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A Molecular Tool for Facilitating Large Scale Seed Production and Genetic Purity Testing of White Onion Lines with High Total Soluble Solids

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Abstract: Owing to the increasing demand from processing industries, there is a need to produce short-day white onion lines with high total soluble solids. Molecular markers, known for their high precision and reproducibility, are more reliable than morphological and biochemical markers for creating DNA fingerprints to test seed genetic purity and identify lines with elevated total soluble solids. Our individual bulb selection methods yielded short-day white onion lines which contain ≥ 18% TSS, which is low as per our requirement. Twenty SSR markers were utilized to characterize 19 short-day white onion lines derived from individual bulb selection till the eighth generation. The overall average number of alleles per simple sequence repeat locus, heterozygosity, and polymorphism information content were found to be 2.45, 0.54, and 0.44, respectively. A simple sequence repeats genotyping-based dendrogram separated all the lines into two clusters. Almost all of the lines in clusters IA and IB exhibit significant levels of total soluble solids, except sub-group IAb-I, which contains modest levels. Our study revealed that AcB-Shweta-tss-9.8 (total soluble solids -9.8%) & HT-GR-1A-M-7TSS>18_21.0 (total soluble solids - 21.0%) are more diversified based on the dendrogram and heat map generated by genotyping and total soluble solids data, respectively. These high total soluble solids containing lines would be extremely advantageous for accelerating molecular breeding of short-day, high total soluble solids containing white onion development using marker-assisted selection. Furthermore, these markers could expedite the selection of bulbs with high total soluble solids (HTSS) content for large-scale seed production and facilitate effective genetic purity testing of seeds.

Keywords: onion, soluble solid content, marker-assisted selection, simple sequence repeat, quality seed production, DNA fingerprint

BACKGROUND

Onion (*Allium cepa* L.) is a major nutraceutical-containing horticulture crop cultivated globally and is also under extensive cultivation in India. As one of the most significant export crops of vegetables from India, it ranks second in production after China [1,2,3,4,5]. Onion has been in high demand globally since it is utilized both as a vegetable and spice both in its fresh and cooked forms [4]. In addition to fleshy onion bulbs, it is available in dehydrated form. Furthermore, it contains many traditional pharmacological components, such as quercetin and other secondary metabolites, which have antibacterial, anti-diabetic, and anti-carcinogenic properties [6,7,8,9]. White onion cultivars seeds and bulb are in high demand due to their processing and industrial applications [10,11,12,13]. Dehydrated products, including flecks, powder, and vinegar, are widely manufactured and sold globally [14,15,16,17]. Given the global market, there's a significant possibility of expanding of processing market [6,18,19]. Dehydration industries require HTSS containing white onion lines with a trait of globose shape bulb, thin neck, pathogen-free, pungent flavor, high genetic purity, and high seed longevity [20]. However, there is a lack of short-day HTSS onion lines that may meet the processing industry requirements [16]. Molecular markers can identify and distinguish between white onions with low total soluble solids (LTSS) and high total soluble solids (HTSS), helping to prevent biopiracy and support the maintenance of seed genetic purity. Several molecular markers, including isozyme, RAPD, SSR, and ILP markers utilized for QTL identification, diversity analysis, and genetic purity testing [9, 13, 10, 11, 12, 21, 22]. Furthermore, these polymorphic and cross-transferable SSRs and other markers are commonly employed for determining genetic diversity, genetic purity of seed, and selecting diverse lines due to their reproducibility, and lack of viability due to environmental influence [23,24]. Furthermore, Roger BT [25] developed HTSS containing genotypes, but they are long-day onions and are not ideal for short-day conditions. A variety of worldwide groups investigated gene mining and molecular breeding but extensive investigations on the HTSS content of short-day onion cultivars are still missing [1, 2, 3, 9, 18, 21, 23, 15, 26]. While our group [27] developed 18% TSS-containing short-day white onion lines using the mass selection breeding approach, but more than 18% TSS-containing white onion lines and their DNA fingerprint for genetic purity testing of seeds are also required. This study aims to develop white onion lines with ≥ 18% total soluble solids (HTSS) using the individual bulb selection breeding method, along with DNA fingerprinting for genetic purity testing of the seeds.

METHODS

Breeding method for the development of ≥ 18% short day HTSS lines by a selection of individual bulb breeding method

Approximately 450 white onion germplasm were brought from different regions of India and conserved at the ICAR-Directorate of Onion Garlic Research, Pune, India (Table 1). These genetic materials have been assessed for TSS. Individual bulbs with TSS of ≥15% had been picked. As a result, selected individual bulbs were multiplied individually through isolation by selfing. By selfing (pollination), till 8th generation bulbs were produced. Selected bulbs were allowed to pollinate in individual cages with manual pollination. After eight generations of selfing, the line was assessed for TSS after 90 days of harvesting with reference to check variety Bhima Shweta (TSS-11.76 %). Overall, 7199 short-day onion bulbs were evaluated for TSS, in which 109 bulbs had TSS ranging from 15 to 21% [19]. Further selections were made for bulbs with TSS levels exceeding 18% TSS. Bulbs with TSS less than 18% were rejected. As a result, 13 HTSS (18-21.4%) and 6 LTSS (9.8 to 10.6%) lines were selected for molecular analysis.

Sample collection, DNA isolation, and genotyping

Nineteen *Allium cepa* genotypes (Table 1) were chosen for the phenotyping (TSS) and genotyping [19]. Five plant young leaves (200mg) were mixed and DNA was extracted [18, 10]. The quality and quantity of DNA were determined on a 1.5% agarose gel and NanoDrop 2000 (Thermo Scientific, USA). To assess the genetic characteristics of the chosen 19 white onion genotypes, 20 SSR markers were applied (Table 2). These markers

Table 1. Description of the samples used for the characterization of high and low total soluble solids (TSS) of white onion genotypes

Sl. no	Variety/ Accession number of individual bulbs	TSS (Total soluble solid) %	Source of collection	Genotypes code
1	HT-GR-2A-M-7(SC),BIGBULB_19.8	19.8	ICAR-DOGR, Breeding Lines	AcHTBB-m-7-tss-19.8
2	HT-GR-2A-M-6 BIIG BULB TSS15-18_19.6	19.6	ICAR-DOGR, Breeding Lines	AcHTBB-m-6-tss-15-18
3	HT-GR-1A-M-7 TSS>18--_21.4	21.4	ICAR-DOGR, Breeding Lines	AcHT-m-7-tss-21.4
4	HT-GR-2B-M-6(SMC)_20.8	20.8	ICAR-DOGR, Breeding Line	AcHT-m-6-tss-20.8
5	HT-GR-1A-M-7 TSS>18_21.2	21.2	ICAR-DOGR, Breeding Line	AcHT-m-7-tss-21.2
6	HT-GR-2B-M-6(SMC)_20.2	20.2	ICAR-DOGR, Breeding Lines	AcHT-m-6-tss-20.2
7	HT-GR-1A-M-7TSS>18_21.0	21.0	ICAR-DOGR, Breeding Lines	AcHT-m-7-tss-21.0
8	HT-GR-2A-M-7(SC)BIGBULB_18.0	18.0	ICAR-DOGR, Breeding Lines	AcHTBB-m-7-tss-18.0
9	HT-GR-2A-M-7(SC)BIGBULB_18.8	18.8	ICAR-DOGR, Breeding Lines	AcHTBB-m-7-tss-18.8
10	HT-GR-2A-M-7(SC)BIGBULB-_19.0	19.0	ICAR-DOGR, Breeding Lines	AcHTBB-m-7-tss-19.0
11	HT-GR-1A-M-7 TSS>18_20.0	20.0	ICAR-DOGR, Breeding Lines	AcHT-m-7-tss-20.0
12	HT-GR-2A-M-6Bigbulb_19.6	19.6	ICAR-DOGR, Breeding Lines	AcHTBB-m-6-tss-19.6
13	HT-GR-1A-M-7 TSS>18-_20.0	20.0	ICAR-DOGR, Breeding Lines	AcHT-m-7-tss-20.0
14	AcB-Shubra-tss-10.0	10.0	ICAR-DOGR, Released variety	AcB-Shubra-tss-10.0
15	AcB-Shubra-tss-10.6	10.6	ICAR-DOGR, Released variety	AcB-Shubra-tss-10.6
16	AcB-Shweta-tss-10.2	10.2	ICAR-DOGR, Released variety	AcB-Shweta-tss-10.2
17	AcB-Shweta-tss-10.0	10.0	ICAR-DOGR, Released variety	AcB-Shweta-tss-10.0
18	AcB-Shweta-tss-9.8	9.8	ICAR-DOGR, Released variety	AcB-Shweta-tss-9.8
19	AcB-Shweta-tss-10.0	10.0	ICAR-DOGR, Released variety	AcB-Shweta-tss-10.0

Table 2. List of primers and their amplification characteristics. Ta, alleles, He and PIC stand for annealing at optimum temperature, heterozygosity and polymorphic information content, respectively

Sl.no.	Markers Name	Forward Primer Sequence(5'-3')	Reverse Primer Sequence(5'-3')	Ta	Observed product size	Alleles	He	PIC
1	ACM 008	GCCGGAAGAGGAGAAGAAGT	CATAATTCCCATGGCTTTGC	50.3	210-400	2	0.482	0.366
2	ACM33	CCTTCTCCCCATTCTCTTCC	ATCATCGTCCTCGTCCTCAT	52.3	250-400	2	0.498	0.374
3	ACM38	ATGCCAGACTACGACAACGA	ACGCCTACCAACCTTCAATG	52.3	200-600	3	0.665	0.591
4	ACM 45	AAAACGAAGCAACAAACAAAA	CGACGAAGGTCATAAGTAGGC	45.1	100-550	2	0.453	0.350
5	ACM 54	GAGTGAGAGGGGAAATGGAA	AAAGATGGTTTGTTGGTGGC	50.3	200-400	2	0.493	0.372
6	ACM 58	GGAGTCACACAACAGAAACACAA	AAGAAGGAATAGAGATGTAGCCGA	53.9	100-200	3	0.614	0.532
7	ACM 60	ATCAGCAGCCTTCCCAGTAA	ATCACACCCGCAAAAGAAT	47.4	190-400	2	0.483	0.366
8	ACM61	GCGTTTGCTGAGAGATTAGGA	TTTCTTGCTGATGATGCTGC	50.3	200-700	2	0.485	0.368
9	ACM 66	CTCCCCGCAACCAGTAATAA	GCTTGGGTTTTGTTTCTCCA	50.3	200-450	2	0.471	0.36
10	ACM069	TTCTGCGCTCTTCCCAGTAT	CAAGCGGTTTGAAAAAGGAG	50.3	200-550	3	0.630	0.564
11	ACM 77	AAATTATGGGCCACCTCCTC	CAAGATTGTCGACTCCCCAT	52.3	100-600	2	0.426	0.335
12	ACM 78	CGCAGAATCTCGTCCTTTTT	AATGGTTTGGAGGTCAGTCG	50.3	190-500	3	0.545	0.451
13	ACM 93	GCCAACAGTTTTCGTAAGTTGA	ATTCTCTTCGGCTTTCGTGA	50.3	100-450	2	0.475	0.362
14	ACM 115	TCCATCTATGCATCTGCCAC	CTATTCTTCCACTGGGGCAA	52.3	210-450	3	0.639	0.562
15	ACM125	AAAAAGGGTTTTATCAGTCGCA	CCGCTGTTGAAATATGGGTT	49.7	100-450	2	0.497	0.374
16	ACM 147	CACTTTCCCGTCTAATCGACA	TTCCCACAATCAAAACACCA	48.2	250-600	3	0.573	0.480
17	ACM 151	TGTCAGACAAGCAACTCCTCC	AGGTGAGGCTTAGATGGGGT	54.4	240-500	3	0.595	0.509
18	ACM 154	CGATGAATACACCGATGACG	CTTGTTTTGGCAGTTGGGAT	50.3	200-400	2	0.489	0.369
19	ACM 168	TGGACTGGCCATGAGACATA	TGCAAGAAGAGAAATTGCCA	48.2	250-500	3	0.657	0.584
20	ACM 229	TACGAGCGGAGGTATGAGC	GCCAGGAAGGCGAGTAGTAA	53.8	220-500	3	0.645	0.572

were obtained from the public domain [9, 15, 18]. PCR amplification was done in a 10µL volume of reaction using 1 unit of Taq DNA polymerase (Thermo Fisher Scientific - US), 1 mM of forward and reverse primer, 0.25 mM of dNTP (Thermo Fisher Scientific - US), and 20ng template DNA. The PCR amplification conditions were followed as described by Jayaswall *et al*. [12]. PCR products were run on 3% agarose gel, and DNA amplification bands was recorded by using GelDoc 2000 (Bio-Rad, USA).

Amplified band analysis

SSR genotyping-based bands were evaluated for the presence (1) or absence (0) of a particular allele [13, 14]. UPGMA clustering method was utilized for the preparation of an SSR genotyping-based dendrogram [26]. Each SSR marker's relevance and reliability were assessed by calculating the heterozygosity (He) and polymorphic information content (PIC) with the PIC calc tool [22].

Quantification of TSS content

Five white onions per line were used to determine total soluble solids by crushing the bulbs, 90 days following harvesting. The total soluble solids were determined using a hand refractometer. Below 18 per cent TSS were labeled LTSS, while ≥ 18 per cent were considered HTSS. In addition, MeV software was used to create a heat map based on TSS values. Further TSS correlation heatmap matrix was matrix was developed by the application of MeV software [8].

RESULT AND DISCUSSION

Simple sequence repeats (SSRs), a second major variant after SNPs play a key role in the expression of agronomically important traits through genome packaging [24]. SSRs are known for their role in structural and functional variation of the genome. Further, onion widely used as a vegetable has have shown increasing production and confirming reasonable profits to the farmers [17]. Hence, SSR markers have been selected due to their significant polymorphism, as demonstrated by numerous prior researches in various crops [2, 3, 9]. A set of twenty polymorphic SSR primers produced separate polymorphic bands in all HTSS and LTSS accessions of white onion lines (Fig. 1). Overall, 49 alleles (an average of 2.45 alleles per SSR marker) were found. This finding is consistent with a recent analysis utilizing AcPIP markers, which showed an average allele of each locus of 2.80 [12]. Furthermore, PIC and heterozygosity ranged from 0.335-0.591 (mean 0.4420) and 0.426-0.665 (mean 0.5407), respectively (Table 2). The average PIC value (0.4420) matches the PIC value of AcPIP markers (0.41) reported by Jayaswall *et al*. [12]. Thus, the current work confirms that the selected 20 SSR markers have polymorphism potential analogous to AcPIP markers, as described by Jayaswall *et al*. [12]. Hence these selected 20 SSR markers could be utilized for testing of genetic purity of seed, hybridity confirmation through molecular breeding [13]. The current study's dendrogram classified HTSS onion genotypes into three groups: IAa, IAb-II, and IIB. Additional LTSS onion genotypes fall within IAb-I (Fig. 2). The correlation heat map matrix of 19 genotypes showed thirteen HTSS lines demonstrated a substantial association for TSS among thirteen individuals, but a negative correlation for TSS with *Allium cepa*_Bhima-Shubra (LTSS) and *Allium cepa*_Bhima-Shweta (LTSS). Furthermore, the *Allium cepa*_Bhima-Shweta (LTSS) and *Allium cepa*_Bhima-Shubra (LTSS) had a positive

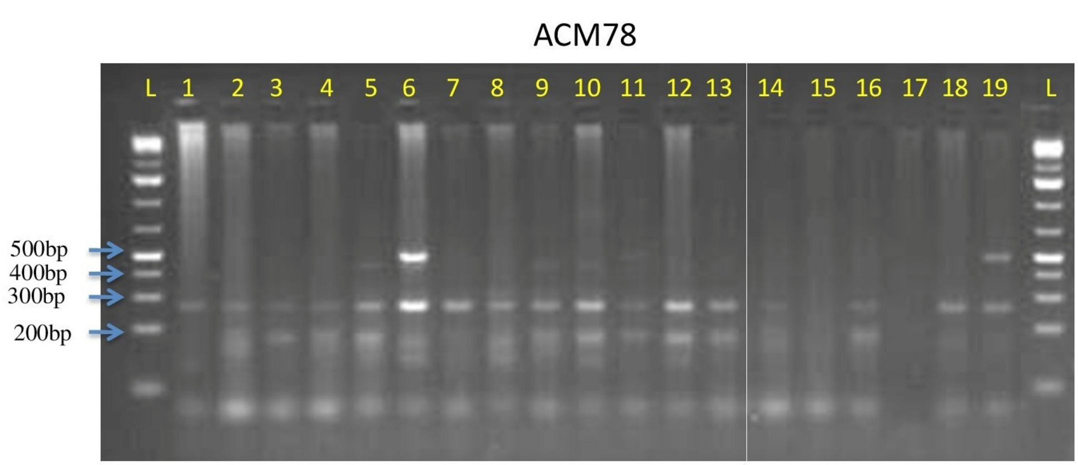

Figure 1. SSR_ACM-078 marker genotyping-based PCR amplification bands of nineteen white onion lines

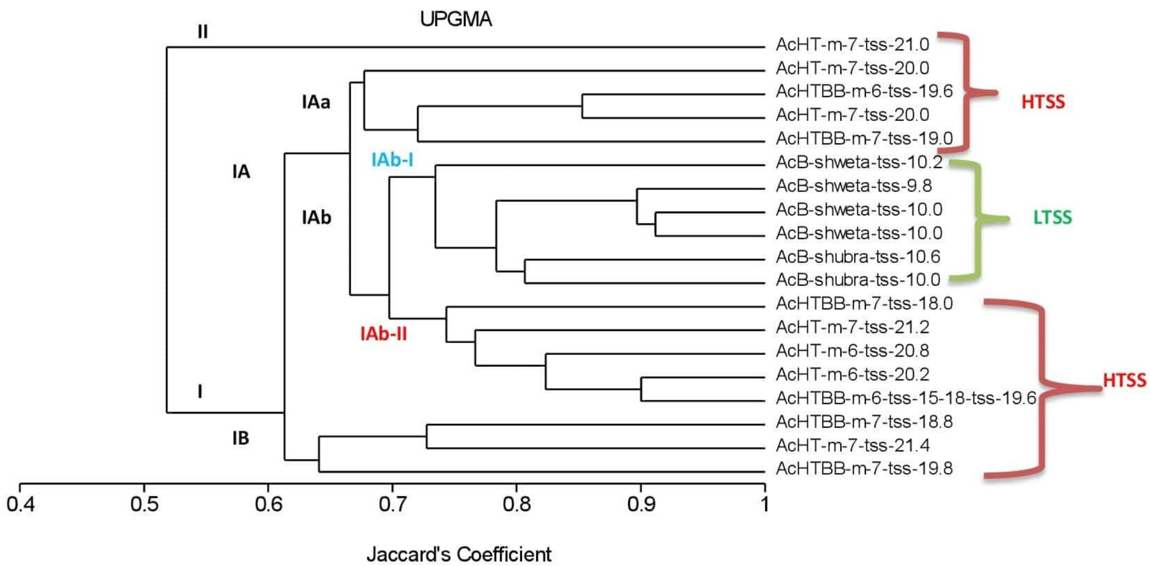

Figure 2. UPGMA twenty SSR marker genotyping-based clustering of nineteen white onion lines

correlation but a negative correlation with those of thirteen HTSS individuals (Fig. 3). Additionally, DNA fingerprinting-based principal component analysis clustered LTSS genotypes in group II and HTSS genotypes in groups I, III, and IV (Fig. 4). Further TSS content-based heat map also classified 19 genotypes in the HTSS and LTSS groups (Fig. 5). DNA fingerprinting-based dendrogram (Fig.1 & 2), correlation matrix (Fig. 3), principal component analysis (Fig. 4), and TSS content-based heat map (Fig. 5) based clustering confirm the clustering consistency of HTSS and LTSS lines. Hence, in comparison to Singh *et al*. [27], these HTSS genotypes are better suited for the

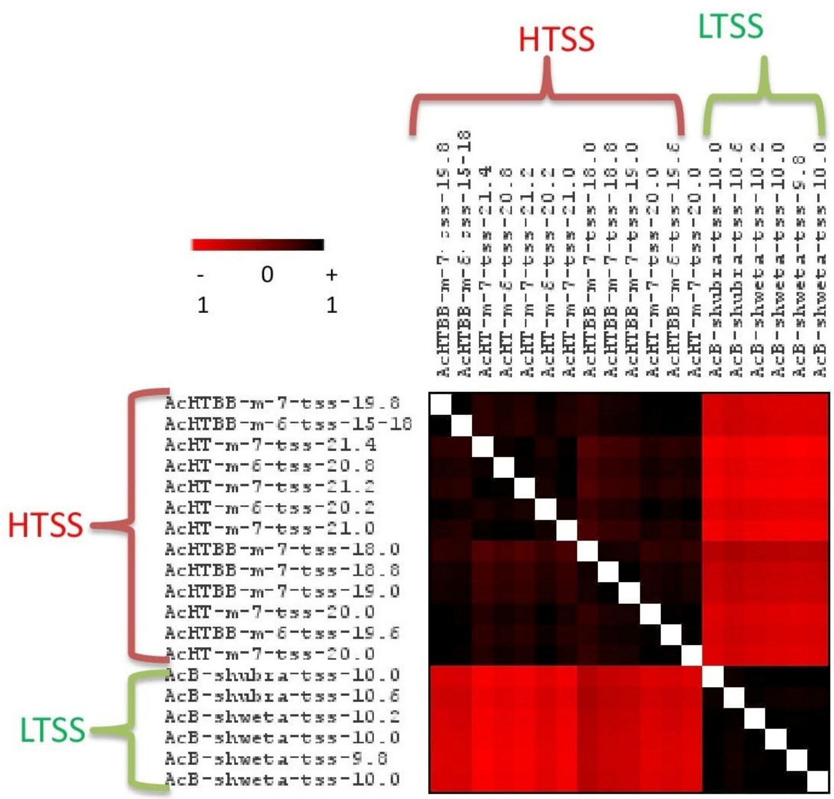

Figure 3. Nineteen white onion lines correlation heatmap matrix

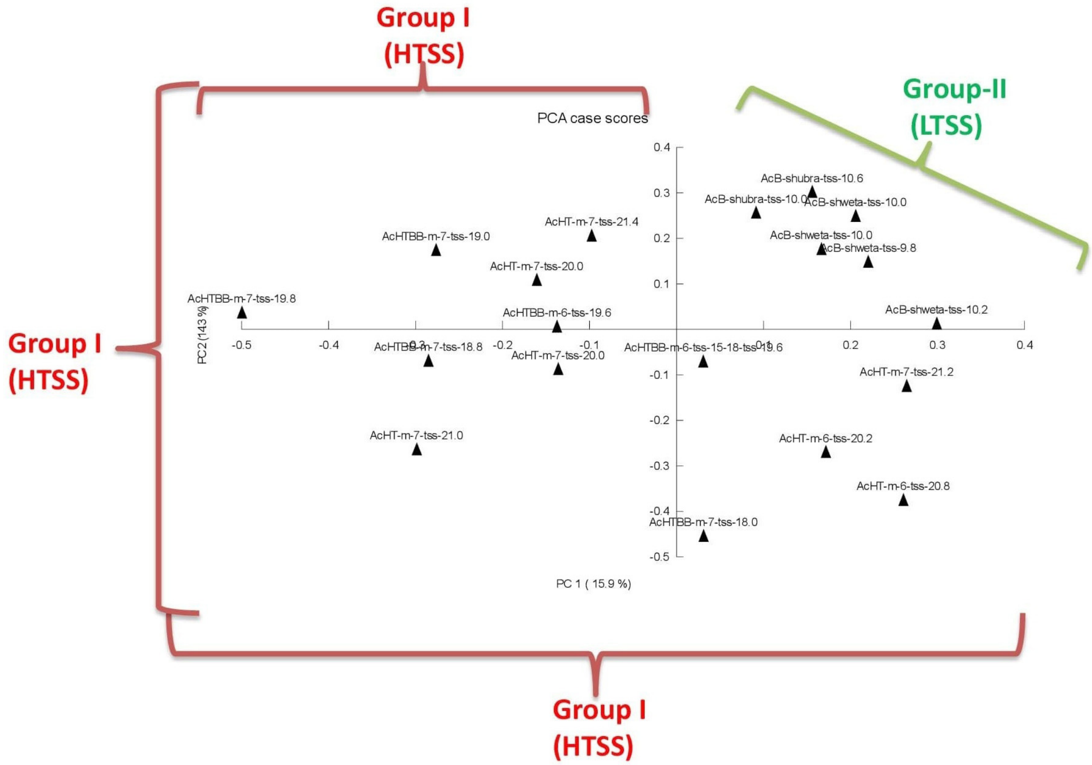

Figure 4. Nineteen white onion lines principal component analysis based on twenty SSR marker genotyping

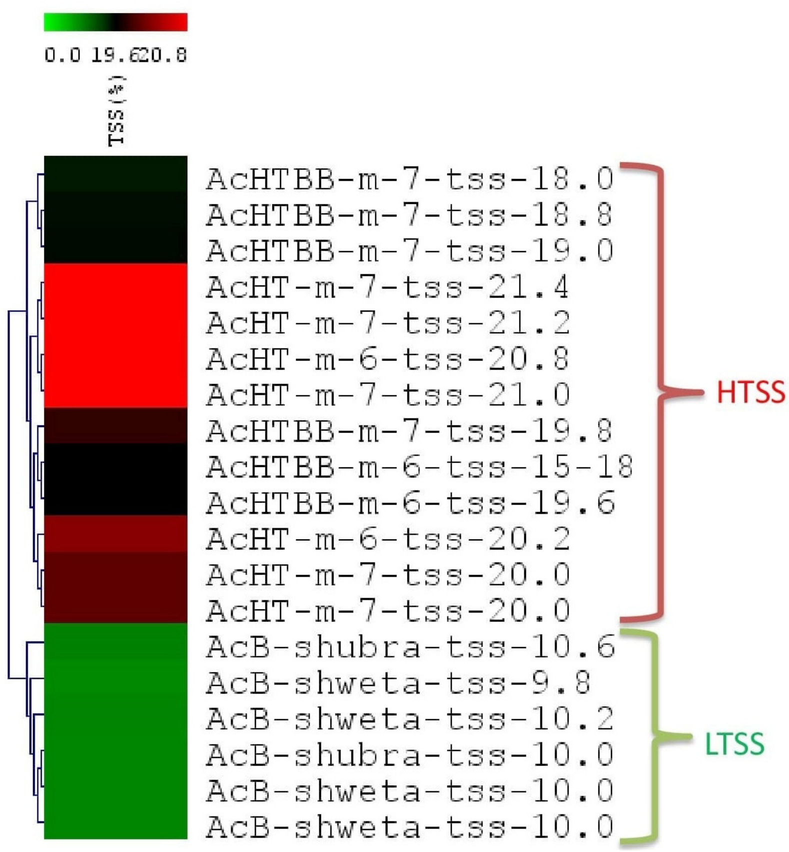

Figure 5. Nineteen white onion lines heat map based on TSS

expedition of the introgression of the HTSS trait into elite cultivar LTSS lines. Further, these 20 selected SSR markers may be utilized for QTL mapping along with allele selection. Further clustering of onion lines based on TSS value further provides an opportunity for the selection of diverse parental groups for molecular breeding of high TSS-containing *Allium cepa* [9, 10, 13,28]. Additionally, these 20 selected polymorphic SSR markers can be used for confirming seed hybridity, selecting HTSS bulbs for seed production, detecting biopiracy, and testing the genetic purity of seeds [28].

CONCLUSION

SSR influences the expression of various trait-associated genes due to their repeat length variability which are responsible for phenotypic variation of plants. In the current study, by application of phenotyping and genotyping, identified top HTSS line HT-GR-1A-M-7 TSS>18--_21.4 (HTSS-21.4%), HT-GR-1A-M-7 TSS>18_21.2 (HTSS-21.2%), HT-GR-1A-M-7TSS>18_21.0 (HTSS-21.0%), HT-GR-2B-M-6(SMC)_20.8 (20.8%), HT-GR-2B-M-6(SMC)_20.2 (HTSS-20.2%) and HT-GR-1A-M-7 TSS>18_20.0 (HTSS-20.0%) could be used for the expedition of the 5G breeding program of onion for introgression of HTSS trait into elite cultivar of onions using these SSR markers. Furthermore, these polymorphic SSR markers could be used to test the genetic purity of both HTSS and LTSS onion seeds. In the future, these marker-assisted selected HTSS bulbs could be employed for the production of genetically pure, high-quality seeds.

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