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Identification of Polymorphic Microsatellite Markers among Potato (*Solanum tuberosum* L.) Genotypes differing in Processing Traits

Soumya Shetty[1*], B.T. Krishnaprasad[2], S. Shyamalamma[1], R.L. Ravikumar[1], H. Amarananjundeswara[3], Geeta Govind[2] and S. K. Pooja[2]

[1]Department of Plant Biotechnology, College of Agriculture, UAS, GKVK, Bengaluru (Karnataka), India.
[2]Division of Agriculture Biotechnology, College of Agriculture, Hassan (Karnataka), India.
[2]Horticulture Research and Extension Centre, Hassan (Karnataka), India.

(Corresponding author: Soumya Shetty)*
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ABSTRACT: Potato (*Solanum tuberosum* L.) is a annual vegetable crop, which belongs to Solanaceae family. In India, there is a huge demand for potato varieties suitable for processing as they have been widely used in agro-based industries. However, certain morphological, bio-chemical and physico-chemical attributes are necessary in potato varieties to meet the requirement for industrial use. In the present study, 35 genotypes of potato differing in processing traits were evaluated for biochemical and physico-chemical properties such as starch, apparent amylose content, amylose: amylopectin ratio and total carotenoids. Highest starch content of 81 per cent was documented in FC-3 with 0.23 percent apparent amylose content and 1:4.5 Amylose: Amylopectin ratio. Further, potato is an auto-tetraploid species with clonal propagation and has got narrow genetic diversity conserved during asexual propagation. Use of molecular markers is most preferred method for the assessment of genetic diversity in this crop. Among the microsatellite markers, SSR (simple sequence repeats) markers are most preferred due to their distribution throughout the genome, co-dominant in nature, low cost and reproducibility. SSR markers linked to processing traits are useful in identifying suitable potato genotype for processing. Hence, in the same genotypes, polymorphic SSR markers were also analysed using known markers *viz*., STM3009, STWAXY, Inh2α, STGBSS, STI007, STI028, STI060, STI014, STI021, STM0019, STM1106 and STM1051 related to processing traits. Among the different SSR primers evaluated, STWAXY, Inh2α and STI060 showed polymorphism. The size of the amplified product varied from 130 base pairs (Inh2α) to 215 base pairs (STM Waxy). In which, ST Waxy characterized as granule bound starch synthase and Inh2α as vacuolar in vertase inhibitor gene, involved in potato starch metabolism. From the study, it was concluded that SSR markers can be used in the identification of potato varieties suitable for processing type with superior tuber quality traits for further food and non food industrial applications.

Keywords: Potato, SSR, polymorphism, physico-chemical, biochemical properties.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a annual vegetable crop, which belongs to Solanaceae family. It is globally grown crops it provides high tuber yield even under variable soil types and weather situations. South America is the primary and secondary centre of origin. Potato has proved its worth in feeding the nations during emergency. It can supplement the food needs of the country in a substantial way. Besides its significance role in human food security, potato is also an vegetable with fascinating genetic traits and cultural history. In India, there is a great scope for potato cultivation suitable for processing and it has opened a new dimension for development of agro-based industries in the country. However, certain morphological, bio-chemical and physico-chemical attributes are necessary in potato varieties to meet the requirement for processing. One of the important production aspects of potato for processing is tuber quality that includes biological (e.g. carbohydrates, proteins and minerals), sensorial traits (e.g. texture, flavour, colour) and industrial traits (e.g. tuber shape, cold sweetening and starch quality). While in the literature the information about the physicochemical and functional properties of potato starch is abundant, there is still little knowledge about the physicochemical and functional properties of Andean tuber starches (Cruz *et al*., 2016). Economic and performance factors make potato starch the best choice for food applications because its pastes have good clarity and a neutral flavor (Gikundi *et al*., 2021). Potatoes are also affordable and can be stored for relatively long periods after harvest without heavy loss of quality, traits that enhanced their relevance for food security (Muthoni *et al.,* 2017; Navarre *et al.,* 2019). Additionally, following rapid shrinkage of arable land due to increasing population and urbanization, crops like potatoes which can produce more food, nutrients, and cash per unit area and time are on high demand (Devaux *et al*., 2020).

Selection of parental materials and understanding of appropriate parents to be used for a particular mating design are key in breeding (Acquaah, 2007). A number of approaches have been used by breeders to select the best parents and cross- combinations. These include progeny tests, combining ability effects, use of mid-parent values, estimated breeding values and genetic diversity (Gopal, 2015). However, to get reliable results with the intricacies of potato genetics and inheritance pattern, various methods need to be combined to aid in the selection of suitable parents (Sharma and Nandineni 2014). High level of genetic diversity possessing different desirable traits is important for crop improvement. This is because selection of parents based on genetic diversity will maximize heterozygosity, broaden the genetic base and produce heterotic progenies (Sun *et al*., 2003).

Potato being an auto-tetraploid species (2n = 4× = 48) with clonal propagation, genetic diversity plays a crucial role in breeding programmes because heterozygosity is found to be conserved during asexual propagation and also hybrids between lines of diverse genetics display greater heterosis and segregants than the closely related parents. Diversity assessment can be done through the use of phenotypic information, pedigree, biochemical and molecular markers (Govindaraj *et al*., 2015). The use of molecular markers is limited in this crop although it can be a most reliable method for assessing genetic diversity. Molecular markers are more preferred once as they are stable and not dependent on environment or developmental stage of the plant. Various molecular markers have been used to estimate genetic diversity in crop plants. Microsatellites are abundant, highly polymorphic, co-dominant and are widely used to detect heterozygosity in several crops. SSR (simple sequence repeats) being co-dominant markers, allows all the alleles except null ones to be observed at each locus using acrylamide gel electrophoresis or sequencing systems.

Therefore, use of microsatellite markers is most reliable method for assessing genetic diversity in this crop. Developing genetic markers, either in the close proximity of the gene or directly based on the gene sequence, enables indirectly steer variation and select genotype suitable for processing. With the objective ofidentification of polymorphism among different potato genotypes using SSR markers associated with processing traits following study was conducted.

MATERIAL AND METHODS

Field experiment was conducted to evaluate different potato genotypes suitable for processing quality traits with economic tuber yield for two seasons during both *Kharif*-2021 and *Rabi*-2021 at Horticulture Research and Extension Centre, Hassan, Karnataka. An experiment was laid out in Randomized Complete Block Design with two replications. A total of 35 different potato genotypes, initially collected from CPRI, Shimla, CPRS, Patna & private hybrids *viz*., AICRP- P-60, AICRP- P-61, AICRP- P-57, AICRP-P-74, AICRP- P-77, AICRP- C-1, AICRP- C-8, AICRP- C-10, AICRP- PH-3, AICRP- C-11, AICRP-C-23, AICRP- P-24, AICRP-P-43, AICRP-P-79, AICRP-P-53, AICRP-P-56, AICRP-C-29, AICRP-RH-2, AICRP-P-72, AICRP-C-13, AICRP-C-20 (check), AICRP-C-24 (check), AICRP-C-17, AICRP-P-73, AICRP-P-81, AICRP-P-14, AICRP-P-1, CYT-1, CYT-2, Patna-1, Patna-2, FC-1, FC-3, FC-5 and FL were evaluated in the study. The land was prepared for the research well before planting by deep summer ploughing and incorporating FYM @ 25 t/ha into the soil followed by rotovator to break the soil clods. The tuber planting was taken up during both seasons by adopting spacing of 60 cm × 20cm with 3 m × 3 m plot size. The recommended dosage of NPK @ 75:75:100 kg/ha was incorporated. From the recommended quantity of nitrogen, 50 per cent of nitrogen was applied at the time of planting and remaining 50 per cent of nitrogen was applied 30 days after planting during earthing-up operation. The recommended package of practices was followed at different stages of crop growth from sowing to till harvesting. Genotypes were evaluated for various biochemical parameters and physico-chemical properties such as chlorophyll, carbohydrates, sugars, proteins, TSS, starch, apparent amylose content, amylose: amylopectin ratio and total carotenoids.

Biochemical parameters: Chlorophyll content was estimated using DMSO method (Shoaf and Lium (1976), total carbohydrates was determined by phenol sulphuric acid method (Nielsen, 2009) and Lowry's method was followed for estimation of total protein content (Lowry *et al*., 1951). Sugars present in the samples were estimated by following the method outlined by Lane and Eynon described by Ranganna (1977). The total soluble solids in percentage value was observed with the help of digital refractometer (0-30).

Physico-chemical parameters:

Sarch Extraction. The residue remained after extraction for sugar was washed for several times using distilled water to ensure that there was no more soluble sugar in the residues. After that, starch content was determined by following the procedure of Kang *et al*. (2009).

Amylose content and Amylose: Amylopetin ratio. The amylose content of samples was determined using the Mccready *et al*. (1950) method. Rapid colorimetric method was used to determine the ratio of amylose: amylopectin (Am:Ap) in acid hydrolyzed starch.

Total carotenoids content. Tubers were cut into pieces and from that, two small fragments were selected. These fragments were further cut into small cubes and frozen in liquid nitrogen, lyophilized and milled. Total carotenoids were extracted from 3 g of freeze-dried tissue in a glass flask with 50 ml of hexane for 24 h in the dark. Total carotenoids concentrations in the solvent were evaluated with a UV- spectrophotometer at 450 nm.

Statistical analysis. The mean, standard error and cumulative differences were applied wherever necessary. The data were analysed using SAS software.

Polymorphic analysis using SSR markers. Genomic DNA was extracted from all 35 potato genotypes from fresh young leaves following the CTAB protocol. In the present study, known SSR markers viz., STM3009, STWAXY, INH2A, STGBSS, STI007, STI028,

STI060, STI014, STI021 STM0019, STM1106 and STM1051 related to processing traits, identified from literature survey were used to screen genotypes. Two different PCR amplification protocols were followed: 1) A regular PCR 2) A touchdown PCR.

PCR conditions for simple sequence repeats: PCR reactions were carried out for all 35 potato genotypes using identified SSR markers. Total PCR reaction volume was 25 µl. Each PCR reaction was composed of 2 µl of each primer, 1 µl of each nucleotide (dNTPs), 1.5 mM $MgCl_2$, 1.5 µl TaqDNA polymerase, 2.5 µl of buffer, 14 µl of nuclease free water and 2 µl genomic DNA as template. List of SSR primers along with sequence information and annealing temperature details were given in Table 1.

Two different PCR amplification protocols were: 1) A regular PCR with initial denaturation at 94°C for four min, followed by 35 cycles of 30 s at 94°C, 30 s at annealing temperature, 30 s at 72°C and a final extension of 10 min at 72°C. 2) A touchdown PCR with initial denaturation at 94°C for four min, followed by 4 touchdown cycles of 30 s at 94°C, at annealing temperature for 30 s, -1°C each cycle and extension at 72°C for 30 s. This was followed by 35 cycles of 30 s at 94°C, 30 s at annealing temperature and 30 s at 72°C. Amplified products were separated by gel electrophoresis in a 2% agarose gel (0.5X TBE buffer at 12 V cm-1 for 20 min) and visualised under UV light after staining with ethidium bromide (1 mg l^{-1}) for 30 min. Most of theloci in potato, a clonally propagated plant, are expected to be heterozygous. Therefore, SSR marker amplifying a single locus can yield up to four bands in potato, which is atetraploid species. Therefore, some bands could appear as more than one copy and consequently denser than other bands.

RESULTS AND DISCUSSION

The processing quality of potato depends on biochemical composition of its variety, reducing sugar, protein, nitrogenous and phenolic compounds. Out of 35 different potato genotypes evaluated for biochemical and physico-chemical properties, AICRP-P-61, AICRP-C-8, AICRP-C-10 AICRP-C-11 AICRP-P-57, FC-3, FC-5, AICRP-PH-3, AICRP-C-23 and AICRP-C-20werefound more suitable for processing quality traits with superior biochemical and physico-chemical properties. During the studies, lowest reducing sugar of 0.19 per cent was recorded in AICRP-P-61 followed by AICRP-C-11, FC-3 and AICRP-C-23 with 0.20 per cent (Table 2). Statistically significant difference ($p<0.05$) were observed among the genotypes for tested biochemical (carbohydrates, sugars, proteins and TSS) and physico-chemical parameters such as starch, apparent amylose content, Am:Ap ratio & carotenoids. Graphical representation of sugars and starch characteristics of potato genotypes illustrated in Fig. 1 and 2.

Highest starch content was documented in FC-3 of 81 per cent with 0.23 per cent apparent amylose content and 1:4.5 Amylose: Amylopectin ratio. Starch has conventionally been used in the food industry to augment the functional properties of various foods. Starch extracted from naturally occurring food sources like potato, is extensively used as an additive in numerous food-related industries as a gelling agent, thickener, stabilizer and also as a substitute for fats in food items. Several physical, chemical and enzymatic modifications have been accomplished to improve the processing operation of potato starch for industrial use. The physicochemical and functional properties of starch system vary with the starch biological origin. The structural characteristics and amylose-to-amylopectin ratio of potato starch also vary among different genotypes. In fact, nutritional and processing quality of potato products (frozen and dry) are greatly affected by their starch characteristics and content. Most of the carbohydrate in the cooked potato flour are found to be gelatinized starch, rather than in soluble form. The starch present in the potato flour may significantly affect its physicochemical properties. Pre-gelatinized starch dispersions have some characteristics of the untreated starch and hence extensively used in the preparation of products like soup mixes, instant puddings and salad dressings etc. (Khrasi *et al*., 2020). Potato quality traits are sometimes under simple genetic control but often they are multigenic, complicating MAS (Slater *et al*., 2014) such as yield, chip color, and specific gravity, which are controlled by many different genes on different chromosomes (Ramakrishnan *et al*., 2015).

Similar research studies on evaluation of different genotypes for biochemical and physico-chemical properties were carried out by Kaur and Aggarwal (2014). Das *et al.* (2021) reported that total starch concentration was affected by cultivar or growing location including environmental conditions and cultural practices followed during the crop growing season. Highest total carotenoids of 4.61 mg/kg was observed in AICRP-C-10 and AICRP-C-23 (Table 3) followed by AICRP-C-20 (4.40 mg/kg). Tatarowska *et al*. (2019) study also showed that the contents of carotenoids in potato tubers are significantly affected by environmental factors that cannot be controlled, as these are influenced by growing location and year of cultivation. Ahmed *et al*. (2018) also conducted genetic diversity studies in potato genotypes for starch and other physico-chemical properties by using microsatellite markers.

Among the twelve different SSR markers used in the present study, the polymorphic bands were observed with three primers *viz.*, STMWaxy, STI060 and Inh2α (Plate 1, 2 & 3). The size of the amplified product varied from 130 base pairs (Inh2α) to 215 base pairs (STMWaxy). Where as, monomorphic bands were documented with STI007, STI014, STGBSS, STI1106 and STM3009. STI021, STI0019 and STM1051 and remaining three primers did not show any amplification (Table 4).

Most of the loci in potato, are expected to be heterozygous. SSR marker amplifying a single locus can yield up to four bands in potato. Therefore, some bands could appear as more than one copy and consequently as denser than others. Similar studies also conducted by Singh *et al*., (2020) to determine the pattern and level of genetic diversity among the selected 20 potato genotypes using 15 SSR markers.

The microsatellites revealed considerable genetic variation among genotypes which can be exploited for further crop improvement in potato.

Table 1: SSR primers details used for polymorphic analysis.

Sr. No.	SSR marker	Sequence	Annealing Temp (^{o}C)
1.	STM3009 F	TCAGCTGAACGACCACTGTTC	49-53 TD
2.	STM3009 R	GATTTCACCAAGCATGGAAGTC	49-53 TD
3.	STWaxy2 F	CCCATAATACTGTCGATGAGCA	50-54 TD
4.	STWaxy2 R	GAATGTAGGGAAACATGCATGA	50-54 TD
5.	inh2α F	AAAGTTGAATTCAAATGAGAAATTTATTC	49-53 TD
6.	inh2α R	ATGGGGTCTCCCTACACGTT	49-53 TD
7.	STI007 F	TATGTTCCACGCCATTTCAG	50-54 TD
8.	STI007 R	ACGGAAACTCATCGTGCATT	50-54 TD
9.	STI028 F	ATACCCTCCAATGGGTCCTT	48-52 TD
10.	STI028 R	CTTGGAGATTTGCAAGAAGAA	48-52 TD
11.	STI060 F	ACTTCTGCATCTGGTGAAGC	50-54 TD
12.	STI060 R	GGTCTGGATTCCCAGGTTG	50-54 TD
13.	STI014 F	AGAAACTGAGTTGTGTTTGGGA	50-54 TD
14.	STI014 R	TCAACAGTCTCAGAAAACCCTCT	50-54 TD
15.	STI021 F	TCATCAAGTCGTCATCAA	56
16.	STI021 R	TCGAATGATCCAAAGCTTCC	56
17.	STM0019 F	AATAGGTGTACTGACTCTCAATG	53
18.	STM0019 R	TTGAAGTAAAAGTCCTAGTATGTG	53
19.	STM1051 F	TCCCCTTGGCATTTTCTTCTCC	60
20.	STM1051 R	TTTAGGGTGGGGTGAGGTTGG	60
21.	STGBSS F	AATCGGTGATAAATGTGAATGC	50-54 TD
22.	STGBSS R	ATGCTTGCCATGTGATGTGT	50-54 TD
23.	STM1106 F	TCCAGCTGATTGGTTAGGTTG	48-52 TD
24.	STM1106 R	ATGCGAATCTACTCGTCATGG	48-52 TD

Table 2: Evaluation of potato genotypes for biochemical parameters.

Sr. No.	Processing hybrids	Total chlorophyll (mg/g)	Total Sugars (%)	Reducing sugars (%)	Carbohydrates (g/100g)	Crude protein (mg/100g)	Total soluble solids (^{0}brix)
1.	**AICRP-P-61**	3.40	0.50	0.19	19	05.48	6.87
2.	**AICRP-C-8**	3.30	0.58	0.23	22	10.45	6.50
3.	**AICRP-C-10**	3.25	0.43	0.23	23	10.14	6.44
4.	**AICRP-C-11**	3.27	0.40	0.20	20	08.89	6.90
5.	**AICRP-P-57**	2.90	0.46	0.21	21	17.69	6.77
6.	**FC-3**	3.50	0.54	0.20	21	06.93	7.00
7.	**FC-5**	3.14	0.50	0.21	21	08.38	7.50
8.	**AICRP-PH-3**	3.20	0.55	0.22	22	10.14	7.25
9.	**AICRP-C-23**	2.88	0.57	0.20	20	08.17	6.80
10.	**AICRP-C-20**	3.45	0.58	0.22	22	12.10	6.30
	Mean	**3.25**	**0.53**	**0.22**	**21.40**	**9.93**	**6.86**
	F- Value	*	*	*	*	*	*
	SEm	**0.25**	**0.04**	**0.03**	**0.32**	**0.92**	**0.24**
	CD	**0.41**	**0.67**	**0.24**	**1.44**	**1.11**	**0.73**

*Significant at p<0.05

Table 3: Evaluation of potato genotypes for physico-chemical parameters.

Sr. No.	Processing hybrids	Starch content (%)	Apparent amylose content (%)	Amylose: Amylopectin ratio (Am: Ap ratio)	Total carotenoids content (mg/kg)
1.	**AICRP-P-61**	75	0.17	1:4	4.32
2.	**AICRP-C-8**	71	0.20	1:4	4.14
3.	**AICRP-C-10**	73	0.21	1:3.6	4.61
4.	**AICRP-C-11**	80	0.20	1:4	4.33
5.	**AICRP-P-57**	77	0.18	1:3.5	4.19
6.	**FC-3**	81	0.23	1:4.5	3.89
7.	**FC-5**	73	0.21	1:3.6	4.16
8.	**AICRP-PH-3**	79	0.20	1:4	4.28
9.	**AICRP-C-23**	74	0.19	1:3.5	4.61
10.	**AICRP-C-20**	78	0.20	1:4	4.40
	Mean	76.20	0.21	1:3.7	4.30
	F- Value	*	*	*	*
	SEm	**0.80**	**0.04**	**0.47**	**0.16**
	CD	**1.99**	**0.26**	**1.51**	**0.71**

*Significant at p<0.05

Table 4: Details of the polymorphic analysis using SSR markers.

Total number of markers	Monomorphic	Polymorphic	Multiple bands	Not amplified
12	6	3	-	3

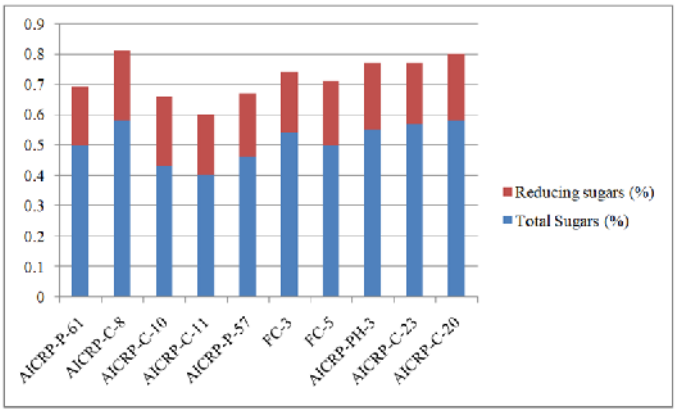

Fig. 1. Total Sugars and reducing sugars (%) in potato genotypes.

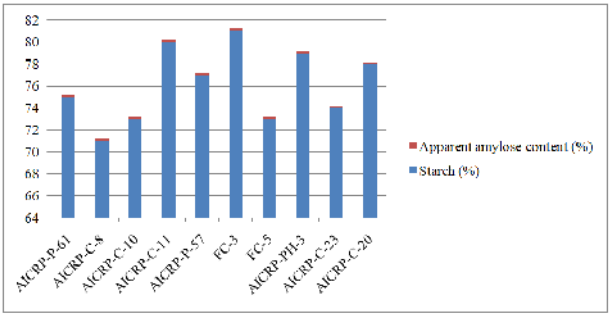

Fig. 2. Starch (%) and apparent amylose content (%) in potato genotypes.

PCR amplification profile for SSR markers

Plate 1: PCR profile for on 3.5 % agarosegel (L: 100 bp ladder, 1 – 30: potato genotypes, Primer: STM Waxy.

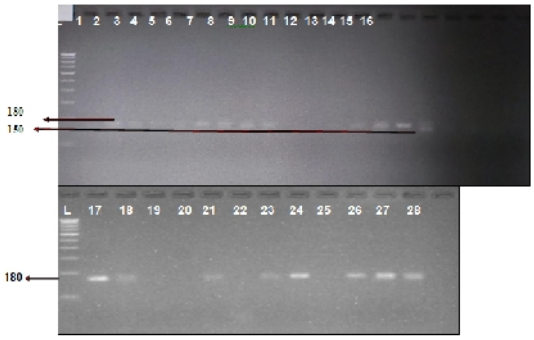

Plate 2: PCR profile for on 3 % agarosegel (L: 100 bp ladder, 1 – 30: potato genotypes, Primer: STI060.

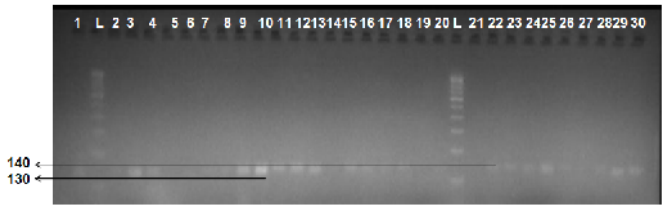

Plate 3: PCR profile for on 3 % agarosegel (L: 100 bp ladder, 1 – 30: potato genotypes, Primer: Inh2α.

Namugga *et al*. (2017) also conducted diversity studies in tetraploid potato genotypes using 16 SSR markers to identify suitable parents for breeding purposes.Results shown that, markers differed significantly in their ability to establish variability amongst the clones. Tillault *et al*. (2019) evaluated twenty potato varieties, including five new genotypes developed in Alberta, Canada were fingerprinted using 10 SSR markers. In the study, STM0037, STM1016, and STM1104 were found to be the best SSR markers to detect genetic differences between potato varieties.

CONCLUSION

In this study, genotypes were initially screened for their biochemical and physico-chemical properties and significant differences were observed among the hybrids. Highest starch content of 81 per cent was documented in FC-3 with 0.23 per cent apparent amylose content and 1:4.5 Amylose: Amylopectin ratio.Usually, starch properties are considered to be a key factor affecting the functional properties of processed potatoes, which are found to be influenced by cultivars and by environmental factors. Further, in the study, polymorphic SSR markers were also analysed using known markers in the working population.Among the SSRs evaluated, only STWaxy, Inh2α and STI060 showed polymorphism. STWaxy is characterized as granule bound starch synthase and Inh2α is characterized as an vacuolar in vertase inhibitor involved in potato starch metabolism. However, potato genotypes that show polymorphism for the SSRs, also display variation in their starch metabolism or not, requires future studies.

As potato starch is widely used in the food industry and other industries like textile and paperbecause of its unique physico-chemical properties, current study on use of SSR markers for the assessment of polymorphism is helpful in the identification of potatohybrids suitable for processing with the superior quality traits.

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