UNIVERSITÀ DEGLI STUDI DI PADOVA

Department of Agronomy, Food, Natural Resources, Animals and Environment(DAFNAE)

Second Cycle Degree (MSc) In Sustainable Agriculture

INSERTIONAL POLYMORPHISMS OF MINIATURE INVERTED-REPEAT TRANSPOSABLE ELEMENTS (hAT-like MITEs) IN INTRONS OF SUGAR BEET.

SUPERVISOR

Prof. Serena Varotto

Prof. Darius Grzebelus

SUBMITTED BY

Pavan Kalyan Ganiginti

Student number-2004964

THE ACADEMIC YEAR 2022/2023

UNIVERSITÀ DEGLI STUDI DI PADOVA

Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente

Corso di laurea magistrale in Agricoltura Sostenibile

INSERTIONAL POLYMORPHISMS OF MINIATURE INVERTED-REPEAT TRANSPOSABLE ELEMENTS (hAT-like MITEs) IN INTRONS OF SUGAR BEET.

Relatore
Prof. Serena Varotto

Prof. Darius Grzebelus

Laureando

Nome: Ganiginti Pavan kalyan

Id number:2004964

ANNO ACCADEMICO 2022/23

CONTENTS

ACKNOWLEDGEMENTS

First and foremost, praises and thanks to God, the Almighty, for giving me the wisdom, strength, support, and knowledge in exploring things and the determination to pursue my study and make this study possible.

I would like to express my deep and sincere gratitude to my research supervisor **prof. Serena Varotto** for the continuous support of this study and research, and her patience, motivation, enthusiasm, and immense knowledge. Her guidance has helped me all the time duringthis research work.

I would also like to sincerely thank my Co-Supervisor **Prof. Darius Grzebelus** for supporting, motivating, and giving me suggestions whenever I needed his help throughout the research work.

Most of all, I am fully indebted to my family members for their constant encouragement, moral support, and love which have accompanied me during the ups and downs in my life.
In the end, I feel great pleasure in expressing my grateful indebtedness and sincere thanks to allmy friends and seniors whom I owe any credit directly or indirectly for this research work.

LIST OF TABLES:

LIST OF FIGURES:

INSERTIONAL POLYMORPHISMS OF MINIATURE INVERTED-REPEAT TRANSPOSABLE ELEMENTS (hAT-like MITEs) IN INTRONS OF SUGAR BEET.

ABSTRACT

Sugar beet (*Beta vulgaris* L.) is an economically important crop due to its high sucrose content in roots and it is the second most important crop after sugar cane for sugar production in the world. The European Union is the world's largest producer of sugar beet 50% of world production, accounting for 22% of sugar. Sugar beet is an obligatory cross-pollinated crop that belongs to the family Amaranthaceae, chromosome number 2n=2x=18. The genome content of sugar beet is 758 Mb, and the repetitive sequence is around 458 Mb, which accounts for 64%. Transposable elements are mainly classified into two classes they are class I (Retro elements) and class II (DNA elements). Non-autonomous, miniature inverted-repeat transposable elements (MITEs) belong to class II TEs. MITEs are the most abundant non-autonomous DNA group that belongs to class II DNA elements in the plant genome. A group of plant *hAT* MITEs belongs to the *Ac/Tam3* family. MITEs act as one of the significant sources of variations that occur in sugar beet. MITE copies inserted within introns can be exploited as potential intron length polymorphism (ILP) markers. PCR can detect ILP markers with primers anchored in exon sequences flanking the target introns. ILP markers are unique owing to their gene specificity, co-dominance, convenience, reliability cost-efficiency. Here, we designed primers for Bvh-ILP (*Beta vulgaris hAT*-like) MITE insertion sites within introns along the sugar beet genome and validated them as candidate ILP markers to develop a set of markers for genotyping the sugar beet. The designed set of Bvh-ILP markers is an easily accessible molecular marker genotyping system based on TE insertion polymorphism.

Keywords-Sugar beet (*Beta vulgaris*), Transposable elements, DNA, PCR, Gel electrophoresis, hAT MITEs, Insertional polymorphism, ILP marker.

1. INTRODUCTION

1.1 SUGAR BEET

Sugar beet (*Beta vulgaris* L. *ssp. Vulgaris*) is one of the economically important crops in the world. It has been cultivated since the 18th century and is extensively used for sugar production (Fehr, 1987). The leading sugar beet producer in the world is Russia (33.9 million tonnes) which contributes 13.73% of world sugar beet production. The other top 5 countries in the world are the (United States of America, Germany, France, and Turkey) accounting for 57.59%. European Union is the world's largest beet sugar producer (50% of the total; (Clarke & Godshall, 1987) it contributes 21.8% of the world's sugar production comes from sugar beets(Dechyeva et al. 2003).

1.2 HISTORY AND ORIGIN

Beet has grown at least 2000 years ago as a garden vegetable native to the Mediterranean region. The first modern sugar beet was grown in the middle of the 18th century from fodder beet in the Silesia region (Germany, now Poland). Beet was commonly used as a leafy vegetable and for medicinal purposes. After 1747, the German chemist Andreas Marggraf demonstrated that the crystals formed after a crude extraction from pulverized beetroots were identical in all properties to sugarcane crystals. Attempts to derive sugar from beets originated from his work and now the roots of sugar beet are more economical for sugar extraction.

1.3 TAXONOMY

Sugar beet is classified as *Beta vulgaris L. ssp. vulgaris* sugar beet group; the second ssp. is *Beta maritima* (L.) Arcang., classified by Linnaeus (1797) as a separate species(Lange et al., 1999). The genus *Beta* L. belongs to the family Amaranthaceae (formerly Chenopodiaceae,) and the chromosome number of sugar beet is (2n=2x=18). All cultivated beets belong to the sub-species *vulgaris* that belong to the species B. *vulgaris.* The cultivars of sugar beet are mainly grown for their high sucrose content in roots which is used for sugar and biomass production (Lange et al., 1999).

1.4 BIOLOGY

Sugar beet is a biennial crop and takes two years from germination to seed set. In the first year, the plant develops a leaf rosette and a beet and then flowers in the second year. The first

year is the vegetative phase where plants' establishment and main production happens. To move into the reproductive phase (year 2) the sugar beet needs a period of vernalization. Sugar beet is an obligatory cross-pollinated species with a genome size of 758 million base pairs. Sugar beet seeds are mainly produced through cytoplasmic male sterility. The selection is mainly based on mass selection (McGrath et al., 1999) sugar beet is developed from progenitors of fodder beet and leaf beet (Fischer, 1989). The ploidy level of genus *Beta* exists in diploid, tetraploid, and hexaploidy with a basic chromosome number of X=9.

1.5. AGRONOMIC PRACTICES

Good agronomic practices fetch higher beet yield and quality. Environmental factors mainly affect the sugar beet yield and quality. environmental factors are solar radiation, temperature, sunshine hours, etc. These factors determine the type of plant growth and sucrose content stored in roots (Petkeviciene, 2009)

1.5.1. CLIMATIC CONDITIONS

Sugar beet is cultivated worldwide, all over the year in all climatic conditions in diverse regions but it requires vernalization for initiation of flowering. The cultivation of sugar beet is increasing in sub-tropical regions nowadays (Linskens et al., 1976). Sugar beet is grown successfully during the winter season and in some areas like the northern hemisphere is planted in the early spring. Mostly it is grown for sugar production it is a by-product like sugar beet pulp and molasses is used as animal feed (Brar et al., 2015). It is mostly grown in latitudes between 30 and 60 N in semi-arid and arid climates as a winter or summer crop in the Mediterranean and other arid regions (Jaggard et al., 1999). Sugar beet emerges faster when air and soil temperature ranges between 15-25 ℃ (G Elias, 2018). For proper growth and sugar accumulation of needs an average temperature of around 20-22 ℃. Temperature above 30 ℃ leads to sugar reduction. Where successful sugar beet industries have developed, diverse adjustments to the physiological limits to crop growth have been made, resulting in many different cropping patterns worldwide (Duke, 2014)

1.5.2. SEEDLINGS

Sugar beet seeds contain very little endosperm for germination and early growth. Seedlings are of 2 types monogerm and multigerm seeds. Monogerm seeds contain only a single embryo while multigerm seeds contain more than 1 embryo. Monogerm seeds are germinated with only 1 seedling while multigerm seeds are germinated with 4-5 seedlings (Hozayn et al.,

2020) found that multigerm seeds produce heavier roots than monogerm seeds Jassem (1982) reported that monogerm seeds contain more sucrose content.

1.5.3. SOIL TYPE AND SOWING METHODS

Sugar beet has grown in all types of fertile soils and is well grown in silt and sandy loam soils with pH 6.5-7.5 (neutral soils).

The sowing method is an important factor in sugar beet influencing yield and quality. The underground part of sugar beet is the main economic component. so, the sowing method and depth of soil Affect its root growth (El-Maghraby et al., 2008)reported that using laser technology leveled soil with deep plowing results in good yield and quality compared with other sowing methods. Direct sowing of sugar beets on ridges is better in yield and quality than flatbed sowing (Garg and Srivastava, 1985). The yield of sugar beet is higher in a ridge or bed sowing compared to a flatbed.

1.5.4. IRRIGATION MANAGEMENT

Water is a crucial factor in growing sugar beet. Sugar beet can suffer from low or high moisture content, so it needs optimal water. Under irrigation limits water, nutrients, and photosynthesis are limited to plants. Over-irrigation leads to less oxygen supply and more disease attacks and loss of nutrients through the root zone (Abdollahian–Noghabi, 1999). In Poland, under conditions of a moderate climate, the average rainfall totals in the growing seasons of sugar beet are in the range of 350–400 mm; however, they are distinguished by great temporal and spatial variability. (Topak et al., 2011)irrigation leads to increased sugar content due to an increase in root yield. For crop establishment, first irrigation is crucial because of the seed's sensitivity to water. Irrigation requirements for sugar beet are moderate 10-12 irrigations are enough for luxuriant growth of sugar beet (Shukla and Awasthi 2013).

1.5.5. NUTRIENT MANAGEMENT

NITROGEN

Nitrogen is the most limiting nutrient in sugar beet. It determines white sugar production by affecting both root and yield quality. Nitrogen fertilizer has a pronounced effect on crops' growth and physiological and chemical properties. Nitrogen promotes excess growth and sugar accumulation in crops (Draycott & Christenson, 2003). Proper nitrogen leads to excess growth and a good yield of sugar. Sugar beet requires balanced growth of nitrogen throughout

the life cycle. Kemp et al. (1994) suggest that the highest root fresh weight is obtained when nitrogen is applied at 360kg/ha.

PHOSPHORUS AND POTASSIUM

Phosphorus and potassium play important roles in sugar beet. Potassium plays an important role in photosynthesis, protein synthesis, and translocation of ions it increases plant growth and yield. It increases the leaf's chlorophyll and sugar beet content (Yu-ying and Hong, 1997).

1.5.6. DISEASES AND MANAGEMENT

So many diseases are occurring in sugar beet, but rhizomania and Cercospora leaf spot are major yield loss occurring diseases.

RHIZOMANIA

Rhizomania disease is probably the most occurring disease in recent years and is more threatening to sugar crops. The first published reports of poorly growing sugar beet crops with symptoms of rhizomania were in Italy in 1952 (Canova,1959). Canova,1966 named rhizomania as root madness that leads to abnormal black necrotic symptoms of roots. Its caused due to association of a protist *Polymyxa betae* and the virus beet necrotic yellow vein mosaic virus or BNYVV ((Tamada et at., 1971; Tamada and Baba, 1973; Tamada, 1975).

VECTOR-*Polymyxa betae* was first identified, named, and described as a parasite of sugar-beet roots by Keskin (1964).

SYMPTOMS

The infection after which viruses were named (Tamada and Baba, 1973) is the yellowing of leaf veins, which eventually become necrotic pale brown in color. Leaves may be pale greenish yellow color and elongated strip-like shape with long petioles upright of leaves which similarly looks like nitrogen deficiency. Roots remain very small, mainly tap root ceases proliferation leading to lateral roots.

MANAGEMENT

CHEMICAL CONTROL

Many fungicides have been tested against rhizomania, which has been extensively reviewed (Schiiufele; 1987; Asher, 1988). Amongst inorganic compounds salts of metallic ions, such as zinc, effectively work in rhizomania.

BIOLOGICAL CONTROL

Biological control of *P. betae* has little attention to date. Seed treatments incorporating the soil rhizobacterium *Pseudomonas fluorescens* to control *P. betae* do not affect the level of infestation or crop yield in field trials (Anon., 1985). More promising results have been achieved with *T. harzianum*.

GENETICAL RESISTANCE

Genetic resistance is the most promising approach to the control of rhizomania in the long term, and the selection of lines resistant or tolerant to the disease has become a major objective in sugar-beet breeding programs in Europe, the USA, and Japan

CERCOSPORA LEAF SPOT

Cercospora leaf spot is the most major and serious disease in sugar beet. The disease is caused by the airborne fungus *Cercospora beticola*.

Signs and symptoms- individual leaf spots initially occur on older leaves and then spread to younger leaves. Leaf spots initially occurred with ash-colored central to brown border oval.

MANAGEMENT

Cultivation and crop rotation will reduce the overwintering inoculum and at least a three-year rotation is needed to reduce the quantity of severely infested crops. Using leaf spot-tolerant cultivars can significantly reduce disease severity and yield loss. Use multiple disease-resistant varieties.

2. CLASSIFICATION OF TRANSPOSABLE ELEMENTS

There are two types of DNA transposable elements based on their coding capacity.

1. AUTONOMOUS(Ac) Autonomous elements have ORFs (open reading frames) that encode proteins for their transposition (Wessler, 2006). Autonomous elements can translocate without the help of any other gene.

2. NON-AUTONOMOUS(Ds) Transposable elements do not encode for protein transposition they depend on autonomous elements, but they can move due to cis sequences, which are required for transposition (Wessler, 2006). Hence, non-autonomous transposons require the help of other autonomous elements to translocate. In plant genomes, there are two classes of transposable elements based on their transposition mechanism (Grzebelus, 2018).

(CLASS 1) RETROTRANSPOSONS

RTS transpose through an RNA intermediate that is then used to produce a complementary DNA (cDNA) by using a reverse transcriptase enzyme named retroviral ribonuclease H (RNase H)(Bennetzen & Wang, 2014). Subsequently, RNA is degraded and from cDNA, the double-stranded DNA sequence is produced and inserted in a new genome position. That is why this mechanism is called a copy-and-paste transposition.

This element is further divided into 5 orders namely.

1. LTR (long terminal repeats) 2. LINEs (Long interspersed nuclear elements) 3. SINEs (Short interspersed nuclear elements) 4. DIRS (Dictyostelium intermediate repeat sequence), and 5. PLE (Penelope-like elements). (Y. Zhang et al., 2017). Because their replicative mechanism can amplify very rapidly and constitute the largest portion of DNA in plant genomes, retrotransposons are frequently located in pericentromeric and intergenic regions. Particularly LTR elements are often responsible for expansion in plant genomes. Especially those comprising *Ty1-Copia* and *Ty3-gypsy* superfamilies have particularly expanded in the genome and usually constitute the major fraction of all plant TEs. (Y. Zhang et al., 2017).

(CLASS 2) DNA TRANSPOSONS

DNA transposons are usually associated with terminal inverted repeats (TIR) and transpose via a DNA intermediate (Feschotte et al., 2002). DNA TE is excised from one site (donor site) and reinserted (target site) in the genome. The departure of the element from the donor site is called "excision" whereas integration of the element into the target site is called "insertion." DNA TE is usually less far-abundant than LTR retrotransposons. DNA transposons are distributed in genic regions, near genes, or UTRs and introns. Only two orders are defined within class 2: TIR (Terminal inverted repeats) and Helitrons. Five superfamilies are present in higher plant genomes *hAT, Mutator, CACTA, PIF/Harbinger*, and *Tc1/mariner*(X. Zhang et al., 2004). TIR transposons transpose by "cut and paste" mechanism. Helitrons, transposing via a "rolling circle "transposition process with replicase /helicase.

2.1. MINITAURE INVERTED TRANSPOSABLE ELEMENTS(MITEs)

The abundant non-autonomous DNA elements known as miniature inverted transposable elements (MITEs) are class II DNA elements that are less than 1000 bp in size, AT-rich, and ubiquitously present in almost all plant genomes. They preferentially insert near, but rarely into,

the genes (Bennetzen & Wang, 2014). Each MITE contains signature structures known as terminal inverted repeats (TIRs ≥10 bp) at either end, flanked by target site duplications (TSD 2–10 bp) (Pritham, 2009). Transposable elements were first discovered by cytogeneticist Barbara McClintock in maize (*Zea mays*) in the year 1940 and named "Controlling elements" (Zhao et al., 2016)

In maize, chromosome breakage was rarely observed, but McClintock observed frequent breakage at a particular locus on chromosome 9 in one special maize line (Zhao et al., 2016). She discovered that two loci are required for the breakage to occur: one locus found at the site of the breakage point is known as Dissociator (*Ds*) while the other which is required to activate the breakage is called Activator (*Ac*). Since the Location of Ac and Ds appears to be variables between generations, McCLINTOCK proposed that they were genetic elements capable of transposition.

The ultimate source of evolution is mutation. As the largest component in plant genomes, Transposable elements (TEs) create numerous types of mutations that cannot be mimicked by other genetic mechanisms when TEs insert into genomic sequences they influence the expression of nearby genes as well as genes unlinked to in the insertion. TEs can duplicate, mobilize, and recombine normal genes or gene fragments it has the potential to create new genes or modify existing genes (Zhao et al., 2016)

MITEs have been shown distributed into almost all genomic regions, although some MITE families tend to closely associate with genes (Guo et al., 2017). Insertion of MITEs near the genic and into various genic regions can impact the regulation of genes and genome evolution (Naito et al., 2009; Oki et al., 2008). Various studies have suggested that MITEs (miniature inverted-repeat transposable elements) play a direct role in transcriptional and post-transcriptional gene modifications by acting as an exon, a source of small RNAs, or providing the transcription start site and the poly(A)-tail (Naito et al., 2009; Sampath et al., 2014). Furthermore, their high copy number and stability make MITEs valuable tools for marker development (Monden et al., 2009; Sampath et al…2014).

2.2. hAT MITEs

hAT MITEs belonging to class II TE have Terminal inverted repeats (TIR) and transpose via the "cut and paste" mechanism. Members of the hAT superfamily have been found in various

distantly related organisms, suggesting their ancient origin, which predates the divergence of plants-fungi and animals(Kempken & Windhofer, 2001).

The founder elements of this superfamily are *hobo* from the fruit fly (*Drosophila melanogaster*), *Ac/Ds* from maize, and *Tam3* from snapdragon (*Antirrhinum majus*;(Coen et al., 1986; McCLINTOCK, 1950; McGinnis et al., 1983). The first letter of the three elements contributes to the name "*hAT*". DTA is the systemic nomenclature of *hAT*(Wicker et al., 2007). Autonomous *hAT* elements have few kilobase pairs in length whereas non-autonomous elements can be short less than 200 base pairs.

Most *hAT* elements have short TIR, less than 30 bp starting with "CA/TA" and ending with "TG/TA ". The target site duplications (TSD) of hAT elements are usually 8bp in length. Since *Ac/Ds* were first identified as transposons in maize they are the best-characterized DNA TE in plants and have been widely used in gene tagging and genomic studies(Kunze & Weil, 2007; Sundaresan et al., 1995; Vollbrecht et al., 2010).

Plant *hAT* transposons belong to the family *Ac/Tam3*. The accelerating analysis of the whole plant genome has significantly increased the information on the abundance variability and evolutionary history of plant *hAT* families (Holligan et al., 2006)

Sequencing of plant genomes often identified the *hAT* superfamily as the largest group of DNA transposons. Autonomous transposons and MITEs of the *hAT* superfamily are widespread among plants, but their abundance, amplification, and diversification differ extensively between taxa (Zhang & Wessler, 2004).

In this study, we showed that the abundance of class II DNA transposable elements may serve as a tool for low-cost and relatively rapid development of gene-derived molecular markers for effective use in sugar beet genotyping studies (Stelmach et al., 2017). Insertions within introns may provide a significant polymorphism. Intron polymorphisms, particularly intron length polymorphisms (ILPs), can be exploited as genetic markers for gene mapping (Wydner et al., 1994) and population genetic surveys (Lessa, 1992). ILPs take advantage of the different rates of evolution of exons and introns that can result in conserved exon nucleotide sequences adjoined to more variable intron sequences. ILPs can be detected by the polymerase chain reaction (PCR) with a pair of primers anchored in the exons flanking the intron of interest (Wang et al., 2005). Indeed, ILP markers are unique due to their gene-specify, codominance, convenience, reliability, and cost-efficiency. Furthermore, ILPs are characterized by high transferability among related plant species. ILP markers are widely used for constructing

genetic maps, diversity analysis, and quantitative trait locus mapping (Gupta et al., 2011; L. Yang et al., 2007). However, to date, studies on the development of ILP markers in plants have been restricted to a few species (X. Chen et al., 2011; S. Gupta et al., 2011; Huang et al., 2010; Muthamilarasan et al., 2014; Wang et al., 2005).

In this study, we performed: i) a genome-wide search for Bvh (*Beta vulgaris hAT*-like) MITEs insertion based on intron length polymorphism markers; ii) validation of candidate ILP markers to develop a panel for genotyping the sugar beet using simple, cost and time efficient PCR; iii) research to find polymorphisms which are useful to confirm homozygosity (e.g., during the production of double haploids).

2.3. DOUBLE HAPLOIDS IN SUGAR BEET

A double haploid (DH) is a diploid genotype that originates when a haploid cell undergoes chromosome doubling. A haploid plant can be generated either spontaneously or artificially by different induction techniques, such as in vivo parthenogenesis or in vitro androgenesis (microspore and anther culture or ovule culture) and gynogenesis (ovule and ovary culture;(Murovec & Bohanec, 2012; Niu et al., 2014). Haploid plant production has various beneficial applications, ranging from plant breeding and genetic manipulation to plant genome/gene mapping(Niu et al., 2014). Additionally, achieving complete homozygosity in a single generation is a significant advantage. Instead in conventional breeding, it takes 6-7 generations to achieve homozygosity(de La Fuente et al., 2013). Doubling the chromosome of a haploid plant is a valuable method to develop pure lines for breeding purposes(Dunwell, 2010). It consists of two stages of haploids and double haploids. Double haploids are achieved mainly by colchicine treatment(Niu et al., 2014).

For sugar beet (*Beta vulgaris L.),* gynogenesis has been exploited for many years due to unresponsiveness to androgenesis. The critical role of haploid and DH plant production in accelerating homozygosity and the need for further improvement in the gynogenesis rate are convincing reasons to focus on doubled haploid induction through unfertilized ovule culture.

3. AIM

To develop a genotyping system by utilizing insertional polymorphisms in introns of sugar beet. It can be used to find polymorphism which is useful to confirm homozygosity (e.g., during the production of double haploids).

4. MATERIAL AND METHODS

4.1 PLANT MATERIAL

Twelve sugar beet (Beta vulgaris) genotypes of diverse origins with different genotypic and phenotypic traits were used for MITEs studies. The varieties were obtained from two sugar beet breeding companies in Poland which are mentioned in the table We obtained the seeds from sugar beet breeding companies and sowed sow them in our greenhouse. Seedlings were collected and DNA was extracted from these 12 genotypes.

Nr	Name	seed	Description	Resistance	Origin
1	Sugar beet1	multigerm	DH	-	KHBC
2	Sugar beet2	multigerm	F3	-	KHBC
3	Sugar beet 3	multigerm	F3	-	KHBC
4	Sugar beet4	multigerm	F4	*Rz1*	KHBC
5	Sugar beet5	multigerm	F4	*Rz1*	KHBC
6	Sugar beet 6	multigerm	F5	*Rz2*	KHBC
7	Sugar beet 7	monogerm	F5	*Cercospora*	KHBC
8	Sugar beet 8	monogerm	F5	*Cercospora*	KHBC
9	Sugar beet 9	monogerm	F5	*Cercospora*	KHBC
10	Sugarbeet 10	monogerm	F4	*Cercospora*	KHBC
11	fodder beet				MHR
12	fodder beet				MHR

Table 1:Data of genotypes and origin.

The 12 DNA samples are collected from 12 genotypes of sugar beet among 6 are multigerm (male parent) sugar beet cultivars 4 are monogerm (female parent) sugar beet cultivars, and 2 are fodder beet (Table 1).

4.2 DNA EXTRACTION

Genomic DNA was extracted from sugar beet seedlings by using CTAB (cetyltrimethylammonium bromide) protocol (Torres et al., 1993). We prepared 140 mg of DNA from each genotype.

CTAB buffer preparation for 100ml:

1. CTAB (Cetyl trimethylammonium bromide)2 gms.

2.1M Tris HCL pH 8-10ml.

3.5M Nacl-28ml

4.0.5 EDTA -4ml

To dissolve CTAB powder, it is necessary to stir and warm up the solution.

DNA extraction of sugar beet.

1. 10 Sugar beet and 2 fodder beet samples were collected for DNA extraction.
2. 200mg of each plant sample was taken and ground with liquid nitrogen.
3. Then transferred those grounded samples into an Eppendorf tube and added 700 µl ofCTAB buffer.
4. The mixture was vertex and incubated at 65℃ for 20 min in a water bath.
5. The samples were then centrifuged at 10000 rpm for 10 min.
6. The supernatant was collected and transferred to another tube and an equal amount of chloroformand isoamyl alcohol was added.
7. The solution was vortexed and centrifuged at 10,000 rpm for 10 min.
8. After centrifuge the solution was divided into an upper aqueous layer and a bottom organiclayer.
9. The upper aqueous layer was transferred into another tube without disturbing thelower organic layer.
10. Then 600 µl of cold ethanol (-20℃) and 150 µl of NaCl were added to the aqueouslayer.
11. This solution was mixed and centrifuged at 13,000 rpm for 10 min.
12. DNA pellets were observed at the bottom of the tube after centrifugation.
13. The supernatant was discarded and washed the DNA pellets with 600 µl of 70% ethanoland centrifuged at 10,000 rpm for 5 min.
14. After the centrifugation ethanol was discarded the pellet was dried and it was added with50 µl of 1X TE buffer.
15. Then stored the samples at 4℃.

4.3 DESIGNING OF PRIMERS

We designed primers by using **National Center for Biotechnology Information** (**NCBI)** website**, https://www.ncbi.nlm.nih.gov/.** The main tool of the NCBI website is the BLAST sequence alignment program.

The BLAST-Basic local alignment search tool is an algorithm used for calculating sequence similarity between biological sequences, such as the nucleotide sequence of DNA and the amino acid sequence of proteins. Sequences in FASTA format were used to get primer

sequences melting temperature and GC% content. CDS of candidate sugar beet gene were used as queries for BLAST searches against the coding sequence between the range of exon and intron.

By using NCBI we designed 66 pairs of primers for our research.

PRIMER NAME	SEQUENCES
Bv_chr3_Hat28_1_1 F	CTGCAGGTCCTTCATTCCCAT
Bv_chr3_Hat28_1_1 R	GGCCTTAACGCCTATGATAGACAC
Bv_chr3_Hat27_1_2 F	CGTTTGAAGCAATCCACGGTC
Bv_chr3_Hat27_1_2 R	CATCATCTCCAGCACCCGAC
Bv_chr4_Hat28_1_3 F	AGGCTCTGTTGTCACCTCTG
Bv_chr4_Hat28_1_3R	TCAGTTGTGGTGCACAGAAGAA
Bv_chr4_Hat28_1_4F	TGCTTCTTCCCACAACATCTCC
Bv_chr5_Hat27_1_5F	TCAGCAGCAGATGGTACAAAGA
Bv_chr5_Hat27_1_5R	CACAGCATGCTTTCTCCAACTC
Bv_chr5_Hat28_1_6F	AAGACGCTTCTTCTTGGCGG
Bv_chr5_Hat28_1_6R	TCAATCGCCATCGACCTTCA
Bv_chr6_Hat24_1_7R	GTGCAGTTCAAGTTTTGGAGGA
Bv_chr6_Hat24_1_7R	AGAGTAGGGTGGTGGCTGAC
Bv_chr6_Hat28_1_8F	CTGGTTATGCATCTACCGAGGG
Bv_chr6_Hat28_1_8R	TGTCTGTGCTGCTTGCCTTTT
Bv_chr6_Hat28_1_9F	GCTTCGCACAACAGATGAGATAG
Bv_chr6_Hat28_1_9R	GGAAGAGTTGGGCCGTGATT
Bv_chr2_hAT|24_1_F	TCGGATTGTGATTCTGTTGGAGT
Bv_chr2_hAT|24_1_R	TTGCTATTGGAGGGTCGCTG
Bv_chr3_hAT|24_2_F	CTTTGTGTCCACTTTCGCTCG
Bv_chr3_hAT|24_2_R	TTTAGGCTCCCTGGCAAATGG
Bv_chr5_hAT|24_3_F	CTGTCATCTTTTTCCACTCTTCAC
Bv_chr5_hAT|24_3_R	ATCCATCAAAGCCAAAACAGCC
Bv_chr5_hAT|24_4_F	GCCTGAAATCAGGTCAAGTGAG
Bv_chr5_hAT|24_4_R	TCAGCCTTCGCTTCTGAACAA
Bv_chr6_hAT|24_5_F	ACCTACATCGTTTGCCCCAG
Bv_chr6_hAT|24_5_R	GGGAACTAAGCCTACCACGC
Bv_chr6_hAT|24_6_F	CGAAGAGAATATTGCTTGGCTTGA
Bv_chr6_hAT|24_6_R	CGAACTGCACAAACCTTGGC
Bv_chr7_hAT|24_7_F	TACCCACTTGGAACTGGAAGG
Bv_chr7_hAT|24_7_R	CTGAAGCTCTTCTCCTGTGACC
Bv_chr7_hAT|24_8_F	TTGTTTGATTCTGATGCTTCTTGGG
Bv_chr7_hAT|24_8_R	CCATATGCCACCAACCAAAGG
Bv_chr8_hAT|24_9_F	TCCTGTGTCCCTCTAACTCCTC
Bv_chr8_hAT|24_9_R	AGAGATATACCCCCGCCGC
Bv_chr8_hAT|24_10_F	TTTGTTCCAGACCTTGCTGAGA
Bv_chr8_hAT|24_10_R	GCCTTTGTTGACTGTATGCCTT

Bv_chr9_hAT|24_11_F	TTTCATCCTCACCATCATTGAAACC
Bv_chr9_hAT|24_11_R	AAGATTTCGCATCTTGACGCC
Bv_chr9_hAT|24_12_F	GAGTGGCAGAGAAGAAGGTGC
Bv_chr9_hAT|24_12_R	CATCAAAAAGGCGAATGTTGCAT
Bv_chr9_hAT 24_13f	TGGGGAGGCTTCCGATAGAC
Bv_chr9_hAT|24_13_R	ACGGCCTAGAAAAGAGGCTG
Bv_chr6_hAT|26_14_F	TTCATGCAGGCTGTTGTAAGAC
Bv_chr6_hAT|26_14_R	TTCTGTGCCTTCTCTTGGAGT
Bv_chr6_hAT|26_15_F	CATTGTCAGTTCTGAAACAGATGA
Bv_chr6_hAT|26_15_R	TGAATCGAGCAGCTTCCACC
Bv_chr7_hAT|26_16_F	GCACATTGGGAATTGAAATGGAAA
Bv_chr7_hAT|26_16_R	TGGCATTATGCTGATGCTATGT
Bv_chr7_hAT|26_17_F	ATGTCCATGTGCTCTGGGTTT
Bv_chr7_hAT|26_17_R	AATGCCGAGCATATTCAACCAC
Bv_chr7_hAT|26_18_F	ATCCACTCAGAGCGAGACTG
Bv_chr7_hAT|26_18_R	CCCATTGTACCTCTCTGGTGT
Bv_chr2_hAT|27_19_F	AGGAGCATACTCCCAGGTCC
Bv_chr2_hAT|27_19_R	TCGTGCAATACCAATGGAGGTG
Bv_chr3_hAT|27_20_F	AAGGGGTATCGTCCAGCAAA
Bv_chr3_hAT|27_20_R	ACTTGATGGGAGATAGGAAAGACAA
Bv_chr3_hAT|27_21_F	AGAACGACTGATGAACTCGACC
Bv_chr3_hAT|27_21_R	TGCATGAGCTCTGGTAATGCT
Bv_chr3_hAT|27_22_F	CATCATGAGCCCACCGTAGA
Bv_chr3_hAT|27_22_R	AGTATGTGGCGTTGCATCAG
Bv_chr4_hAT|27_23_F	CAGGATGGTTAGCAGCGAGAT
Bv_chr4_hAT|27_23_R	TCAGGGACGACCTTGTTCTC
Bv_chr4_hAT|27_24_F	ATCCGGGCACACAATAAACCA
Bv_chr4_hAT|27_24_R	TTAAGACCCTGCATGGAAGGAG
Bv_chr5_hAT|27_25_F	TTATTGCGTTTCGAGAGCCG
Bv_chr5_hAT|27_25_R	CACACAGTATTGCTGCCTGG
Bv_chr6_hAT|27_26_F	TTGCCAATGAAGATGAAGCGATG
Bv_chr6_hAT|27_26_R	CAAGCGTTACAACAGCCCAT
Bv_chr7_hAT|27_27_F	GTGAGGCTAGCTAGTGGCAAA
Bv_chr7_hAT|27_27_R	CCAGTGAGTTCCCGGTACAT
Bv_chr7_hAT|27_28_F	GGGAACTGGAGCCAACTTCA
Bv_chr7_hAT|27_28_R	TGGAAAAGGACAATGGTATTGGT
Bv_chr7_hAT|27_29_F	TTGTGCTTGGCTTGCTTGAC
Bv_chr7_hAT|27_29_R	GTCGGTAGCAGCAGGATCTC
Bv_chr7_hAT|27_30_F	GATCCACCGGAGCTGTAAGG
Bv_chr7_hAT|27_30_R	GGCCTTGAAACAAGGTGTTCG
Bv_chr7_hAT|27_31_F	ACCTTGGATTGATAGGGGCAC
Bv_chr7_hAT|27_31_R	GGAGTAGGCCCATCTGGCTT
Bv_chr8_hAT|27_32_F	TTTCAACCATCCAGCCCCTC
Bv_chr8_hAT|27_32_R	ACAAATCGCGGTGGAGGAAT

Bv_chr1_hAT|28_33_F	TCTACAAACAGGCAACAGGAT
Bv_chr1_hAT|28_33_R	CCATGTTTAACTTCAAGTGCCC
Bv_chr1_hAT|28_34_F	TAAGCCAGCATCAGAGTCAGC
Bv_chr1_hAT|28_34_R	TTCTTCGACTTAGGCAGCAAGT
Bv_chr2_hAT|28_38_F	TCGAAGAACCATCTGTCCACTG
Bv_chr2_hAT|28_38_R	TTGCTATTGGAGGGTCGCTG
Bv_chr2_hAT|28_39_F	AAAGCAGAAATGGCCCGAGAG
Bv_chr2_hAT|28_39_R	TTCCAAGCTGAGCCCACG
Bv_chr3_hAT|28_42_F	TCAAGGGTCGTATATGGTGAATTGT
Bv_chr3_hAT|28_42_R	TAAATGAGCAACGGTGTTTCCC
Bv_chr3_hAT|28_43_F	GGTTAGCGAAGCAAATCCCA
Bv_chr3_hAT|28_43_R	GTTTCTGCAGCAGCATCTGAC
Bv_chr3_hAT|28_44_F	AGCTTGGGATGACTACTCAGG
Bv_chr3_hAT|28_44_R	AGCAATGTGTGACTCCTAAAGT
Bv_chr4_hAT|28_50_F	CTCTCATGGTGCGCCAACTG
Bv_chr4_hAT|28_50_R	GTCTTAGCCCTCCTATGGGC
Bv_chr4_hAT|28_51_F	CATTGCTGAATGCAGGGGTTAC
Bv_chr4_hAT|28_51_R	AGCTTTCAGGCAAGCTCAAAC
Bv_chr5_hAT|28_53_F	CATCACCCCGCAACTTCATTC
Bv_chr5_hAT|28_53_R	ACAGTTGACCGAAATTGATGTCTC
Bv_chr5_hAT|28_54_F	GTTTCAACCGCGAGTTTCCT
Bv_chr5_hAT|28_54_R	CTTCACAACCTCTAGCGCAGG
Bv_chr6_hAT|28_57_F	TTGTGATAGCTGAGGCCAGA
Bv_chr6_hAT|28_57_R	TCATTCATAAGCTCCCAAGCCAT
Bv_chr6_hAT|28_58_F	CAGGATTCTCTCCGGCTGTT
Bv_chr6_hAT|28_58_R	GCCAGATGGAATCTAGGGCTC
Bv_chr6_hAT|28_59_F	CCACCCCATCTTCAGATTGCC
Bv_chr6_hAT|28_59_R	ATTCAAAGAACGGACAGCAGC
Bv_chr6_hAT|28_60_F	CTGGAACAGGCGGATGAACATA
Bv_chr6_hAT|28_60_R	CAGCATACCCCATGGTTCTGG
Bv_chr6_hAT|28_61_F	TGGGGAGTAAACCTCGCCTT
Bv_chr6_hAT|28_61_R	AAGCCTTGTTTGCTGATCTAGG
Bv_chr6_hAT|28_62_F	TCACCAAACTCTCAACTTGAACC
Bv_chr6_hAT|28_62_R	TGAGACTAACTCTATTTGCCACCA
Bv_chr6_hAT 28_70_F	ACGTTGCATTGTCATCGAGC
Bv_chr7_hAT|28_70_R	ATCGACAATTCAGTCCGCAGT
Bv_chr7_hAT|28_71_F	ATCGGACACGACACGACACC
Bv_chr7_hAT|28_71_R	CCAAATGCAAATTCAGTGAGAGGT
Bv_chr7_hAT|28_72_F	AAACTACATCTGTCTGGCTGGA
Bv_chr7_hAT|28_72_R	AAGTGAAGAGTGTCCCGTCC
Bv_chr7_hAT|28_73_F	TGTCGTGTAATTCCTGGGGG
Bv_chr7_hAT|28_73_R	ACGTGAAGCTCTTGAAGGCG
Bv_chr9_hAT|28_81_F	CAGCCATCCGAGGTTTGCAT
Bv_chr9_hAT|28_81_R	TGCAGTTATATATACTTCCTTCGCC

Bv_chr9_hAT|28_82_F	TACCTACAGATAGTGGGCTGTCA
Bv_chr9_hAT|28_82_R	ATTCGGGGGAAAATAGGGGGAT
Bv_chr9_hAT|28_83_F	AGTCTTTCTTCTTTCTTGCGAGC
Bv_chr9_hAT|28_83_R	GAGAAGATGTTATACAAGCTGAACA
Bv_chr9_hAT|28_84_F	GGGGAATTTATCGTGCGAGC
Bv_chr9_hAT|28_84_R	AGTTGACACAAGTGATCCTCGG

4.4 PCR ANALYSIS

A total of 12 genotypes were used in PCR reactions with 66 different pairs of primers. (Table 2).

PCR	CONCENTRATION	Volume per 1 reaction	Volume per 13 reactions
Water		6.65 µl	86.45 µl
Dream Taq buffer (Green)	10x	1 µl	13 µl
Forward primer	10µM	0.5 µl	6.5 µl
Reverse primer	10µM	0.5 µl	6.5 µl
DNTP	10mM	0.25 µl	3.25 µl
Dreamtaq polymerase	5µ/µl	0.1 µl	1.3 µl
DNA	100x	1µl	12 µl

Table 2: PCR reactions for microsatellite and other molecular markers.

We prepared the master mix for 13 samples; we then added DNA only to 12 samples and we kept the 13th samples as a control.

PCRs were performed in Eppendorf thermal cycler as shown in Table 3

STEPS	TEMPERATURE	TIME	CYCLES
1.PRE DENATURATION	95	2 minutes	30 cycles
2. DENATURATION	95	15 seconds	
3. ANNEALING*	60	15 seconds	
4. EXTENSION	68	1 minute	
5. FINAL EXTENSION	68	1 minute	
6. PCR TEMPERATURE	10	Constant temperature	

Table 3: PCR protocol followed for microsatellite primers.

*We run PCR for 90 minutes and the Annealing temperature changes from primer to primer based on the NCBI results of each primer.

4.5 GEL ELECTROPHORESIS

4.5.1. BUFFER PREPARATION

We need to prepare 1XTBE (Tri's base) buffer with pH 8.2-8.4 was prepared from 5X TBE buffer by dilution in a (4:1) ratio of water and 5X buffer.

4.5.2 GEL PREPARATION

The concentration of a gel is 1% agarose. After preparation of a 1XTBE buffer of 300 ml in a conical flask and add 3 gms of agarose gel which is equal to 1% of gel concentration and keep in the oven for 2-3 minutes and make sure the gel is clear without any clumps and cool it down under tap water by stirring for few minutes. Then add Midori strain of 12-14 µl into the flask and stir it well.

4.5.3. POURING OF GEL

Pour the cooling gel into the casting tray which is already mounted with combs and do not disturb the tray for one hour to solidify the gel for loading samples.

4.5.4. ELECTROPHORESIS

The solidified gel is placed into a chamber filled with 1xTBE Buffer. The gel is positioned so that the chamber wells are closest to the negative electrode of the chamber**.** The gel chamber wells are loaded with DNA samples and a 1kb DNA ladder is also loaded as a reference for sizes. We set the voltage as 120 and time as 90 minutes and the current as turning on the power supply sets up the electric field and DNA samples will start migrating from the negative to the positive side of the chamber.

4.5.5. VISUALIZING THE DNA

Gels were exposed to UV light and pictures are taken DNA bands are visualized from each lane. The 1kb DNA ladder that was loaded is also visualized and the length of DNA bands can be estimated by reference as a 1kb ladder.

4.6 STATISTICAL ANALYSES

4.6.1. DATA ANALYSIS

The Bvh-ILP markers are scored manually according to the size (bp) of the amplified fragments of the Bvh-ILP 66 markers of all individual samples from the tested populations based on visual inspection of electrophoretic patterns, and polymorphic information content.

4.6.2. GENETIC DISTANCE

Genetic distance (GD) is one of the important measures to understand the diversity of the parents, GD should be optimum to exploit the heterosis or breeding. Hence, the genetic distance was calculated from 12 genotypes based on the molecular markers data from the software package GENALEX (6.05).

4.6.3. AMOVA

Analysis of molecular variance (AMOVA) is a statistical model for the molecular algorithm in a single species, typically biological. The method was developed by (Laurent Excoffier, Peter Smouse, and Joseph Quattro1992).

AMOVA is used to calculate the level of genetic differentiation among different populations. It uses molecular markers and tells us the difference between populations, and within populations, AMOVA was performed using GenALEx 6.5 software. Simply calculates population differentiation based on the genotypic variance. Probability values were estimated by 9999 permutations to determine whether the partitioning of variance components was significant.

4.6.4. PRINCIPAL COORDINATE ANALYSIS

The principal coordinate analysis method is used to visualize and explore similarities or dissimilarities of the data. The data can be generated from quantitative or semi-quantitative or qualitative variables. Principle coordinate analysis was carried out from 3 populations from 66 markers data using the software package GenALEx 6.5.

4.6.5. GENETIC PARAMETERS AND ALLELIC DIVERSITY

Genetic parameters and allelic diversity estimated from the software package GenALEx (version 6.503), estimating basic statistics viz., allelic richness as determined by the total number of the detected alleles, number of alleles per locus (Na), number of effective alleles (Ne), gene diversity as determined by observed heterozygosity (Ho), expected heterozygosity

(He), the occurrence of unique, rare, common alleles & Shannon's information measure (I) and the inbreeding coefficient as shown by Fixation index (F).

4.6.6. FST VALUE

The degree of similarity dissimilarity value is calculated from the GenALEx (6.50) software package. Values can range from 0 to 1. High FST shows a considerable degree of differentiation between genotypes.

5) RESULTS

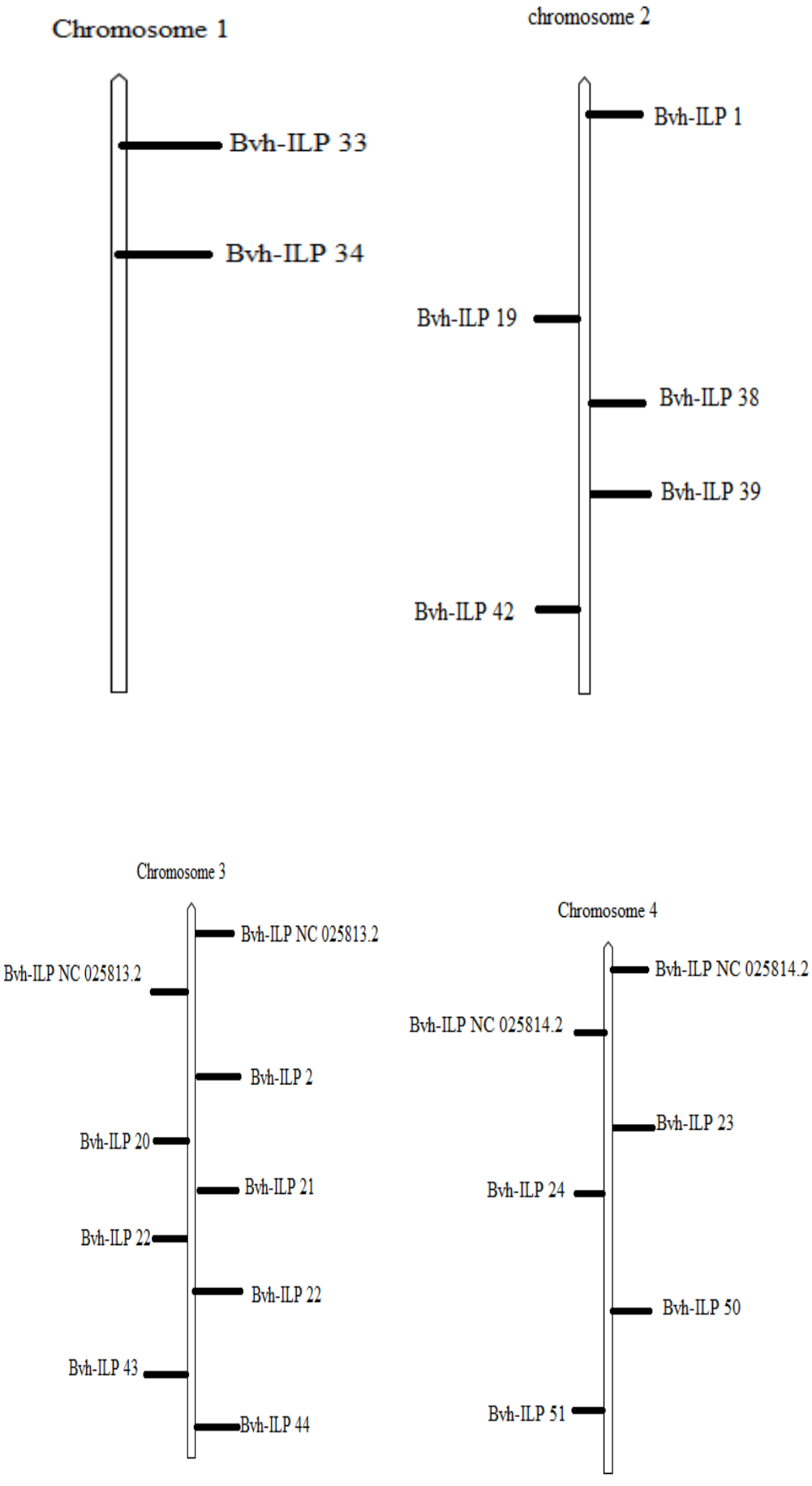
A total of sixty-six pairs of primers were used with 12 genotypes for the study. Forty primers were monomorphisms, twenty pairs were polymorphisms, and the remaining six have no results/ a few empty spots, this may be due to some insertions or deletions, or maybe the primers were not working for the DNA. (Table 4)

INSERTION SITES	NUMBER
POLYMORPHISMS	20
MONOMORPHIC	40
NO RESULT/Few Spots Empty	6
TOTAL	66

Table 4: BV-hAT insertion sites and their polymorphism.

5.1 DEVELOPMENT AND VALIDATION OF CANDIDATE Bvh -ILP MARKERS

Insertion sites of 66 Bvh-ILP MITEs within introns of annotated genes were chosen to develop *Beta vulgaris hAT-like* intron length polymorphisms (Bvh-ILP) markers. These markers are evenly distributed throughout the genome (Figure 1). The number of Bvh-ILP insertion sites is evaluated and varied per chromosome from 1 to 9 with the highest number of insertion sites in chromosome 7, with 14 insertions, and in chromosome 6, with 14 insertions with an average of 18.48. the lowest number of insertions was in chromosome 1 with 2 insertions.


Chromosome 1
Bvh-ILP 33
Bvh-ILP 34
chromosome 2
Bvh-ILP 1
Bvh-ILP 19
Bvh-ILP 38
Bvh-ILP 39
Bvh-ILP 42
Chromosome 3
Bvh-ILP NC 025813.2
Bvh-ILP NC 025813.2
Bvh-ILP 2
Bvh-ILP 20
Bvh-ILP 21
Bvh-ILP 22
Bvh-ILP 22
Bvh-ILP 43
Bvh-ILP 44
Chromosome 4
Bvh-ILP NC 025814.2
Bvh-ILP NC 025814.2
Bvh-ILP 23
Bvh-ILP 24
Bvh-ILP 50
Bvh-ILP 51

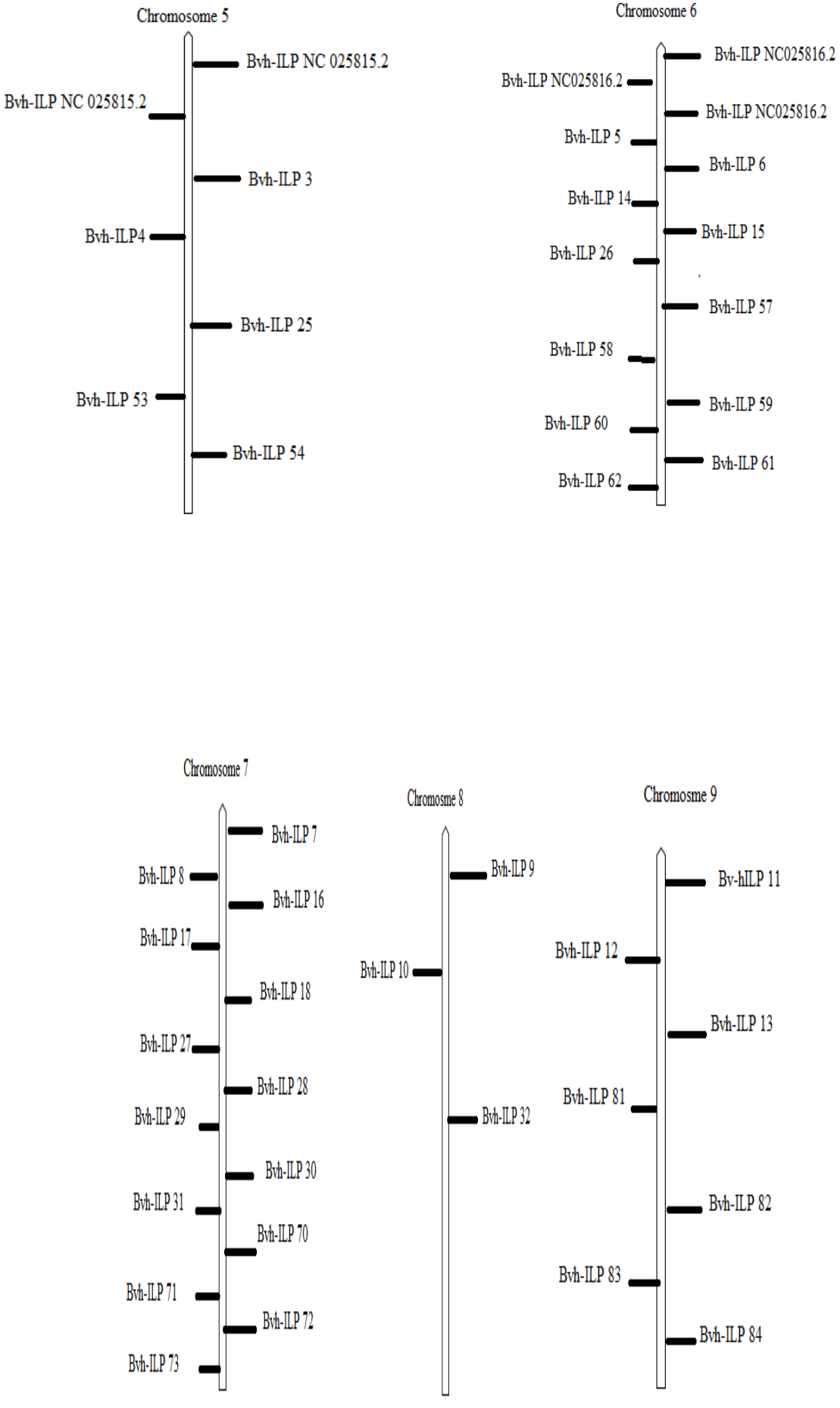

Chromosome 5
Bvh-ILP NC 025815.2
Bvh-ILP NC 025815.2
Bvh-ILP 3
Bvh-ILP4
Bvh-ILP 25
Bvh-ILP 53
Bvh-ILP 54
Chromosome 6
Bvh-ILP NC025816.2
Bvh-ILP NC025816.2
Bvh-ILP NC025816.2
Bvh-ILP 5
Bvh-ILP 6
Bvh-ILP 14
Bvh-ILP 15
Bvh-ILP 26
Bvh-ILP 57
Bvh-ILP 58
Bvh-ILP 59
Bvh-ILP 60
Bvh-ILP 61
Bvh-ILP 62
Chromosome 7
Bvh-ILP 7
Bvh-ILP 8
Bvh-ILP 16
Bvh-ILP 17
Bvh-ILP 18
Bvh-ILP 27
Bvh-ILP 28
Bvh-ILP 29
Bvh-ILP 30
Bvh-ILP 31
Bvh-ILP 70
Bvh-ILP 71
Bvh-ILP 72
Bvh-ILP 73
Chromosme 8
Bvh-ILP 9
Bvh-ILP 10
Bvh-ILP 32
Chromosme 9
Bv-hILP 11
Bvh-ILP 12
Bvh-ILP 13
Bvh-ILP 81
Bvh-ILP 82
Bvh-ILP 83
Bvh-ILP 84

FIGURE 1 | Physical genomic distribution of the 66 developed Bvh-ILP markers on nine chromosomes of the Sugar beet genome. The vertical bars correspond to the position of introns harboring Bvh -insertions, selected for the development of ILP markers.

Upon PCR amplification of 66 markers, 17 markers of Bvh-ILP showed polymorphism with two allelic variants, only 3 showed polymorphism with an additional variant, 36 markers showed monomorphism, and 9 had few empty spots or no amplification markers shown in (table 5). A high number of insertion sites are observed in chromosome 6 and chromosome 7 with 14 insertions in this chromosome 6 had a high number of monomorphic insertions in 11 markers and only 1 marker is polymorphic with two allelic variants. Chromosome 7 shows high polymorphic insertion sites in 7 markers and monomorphic in 6 markers. A low number of insertions are present in chromosome 1 in only 2 markers with monomorphic (Table 5).

Finally,66 Bvh-ILP markers (table 5) markers showing polymorphisms were chosen for developing a panel for genotyping the sugar beet.

Chromosome	Number of insertion sites	Polymorphic with two allelic variants	Polymorphic with an additional variant	Monomorphic	NO Amplification
1	2	-	-	2	-
2	5	2	1	2	-
3	8	3	1	4	-
4	6	1	-	4	1
5	7	1	1	4	-
6	14	1	-	11	2
7	14	7	-	6	1
8	3	2	-	-	1
9	7	-	-	3	4
TOTAL	66	17	3	36	9

Table 5 Results of the experimental validation of developed candidate Bvh-ILP markers.

5.2) AMPLIFICATION OF Bvh-ILP MARKERS IN 12 SUGAR BEET CULTIVARS:

MARKER NAME	GENOTYPES											
	SB1	SB2	SB3	SB4	SB5	SB6	SB7	SB8	SB9	SB10	FB1	FB2
Bvh-ILP 2	h/e	H/o	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	H/o	h/o
Bvh-ILP 1	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	H/0
Bvh-ILP 38	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	H/0
Bvh-ILP 20	h/e	h/o	h/e	h/o	h/o	h/e	h/e	h/e	h/e	h/e	h/o	h/o
Bvh-ILP 43	h/e	H/o	h/o	h/e	h/e	h/e	h/e	h/e	h/e	h/e	H/o	H/o
Bvh-ILP 44	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	H/0
Bvh-ILP 24	h/o	h/o	h/o	h/adv	h/o	h/o	h/o	h/o	h/e	h/o	h/e	H/o
Bvh-ILP 53	h/e	H/o	h/e	h/e	H/o	h/o	h/o	h/o	h/o	h/o	h/e	h/e
Bvh-ILP 54	h/e	h/e	h/e	h/e	h/e	h/e	h/o	h/o	h/o	h/o	h/o	h/o
Bvh-ILP 58	h/o	h/o	h/o	h/o	h/o	h/e	h/e	h/o	h/o	h/o	h/o	h/o
Bvh-ILP 16	H/o	h/o	h/o	h/e	h/e	h/o	h/e	h/o	h/o	h/e	h/e	h/e
Bvh-ILP 28	H/o	h/e	H/o	h/e	h/e	h/e	h/e	h/e	h/e	h/e	H/o	H/0
Bvh-ILP 31	h/e	H/adv	H/adv	h/e	h/e	h/e	h/e	h/o	h/o	H/adv	h/o	H/adv
Bvh-ILP 73	H/o	H/o	h/e	h/e	h/e	h/e	h/e	h/e	H/o	h/o	H/o	h/e
Bvh-ILP 9	H/o	h/e	h/e	h/o	h/o	h/e	h/e	h/e	h/e	h/adv	H/o	h/e
Bvh-ILP 10	H/o	H/o	h/adv	h/adv	h/adv	h/e	h/e	h/e	h/0	h/o	h/0	h/0
Bvh-ILP 30	h/e	h/e	h/e	h/o	h/o	H/0	H/o	H/o	H/o	H/o	H/o	H/o
Bvh-ILP 71	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/e	h/o	h/o
Bvh-ILP 7	h/e	h/e	h/e	h/e	h/o	h/e	h/e	h/e	h/e	h/e	h/e	h/e
Bvh-ILP 61	h/e	h/e	h/o	h/o	h/adv	h/o	h/o	h/adv	h/e	h/e	h/e	h/e
BVh-ILP 18	h/e	H/o	h/o	h/e	H/o	h/e	h/e	h/e	h/e	h/e	h/o	h/o

Table 6 Results of polymorphisms of Bvh-ILP marker of 12 genotypes.

***Bvh-ILP (Beta vulgaris (hAT) Intron length polymorphism, SB (Sugar beet), FB (Fodder beet).**

***h/e (homozygous empty), h/o (homozygous occupied), H/o (Heterozygous occupied), H/adv (Heterozygous with additional variant), h/adv (homozygous additional variant).**

The Bvh-ILP Markers Profiles are scored manually. Each allele was scored as A (Empty insertion site), B (Occupied insertion site), and C (Additional Variant).

Some markers mentioned here in our results are: 1) Bvh-ILP 1 2) Bvh-ILP 9 3) Bvh-ILP 16 4) Bvh-ILP 315) Bvh-ILP 44 6) Bvh-ILP 24 7) Bvh-ILP NC 025816.2 8) Bvh-ILP 3 are with 12 DNA lanes are named from 1to 12 and 1kb ladder with 250 to 10,000 base pairs with 14 DNA fragments are marked.

A) Amplification of the **Bvh-ILP 1** marker is polymorphic as it reveals products of different sizes. Plants in lanes 1-11 are homozygous for the empty site, and the plant in lane 12 is heterozygous and represents the site occupied with *hAT* MITE. **Bvh-ILP 1** positions on chromosome 2 of *Beta vulgaris*.

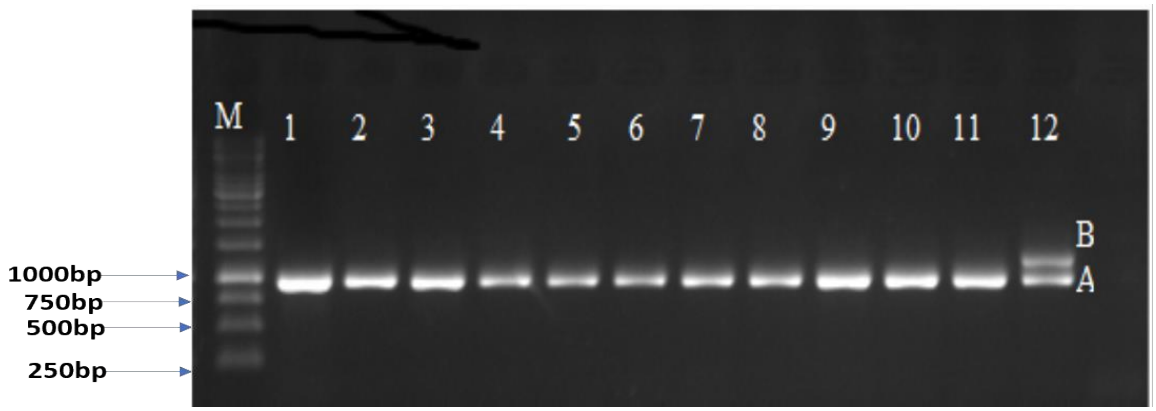
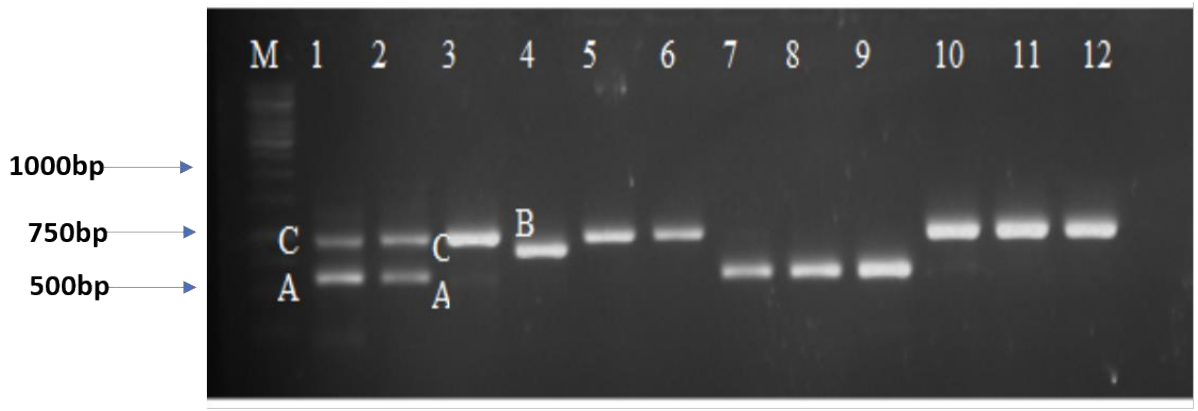


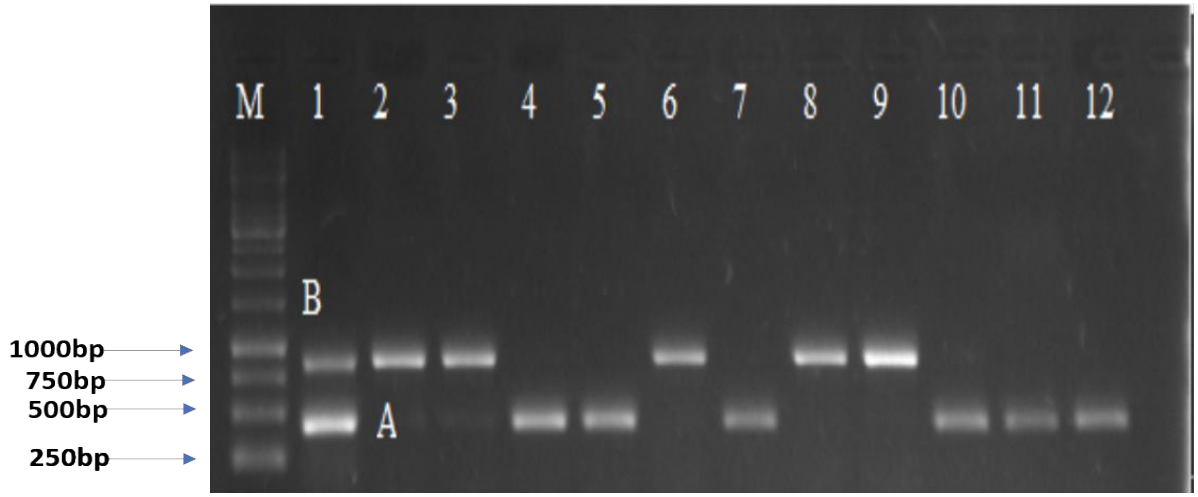
Figure 2) Electrophoretic profile of PCR products using Bvh-**ILP 1** primer for 12 sugar beet genotypes.

B) Amplification of the **Bvh-ILP 9** marker reveals polymorphism. Plants on lanes 1 and 2 are heterozygous, plants on lanes 3,5,6,10,11, and 12 are homozygous for the full site, plants on lanes 7,8,9 a homozygous for the empty, site and plant on lane 4 is homozygous for an additional variant. **Bvh-ILP 9** positions on chromosome 8 of *Beta vulgaris*.



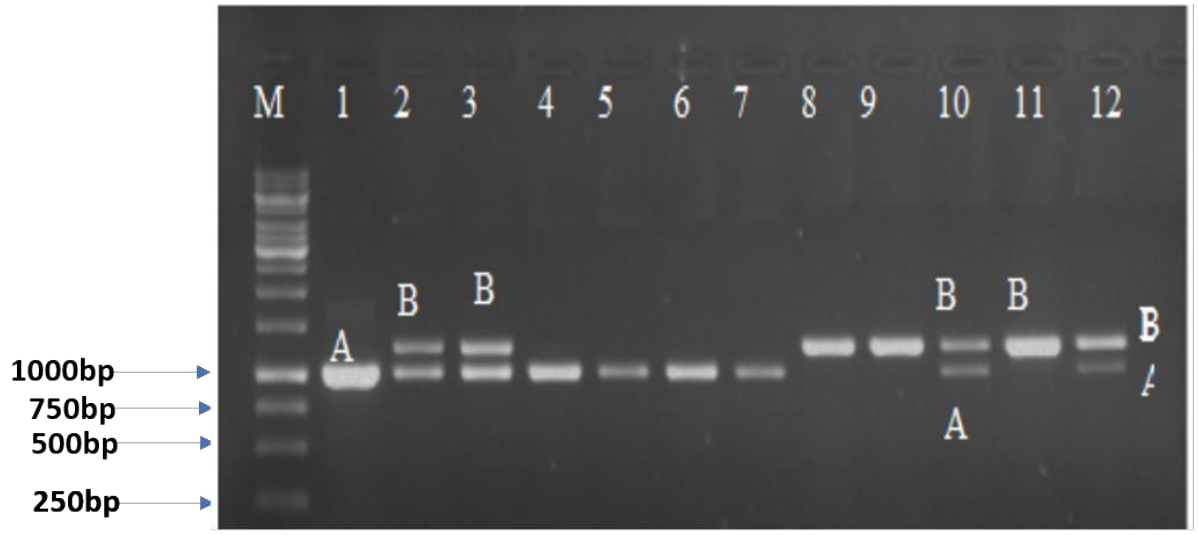
(Figure 3) Electrophoretic profile of PCR products using **Bvh-ILP 9** primer for 12 sugar beet genotypes.

C)Amplification of **Bvh-ILP 16** marker is polymorphism. Plants on lane 1 are heterozygous, plants on lane 2,3,6,8,9 are homozygous with the occupied site, and plants on lane *4,5,7,10,11,12* is homozygous with the empty site. **Bvh-ILP 16** belongs the chromosome 7 of *Beta vulgaris*. (Figure 3)



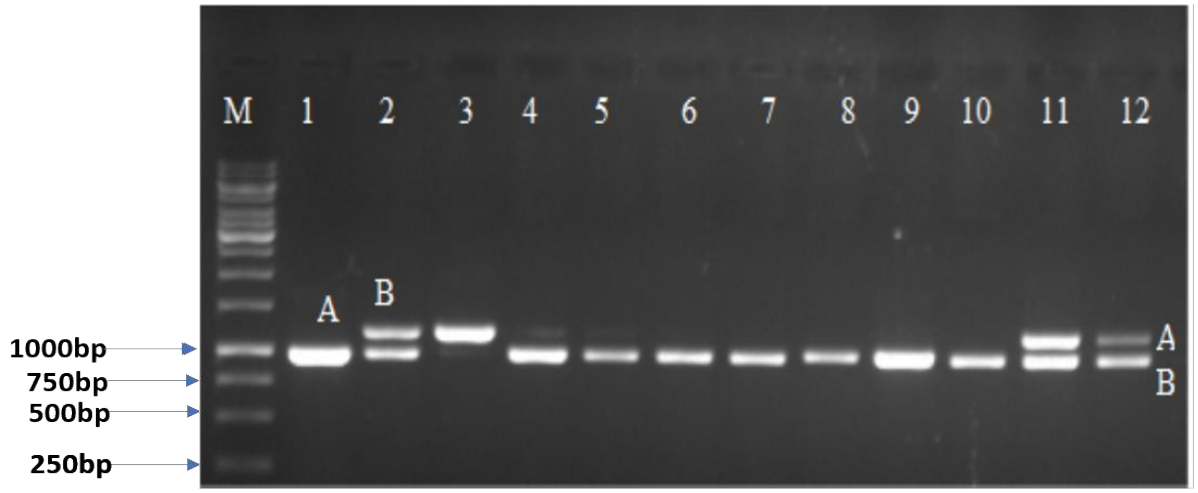
(Figure 4) Electrophoretic profile of PCR products using **Bvh-ILP 16** primer for 12 sugar beet genotypes.

D)Amplification of **Bvh-ILP 31** polymorphism. Plants on lanes 2,3,10 and 12 are heterozygous, plants on lanes 8,9, and 11 are homozygous with the occupied site, and plants on lanes 1,4,5,6, and 7 are homozygous with the empty site. **Bvh-ILP 31** belongs to chromosome 7 of Beta vulgaris. (Fig 4)



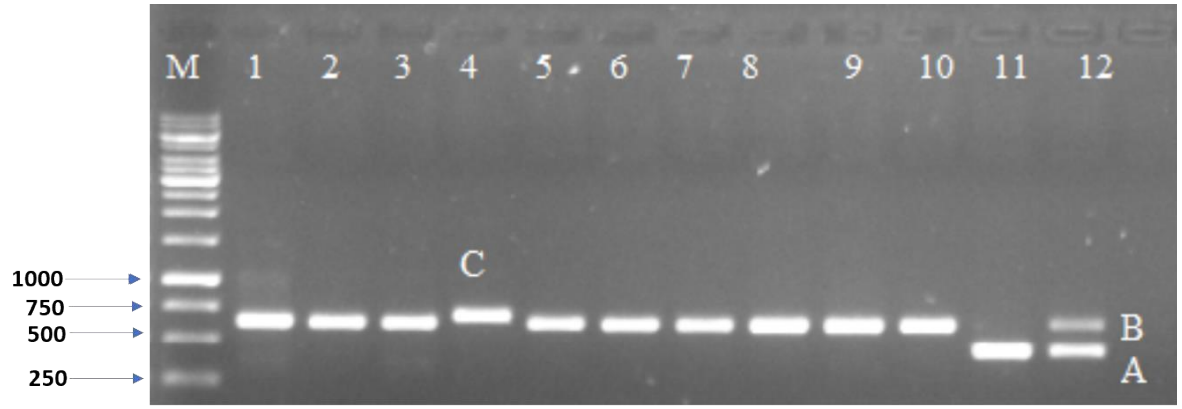
(Figure 5) Electrophoretic profile of PCR products using **Bvh-ILP 31** primer for 12 sugar beet genotypes.

E) Amplification of **Bvh-ILP 44 showed** polymorphism. Plants on lanes 2,11 and 12 are heterozygous, plant on lane 3 is homozygous with the occupied site, and plants on lane 1,4,5,6,7,8,9,10 are homozygous with the empty site. **Bvh-ILP 44 belongs** to chromosome 3 of Beta vulgaris. (Fig 5)



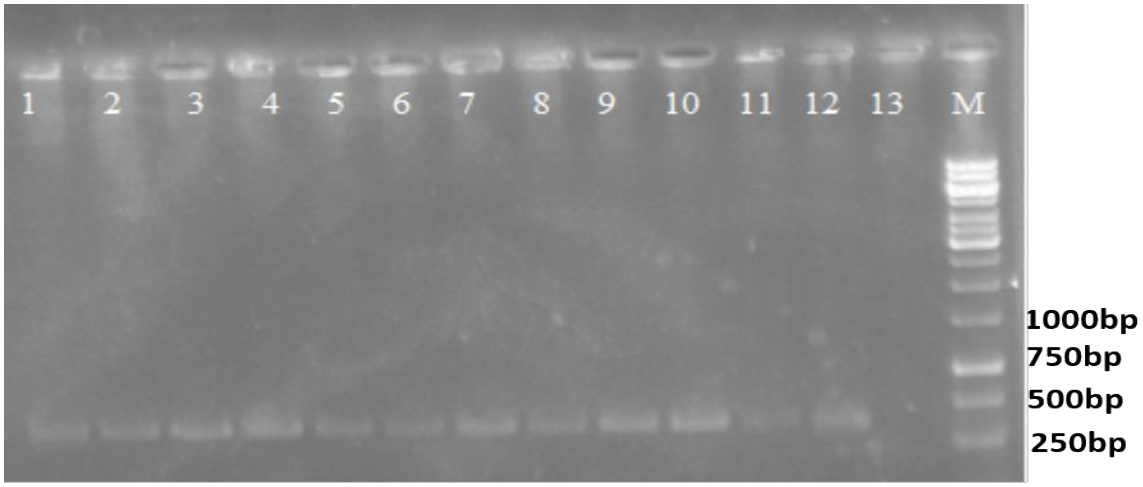
(Figure 6) Electrophoretic profile of PCR products using **Bvh-ILP 44** primer for 12 sugar beet genotypes.

F) Amplification of **Bvh-ILP 24** showed polymorphism. The plant on lane 12 is heterozygous, the plant on lane 4 is a homozygous additional variant, plants on lanes 1,2,3,5,6,7,8,9,10 are homozygous occupied site and lane 11 is homozygous empty site. **Bvh-ILP 24** belongs to chromosome 4 of Beta vulgaris. (Fig 6)



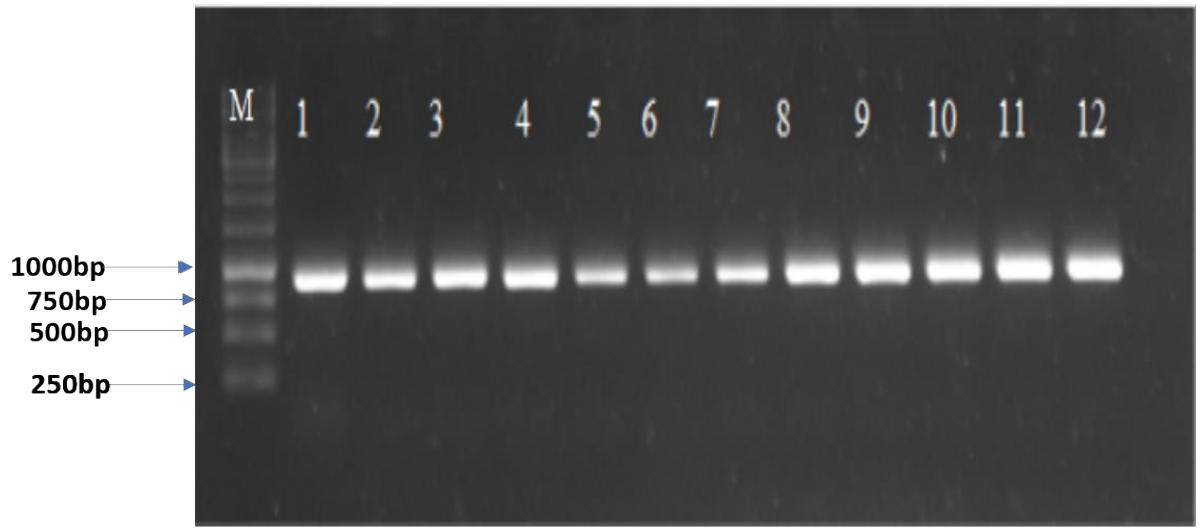
(Figure 7) Electrophoretic profile of PCR products using Bvh-**ILP 24** primer for 12 sugar beet genotypes.

G) There is no amplification of this marker since primers are not working with this DNA **NC_025816.2** belongs to chromosome 5 of beta vulgaris. (Figure 7).



(Figure 8) Electrophoretic profile of PCR products using **Bvh-ILP NC_025816.2** primer for 12 sugar beet genotypes.

H) Amplification Bvh-**ILP 3** is monomorphic and observed on all lanes. It is homozygous with an empty site and it belongs to chromosome 5 of beta vulgaris.



(Figure 9) Electrophoretic profile of PCR products using **Bvh-ILP 3** primer for 12 sugar beet genotypes

MARKER CLASS	Range of intron length (BP)	Number of Bvh-ILP	Number of positively validated Bvh-ILP markers
I	200-400	2	0
II	400-600	11	1
III	600-800	17	7
IV	800-1000	21	10
V	1000-1200	4	2
VI	1200-1400	2	0

Table 7 The intron length-based classification of candidate Bvh-ILP marker.

In Bvh-ILP insertions, the length of introns harboring the selected Bvh insertions varied from 200 to 1400 base pairs (bp). Based on the length of amplified introns, the developed markers are divided into six classes i.e. I, II, III, IV, V, and VI, with intron ranging from 200 to 1400 bp, each class interval with 200 bp. (Table 7).

Introns belonging to class II, III, and IV markers were most abundant occupied with 86% which are successfully amplified indicating the most suitable length of introns considered for Bvh-ILP markers. Class, I am shorter in length, meaning that no MITEs are present in these markers. Class VI is 1200-1400 bp longer than the polymorphic length, which means that they have additional insertions. Class IV (800-1000) showed the highest percentage of 47.6% and class III (600-800) showed 36.8% total of 84.4% of class III&IV of successful amplification rate, indicating that the most suitable length of introns considered for Bvh-ILP markers. In classes V and VI classes, there is no successful amplification, so we did not consider these classes for further analysis.

5.3 GENETIC DISTANCE

Genetic distance (GD) is one of the most important measures to understand the diversity of the parents. GD should be optimum to exploit the heterosis segregation in crop breeding. Hence, the genetic distance was calculated from 12 genotypes' molecular marker data (Table 8).

SB1	SB2	SB3	SB4	SB5	SB6	SB7	SB8	SB9	SB10	FB1	FB 2	
0	31	34	44	37	46	54	56	42	46	53	56	**SB1**
31	0	25	31	46	57	73	55	49	63	44	49	**SB2**
34	25	0	46	47	58	70	70	50	58	49	56	**SB3**
44	31	46	0	29	60	68	64	60	58	41	46	**SB4**
37	46	47	29	0	45	53	65	45	39	34	45	**SB5**
46	57	58	60	45	0	24	28	32	34	45	40	**SB6**
54	73	70	68	53	24	0	28	32	26	53	48	**SB7**
56	55	70	64	65	28	28	0	24	40	47	44	**SB8**
42	49	50	60	45	32	32	24	0	26	33	36	**SB9**
46	63	58	58	39	34	26	40	26	0	37	32	**SB10**
53	44	49	41	34	45	53	47	33	37	0	17	**FB1**
56	49	56	46	45	40	48	44	36	32	17	0	**FB2**

Table: 8 Results of Genetic distance of 12 genotype populations

***SB (sugar beet) FB (Fodder beet)**

The highest genetic distance is 73 between (sugar beet 2) and (Sugar beet 7) and the lowest genetic distance between (Fodder beet 1) to (fodder beet 2) is 17.

5.4 AMOVA (ANALYSIS OF MOLECULAR VARIANCE)

Analysis of molecular variance (AMOVA) is a statistical model for the molecular algorithm in a single species typically biological. The method was developed by (Laurent Excoffier, Peter Smouse, and Joseph Quattro1992). AMOVA is used to calculate the level of genetic differentiation among different populations. It uses molecular markers and tells us the difference between populations, and within populations. The population was divided into 3 classes and 12 genotypes of which 6 are sugar beet (multigerm) and 4 are sugar beet (monogerm) and 2 are fodder beet. The percentages of different populations and differences between populations and within individual populations are explained in the pie chart (Figure 10).

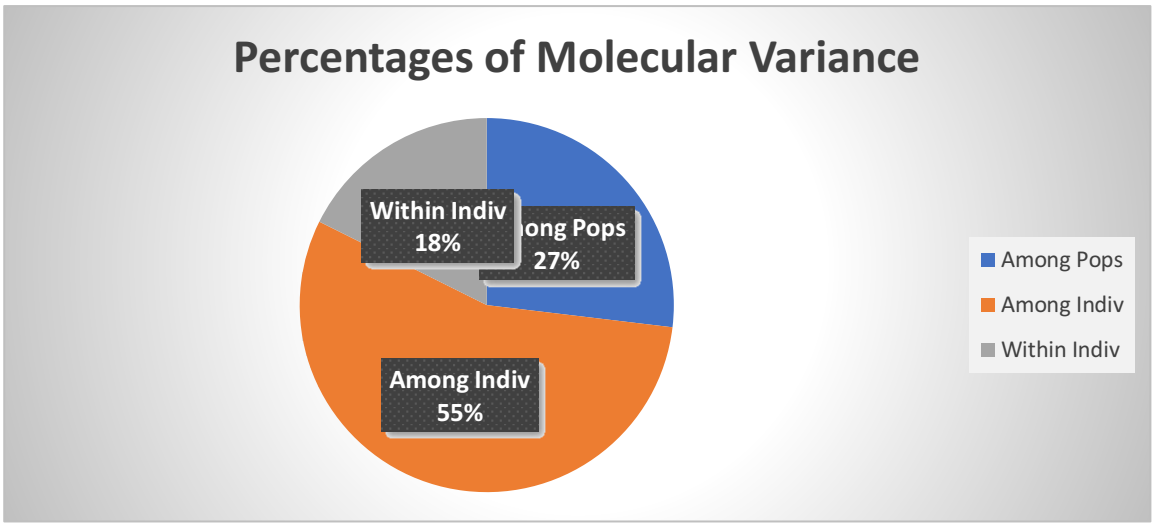


Figure 10. Percentage of molecular variance (AMOVA)

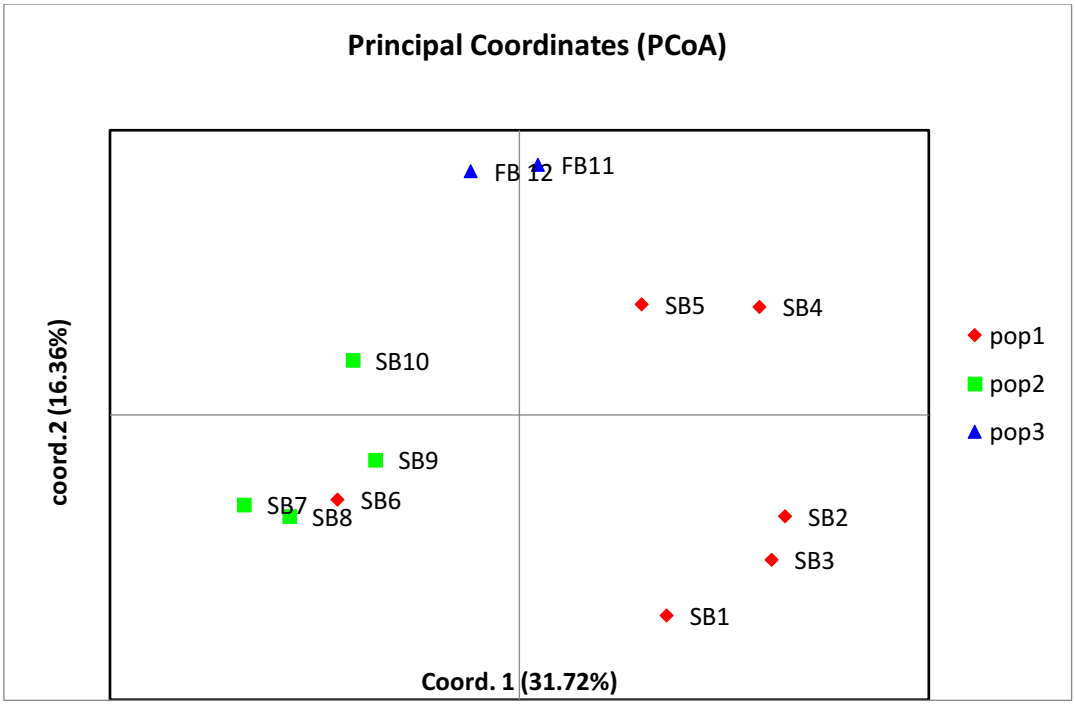
The pie chart of AMOVA (Figure I) is clearly showing the results of the research the genetic difference between populations is 27% off 3 populations which are (population 1) sugar beet (multigerm), (population 2) sugar beet(monogerm) and (population 3) is fodder beet. Within individuals, 18% is low variation but among individuals, the population is high which is 55% variation. Which is clearly shown in (table 9)

Source	df	SS	MS	Est. Var.	%
Among Pops	2	44.625	22.313	1.842	27%
Among induvial	9	79.250	8.806	3.799	55%
Within Induvial	12	14.500	1.208	1.208	18%
Total	23	138.375		6.849	100%

Table 9 :Amova indicates the percentage of variation.

5.5 PRINCIPLE COORDINATE ANALYSIS (PCOA)

The principal coordinate analysis method is used to visualize and explore similarities or dissimilarities of the genetic material based on quantitative, semi-quantitative, or qualitative variables. Sixty-six Bvh-ILP markers were screened for three populations from 12 genotypes. The molecular data is shown (Figure 11).



(Figure 11) PCoA of 12 sugar beet genotypes based on 66 Bvh-ILP markers.

The diversity of 12 Principal coordinate analyses was revealed by PCoA (Figure 10). PCoA indicates that using the first 3 axes 60.57% of the total variation is explained with the 1 st,2^{nd,} and 3rd axis explaining the 1st axis with (31.72%),2nd axis with (16.36%) and 3rd axis with (12.48%). The results in (figure 10) clearly show the separation of sugar beet and fodder beet accessions along coordinate 2 and monogerm vs. multigerm sugar beets along coordinate 1 (with an exception of multigerm SB6 grouping with monogerm accessions). A more detailed inspection suggests that sugar beet population 1 is highly diverse. We can see SB1 present in one quadrant and SB5 present in another quadrant. In population 2 the diversity is less we can see SB, 8, and 9 are present in the same quadrant, while SB10 is slightly more diverse. It indicates the distance and diversity are very less compared to population 1.

6. DISCUSSION

Sugar beet (*Beta Vulgaris)* is a biennial, cross-pollinated plant belonging to the Amaranthaceae family. Sugar beet cultivars are developed from progenitors of fodder beet and leaf beet. DNA content (C value) of *Beta vulgaris* is reported as 714-758 million base pairs per haploid genome. The nine chromosomes of sugar beet are morphologically similar: mitotic metaphase centromeres are either metacentric or sub-metacentric (Arumuganathan & Earle, 1991). Highly repetitive DNA sequences are>60% of the beet genome(Zakrzewski et al., 2017). TE are discrete segments of DNA capable of changing their genomic location through

transposition. Transposable elements have been recognized as major drivers of the evolution of eukaryotic organisms. Introns are most abundant in eukaryotic organisms and are found in several gene components.

The popularity of intron length polymorphism (ILP) is growing because it not only offers similar benefits to SSR but also shows certain unique qualities, including direct representation of variation within specific genes and subspecies(Wang et al., 2005). Like SSRs, when primers were designed in flanking exons to amplify introns by PCR, cross-species amplification became possible.

In this study, a total of 66 Bvh-ILP markers (*Beta vulgaris(hAT)* intron length polymorphism) were used to find polymorphisms that are useful to confirm homozygosity to produce double haploids in sugar beet. The use of markers in MAS is becoming increasingly interesting for mapping populations, association mapping, and sequence information derived from genomic research. Genetic distances based on molecular marker information are used to evaluate and compare genetic resources available to breeders. In 66 Bvh-ILP markers, we got 20 polymorphic markers and 40 monomorphic markers. The 20 polymorphic markers can be useful in the production of double haploids. Sugar beet is highly heterozygous so we need to identify homozygous genotypes by using Bvh-ILP markers. Then those homozygous genotypes can be used as parental lines in a hybrid development program. Due to its polymorphic of Bvh-ILP markers are useful to distinguish between homozygous and heterozygous lines.

6.1. DEVELOPMENT OF Bvh-ILP MARKER

The effective utilization and conservation of plant genetic resources require a thorough characterization of genetic structure. Because of its ability to distinguish genotypes at the DNA level, molecular marker analysis is considered the most reliable and efficient method for this characterization. Several molecular marker-based methods (RAPD, AFLP, SSR, ISSR, etc.) are currently in use and allow genotypes to be clustered according to their genetic relationships (Eröz Poyraz et al., 2012; Hu et al., 2005; Ojagh & Akhundova, 2010).

In the present investigation, it is a novel PCR technique with an ILP (Intron length polymorphism) marker was used to construct genetic profiles for comparison of the genetic diversity of 12 sugar beet genotypes by using 66 Bvh-ILP of diverse origin.

In our experiment, we screened 66 Bvh-ILP markers the level of polymorphism markers is 20 which accounts for (30.3%) and monomorphism is 40 which accounts for (60.6%). The level of polymorphism high in the ISSR marker (97.2%) was slightly higher than the RAPD marker (93%)(Izzatullayeva et al., 2014). Similar results were also reported by Mathur et al. (2013) for Arbus precatorius L. and by Srivastava et al. (2007) for Beta vulgaris L. In contrast, RAPD and ISSR are dominant markers that were found to be more polymorphic than Bvh-ILP markers and are co-dominant in rice bean landraces (Muthusamy et al., 2008).

6.2. AMPLIFICATION OF MARKERS

In all 66 Bvh-ILP markers, the amplification tested with Bvh-ILP markers resulted in PCR products that varied in size and number. In most cases, the quantity and number of amplification products were the same in regular and touch-down versions of PCR. Amplification with 66 primers is good.

The amplification of Bvh-ILP elements facilitated the selection of evenly distributed insertion sites for analysis, as well as equal coverage of the genome with the developed markers.90.9% of the candidate markers, are successfully amplified, and 78.5 % of them identified as Bvh-ILP

insertion polymorphisms. The successful amplification is higher in comparison with other ILP markers in other plants, such as Daucus carota L (62.7%Stelmach et al.), Vigna unguiculata (89%;(S. K. Gupta et al., 2012), Glycine max (88.2%:(Shu et al., 2010), Solanum Lycopersicon (71%; Wang et al., 2005). Probably, because of a high percentage of ambiguous amplification of introns longer than 1200-1400 bp, the length of the intron is considered the main cause of PCR failure, and generally, the successful amplification rate decreases with greater length of intron (S. K. Gupta et al…2012).

6.3. GENETIC DISTANCE

Genetic variability in germplasm lines is a prerequisite for any crop improvement and Sugar beet is not an exception for it. As variability increases there will be an increase in genetic distance between germplasm accessions. Genetic distance and Genetic diversity are directly proportional to each other and which in turn is necessary to get higher diversity. One way of estimating diversity is to calculate the genetic distance of the parents either based on morphological observations or molecular markers diversity (Pavani et al., 2018) using morphological and molecular marker data to estimate genetic distance among the parents of

the hybrids. Recently molecular markers have been used to measure genetic distance among beta species (Shen et al., 1996).

In the present study, genetic distance was calculated based on morphological markers data. Observations showed that genetic distance is high between sugar beet 2 (multigerm) and sugar beet 7 (monogerm) and lower genetic distance in (fodder beet 1) and (fodder beet 2). Isozyme polymorphism indicated significant differences between fodder beet and sugar beet, but not between monogerm sugar beet and multigerm sugar beet (Nagamine et al., 1989a). Restriction fragment length polymorphism (RFLP) analyses showed large amounts of genetic variability between cultivars within *Beta vulgaris* (Mita et al., 1991).

6.4. AMOVA (ANALYSIS OF MOLECULAR VARIANCE)

The AMOVA indicates that most (55%) of molecular variations are indicated among individuals and the population is (27%) and only (18%) among individuals. as compared with SSR markers agreement is the same among the population (Taški-Ajduković et al., 2017) these results are similar to those (Abbasi et al., 2014). The gene diversity within individual plants increases in the breeding system where separate gene pools are used for the development of paternal and maternal parents (Viard et al. 2002)

AMOVA results demonstrated that molecular variation was mainly found among individuals, as expected for an outcrossing species (Galewski & McGrath, 2020).

6.5. PCOA (PRINCIPAL CO-ORDINATE ANALYSIS)

In PCoA based on estimates between all pairs of sugar beet, and fodder beet inbreds, the first two principal co-ordinates explained 1st co-ordinates (31.72%) and co-ordinate 2 (16.36%) of molecular variance. This agreement with SSR markers in sugar beet the principal co-ordinates of two axis is 17.6% and 7.6% molecular variance (Li et al., 2010)This observation is with clustering of populations the 3 populations are 1) sugar beet (multigerm) 2) sugar beet (monogerm) 3)fodder beet is present in different clusters, as a result, this strong separation of genotypes is not expected because all belongs to sugar beet but due to this monogerm lines are used as the female parent and multigerm lines are used as the male parent in F1 hybrids, this lead to strong separation of the heterotic pool. This type of selection leads to a differentiated population (Duvick et al.,2010).

In this study in all 66 Bvh-ILP markers, we performed the 20 markers which are hAT-inserted polymorphic markers of a minimum of 2 to 4 polymorphisms observed in these markers of

sugar beet. which are used to confirm homozygosity for the production of double haploids. Double haploids save time and are cost-efficient compared to conventional breeding because double haploids have few crossing-overs and have great chances of inheriting favorable traits. Integrating marker-assisted selection with double haploids allows for rapidly producing pure lines.

In this experiment, we used ILP(intron length polymorphism) markers which are a novel and unique technique for marker-assisted selection and determining genetic diversity given the tremendous advantages they offer in terms of subspecies specificity, neutrality (no phenotypic effect), and the ability to perform assay variation within genes.

7. CONCLUSION

The present study reports the development of novel gene-based 66 ILP markers that are evenly distributed in nine sugar beet chromosomes. In this study, we found several markers present in each chromosome, and an equal number of chromosomes are present in chromosomes 6 and 7 with 14 markers each which contributes 42.42%. And the lowest number of markers are present in chromosome 1 with 2. After PCR validation amplifications of 66 Bvh-ILP markers, we get 40 monomorphisms (60.6%) and 20 polymorphisms (30.3%), and 6 are not amplified well. In those 60 amplified markers, the specific intron length of 600-1000 bp range of 48(72%) of markers shows good amplification.

By using Bvh-ILP markers we performed a genome-wide search of *Beta vulgaris* MITEs insertions based on intron length polymorphisms, validation of ILP markers to develop a panel for genotyping by using PCR, and find polymorphisms that are useful to confirm homozygosity (e.g.: during the production of double haploids). The study provides an important functional marker resource trait for future trait discovery and improvement for the genomic-assisted breeding of sugar beet.

8. BIBLIOGRAPHY

Abbasi, Z., Arzani, A., & Majidi, M. M. (2014). Evaluation of genetic diversity of sugar beet (Beta vulgaris L.) crossing parents using agro-morphological traits and molecular markers. *Journal of Agricultural Science and Technology*, *16*(6).

Arumuganathan, K., & Earle, E. D. (1991). Nuclear DNA content of some important plant species. *Plant Molecular Biology Reporter*, *9*(3). https://doi.org/10.1007/BF02672069

Benjak, A., Boué, S., Forneck, A., & Casacuberta, J. M. (2009). Recent amplification and impact of MITEs on the genome of grapevine (Vitis vinifera L.). *Genome Biology and Evolution*, *1*. https://doi.org/10.1093/gbe/evp009

Bennetzen, J. L., & Wang, H. (2014). The contributions of transposable elements to the structure, function, and evolution of plant genomes. In *Annual Review of Plant Biology* (Vol. 65). https://doi.org/10.1146/annurev-arplant-050213-035811

Brar, N. S., Dhillon, B. S., Saini, K. S., & Sharma, P. K. (2015). Agronomy of sugar beet cultivation-A review. *Agricultural Reviews*, *36*(3). https://doi.org/10.5958/0976-0741.2015.00022.7

Chen, J., Hu, Q., Zhang, Y., Lu, C., & Kuang, H. (2014). P-MITE: A database for plant miniature inverted-repeat transposable elements. *Nucleic Acids Research*, *42*(D1). https://doi.org/10.1093/nar/gkt1000

Chen, X., Zhang, G., & Wu, W. (2011). Investigation and utilization of intron length polymorphisms in conifers. *New Forests*, *41*(3). https://doi.org/10.1007/s11056-010-9229-5

Clarke, M. A., & Godshall, M. A. (1987). Chemistry and Processing of Sugar Beet and Sugarcane. In *Sugar Sciences* (Vol. 9).

Coen, E. S., Carpenter, R., & Martin, C. (1986). Transposable elements generate novel spatial patterns of gene expression in antirrhinum majus. *Cell*, *47*(2). https://doi.org/10.1016/0092-8674(86)90451-4

de La Fuente, G. N., Frei, U. K., & Lübberstedt, T. (2013). Accelerating plant breeding. In *Trends in Plant Science* (Vol. 18, Issue 12). https://doi.org/10.1016/j.tplants.2013.09.001

Dechyeva, D., Gindullis, F., & Schmidt, T. (2003). Divergence of satellite DNA and interspersion of dispersed repeats in the genome of the wild beet Beta procumbens. *Chromosome Research*, *11*(1). https://doi.org/10.1023/A:1022005514470

Draycott, A. P., & Christenson, D. R. (2003). Nutrients for sugar beet production. *Soil-Plant Relationships*.

Dunwell, J. M. (2010). Haploids in flowering plants: Origins and exploitation. *Plant Biotechnology Journal*, *8*(4). https://doi.org/10.1111/j.1467-7652.2009.00498.x

Duvick, D. N., Smith, J. S. C., & Cooper, M. (2010). Long-Term Selection in a Commercial Hybrid Maize Breeding Program. In *Plant Breeding Reviews*. https://doi.org/10.1002/9780470650288.ch4

El-Maghraby, S. S., Gomaa, M. A., Rehab, I. F., & Hassan, H. M. S. (2008). Response of sugar beet to some mechanical management practices, irrigation, and plant densities. *Sugar Tech*, *10*(3). https://doi.org/10.1007/s12355-008-0040-y

Eröz Poyraz, I., Sözen, E., Ataşlar, E., & Poyraz, I. (2012). Determination of genetic relationships among Velezia L. (Caryophyllaceae) species using RAPD markers. *Turkish Journal of Biology*, *36*(3). https://doi.org/10.3906/biy-1012-177

Fehr, W. (1987). Principles of Cultivar Development: Crop Species. *Agronomy Books*.

Feschotte, C., Jiang, N., & Wessler, S. R. (2002). Plant transposable elements: Where genetics meets genomics. In *Nature Reviews Genetics* (Vol. 3, Issue 5). https://doi.org/10.1038/nrg793

Fischer, H. E. (1989). Origin of the "Weisse Schlesische Rübe" (white Silesian beet) and resynthesis of sugar beet. *Euphytica*, *41*(1–2). https://doi.org/10.1007/BF00022414

G Elias, S. (2018). The Importance of Using High-Quality Seeds in Agriculture Systems. *Agricultural Research & Technology: Open Access Journal*, *15*(4). https://doi.org/10.19080/artoaj.2018.14.555961

Galewski, P., & McGrath, J. M. (2020). Genetic diversity among cultivated beets (Beta vulgaris) was assessed via population-based whole genome sequences. *BMC Genomics*, *21*(1). https://doi.org/10.1186/s12864-020-6451-1

Grzebelus, D. (2018). The functional impact of transposable elements on the diversity of plant genomes. In *Diversity* (Vol. 10, Issue 2). https://doi.org/10.3390/d10020018

Guo, C., Spinelli, M., Ye, C., Li, Q. Q., & Liang, C. (2017). Genome-Wide Comparative Analysis of Miniature Inverted Repeat Transposable Elements in 19 Arabidopsis thaliana Ecotype Accessions. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-02855-1

Gupta, S. K., Bansal, R., & Gopalakrishnan, T. (2012). Development of intron length polymorphism markers in cowpea [Vigna unguiculata (L.) Walp.] and their transferability to other Vigna species. *Molecular Breeding*, *30*(3). https://doi.org/10.1007/s11032-012-9722-y

Gupta, S., Kumari, K., Das, J., Lata, C., Puranik, S., & Prasad, M. (2011). Development and utilization of novel intron length polymorphic markers in foxtail millet (Setaria italica (L.) P. Beauv.). *Genome*, *54*(7). https://doi.org/10.1139/g11-020

Holligan, D., Zhang, X., Jiang, N., Pritham, E. J., & Wessler, S. R. (2006). The transposable element landscape of the model legume Lotus japonicus. *Genetics*, *174*(4). https://doi.org/10.1534/genetics.106.062752

Hozayn, M., Abd-Elmonem, A. A., & Samaha, G. M. (2020). The physiological effect of pre-soaking with tryptophan on sugar beet (Beta vulgaris L.) productivity under different levels of salinity stresses. *Bulletin of the National Research Centre*, *44*(1). https://doi.org/10.1186/s42269-020-00324-w

Hu, D., Zhang, Z., Zhang, D., Zhang, Q., & Li, J. (2005). The genetic relationship of ornamental peaches was determined using AFLP markers. *HortScience*, *40*(6). https://doi.org/10.21273/hortsci.40.6.1782

Huang, M., Xie, F. M., Chen, L. Y., Zhao, X. Q., Jojee, L., & Madonna, D. (2010). Comparative Analysis of Genetic Diversity and Structure in Rice Using ILP and SSR Markers. *Rice Science*, *17*(4). https://doi.org/10.1016/S1672-6308(09)60025-1

Izzatullayeva, V., Akparov, Z., Babayeva, S., Ojaghi, J., & Abbasov, M. (2014). The efficiency of using RAPD and ISSR markers in the evaluation of genetic diversity in sugar beet. *Turkish Journal of Biology*, *38*(4). https://doi.org/10.3906/biy-1312-35

Jaggard, K. W., Clark, C. J. A., & Draycott, A. P. (1999). The weight and processing quality of components of the storage roots of sugar beet (Beta vulgaris L). *Journal of the Science of Food and Agriculture*, *79*(11). https://doi.org/10.1002/(SICI)1097-0010(199908)79:11<1389: AID-JSFA377>3.0.CO;2-B

Kempken, F., & Windhofer, F. (2001). The hAT family: A versatile transposon group common to plants, fungi, animals, and man. In *Chromosoma* (Vol. 110, Issue 1). https://doi.org/10.1007/s004120000118

Kunze, R., & Weil, C. F. (2007). The hAT and CACTA Superfamilies of Plant Transposons. In *Mobile DNA II*. https://doi.org/10.1128/9781555817954.ch24

Lange, W., Brandenburg, W. A., & de Bock, T. S. M. (1999). Taxonomy and autonomy of beet (Beta vulgaris L.). *Botanical Journal of the Linnean Society*, *130*(1). https://doi.org/10.1006/bojl.1998.0250

Lessa, E. P. (1992). Rapid surveying of DNA sequence variation in natural populations. *Molecular Biology and Evolution*, *9*(2). https://doi.org/10.1093/oxfordjournals.molbev.a040723

Li, J., Schulz, B., & Stich, B. (2010). Population structure and genetic diversity in elite sugar beet germplasm investigated with SSR markers. *Euphytica*, *175*(1). https://doi.org/10.1007/s10681-010-0161-8

Linskens, H. F., Martens, M. J. M., Hendriksen, H. J. G. M., Roestenberg-Sinnige, A. M., Brouwers, W. A. J. M., van der Staak, A. L. H. C., & Strik-Jansen, A. M. J. (1976). The acoustic climate of plant communities. *Oecologia*, *23*(3). https://doi.org/10.1007/BF00361233

McClintock, B. (1950). The origin and behavior of mutable loci in maize. *Proceedings of the National Academy of Sciences of the United States of America*, *36*(6). https://doi.org/10.1073/pnas.36.6.344

McGinnis, W., Shermoen, A. W., & Beckendorf, S. K. (1983). A transposable element inserted just 5′ into a Drosophila glue protein gene alters gene expression and chromatin structure. *Cell*, *34*(1). https://doi.org/10.1016/0092-8674(83)90137-X

McGrath, J. M., Derrico, C. A., & Yu, Y. (1999). Genetic diversity in selected, historical US sugarbeet germplasm and Beta vulgaris ssp. maritima. *Theoretical and Applied Genetics*, *98*(6–7). https://doi.org/10.1007/s001220051157

Menzel, G., Krebs, C., Diez, M., Holtgräwe, D., Weisshaar, B., Minoche, A. E., Dohm, J. C., Himmelbauer, H., & Schmidt, T. (2012). Survey of sugar beet (Beta vulgaris L.) hAT transposons and MITE-like hATpin derivatives. *Plant Molecular Biology*, *78*(4–5). https://doi.org/10.1007/s11103-011-9872-z

Mita, G., Dani, M., Casciari, P., Pasquali, A., Selva, E., Minganti, C., & Piccardi, P. (1991). Assessment of the degree of genetic variation in beet based on RFLP analysis and the taxonomy of Beta. *Euphytica*, *55*(1). https://doi.org/10.1007/BF00022552

Monden, Y., Naito, K., Okumoto, Y., Saito, H., Oki, N., Tsukiyama, T., Ideta, O., Nakazaki, T., Wessler, S. R., & Tanisaka, T. (2009). High potential of a transposon mPing as a marker system in japonica × japonica cross in rice. *DNA Research*, *16*(2). https://doi.org/10.1093/dnares/dsp004

Murovec, J., & Bohanec, B. (2012). Haploids and Doubled Haploids in Plant Breeding. In *Plant Breeding*. https://doi.org/10.5772/29982

Muthamilarasan, M., Venkata Suresh, B., Pandey, G., Kumari, K., Parida, S. K., & Prasad, M. (2014). Development of 5123 intron-length polymorphic markers for large-scale genotyping applications in foxtail millet. *DNA Research*, *21*(1). https://doi.org/10.1093/dnares/dst039

Muthusamy, S., Kanagarajan, S., & Ponnusamy, S. (2008). The efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (Vigna umbellata) landraces. *Electronic Journal of Biotechnology*, *11*(3). https://doi.org/10.2225/vol11-issue3-fulltext-8

Naito, K., Zhang, F., Tsukiyama, T., Saito, H., Hancock, C. N., Richardson, A. O., Okumoto, Y., Tanisaka, T., & Wessler, S. R. (2009). Unexpected consequences of a sudden and massive transposon amplification on rice gene expression. *Nature*, *461*(7267). https://doi.org/10.1038/nature08479

Niu, Z., Jiang, A., Abu Hammad, W., Oladzadabbasabadi, A., Xu, S. S., Mergoum, M., & Elias, E. M. (2014). Review of doubled haploid production in durum and common wheat through wheat × maize hybridization. *Plant Breeding*, *133*(3). https://doi.org/10.1111/pbr.12162

Ojagh, J., & Akhundova, E. (2010). Genetic diversity in doubled haploid wheat based on morphological traits, gliadin protein patterns, and RAPD markers. *African Journal of Agricultural Research*, *5*(13).

Oki, N., Yano, K., Okumoto, Y., Tsukiyama, T., Teraishi, M., & Tanisaka, T. (2008). A genome-wide view of miniature inverted-repeat transposable elements (MITEs) in rice, Oryza sativa ssp. japonica. *Genes and Genetic Systems*, *83*(4). https://doi.org/10.1266/ggs.83.321

Pavani, M., Sundaram, R. M., Ramesha, M. S., Kavi Kishor, P. B., & Kemparaju, K. B. (2018). Prediction of heterosis in rice based on the divergence of morphological and molecular markers. *Journal of Genetics*, *97*(5). https://doi.org/10.1007/s12041-018-1023-8

Petkeviciene, B. (2009). The effects of climate factors on sugar beet early sowing timing. *Agron. Res*, *7*(I).

Piégu, B., Bire, S., Arensburger, P., & Bigot, Y. (2015). A survey of transposable element classification systems - A call for a fundamental update to meet the challenge of their diversity and complexity. In *Molecular Phylogenetics and Evolution* (Vol. 86). https://doi.org/10.1016/j.ympev.2015.03.009

Pritham, E. J. (2009). Transposable elements and factors influencing their success in eukaryotes. In *Journal of Heredity* (Vol. 100, Issue 5). https://doi.org/10.1093/jhered/esp065

Sampath, P., Murukarthick, J., Izzah, N. K., Lee, J., Choi, H. il, Shirasawa, K., Choi, B. S., Liu, S., Nou, I. S., & Yang, T. J. (2014). Genome-wide comparative analysis of 20 miniature inverted-repeat transposable element families in Brassica rapa and B. oleracea. *PLoS ONE*, *9*(4). https://doi.org/10.1371/journal.pone.0094499

Shu, Y., Li, Y., Zhu, Y., Zhu, Z., Lv, D., Bai, X., Cai, H., Ji, W., & Guo, D. (2010). Genome-wide identification of intron fragment insertion mutations and their potential use as SCAR molecular markers in the soybean. *Theoretical and Applied Genetics*, *121*(1). https://doi.org/10.1007/s00122-010-1285-x

Stelmach, K., Macko-Podgórni, A., Machaj, G., & Grzebelus, D. (2017). Miniature inverted-repeat transposable element insertions provide a source of intron-length polymorphism markers in the carrot (Daucus carota L.). *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.00725

Sundaresan, V., Springer, P., Volpe, T., Haward, S., Jones, J. D. G., Dean, C., Ma, H., & Martienssen, R. (1995). Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. *Genes and Development*, *9*(14). https://doi.org/10.1101/gad.9.14.1797

Taški-Ajduković, K., Nagl, N., Ćurčić, Ž., & Zorić, M. (2017). Estimation of genetic diversity and relationship in sugar beet pollinators based on SSR markers. *Electronic Journal of Biotechnology*, *27*. https://doi.org/10.1016/j.ejbt.2017.02.001

Topak, R., Süheri, S., & Acar, B. (2011). Effect of different drip irrigation regimes on sugar beet (Beta vulgaris L.) yield, quality, and water use efficiency in Middle Anatolian, Turkey. *Irrigation Science*, *29*(1). https://doi.org/10.1007/s00271-010-0219-3

Torres, A. M., Weeden, N. F., & Martín, A. (1993). Linkage among isozyme, RFLP, and RAPD markers in Vicia faba. *Theoretical and Applied Genetics*, *85*(8). https://doi.org/10.1007/BF00215032

Viard, F., Bernard, J., & Desplanque, B. (2002). Crop-weed interactions in the Beta vulgaris complex at a local scale: Allelic diversity and gene flow within sugar beet fields. *Theoretical and Applied Genetics*, *104*(4). https://doi.org/10.1007/s001220100737

Vollbrecht, E., Duvick, J., Schares, J. P., Ahern, K. R., Deewatthanawong, P., Xu, L., Conrad, L. J., Kikuchi, K., Kubinec, T. A., Hall, B. D., Weeks, R., Unger-Wallace, E., Muszynski, M., Brendel, V. P., & Brutnell, T. P. (2010). Genome-wide distribution of

transposed Dissociation elements in maize. *Plant Cell*, *22*(6). https://doi.org/10.1105/tpc.109.073452

Wang, X., Zhao, X., Zhu, J., & Wu, W. (2005). Genome-wide investigation of intron length polymorphisms and their potential as molecular markers in rice (Oryza sativa L.). *DNA Research*, *12*(6). https://doi.org/10.1093/dnares/dsi019

Wessler, S. R. (2006). Transposable elements and the evolution of eukaryotic genomes. In *Proceedings of the National Academy of Sciences of the United States of America* (Vol. 103, Issue 47). https://doi.org/10.1073/pnas.0607612103

Wicker, T., Sabot, F., Hua-Van, A., Bennetzen, J. L., Capy, P., Chalhoub, B., Flavell, A., Leroy, P., Morgante, M., Panaud, O., Paux, E., SanMiguel, P., & Schulman, A. H. (2007). A unified classification system for eukaryotic transposable elements. In *Nature Reviews Genetics* (Vol. 8, Issue 12). https://doi.org/10.1038/nrg2165

Wydner, K. S., Sechler, J. L., Boyd, C. D., & Passmore, H. C. (1994). Use of an intron length polymorphism to localize the tropoelastin gene to mouse chromosome 5 in a region of linkage conservation with human chromosome 7. *Genomics*, *23*(1). https://doi.org/10.1006/geno.1994.1467

Xin, Y., Ma, B., Xiang, Z., & He, N. (2019). Amplification of miniature inverted-repeat transposable elements and the associated impact on gene regulation and alternative splicing in mulberry (Morus notabilis). *Mobile DNA*, *10*(1). https://doi.org/10.1186/s13100-019-0169-0

Yang, L., Jin, G., Zhao, X., Zheng, Y., Xu, Z., & Wu, W. (2007). PIP: A database of potential intron polymorphism markers. *Bioinformatics*, *23*(16). https://doi.org/10.1093/bioinformatics/btm296

Zakrzewski, F., Schmidt, M., van Lijsebettens, M., & Schmidt, T. (2017). DNA methylation of retrotransposons, DNA transposons and genes in sugar beet (Beta vulgaris L.). *Plant Journal*, *90*(6). https://doi.org/10.1111/tpj.13526

Zhang, X., Jiang, N., Feschotte, C., & Wessler, S. R. (2004a). PIF - and Pong -Like Transposable Elements: Distribution, Evolution, and Relationship with Tourist -Like Miniature Inverted-Repeat Transposable Elements. *Genetics*, *166*(2). https://doi.org/10.1093/genetics/166.2.971

Zhang, X., Jiang, N., Feschotte, C., & Wessler, S. R. (2004b). PIF- and Pong-Like Transposable Elements: Distribution, Evolution, and Relationship with Tourist-Like Miniature Inverted-Repeat Transposable Elements. *Genetics*, *166*(2). https://doi.org/10.1534/genetics.166.2.971

Zhang, X., & Wessler, S. R. (2004). Genome-wide comparative analysis of the transposable elements in the related species Arabidopsis thaliana and Brassica oleracea. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(15). https://doi.org/10.1073/pnas.0401243101

Zhang, Y., Fan, C., Li, S., Chen, Y., Wang, R. R. C., Zhang, X., Han, F., & Hu, Z. (2017). The diversity of sequence and chromosomal distribution of new transposable element-

related segments in the rye genome was revealed by FISH and lineage annotation. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.01706

Zhao, D., Ferguson, A. A., & Jiang, N. (2016). What makes up plant genomes: The vanishing line between transposable elements and genes. In *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms* (Vol. 1859, Issue 2). https://doi.org/10.1016/j.bbagrm.2015.12.005