Electronic Journal of Plant Breeding,
ISSN 0975-928X Vol 7 No 2 DOI: 10.5958/0975-928X.2016.00033.8

# Profiling of sugar beet genotypes for agronomical, sugar quality and forage traits and their genetic diversity analysis using SSR markers

**Surinder K Sandhu*[1], Navraj Kaur Sarao[2], Meenakhsi Goyal[1], SK Uppal[3],Pritpal Singh[1,] Satveer kaur[2] and Jaspreet Kaur[2]**

[1] Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana
[2] School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana Punjab
[3]Department of Chemistry, Punjab Agricultural University, Ludhiana
*Corresponding author surindersandhu@pau.edu

(Received: April 2015, Accepted: September 2015)

**Abstract**
Earlier studies have well established the cultivation of sugar beet as winter crop in Indian subtropics and indicated its potential in augmentation of the raw material for sugar production with good profitability. A set of sugarbeet genotypes obtained, were therefore, evaluated for their economic traits and sugar yield at two feasible crop durations. Out of 15, three genotypes viz., Kaveri, Shubra and LKC-2010 have performed well and suggested to exploit commercially with crop duration of 210 days. As sucrose content increased in all the genotypes (though per cent increase varied among genotypes) from April to May, it is recommended to harvest sugarbeet in mid May for better returns. Forage quality parameters theoretically indicated the suitability of sugarbeet tops after harvest to use as fodder, further boosting its acceptability along with shorter crop duration and lesser requirement of resources crop inputs as compared to sugarcane, Thus, sugarbeet appears agriculturally feasible, less capital intensive and holds great promise. Molecular characterization of sugarbeet genotypes was done using 14 polymorphic simple sequence repeat (SSR) markers. Unique DNA profiles of all the genotypes could be created using a set of six polymorphic SSR markers. The dendrogram depicting the genetic relationships classified the genotypes in two diverse clusters. This SSR marker based diversity would facilitate sugarbeet hybridization programme involving diverse parents.

***Key words*:** Acid detergent fibre**,** Ash content, crude protein, *in vitro* dry matter digestibility, molecular characterization, sugar beet.

## Introduction

Sugar beet (*Beta vulgaris* L.), primarily a crop of the temperate region, has recently extended its scope to the subtropics. It has emerged as an ideal candidate for crop diversification, for its bioethanol production potential and to revive the sugar industry as this crop has the capacity to produce the same amount of sugar within short duration even in half of the time of sugarcane (Pathak *et al*., 2014). In India, Sugar industry is the second most important agro-based industry with which the farming community is directly associated. India happens to be among the few fortunate countries blessed with the agroclimatic diversity to be capable of cultivating both the major sugar crops of the world. Various studies have well established the cultivation of sugarbeet as winter crop in Indian subtropics. This will help in augmentation of the raw material for sugar production with good profitability coupled with water economy. The emerging bio-fuel scenario in the country has brought the potential of sugarbeet much closer to realization. It has opened new vistas for alternate industrial uses other than the traditional sugar manufacture. It occupies field only for 6-7 months and yields nearly as much sugar as a 12-month sugar crop in North India. It produces about 60-75 tonnes of roots per hectare with sugar content of 15-16% and 10-11% sugar recovery on an average. The proposed study has been envisaged to assess the potential of sugarbeet in sub-tropics to enhance the economic viability of sugar mills of the state by increasing the milling period by about two months or even more, using sugar beet as a feedstock. Concomitantly, an optimum utilization of the capital, machinery and man power will result in better efficiency of the mills. Moreover, to enhance economic viability, sugar factories need to be converted to sugar complexes with product diversifications like biofuel, co-generation etc. Sugar

beet is an excellent supplementary and complementary crop which can meet requirements of biofuel ethanol through all sugar mills by extending the season from current 4-5 months to 8-10 months if managed well. The preliminary studies conducted during 2002-03 and 2003-04 with monogerm genotypes indicated that sugar beet can be grown successfully in subtropics both as pure crop as well as intercrop in autumn cane. In 2012-13, Punjab Agricultural University, Ludhiana has acquired the seed of 15 monogerm genotypes from Indian Institute of Sugarcane Research, Lucknow (UP) and evaluated under autumn sown (October to May) conditions for yield and its components and quality traits. Efforts were made to study the genetic diversity in these genotypes using SSR markers for their utilization as parents for future breeding programme.

Sugarcane tops have been extensively used as animal feed as a part of cultural practice. Likewise, efforts were made to analyze the fodder quality of sugar beet leaves. Various biochemical parameters like nitrate content, crude protein (CP), crude fibre (CF), ash content and *in vitro* dry matter digestibility (IVDMD) are major determinants of fodder quality. Nitrate is a normal constituent of plants consumed from soil but if higher than normal amounts of nitrate are consumed, an accumulation of nitrite may occur in the rumen and absorbed into the bloodstream that results in conversion of hemoglobin to methanoglobin, and blocks the transfer of oxygen**.** CP determines the amount of protein within that product, CF identifies the insoluble fibre within a plant cell wall and is comprised of cellulose and lignin and IVDMD measures the digestibility limit of feed.

Efforts were made to study the genetic diversity in these sugarbeet genotypes using SSR markers for their utilization as parents for future breeding programme. For the assessment of genetic diversity, various types of approaches have been employed. Conventionally the assessment of genetic diversity was based on morphological markers which mainly comprise the traits of agronomic interest and employing different statistical approaches. However, morphological characterization alone does not portray the reliable genetic relationship among the genotypes this may happen due to environmental interactions, unknown genetic control of these traits and inadequate sampling of genome (Yun *et al.,* 2003). The molecular markers like RAPD, AFLP, and SSR have been efficiently employed in genetic diversity studies in sugar beet (Laurent *et al.* 2007; Smulders *et al* 2010; Richards *et al* 2004). Among the various types of molecular markers, SSR (simple sequence repeats) markers have been gained considerable importance in plant genetics and breeding owing to their hyper variability, wide genomic distribution, co-dominant inheritance, reproducibility, multi-allelic nature and chromosome specific location (Kalia *et al.* 2011). Hence, the present study was planned with objectives of agronomic evaluation of sugar beet genotypes for economic traits; biochemical assay of sugar beet genotypes for their sucrose quality; assessment of genetic diversity of sugar beet genotypes based on EST-SSR markers and biochemical assay of sugar beet leaves for their fodder quality.

**Material and Methods:**

**Agronomic evaluation of sugar beet genotypes for agronomic and quality traits under field conditions:**

The seeds of fifteen sugarbeet genotypes were obtained from Indian Institute of Sugarcane Research, Lucknow (UP), India. The field experiment was conducted in randomized complete block design with three replications at University Seed Farm, Ladhowal, Ludhiana in October, 2012. Each genotype was planted in plot of six rows spaced at 60 cm and plants at 20 cm. The crop was raised as per recommended practices. The crop was harvested in May 14, 2013. The field data were recorded for number of plants in each plot, top weight, beet root weight on plot basis, and beet root length and root diameter on mean basis of ten plants. For quality studies, beet roots were harvested, cleaned, shredded and sucrose % beet root was find out using polarimeter at 180 and 210 days after planting. The sugar yield was calculated as multiple of beet root yield at harvest and sucrose percent.

**Forage quality of sugarbeet leaves:**

The leaves of randomly selected five plants of each genotype were dried in an oven at 60-65$^0$C temperature to a constant weight. The samples were ground to pass through 1 mm sieve and stored in air tight containers for analysis. Nitrate- N was determined by the method as advocated by Cataldo *et al.* (1975).The crude protein, crude fibre and ash content of these samples were determined as per AOAC. (1990). The samples were also analysed for *in vitro* dry matter digestibility (Tilley and Terry, 1963).

**Molecular characterization of sugarbeet genotypes:**

Fifteen genotypes of sugar beet were characterized using 19 EST-SSR markers. DNA was extracted from the leaves of two week seedlings using a modified CTAB (Cetyl Trimethyl Ammonium Bromide) method (Maguire *et al.*, 1994). SSR sequences were amplified through polymerase chain reaction (PCR) using SSR primer specific for sugar beet as listed in

Table 1. PCR amplification (25 ul total volume) was performed in 2.0 µl of 10 X PCR buffer, 2.5 µl of dNTPs (1mM), 1.25 µl of each of the forward and reverse primers (5 µM each), 0.2 µl of Taq polymerase (5 units/µl), 5.0 µl of DNA (15 ng) and distilled deionized water using an Eppendorf thermal cycler. The PCR profile consisted of initial denaturation at 94°C for 3 min and subsequent 30 cycles each with denaturation at 94°C for 1 min, primer annealing at 58 °C for 1 min and primer extension at 72°C for 1 min. Final extension step was performed at 72°C for 5 min. PCR products were run on 2.5% agarose gel containing ethidium bromide (5µL/100ml) and electrophoresed at 140-150 V for 2-3hrs in 1X TBE buffer. The bands were visualized under UV light and photographed using photo documentation system. SSR alleles were scored for presence and absence of the SSR bands. Lanes showing clear and distinct bands were given a score of 1, those showing no bands a score of 0 and missing data a score of nine.

***Data Analysis***

Polymorphism information content (PIC) for each SSR marker was determined as per the procedure outlined by Senior *et al.* (1998).

$$PIC = 1-\sum_{i=1}^{n} (P_{ij})^2$$

Where $P_{ij}$ is the frequency of $j^{th}$ allele in $i^{th}$ primer and summation extends over 'n' patterns. Genetic similarity coefficients between various genotypes (in pair-wise comparisons) were calculated from the SSR data matrix using dice coefficient and the resulting genetic similarity matrix was analysed using NTSYS-PC version 2.02 to produce an agglomerative heirarchical classification (Rohlf, 1989) by employing Unweighted Pair Group Method using Arithmatic Averages (UPGMA). Microsatellite marker amplification profile for all the genotypes was also analyzed by using computer software DARwin 5.0 Programme (Perrier and Jacquemoud-Collet, 2006).

**Results and Discussion:**

**Morphological and biochemical evaluation of sugar beet genotypes:**

Sugar beet genotypes were evaluated for their mean genotypic differences for economically important traits. The analysis of variance determined critical differences among genotypes. Since sugarbeet is used for sugar extaction from its roots, the traits governing root yield viz. root diameter, root length and single root weight were of major importance. Based on Turkey's test, the genotypes marked with different letters were statistically significant from each other at 5% level of significance (Table 2). The genotype Kaveri has highest root diameter (10.50cm) with mean root weight 830 gm and the highest root yield (98 t/ha). LKC-2010 yielded 91.54 t/ha with single root weight of 1.10 Kg. Other genotypes *viz.*, LKC-4, Hilima, LKC-2007, LKC-LB and Shubra had high and statistically similar root yield. The root length and root diameter were negatively correlated to each other (r -0.181). The root length and single root weight had statistically significant positive correlation with root yield having r value 0.589 and 0.561, respectively. Fig.1 graphically depicted variability in sugarbeet genotypes for mean root weight (bars exhibiting standard error) and thereby inferred the scope of selection of elite genotypes for majotr economic trait. The commercial value of sugarbeet for sugar extraction depends upon root weight and sucrose content in roots. The sucrose content is highly influenced with the maturity span of sugarbeet. Therefore, the genotypes were evaluated for sucrose content in months of April (180 days after planting) and May (210 days after planting). Table 3 described the sucrose content of genotypes at these two stages with 30 days interval. Further data could not be collected as after first fortnight of May, the temperature increase steadily leading to rotting of beet roots. Gross sugar is calculated as multiple of beetroot yield at harvest and its sucrose content. Genotypes Kaveri, Hilima and Shubra recorded good sugar yield in both the months (April and May). LK-2007 has greater increase in sucrose content after one month period than other genotypes. As revealed in Fig. 2, all the genotypes exhibited higher sucrose content at 210 days that that at 180 days, it is suggested that to fetch maximum sugar recovery, it is better to delay the harvesting till first week of May under Punjab conditions as no decay was observed in beet roots till this period. Afterwards, rise in temperatures may disrupt the sugar recovery too. A study was conducted by Kapur et al., 2000 to determine how sugarbeet cultivars respond to high temperatures in Uttar Pradesh, India during 1992-96. The indigenously bred cultivars showed greater tolerance to high temperature. Individual root weight increased, reflecting the ability of sugarbeet to grow in spite of the high diurnal temperature, aided by moisture availability and milder night temperatures. However, these environmental conditions were not conducive to sugar accumulation since sugar content declined from April/May to May/June. While investigating the feasibility of sugarbeet cultivation at Bangladesh, Islam et al., 2012 reported lower yield potential of sugarbeet genotypes at 180 days after sowing (DAS) compared to 165 DAS. Severe leaf shedding and drying up of roots in the later stage might be reason for yield reduction. They observed nine sugarbeet genotypes with more than 10 per cent

sucrose and suggested to consider as sugar producing genotypes.

**Forage quality of sugarbeet leaves**

The feasibility to explore the use of sugarbeet leaves as fodder after harvest was explored based on five biochemical determinants. The crude protein content in forage crops has direct relationship with palatability and digestibility of feed. The data exhibited high protein content in the leaves of all genotypes varying from 24.5 to 27.8%. The genotypes LK-8, LKC-2010 and Shubra were superior to other genotypes in relation to crude protein. The significant differences in crude protein content of various sugar beet genotypes have also been confirmed by Singh and Garg, 2013. Dietary fibre plays an important role in ruminants to maximize the dry matter intake and stimulate chewing activity and rumen fermentation (Nadeem and Sufyan, 2005). Providing adequate fibre, while attempting to meet energy needs, can be a challenge particularly in rations for early lactation animals. Higher crude fibre (CF) content in forage crops however has adverse effect on forage quality as it affects the digestibility. The data presented in table 4 showed CF content varied from 6-17% in all genotypes with Hilima had the highest figure. The lowest value for crude fibre contents was recorded by LS-6.

Sugar beet genotypes exhibited significant variations for ash contents. LK-8 reported highest ash content (23 %) over all tested genotypes followed by IISR-COMP1. The significant variations in ash contents among tested genotypes suggested differences in nutrient absorption from soil and utilization within the plants. Forage digestibility is one of the most important characteristics of forage nutritional value. *In vitro* dry matter digestibility (IVDMD) in the leaves was ranged from 80.0 to 93.6%. The suitability of a particular genotype for forage quality can give extra benefit to growers and will help to boost the acceptability of this crop.

Nitrate N in sugar beet genotypes varied from 900-1800 ppm and the highest level was found in LKC 2010.The nitrate N values were within the permissible limits i.e.<2000 ppm but sole feeding is not advisable in the genotypes having nitrate N values >1000 ppm (Andrew and Kumar, 1992). Pathak et al., 2014 also reviewed beet-tops a highly nutritious cattle-feed with a potential of improving milk yield in cows. He also observed some antinutritional components in beet tops and advised only sun-dried tops 100 kg + 60 g finely ground lime to be used as cattle feed. Analysis of variance indicated availability of significant genotypic differences among genotypes for economic traits. Amongst all the genotypes studied, Kaveri has the highest root yield and fairly good forage quality traits but sucrose content was moderate. Shubra has high root yield (though lower than Kaveri and LKC-2010) but the highest sucrose content at harvest and high forage quality traits. LKC-2007 has the second highest sucrose content a harvest (16.27) but its root yield was significantly lower than Kaveri, Shubra and LKC-2010. Since sugar recovery is governed by both root yield and sucrose content, it is better to consider sugar yield as criterion to determine the commercial feasibility of genotypes.

**Molecular characterization of sugarbeet genotypes:**

All the 19 EST-SSR markers used for cultivar identification and diversity analysis of 15 genotypes of sugar beet, showed clear and consistent amplification profile. Among these, five markers (FDSB1300, FDSB1027, SB15, FDSB1011 and FDSB502) showed monomorphic pattern. The remaining 14 showed polymorphic pattern and amplified a total of 31 alleles. Laurent *et al* (2007) concluded that EST-SSR could be valuable tools for diversity studies in sugar beet. The number of alleles ranged from 2 to 3 with an average of 2.2 alleles per locus. Of the 14 polymorphic SSR markers, three (SB07, SB04, FDSB1033) showed three alleles and for the remaining eleven (FDSB957, FDSB1002, FDSB1023, SB06, BvGTT1, FDSB568, FDSB990, FDSB1250, FDSB1007, FDSB1001 and FDSB1427) amplified two alleles (Fig. 3). Some genotypes failed to show amplification hence, revealed no bands (null allele) for a specific SSR primer. Laurent *et al.* (2007) observed 1-8 alleles while characterizing 31 accessions using twenty EST-SSR whereas, Smulders *et al* (2010) found 2-21 alleles and Richards *et al* (2004) reported 2-11 alleles for their microsatellite markers in sugar beet. EST-SSRs are characterized by a more elevated level of polymorphism than standard genomic SSR (Laurent *et al.* 2007). Similarly, in the present study nineteen EST-SSRs used for genotyping15 genotypes showed a high level of polymorphism (73.7%) underlining a considerable amount of genetic variation present between these genotypes.Unique DNA profiles of all the genotypes could be created using a set of six (FDSB957, FDSB1002, FDSB1023, BvGTT1, FDSB1001, FDSB1427) polymorphic primers. Therefore, SSR markers used in the present study could precisely distinguish all the fifteen genotypes from each other and thus, these SSR markers can be further used to differentiate the future genotypes from the existing ones. Identification of the genotypes using PCR based co-dominant markers such as SSR offer excellent opportunities for supplementing and refining

morphological descriptors (Chakravarthi and Naravaneni, 2006 and Jalaludin *et al*., 2007). If accepted, the DNA marker based DUS testing will effectively augment the process of discrimination of the candidate varieties and hybrids.

The PIC values which is a measure of allelic diversity at a locus ranged from 0.23 (FDSB1007) to 0.63 (FDSB1033, SB07), with an average value of 0.45 across all genotypes. In the present study, PIC values were comparatively higher (0.63) than that reported (0.59) by Laurent et al. (2007) using the same set of EST-SSRs. The dendrogram (Fig.4) depicting the genetic relationships as revealed by NTSYS-pc 2.02 and the tree diagram (Fig. 5) generated using DARwin 5.0 programme classified the genotypes in to three main clusters. Seven genotypes viz. LK-4, LS-6, LKC-2000, LK8, LKC-10, SHUBRA and LKC-2010 were grouped in cluster I whereas, LKC-2006 and LK-27 were in cluster II. Third cluster was further divided in to two sub clusters. Three genotypes viz., Kaveri, LKC-LB and Hilima were in sub-cluster I while, genotypes LKC-2007, LKC-HB and IISRCOMP-1 were grouped in sub-cluster II. Genotypes viz. LKC-HB and IISR COMP-1 showed the highest genetic similarity having similarity coefficient of 0.95 and were closely related. The clustering pattern of genotypes generated by both Programmes (NTSYS-pc 2.02 and DARwin 5.0) was similar. Though, the genotypes revealed high genetic similarity in cluster analysis but could be distinguished from each other using a set of five SSR primers. This SSR marker based diversity among sugar beet genotypes will facilitate the optimal use of genetic resources for breeding.

**References**

AOAC (1990) Official methods of analysis, 13th edn, Association of Official Analytical Chemists, Washington, D.C.

Cataldo DA, Maroon, M, Schradar, LE and Youngs, VL (1975) Rapid calorimetric determination of nitrate in plant tissues by nitration of salicylic acid. Commun. Soil. Sci. Plant analysis 6 (1): 71-80

Chakravarthi BK and Naravaneni R (2006) SSR marker based DNA fingerprinting and diversity study in rice (*Oryza stiva* L.). African Journal of Biotechnology 5(9): 684–688

Islam MS, Ahmad, S, Uddin, MN and Satat, MA (2012) Evaluation of tropical sugarbeet genotypes under Banglasdesh conditions. Bangladesh J. Agril.Res. 37(4):721-728

Georing HK and Van Soest, PJ (1970) Forage fibre analysis. Apparatus, reagents, procedure and some applications. Agriculture Hand Book No. 379, USDA Washington, DC

Islam, MS, Ahmad S, Uddin MN and Satat MA (2012) Evaluation of tropical sugarbeet genotypes under Banglasdesh conditions. Bangladesh J. Agril.Res. 37(4):721-728

Jalaluddin M, Nakai H, Yamamoto T (2007) Genetic diversity and DNA fingerprinting of some modern Indica and Japonica rice. SABRAO 39:43–52

Kalia RK, Rai M, Kalia K, Singh S and Dhawan, RAK (2011) Microsatellite markers: an overview of the recent progress in plants. Euphytica .177:309–334

Laurent V, Devaux P, Thiel T, Viard F, Mielordt S, Touzet P, Quillet MC: Comparative effectiveness of sugar beet microsatellite markers isolated from genomic libraries and GenBank ESTs to map the sugar beet genome**.** *Theor Appl Genet* 2007, 115:793-805

Maguire TL, Collins GG and Sedgley M (1994) A modified CTAB DNA extraction procedure for plants belonging to the family proteaceae. Plant Molecular Biology Reporter 12:106-109.

Nadeem A and Sufyian AB (2005) Partial replacement of forage fiber with non forage fiber in ruminant ration: A review. Pakistan V J :25 (2) 92-98

Pathak A D, Kapur Raman, Solomon S, Kumar Rajesh, Srivastava Sangeeta and Singh P R . 2014. Sugar Beet: A Historical Perspective in Indian Context. Sugar Tech (Apr-June 2014) 16(2):125–132 DOI 10.1007/s12355-014-0304-7

Perrier X and Jacquemoud-Collet JP (2006) DARwin Software http://darwin.cirad.fr/darwin.

Raman Kapur, Srivastava H M, Saxena VK Bhatnagar S (2000) Adaptability studies for high temperature tolerance of sugarbeet varieties in subtropical India. Crop improvement 2000 Vol. 27 No. 2 pp. 178-186

Richards CM, Brownson M, Mitchell SE, Kresovich S, Panella L: Polymorphic microsatellite markers for inferring diversity in wild and domesticated sugar beet (*Beta vulgaris*)**.** *Mol Ecol Notes* (2004) 4:243-245

Rohlf FJ (1989) *NTSYS-pc Numerical Taxonomy and Multivariate Analysis System, Version 2.02.* Setauket, NY: Exeter Publications.

Senior ML, Murphy JP, Goodman MM and Stuber CW (1998) Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. *Crop Science* 38:1088–1098

Singh D and Garg AK (2013) Evaluation of beet varieties for forage yield and quality parameters. *Range Mgmt. & Agroforestry* 34 (2): 182-185

Smulders Marinus JM, G Danny Esselink, Isabelle Everaert, Jan De Riek and Ben Vosman (2010) Characterisation of sugar beet (*Beta vulgaris* L. ssp. vulgaris) varieties using microsatellite markers *BMC Genetics* 11:41

Singh, D and Garg, AK 2013 Evaluation of beet varieties for forage yield and quality parameters. *Range Mgmt. & Agroforestry* 34 (2): 182-185

Tillay, JMA and Terry, RA 1963 A two stage technique for the *in vitro* digestion of crops. *British J. Grassland Soc.* 18:104-111

Yun-Jiang Cheng, Wen-Wu Guo, Hua-Lin Yi, Xiao-Min Pang, Xiuxin Deng (2003). An efficient protocol for genomic DNA extraction from *Citrus* Species. Pl Molecular Biology Reporter. 21: 177

**Table 1** List of EST-SSR markers used

| SSR markers | Primers R | Primers F |
|---|---|---|
| FDSB1300 | AATTTAAACGCGAGAGCAGC | TCAGCTTCTGGGCTTTTTGT |
| FDSB1027 | CAGGCATGAGTAGCATGAACTAAAG | GCTGGATGCTGACAACTATGAAAC |
| FDSB957 | TCAATCCATCTCTATTCTCTCCG | GTCATGGTTGGTCGATCCTT |
| FDSB1002 | GAAAACGGAGTTCAGTCAGGGA | CCTTAAACCTAAAAACGCCAGC |
| FDSB1023 | TCTCTCTCCCCCTAAAAGTTCA | GTAGCTAGTTCAGCAATCTTCGC |
| SB06 | AAATTTTCGCCACCACTGTC | ACCAAAGATCGAGCGAAGAA |
| SB07 | TGTGGATGCGCTTTCTTTTC | ACTCCACCCATCCACATCAT |
| SB04 | ACCGATCACCAATTCACCAT | GTTTTGTTTTGGGCGAAATG |
| SB15 | CACCCAGCCTATCTCTCGAC | GTGGTGGGCAGTTTTAGGAA |
| BvGTT1 | CAAAAGCTCCCTAGGCTT | ACTAGCTCGCAGAGTAATCG |
| FDSB568 | TTCTGGGGATGATTTCTTCG | CCGGGACAGAGAGAACAGAG |
| FDSB1011 | CAACTTATTTAAGCCTTTTAGTGC | GATCCATTTATTTCGTGTTGA |
| FDSB502 | GCAAAAACCCAAAACCCTTT | TTTCTCTCTCCTCCTCTTCCTC |
| FDSB990 | TCTCACCTGAAATCCGAACC | CCATCCGTAACTCGGTGACT |
| FDSB1250 | TTCACCGCCTGAATCTTTTC | CGACGAAGAATCGGGTAAAA |
| FDSB1007 | ATTAGAATAGCATCAATTGTGG | CCTTATAGTTGGAATTGAGAAA |
| FDSB1001 | ACTTCAACCACTATCACAAAGTGAG | ATCTTATGCTGCCATGACCA |
| FDSB1427 | TTGAAGGCTCACCTCAAACAAA | CTGTTGCTGTTGCTGTTGCT |
| FDSB1033 | GCTGAGATGATGTTTGTTAGGGC | TTCAAATCGCCATCTCCCAG |

**Table 2** Genotypic mean differences for different traits in sugarbeet germplasm

| | **Morphological traits** | | | |
|---|---|---|---|---|
| Genotype | Root Diameter (cm) | Root length (cm) | Single root weight (Kg) | Root weight (tons/ha) |
| LKC-2010 | 9.67$^{cdef}$ | 26.00$^{def}$ | 1.10$^{a}$ | 91.54$^{b}$ |
| LK-4 | 10.08$^{abcde}$ | 29.15$^{abc}$ | 0.83$^{bcd}$ | 82.58$^{c}$ |
| LK-8 | 9.50$^{ef}$ | 26.28$^{def}$ | 0.80$^{bcd}$ | 67.65$^{d}$ |
| LKC-2000 | 8.62$^{gh}$ | 26.89$^{cdef}$ | 0.60$^{d}$ | 68.27$^{d}$ |
| LK-27 | 9.76$^{bcdef}$ | 25.14$^{f}$ | 0.97$^{ab}$ | 72.51$^{d}$ |
| LKC-HB | 9.70$^{cdef}$ | 30.39$^{cd}$ | 0.70$^{abc}$ | 68.76$^{d}$ |
| LKC-10 | 9.64$^{def}$ | 28.08$^{bcde}$ | 0.73$^{bcd}$ | 72.75$^{d}$ |
| LKC-2006 | 9.70$^{cdef}$ | 25.39$^{ef}$ | 0.73$^{bcd}$ | 74.73$^{d}$ |
| LS-6 | 9.30$^{f}$ | 29.68$^{ab}$ | 0.70$^{cd}$ | 73.57$^{d}$ |
| IISR Comp-1 | 9.24$^{fg}$ | 31.44$^{a}$ | 0.70$^{cd}$ | 68.29$^{d}$ |
| Hilima | 10.30$^{abc}$ | 24.43$^{f}$ | 0.70$^{cd}$ | 78.93$^{cd}$ |
| LKC-2007 | 8.59$^{h}$ | 28.59$^{bcd}$ | 0.67$^{cd}$ | 78.47$^{cd}$ |
| LKC-LB | 10.26$^{dcef}$ | 26.04$^{def}$ | 0.87$^{abc}$ | 83.41$^{cd}$ |
| Kaveri | 10.50$^{a}$ | 29.21$^{abc}$ | 0.83$^{bcd}$ | 98.68$^{a}$ |
| Shubra | 10.39$^{ab}$ | 28.46$^{bcd}$ | 0.83$^{bcd}$ | 84.01$^{cd}$ |
| CD* (0.05) | 0.63 | 2.74 | 0.25 | 4.60 |

*Tukey's studentized range (HSD) test
Same letters indicate non-significant differences among genotypes.

**Table 3** Performance of sugar beet genotypes for sucrose content and sugar yield

| Genotype | Beet root yield (t/ha) | Sucrose content (%) at 180 days | Sucrose content (%) at 210 days | % increase in Sucrose % Beet root at 210 days | Gross sugar (t/ha, at harvest) |
|---|---|---|---|---|---|
| LKC-2010 | 91.54 | 12.24$^{abc}$ | 14.87$^{bcd}$ | 21.49 | 13.61 |
| LK-4 | 82.58 | 11.12$^{abc}$ | 13.93$^{bcd}$ | 25.27 | 11.50 |
| LK-8 | 67.65 | 10.17$^{c}$ | 15.18$^{abc}$ | 49.26 | 10.27 |
| LKC-2000 | 68.27 | 11.97$^{abc}$ | 14.10$^{cd}$ | 17.79 | 9.63 |
| LK-27 | 72.51 | 11.69$^{abc}$ | 15.44$^{abc}$ | 32.08 | 11.20 |
| LKC-HB | 68.76 | 11.85$^{abc}$ | 15.51$^{abc}$ | 30.89 | 10.66 |
| LKC-10 | 72.75 | 11.47$^{abc}$ | 12.89$^{d}$ | 12.38 | 9.38 |
| LKC-2006 | 74.73 | 11.59$^{abc}$ | 15.88$^{abc}$ | 37.01 | 11.87 |
| LS-6 | 73.57 | 13.28$^{ab}$ | 14.58$^{bcd}$ | 9.79 | 10.73 |
| IISR Comp-1 | 68.29 | 12.35$^{abc}$ | 14.14$^{cd}$ | 14.49 | 9.66 |
| Hilimia | 78.93 | 12.40$^{abc}$ | 15.65$^{abc}$ | 26.21 | 12.35 |
| LKC-2007 | 78.47 | 10.51$^{bc}$ | 16.27$^{ab}$ | 54.80 | 12.77 |
| LKC-LB | 83.41 | 11.07$^{bcd}$ | 14.27$^{bcd}$ | 28.91 | 11.90 |
| Kaveri | 98.68 | 12.47$^{abc}$ | 15.68$^{abc}$ | 25.74 | 15.47 |
| Shubra | 84.01 | 13.81$^{aa}$ | 17.02$^{a}$ | 23.24 | 14.30 |
| CD* (0.05) | 4.60 | 2.97 | 2.11 | - | - |

*Tukey's studentized range (HSD) test
Same letters indicate non-significant differences among genotypes.

**Table 4** Performance of sugarbeet leaves for forage quality traits

| Genotypes | Forage quality traits | | | | |
|---|---|---|---|---|---|
| | Nitrate content $NO_3$-N | Crude protein % | Acid detergent fibre **%** | Ash content **%** | *In vitro* dry matter digestibility % |
| LKC-2010 | 2000$^{bcd}$ | 27.8$^{c}$ | 13$^{efg}$ | 17.6$^{abc}$ | 81.6$^{d}$ |
| LK-4 | 900$^{bcd}$ | 2.50$^{a}$ | 8.0$^{cde}$ | 17.6$^{a}$ | 93.2$^{i}$ |
| LK-8 | 1400$^{a}$ | 27.8$^{a}$ | 11.0$^{bc}$ | 23.0$^{bcd}$ | 82.2$^{d}$ |
| LKC-2000 | 1400$^{cde}$ | 25.4$^{c}$ | 9.00$^{h}$ | 21.2$^{d}$ | 91.5$^{a}$ |
| LK-27 | 900$^{e}$ | 21.8$^{d}$ | 12.0$^{fg}$ | 17.00$^{bcd}$ | 83.2$^{ab}$ |
| LKC-HB | 1000$^{cbd}$ | 21.8$^{c}$ | 9.0$^{g}$ | 16.40$^{abcd}$ | 84.90$^{g}$ |
| LKC-10 | 1400$^{bcd}$ | 25.4$^{c}$ | 7.0$^{g}$ | 15.40$^{d}$ | 89.2$^{f}$ |
| LKC-2006 | 1400$^{b}$ | 25.4$^{a}$ | 7.0$^{cde}$ | 18.2b$^{cd}$ | 87.8$^{e}$ |
| LS-6 | 1200$^{e}$ | 25.4$^{e}$ | 6.0$^{bcd}$ | 16.2$^{bcd}$ | 93.6$^{bcd}$ |
| IISR Comp-1 | 900$^{e}$ | 25.4$^{d}$ | 10.07$^{b}$ | 21.4$^{bcd}$ | 90.4$^{e}$ |
| Hilima | 1400$^{de}$ | 22.5$^{e}$ | 17.0$^{efg}$ | 13.4$^{cd}$ | 80.0$^{h}$ |
| LKC-2007 | 1000$^{e}$ | 26.0$^{c}$ | 13.0$^{def}$ | 18.2$^{ab}$ | 88.0$^{abc}$ |
| LKC-LB | 1000$^{e}$ | 22.5$^{b}$ | 11.0$^{bc}$ | 16.0$^{abcd}$ | 82.6$^{f}$ |
| Kaveri | 1000$^{e}$ | 22.5$^{d}$ | 14.0$^{cde}$ | 17.40$^{d}$ | 80.4$^{cd}$ |
| Shubra | 1600$^{bcd}$ | 27.8$^{d}$ | 11.0$^{a}$ | 17.60$^{d}$ | 84.8$^{bcd}$ |
| CD* (0.05) | 0.03 | 0.52 | 2.01 | 4.81 | 1.08 |

*Tukey's studentized range (HSD) test
Same letters indicate non-significant differences among genotypes.

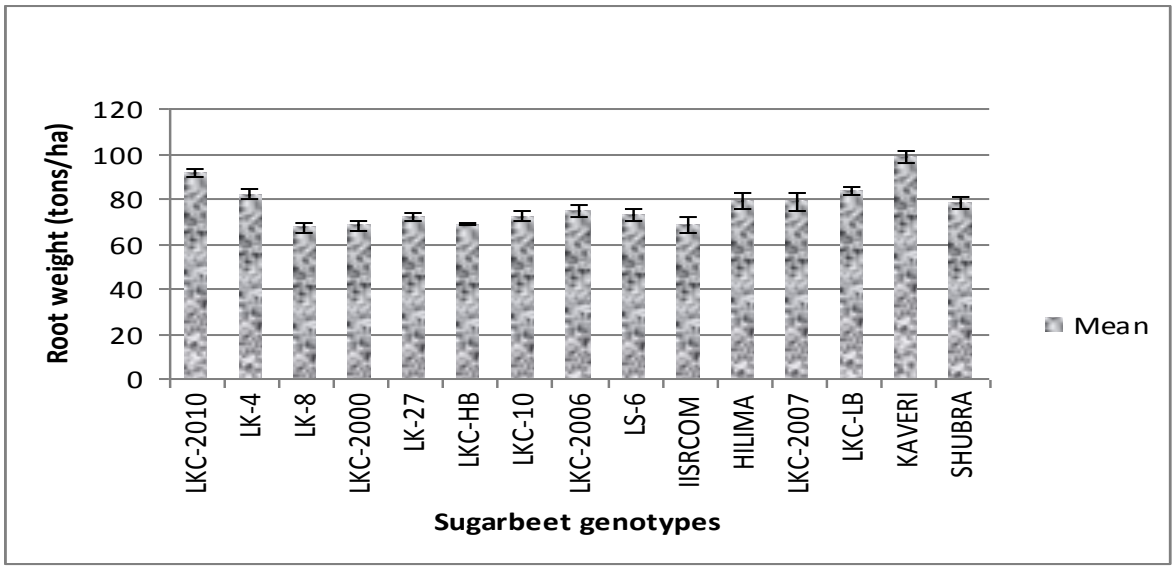


**Fig. 1** Variability in sugarbeet genotypes for root weight

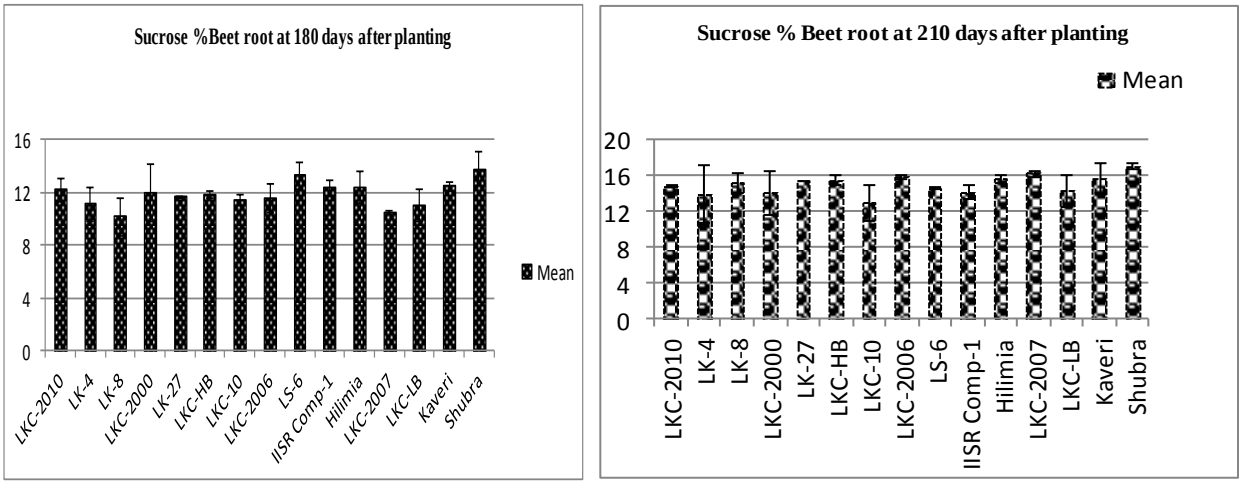


**Fig. 2** Sucrose content (%) in sugarbeet genotypes at different stages

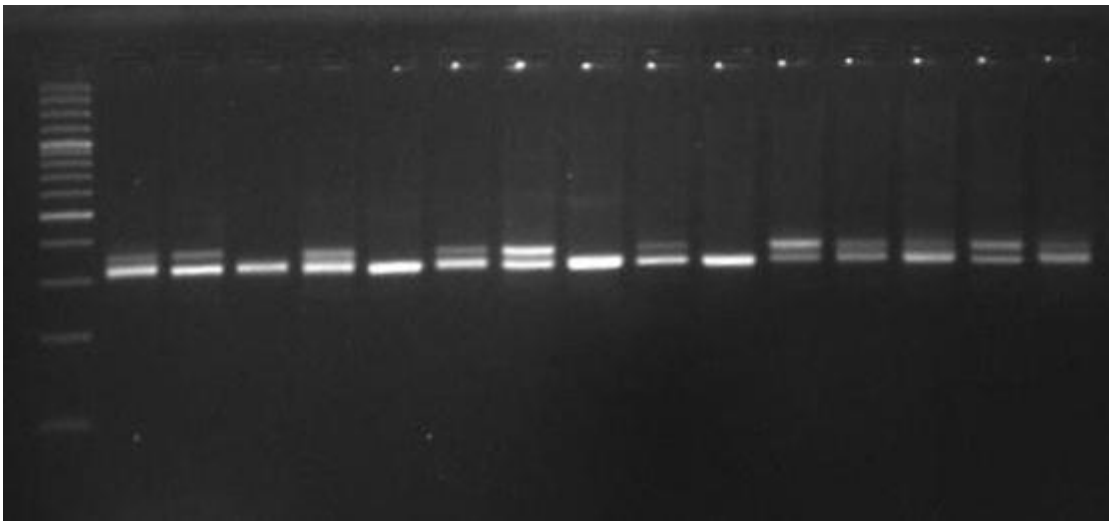

**Fig. 3** Amplification of genomic DNA of 15 genotypes of sugarbeet using FDSB957 marker on agarose gel

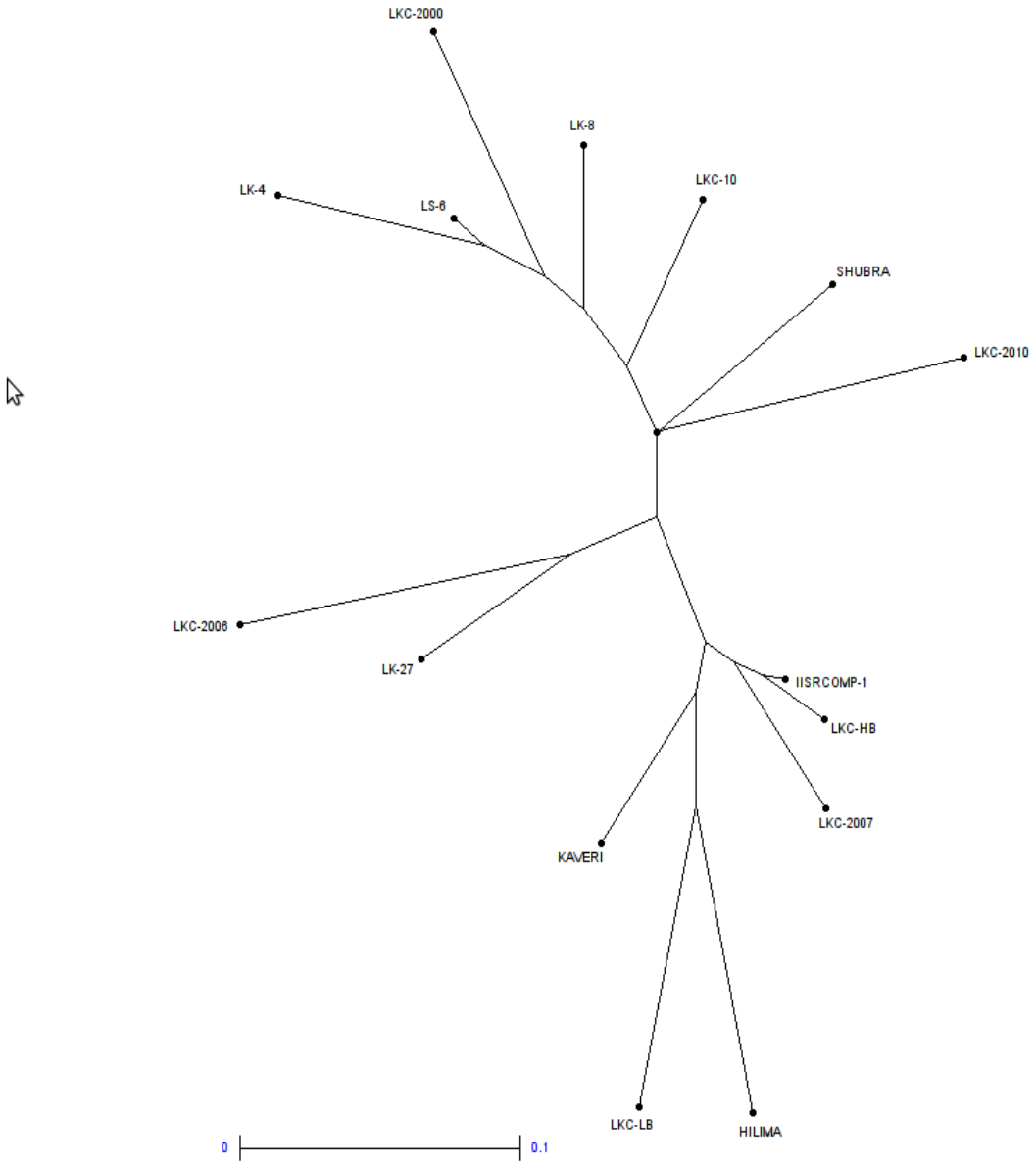


**Fig. 4** Phylogenetic tree diagram depicting genetic relationships among 15 sugarbeet genotypesbased on SSR data using the computer program Darwin 5.0.

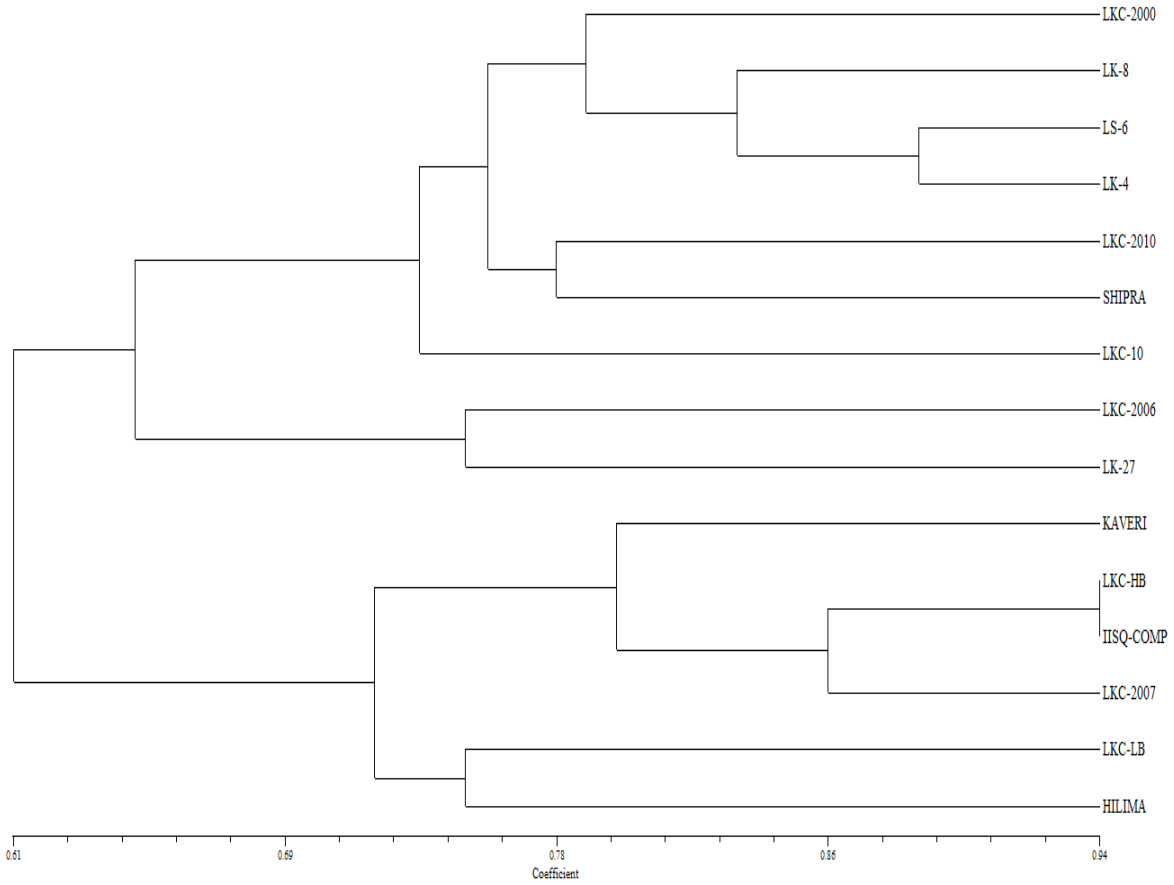


**Fig. 5** Dendrogram depicting genetic relationships among 15 genotypes based on SSR data using UPGMA (Dice Coefficient)