

HYBRID PURITY ANALYSIS OF NSL COMMERCIAL TOMATO HYBRIDS USING SSR MARKERS

J.AKHILA

Master of Science, Department of Biotechnology, Vikrama Simhapuri University, Nellore, India

Abstract: *Exploitation of the complete potential of any hybrid needs the possessing of genetically High-purity seeds. SSR microsatellite markers(Brondani et al. 2006; Acuna et al. 2012; He et al. 2012; Faria et al. 2010) were used to test seed genetic purity (of two commercial hybrid tomatoes (*Lycopersicon esculentum* L.) varieties ‘NTH 1813’ and ‘SONA’. 35 SSR markers were used to detect the polymorphic pattern between their parental lines. F1 hybrids (Marschall S. 2006) mainly showed a complementary pattern of both parents, which could be very useful to distinguish the F1 from its parental lines (Peter Abramoff, Thomson 1994). The number of SSR primers was showed monomorphic DNA bands with parental lines. Three primers showed polymorphic bands with two commercial tomato varieties. Toms2 and toms236 were showed polymorphic DNA bands with NTH1813 and MT8 showed polymorphic DNA bands with SONA.*

Keywords: *hybrid, purity, SSR markers, complementary, F1 hybrid, parental lines, polymorphic bands.*

I. INTRODUCTION

Tomato (*Solanum lycopersicum*) is the world’s largest vegetable crop. Tomato (Muller CH, 1940) is one of the most important vegetable plants in the world. It was originated in “Western South America”, and domestication is thought to have occurred in Central America. Because of its importance as food, a tomato has been bred to improve productivity, fruit quality, and resistance to “biotic” and “abiotic stresses” (Shao et al., 2008).

Tomato has been widely used not solely as food, but also as research material. The tomato plant has many interesting features such as fleshy fruit, a sympodial shoot (Schmitz G, Theres K.1999) and compound leaves, which other model plants (e.g., rice and *Arabidopsis*) do not have. Most of those traits are agronomically vital and can't be studied in mistreatment alternative model plant systems. There are unit “13 recognized wild tomato species (Peralta, et al 2008) that show a good form of phenotypes and might be crossed with the cultivated tomato. These wild tomatoes are important to breeding, as sources of desirable traits, and for evolutionary studies. Current progress of the tomato genome sequence project has generated useful information to help in the study of tomato.

A molecular marker could be a sequence or deoxyribonucleic acid sequence with a renowned location on a body and related to a specific sequence or attribute. It will be delineated as a variation, which can arise thanks to mutation or alteration within the genomic loci that may be ascertained. differing kinds of PCR bases markers (Welsh and McClelland, 1990) are developed. fragment Length Polymorphism (RFLP), Random Amplified Polymorphic deoxyribonucleic acid (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeat (ISSRs), Sequence characterised Amplified Regions (SCARs), Sequence Tag Sites (STSs), Cleaved Amplified Polymorphic Sequences (CAPS), microsatellite or straightforward Sequence Repeats (SSRs), Expressed Sequence Tags (ESTs), Single ester Polymorphism (SNPs), Variable variety bicycle Repeats (VNTR), Short bicycle Repeats (STR) and begin sequence Targeted Polymorphism (SCOT).

Microsatellites or simple sequence repeats (SSRs) are simple sequences of tandemly repeats which can presently be a short motif of di-nucleotides or tri-nucleotides, or tetra-nucleotides repeated and contains in 1-6 base pairs (bp) in length. On the chromosome, SSRs occur once in every 18.8 kb, on the average, with one SSR per 23.8 kb and 16 kb on short and long arms, respectively. In genetic engineering, SSRs have played an important role in genome evolution. SSRs have become a popular type of co-dominant molecular marker in genetic analysis and plant breeding application and also been useful in integrating genetic, physical, and sequence-based maps of rice, and provided breeders and geneticists with an efficient tool to link phenotypic and genotypic variations. Therefore, they represent new sources of informative genetic markers.

II. REVIEW OF LITERATURE

3 DNA molecular marker systems, RAPD, ISSR, and SSR, were used to check seed genetic purity of a hybrid tomato ‘Hezuo906’. Genetic relationships of the F1 hybrid and their 2 oldsters were analyzed with 218 RAPD random decamer primers, fifty-four ISSR primers, and forty-nine SSR primers. Among the 321 primers screened, 2 dominant RAPD primers and 2 codominant SSR primers were hand-picked for testing the genetic purity of the F1 seeds. a complete of 208 hybrid people were investigated with the chosen primers. The combined results of the marker analyses confirmed that 10 of 208 ‘Hezuo906’ were false hybrids, and also the overall genetic purity of the F1 hybrid seed ton was ninety-five .1%. This study showed that the molecular markers are a unit extremely economical and reproducible for genetic purity testing of tomato business hybrid seeds (Liu.L et.al., 2008).

Salt stress was applied to tomato business genotypes to visualize adverse effects on their composition traits. 3 were saline tolerant (San Miguel, Romelia and Llanero), 2 were gently tolerant (Perfect peel HF1 and Heinz 1350) whereas the remaining were sensitive. Genotyping cultivars victimization nineteen polymorphic SSRs out of twenty-five tested created a whole of seventy alleles with a mean of 3.68 alleles per locus and PIC values starting from zero.22 (SSR 26, 92, sixty-six and TG35) to zero.82 (SSR 356). Principal component analysis (PCA) showed a pair of contrastive panels discriminating tolerant and sensitive groups and one panel with scattered genotypes. Structure analysis clustered genotypes among three groups in accordance with their salt stress behavior. The success of tomato salt-tolerance breeding programs could also be accrued through molecular characterization of diversity among business cultivars that adapt otherwise to worry conditions. to the present finish, we tend to combined phenotypes and SSR marker-genotypes to hunt sources of salt tolerance which may be tomato species-specific. we tend to integrate and delineate genotype-phenotype associations from multiple loci into a multi-layer network illustration. it's a general browse linking discriminating genotypes to salt stress phenotypes, which can guide methods for the introgression of valuable traits in target tomato varieties to beat salinity. (Charfeddine Gharsallah et.al.,2016).

Simple sequence repeats or SSRs is a type of DNA-based that promises to be a reliable marker for hybridity testing plants. In this study, a simple procedure for determining hybridity using tomato seedlings has been developed. Three tomato F1 hybrids developed by the Institute of Plant Breeding-UPLB-CA were used, namely: F1-6137, F1-6174, and F1-6193. F1-6137 and F1-6174 are new variety releases for off-season tomato production and are named "Assunta" and "Ara", respectively.27 SSR primer pairs were screened initially for polymorphism using bulked DNA samples from each of the parents and hybrids. Two SSR primers were found polymorphic Primer 1 (for F1-6137 and F1-6193) and Primer 2 (for F1-6174). These markers were then used to successfully assess and validate the genetic purity or hybridity of the F1 progenies in each of the three hybrids. The results of this study have demonstrated that SSR marker technology is a highly reproducible and reliable early detection procedure for hybrid purity in tomato varieties. Compared to the traditional field testing procedure such as grow-outs, the use of SSR markers could potentially save a lot of time, money and effort. So far, this is the first report of successful development and utilization of SSR marker for hybridity testing for improved tomato varieties in the Philippines (Caguiat, PI et.al., 2003).

III. MATERIALS AND METHODS

a. plant materials:

For the purpose of molecular identification, two tomato hybrids NTH1813, SONA released for commercial cultivation in different parts of India and their parental lines were selected. The F1 seeds of these hybrids and their parental lines were obtained from Tomato Section, Nuzivedu seeds, Medchal, Secunderabad. Seeds of the above-mentioned hybrids were grown in the greenhouse. A random sample of 400 seeds of each of the hybrid representing the commercial F1 seed lot was used for testing their genetic purity.

b. Molecular analysis:

Plant deoxyribonucleic acid was isolated from seedlings victimization CTAB (Van der Beek et al., 1999) protocol as follows: concerning 0.1 g of young leaf tissue for every sample was homogenized in atomic number 7, and incubated at 60°C for 30–45 min with 500 µL of CTAB buffer (1.0M pH 8.0 Tris-HCL, 3ML NaCl, 0.5 EDTA, 1% PVP-360). Then 500µL 24:1 of chloroform: isoamyl alcohol mixture was further and homogenized completely for 5min. After the natural process (5 min, 13000 rpm), the aqueous layer was pipetted into a new Eppendorf tube and an approximately equal volume of cold ethanol was added. After storage at -20°C for 30–60 min, precipitated polymer was centrifuged, vacuum dried and at last hold on in TE buffer. For the process, deoxyribonucleic acid from the majority leaf samples of two to five individual plants was used. Quantification of DNA was accomplished by analyzing the DNA on UV spectra photometer analyzer and also on 0.8 % Agarose gel using diluted uncut lambda DNA as standard. DNA was diluted in TE buffer to an amount of roughly 25ng/µL for PCR analysis. The sequence data for the primer pairs were obtained from the publications of Wu dialect & Tanksley (1993), and Temnykh et al. (2000) from sequence data obtained from deoxyribonucleic acid libraries and printed sequence knowledge (www.gramene.org)..

c. PCR amplification:

35 SSR primer pairs were selected for this study. PCR was performed in an exceeding volume of the reaction mixture was twenty µL containing 30ng of model deoxyribonucleic acid, 1 x PCR buffer with 1.5mM of MgCl₂, 0.2 mM of each dNTPs, 10 pmol of each primer and 1U of Taq DNA polymerase. PCR was carried out in a Thermal Cycler was used and programmed for 35 cycles of 95°C (5 min), 94°C (1 min) 56°C (30 Sec.), 72°C (1 min) then followed by final extension at 72°C for five min. PCR products (10–15 µL) were used for electrophoresis and the amplicons were resolved on 1 % agarose gel stained with ethidium bromide at 1 µg/mL and visualized under UV in a gel documentation system and impurities were known supported deviations in expected amplification pattern.

Table 1: Composition of CTAB extraction buffer

S.NO.	Stock	Volume (1L)
1	Tris HCL	15.76g
2	EDTA* (pH 8.0)	20mM
3	CTAB*	2%
4	Nacl	81.8g
5	PVP – 40*	2%
6	DIECO*	0.69g

Table 2: Composition of PCR master mix (25µL)

S No.	Contains	Volume in µL	PCR concentration
1	Milli Q Water	14.7	
2	PCR buffer	2.5	1X
3	dNTP's	2.5	0.2 mM
4	Primers (F/R)	4(each)	10pico mol (each)
5	Taq enzyme	0.3	0.5 unit
6	Template DNA	1	50 ng/µl

Table 3: Reaction condition

PCR steps	Temperature	Time
Initial denaturation	94 ⁰ C	7 min
Final denaturation	94 ⁰ C	30 sec
Annealing	55 ⁰ C	30 sec
Initial extension	72 ⁰ C	1 min
Final extention	72 ⁰ C	10 min
Hold at	4 ⁰ C	

III. RESULTS

Fig 1: DNA quality checking gel

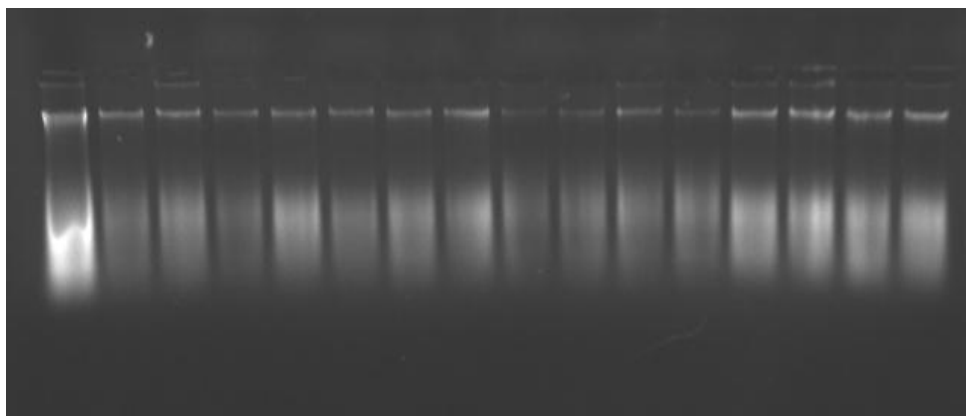


Fig 2: Screening of parental lines using monomorphic markers-1

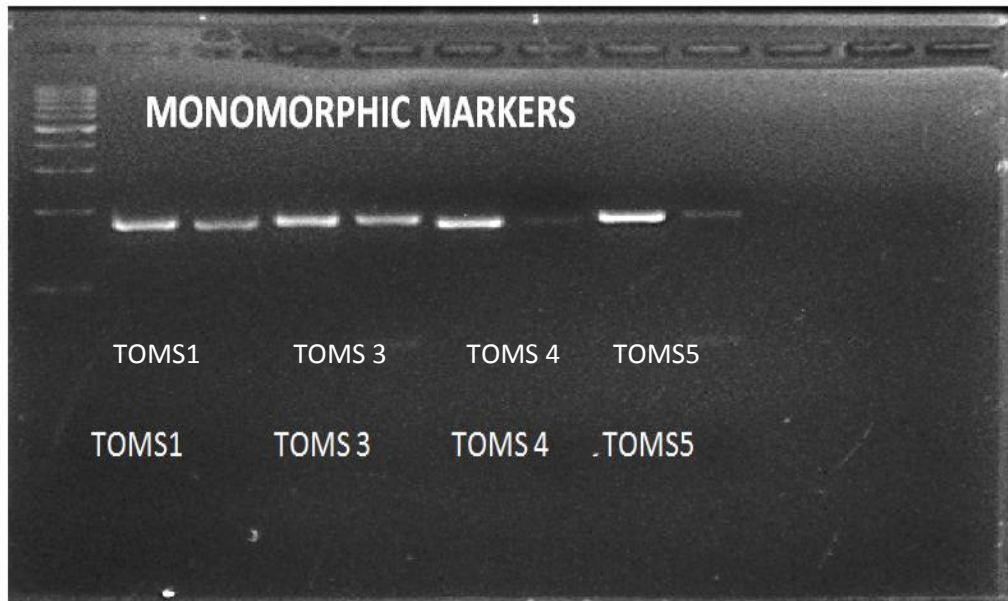


Fig 3: Screening of parental lines using monomorphic markers-2

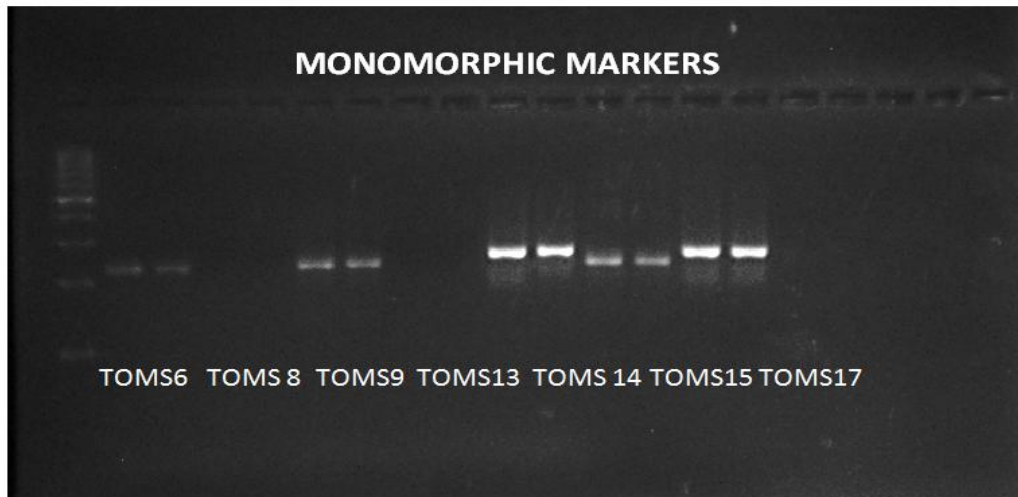


Fig 4: Screening of parental lines using monomorphic markers-3



Fig 5: Screening of parental lines using polymorphic markers -1

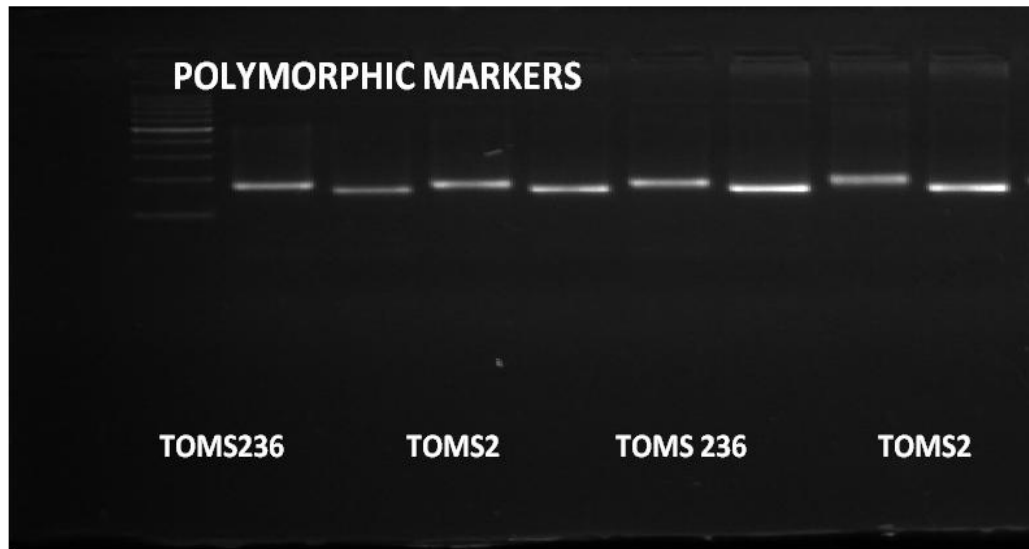


Fig 6: Screening of parental lines using polymorphic markers -2

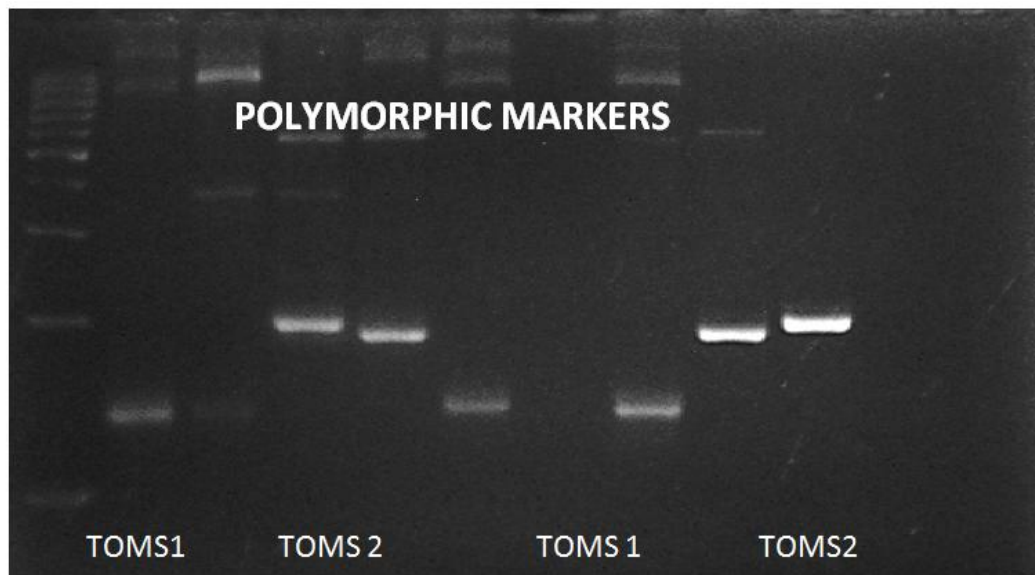


Fig 7: Identification of single polymorphic markers among different monomorphic markers

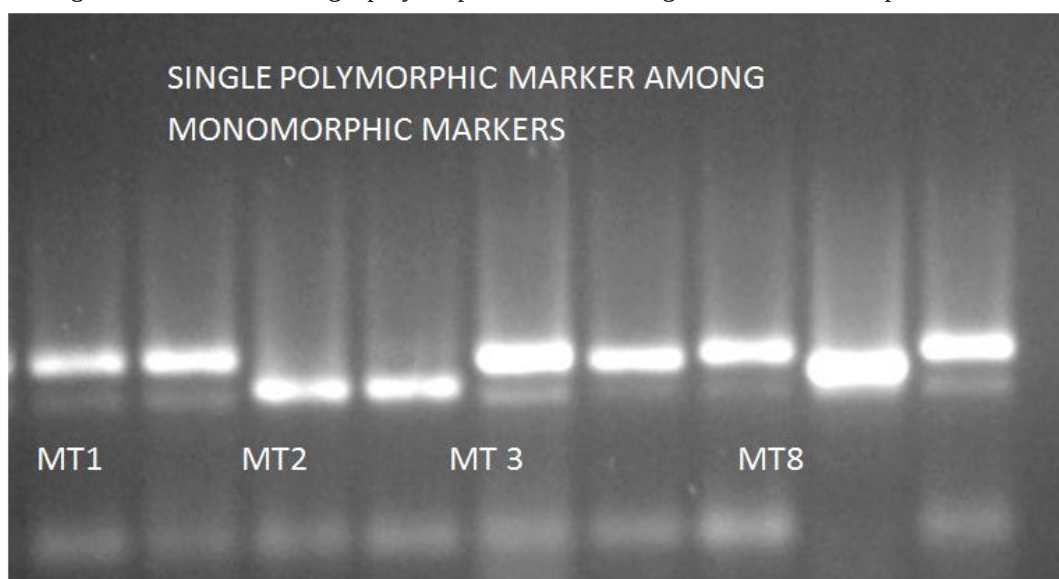


Fig 8: Screening of parental lines using polymorphic markers -3

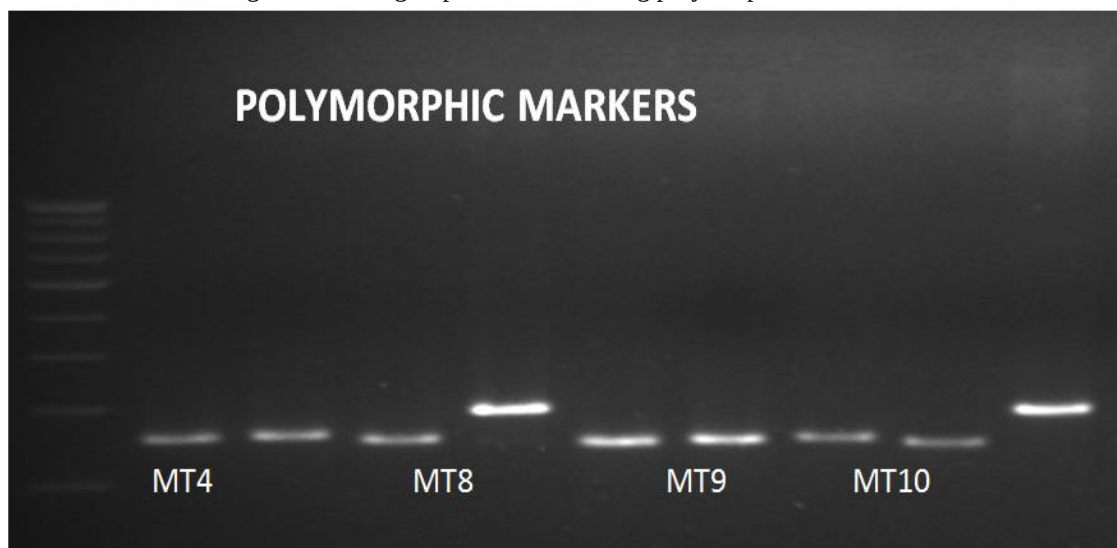


Fig 9: Screening of NTH1813 hybrid using polymorphic marker TOMS23

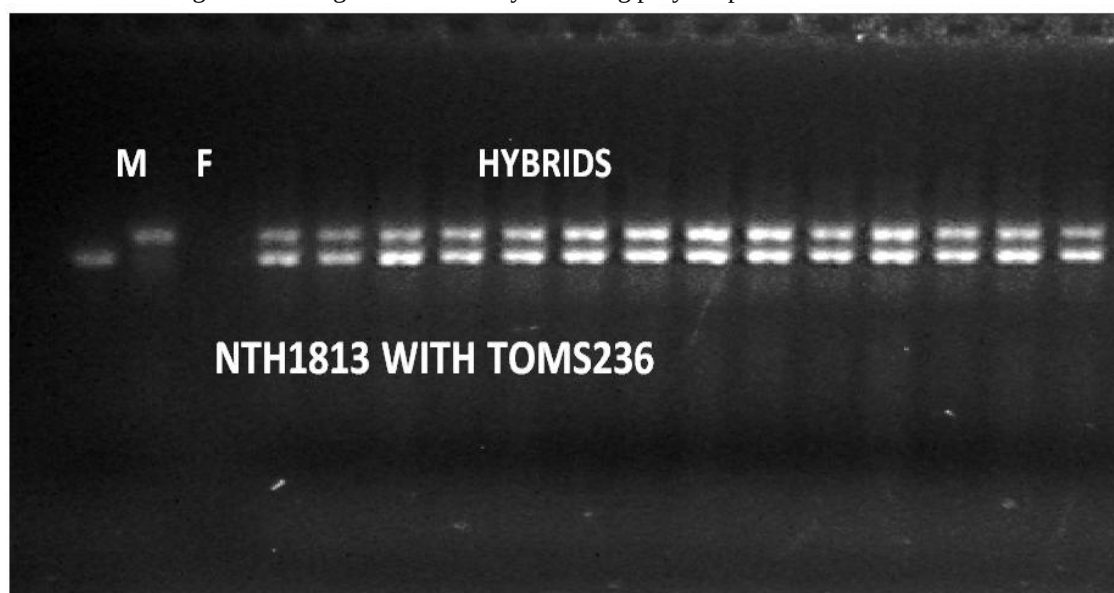


Fig 10: Screening of SONA hybrid using polymorphic marker MT8



Table 1: DNA samples showing monomorphic & polymorphic with SSR primers

S NO	PRIMER	DNA SAMPLES	
		NTH 1813	SONA
1	Toms1	Monomorphic	Monomorphic
2	Toms2	Polymorphic	Polymorphic
3	Toms 3	Monomorphic	Monomorphic
4	Toms4	Monomorphic	Monomorphic
5	Toms5	Monomorphic	Monomorphic
6	Toms6	No amplification	No amplification
7	Toms7	Monomorphic	Monomorphic
8	Toms8	Monomorphic	Monomorphic
9	Toms9	No amplification	No amplification
10	Toms10	Monomorphic	Monomorphic
11	Toms11	Monomorphic	Monomorphic
12	Toms12	Monomorphic	Monomorphic
13	Toms13	No amplification	No amplification
14	Toms14	Monomorphic	Monomorphic
15	Toms15	Monomorphic	Monomorphic
16	Toms16	Monomorphic	Monomorphic
17	Toms17	Monomorphic	Monomorphic
18	Toms18	Polymorphic	Polymorphic
19	Toms19	Monomorphic	Monomorphic
20	Toms20	No amplification	No amplification
21	Toms21	Monomorphic	Monomorphic
22	Toms22	Monomorphic	Monomorphic
23	Toms23	No amplification	No amplification
24	Toms24	Monomorphic	Monomorphic
25	Toms25	Monomorphic	Monomorphic
26	Toms26	Polymorphic	Monomorphic
27	MT 1	Monomorphic	Monomorphic
28	MT 2	Monomorphic	Monomorphic
29	MT 3	Monomorphic	Monomorphic
30	MT 4	No amplification	No amplification
31	MT 5	Monomorphic	Monomorphic
32	MT 6	Monomorphic	Monomorphic
33	MT 7	Monomorphic	Monomorphic
34	MT 8	Monomorphic	Polymorphic
35	MT 9	Monomorphic	Monomorphic
36	MT 10	Monomorphic	Monomorphic

IV. DISCUSSION

In the present study, two hybrid tomato genotypes were characterized on the basis of molecular markers and they are discussed hereunder.

Characterization based on Microsatellite Markers:

Molecular markers have well-tried to be powerful tools within the assessment of genetic variation and within the elucidation of genetic relationships among and among the species.

The genotypes under study were hence subjected to molecular markers analysis for characterization. The major advantage of the molecular markers over the conventional markers lies in their ability to cover the whole genome and this being able to distinguish even closely related varieties which are otherwise non-distinguishable. Moreover, the stability of these markers over different environments, no stage specificity, and advent of rapid and workable techniques like microsatellites for percentage analysis (Dakin, EE; Avise, JC (2004). makes molecular techniques convenient for characterization and also for testing distinctness of varieties and also for future protection.

Varietal identification can be done through various methods like morphological, biochemical and/or molecular analysis. Since the morphological method involves more time and influenced by the environment, isozymes (R. L. Hunter and Clement Markert 1957) came in to use for characterization of cultivated varieties and their wild relatives in tomato. Though morphological and isozyme markers have been employed in assessing the genetic diversity of a species, the accuracy is questionable. The availability of the low range of morphological and organic chemistry markers, their poor or unknown genetic control, environmental influence on the phenotypic expression, stage-specific expression, and procedural difficulties are known impediments in using as genetic markers in genetic purity studies.

In the present study, two tomato genotypes were analyzed using 35 SSR primers pairs of which 3 SSR primers were found to be polymorphic. A maximum number of primer pairs showed monomorphic patterns. The primer “toms 236” and “toms 2” showed a polymorphic pattern with NTH 1813 and primer” MT 8” showed a polymorphic pattern with SONA. Many primer pairs were not showed any amplified product with PCR i.e, toms6, toms9, toms13, toms 20, toms 23 and MT 4. And rest of the remaining primers showed monomorphism.

V. CONCLUSION

This study concludes that the simple sequence markers are a sensitive and unique tool for identification of molecular differences for closely related or distinct tomato varieties and also useful for identification of new varieties. The allelic diversity determined by the microsatellite markers has enabled the detection of differences between two NSL commercial tomatoes hybrids studied. However, the microsatellite marker panel could still be improved upon through further screening for polymorphic markers to enhance its discrimination power.

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