين محتوى السكر ومحتوى النشا. استنادًا إلى إمكانية نقل مؤشرات SSR بين الأنواع، تم استخدام ستة بوادئ SSR تميز التباين في تراكم النشا في البطاطا الحلوة لتقييم التنوع الوراثي بين مستنسخات البطاطس المختارة. وقد انتج التحليل أنماطا متميزة وقابلة للتكرار تحتوي على 68 نمطا، منها 62 نمط متعدد الأشكال. عكست البادئات المستخدمة قوة تحليل عالية ظهرت في PIC قدرها 0.457 في المتوسط وتضخيم 44 حزمة فريدة، موزعة في جميع clones التى تمت دراستها باستثناء واحدة تحتوي على محتوى نشاء أعلى، مقارنة بالصنف الأصلي. تعد هذه البصمة الوراثية شرطًا أساسيًا لتحديد clones ذات القدرة المميزة على تراكم النشا، والتي تم تناولها في هذه الدراسة، في برامج التربية التي تستهدف تحسين تراكم النشا في درنات البطاطس. من ناحية أخرى، فشلت البادئات المستخدمة في إنشاء علاقة نسبية تتوافق مع محتوى النشا في clonesالموجهة. يمكن أن يعزى ذلك إلى استخدام عدد قليل من البادئات التي لم تغطي نسبة كبيرة من الجينوم المستهدف.