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Hybridity testing of tomato F_1 progenies derived from parents with varying fruit quality and shelf life using single nucleotide polymorphism (SNPs)

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ABSTRACT

The demand for tomato is increasing day by day mostly because of the increased per capita fresh fruit consumption. Nonetheless as a perishable fruit crop, it has relatively short life after ripening thus experiences remarkable post-harvest losses. The study was to select true F_1 hybrids using SNPs for the development of inbred lines with long shelf life character through marker assisted backcrossing. Nine out of the one hundred and forty SNP markers failed, 93 were uninformative and 38 were polymorphic and spread across the 12 chromosomes. The major allele frequency, gene diversity, heterozygosity and Polymorphic Information Content (PIC) for each locus were calculated for SNP markers using Power Marker 3.5. The mean value of the major allele frequency was 0.673, ranging between 0.529 and 0.794. The average gene diversity and Heterozygosity values were 0.419 and 0.125 respectively. The PIC ranged from 0.273 (Sly11-Rx4) to 0.375 (Sly04-9) with a mean of 0.329. Of the 31 polymorphic SNPs, two SNPs (Sly04-9 and Sly07-2) exhibited the maximum PIC and a gene diversity value of 0.50. The markers Sly11-13 and Sly11-Rx4 were found to be the least informative with PIC and gene diversity values equaling 0.327 and major allele frequency of 0.794. The maximum heterozygosity was observed with SNP markers Sly03-8, Sly06-7, Sly08-1, Sly08-8, while the minimum heterozygosity was observed with SNPs marker Sly02-9, Sly06-1, Sly10-4, Sly10-5, Sly11-13, Sly11-Rx4, and Sly12-9.The SNPs tested for shelf life genes *rin* (Sly05-rin1 and Sly05-rin2) and *nor* (Sly10-nor) were monomorphic within the seven different plants (parentals). The SNP (Sly10-alc) tested for the *alc* gene was however, polymorphic within the seven lines. The result indicates that only one SNP (Sly10-alc) is a functional SNP to detect a long shelf life gene. Tomato genotypes CSIR/CRI-P002 and CSIR/CRI-ATS064 were however, polymorphic for *alc*. The other SNPs for *rin* and *nor* were not useful and new SNPs are needed to be redesigned. The molecular test revealed two superior F_1 hybrids which were selected for evaluation as

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lines for improvement. The advantage is the reduction in cost, time, labor and field space requirement.

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Introduction

Tomato is one of the most important vegetable crops cultivated and consumed worldwide. The production of tomato is an important source of income for small holder farmers who produce the bulk of tomatoes especially in Ghana. An important challenge for fresh market tomato is post-harvest losses. Post-harvest losses in quality and quantity are due to natural perishability, precarious transportation (distance and poor roads), inappropriate packing materials; vehicles convey the fruits and poor storage conditions. The fresh market tomatoes are mainly marketed in the open markets, local stores, road sides and farm gate. A key limitation in tomato fruit marketing is the premature ripening and softening during transportation. This predisposes the crop to rapid post-harvest softening and poor shelf life, leading to great losses which result in low returns to growers, processors and traders. In terms of foreign exchange earnings, the whole country suffers [4]. Postharvest losses of tomatoes can be as high as 25–42% globally [4] and up to 50% in developing countries contributing to higher market prices of the commodity [7]. Kitinoja and Kader [15] reported postharvest losses in Ghana is up to 30%. Firm tomato fruits are demanded by growers, shippers, and processors to enable the fruit withstand the rigors of shipping and reach the consumer in acceptable condition [3]. Development of firm tomato varieties is critical for reduction in post-harvest loses of fresh market tomatoes in Ghana. Extending the shelf life of tomato is very essential for both successful marketing and reduction of great losses in terms of quality and quantity. This would allow farmers and other stakeholders particularly wholesalers and retailers more time to market their produce before quality is degraded. The use of ripening mutant genes in the heterozygous condition offers the possibility of picking fruit at early stages of red pigment development, thereby ensuring harvest of mature fruit, and yet having adequate shelf life [16,19,30]. The gene mutations alter respiration, ethylene evolution, pectolytic enzyme activity, pigment concentration and ultimately shelf life of the fruit. The effect is more pronounced in homozygotes, where fruits do not develop normal color even with the treatment of ethylene. In heterozygotes, the gene expression is additive and develop acceptable color with 100 to 400 percent increase of shelf life [8].

Tomato breeding has resulted in dramatic changes in tomato fruit attributes, plant architecture and productivity. Breeders place emphasis mainly on yield, fruit size, fruit appearance (lack of defects and attractive color), disease resistance and more recently fruit firmness and shelf life [25]. In addition, total soluble solids are an important fruit quality trait to tomato breeders following their high requisite in both fresh market sales and the processing of tomatoes [20]. Traditional plant breeding involves crossing of pure lines selected for favourable alleles to produce plants, inbred parental lines, with desirable traits of interest such as higher yield, pest and disease resistance, nutrition or fruit quality and other postharvest qualities. With the advancements in genetics, molecular biology and tissue culture, plant breeding is now increasingly being carried out using molecular genetic tools as well [29]. Selection of suitable parents is a key issue in hybridization to create new variation that combines the desired traits of the parents. To confirm that the F_1 progenies or hybrids are true crosses produced by crossing parents having different genetic characteristics it is important to establish the hybridity of the progenies. During this process, plant breeders depend on lines with superior phenotypic characters, which is time consuming and sometimes difficult to achieve with classical methods [5].

Current molecular techniques enable scientists to carry out these hybridity tests [32]. The process of molecular breeding in crops was enabled by the transformation of molecular markers as resulted in both the pace and precision of plant genetic analysis. Remarkable advances in the evolution of marker systems and their respective detection platform have been witnessed in the last three eras. The abundance of single nucleotide polymorphisms (SNPs) in the genomes coupled with high-throughput detection during recent years have made them popular in molecular genetics [18]. Innovative sequencing technologies have revealed satisfactory variation to examine the effect of human selection on an entire plant genome. Single Nucleotide Polymorphisms (SNPs) are a major form of sequence variation, scattered throughout the genome and are stable markers for genetic analysis. SNPs have become a marker system of choice for genetic analysis in plant species. In tomato, SNPs have been discovered using several methods. Over 7000 SNPs have been reported for tomatoes by the SolCap project (http://solcap.msu.edu/) and are used in hybridity tests for both small and large scales (Personal communication, M. Massoudi, 2018) The study was designed with the primary objective of examining the hybridity of tomato F_1 progenies using SNPs and SNP-based markers for each chromosome and identifies true F_1 progeny/ies for further development of long shelf life inbreds using marker-assisted backcrossing (MABC). Thus it attempted to use SNPs to identify true hybrids in crosses among 7 tomato parental lines and screen the markers for possibility of identifying those that will be associated with improved shelf life of the crop.

Table 1a
Characteristics of three ripening gene mutants of *Solanum lycopersicum*.

Gene	accession name	Allele	Locus name	Phenotype	Chromosome	Arm	Marker type	Mutant type
Rin	LA1795	–	ripening Inhibitor	Fruits green at maturity, later turns bright yellow, retard ripening	5	L	Morph	SPON
Alc	LA3134	–	alcobaca	Greatly delayed fruit ripening processes; yellow fruit color	10	S	Morph	SPON
Nor	LA3770	2	non-ripening	Very retarded pigmentation, non-softening, and crack resistance of fruits.	10	S	Morph	–

Source: Tomato Genetic Resource Center (2017).
L=Long, *S*=Short, Morph=Morphological, SPON=Spontaneous.

Table 1b
Characteristics of the four CSIR/CRI tomato lines/varieties.

Characteristics\Variety	CSIR/CRI-P005	CSIR/CRI-P002	CSIR/CRI-P082	CSIR/CRI-ATS60
Plant habit	Semi determinate	Semi determinate	Indeterminate	Semi determinate
Leaf type	Regular	Regular	Regular	Regular
Leaf attitude	Semi erect	Horizontal	Horizontal	Horizontal
Exterior color (immature fruit)	Light green	Green	Green	Light green
Exterior color (mature fruit)	Red	Red	Red	Red
Fruit shape	High rounded	Bell shaped	Flattened	Rounded
Fruit size	Intermediate (5.1–8 cm)*	Intermediate (5.1–8 cm)*	Large (8.1–10 cm)	Intermediate (5.1–8 cm)*
Fruit blossom end shape	Flat	Flat	Indented	Flat
Blossom end scar condition	closed	both (open & closed)	Open	closed
Protein (mg/fruit)	0.75 ± 0.017	0.35±0.003	–	–
Ascorbic acid (mg/100 g)	26.02 ± 0.16	30.09 ± 1.26	–	–
Titratable Acidity	3.02 ± 0.084	3.39 ± 0.004	–	–
Brix	6.3	6.6	5.1	5.2
Yield	29 t/ha	27 t/ha	20 t/ha	18 t/ha
*Adaptability	Both seasons	Both seasons	Both seasons	Dry season

Source: CSIR-CRI, Kwadaso (2017).
* Easily established with good/high yield.

Materials and methods

Genetic materials

Seven tomato (*S. lycopersicum*) genotypes, including three ripening gene mutants (*rin, nor, alc*) from the Tomato Genetic Resource Center (TGRC), UC Davis, USA (Table 1a) and four superior tomato varieties (CRI-P002, CRI-P005, CR1-ATS60, CRI-PO82) from CSIR-Crops Research Institute (CSIR-CRI) (Table 1b) were chosen as parents in this study. The ripening mutant genes (*rin, nor,* and *alc)* have the potential to delay fruit ripening processes and prolong shelf life but lacks good agronomic and economically important traits. The CSIR-CRI genotypes were selected based on their agronomic and economic important traits. They however, lack good shelf life and firmness and it is unknown if they have disease resistant genes.

Development of F_1 crosses

The three ripening gene mutants (*rin, nor, alc*) were used as males whereas four superior tomato varieties (CRI-P002, CRI-P005, CR1-ATS60, CRI-PO82) were used as females and crossed to produce 12 F_1 progenies (Table 2) in 2017 at the Kwadaso station of the CSIR-Crops Research Institute, Ghana.

The block was set up in a screen house at the CSIR-Crops Research Institute, Kumasi, Ghana. Two rows were planted for each parent. Twelve seedlings were planted per genotype. Standard agronomic practices such as manual weeding, spraying, fertilizer application etc. were regularly done. The crosses were done on the 6th of February 2017. To ensure that enough F_1 seeds were available for evaluation at two locations, the crosses were repeated in June 2017.

Hand pollination was carried out using forceps, a bottle of 70% ethanol to clean the pollinating forceps between crosses. On each flower cluster, a well-developed flower that has not yet opened was chosen. Preference was given to flowers that showed a little yellowing of the petals as these should be receptive to pollen and set fruit the same day they are pollinated [23]. Two or more sepals were then pulled to expose the petals on one side of the flower. The sides of the petals were further pinched between the thumb and forefinger and gently rocked side to side after which the petals and anthers were pulled straight off (away from the flower). At this stage, all older flowers were pulled off to prevent shedding of pollen onto the freshly emasculated flower. Using the forceps, the anther cone was sliced opened laterally and the mature flower from the male parent (pollen donor) was shook and put on a black material to see the whitish clump of pollen. The pollen was

Table 2
Tomato genotypes and F_1 progenies derived.

Actual name	Designated code	Source
CSIR/CRI-P002	A	Ghana
CSIR/CRI-P005	B	Ghana
CSIR/CRI-P082	D	Ghana
CSIR/CRI-ATS60	E	Ghana
Alc-LA3134	1	USA
Rin-LA1795	4	USA
Nor-LA3770	8	USA
F_1 (CSIR/CRI-P002 x Alc-LA3134)	A1	USA/Ghana
F_1 (CSIR/CRI-P005 x Alc-LA3134)	B1	USA/Ghana
F_1 (CSIR/CRI-P082 x Alc-LA3134)	D1	USA/Ghana
F_1 (CSIR/CRI-ATS06 x Alc-LA3134)	E1	USA/Ghana
F_1 (CSIR/CRI-P002 x Rin-LA1795)	A4	USA/Ghana
F_1 (CSIR/CRI-P005 x Rin-LA1795)	B4	USA/Ghana
F_1 (CSIR/CRI-P082 x Rin-LA1795)	D4	USA/Ghana
F_1 (CSIR/CRI-ATS06 x Rin-LA1795)	E4	USA/Ghana
F_1 (CSIR/CRI-P002 x Nor-LA3770)	A8	USA/Ghana
F_1 (CSIR/CRI-P005 x Nor-LA3770)	B8	USA/Ghana
F_1 (CSIR/CRI-P082 x Nor-LA3770)	D8	USA/Ghana
F_1 (CSIR/CRI-ATS06 x Nor-LA3770)	E8	USA/Ghana
Local (farmers' check)	C	Ghana

Plate 1. Harvesting of tomato leaf samples.

at that moment applied to the emasculated flower. Enough pollen was applied to cover the entire stigma. Lastly, a tag was tied around the flower to indicate that it was hand pollinated.

DNA extraction

Fresh leaf tissues were harvested from three random plants of each tomato genotype grown at Kwadaso station of the CSIR-Crops Research Institute, Ghana and placed in known positions of 96 deep-well plate supplied by Ag-Biotech, Inc (Monterey, CA). The plant materials selected were apparently from disease–free and pest-free sources, free of federal noxious weeds and soils. A leaf sample of approximately 1.5 sq. cm was put into each well and the location of each sample was recorded before they were shipped to Ag-Biotech, Inc. via USDA-APHIS office in the USA (Plates 1 and 2).

A modified CTAB protocol was used to extract genomic DNA of approximately 50 mg. of young leaf tissue from each line or F_1 plant including the parents [10,26].

SNP DNA marker analysis

DNA of approximately 50 ng/μL was prepared from each sample for SNP genotyping. An optimized subset of 140 SNPs markers that were derived from the 7725 SNP array developed by the Solanaceae Coordinated Agricultural Project (SolCap) were used [21,27]. The polymorphism rates between the tomato lines aided in the selection of the markers. The tomato genotypes or lines were screened using the 140 SNPs with a minimum of 10 SNPs for each tomato chromosome.

One hundred and forty (140) SolCap SNPs were converted to allele-specific primers by Ag-Biotech, Inc. In addition to the SolCap SNPs, 13 SNP primers linked to various tomato resistance genes were also included. For the shelf-life gene, *nor,* sequences close to the RFLP markers TG395 and CT16 (trade secret, Ag-Biotech, Inc., personal communication, D. P. Maxwell,

Plate 2. Plates for leaf samples.

2018) were converted to SNP-based markers. For *alc* and *rin*, information from Yogendra and Gowda [33] and Vrebalov et al. [31] was used to design KASP primer sets by Ag-Biotech, Inc. Two SolCap markers were converted for *rin*, and these were called *rin*1 and *rin*2 (chr05). Each allele-specific primer set was composed of two fluorescent-dye labeled primers and one common primer. These primers were used in a reaction mixture for PCR and after about 33 cycles; the fluorescence was detected and used to determine the genotype of the plant from which the DNA had been extracted. The SNP-based detection method was similar to the KASP genotyping platform (LGC Genomics, Beverly, MA, USA). The presence of both alleles from their corresponding parents was identified as true F_1 progenies.

Data analysis

The major allele frequency, gene diversity, heterozygosity and Polymorphic Information Content (PIC) for each locus were calculated for SNP markers using Power Marker 3.5 [28]. Data from the SNP genotyping was grouped into three categories; Combined data for all SNPs and three mutant (Alc, Rin and Nor) data sets for specific SNP markers. The data was then formated as required by DARwin software. Dissimilarity matrix was generated using the Euclidean method in DARwin and subjected to clustering using the complete linkage method in DARwin [22]. This was done for all the data sets. Principal component analysis (PCA) was also carried out on the combined data using the software XLSTAT [1,34] to visualize further the display of parents and hybrids.

Results

Polymorphic SNP DNA markers

Of the 140 SNPs tested on four Ghana breeding lines and three TGRC lines with different genes for extended shelf life, nine SNPs failed. However, of the 131 that gave results, 38 were found to be polymorphic among the lines tested and 93 were uninformative (monomorphic). The SNPs that were found to be polymorphic were spread across the entire tomato genome consisting of 12 chromosomes. More than 15 SNPs were polymorphic for chromosomes 1, 10 and 11. On the other hand fewer polymorphisms were found on chromosomes 3, 5 and 12 (Table 3). Fig. 1 shows the SNP polymorphism screening among the tomato parental genotypes for Sly02-8.

Analysis of the genetic diversity of SNP markers (For: Ghana lines x alc)

The summary of genetic diversity statistics for 31 SNPs on Ghana lines x alc is presented in Table 4. The mean value of the major allele frequency was 0.673, ranging between 0.529 and 0.794. The average gene diversity and heterozygosity values were 0.419 and 0.125 respectively. The PIC ranged from 0.273 (Sly11-Rx4) to 0.375 (Sly04-9) with a mean of 0.329. Of the 31 polymorphic SNPs, two SNP primers (Sly04-9 and Sly07-2) exhibited the maximum PIC and a gene diversity value 0.50 (Table 4.7). The primers Sly11-13 and Sly11-Rx4 were found to be the least informative with PIC and gene diversity values equaling 0.327 and major allele frequency of 0.794 (Table 4), SNP markers which showed low PIC values in the present study may not be considered for future studies on genetic diversity. The maximum heterozygosity was observed with SNP markers Sly03-8, Sly06-7, Sly08-1, Sly08-8, while the minimum heterozygosity was observed with SNPs marker Sly02-9, Sly06-1, Sly10-4, Sly10-5, Sly11-13, Sly11-Rx4, and Sly12-9.

Table 3
List of SNPs markers evaluated for polymorphism among the seven lines.

Entry No.	Primer Name	Chromosome Number	Position (million nt)	Result
1	Sly01-1	1	0.5	Failed
2	Sly01-2	1	2	Monomorphic
3	Sly01-3	1	3	Failed
4	Sly01-4	1	8	Monomorphic
5	Sly01-5	1	12	Monomorphic
6	Sly01-6	1	34	Monomorphic
7	Sly01-7	1	41	Monomorphic
8	Sly01-8	1	58	Monomorphic
9	Sly01-9	1	62	Polymorphic
10	Sly01-10	1	76	Polymorphic
11	Sly01-11	1	80	Polymorphic
12	Sly01-12	1	85	Polymorphic
13	Sly01-13	1	90	Polymorphic
14	Sly01-14	1	97	Monomorphic
15	Sly02-1	2	2	Polymorphic
16	Sly02-2	2	7	Monomorphic
17	Sly02-3	2	10	Monomorphic
18	Sly02-4	2	14	Monomorphic
19	Sly02-5	2	20	Monomorphic
20	Sly02-6	2	30	Monomorphic
21	Sly02-7	2	35	Polymorphic
22	Sly02-8	2	44	Polymorphic
23	Sly02-9	2	48	Polymorphic
24	Sly02-10	2	50	Failed
25	Sly03-1	3	0.5	Monomorphic
26	Sly03-2	3	2	Monomorphic
27	Sly03- 3	3	7	Monomorphic
28	Sly03-4	3	12	Monomorphic
29	Sly03-5	3	25	Monomorphic
30	Sly03-6	3	39	Monomorphic
31	Sly03-7	3	52	Monomorphic
32	Sly03-8	3	60	Polymorphic
33	Sly03-9	3	62	Monomorphic
34	Sly03-10	3	64	Monomorphic
35	Sly04-1	4	1	Failed
36	Sly04-2	4	2	Monomorphic
37	Sly04-3	4	5	Monomorphic
38	Sly04-4	4	10	Monomorphic
39	Sly04-5	4	17	Monomorphic
40	Sly04-6	4	29	Polymorphic
41	Sly04-7	4	44	Polymorphic
42	Sly04-8	4	51	Monomorphic
43	Sly04-9	4	57	Polymorphic
44	Sly04-10	4	62	Monomorphic
45	Sly04-11	4	64	Monomorphic
46	Sly05-1	5	4	Monomorphic
47	Sly05-rin1	5	5	Monomorphic
48	Sly05-rin2	5	5	Monomorphic
49	Sly05-4	5	6	Monomorphic
50	Sly05-5	5	10	Polymorphic
51	Sly05-6	5	23	Monomorphic
52	Sly05-7	5	40	Monomorphic
53	Sly05-8	5	54	Monomorphic
54	Sly05-9	5	59	Polymorphic
55	Sly05-10	5	62	Monomorphic
56	Sly05-Rx3	5	63	Monomorphic
57	Sly05-12	5	64	Monomorphic
58	Sly06-1	6	2	Polymorphic
59	Sly06-Mi	6	2	Monomorphic
60	Sly06-Ty1	6	30	Monomorphic
61	Sly06-Ty3	6	31	Monomorphic
62	Sly06-3	6	3	Monomorphic
63	Sly06-4	6	7	Monomorphic
64	Sly06-5	6	12	Monomorphic
65	Sly06-6	6	29	Polymorphic
66	Sly06-7	6	37	Polymorphic
67	Sly06-8	6	42	Monomorphic
68	Sly06-9	6	46	Monomorphic
69	Sly07-1	7	2	Polymorphic

(*continued on next page*)

Table 3 (*continued*)

Entry No.	Primer Name	Chromosome Number	Position (million nt)	Result
70	Sly07-2	7	3	Polymorphic
71	Sly07-3	7	6	Monomorphic
72	Sly07-4	7	10	Monomorphic
73	Sly07-5	7	32	Monomorphic
74	Sly07-6	7	48	Monomorphic
75	Sly07-7	7	56	Monomorphic
76	Sly07-13	7	61	Monomorphic
77	Sly07-9	7	63	Polymorphic
78	Sly07-10	7	65	Monomorphic
79	Sly08-1	8	0.5	Polymorphic
80	Sly08-2	8	2	Monomorphic
81	Sly08-3	8	8	Monomorphic
82	Sly08-4	8	14	Failed
83	Sly08-5	8	28	Monomorphic
84	Sly08-6	8	39	Monomorphic
85	Sly08-7	8	46	Monomorphic
86	Sly08-8	8	50	Polymorphic
87	Sly08-9	8	56	Polymorphic
88	Sly08-10	8	61	Monomorphic
89	Sly09-1	9	0.25	Polymorphic
90	Sly09-2	9	0.5	Polymorphic
91	Sly09-3	9	2	Monomorphic
92	Sly09-4	9	5	Monomorphic
93	Sly09-Tm2a	9	14	Failed
94	Sly09-5	9	16	Failed
95	Sly09-6	9	35	Monomorphic
96	Sly09-7	9	50	Monomorphic
97	Sly09-Frl	9	65	Monomorphic
98	Sly09-8	9	67	Monomorphic
99	Sly09-Ph3	9	72	Monomorphic
100	Sly09-10	9	72	Monomorphic
101	Sly10-1	10	1	Polymorphic
102	Sly10-nor	10	1	Monomorphic
103	Sly10-alc	10	1	Polymorphic
104	Sly10-4	10	3	Polymorphic
105	Sly10-5	10	4	Polymorphic
106	Sly10-6	10	11	Monomorphic
107	Sly10-7	10	24	Monomorphic
108	Sly10-8	10	35	Monomorphic
109	Sly10-9	10	47	Polymorphic
110	Sly10-10	10	51	Monomorphic
111	Sly10-11	10	57	Polymorphic
112	Sly10-12	10	60	Monomorphic
113	Sly10-Ph2	10	65	Monomorphic
114	Sly11-1	11	0.25	Monomorphic
115	Sly11-2	11	0.5	Monomorphic
116	Sly11-3	11	3	Monomorphic
117	Sly11-4	11	8	Monomorphic
118	Sly11-5	11	9	Monomorphic
119	Sly11-6	11	16	Polymorphic
120	Sly11-7	11	30	Monomorphic
121	Sly11-8	11	40	Monomorphic
122	Sly11-9	11	40	Monomorphic
123	Sly11-10	11	49	Monomorphic
124	Sly11-11	11	49	Polymorphic
125	Slyl1-12	11	52	Monomorphic
126	Sly11-i2	11	52	Monomorphic
127	Sly11-Ty2	11	52	Monomorphic
128	Sly11-13	11	54	Polymorphic
129	Slyll-Rx4	11	54	Polymorphic
130	Sly11-14	11	56	Monomorphic
131	Sly12-Bw12	12	3	Monomorphic
132	Sly12-2	12	5	Monomorphic
133	Sly12-3	12	13	Monomorphic
134	Sly12-4	12	30	Monomorphic
135	Sly12-5	12	36	Monomorphic
136	Sly12-6	12	41	Failed
137	Sly12-7	12	46	Failed
138	Sly12-8	12	54	Monomorphic
139	Sly12-9	12	63	Polymorphic
140	Sly12-10	12	66	Polymorphic

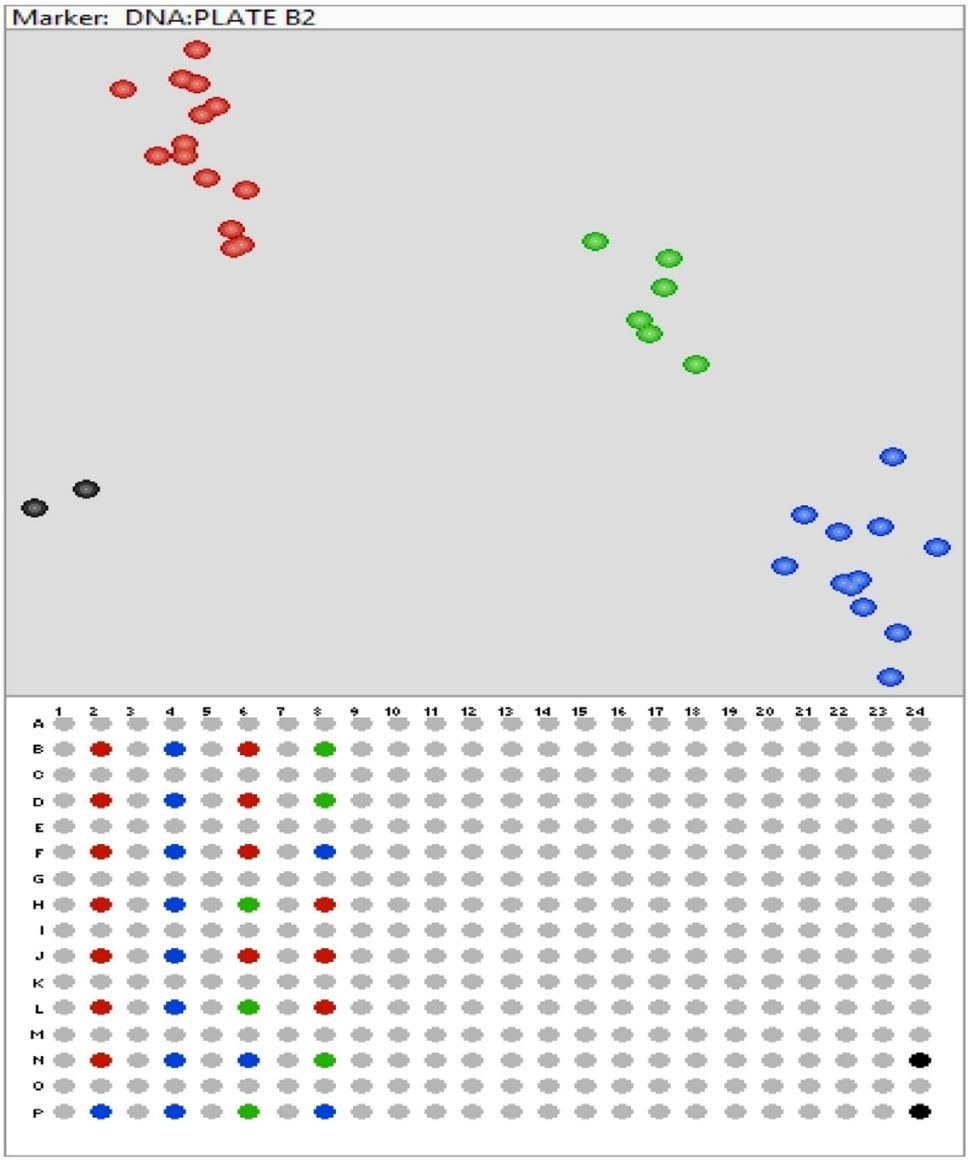


Fig. 1. KASP assay for SNP Sly02-8 showing the SNP genotype of 19 samples. Red dots = *Y*: Y genotype, Green dots = *X*: Y genotype, Blue dots = *X*: X genotype. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Putative F_1 progenies based on polymorphic SNP markers

Of the 38 SNP markers that were polymorphic only 36 were able to discriminate between the seven parents and 12 hybrids (Fig. 2). SNP marker, Sly03-8 however, gave the highest success rate of 83.3% to distinguish between the parents and hybrids while SNP markers; Sly06-6, Sly10-4, Sly11-6 and Sly12-2 gave the lowest percentage (Fig. 2). Seven SNP markers comprising Sly01-13, Sly02-8, Sly03-8, Sly04-9, Sly07-2, Sly08-9 and Sly11-11 had more than 50% success rate in hybrid identification across the multiple crosses and parents.

Fig. 3 displays percentage of SNPs that identified each of the F_1 hybrids as true. Out of the 36 SNPs that distinguished between the parents and hybrids, the highest number of SNPs representing 31% (18 SNPs) identified F_1 (Alc-LA3134 x CSIR/CRI-P002) and F_1 (Alc-LA3134 x CSIR/CRI-ATS06) as true hybrids. The least number of SNPs representing 5% identified F_1 (Alc-LA3134 x CSIR/CRI-P005) as true hybrid.

Fig. 4 indicates clustering of parents and F_1 hybrids as revealed by the SNPs. It groups the genotypes into three clusters and the parents are clearly divergent from each other. Tomato F_1 hybrids; B4, D1 and B1 are in the same cluster group with Ghana lines used as females. In the green cluster where we have F_1s comprising D8, B8 and A8 very few markers identified F_1s as successful or true as per closeness to the cluster comprising the female parents in the circular route. The F_1s in the

Table 4
Summary of statistics calculated for genetic diversity based on 31 informative SNP.

Marker	Major allele frequency	Gene diversity	Heterozyosity	PIC
Sly01-11	0.559	0.493	0.059	0.372
Sly01-13	0.529	0.498	0.118	0.374
Sly02-8	0.529	0.498	0.118	0.374
Sly02-9	0.765	0.369	0.000	0.295
Sly03-8	0.706	0.415	0.235	0.329
Sly04-6	0.625	0.469	0.125	0.359
Sly04-7	0.588	0.484	0.118	0.367
Sly04-9	0.500	0.500	0.177	0.375
Sly05-5	0.765	0.359	0.118	0.295
Sly05-9	0.735	0.389	0.177	0.314
Sly06-1	0.794	0.327	0.059	0.274
Sly06-6	0.706	0.415	0.118	0.329
Sly06-7	0.706	0.415	0.235	0.329
Sly07-1	0.735	0.389	0.176	0.313
Sly07-2	0.500	0.500	0.176	0.375
Sly07-9	0.765	0.359	0.117	0.295
Sly08-1	0.647	0.456	0.235	0.352
Sly08-8	0.706	0.415	0.235	0.329
Sly08-9	0.75	0.375	0.125	0.304
Sly09-1	0.765	0.359	0.117	0.295
Sly10-1	0.765	0.359	0.117	0.295
Sly10-alc	0.765	0.359	0.117	0.295
Sly10-4	0.559	0.493	0.058	0.371
Sly10-5	0.559	0.493	0.058	0.371
Sly10-11	0.765	0.359	0.117	0.295
Sly11-6	0.559	0.493	0.176	0.371
Sly11-11	0.529	0.498	0.117	0.374
Sly11-13	0.794	0.327	0.058	0.273
Sly11-Rx4	0.794	0.327	0.058	0.273
Sly12-9	0.559	0.493	0.058	0.371
Sly12-10	0.735	0.389	0.176	0.313
Mean	**0.673**	**0.419**	**0.125**	**0.329**

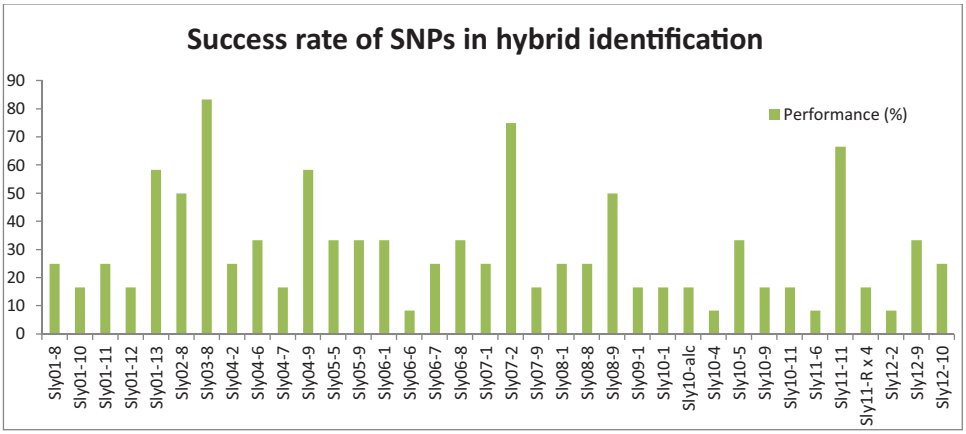


Fig. 2. SNPs panel performance in hybrid identification.

blue cluster comprising A1, E1, A4, D4, E4 and E8 nonetheless, had higher number of SNPs contributing to successful hybrids than the F_1s in the green cluster. Besides, in the same cluster the F_1s such as A1 and E1 which are positioned in the middle of the circle route is between the two parents had higher number of SNPs compare with other A4, D4, E4. E8.

Fig. 5 displays results of the Principal Component Analysis (PCA) showing separation of the male parents (Nor-LA3770, Rin-LA1795 and Alc-LA3134) from the female parents (CSIR/CRI-P005, CSIR/CRI-P002, CSIR/CRI-P082 and CSIR/CRI-ATS06) with majority of F_1s being close to the female parents. The F_1s however, do not concede with the females. The F_1s are also farther away from the males. The F_1 placement on the PCA is dependent on the proportion of SNPs that identified it as true. At least five F_1s were distant from the females with the rest being closer to the females at different remoteness.

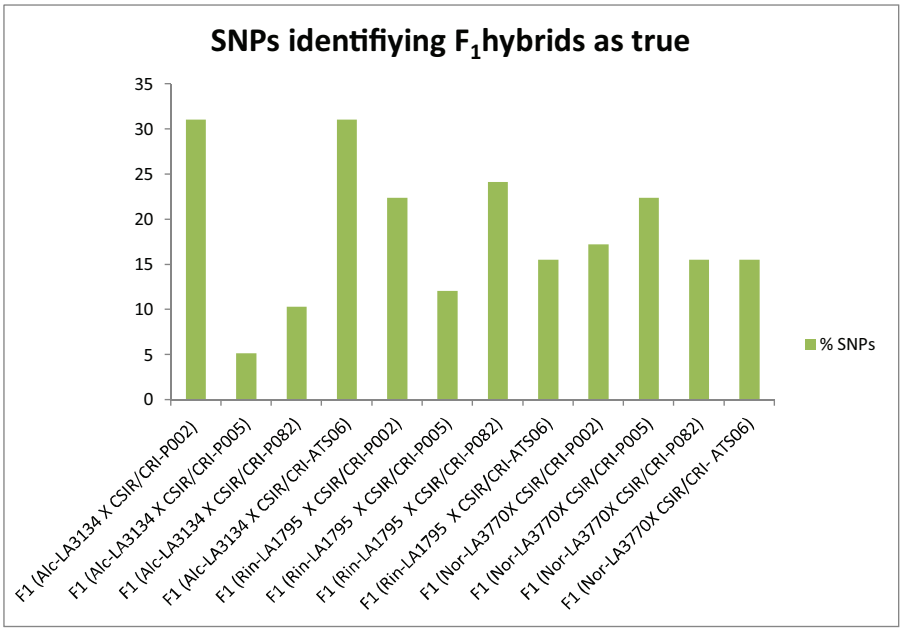


Fig. 3. Percentage SNP markers identifying F_1 hybrids as true.

Table 5
SNPs screening for ESL associated with parental lines using *rin, nor* and *alc* SNPs.

Tomato genotype (Parental)	Sly05-rin 1	Sly05-rin 2	Sly10-nor	Sly10-alc
CSIR/CRI-P002	MM	MM	MM	PM
CSIR/CRI-P005	MM	MM	MM	MM
CSIR/CRI-P082	MM	MM	MM	MM
CSIR/CRI-ATS06	MM	MM	MM	PM
LA1795 (RIN)	MM	MM	MM	MM
LA3134 (ALC)	MM	MM	MM	PM
LA3770 (NOR)	MM	MM	MM	MM

MM = M onomorphic; PM = Polymorphic; ESL = Extended Shelf Life.

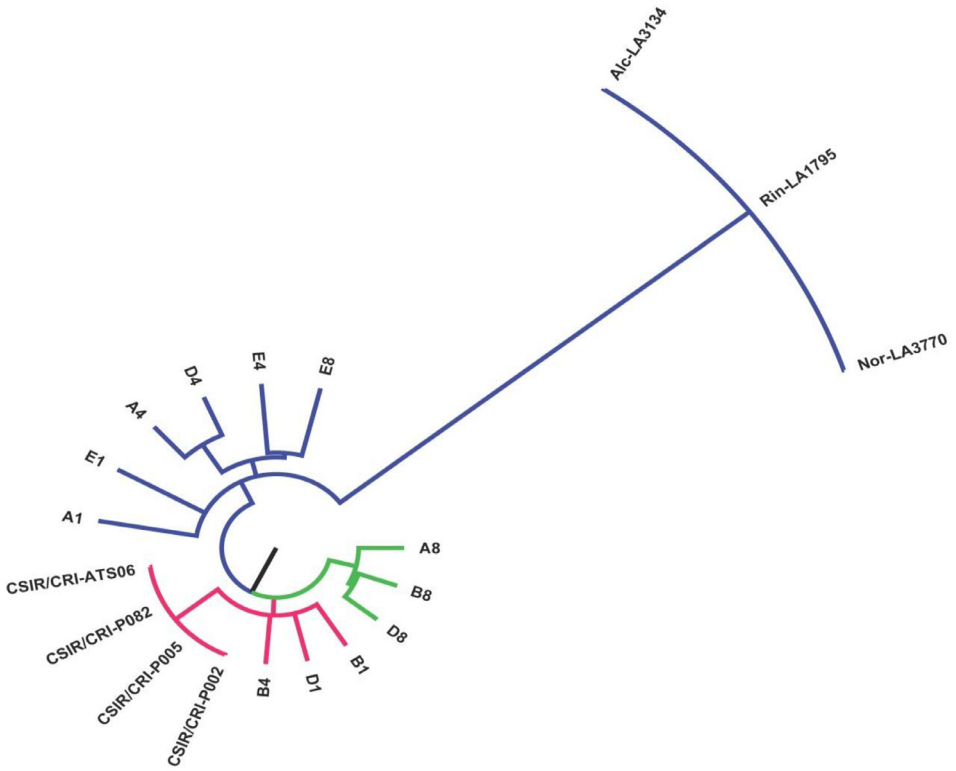
Screening for shelf life genes using rin, nor and alc SNPs markers

The SNPs tested for shelf life genes *rin* and *nor* were monomorphic for the seven different plants (parental). The SNP (Sly10-alc) tested for the *alc* gene was however, polymorphic within the seven lines. The result indicates that only one SNP (Sly10-alc) is a functional SNP to detect a long shelf life gene. Tomato genotypes CSIR/CRI-P002, CSIR/CRI-ATS06 and Alc-LA3134 were however, polymorphic for *alc* (Table 5). Fig. 6 shows clustering of parents and F_1 hybrids as revealed by Sly10-alc. With three cluster groups, two of the F_1s (B1 and D1) are in the same group of the female parents while A1 and E1 are neither in the female nor male cluster group.

The other SNPs used to discriminate between the two groups (mutant and without mutant) did not flank the region of chromosome where they are located thus was not useful in this study and new SNPs are needed to be redesigned. Figs. 7 and 8 reveals clustering of parents and F_1 hybrids of nor and rin mutants that failed in discriminating between the parents and hybrids. In each of these figures, only two clusters were noticed. All the F_1s are in the same group with the female parents singling out the male parent (mutant) in another group.

Screening for resistance genes

Of the seven different plants that were tested for resistance genes using 13 SNPs from Ag-Biotech Inc., only CSIR/CRI-ATS60 **[E]** had a resistance gene for Rx4, bacterial spot (Sly11-Rx4; Table 6). No resistance genes were detected for Fusarium crown and root rot, bacterial wilt, Fusarium wilt, late blight, root-knot nematode, or geminivirus-resistant genes.



Parents	CSIR/CRI-P002	=	[A]	Alc-LA31347	=	[1]
	CSIR/CRI-P005	=	[B]	Rin-LA1795	=	[4]
	CSIR/CRI-P082	=	[D]	Nor-LA3770	=	[8]
	CSIR/CRI-ATS06	=	[E]			

F_1s	F_1 (CSIR/CRI-P002 x Alc-LA3134)	=	[A1]
	F_1 (CSIR/CRI-P005 x Alc-LA3134)	=	[B1]
	F_1 (CSIR/CRI-P082 x Alc-LA3134)	=	[D1]
	F_1 (CSIR/CRI-ATS06 x Alc-LA3134)	=	[E1]
	F_1 (CSIR/CRI-P002 x Rin-LA1795)	=	[A4]
	F_1 (CSIR/CRI-P005 x Rin-LA1795)	=	[B4]
	F_1 (CSIR/CRI-P082 x Rin-LA1795)	=	[D4]
	F_1 (CSIR/CRI-ATS06 x Rin-LA1795)	=	[E4]
	F_1 (CSIR/CRI-P002 x Nor-LA3770)	=	[A8]
	F_1 (CSIR/CRI-P005 x Nor-LA3770)	=	[B8]
	F_1 (CSIR/CRI-P082 x Nor-LA3770)	=	[D8]
	F_1 (CSIR/CRI-ATS06 x Nor-LA3770)	=	[E8]

Fig. 4. Clustering of parents and F_1 hybrids as revealed by the SNPs.

Discussion

SNP markers and genetic diversity

In this study, 38 polymorphic SNP loci were used to examine tomato genotypes so as to identify true F_1 progenies. SNP markers allowed us to determine the genetic variability present between the genotypes used in this study. Although the parental genotypes utilized have contrasting background, very low polymorphisms were found when screened with the SNPs. This can be attributed to the monoecious and self-pollinating nature of tomato. The data gathered in this study show

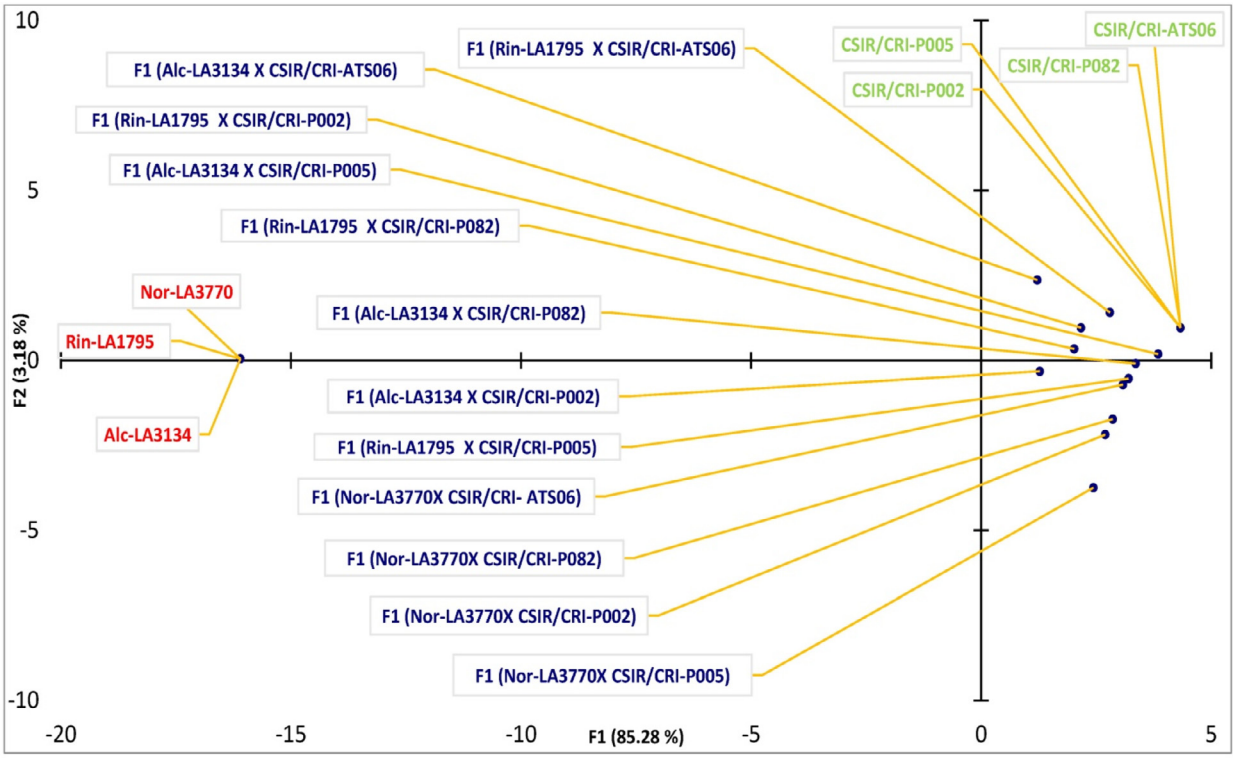


Fig. 5. Principal Component Analysis (PCA) of tomato F_1s and parents.

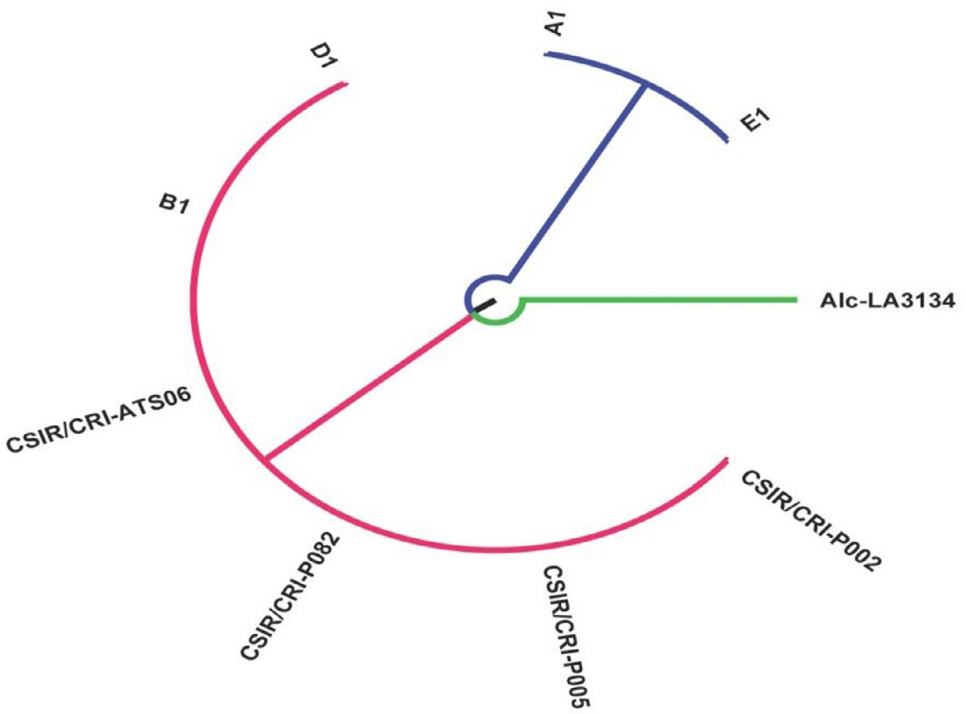


Fig. 6. Clustering of parents and F_1 hybrids as revealed by the Sly10-alc.

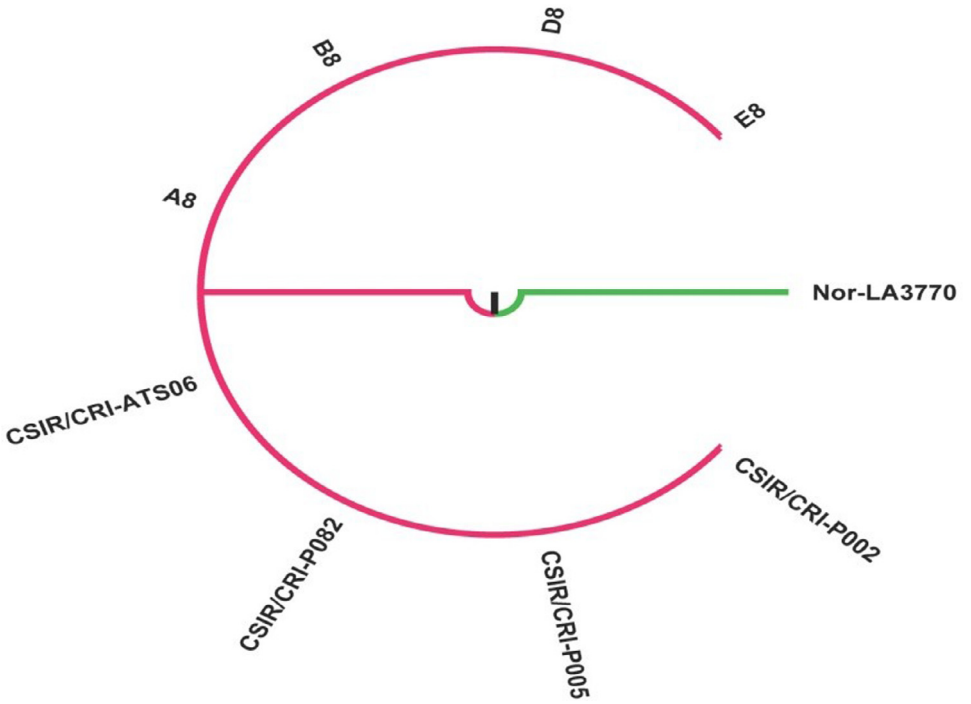


Fig. 7. Clustering of parents and F_1 hybrids as revealed by the Sly10-nor.

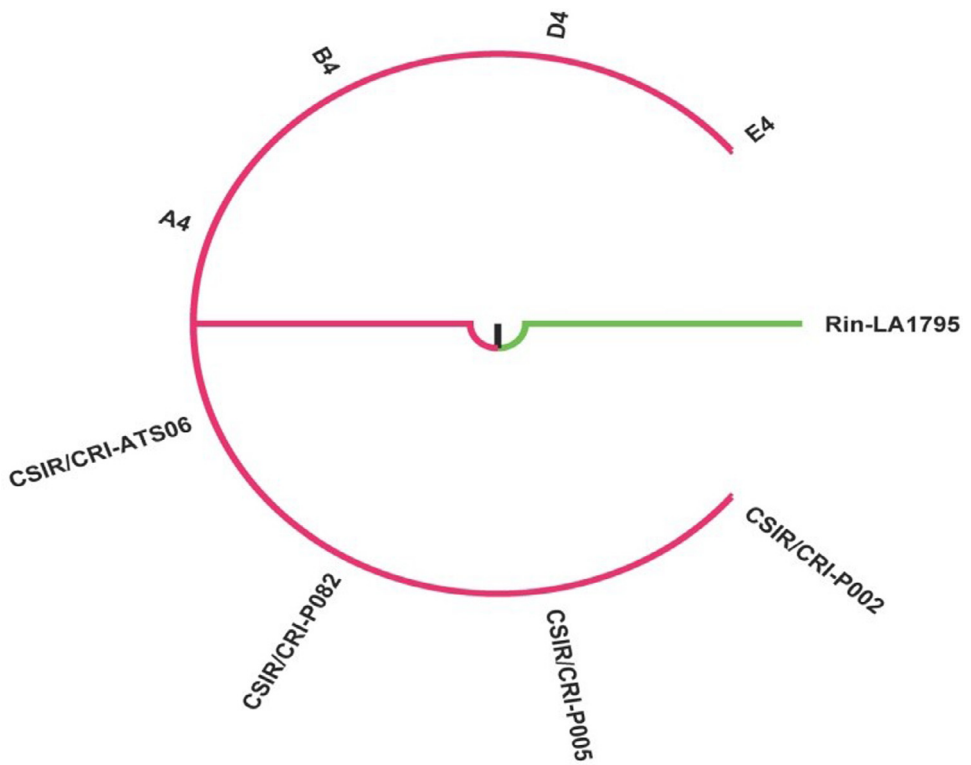


Fig. 8. Clustering of parents and F_1 hybrids as revealed by the Sly05-rin 1 and Sly05-rin 2.

Table 6
SNPs screening for resistance genes on tomato parental genotypes.

Resistance genes/ESL	A	B	D	E	1	4	8
Frl resistance to Fusarium crown & root rot	–	–	–	–	–	–	–
Bw12, resistance to bacterial wilt	–	–	–	–	–	–	–
Rx4, bacterial spot	–	–	–	+	–	–	–
I2, Fusarium wilt race 2	–	–	–	–	–	–	–
Ph2, late blight resistance	–	–	–	–	–	–	–
Ph3, late blight resistance	–	–	–	–	–	–	–
I3, Fusarium wilt race 3	–	–	–	–	–	–	–
Mi, root knot nematode resistance	–	–	–	–	–	–	–
Ty1, TYLCV and other geminiviruses	–	–	–	–	–	–	–
Ty2, TYLCV and other geminiviruses	–	–	–	–	–	–	–
Ty3, TYLCV and other geminiviruses	–	–	–	–	–	–	–
Rx3, bacterial Spot resistance	–	–	–	–	–	–	–

A = CSIR/CRI-P002; **B**= CSIR/CRI-P005; **D** =CSIR/CRI-P082; **E** =CSIR/CRI-ATS60; **1**= Alc-LA3134; **4**= Rin-LA1795; **8** = Nor-LA3770; - =absent; + =present.

that it is possible to accurately and rapidly determine true hybrids in tomato F_1s using SNPs and simultaneously do away with the inaccurate and large morphological observations. The use of SNPs in this study was meant to increase selection efficiency thereby reducing F_1 progenies/hybrids that need not be carried over into the next experiment. This implies that breeders can discard false F_1s early and save time and other valuable resources [6]. It takes only two to three weeks to obtain the SNP data from the time seeds are planted; in contrast, morphological observation for identifying hybrids may take months or several growing seasons. With the use of molecular markers target traits can be identified from the true F_1 progenies for efficient selection and accurate decision making. In addition, these data can supplement other data obtained through genetic diversity analyses and association studies if the same materials were used. The genetic divergence study helps the breeder to concentrate on possible promising parent combinations.

Significant differences among the genotypes were present considering the allelic richness per locus. Polymorphic Information Content (PIC) values of each marker were used as decisive factor for the informativeness of each marker in resolving the diversity of tomato genotypes. Based on PIC value grouping, all the SNPs were classified as reasonably informative, suggesting their potential use for linkage disequilibrium and association mapping studies. However, SNP markers which showed low PIC values may not be considered for future studies on genetic diversity. The maximum number of SNP markers observed on chromosomes 1, 10 and 11 could be ascribed to the fact that they harbor most of the fruit quality related genes. This however, disagrees with Doganlar et al. [9]; Labate and Robertson [17] and Sim et al. [27] who in other independent studies described chromosome 9 as the anchorage for most of the disease resistance and fruit quality related genes.

Putative F_1 progenies based on polymorphic SNP markers

For an effective validation of hybrid, the markers used should be able to differentiate clearly the heterozygotes. An organism is considered as a hybrid when gene segments from its paternal parent is introgressed into certain parts of its genome and still have chromosomal segments which are of maternal origin. In this study, three genes (*rin, nor, alc*) that were converted for SNP markers and utilized in the crosses produced 12 F_1 progenies. SNP marker Sly10-alc, (*alc* -shelf life gene) identified true F_1 hybrids or progenies with the shelf life gene out of the putative F_1 progenies. The molecular analysis revealed that among the four crosses involved with *alc* gene, the progenies from two crosses (Alc-LA3134 x CSIR/CRI-P002 and Alc-LA3134 x CSIR/CRI-ATS06) were confirmed as true hybrids. This was noticeable in the cluster analysis at Fig. 6 where progenies from the two crosses cluster into one group as true hybrids. However, some selfed individuals as well as other putative F_1s were observed during the testing of hybrids. It was therefore not surprising to find some of the F_1s in the same cluster with the female parents thus demonstrating crosses were unsuccessful. According to Ahmad et al. [2] identification of true hybrid is therefore essential for advancing into the next generation or developing a segregating population since even a low number of selfed plants can mislead subsequent genetic studies including inheritance or genetic mapping. The SNP marker Sly10-alc has been validated using an F_2 population in another study. It established a locus linked to Sly10-alc is significantly associated with *P*-value of 0.0001and has effect of increasing shelf life by 22 days (unpublished).

The F_1 progenies obtained from this study can be classified into four groups namely expected progenies, unexpected progenies, true F_1 progenies and false F_1 progenies. The expected progenies exemplified progenies that produced heterozygotes F_1s from polymorphic parents based on the SNP markers. On the other hand progenies that produced heterozygotes F_1s from monomorphic parents are grouped as unexpected. Successful progenies were distinguished when screened with the SNP marker Sly10-alc (shelf life gene) and progenies from monomorphic SNPs between parents (Ghana lines and Alc-LA323) that produced heterozygotes F_1s represented true F_1 and false F_1 respectively.

The presence of both alleles from crossing female and male polymorphic parents indicates that the crosses were successful for SNP markers that produced heterozygotes F_1 progenies. This is based on individuals consistently being genotyped as heterozygous such as expected. The progenies that were selected as true hybrids displayed as such. Since a single seed can be formed from one fertilization event, it can therefore be assumed that a progeny is indeed a product of cross-pollination

given that in certain part of its genome, it contains segments that are inherited from its male parent. In subsequent studies, SNP markers that were monomorphic would not be used. This is in agreement with Graves et al. [14] who indicated that for individuals that consistently did not satisfy the expected heterozygous SNP genotype, with regards to KASP genotyping assays for which both parents were homozygous for opposite alleles should be removed from ensuing studies. For monomorphic parents that produced heterozygotes F_1 progenies, it is possible that some of the early–maturing stigmas were exposed to other pollen within the greenhouse rather than the intended ones. This could explain why some of the progenies were identified as unexpected genotypes.

According to Glaszmann et al. [13] the major requirement of any breeding program is to ensure that accurate crosses are made. In this study some progenies had SNP genotypes matching the female parent and were determined to be selfs. This was evident in Figs. 4–8 and explains why they were clustered into the same group with the female parents. According to Fang et al., [11] and Gedye et al. [12] since the likelihoods of self-cross pollination always exist; it is therefore warranted that true crosses/selfs are confirmed using molecular techniques. This implies that same maternal features in terms of exhibiting maternal allele in all the markers used in hybridity screening can be said to be a product of self-pollination indicating unsuccessful cross-pollination. Some of the putative F_1s that demonstrated false declined to detect the 'X' allele even in the crosses where the presence of the 'X' allele was the only option. On the other hand some SNP markers detected more heterozygotes than expected showing heterozygotes even in crosses where both parents were homozygotes for the same allele. The discrepancies made the information from such markers unreliable implying cross pollination from another source is most likely the problem. In all these the 36 SNPs especially the seven markers ($>$ 50% success rate) that distinguished hybrids in most of the crosses could be useful in other studies. These selected SNPs have been validated in our marker assisted backcrossing project (unpublished).

Screening for shelf life genes using rin, nor and alc SNPs markers

The study was to identify SNPs that can differentiate between parents with mutant genes for shelf life and the parent without the genes. Although, the mutant alleles of these three genes have similar effect on extending shelf life as well as maintenance of firmness, only SNP marker; Sly 10-alc was polymorphic for shelf life genes on the tomato genotypes used for this study. Thus leaving *rin* and *nor* SNP markers non-informative hence limiting their further usage in the study. Nonetheless it does not mean that *rin* and *nor* genes are not important for shelf life but the SNPs used did not flank the region of chromosome where they are located. This further explains why all the F_1s for both rin and nor SNPs were grouped in the same cluster with the females thus signifying crosses were not successful. For the *alc* and *rin* genes, information from Yogendra and Gowda [33] and Vrebalov et al. [31] was used to develop SNP-based KASP primer sets by Ag-Biotech, Inc. In the case of *nor,* SolCap markers (trade secret, AgBiotech, Inc., personal communication, D. P. Maxwell, 2018) close to the RFLP markers TG395 and CT16 were converted to SNP-based markers.

The Kompetitive Allele Specific PCR (KASP) primer set for the *alc* gene was successful as the SNP used was in the *alc* gene. The KASP primer sets for *nor* and *rin* from Ag-Biotech, Inc. were designed from SOLCAP SNPs near the *nor* and *rin* regions on chromosomes 10 and 5 respectively, but had not been verified. The *nor* is positioned 1 million nt whereas *rin* is 5 million nt. Thus, this first test showed that they were not useful for detection of the *nor* and *rin* genes. Other KASP primer sets will need to be designed and tested. It is always a major effort to develop KASP primers that will be tightly linked to a particular gene. It involves using information from the literature, then sometimes sequencing various genotypes to develop of KASP primers and then testing the KASP primers on known genotypes (personal communication, D. P. Maxwell, 2018). Sometimes they work and sometimes new primers have to be designed. So the latter is the case for *nor* and *rin* primer sets.

Screening for resistance genes

In this study, a disease resistance gene called RX4 for resistance to Bacteria spot was detected only on CSIR/ATS06 tomato parent. Plant diseases do not only have impact on yield but also on fruit quality. According to Ritchie [24], our understanding of host-pathogen interactions and molecular basis of the gene for gene model is more enhanced through the study of this bacterial pathogen. Thus identifying a bacteria spot resistant gene in one of the parents is important for our future breeding work. Additional efforts are however, needed to introgress other important resistance genes such as Ty-1, Ty-2, Ty-3 of geminivirus; MI of root knot nematode; I2 for Fusarium wilt race 2 and Bacteria wilt which are of economic importance in Ghana. The use of these SNPs show the power of marker assisted breeding as an important tool and a necessity for future breeding efforts.

Conclusion

This study was to select true F_1 hybrids using SNPs for possible development of long shelf life inbreds using marker assisted backcrossing (MABC). This study revealed that SNP markers can be utilized to successfully distinguish honest to goodness cross breeds in tomato plants. Screening SNP markers for polymorphism demonstrated that distinctive tomato genotypes can be signified by different alleles which are useful in perceiving genuine hybrids among descendants. The utilization of SNP markers conveyed an early location technique to choose the *alc* gene and screen out plants even at a beginning time of development of inbreds. The molecular test revealed two superior F_1 hybrids which were selected for

further evaluation and improvement. The advantage is not advancing putative F1s that are false coupled with the reduction in cost, time, labor and field space requirement

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