T.R.
VAN YÜZÜNCÜ YIL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL BIOTECHNOLGY

ASSESSMENT OF GENETIC DIVERSITY OF GLOBAL SUGAR BEET GERMPLASM THROUGH SILICO DART AND SNP MARKERS COVERING WHOLE GENOME

Ph.D. THESIS

Noor ALQALUS
Supervisor: Prof. Dr. Mehtap YILDIZ
Second Supervisor: Prof. Dr. Faheem Shahzad BALOCH

VAN – 2025

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This project was supported by Van YYU Scientific Research Projects Coordination Unit with the project number of FBA-2022-10150

VAN – 2025

ACCEPTANCE AND APPROVAL PAGE

This thesis entitled “Assessment of Genetic Diversity of Global Sugar Beet Germplasm Through SilicoDArT and SNP Markers Covering Whole Genome” presented by Noor ALQALUS under supervision of Prof. Dr. Mehtap YILDIZ in the department of Agricultural Biotechnology has been accepted as a Ph.D. thesis according to Legislations of Graduate Higher Education on …...../...../..... with majority of votes members of jury.

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ETHICAL DECLARATION

I declare that all the information in this thesis has been obtained and presented within the framework of ethical behavior and academic rules, and that in this thesis, which has been prepared in accordance with the thesis writing rules, all kinds of statements and information that do not belong to me have been fully cited.

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ABSTRACT

ASSESSMENT OF GENETIC DIVERSITY OF GLOBAL SUGAR BEET GERMPLASM THROUGH SILICO DART AND SNP MARKERS COVERING WHOLE GENOME

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March 2025, 83 pages

Despite the relatively brief domestication history of *Beta vulgaris* ssp. *vulgaris*, our knowledge of its genomic diversity is still limited, hindering advancements in breeding efforts. To tackle this challenge, the genetic diversity of 94 genotypes originating from 16 countries was analyzed using 4609 SNP markers and 6950 SilicoDArT markers.

The investigated germplasm shows a high degree of genetic diversity. However, the SNP markers data set had higher diversity values than the SilicoDArT marker system. However, the SNP markers data set had higher diversity values than the SilicoDArT marker system. Obtained data from SNP markers revealed three main clusters: S-I, S-II, and S-III. Cluster S-I was the smallest group, with 35 accessions and 51% of them having USA origin. European accessions shared 14% of the cluster. Furthermore, accession from the USA was grouped in Cluster S-III-B, with over 80% from Middle East countries.

Results obtained from using SilicoDArT markers revealed that the most distinct accessions were NSL 176303 (Serbia) and PI 140355 (Montenegro). The germplasm was divided into D-I and D-II. First cluster containing 90% of accessions originated from the USA. The D-II cluster was diverse, with accessions from the UK, Türkiye, Iran, and Iraq. The SNP-based clustering supported the D-I cluster, and the SilicoDArT markers-based PCA clustering results agreed with the UPGMA and STRUCTURE results. The AMOVA results confirmed that genetic variation in the germplasm was largely due to differences among accessions or within the clusters (rather than among countries), accounting for 74.5-77.6% of the total variation.

Finally, the study identified Ames 2644 and Ames 8297 as the most genetically distinct genotypes, which are ideal for breeding sugar beet.

Keywords: Genetic diversity, SilicoDArT, SNPs, Sugar beet

ÖZET

ŞEKER PANCARINDA SİLİKO DART SNP MARKIRLARI KULLANILARAK GENETİK FARKLILIK VE POPÜLASYON YAPISININ ANALİZİ

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Şeker pancarının (*Beta vulgaris* ssp. *vulgaris)* nispeten kısa evrimsel geçmişi göz önüne alındığında, genotip/çeşitler arasındaki genetik çeşitlilik ve işlevsel genler hakkındaki bilgimiz sınırlıdır ve bu da ıslah çalışmalarındaki ilerlemeleri yavaşlatmıştır. Bu sorunu ele almak için, 4609 SNP ve 6950 SilicoDArT markırı kullanılarak 16 ülkeden toplam 94 genotipte popülasyon yapısı ve genetik çeşitliliği araştırılmıştır.

Bu tez çalışmasında şeker pancarı genotipleri arasında yüksek düzeyde genetik çeşitliliğin var olduğu tespit edilmiştir. Ancak, SNP markırlarına ait veri seti, SilicoDArT markır sistemine kıyasla daha yüksek çeşitlilik değerlerine sahip olmuştur. Elde edilen veri ile yapılan analizlerde şeker pancarı çeşit/genotipleri üç ana kümeye ayrılmıştır: S-I, S-II ve S-III. S-I kümesi, 35 şeker pancarı çeşit/genotipini kapsamaktadır ve bunların %51'i ABD kökenlidir. Avrupa genotipleri kümenin %14'ünü oluşturmaktadır. Ayrıca, ABD orijinli çeşit/genotipleri de içeren S-III-B Kümesinin %80'den fazlası Orta Doğu ülkeleri orijinli genotiplerden oluşmaktadır.

SilicoDArT markırlarının kullanımından elde edilen sonuçlar, NSL 176303 (Sırbistan) ve PI 140355'te (Karadağ) genotiplerinin en uzak genotipler olduğunu ortaya koymuştur. Şeker pancarı çeşit/genotipleri, D-I ve D-II olmak üzere iki gruba ayrılmıştır, D-I kümesi ABD'den gelen çeşit/genotiplerin %90'ını içermekte. D-II kümesi, İngiltere, Türkiye, İran ve Irak'tan gelen çeşit/genotipleri kapsayarak çeşitlilik göstermektedir. SNP tabanlı kümeleme, D-I kümesini desteklemiştir ve SilicoDArT markırlarına dayalı PCA kümeleme sonuçları, UPGMA ve STRUCTURE sonuçlarıyla örtüşmektedir. AMOVA sonuçları, şeker pancarı germplazmındaki genetik varyasyonların büyük ölçüde genotipler arasındaki veya kümeler içindeki farklılıklardan kaynaklandığını ve toplam varyasyonun %74.5-77.6'sını oluşturduğunu doğrulamıştır.

Sonuç olarak, Ames 2644 ve Ames 8297 numaralı genotiplerin genom düzeyindeki analizler sonucunda elde edilen yüksek farklılığı, bu genotiplerin şeker pancarı ıslahında kullanımı için ideal olacağını düşündürmektedir.

Anahtar kelimeler: DArT, Genetik çeşitlilik, SNPs, Şeker pancarı

ACKNOWLEDGMENT

First and foremost, I extend my deepest gratitude to my supervisor, Prof. Dr. Mehtap YILDIZ for her unwavering support, insightful critiques, precious advice, and patience throughout my PhD journey. Her dedication to academic excellence and keen attention to detail have played a crucial role in shaping this dissertation and have continuously inspired me both in my research and daily life.

I would like to thank my second supervisor Prof. Dr. Faheem Shahzad BALOCH, and my appreciation to Prof. Dr. Pablo CAVAGNARO, for taking in response the statistical analysis of the data.

Many thanks to Gokhan SADIK and Mehdi AL KHFAJI for their help in the lab and during my study.

My heartfelt thanks and best gratitude are also extended to the member of the examination committee for evaluating the content of the thesis and offering their valuable criticisms.

I gratefully acknowledge the support of the Van Yuzuncu Yil University Institute of Natural And Applied Sciences for allowing me to complete my work.

I would like to extend my sincere gratitude to the Scientific Research Projects Unit (BAP) of Van Yuzuncu Yil University which has provided financial support for this research under the project FBA-2022-10150. Their generous contributions have made it possible to conduct this study and have significantly contributed to its successful completion. The financial support has allowed for the procurement of necessary research materials, access to specialized equipment, and the opportunity to engage in valuable research experiences.

Last but not least, I would like to thank my husband and family, whose guidance, support, and love have led me to become who I am today, and without which none of my achievements, including this thesis, would have been possible.

2025

Noor ALQALUS

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SYMBOLS AND ABBREVIATIONS

Some symbols and abbreviations used in this thesis are presented below, along with their descriptions.

Symbols	Description
bp	Base pairs
cm	Centimeter
g	Gram
m	Meter
mg	Milligram
mol	Mole
ng	Nanogram
nm	Nanometer
pg	Picogram
°C	Degree Celsius
µl	Microliter

Abbreviations	Description
AMOVA	Analysis of Molecular Variance
CMS	Cytoplasmic Male Sterility
CTAB	Cetyltrimethylammonium bromide
DArT	Diversity Arrays Technology
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic Acid
FASTSTRUCTURE	Population structure analysis tool
GBS	Genotyping By Sequencing
GATK	Genome Analysis Toolkit
GWAS	Genome-Wide Association Study
ISO	The International Organization for Standardization

ISSR	Inter-Simple Sequence Repeat
MAS	Marker-Assisted Selection
MAF	Minor Allele Frequency
NTSYS	Numerical Taxonomy and Multivariate Analysis System
NGS	Next-Generation Sequencing
LCR	Ligation Chain Reaction
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PIC	Polymorphism Information Content
RAD	Restriction-site Associated DNA
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
QTL	Quantitative Trait Locus
QTLs	Quantitative Trait Loci
SLAF	Specific-Locus Amplified Fragment
SMS	Single Molecule Sequencing
SMRT	Single Molecule Real-Time Sequencing
SNPs	Single Nucleotide Polymorphisms
SSCP	Single-Strand Conformation Polymorphism
SSR	Simple Sequence Repeat
TAE	Tris-Acetate-EDTA buffer
TGS	Third-Generation Sequencing
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
USDA	United States Department of Agriculture

1. INTRODUCTION

1.1 Sugar Beet

Throughout history, sugar has played a crucial role as a vital nutrient, acting both as a tonic and a key source of dietary energy. As a significant agricultural commodity, the crop is primarily utilized in the sugar industry and stands as the second-largest source of sugar production, following sugarcane (Eggleston, 2019; Akyuz and Ersus, 2021). Currently, sugar beetroot is expected to represent 20-30% of sugar production worldwide. Sugar is the primary commodity obtained from sugar beet; nevertheless, its processing yields other by-products such as molasses, pomace, and ethyl alcohol (Wang et al., 2023). Furthermore, sugar beet leaves are nutritionally valuable, being rich in protein and containing a balanced profile of amino acids. Considering the essential function of sugar beet in human nutrition, it is crucial to emphasize both the amount and quality of its production (Hemayati et al., 2024).

Sugar beet has its origins traced back to 8500 B.C. along the coastal regions of Europe. Initially, prehistoric humans utilized its leaves as an edible resource. Although the method of extracting sugar from its roots was discovered in 1705, it remained underutilized until later. Scientist Andreas Marggraf was the first to identify that crushed sugar beet roots contained crystals identical to those found in sugarcane stalks (Pathak et al., 2022).

In 1811, sugar beet became an important crop as a source of sugar when the British Empire blocked the supply of sugar cane from the West Indies by the French. This made Napoleon think about the cultivation and growing of sugar beet in France. In 1840, the first factory of producing sugar from sugar beet was established in the United States, this was after the revival of factories and the introducing sugar beet to North America in 1840. Furthermore, interest in the crop increased by other countries, Russia and Ukraine started to grow and cultivate the crop in 1850, and by 1950 India introduced sugar beet as a new cash crop (Pathak et al., 2022).

The Sugar beet (*Beta vulgaris* L.) crop is a member of the Amaranthaceae family and is the world's second most important sugar crop after *Saccharum officinarum* L. (Sugarcane). Its family comprises approximately 1,400 species across

105 genera (Henry, 2010). This herbaceous dicot family includes economically vital species such as *Beta vulgaris* (sugar beet, fodder beet, red beet, Swiss chard) and *Spinacia oleracea* (spinach).

Sugar beet was first cultivated as a garden vegetable over 2,000 years ago, likely derived from various *Beta* species native to the Mediterranean region. It became a staple in European cuisine from the Middle Ages onward. The modern sugar beet was bred from a white-rooted Silesian beet with high sugar content in the early 18th century. By the late 18th century, sugar beet had been selectively bred from high-sugar-content fodder beets, with the first commercial sugar processing occurring in 1802 (Draycott, 2006; Biancardi et al., 2010).

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The plant features a short stem consisting of petioles, which attach to the leaves, and leaf blades. The crown, often considered a shortened stem section, is viewed as part of the root. Its leaves are smooth and large and grow in a rosette formation from the crown, with their number influenced by genetic and environmental conditions. The shoot also produces a long, loose, spike-like inflorescence. The plant produces dark green leaves, ovate in shape, and taper into broad petioles. During the initial growing season, new leaves appear continuously in a spiral pattern around the plant's crown. The uppermost leaves are smaller and have blades shaped from rhombic to narrowly lanceolate (Biancardi et al. 2010; Marlander et al., 2011) (Figure 1.1). The sugar beet's root is a white, cone-shaped taproot that is thick near the top and gradually narrows downward. The root structure is composed of the crown, neck, and true root. The crown, a compressed portion of the stem, contains leaf buds that give rise to the leaves. The neck, located just below the crown, is the broadest root section and contains a thickened hypocotyl. The true root, which develops from multiple cambial rings, extends downward. The main root is diarch, featuring two vertical grooves where lateral

roots emerge. Sucrose primarily accumulates in the vascular rings of the phloem. On average, the root contains 75% water, and sugar is the greatest solid ingredient in the root (20%), followed by pulp (5%) and non-sugars (2.6%). Concentric rings are observed at the transition between the hypocotyl and crown (Misra and Shrivastava, 2022).

Flowering sugar beet requires vernalization followed by extended daylight exposure, aligning with overwintering and increasing day length in spring. Vernalization is essential, as without it, plants continue its vegetative growth indefinitely without flowering. The optimal temperature for this process is 5–10°C, with a 40-day cold exposure needed for reproductive development (Sparkes, 2003). Sugar beet flowers are small and grow in dense, spike-like formations. The plant produces male, female, and hermaphroditic flowers, with the latter being circular, green, and consisting of five tepals fused at the base. Flower size ranges from 3–5 × 2–3 mm, five stamens in each flower with a semi inferior shpe ovary having two to three stigmas. While primarily wind-pollinated, sugar beet flowers can also be pollinated by insects. The fruit is a nut, and the flower clusters harden and fuse to form the fruit. The fruit is encased by the perianth, which is leathery and curved inward (Shultz, 2003). Typically, each fruit holds up to five seeds. Sugar beet seeds are small (1–2 mm in diameter), circular, dark brown, and lightweight, with 1,000 seeds weighing between 1.5 and 6 grams. The seed structure consists of a seed ball containing two or more seeds, with monogerm seeds housing a single embryo and multigerm seeds containing multiple embryos (Misra and Shrivastava, 2022).

This crop is a monoecious, hermaphroditic plant which undergoes cross-pollination, exhibits self-incompatibility which prevents self pollination. The majority of sugar beet cultivars, up to 78.1%, consist of plants that are self incompatible which fail to produce seeds in isolation after one or two inbreeding cycles. However, self-compatible (self-fertile) plants make up 4.3–12.9% of varietal populations, with pollen fertility ranging from 83.6–98.2%. While self-fertile strains tend to yield higher sugar content and crop production, some evidence suggests that inbreeding depression is not entirely mitigated (Zhuzhzhalova et al., 2023).

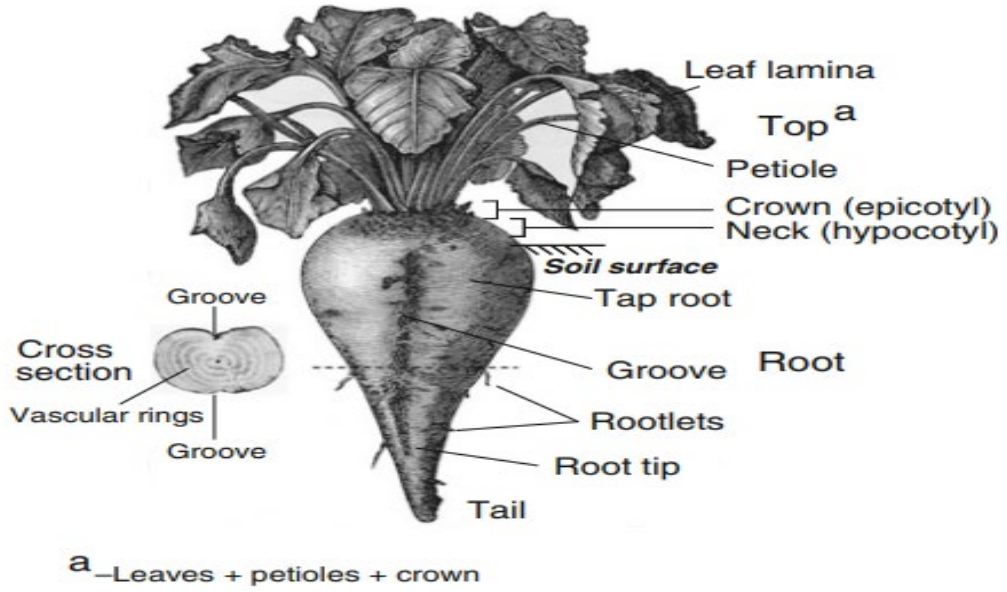

Figure 1.1 The morphological characters of sugar beet plant, (Biancardi et al., 2010)

The byproducts from sugar beet industry have a significant amounts of sugar beet pulp produce. This has traditionally been utilized as livestock feed. Recent progress in biotechnological valorization has highlighted sugar beet as a promising feedstock for the production of various high-value bioproducts, including biohydrogen, biodegradable plastics, and key platform chemicals such as lactic acid, citric acid, alcohols, microbial enzymes, single cell proteins, and pectic oligosaccharides (Usmani et al., 2022). Furthermore, sugar beet is a key source for bioethanol production, with its by-products gaining attention due to the increasing focus on fully bio based resources and the development of second generation biofuels and biorefineries (Martani et al., 2020; Isler-Kaya and Karaosmanoglu, 2022).

The genus of sugar beet, *Beta* L., have four sections. The subspecies *vulgaris*, belonging to the species vulgaris and the section *Beta*, includes all cultivated beets (see Table 1.1). Wild beets have been identified as possessing traits beneficial for cultivated varieties, particularly disease resistance (Biancardi et al., 2010). Early farmers likely selected beets with delayed bolting and flowering, as wild species typically flower 2-3 months after emergence. This selection allowed for an extended growing season and the use of leaves as a food source (McGrath et al., 2007).

Table 1.1 The *Beta* genus taxonomy (Letschert, 1993; Ford-Lloyd et al., 2005)

Chromosome number	*Beta* genus	Species	Sub species
	Section *Beta*		
2*x* = 18	*vulgaris* L.	*vulgaris*	Sugar beet
			Garden beet
			Leaf beet
			Fodder beet
3*x* = 27		*maritima*	
4*x* = 36		*adanensis*	
2*x* = 18; 4*x* = 36	*macrocarpa* Guss.		
2*x* = 18	*patula* Ait		
	Section *Corollinae*		
2*x* = 18; 4*x* = 36	*Beta lomatogona* Fisc. et May.		
2*x* = 18	*Beta macrorhiza* Stev.		
4*x* = 36	*Beta corolliflora* Zos. ex Buttler		
4*x* = 36	*Beta intermedia* Bunge		
4*x* = 36; 6*x* = 54	*Beta trigyna* Waldst. et Kit.		
	Section *Nanae*		
2*x* = 18	*Beta nana* Boiss. et Heldr.		
	Section *Procumbentes*		
2*x* = 18	*Beta procumbens* Sm.		
2*x* = 18	*Beta webbiana* Moq.		
4*x* = 36	*Beta patellaris* Moq.		

A major challenge in the sugar beet industry is the need to breed monogerm hybrids using cytoplasmic male sterility (CMS). Over 65 years ago, naturally occurring monogerm mutant plants were identified, serving as the foundation for developing monogerm beetroot varieties and hybrid components (Karakotov et al., 2021).

The gene pool of white fodder beet is thought to have provided the genetic basis for early sugar beet varieties. This limited germplasm base may have resulted in a narrower genetic diversity for sugar beet compared to other open-pollinated crops (Lewellen, 1992). In the early 1900s, efforts began to screen exotic and wild beet germplasm for disease resistance, driven by increasing pest and disease pressures. Historically, beet was cultivated as a vegetable or fodder crop, with the selection of sugar beet beginning in the late 18th century. Through long-term selective breeding, the

sugar content of beets increased from around 4% to over 18% today (Biancardi et al., 2010).

The crop's industrial diploid strain (2n = 18 chromosomes) genome has been sequenced enlightening a genome size between 714 and 758 megabases and identifying 27421 predicted protein-coding genes (Dohm et al., 2014). Breeding efforts have also led to the development of triploid and tetraploid cultivars, and the chloroplast genome has been successfully sequenced as well (Li et al., 2014). Genome sequencing is anticipated to significantly advance the discovery of genes linked to key agronomic traits, thereby enhancing molecular breeding strategies and expanding the crop's role in energy biotechnology (Zicari et al., 2019). During the 1980s and 1990s, increased interest in wild germplasm as a means of improving sugar beet underscored the importance of conserving wild *Beta* genetic resources.

1.2 Next-Generation Sequencing (NGS)

These technologies have reformed biological research, building on the foundational work from the previous decade that used for establishing genome reference sequences for humans and many model organisms. These advancements have been driven by two main factors: the significant reduction in costs and a remarkable increase in data production capacity. The introduction of NGS technologies between 2004 and 2006 transformed biomedical research, leading to a dramatic increase in sequencing data output (Mardis, 2017). This surge in data production is largely attributed to innovations in nanotechnology, which enabled massively parallel sequencing of single DNA molecules. The combination of high throughput and the ability to sequence single DNA molecules are defining characteristics of NGS, regardless of the specific sequencing platform used (Hu et al., 2021).

The sequencing techniques developed by Sanger, as well as Maxam and Gilbert, are classified as first-generation sequencing technologies (Thudi et al., 2012). Sanger's method, commonly called terminator sequencing, utilizes dideoxy nucleotides (ddNTPs) as terminators alongside deoxynucleotides (dNTPs) to generate DNA fragments of varying lengths (Kchouk et al., 2017). Another first-generation approach, Maxam–Gilbert sequencing, also known as the chemical degradation method, involves

nucleotide cleavage using chemicals, making it particularly suitable for analyzing small nucleotide polymers. Sanger's sequencing technique provided the foundation for future sequencing technologies, earning him recognition as the father of sequencing (Pillai et al., 2016). First-generation sequencing is slow, expensive, and has low throughput. It sequences short DNA fragments but struggles with GC-rich or repetitive regions, resulting in incomplete coverage. Its low sensitivity to rare mutations also limits its use in cancer genomics (Metzker, 2010; Heather and Chain, 2016; Shendure et al., 2017).

To address the limitations of first generation sequencing, second generation sequencing, also known as next generation sequencing, emerged by 2005. A defining feature of NGS is the isolation of DNA and the creation of single stranded DNA libraries through the fragmentation of sample DNA. Different commercial platforms utilize unique adaptor chemistries for the amplification of DNA fragments. These modified DNA libraries are then amplified using polymerase chain reaction methods, either on beads or glass slides. During sequencing, single-stranded amplified DNA is converted into double-stranded DNA by incorporating complementary nucleotides according to the Adenine, Thymine, Guanine, Cytosine template during individual flow cycles. Sequencing instruments detect the signals generated by complementary base pairing specific to the DNA template. NGS can involve short-read or long-read sequencing, both of which enable the large-scale parallel sequencing of millions of DNA strands simultaneously (Goodwin et al., 2016). However, NGS has some limitations: (i) short reads struggle to resolve certain genomic features, and (ii) genome assembly is challenging due to short read lengths. Additionally, the PCR amplification step increases both expense and procedure duration (Pillai et al., 2016).

The introduction of the Third Generation Sequencing technologies (TGS) was to reduce the limitation of NGS. Unlike NGS, where sequencing halts after each base incorporation, TGS features single-molecule sequencing (SMS) and real-time sequencing (Schadt et al., 2011). The first SMS technology was commercialized by Helicos Biosciences and functioned similarly to Illumina sequencing but without bridge amplification (Pushkarev et al., 2011). However, due to its slow processing speed, high cost, and short read lengths (32 bp), it was not widely adopted. The first true TGS platform, single-molecule real-time (SMRT) sequencing, was launched in 2011 by Pacific Biosciences (PacBio) (Eid et al., 2009). More recently, Oxford Nanopore

Technologies (ONT) introduced nanopore sequencing (Jain et al., 2015). A key distinction of SMRT and nanopore sequencing is the absence of PCR amplification, the real-time sequencing process, and the ability to generate long reads.

1.3 Diversity Arrays Technology (DArT)

These markers were introduced in the early 2000s, represent a relatively recent hybridization-based genotyping approach implemented on a microarray platform for the rapid and simultaneous detection of DNA polymorphisms (Kilian et al., 2012). DArT primarily identifies dominant markers, which are largely derived from single nucleotide polymorphisms (SNPs) at restriction sites across hundreds to thousands of random genomic loci (Wenzl et al., 2004). Unlike SSR- and SNP-based markers, DArT markers are dominant and scored as either present or absent, providing less genetic information per locus. Although widely applied in the genotyping of various plant species, the sequence characteristics and genome-wide distribution of DArT markers remain largely unexplored (Hong et al., 2009; Petroli et al., 2012).

The DArT process consists of five key steps: (1) constructing a genomic library (genomic representation), (2) printing the genomic library onto microarrays, (3) labeling genomic representations, (4) hybridizing the labeled genomic representation onto the microarray followed by washing, and (5) scanning and analyzing the data (Wittenberg, 2007). The current format of DArT is shown in figure 1.2 as explained by Kilian et al. (2012). One major advantage of DArT is its high-throughput capability, enabling the parallel analysis of a comprehensive set of markers covering the entire genome across multiple genomic samples. Furthermore, since the technology relies on cloned DNA fragments, the resulting sequences can be analyzed and shared with the global research community. It is also readily accessible, and assays can be performed by skilled providers at an affordable cost. However, an essential step for DArT analysis is the creation and validation of a diagnostic DArT array prior to its implementation (Tinker et al., 2009).

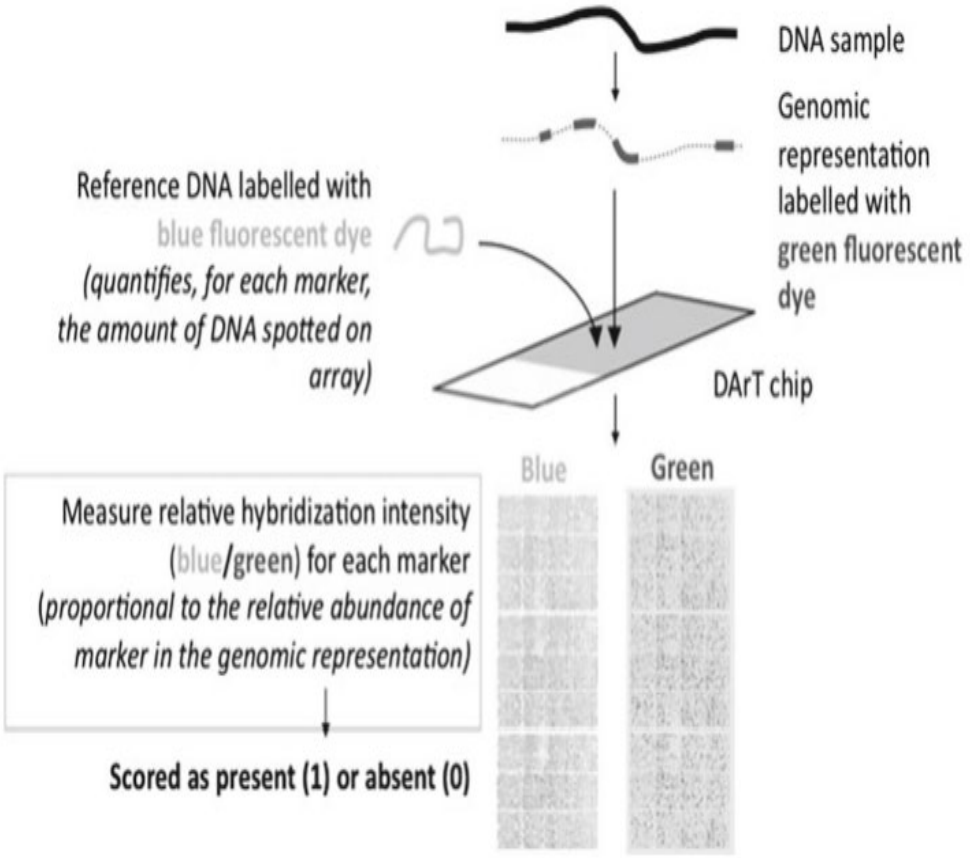

Figure 1.2 The current format of DArT assay (Kilian et al., 2012)

DArT markers have been successfully utilized in genetic diversity analysis, linkage mapping, and assessing population structure across various crop species. Their use in minor crops is also expanding, as they offer a powerful tool for gene discovery and molecular breeding without requiring prior sequence data, thanks to their whole-genome coverage. The first application of DArT was in evaluating genetic diversity in cultivated rice (Jaccoud et al., 2001). Since then, it has been applied to barley , grand eucalyptus (Lezar et al., 2004), cassava (Xia et al., 2005), and wheat (Akbari et al., 2006), and has also been validated in the model plant (Wittenberg et al., 2005).

1.4 Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms are DNA sequence variations resulting from point mutations, leading to different alleles with alternative bases at a specific nucleotide position within a locus (Mathur et al., 2018). Due to their high prevalence, codominant inheritance, and typically biallelic nature, SNPs are widely used in genome-wide association studies and are ideal for high-throughput screening. The study of base substitutions, which form the basis of SNPs, has been ongoing since DNA sequencing was first introduced in 1977. However, it was not until the late 1990s, with the development of gene chip technology, that SNP genotyping could be conducted efficiently across large sample sets. As the most abundant form of polymorphism in any organism, SNPs have become integral to molecular marker development. Their compatibility with automation and ability to detect hidden polymorphisms undetected by other markers make them invaluable tools in genetic research (Agrawal and Shrivastava, 2014).

SNPs are frequently used as primary markers for constructing high-density genetic maps due to their high frequency in genomes (Jehan and Lakhanpaul, 2006). The selection of SNPs facilitates the identification of desirable traits in large populations, enabling the efficient enhancement of crops using new-generation technologies, which are more cost-effective than traditional methods. Because these traits are genetically controlled, phenotypic experiments can be conducted more quickly, allowing breeders to select desirable traits early and propagate advantageous alleles across numerous populations (Morgil et al., 2020).

Analytical methods for SNP genotyping require sequence data to develop allele-specific PCR primers or oligonucleotide probes. SNPs and their surrounding sequences can be identified either by constructing and sequencing DNA libraries or by searching existing sequence databases. Once SNPs are pinpointed and suitable primers are created, high-throughput automation becomes a significant advantage. Common approaches for SNP detection include multiplex PCR, hybridization to oligonucleotide microarrays, and automated sequencer analysis. SNP analysis is particularly valuable for distinguishing cultivars in crops where genetic variation is limited, such as

cultivated tomatoes. Moreover, SNPs contribute to the enrichment of linkage maps, facilitating the identification of key genetic traits (Agrawal and Shrivastava, 2014).

Despite technological advancements, SNP genotyping remains complex and requires specialized equipment. Traditional genotyping methods include direct sequencing, single-base sequencing, allele-specific oligonucleotide analysis, denaturing gradient gel electrophoresis (DGGE), single-strand conformation polymorphism (SSCP), and ligation chain reaction (LCR). Each technique has unique advantages and limitations, making them suitable for SNP genotyping, particularly in smaller laboratories with limited budgets and personnel. However, large-scale SNP marker analysis depends on access to expensive, state-of-the-art equipment (Agrawal and Shrivastava, 2014).

The advent of next-generation sequencing (NGS) technologies—such as those from 454 Life Sciences, HiSeq, SOLiD, and Ion Torrent—has addressed the challenges of low throughput and high costs in SNP discovery. Transcriptome resequencing with NGS allows for the rapid and cost-effective identification of SNPs within genes while avoiding highly repetitive genomic regions. Additionally, genome complexity reduction techniques like Complexity Reduction of Polymorphic Sequences (CRoPS) and Restriction Site Associated DNA (RAD) improve computational efficiency by filtering out duplicated SNPs. These methods are effective for SNP identification in crops, even without a reference genome sequence (Dwiningsih et al., 2020).

In plants, SNPs are increasingly replacing simple sequence repeats (SSRs) as the preferred DNA marker for plant breeding and genetics applications. This shift is driven by their higher abundance, stability, automation potential, efficiency, and cost-effectiveness (Duran et al., 2009). SNPs are the most common form of genetic variation in eukaryotic genomes and are found in both coding and noncoding regions of nuclear and plastid DNA. For instance, SNP-based resources developed for rice research include large SNP datasets, tools for identifying informative SNPs for specific applications, and custom-designed SNP assays for marker-assisted and genomic selection (Kim et al., 2010). In sugar beet, these markers associated with traits such as low bolting tendency, skin roughness, root yield, sugar yield, growth vigor, plant height, crown size, and flesh coarseness have been identified and can be utilized for marker-assisted breeding and selection (Ravi et al., 2021; Wang et al., 2023).

SNPs play a crucial role in assessing genetic diversity, which is essential for understanding the relationships between different varieties, improving crops, and conserving germplasm. Over the years, SNPs have been used to study diversity within specific genes or genomic regions, providing insights into phylogenetic relationships among species. The development of new and third-generation sequencing technologies has enabled large-scale SNP-based genetic diversity analysis, which is beneficial for conserving genetic diversity in domesticated populations. In plant phylogenetic and evolutionary studies, gene sequences have traditionally been the focus, making the knowledge of SNPs highly valuable (Lasky et al., 2012).

2. LITERATURE REVIEW

Richards et al. (2004) assessed two sugar beet assessions using 8 microsatellite loci for characterization. They found high allele diversity and polymorphism information content (PIC). They suggested that microsatellite markers might help allow more research in studying the diversity of wild accessions population and within core collections of sugar beet.

Fenart et al. (2008) conducted a genetic analysis of 1640 weed beets, wild sea beets, and 35 diploid cultivars using four mitochondrial minisatellite loci, one chloroplastic PCR-RFLP marker, and five nuclear microsatellite loci. Their research identified significant genetic differences between wild sea beets and related groups. Based on their findings, they categorized the examined germplasm into four distinct forms: cultivated, wild, ruderal, and weed.

Li et al. (2010) analyzed 289 diploid inbred sugar beet lines to assess genetic diversity, population structure, and linkage disequilibrium within the germplasm. Using SSR markers, they identified two distinct subgroups within elite sugar beet germplasm. Their findings suggest that association mapping could be a valuable tool for sugar beet breeding.

Smulders et al. (2010) characterized triploid and diploid sugar beet genotypes through 12 high-quality marker patterns. Using 30 individual plants per variety for genotyping 30, they developed 25 new microsatellite markers for genetic characterization. Their results demonstrated that all tested varieties could be distinguished using the 12 markers, which are useful for genetic mapping and molecular breeding.

Li et al. (2011) developed a genome-wide distribution map of genetic diversity and linkage disequilibrium to enhance germplasm organization and genome-wide association mapping in sugar beet. They analyzed 502 diploid inbred lines from pollen parent heterotic gene pools, comprising 238 sugar-type and 264 yield-type inbreds, using 328 SNP markers. Their findings revealed two distinct subgroups within elite sugar beet germplasm. They also proposed that population genetic approaches could aid in identifying candidate genes under selection, underscoring the need for a substantial

increase in the number of markers to improve the effectiveness of genome-wide association mapping.

Nagl et al. (2011) assessed genetic variation in sugar beet using RAPD markers. Their study involved 12 sugar beet genotypes tested with 8 RAPD primers and 5 primer mixtures, resulting in stable and reproducible bands across all samples. They identified 44 polymorphic and 14 monomorphic loci, with an average of 6.13 bands per primer. Based on their findings, they proposed that RAPD markers could effectively analyze genetic diversity, particularly in genotypes with high homology and homozygosity.

Simko et al. (2012) genotyped 54 diploid sugar beet accessions (*Beta vulgaris* L. ssp. *vulgaris*) using 702 DArT, 34 SNP, and 30 SSR markers from five hybrid seed companies. Their analysis revealed three distinct populations, with clustering patterns observed through population structure analysis. They concluded that selecting an appropriate marker system and increasing the number of marker loci is essential for accurately assessing sugar beet genetic diversity.

Abbasi et al. (2014) evaluated genetic diversity of 168 genotypes of sugar beet (8 diploid pollinators pollen parents and 4 diploid male sterile lines) under salinity and drought stress using 18 microsatellite SSR markers and agro morphological traits. They found the efficacy of SSR markers for sugar beet crossing parent's genetic diversity evaluating.

Dohm et al. (2014) extracted and sequenced genomic DNA from root and leaf samples of sugar beet, utilizing single-nucleotide polymorphism (SNP)-based markers for genome region design. Their study included four sugar beet accessions and sea beet (*Beta vulgaris* ssp. *maritima)* to analyze intraspecific genomic variation. The reference genome revealed over seven million variable sites and regions of low variability, suggesting the effects of artificial selection.

Ghasemi et al. (2014) employed Random Amplified Polymorphic DNA (RAPD) markers to assess the genetic diversity of 13 sugar beet genotypes. Among 40 tested primers, only 10 generated polymorphic, clear, and reproducible bands. Their analysis differentiated the 13 genotypes into distinct groups, demonstrating that RAPD markers can efficiently identify cultivar diversity within a short time.

Izzatullayeva et al. (2014) conducted a comparative analysis of genetic variation in 42 sugar beet accessions using 12 RAPD and 12 ISSR markers. The RAPD primers

generated 204 amplification products, while the ISSR primers produced 178 fragments, with 190 and 173 being polymorphic, respectively. Their findings showed high genetic diversity indices (0.86 for RAPD and 0.91 for ISSR), indicating that both marker systems were equally effective for evaluating genetic variation in sugar beet accessions.

Stevanato et al. (2014) evaluated SNPs for genetic diversity of sugar beet. They offered 192 SNPs for 150 plants of 15 sugar beet genotypes (5 varieties, 5 cytoplasmic male sterile, and 5 pollinators). They suggested that the markers they evaluated have the potential to distinguish the studied plant material effectively. Furthermore, commercial cultivars and pollinator used in this study had lower polymorphism degree than the cytoplasmic male sterile genotypes. Furthermore, STRUCTURE and Principal Coordinate Analysis clustered the studied genotypes into three distinct subpopulations.

Ren et al. (2015) studied F_2 population of 144 plants of Watermelon (*Citrullus lanatus*) to identify the important agronomic traits and provide new genetic and genomic information that might be used in this species research. They used DArTSeq for F_2 population genotyping, and for genomic complexity reduction, they selected *PstI* and *MseI* restriction enzymes. They utilized a map construction method that produced a large number of SNPs (3,465) markers by sequencing DArT leading to the development of high-density and accurate genetic linkage map.

Andrello et al. (2016) conducted an experiment using 4436 DArT markers to examined the genetic diversity of 1264 accessions of *Beta* (*Beta vulgaris* subsp. *maritima*, *B. vulgaris* subsp. *adanensis*, *B. macrocarpa*, *B. patula* and *B. vulgaris* subsp. *vulgaris*). The results of their experiment showed that some markers were significantly associated with environmental variables in *B. vulgaris* subsp. *maritima*. Furthermore, in developing cultivars for abiotic tolerance; frost, and drought, DArT markers might be useful.

Ries et al. (2016) developed an innovative approach to identify causative loci for phenotypic traits within weeks of harvesting plant samples. Their method eliminated the need for prior genetic knowledge or additional sequencing of single offspring genotypes or parental lines. By adapting a mapping-by-sequencing approach, they demonstrated that phenotype-based pooling of diverse accessions from breeding panels, followed by direct allele frequency distribution analysis, is an effective strategy for gene identification in crop species. To ensure reliable results, a sequencing depth of at least

30-fold coverage was required, with increased coverage to 50- or 70-fold reducing the likelihood of missing critical genomic regions. Additionally, extensive post-processing steps—including sequence data refinement, read mapping, variation calling, and precise allele frequency estimation, particularly for insertions and deletions (InDels)—were essential for success. The GATK toolkit proved to be a flexible and adaptable tool for managing these processing steps in crop genome analysis.

Srivastava et al. (2017) amplified 13 genomic DNA of *Beta vulgaris* L. genotypes using 14 microsatellite markers in order to identify genetic variation and diversity. To analyze and categorize the heterozygous groups, they employed NTSYS software and amplified bands on the gel. They indicated the usefulness of microsatellite markers for exploring molecular genetic diversity with low cost in sugar beet for obtaining new genetic combinations.

Taski-Ajdukovic et al. (2017) studied 26 SSR primers for genetic diversity investigation of 140 individual samples from 12 diploid sugar beet pollinators as pollen parents and 2 cytoplasmic male sterile as seed parents. They find out that 129 alleles with a mean of 3.2 alleles per SSR marker and heterozygosity mean was 0.30. These results show pollinator selection as a suitable parental might be improved using this method.

Wang et al. (2018) developed a high-density genetic map of sugar beet by generating genotype data through Specific-Locus Amplified Fragment (SLAF) sequencing. They constructed an ultra-dense genetic map based on an F1 population derived from a cross between two distinct diploid lines: 3a (high-yield, low-sugar, sterile, monogerm) and 3b (low-yield, high-sugar, pollinated, polyembryonic). Using Illumina high-throughput sequencing, they generated 201.10 million high-quality paired-end reads, developing 171,637 SLAFs, of which 48,478 were polymorphic. Ultimately, 3,287 polymorphic SLAFs were mapped into nine linkage groups. They suggested that this genetic map will enhance the fine mapping of QTLs and marker-assisted selection (MAS) in sugar beet breeding.

Galewski and McGrath (2020) introduced a categorized approach for characterizing genetic diversity in cultivated sugar beet. They employed pooled sequencing of accessions representing different crop type lineages, incorporating a range of phenotypic variations such as leaf and root traits, sucrose accumulation, water

content, pigment distribution, and disease resistance. Their results revealed extensive genetic variation both within and between crop types. Additionally, while demographic history was shared within each type, principal component analysis (PCA) showed strong differentiation between crop types.

3. MATERIALS AND METHODS

3.1 Plant Materials

The sugar beet genotypes included in the project consist of 94 accessions and registered varieties collected from the world obtained from The United States Department of Agriculture (USDA) were used as plant material. Detailed information about the genotypes to be used is given in Table 3.1. Sugar beet genotypes were planted under field conditions in the experimental field of the Field Crops Department, Van Yüzüncü Yıl University, Faculty of Agriculture on 4 April, 2022. Plants were monitored daily and irrigated when it was necessary (Figure 3.1). All weeds were removed around plants and within lines. A well-developed plant from each genotype was harvested on 25 October 2022, then labeled, and leaf samples were taken from them and kept in Van Yüzüncü Yıl University Faculty of Agriculture Agricultural Biotechnology Laboratory for two days at -85 °C and then dried in a lyophilized for two days. Samples dried in the lyophilized were ground in the Tissue Analyzer device. For scoring genotypes' morphological variation, photos were taken for each genotype at harvesting (Figure 3.2, 3, 4, 5).

Table 3.1 Origins of sugar beet genotypes that will be used in the study

#	Accessions No.	Origin	Scientific name
1	Ames 2644	Utah, USA	*Beta vulgaris* L. subsp. *vulgaris*
2	Ames 2658	Utah, USA	*Beta vulgaris* L. subsp. *vulgaris*
3	Ames 2661	Utah, USA	*Beta vulgaris* L. subsp. *vulgaris*
4	Ames 2662	Utah, USA	*Beta vulgaris* L. subsp. *vulgaris*
5	Ames 3039	California, USA	*Beta vulgaris* L. subsp. *vulgaris*
6	Ames 3047	California, USA	*Beta vulgaris* L. subsp. *vulgaris*
7	Ames 3049	California, USA	*Beta vulgaris* L. subsp. *vulgaris*
8	Ames 3060	Denmark	*Beta vulgaris* L. subsp. *vulgaris*
9	Ames 4375	No info	*Beta vulgaris* L. subsp. *vulgaris*
10	Ames 8281	England, UK	*Beta vulgaris* L. subsp. *vulgaris*
11	Ames 8283	England, UK	*Beta vulgaris* L. subsp. *vulgaris*
12	Ames 14432	Plovdiv, Bulgaria	*Beta vulgaris* L. subsp. *vulgaris*
13	NSL 6320	Illinois, USA	*Beta vulgaris* L. subsp. *vulgaris*
14	NSL 28024	Wyoming, USA	*Beta vulgaris* L. subsp. *vulgaris*
15	NSL 28714	Wyoming, USA	*Beta vulgaris* L. subsp. *vulgaris*
16	NSL 28716	Wyoming, USA	*Beta vulgaris* L. subsp. *vulgaris*
17	NSL 86577	Colorado, USA	*Beta vulgaris* L. subsp. *vulgaris*

Table 3.1 Origins of sugar beet genotypes that will be used in the study (continued)

#	Accessions No.	Origin	Scientific name
18	NSL 176303	Former Serbia and Montenegro	*Beta vulgaris* L. subsp. *vulgaris*
19	PI 105335	China	*Beta vulgaris* L. subsp. *vulgaris*
20	PI 113306	China	*Beta vulgaris* L. subsp. *vulgaris*
21	PI 117117	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
22	PI 120694	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
23	PI 120695	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
24	PI 120706	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
25	PI 124528	India	*Beta vulgaris* L. subsp. *vulgaris*
26	PI 140350	Iran	*Beta vulgaris* L. subsp. *vulgaris*
27	PI 140353	Iran	*Beta vulgaris* L. subsp. *vulgaris*
28	PI 140354	Iran	*Beta vulgaris* L. subsp. *vulgaris*
29	PI 140355	Iran	*Beta vulgaris* L. subsp. *vulgaris*
30	PI 140356	Iran	*Beta vulgaris* L. subsp. *vulgaris*
31	PI 140358	Iran	*Beta vulgaris* L. subsp. *vulgaris*
32	PI 140360	Iran	*Beta vulgaris* L. subsp. *vulgaris*
33	PI 140361	Iran	*Beta vulgaris* L. subsp. *vulgaris*
34	PI 141919	No info	*Beta vulgaris* L. subsp. *vulgaris*
35	PI 142808	Iran	*Beta vulgaris* L. subsp. *vulgaris*
36	PI 142809	Iran	*Beta vulgaris* L. subsp. *vulgaris*
37	PI 142812	Iran	*Beta vulgaris* L. subsp. *vulgaris*
38	PI 142814	Iran	*Beta vulgaris* L. subsp. *vulgaris*
39	PI 142815	Iran	*Beta vulgaris* L. subsp. *vulgaris*
40	PI 142817	Iran	*Beta vulgaris* L. subsp. *vulgaris*
41	PI 142818	Iran	*Beta vulgaris* L. subsp. *vulgaris*
42	PI 142820	Iran	*Beta vulgaris* L. subsp. *vulgaris*
43	PI 142821	Iran	*Beta vulgaris* L. subsp. *vulgaris*
44	PI 142823	Iran	*Beta vulgaris* L. subsp. *vulgaris*
45	PI 144675	Iran	*Beta vulgaris* L. subsp. *vulgaris*
46	PI 148625	Iran	*Beta vulgaris* L. subsp. *vulgaris*
47	PI 164659	India	*Beta vulgaris* L. subsp. *vulgaris*
48	PI 164671	India	*Beta vulgaris* L. subsp. *vulgaris*
49	PI 164805	India	*Beta vulgaris* L. subsp. *vulgaris*
50	PI 164968	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
51	PI 165062	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
52	PI 165485	India	*Beta vulgaris* L. subsp. *vulgaris*
53	PI 169014	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
54	PI 169017	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
55	PI 169029	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
56	PI 169032	Turkey	*Beta vulgaris* L. subsp. *vulgaris*

Table 3.1 Origins of sugar beet genotypes that will be used in the study (continued)

#	Accessions No.	Origin	Scientific name
57	PI 171508	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
58	PI 171516	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
59	PI 171518	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
60	PI 171519	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
61	PI 172733	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
62	PI 176875	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
63	PI 179176	Iraq	*Beta vulgaris* L. subsp. *vulgaris*
64	PI 179180	Turkey	*Beta vulgaris* L. subsp. *vulgaris*
65	PI 193458	Ethiopia	*Beta vulgaris* L. subsp. *vulgaris*
66	PI 256052	Afghanistan	*Beta vulgaris* L. subsp. *vulgaris*
67	PI 256053	Afghanistan	*Beta vulgaris* L. subsp. *vulgaris*
68	PI 590616	Hauts-de-France, France	*Beta vulgaris* L. subsp. *vulgaris*
69	PI 590621	Wyoming, USA	*Beta vulgaris* L. subsp. *vulgaris*
70	PI 590697	Maryland, USA	*Beta vulgaris* L. subsp. *vulgaris*
71	PI 590808 INBRED	Utah, USA	*Beta vulgaris* L. subsp. *vulgaris*
72	PI 590812 ANNUAL	Utah, USA	*Beta vulgaris* L. subsp. *vulgaris*
73	PI 596528	Colorado, USA	*Beta vulgaris* L. subsp. *vulgaris*
74	PI 610286	Chile	*Beta vulgaris* L. subsp. *vulgaris*
75	PI 610287	Chile	*Beta vulgaris* L. subsp. *vulgaris*
76	PI 610291	Argentina	*Beta vulgaris* L. subsp. *vulgaris*
77	PI 610323 MS	California, USA	*Beta vulgaris* L. subsp. *vulgaris*
78	PI 610417	California, USA	*Beta vulgaris* L. subsp. *vulgaris*
79	PI 611059	Plovdiv, Bulgaria	*Beta vulgaris* L. subsp. *vulgaris*
80	PI 611060	China	*Beta vulgaris* L. subsp. *vulgaris*
81	PI 611062	Greece	*Beta vulgaris* L. subsp. *vulgaris*
82	PI 142816	Iran	*Beta vulgaris* L. subsp. *vulgaris*
83	PI 633934	California, USA	*Beta vulgaris* L. subsp. *vulgaris*
84	Ames 8302	England, UK	*Beta vulgaris* L. subsp. *vulgaris*
85	Ames 8295	England, UK	*Beta vulgaris* L. subsp. *vulgaris*
86	Ames 8294	England, UK	*Beta vulgaris* L. subsp. *vulgaris*
87	Ames 8298	England, UK	*Beta vulgaris* L. subsp. *vulgaris*
88	NSL 176412	Former Serbia and Montenegro	*Beta vulgaris* L. subsp. *vulgaris*
89	Ames 8297	England, UK	*Beta vulgaris* L. subsp. *vulgaris*
90	Ames 8286	England, UK	*Beta vulgaris* L. subsp. *vulgaris*
91	Ames 8287	England, UK	*Beta vulgaris* L. subsp. *vulgaris*
92	Ames 8288	England, UK	*Beta vulgaris* L. subsp. *vulgaris*
93	Ames 8291	England, UK	*Beta vulgaris* L. subsp. *vulgaris*
94	Ames 8292	England, UK	*Beta vulgaris* L. subsp. *vulgaris*

Figure 3.1 Watering, monitoring, and observing the plants

Figure 3.2 Photographs of the accessions after harvest; Ames 2644-USA1, Ames 2658-USA2, Ames 2661-USA3, Ames 2662-USA4, Ames 3039-USA5, Ames 3047-USA6, Ames 3049-USA7, Ames 3060-Denmark, Ames 4375- No info, Ames 8281, Ames 8283-UK2, Ames 14432-Bulgaria1, NSL 6320-USA8, NSL 28024- USA9, NSL 28714-USA10, NSL 28716-USA11, NSL 86577-USA12, NSL 176303-Serbia1, PI 105335-China1, PI 113306-China2, PI 117117-Turkey1, PI120694-Turkey2, PI 120695-Turkey3, PI 120706-Turkey4, PI 124528-India1

Figure 3.3 Photographs of the accessions after harvest; PI 140353-Iran2, PI 140354-Iran3, PI 140355-Iran4, PI 140356-Iran5, PI 140358-Iran, PI 140360-Iran7, PI 140361-Iran8, PI 141919-No info, PI 142808-Iran9, PI 142809-Iran10, PI 142812-Iran11, PI 142814-Iran12, PI 142815-Iran13, PI 142817-Iran14, PI 142818-Iran15, PI 142820-Iran16, PI 142821-Iran17, PI 142823-Iran18, PI 144675-Iran19, PI 148625-Iran20,PI 164659-India2, PI 164805-India4, PI 164968-Turkey5, PI 165062-Turkey6, PI 165485-India5

Figure 3.4 Photographs of the accessions after harvest; PI 169014-Turkey7, PI 169017-Turkey8, PI 169029-Turkey9, PI 169032-Turkey10, PI 171508-Turkey11, PI 171516-Turkey12, PI 171518-Turkey13, PI 171519-Turkey14, PI 176875-Turkey16, PI 179176-Iraq, PI 179180-Turkey17, Turkey17-Ethiopia, PI 256052-Afghanistan1, PI 256053-Afghanistan2, PI 590616-France, PI 590621-USA13, PI 590697-USA14, PI 590808 INBRED-USA15, PI 590812 ANNUAL-USA16, PI 596528-USA17, PI 610286-Chile1, PI 610287-Chile2, PI 610323 MS-USA18, PI 610417-USA19, PI 61105- Bulgaria2

Figure 3.5 Photographs of the accessions after harvest; PI 611060-China3, PI 611062-Greece, PI 142816-Iran21, PI 633934-USA20, Ames 8302-UK3, Ames 8295-UK4, Ames 8294-UK5, NSL 176412-Serbia2, Ames 8297-UK7, Ames 8286-UK8, Ames 8287-UK9, Ames 8288-UK10, Ames 8291-UK11

3.2 Molecular Analysis

3.2.1 DNA Isolation

Leaf samples from the 94 genotypes were collected and stored in a deep freezer at -85 °C for two days, then placed in freeze-dryers for 48 hours. Samples were then mashed and ground by TissueLyser (QIAGEN-Tissue LyserII) for 3 minutes to make a fine powder then took (25-30mg) of fine powder used for DNA extraction. DNA isolation from freeze-dried and ground samples in a lyophilizer was performed following the CTAB method reported by Doyle and Doyle (1990) and Boiteux et al. (1999) with minor modifications, and a unique protocol suggested by Diversity Arrays Technology (Figure 3.6).

The extraction begins by grinding tissue in a 2 ml tube until the volume reaches approximately 200 ml (up to the 200 ml in the tube). A water bath is preheated to 60–65 °C. Next, 1 ml of Extraction Buffer (EB) and 1 µl of RNAse are added to the tube, followed by thorough mixing via vortexing. The tube is then incubated in the water bath for 30 minutes, during which the CTAB (which is a detergent commonly used in molecular biology for isolating high-quality DNA, particularly from plant tissues) is also warmed. Once cooled, 1000 µl of chloroform/isoamyl alcohol (CI) is added to the tube and mixed thoroughly. The mixture is centrifuged at maximum speed (14000 rpm) for 5 minutes, and the top phase (approximately 800 µl) is carefully transferred to a new 2 ml tube. Subsequently, 100 µl of cooled CTAB is added and mixed, followed by the addition of 1000 µl of CI, which is also mixed thoroughly. The mixture is centrifuged again at maximum speed for 5 minutes, and the top phase (approximately 900 µl) is transferred to another new 2 ml tube. To the transferred phase, 1000 µl of Precipitation Buffer (PB) is gently mixed in, and the solution is incubated at room temperature for 30 minutes. The mixture is then centrifuged for 10 minutes to collect the pellet. The supernatant is discarded, and the water bath is adjusted to 50–60 °C. To dissolve the pellet, 400 µl of 1 M NaCl is added, and the tube is incubated in the water bath for approximately 20 minutes. Following this, 1000 µl of cold 95% ethanol (ETOH) is added and mixed thoroughly. The sample is stored overnight at -20 °C or incubated for 20–30 minutes at room temperature before proceeding. The solution is centrifuged for 10 minutes at maximum speed, and the supernatant is discarded. Next, 500 µl of 70% ethanol is added, and the sample is centrifuged again for 5 minutes at maximum speed. The supernatant is discarded, and the pellet is dried in a 37 °C incubator for 30 minutes with the tubes left open. Finally, the pellet is dissolved in 100 µl of TE buffer or H_2O, with the tubes closed, and incubated for 15–20 minutes. The dissolved pellet is stored at -20 °C for further use.

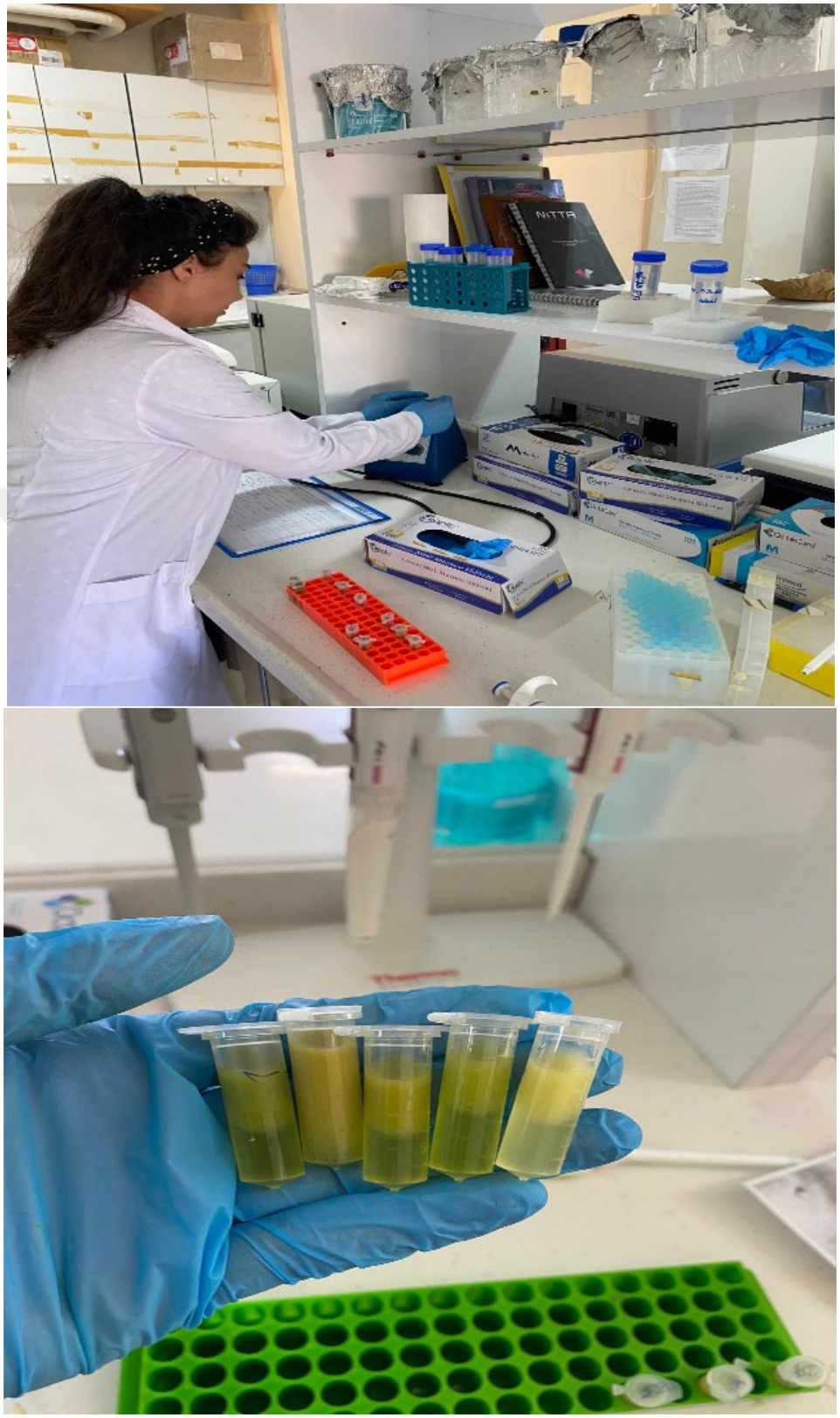

Figure 3.6 DNA extraction from plant samples

3.2.2 Testing DNA Quantity and Purity

To assess the quality, quantity, and purity of the sugar beet genotypes' DNA samples, the following steps were performed.

3.2.2.1 Amount and Purity

The amount and purity of DNA to be used in PCR reactions are extremely important. To measure DNA concentration, a Nanodrop spectrophotometer (Thermo Scientific-Nanodrop2000) was used. The device was initialized and calibrated according to the manufacturer's instructions using 1 µl of nuclease-free water as the blank. Following calibration, 1 µl of each DNA sample was loaded onto the measurement pedestal, ensuring no air bubbles or contaminants were present. The Nanodrop software was set to DNA measurement mode (260/280 nm), and the absorbance readings were recorded (Table 3.2). The concentration of DNA in each sample was displayed in ng/µl, along with the 260/280 ratio, which was used to assess the purity of the DNA. Between measurements, the pedestal was cleaned thoroughly with lint-free tissue and nuclease-free water to avoid cross-contamination. Finally, the samples were diluted to 25 ng/µl for PCR reactions.

Table 3.2 DNA samples results

#	Accessions No.	DNA sample No.	DNA concentration
1	Ames 2644	sb4	537.5
2	Ames 2658	sb5	517.5
3	Ames 2661	sb6	1259
4	Ames 2662	sb7	1476.5
5	Ames 3039	sb8	2033.5
6	Ames 3047	sb9	3305.7
7	Ames 3049	sb10	2391.6
8	Ames 3060	sb11	2089.9
9	Ames 4375	sb12	1346.7
10	Ames 8281	sb14	329.1

Table 3.2 DNA samples results (continued)

#	Accessions No.	DNA sample No.	DNA concentration
11	Ames 8283	sb15	267.6
12	Ames 14432	sb16	1291.6
13	NSL 6320	sb17	281.5
14	NSL 28024	sb19	224.9
15	NSL 28714	sb20	511.8
16	NSL 28716	sb21	1379.1
17	NSL 86577	sb22	484.5
18	NSL 176303	sb23	241
19	PI 105335	sb24	578.9
20	PI 113306	sb25	780.4
21	PI 117117	sb26	199.6
22	PI 120694	sb27	904.6
23	PI 120695	sb28	256.1
24	PI 120706	sb29	295.7
25	PI 124528	sb30	3495.8
26	PI 140350	sb31	1260
27	PI 140353	sb32	493.2
28	PI 140354	sb33	395.4
29	PI 140355	sb34	601.9
30	PI 140356	sb35	155.8
31	PI 140358	sb36	688
32	PI 140360	sb37	3598.7
33	PI 140361	sb38	319.3
34	PI 141919	sb39	4212
35	PI 142808	sb40	626.4
36	PI 142809	sb41	2659.4
37	PI 142812	sb42	1043.8
38	PI 142814	sb43	815.2
39	PI 142815	sb44	2391.9

Table 3.2 DNA samples results (continued)

#	Accessions No.	DNA sample No.	DNA concentration
40	PI 142817	sb46	333.4
41	PI 142818	sb49	129.5
42	PI 142820	sb50	601.3
43	PI 142821	sb51	682.5
44	PI 142823	sb52	633
45	PI 144675	sb53	117.8
46	PI 148625	sb54	5227.2
47	PI 164659	sb55	822
48	PI 164671	sb56	409.7
49	PI 164805	sb57	787.4
50	PI 164968	sb58	198.3
51	PI 165062	sb59	148.7
52	PI 165485	sb60	322.3
53	PI 169014	sb61	765.9
54	PI 169017	sb63	549
55	PI 169029	sb64	1417.5
56	PI 169032	sb65	3015.7
57	PI 171508	sb66	1921.9
58	PI 171516	sb67	944.3
59	PI 171518	sb68	804.4
60	PI 171519	sb69	732.8
61	PI 172733	sb70	3555.5
62	PI 176875	sb71	6144.5
63	PI 179176	sb73	413.1
64	PI 179180	sb74	3619.2
65	PI 193458	sb75	218.7
66	PI 256052	sb76	6001.8
67	PI 256053	sb77	3430
68	PI 590616	sb78	1532

Table 3.2 DNA samples results (continued)

#	Accessions No.	DNA sample No.	DNA concentration
69	PI 590621	sb79	592.9
70	PI 590697	sb80	2140.7
71	PI 590808 INBRED	sb81	719.4
72	PI 590812 ANNUAL	sb82	3801.1
73	PI 596528	sb83	735.7
74	PI 610286	sb84	3221.1
75	PI 610287	sb85	2328.8
76	PI 610291	sb86	930
77	PI 610323 MS	sb87	823.6
78	PI 610417	sb88	2295.2
79	PI 611059	sb89	1026.4
80	PI 611060	sb90	2707.3
81	PI 611062	sb91	4546
82	PI 142816	sb92	687.9
83	PI 633934	sb93	3884.3
84	Ames 8302	sb94	3331.3
85	Ames 8295	sb95	3132.9
86	Ames 8294	sb97	2739.2
87	Ames 8298	sb99	200.7
88	NSL 176412	sb100	105.4
89	Ames 8297	sb101	1654.8
90	Ames 8286	sb102	2510.3
91	Ames 8287	sb103	1154.1
92	Ames 8288	sb104	657
93	Ames 8291	sb105	1144.8
94	Ames 8292	sb106	1010.1

3.2.2.2 Polymerase Chain Reaction – PCR

To check the quality of the DNA samples, PCR was carried out on the DNA samples of all genotypes following a protocol that involves three main stages; stage one is an initial denaturation step, stage 2 involves 45 cycles of denaturation, annealing, and extension, the final stage comprises a final extension to ensure complete elongation of all amplified fragments, followed by a holding step to preserve the PCR products. Each PCR reaction was set up with a volume of 25 µl per tube, as specified in Table 3.3. The reactions were conducted using a Thermal Cycler (Applied Biosystems-Veriti). The steps and details of the polymerase chain reaction are outlined in Table 3.4.

Table 3.3 Polymerase chain reaction component

PCR Component	Amount used (µl)
dH_2O	13.53
Primer (5 mM)	3
Taq Buffer	2
$MgCl_2$ (25 mM)	2.45
dNTP (2.5 mM)	2
Taq Polymerase (SUFermentas)	0.2
gDNA (25 ng/µl)	2
Total Reaction Volume	25

Table 3.4 PCR Cycle for iPBS Retrotransposon

Steps	Temperature / Duration / Cycles
Initial Denaturation	95°C / 4 minutes / 1 cycle
Denaturation	95°C / 15 seconds / 30 cycles
Annealing	50–65°C / 1 minute / 30 cycles
Extension	68°C / 1 minute / 30 cycles
Final Extension	72°C / 5 minutes / 1 cycle
Hold	+4°C

3.2.2.3 Agarose Gel Electrophoresis

PCR products obtained were run on a 2% agarose gel which was prepared by dissolving 3 g of agarose in 150 ml of 1X TAE buffer, with the agarose completely dissolved by heating in a microwave oven. Once the solution was cooled, 25 µl of ethidium bromide was added as a staining agent, thoroughly mixed, and allowed to cool further before being poured into gel casting templates fixed with a comb. The gel was left to solidify, after which the comb was removed, and the gel was placed in the electrophoresis unit. PCR products were mixed with 3 µl of agarose gel loading dye and carefully loaded into the wells (Figure 3.7). The size of the separated bands was determined using a DNA marker in the range of 200-20000 base pairs. Electrophoresis was run at 120 volts for 4 hours, after which the bands were visualized under UV light and photographed (Figure 3.8 a,b).

Figure 3.7 DNA preperation for gel electrophoresis

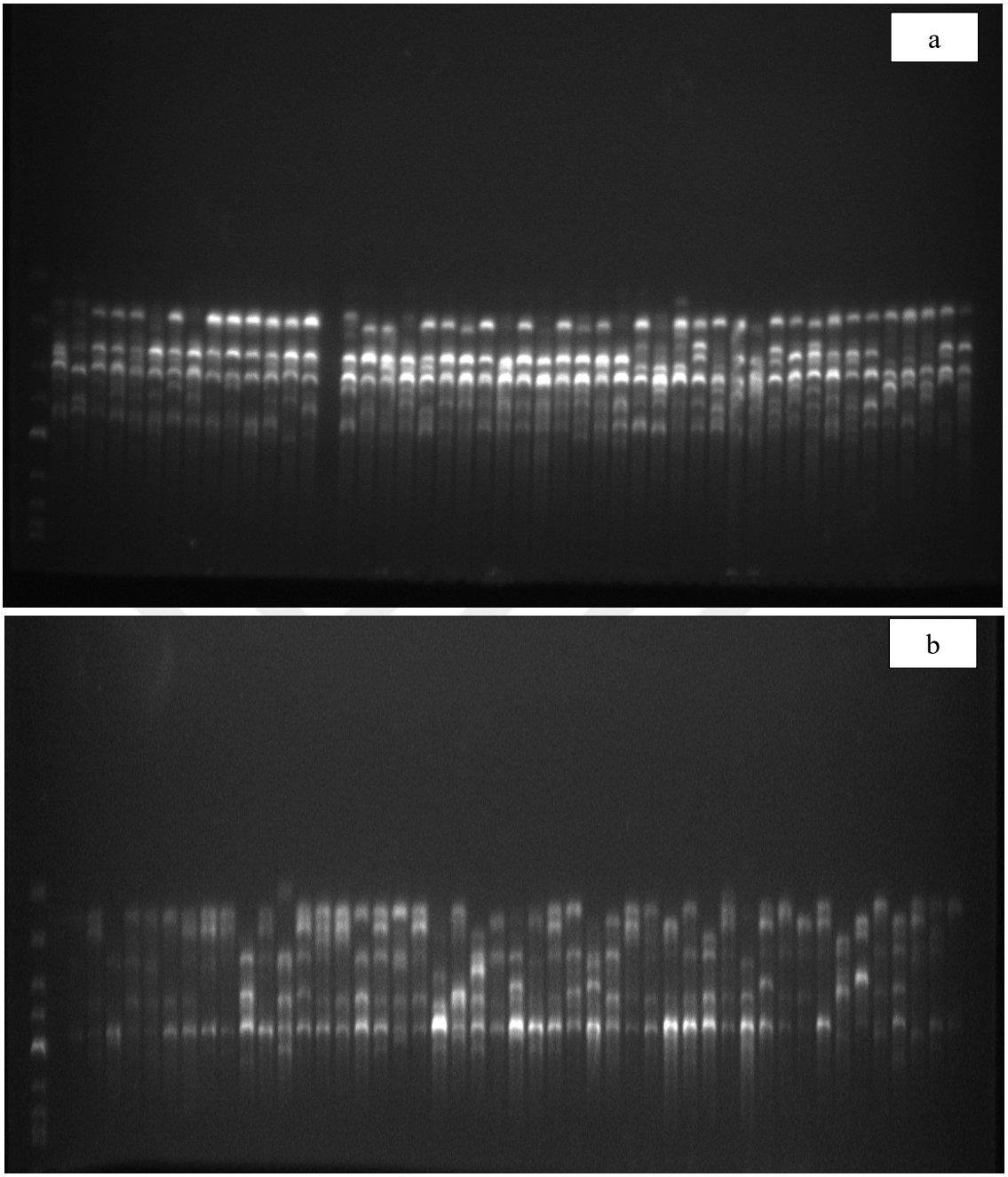

Figure 3.8 PCR gel image obtained using iPBS primer

3.2.3 Molecular Marker

100 µL of genomic DNA samples isolated from sugar beet genotypes with a concentration of 50 ng/µL for each sample was sent to Diversity Arrays Technology Pty Ltd for sequencing and marker genotyping. Genotyping was done by service procurement and thousands of markers (at least 100000 up to 1 million) were obtained

with GBS. DArTSeq (Diversity Array Technology Sequencing) is a method based on methyl filtration technology and next-generation sequencing platforms, reducing the complexity of genomic data and determining its genotypic characterization. By using the DArTseq method, two different data types were obtained; SNPs and SilicoDArT markers. SilicoDArT markers contain the "presence/absence variation – PAV" value found in restriction fragments, and SNPs represent nucleotide polymorphisms of restriction fragments.

3.3 Statistical Analysis

The SNP and SilicoDArT marker data were analyzed separately. Raw data were loaded and filtered using version 4.2 of R software (R Core Team, 2021) and the dartR package v2 (Gruber et al., 2022, Mijangos et al., 2022) with the following criteria: all SNPs and SilicoDArT markers with more than 5% missing data were excluded, as well as markers absent in all individuals of at least one population, with populations defined by the accessions' countries of origin. Markers with a reproducibility score (RepAvg) below 100% and those derived from the same DNA fragment (considered redundant and uninformative) were also removed. Additionally, SNPs with a minor allele frequency (MAF) lower than 5% were discarded. The filtered SNP and SilicoDArT data were then utilized for genetic analyses of the sugar beet germplasm collection.

Simple agglomerative hierarchical clustering was conducted using the poppr R package (Kamvar et al., 2014; 2021). Pairwise genetic dissimilarity (GD) values were calculated among accessions using Hamming distance with the 'bitwise.dist' function. Following this, a distance matrix was generated and used to create dendrograms via the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) using 'aboot' and visualized with the 'ggtree' package (Yu et al., 2017; 2021). Principal component analysis (PCA) was performed with the 'gl.pcoa' function in dartR v2, and the first two principal coordinates were plotted. The genetic structure of the populations was analyzed using Bayesian clustering algorithms from the fastSTRUCTURE software (Raj et al., 2014), a version of STRUCTURE (Pritchard et al., 2000) designed to handle genomic SNP matrix data. Distruct barplots were generated in R using the 'pophelper' package (Francis, 2020). The optimal number of populations (K) was

determined using the post hoc methods proposed by Evanno et al. (2005) by running fastSTRUCTURE with 100 replicates of K ranging from 1 to 15, with the most parsimonious model selected based on their mean likelihood and delta K. Analysis of molecular variance was performed using the pegas AMOVA as implemented in dartR (Mijangos et al., 2022) considering i) countries and ii) clusters inferred from the UPGMA tree as subpopulations. General genetic statistics were calculated separately for populations (countries) and UPGMA genetic clusters using the ‘popgen’ function in the snpReady package (Granato and Fritsche-Neto 2018; Granato et al., 2018).

4. RESULTS

3.4 Analysis of Molecular Variance (AMOVA)

There were 45063 silicoDArT markers and 41080 raw SNP markers called in total. The number of selected SNPs markers were higher than SilicoDArT markers (224515), with 823874. With the exception of the communities' geographic origins, all of the called markers were polymorphic; 12497 SNPs and 25394 silicoDArT markers were present in at least one in each population. Of the mentioned markers, 19614 and 8676 SNPs and 24629 silicoDArT markers were found. We chose the markers with MAF > 5% from the latter group, yielding 6950 silicoDArT markers and 4609 SNPs in total that were utilized for further genetic analysis in the sugar beet germplasm.

To assess population differentiation using molecular markers, Analysis of Molecular Variance (AMOVA) was performed with 11559 markers, including 4609 SNP markers and 6950 silicoDArT markers. The raw data was processed in R (version 4.2, R Core Team, 2022) using the dartR package v2 (Mijangos et al., 2022). The 94 genotypes analyzed were grouped into 22 distinct clusters, and allele frequency distributions were examined both within and among these groups.

The results revealed significant genetic variation among groups ($P \leq 0.001$), indicating a strong differentiation pattern within the sugar beet collection. Additionally, all variance components were highly significant ($P < 0.001$) across different levels of genotype comparison, as demonstrated by the AMOVA results (Tables 4.1 and 4.2).

These findings confirm the presence of substantial genetic variability, suggesting that the sugar beet accessions studied exhibit notable differentiation, which may be valuable for breeding and conservation efforts.

Table 4.1 The statistics analysis AMOVA for SNP data

	df	SSD	MSD	Variance component	Statistics	P.
Pop. Names	21	1.190537	0.05669223	0.0068847	0.189428	<0.0001
Error	72	2.121132	0.02946017	0.0294602		
Total	93	3.311669	0.03560934			

Table 4.2 The statistics analysis AMOVA for silicoDArT data

	df	SSD	MSD	Variance component	Statistics	P.
Pop. Names	21	2.406482	0.11459440	0.012944	0.1695559	<0.0001
Error	72	4.564509	0.06339596	0.063396		
Total	93	6.970992	0.07495690			

The AMOVA was performed based on populations in UPGMA and structure analysis. AMOVA based on the result of the structure revealed higher variety among the population ($p<0.001$). The PIC values were observed for SNPs (0.24) and for SilicoDArT (0.18) also. The gene diversity (GD) for the SNP marker was (0.30), and for the SilicoDArT was (0.21). The minor allele frequency (MAF) was 0.22 for SNP, and 0.13 for SilicoDArT markers. Investigated genetic variation between the genotypes based on country grouping demonstrated that average effective (Ne) allele values were 134.58 and -98.62, for both SNP, and SilicoDArT markers respectively (Table 3). The highest local inbreeding coefficient (F) was found in the SNP marker (0.28), while the SilicoDArT marker showed the lowest value of F (-0.21) (Table 4.3).

Table 4.3 Genetic diversity analysis for the SNP, and silicoDArT data

Value	SNP	SilicoDArT
GD	0.31	0.30
PIC	0.25	0.25
MAF	0.22	0.22
H0	0.20	0.45
F	0.35	-0.48
Ne	134.58	-98.62
Va	3894.17	6972.85
Vd	1424.96	2574.82
Number of genotype	94	94
Number of Markers	45063	41080

GD: gene diversity, PIC: Polymorphic Information Content, MAF: minimum allele frequency, Ho: Observed heterozygosity, F: Inbreed coefficient, Ne: Effective number of alleles, Va: additive variance, Vd: dominance variance.

3.5 Principal Component Analysis (PCA)

Ensuring the accuracy of imputed molecular markers (SNP and SilicoDArT) is essential before proceeding with further genetic analyses. To evaluate genetic relationships among sugar beet genotypes, Principal Component Analysis (PCA) was conducted. The analysis was performed separately for SNP and SilicoDArT markers (Figure 4.1, A and B) using a wrapper function from the dartR package (Mijangos et al., 2022). This method optimizes the linear correlation between distance matrices and low-dimensional space representation.

PCA was carried out on 45063 markers and 41080 SilicoDArT markers to examine genetic relationships both within and among groups. The resulting PCA plots were generated using the first two principal components (PC1 and PC2), based on data from 94 genotypes.

The PCA results using SNPs marker (Figure 4.1, A) identified three distinct clusters (S-I, S-II, and S-III), with PC1 and PC2 accounting for 8.9% and 4.6% of the total genetic variation, respectively. These findings highlight clear genetic differentiation within the sugar beet collection and provide valuable insights into population structure and diversity. Samples in S-I showed tight clustering along PC1, suggesting a genetically homogeneous group. This may correspond to cultivated populations or breeding lines with limited genetic variation. This group included 19 accessions from Iran, 10 from Türkiye, 10 from UK, 2 from Afghanistan, and 1 from Iraq. S-II was located in the lower portion of the PCA plot and appeared well-separated from S-I and S-III. This cluster consisted of 18 accessions of mixed origins: Turkey (4), India (4), the US (2), and several other geographical origins with less than two accessions per country. This distinct grouping indicates a genetically differentiated population, potentially arising from geographic or reproductive isolation. S-III, positioned on the left side of the plot, exhibited a broader spread compared to S-I and S-II, suggesting greater genetic diversity. This cluster includes 26 accessions from various European sources as well as the majority of US accessions (15). This cluster may represent wild or introgressed populations with high allelic variation. The clustering pattern indicates significant genetic structuring within the analyzed populations,

reflecting the impact of breeding practices, geographic isolation, and possibly environmental adaptations.

The PCA using silicoDArTs is presented in Figure 4.1 B, and showed two major clusters, D-I and D-II, separated along PC1 (7.2% of the total variation) and PC2 (4.1%). Cluster D-I: This cluster was the largest and most dispersed, suggesting a genetically diverse population with varying genetic backgrounds. The spread along PC1 indicates that D-I likely represents a composite population, possibly involving gene flow between cultivated and wild relatives. D-II formed a compact grouping on the left side of the PCA plot, indicative of a genetically uniform population. This may correspond to a subset of populations subjected to selective breeding or isolated evolution. The observed differentiation between D-I and D-II reflects limited gene flow and potentially distinct evolutionary or ecological trajectories among the populations.

Across both PCA analyses, clear genetic structuring was observed, with clusters varying in genetic diversity and composition. The higher diversity in S-III and D-I suggests their potential as reservoirs of unique alleles, while the homogeneity of S-I, S-II, and D-II may reflect selective breeding or adaptation to specific environments. These results underscore the importance of genetic diversity in population differentiation and provide a basis for understanding evolutionary relationships and guiding conservation efforts.

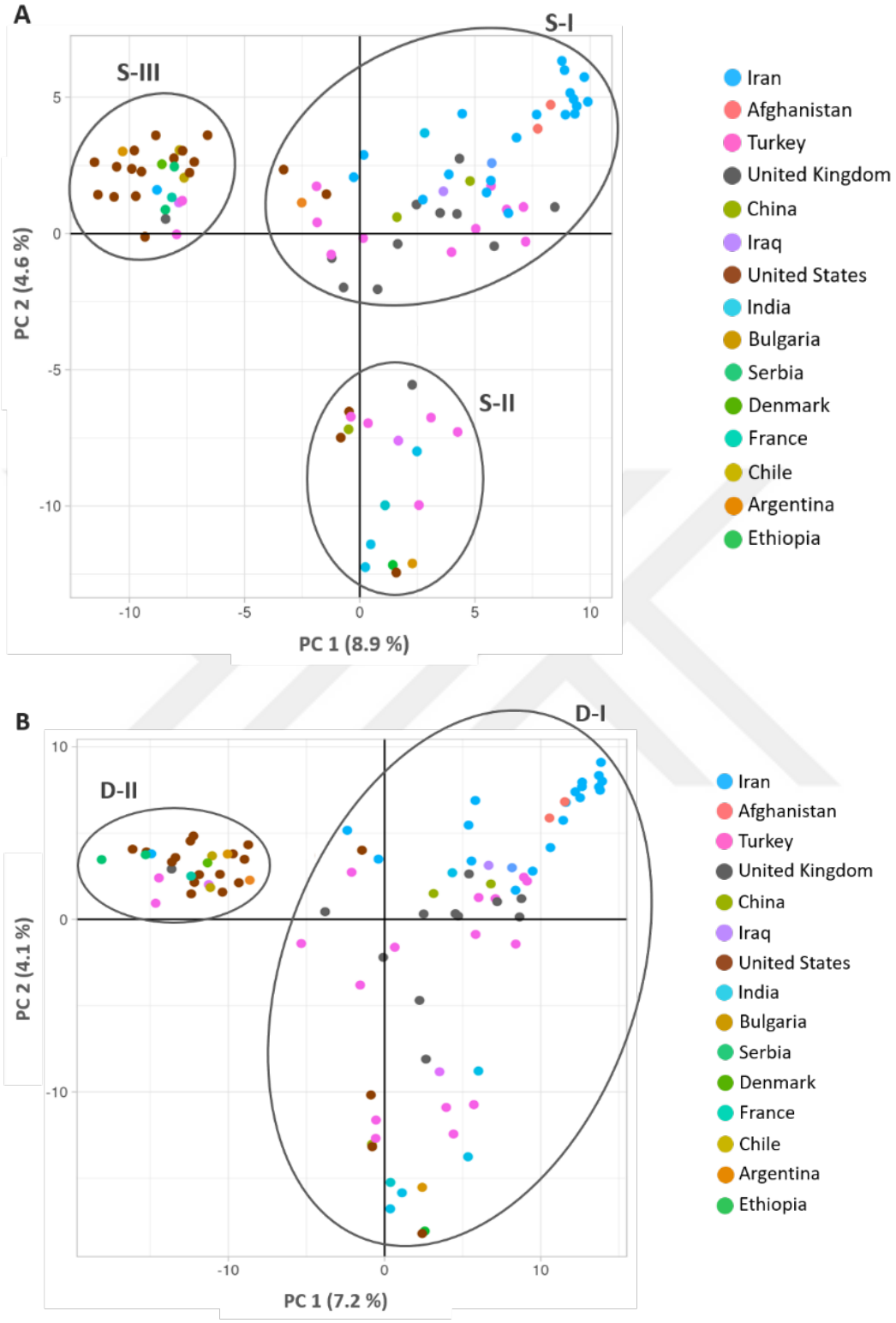

Figure 4.1 Genetic clustering of 94 sugar beet accessions by country of origin based on principal coordinate analysis of 4609 SNPs (A) and 6950 silicoDArT markers (B)

3.6 Genetic Distance and Population Diversity Analysis

To check and confirm genetic variations for each group, UPGMA tree was constructed, visualized, and the results showed clear diversity among genotypes from each group (Figure 4.2, A and B, 4.3 A and B). Overall, a substantial correlation was observed between the UPGMA clusters S-III-B, S-III-A, and S-II, and the SNP-based PCA clusters S-I (materials primarily from the Middle East), S-II (materials of mixed origins), and S-III (materials primarily from the US), respectively.

Relationships between genotypes were detected, first using 4609 SNP markers, and second using 6950 markers of SilicoDArT. When comparing the clustering results from each marker type (SNP and SilicoDArT) with those obtained using the entire marker set, a high degree of consistency was observed, with only rare discrepancies in genotype classification. However, it is important to consider that differences in marker characteristics and the number of markers used for each type may have contributed to slight variations in the detected genetic structure. These minor discrepancies highlight the potential influence of marker selection on genetic analyses but do not significantly affect the overall population structure findings.

In Figure 4.2 A the clusters S-I, S-II, and S-III, indicate that groups revealed by clustering the accessions at a GD < 0.145 (indicated by the vertical dashed blue line). Cluster S-III was further classified into two sub-clusters, S-III-A and S-III-B. Horizontal bold and dashed lines separate genetic clusters and sub-clusters, respectively. Branch support (>50%) is based on 1000 bootstrap replications and shown as a percentage. Figure 4.2 B, created based on the genetic structure of the sugar beet accessions with varying optimal population numbers (K=2, K=3, and K=4), illustrates each accession as a horizontal bar divided into two (K=2), three (K=3), or four-colored segments (K=4), representing their relative membership to the respective clusters. Results from post hoc analyses of the optimal K, testing K values from 1 to 15, are presented in Fig 4.2. ISO country codes are as follows: AF: Afghanistan; AR: Argentina; BG: Bulgaria; CL: Chile; CN: China; CS: Serbia and Montenegro; DK: Denmark; FR: France; GB: United Kingdom; GR: Greece; IN: India; IQ: Iraq; IR: Iran; ET: Ethiopia; TR: Türkiye; US: United States of America. For two accessions, information on the country of origin was ‘not available’ (n.a.).

In Figure 4.3, A clusters D-I and D-II grouped accessions with GD < 0.175 (indicated by the vertical dashed blue line). Horizontal black lines separate genetic clusters, and four subclusters within D-II are indicated with letters A-D. Branch support (>50%) is based on 1000 bootstrap replications and shown as a percentage. Figure 4.3, B which shows the estimated genetic structure for the sugar beet collection. Each accession is represented by a horizontal bar partitioned into two colored segments (K=2), indicating their relative membership to the two clusters. ISO country codes are as follows: AF: Afghanistan; AR: Argentina; BG: Bulgaria; CL: Chile; CN: China; CS: Serbia and Montenegro; DK: Denmark; FR: France; GB: United Kingdom; GR: Greece; IN: India; IQ: Iraq; IR: Iran; ET: Ethiopia; TR: Türkiye; US: United States of America. For two accessions, information on the country of origin was ‘not available’ (n.a.).

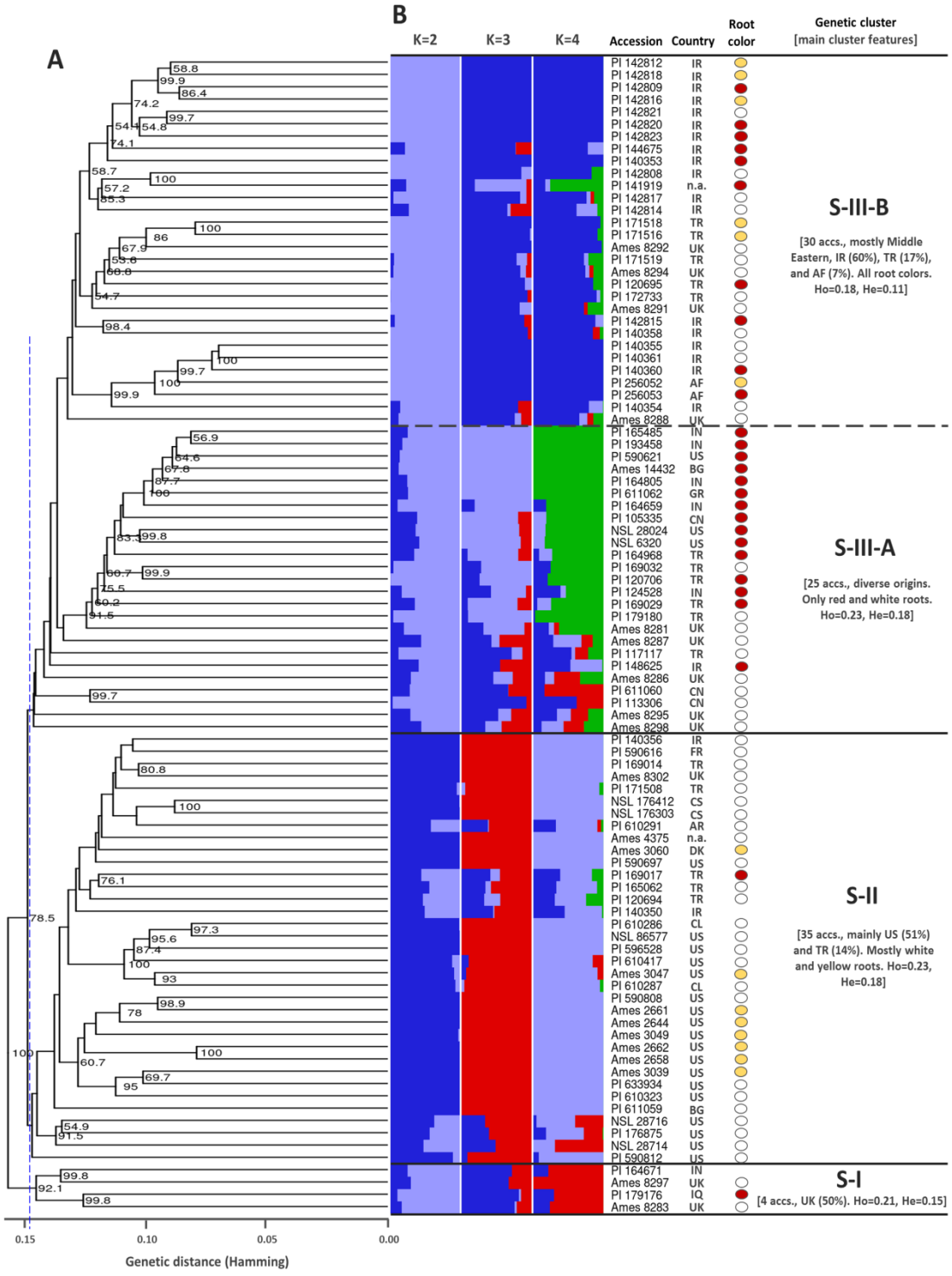

Figure 4.2 Genetic relationships and population structure for 94 sugar beet accessions based on 4609 SNP markers. (A) UPGMA dendrogram based Hamming genetic distance (GD). (B) Genetic structure of the sugar beet accessions considering different optimal number of populations (i.e., K=2, K=3, and K=4)

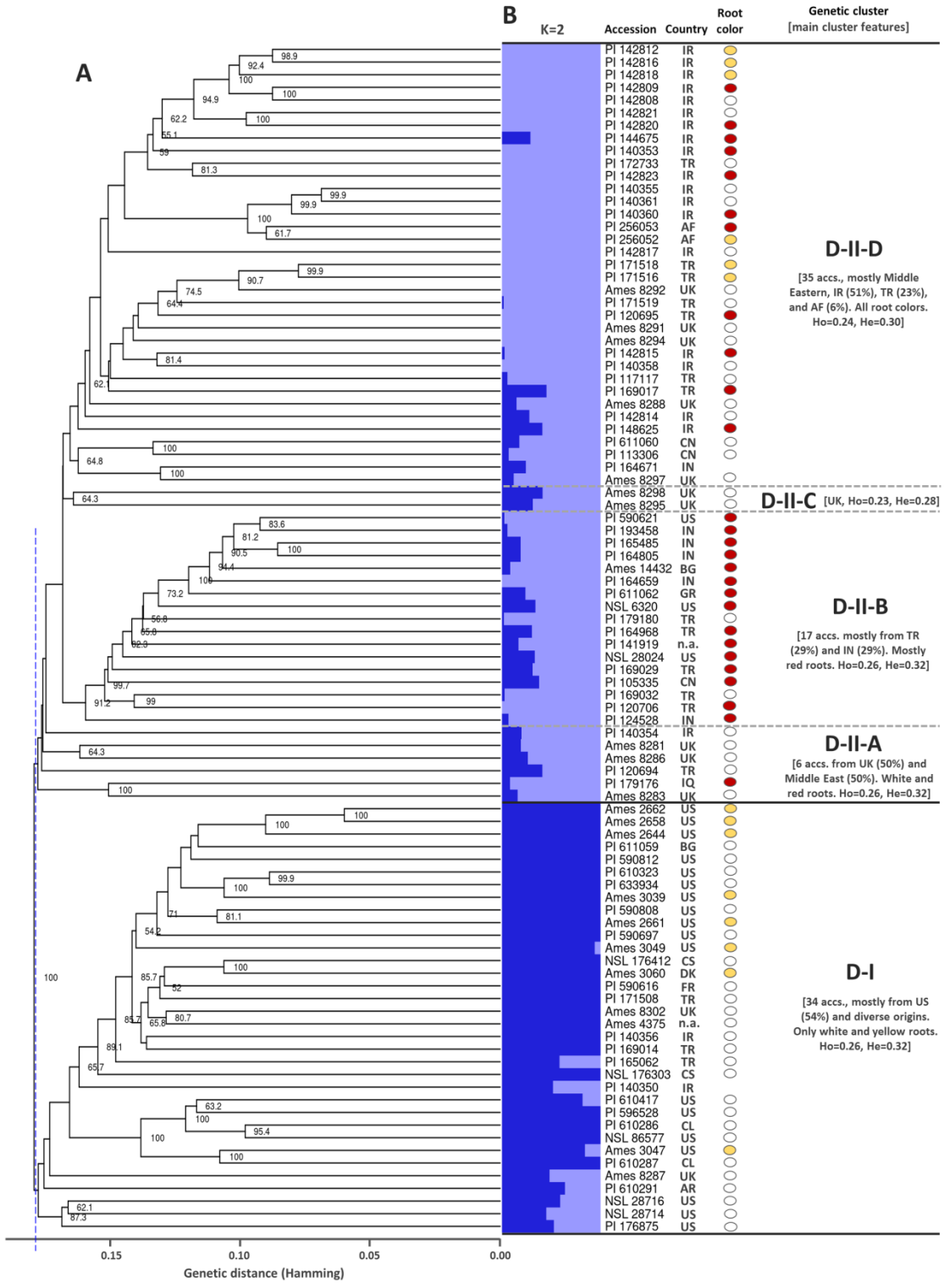

Figure 4.3 Genetic relationships and population structure for 94 sugar beet accessions based on 6950 silicoDArT markers. (A) UPGMA dendrogram based Hamming genetic distance (GD). (B) Estimated genetic structure for the sugar beet collection

The analysis of leaf characteristics among the 94 sugar beet accessions revealed significant variations in blade color, shape, vein coloration, margin undulation, and leaf length (Table 4.4). The majority of accessions exhibited green leaf blades, while a few displayed red or a combination of green and red pigmentation, suggesting potential genetic differences in pigmentation. Most accessions had broad leaf blades, with fewer showing medium or narrow shapes. Red coloration of veins was generally weak or absent in most accessions, though some displayed medium to very strong red pigmentation, particularly in accessions such as PI 590621. The undulation of leaf margins varied, with weak undulation being the most common, while a few accessions exhibited medium to strong undulation. Leaf length ranged from as short as 3 cm in Ames 8297 and Ames 8286 to as long as 36 cm in PI 142821, with broader leaf blades generally corresponding to longer leaf lengths. Additionally, some accessions, including PI 140350, PI 164671, and PI 610291, were marked as "No Plant", indicating unavailable data. Overall, while most accessions shared common leaf traits, specific accessions exhibited distinct characteristics, highlighting the diversity within the sugar beet germplasm.

Table 4.4 Leaf characteristics of the 94 sugar beet accessions markers

Accessions No.	Blade color	Blade shape	Red coloration of veins	Undulation of margins	Length cm
Ames 2644	Green	Narrow	Weak	Weak	19
Ames 2658	Green	Narrow	Weak	Medium	13
Ames 2661	Green	Broad	Weak	Strong	22
Ames 2662	Green	Medium	Weak	Medium	20
Ames 3039	Green	Broad	Weak	Weak	18
Ames 3047	Green	Broad	Weak	Medium	22
Ames 3049	Green	Medium	Weak	Weak	27
Ames 3060	Green	Medium	Weak	strong	27
Ames 4375	Green	Medium	Medium	Medium	12
Ames 8281	Green	Medium	Absent	Absent	5
Ames 8283	Green	Narrow	Absent	Medium	11
Ames 14432	Red	Medium	Strong	Medium	17
NSL 6320	Green	Broad	Weak	Weak	14
NSL 28024	Green/Red	Broad	Strong	Weak	14
NSL 28714	Green	Broad	Absent	Medium	20
NSL 28716	Green	Broad	Absent	Medium	18
NSL 86577	Green	Medium	Absent	Absent	16

Table 4.4 Leaf characteristics of the 94 sugar beet accessions markers (continued)

Accessions No.	Blade color	Blade shape	Red coloration of veins	Undulation of margins	Length cm
NSL 176303	Green	Broad	Absent	Weak	15
PI 105335	Red	Broad	Strong	Medium	15
PI 113306	Green	Broad	Absent	Weak	21
PI 117117	Green	Broad	Absent	Absent	17
PI 120694	Green	Broad	Absent	Absent	20
PI 120695	Green	Broad	Weak	Weak	18
PI 120706	Green	Broad	Absent	Weak	27
PI 124528	Green	Broad	Medium	Weak	24
PI 140350	No Plant	-	-	-	-
PI 140353	Green	Broad	Medium	Weak	14
PI 140354	Green	Broad	Weak	Weak	21
PI 140355	Green	Broad	Absent	Weak	14
PI 140356	Green	Broad	Absent	Weak	24
PI 140358	Green	Broad	Medium	Weak	13
PI 140360	Green	Broad	Absent	Weak	19
PI 140361	Green	Broad	Absent	Weak	25
PI 141919	Green	Broad	Medium	Weak	11
PI 142808	Green	Broad	Absent	Weak	17
PI 142809	Green	Broad	Medium	Weak	31
PI 142812	Green	Broad	Absent	Weak	30
PI 142814	Green	Broad	Absent	Weak	18
PI 142815	Green	Broad	Absent	Weak	29
PI 142817	Green	Broad	Absent	Weak	18
PI 142818	Green	Broad	Absent	Weak	26
PI 142820	Green	Broad	Absent	Weak	22
PI 142821	Green	Broad	Absent	Weak	36
PI 142823	Green	Broad	Weak	Weak	20
PI 144675	Green	Broad	Weak	Weak	32
PI 148625	Green	Broad	Absent	Weak	23
PI 164659	Green/Red	Broad	Strong	Medium	9
PI 164671	No Plant	-	-	-	-
PI 164805	Green/Red	Broad	Strong	Medium	19
PI 164968	Green/Red	Broad	Very Strong	Medium	20
PI 165062	Green	Broad	Absent	Medium	20
PI 165485	Green/Red	Broad	Very Strong	Medium	13
PI 169014	Green	Broad	Absent	Strong	18
PI 169017	Green	Broad	Medium	Medium	28
PI 169029	Green/Red	Broad	Very Strong	Medium	28
PI 169032	Green	Medium	Absent	Weak	8
PI 171508	Green	Broad	Absent	Medium	16
PI 171516	Green	Broad	Absent	Medium	9
PI 171518	Green	Broad	Absent	Weak	24

Table 4.4 Leaf characteristics of the 94 sugar beet accessions markers (continued)

Accessions No.	Blade color	Blade shape	Red coloration of veins	Undulation of margins	Length cm
PI 171519	Green	Broad	Absent	Weak	20
PI 172733	No Plant	-	-	-	-
PI 176875	Green	Broad	Absent	Medium	22
PI 179176	Green	Broad	Medium	Medium	8
PI 179180	Green	Broad	Absent	Medium	17
PI 193458	Green/Red	Broad	Strong	Medium	16
PI 256052	Green	Narrow	Absent	Weak	12
PI 256053	Green	Broad	Absent	Weak	12
PI 590616	Green	Narrow	Absent	Weak	4
PI 590621	Red	Broad	Very Strong	Medium	11
PI 590697	Green	Broad	Absent	Medium	18
PI 590808 INBRED	Green	Broad	Absent	Medium	20
PI 590812 ANNUAL	Green	Broad	Absent	Medium	10
PI 596528	Green	Broad	Absent	Weak	21
PI 610286	Green	Broad	Absent	Medium	22
PI 610287	Green	Broad	Absent	Medium	13
PI 610291	No Plant	-	-	-	-
PI 610323 MS	Green	Broad	Absent	Medium	20
PI 610417	Green	Broad	Absent	Medium	11
PI 611059	Green	Broad	Absent	Medium	27
PI 611060	Green	Broad	Absent	Weak	5
PI 611062	Red	Broad	Very Strong	Medium	18
PI 142816	Green	Broad	Absent	Absent	27
PI 633934	Green	Broad	Absent	Medium	20
Ames 8302	Green	Broad	Absent	Medium	25
Ames 8295	Green	Broad	Absent	Medium	22
Ames 8294	Green	Broad	Absent	Medium	18
Ames 8298	No Plant	-	-	-	-
NSL 176412	Green	Broad	Absent	Medium	16
Ames 8297	Green	Narrow	Absent	Weak	3
Ames 8286	Green	Narrow	Absent	Weak	3
Ames 8287	Green	Broad	Absent	Weak	5
Ames 8288	Green	Broad	Medium	Weak	15
Ames 8291	Green	Broad	Absent	Weak	17
Ames 8292	No Plant	-	-	-	-

The evaluation of root characteristics among the 94 sugar beet accessions revealed substantial variation in root shape, tip shape, external color, length, width, and weight (Table 4.5). Root shape in the longitudinal section varied, with the most common categories being types 1 to 6, indicating different morphological traits. Tip shape also

exhibited diversity, ranging from type 1 (pointed) to type 5 (blunt or rounded)**.** External root color was predominantly white, though several accessions displayed yellow or reddish-purple pigmentation, with the latter being particularly notable in accessions such as PI 14432 and PI 120695. Root length ranged from as short as 7 cm in NSL 176412 to as long as 70 cm in PI 176875, highlighting significant differences in growth potential. Similarly, root width varied widely, with some accessions exhibiting narrow roots, such as 2 cm in Ames 8286, while others, such as PI 611059, had a width of 39 cm**.** Root weight also displayed considerable variation, ranging from 10 gr in Ames 8297, Ames 8286, and PI 611060 to 2676 gr in PI 144675, indicating significant differences in biomass accumulation. Additionally, some accessions, such as PI 140350, PI 164671, and PI 610291**,** were marked as "No Plant", signifying missing or unavailable data. The diversity in root characteristics across accessions suggests potential for selection in breeding programs aimed at optimizing root morphology, size, and biomass for improved yield and adaptability.

Table 4.5 Root characteristics of the 94 sugar beet accessions markers

Accessions No.	Shape in longitudinal section	Shape of tip	External color	Length cm	Width cm	Weight gr
Ames 2644	4	1	Yellow	16	5	118
Ames 2658	3	2	Yellow	37	8.5	307.4
Ames 2661	6	1	Yellow	23	8	606.3
Ames 2662	5	1	Yellow	27	7.5	184.3
Ames 3039	4	1	Yellow	28	6.5	369.3
Ames 3047	4	1	Yellow	44	6.5	465.5
Ames 3049	4	1	Yellow	29	11	120.7
Ames 3060	3	4	Yellow	18	9	856.3
Ames 4375	6	1	White	30	13	1300
Ames 8281	4	1	White	12	2	20
Ames 8283	6	1	White	28	6	74
Ames 14432	3	2	Reddish purple	21	12	393
NSL 6320	3	3	Reddish purple	28	10	308
NSL 28024	3	2	Reddish purple	14	7	106
NSL 28714	5	3	White	36	9	412
NSL 28716	1	4	White	24	11	572
NSL 86577	5	1	White	22	11	546

Table 4.5 Root characteristics of the 94 sugar beet accessions markers (continued)

Accessions No.	Shape in longitudinal section	Shape of tip	External color	Length cm	Width cm	Weight gr
NSL 176303	3	2	White	28	14	555
PI 105335	6	1	Reddish purple	20	4	66
PI 113306	3	1	White	31	7	376
PI 117117	5	1	White	24	8	272
PI 120694	3	3	White	20	17	1150
PI 120695	1	3	Reddish purple	28	14	830
PI 120706	2	2	Reddish purple	21	13	508
PI 124528	2	2	Reddish purple	21	14	952
PI 140350	No Plant	-	-	-	-	-
PI 140353	2	2	Reddish purple	17	11	314
PI 140354	1	3	White	16	13	584
PI 140355	2	3	White	26	18	692
PI 140356	1	5	White	21	9	310
PI 140358	3	2	White	19	14	785
PI 140360	2	3	Reddish purple	24	21	965
PI 140361	3	1	White	18	12	278
PI 141919	3	2	Reddish purple	18	10	222
PI 142808	2	3	White	24	18	820
PI 142809	1	3	Reddish purple	30	25	1328
PI 142812	1	3	Yellow	42	32	1158
PI 142814	4	4	White	21	10	344
PI 142815	2	4	Reddish purple	34	28	2430
PI 142817	3	4	White	23	8	192
PI 142818	2	2	Yellow	20	23	812
PI 142820	1	4	Reddish purple	27	11	452
PI 142821	1	3	White	27	24	1514
PI 142823	3	4	Reddish purple	30	7	76
PI 144675	1	4	Reddish purple	40	36	2676
PI 148625	2	4	Reddish purple	24	22	1476
PI 164659	3	1	Reddish purple	17	11	482
PI 164671	No Plant	-	-	-	-	-
PI 164805	2	2	Reddish purple	20	14	580
PI 164968	2	2	Reddish purple	37	18	1078
PI 165062	5	1	White	37	14	569
PI 165485	3	1	Reddish purple	15	8	188
PI 169014	2	4	White	28	16	718
PI 169017	2	4	Reddish purple	33	16	1138
PI 169029	3	4	Reddish purple	35	13	873
PI 169032	3	4	White	17	7	740
PI 171508	3	4	White	31	16	310
PI 171516	3	1	Yellow	20	8	158
PI 171518	2	3	Yellow	30	16	1132
PI 171519	2	4	White	31	17	1088
PI 172733	No Plant	-	-	-	-	-
PI 176875	4	4	White	70	10	708
PI 179176	5	1	Reddish purple	13	4	26
PI 179180	5	4	White	33	5	127

Table 4.5 Root characteristics of the 94 sugar beet accessions markers (continued)

Accessions No.	Shape in longitudinal section	Shape of tip	External color	Length cm	Width cm	Weight gr
PI 193458	2	4	Reddish purple	20	13	308
PI 256052	3	4	Yellow	26	8	120
PI 256053	2	4	Reddish purple	28	14	532
PI 590616	3	4	White	21	6	74
PI 590621	3	2	Reddish purple	21	7	114
PI 590697	3	4	White	26	11	468
PI 590808 INBRED	3	4	White	22	9	622
PI 590812 ANNUAL	4	4	White	19	4	28
PI 596528	4	4	White	32	10	538
PI 610286	3	4	White	27	10	718
PI 610287	3	4	White	33	9	340
PI 610291	No Plnat	-	-	-	-	-
PI 610323 MS	3	4	White	31	8	374
PI 610417	3	4	White	23	6	100
PI 611059	2	4	White	13	39	898
PI 611060	5	4	White	17	2	10
PI 611062	1	3	Reddish purple	21	5	72
PI 142816	1	4	Yellow	31	21	980
PI 633934	4	4	White	35	12	786
Ames 8302	4	4	White	26	11	570
Ames 8295	4	3	White	26	13	508
Ames 8294	5	4	White	19	7	256
Ames 8298	No Plant	-	-	-	-	-
NSL 176412	4	4	White	7	28	374
Ames 8297	4	4	White	17	3	10
Ames 8286	6	2	White	16	2	10
Ames 8287	4	4	White	20	5	104
Ames 8288	4	3	White	20	5	322
Ames 8291	2	4	White	20	3	10
Ames 8292	No Plant	-	-	-	-	-

As it shown in Figure 4.2, 4.3, and Table 4.6, by using UPGMA dendrogram-based hamming genetic distance, the plants were divided into four main clusters, S-I, S-II, S-III A, and S-III B, by using SNP marker, and D-I, D-II by using SilicoDArT, and when we linked these results with the morphological traits were studied that all the plants vary in the studied traits (Flowering, blade color, external color of root, and root shape).

The Table 4.6 highlights how different genetic markers (SNP and SilicoDArT) are associated with specific trait variations in sugar beet genotypes. For each trait, the table shows the percentage distribution across variations in specific clusters, reflecting

the prevalence of these traits within each marker type. Furthermore, the clusters show distinct trait distributions, suggesting variability in marker efficiency or association with specific genetic traits. The flowering trait ranged from (36.40-24.00) % among the clusters, and for the non-flowering was ranged from (76.00-63.60) % among the clusters. For the green blade color was ranged from (100.00-54.16) %, the red color was ranged from (16.67-0.00) %, and for the green/red was ranged (29.17-0.00) %. The leaf external color was in three colors (white, reddish, and yellow), the white color was ranged from (75.00-37.50) %, the reddish color was ranged from (62.50-0.00) %, and the yellow color ranged from (76.67-0.00) %. The shape in the longitudinal section, was observed in six shapes. The first was Transverse narrow elliptic ranged between (29.03-0.00) %, the second was transverse medium elliptic ranged between (32.25-0.00) %, the third was circular ranged between (40.00-0.00) %, the fourth was obovate ranged between (34.38-8.93) %, the fifth shape was narrow oblong was ranged between (33.30-3.22) %, and finally the sixth shape was very narrow obovate was ranged between (33.30-3.22) %.

SNP and SilicoDArT markers were used to identify genetic variations and their association with phenotypic traits. For instance, under the Flowering trait, the percentage of flowering genotypes varies between SNP clusters (e.g., 25% in S-I vs. 36.4% in S-III B) and SilicoDArT clusters (e.g., 29.42% in D-1 vs. 30% in D-2). Under the Blade Color trait, green color dominates across all clusters, but mixed red/green color is observed more in SNP cluster S-III A (29.17%) and SilicoDArT D-2 (5.36%).

Root external color shows notable differences between markers. SNP: White root color is highly prevalent (e.g., 66.67% in S-I), but reddish and yellow roots vary significantly (e.g., reddish in S-III A is 62.5%, while yellow is 0% in S-II but 26.67% in S-III A). SilicoDArT: D-1 and D-2 clusters also display distinct patterns, such as 75% white roots in D-1 compared to 48.21% in D-2. Shape in longitudinal section has multiple categories (1 to 6). The distribution across SNP and SilicoDArT markers varies, highlighting the genetic diversity linked to this trait. For instance, in SNP clusters, category 3 (circular) is prominent in S-II (40%) and S-III B (41.67%). In SilicoDArT clusters, D-2 has the most significant proportion for category 3 (33.93%).

The difference in plant characteristics might be linked to genetic variations associated with another trait. Moreover, the cluster S-I contain three countries UK,

India, and Iraq, This similarity may go back to the period of World War I, in which the United Kingdom invaded Iraq, With the help of Indian recruits, who played a role in transporting some of these plants between these three countries. Furthermore, S-III B contain three main countries (Iran, Turkey, and Afghanistan), in addition to UK, and USA, the similarity between these three countries is due to the commercial relationship and common history between them. As for the United Kingdom and the United States of America, they are considered among the countries that claim to obtain this plant to study it, perhaps, and then attribute it genetically to them over time.

Table 4.6 The percentage of each studied trait in sugar beet genotypes both the SNP, and SilicoDArT markers

Traits	Variation	SNP				SilicoDArT	
		S-I	S-II	S-III A	S-III B	D-1	D-2
Flowering	Yes	25.00 %	28.12 %	24.00 %	36.40 %	29.42 %	30.00 %
	No	75.00 %	71.88 %	76.00 %	63.60 %	70.58 %	70.00 %
Blade Color	Green	100.00 %	100.00 %	54.16 %	100.00 %	100.00 %	80.35 %
	Red	0.00 %	0.00 %	16.67 %	0.00 %	0.00 %	7.15 %
	Green/red	0.00 %	0.00 %	29.17 %	0.00 %	0.00 %	12.50 %
Root external color	White	66.70 %	70.00 %	37.50 %	48.40 %	75.00 %	41.07 %
	Reddish	33.30 %	3.33 %	62.50 %	32.25 %	0.00 %	48.21 %
	Yellow	0.00 %	26.67 %	0.00 %	19.35 %	25.00 %	10.72 %
Shape in longitudinal section	1	0.00 %	3.33 %	4.17 %	29.03 %	6.25 %	16.08 %
	2	0.00 %	10.00 %	25.00 %	32.25 %	6.25 %	30.35 %
	3	0.00 %	40.00 %	37.50 %	22.58 %	34.38 %	30.35 %
	4	33.30 %	30.00 %	12.50 %	9.70 %	34.38 %	8.93 %
	5	33.30 %	13.33 %	12.50 %	3.22 %	12.50 %	8.93 %
	6	33.30 %	3.33 %	8.33 %	3.22 %	6.25 %	5.36 %

Shape in longitudinal section;1) transverse narrow elliptic 2) transverse medium elliptic 3) circular 4) obovate 5) narrow oblong 6) very narrow obovate.

3.7 Defining Population Structure

A major challenge in association studies is the potential for population stratification to introduce false positives (Zhao et al., 2007a). To address this issue, various biometrical models have been developed for detecting and accounting for population structure (Zhu et al., 2008). A widely used technique, offered by Pritchard et al. (2000), employs molecular marker data within a Bayesian framework to estimate group membership probabilities for genotypes. In sugar beet, population structure and

intraspecific differentiation were analyzed using FASTSTRUCTURE (Pritchard et al., 2000; Raj et al., 2014; Stephens and Pritchard, 2014).

To determine the optimal number of populations (K), the admixture model was applied, correlating allele frequencies across individuals. Structure analysis involved running at least 100 replicates of K values ranging from 1 to 15, selecting the most parsimonious model based on the median likelihood (L50). The Delta K (ΔK) method which, evaluates the rate of change in the log probability of data across successive K values, was used to determine the most suitable number of genetic clusters (Evanno et al., 2005). The FASTSTRUCTURE analysis identified an optimal K value of 2, indicating that the sugar beet genotypes can be effectively categorized into two distinct genetic groups.

To validate these results, upgma and PCA were conducted as complementary approaches. PCA revealed significant genetic diversity, particularly among landrace groups. Turkish and foreign sugar beet varieties exhibited close associations, with admixture patterns supporting their relatedness within the broader gene pool. When comparing different methodologies, the substructure identified in sugar beet collections aligned with the FASTSTRUCTURE-determined K value. Additionally, tree clustering analysis produced groupings that closely matched the K3 structure bar plot, indicating strong agreement between methods.

Overall, population structure analysis confirmed that sugar beet genotypes can be effectively classified based on geographical origin. However, Bayesian approaches like STRUCTURE rely on predefined models and assumptions, which necessitate careful interpretation of results. To ensure a robust analysis, distance-based methods such as factorial analysis, which do not impose prior assumptions on the data, should be used to cross-validate findings.

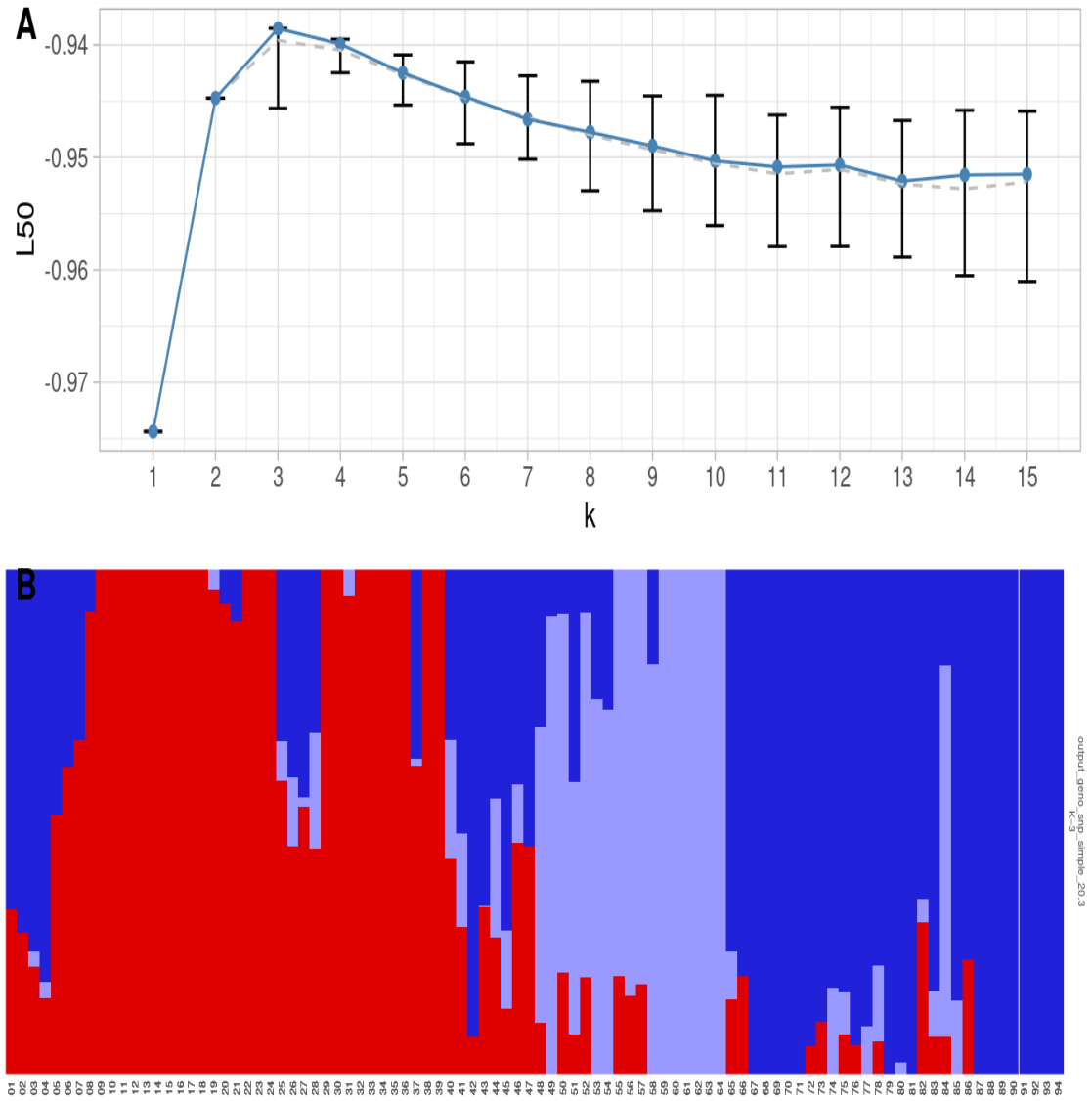

Figure 4.4 The structure using SNP data. A) Median likelihood of structure models ranging from 1 to 15 clusters, based on 100 replicates. B) Structure barplot (distruct) of individuals using K = 3

5. DISCUSSION

This study, while accounting for the limitations present in existing literature, aimed to assess the population structure and genetic diversity of global sugar beet germplasm sourced from 16 countries. The evaluation utilized whole-genome data derived from silicoDArT and SNP markers. Out of the extensive number of SNP and DArT markers identified through GBS analysis, fewer than 4609 SNPs and 6950 DArT markers were employed for genetic diversity assessment, using specific filtering criteria. This approach holds significant importance for sugar beet breeding, as future research on marker-assisted breeding will largely rely on the identification and application of high-throughput markers spanning entire genomes.

The findings of this study provide a detailed understanding of sugar beet germplasm diversity, population structure, and the utility of various molecular markers in breeding programs. These results align with and build upon prior research that has utilized a combination of phenotypic traits and molecular markers to improve sugar beet breeding outcomes. Consequently, the resulting dataset will serve as a valuable resource for future marker-assisted breeding efforts in sugar beet. Several diversity metrics, including genetic diversity, PIC, MAF, observed and expected heterozygosity, and the mean inbreeding coefficient, revealed a substantial level of conserved genetic variation within the USDA sugar beet germplasm.

The AMOVA analysis revealed significant genetic variation among and within populations of the sugar beet germplasm studied driven by geographic, reproductive, and genetic factors, indicating a clear pattern of population differentiation ($p < 0.001$). Both SNP and SilicoDArT markers proved effective in capturing genetic diversity, with complementary strengths that can be leveraged for breeding and conservation. The variance components for both SNP and SilicoDArT markers were significant, supporting the robustness of these findings. Specifically, SNP markers explained a variance component of 0.0069, while SilicoDArT markers contributed slightly higher at 0.0129. These results align with previous studies that reported significant population differentiation in sugar beet populations due to breeding practices, geographical isolation, and genetic drift (Hohmann et al., 2016; McGrath et al., 2021). The high F-statistic values, 0.189 for SNP markers and 0.170 for SilicoDArT markers, further

underscore the substantial genetic differentiation among populations. Similar levels of differentiation were reported by Panella et al. (2014), who found high genetic variance among sugar beet populations across different geographic regions, highlighting the role of distinct selection pressures in shaping genetic diversity.

The genetic diversity indices, including gene diversity (GD), polymorphic information content (PIC), and minor allele frequency (MAF), were nearly identical for SNP and SilicoDArT markers, with values of 0.31 and 0.30 (GD), 0.25 (PIC), and 0.22 (MAF), respectively. This consistency suggests that both marker systems are effective in capturing the genetic diversity of the germplasm under study. However, differences in observed heterozygosity (H_0) and inbreeding coefficients (F) highlight the distinct properties of these marker systems. The higher H_0 value for SilicoDArT markers (0.45) compared to SNPs (0.20) and the negative F value (-0.48) for SilicoDArT markers indicate that these markers may better capture heterozygosity, possibly due to their dominance and ability to detect both homozygous and heterozygous loci (He et al., 2014). In contrast, the positive F value for SNP markers (0.35) suggests a higher level of inbreeding within populations. This is consistent with previous research indicating that SNP markers are more effective at detecting inbreeding due to their co-dominant nature and higher resolution (Alheit et al., 2011).

The effective number of alleles (Ne) differed significantly between SNP (134.58) and SilicoDArT (-98.62) markers, reflecting the distinct genetic architectures captured by each marker type. While the negative Ne for SilicoDArT markers might indicate an issue with allele frequency distribution or population structure assumptions, the positive and high Ne for SNP markers support their utility in capturing genetic variability within populations. This is consistent with findings by Kuleung et al. (2004), who observed a higher Ne value in SNP-based studies due to their finer resolution.

The variance attributable to additive (Va) and dominance (Vd) effects also highlighted the distinct contributions of SNP and SilicoDArT markers. The higher Va for SilicoDArT markers (6972.85) compared to SNPs (3894.17) may indicate a stronger contribution of these markers to capturing population-wide additive genetic variation. This is in line with the findings of Biscarini et al. (2016), who reported that dominant markers are particularly effective for detecting additive genetic effects in structured populations.

The significant genetic variation observed among populations has critical implications for breeding and conservation. Populations with high genetic diversity, as indicated by GD values, provide a valuable reservoir of alleles for breeding programs aimed at improving stress tolerance, disease resistance, and yield in sugar beet (Raggi et al., 2019). Conversely, populations with high inbreeding coefficients may require strategies to enhance genetic diversity, such as introducing diverse germplasm or implementing crossbreeding programs. The high observed heterozygosity in SilicoDArT markers suggests their utility in detecting outcrossing events and gene flow, which can be leveraged in breeding programs to maintain or increase genetic diversity. Additionally, the results emphasize the importance of maintaining populations with high genetic variation for conservation purposes, as they serve as a genetic buffer against environmental and disease pressures (Wen et al., 2020).

This study suggests that exploring correlations may provide a foundation for the indirect selection of desirable traits in sugar beet breeding. PCA revealed that the first five principal components captured the majority of the information across 20 phenotypic traits, with the first principal component emphasizing above-ground plant characteristics. This research offers theoretical insights for variety selection and germplasm innovation. The primary criterion for evaluating the success rate of variety assignment, population structure, and clustering is the ability to group varieties into the same populations as those identified using a combined set of all markers. It was assumed that an infinite number of markers would perfectly characterize population structure and that any sufficiently large marker set would yield similar results (Van Hintum et al., 2007). The progression of success-rate curves supports this assumption, demonstrating that a substantial number of markers, regardless of type, can produce the same variety groupings as the full marker set. The empirical results align with modeling simulations, which show that clustering error rates decrease significantly with an increasing number of marker loci (Guillot et al., 2010).

The PCA analysis revealed significant genetic structuring and diversity among sugar beet accessions, reflecting the influence of geographic origins, breeding practices, and evolutionary history. The complementary use of SNP and SilicoDArT markers provided a comprehensive understanding of genetic relationships, offering valuable insights for breeding and conservation strategies. These findings contribute to the

growing body of knowledge on sugar beet genetic diversity and highlight the importance of maintaining genetic variation in crop improvement programs.

The clustering patterns observed in PCA were largely consistent with geographical origins and breeding histories. For example, S-I comprised tightly grouped genotypes from Iran, Türkiye, the UK, and a few other regions, suggesting limited genetic variation possibly due to selective breeding or shared ancestry. Similarly, the genetic homogeneity in D-II supports the notion of a genetically uniform group shaped by breeding practices. These findings are consistent with previous studies that reported strong genetic structuring in sugar beet populations due to breeding and geographic isolation (Hohmann et al., 2016; McGrath et al., 2021). Clusters S-III (SNP-based) and D-I (SilicoDArT-based) exhibited higher genetic diversity compared to other groups. S-III included accessions from the US and Europe, regions known for their diverse sugar beet germplasm (Grimmer et al., 2007). Similarly, the wide spread of D-I along PC1 in the SilicoDArT-based PCA suggests the presence of alleles from both wild and cultivated relatives, reflecting greater genetic variation. The high diversity in these clusters aligns with findings by Wen et al. (2020), who reported that sugar beet populations in the US and Europe harbor significant genetic variation due to introgressions from wild relatives. While both SNP and SilicoDArT markers revealed consistent patterns of genetic structuring, slight differences in clustering were observed. For instance, S-II (SNP-based) and D-II (SilicoDArT-based) shared similarities in genetic homogeneity, but the substructuring of populations differed slightly. These differences could be attributed to the nature of the markers. SNPs, being co-dominant markers, provide finer resolution for distinguishing closely related individuals, while SilicoDArT markers, which are dominant, tend to capture broader genetic differences. Similar discrepancies between marker systems have been reported in other crop species (He et al., 2014; Kuleung et al., 2004). However, the congruence in overall clustering patterns across marker systems underscores their reliability in assessing genetic relationships. Studies comparing marker systems in other crops, such as wheat and maize, also found high correlations between SNP and dominant markers in detecting genetic diversity (Alheit et al., 2011; Biscarini et al., 2016).

The UPGMA dendrograms based on both SNP and SilicoDArT markers demonstrated clear clustering patterns. The SNP-based UPGMA tree revealed three

major clusters, S-I, S-II, and S-III, with further sub-clustering in S-III-A and S-III-B. These findings were supported by bootstrap values greater than 50% across most branches, suggesting robust phylogenetic relationships. The clustering of accessions was consistent with their geographical origins, as S-I primarily contained materials from the Middle East, S-II consisted of mixed-origin materials, and S-III predominantly grouped U.S. materials. These results align with previous studies emphasizing the role of geographic origin in shaping genetic diversity in sugar beet germplasm (McGrath et al., 2021; Hohmann et al., 2016).

The genetic structure analysis further supported the UPGMA clustering, as population subdivisions were evident at different levels of K. For instance, at K=2, accessions were broadly divided into two major clusters, while finer subdivisions were evident at K=3 and K=4. The presence of distinct genetic clusters reflects the effects of breeding history and geographical isolation, which have been reported in earlier studies on sugar beet diversity (Grimmer et al., 2007; Wen et al., 2020).

In this study, achieving a 100% success rate for clustering required approximately three times more SNP markers. However, selecting only highly polymorphic loci significantly reduced the number of markers needed to reach the same success rate (Jones et al., 2007). The ΔK criterion proposed by Evanno et al. (2005) yielded the highest value, as this method is known to detect the primary structure level (Lia et al., 2009), which primarily distinguishes genotypes. Our findings align with previous research suggesting that using a single covariate in association models cannot fully account for genotype stratification (Mezmouk et al., 2011). Moreover, Evanno et al. (2005) indicated that partially sampled genotypes result in a lower ΔK at the true K. Similarly, Kalinowski (2011) demonstrated that unbalanced sample sizes may exacerbate errors. Evanno et al. (2005) further noted that the actual number of groups is best identified by the modal value of ΔK, a measure based on the second-order rate of change in the likelihood function with respect to K.

The SilicoDArT markers provided comparable but slightly distinct clustering results. The two major clusters, D-I and D-II, were consistent with the SNP-based clusters but revealed additional sub-structuring within D-II (subclusters A–D). These differences could be attributed to the inherent nature of the markers used. SilicoDArT markers, being dominant markers, often capture different aspects of genetic variation

compared to SNP markers, which are codominant and provide finer resolution. Such discrepancies have been previously reported in genetic studies comparing marker systems (He et al., 2014; Kuleung et al., 2004). Notably, the high congruence between SNP-based and SilicoDArT-based results suggests that both marker systems are reliable for studying genetic relationships in sugar beet. However, the slight differences in clustering patterns highlight the importance of considering multiple marker systems to obtain a comprehensive understanding of genetic diversity.

Understanding population structure is critical for interpreting genetic diversity and avoiding false positives in association studies (Zhao et al., 2007b). In this study, Bayesian clustering analysis using FASTSTRUCTURE revealed an optimal population structure of two clusters (K = 2), as determined by the ΔK method proposed by Evanno et al. (2005). This methodology has been widely used for genetic studies, offering reliable insights into the substructure of populations by maximizing the likelihood of observed genetic data.

The high diversity observed among genotypes, particularly in landrace groups, aligns with findings from other studies that highlight the genetic richness of landraces compared to modern cultivars (Barbosa et al., 2021). The clustering of Turkish and foreign varieties, which showed admixture and close genetic associations, reflects historical gene flow and breeding practices that likely influenced genetic structure. These results are consistent with the work of McGrath et al. (2021), who reported significant genetic intermixing in global sugar beet germplasm collections. To validate the results from FASTSTRUCTURE, complementary analyses such as principal component analysis (PCA) and UPGMA were employed. These distance-based methods provided consistent results, further supporting the reliability of the Bayesian approach. Notably, the clustering at K = 3 in the structure analysis corresponded closely with tree clustering results, indicating a high degree of agreement between the methodologies. Distance-based methods, such as PCA, have the advantage of being model-independent and thus free from the assumptions inherent in Bayesian approaches. This independence makes them a valuable cross-validation tool for population structure analysis, as highlighted by recent studies in crop genetics (Wen et al., 2020). The landrace groups, which displayed high diversity in PCA, further underline the importance of considering multiple approaches to capture the full complexity of population structure.

Monteiro et al. (2017) explored high-throughput genotyping approaches and PCA-based population structure analyses, which revealed clear clustering patterns and substantial gene flow between sugar beet populations. Their methodology parallels this study's PCA findings, which showed that the first principal component explained most of the above-ground phenotypic variation. This reinforces the validity of using PCA as a tool for grouping germplasm based on key phenotypic and genotypic features. Longin et al. (2015) employed genome-wide association studies (GWAS) to link genetic loci with agronomically important traits in sugar beet. Their findings support this study's proposition that correlations between genetic markers and phenotypic traits can serve as a foundation for indirect selection in breeding programs. The use of PCA and PCoA to cluster genotypes into distinct groups in this study complements Longin et al. (2015) findings on the practical application of genetic diversity analyses for trait improvement. Meyer et al. (2011) conducted a comparative evaluation of DArT, SSR, and SNP markers, concluding that SNPs provide higher resolution and polymorphism rates compared to other marker systems. Consistent with this, our study observed that SNP markers yielded better diversity indices than silicoDArT markers, supporting Meyer et al. (2011) assertion about the superiority of SNPs for genetic studies. This suggests that SNP-based genotyping remains a robust choice for characterizing sugar beet populations. Panella et al. (2014) combined molecular and phenotypic data to evaluate sugar beet hybrid breeding strategies. They emphasized the importance of molecular markers in identifying superior parental lines and predicting hybrid performance. Similarly, this study's findings, such as the clustering patterns observed between breeding lines and landraces, provide valuable insights for hybrid design and germplasm utilization in sugar beet breeding.

Genotypes within the same cluster are likely to share genetic similarities, making them suitable candidates for selection in breeding strategies aimed at preserving or enhancing specific traits. Conversely, individuals from different clusters may be used for hybridization to increase genetic diversity and heterosis, as demonstrated in studies on other crops such as maize and wheat (Franco et al., 2018; Zhou et al., 2021). Moreover, the observed admixture between Turkish and foreign varieties suggests that these populations could serve as bridges for gene flow, potentially enhancing genetic diversity in breeding programs. This is particularly important for addressing challenges

such as climate change and disease resistance, where diverse genetic resources are key to developing resilient varieties (Hohmann et al., 2016).

In clustering and structure analyses, all varieties are assigned to a predefined number of populations or clusters. Interestingly, when detecting population structure, STRUCTURE analysis required the same or fewer loci compared to clustering methods. This observation is valuable for determining the appropriate analytical method when marker availability is limited. However, these findings are based on a single set of hybrid varieties with a highly structured population. Additional studies on populations with both similar and different structures are necessary to validate the differences observed in STRUCTURE results.

Typically, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Principal Component Analysis (PCA) are used as complementary methods to validate STRUCTURE findings. In PCA, the genotypes displayed considerable diversity, particularly within the landrace groups. The results demonstrated low differentiation between groups, indicating significant gene flow among them. PCA analysis of the plant material classified the genotypes into three major categories, and the clustering pattern of breeding lines and improved cultivars was further supported by the short pairwise genetic distances between populations. These findings are consistent with previous molecular marker studies. For example, Rispali et al. (2023) conducted PCoA on 325 accessions using DArT markers, identifying three genetic clusters. A detailed analysis of these clusters further revealed notable associations, reinforcing the genetic structure observed in the current study. In addition, McGrath et al. (2007) emphasized the critical role of molecular markers such as SSRs, SNPs, and DArTs in sugar beet breeding, particularly for enhancing disease resistance and yield traits. Their work underscores the importance of integrating high-throughput marker systems for efficient genetic analysis and marker-assisted selection. Similarly, this study's use of SNP and silicoDArT markers aligns with McGrath's recommendation for deploying high-resolution marker systems to capture population structure and diversity accurately.

The combined insights from these studies and the current findings highlight the importance of leveraging diverse molecular markers and analytical approaches in sugar beet breeding programs. By integrating high-throughput genotyping techniques such as SNP and silicoDArT markers with robust statistical tools like PCA and STRUCTURE,

breeders can gain a deeper understanding of genetic diversity and population structure. This facilitates the selection of superior germplasm, the design of effective hybrid combinations, and the identification of genetic loci associated with key agronomic traits. The broader application of these approaches across structured and diverse populations will further enhance sugar beet breeding efforts, enabling the development of cultivars with improved yield, disease resistance, and adaptability.

6. CONCLUSION

Sugar, a vital component of human diets, is derived primarily from sugar cane and sugar beet, with the latter's production highly influenced by climatic conditions. Consequently, characterizing sugar beet germplasm is essential for developing improved cultivars. This study evaluated the genetic diversity and population structure of international sugar beet germplasm from 16 countries using SNP and SilicoDArT markers.

The findings revealed a considerable level of genetic diversity within the USDA sugar beet germplasm, with SNP markers exhibiting higher diversity values compared to SilicoDArT markers. The lower diversity observed could be attributed to differences in germplasm composition, the higher number of SNP and SilicoDArT markers used, and similarities in geographic collections relative to previous studies.

The study identified Ames 2644 and Ames 8297 as the most genetically distinct genotypes, making them ideal candidates for sugar beet breeding and future genetic research in this species. Genetic distances between accessions were highlighted as crucial for breeding efforts. Germplasm was categorized into three main clusters: S-I, S-II, and S-III. The smallest cluster, S-I, included 35 accessions, 51% of which were of USA origin, while 14% were European. The historical origins of sugar beet in Germany in the 18th century and its global dissemination were noted, with USDA germplasm collection and breeding activities contributing to genetic similarities between USA, Turkish, and European accessions. S-III emerged as the largest cluster, encompassing 55 accessions from the Middle East, Southeast, and East Asia.

SilicoDArT marker analysis identified genetically distinct genotypes in NSL 176303 (Serbia) and PI 140355 (Montenegro), differing from SNP marker results. This underscores the necessity of prioritizing research on specific genotypes and utilizing both marker systems in breeding programs. Germplasm was further classified into two groups, D-I and D-II, with D-I containing 90% of USA accessions and resembling SNP-based UPGMA clustering. In contrast, the D-II cluster exhibited greater diversity, with accessions from the UK, Türkiye, Iran, and Iraq. The SNP-based clustering aligned with D-I, and the SilicoDArT markers-based PCA clustering supported UPGMA and STRUCTURE results.

AMOVA confirmed that genetic variation was primarily due to differences within clusters. For SNPs, the majority of genetic variation was observed among the accessions (i.e., within clusters), making up 71.3% of the total variation, while variation among clusters accounted for 28.7%. Similarly, for silicoDArTs, most genetic variation occurred among the accessions (i.e., within clusters), ranging from 74.5% to 77.6% of the total variation, depending on the model, whereas variation among clusters ranged from 22.4% to 25.5%. Comparable results were obtained when countries were treated as subpopulations. This study underscores the importance of selecting appropriate markers for genetic diversity assessment and marker-assisted breeding. The successful application of GBS-derived SNPs and SilicoDArT markers demonstrates their effectiveness in assessing genetic diversity and advancing breeding programs for sugar beet and other crops.

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EXTENDED TURKISH SUMMARY
(GENİŞLETİLMİŞ TÜRKÇE ÖZET)

ŞEKER PANCARINDA SİLİKO DART SNP MARKIRLARI KULLANILARAK GENETİK FARKLILIK VE POPÜLASYON YAPISININ ANALİZİ

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Mart 2025, 83 sayfa

Şeker, insan diyetinin önemli bir bileşenidir ve iki şeker bitkisinden elde edilir: şeker kamışı ve şeker pancarı. Şeker pancarı üretimi iklim koşullarından büyük ölçüde etkilenir ve bu da bitki genetik kaynaklarının yeni çeşitlerin geliştirilmesi için çok önemli bir hale getirir. Şu anda şeker pancarının dünya çapında şeker üretiminin %20-30'unu kapsadığı tahmin edilmektedir. Şeker, şeker pancarından elde edilen birincil üründür ancak işlenmesi ile pekmez, posa ve etil alkol gibi diğer yan ürünleri de sağlamaktadır. Ayrıca şeker pancarı yaprakları, protein açısından zengin olması ve dengeli bir amino asit profili içermesi sebebiyle besin açısından değerlidir. Şeker pancarının insan beslenmesindeki temel işlevi düşünüldüğünde hem üretim miktarını hem de kalitesini vurgulamak çok önemlidir.

2000'li yılların başında geliştirilen Diversity Arrays Technology (DArT) belirteçleri, mikroarray tabanlı bir hibrid yöntemi kullanarak hızlı DNA polimorfizmi tespiti sağlayan bir genotipleme yaklaşımı sunmaktadır. SNP ve SSR belirteçlerinden farklı olarak, DArT belirteçleri dominant özellik taşır ve yalnızca var veya yok olarak değerlendirilir, bu da her lokustan daha az genetik bilgi sağlamasına neden olur. Bitki genotiplemede yaygın olarak kullanılsa da genom genelindeki dağılımı büyük ölçüde keşfedilmemiştir. Tek nükleotid polimorfizmleri (SNP'ler), nokta mutasyonları sonucu oluşan DNA dizisi varyasyonlarıdır ve organizmalardaki en yaygın polimorfizm türüdür. Kodominant yapıları ve yüksek yaygınlıkları nedeniyle SNP'ler, genom çapında ilişkilendirme çalışmaları ve yüksek verimli taramalar için yaygın olarak kullanılmaktadır. 1990'ların sonlarında geliştirilen gen çip teknolojisi, SNP genotiplemesini büyük örnek grupları üzerinde verimli bir şekilde gerçekleştirmeyi mümkün kılmıştır. Otomasyona uyumlulukları ve diğer belirteçlerin tespit edemediği

gizli polimorfizmleri saptama yetenekleri sayesinde SNP'ler, genetik araştırmalarda vazgeçilmez araçlar haline gelmiştir.

Bu çalışma, SNP ve SilicoDArT belirteçlerini kullanarak 16 ülkeden gelen uluslararası şeker pancarı germplazmasının genetik çeşitliliğini ve popülasyon yapısını değerlendirmiştir. Elde edilen sonuçlar, USDA şeker pancarı genetik kaynaklarının iyi düzeyde genetik çeşitliliğe sahip olduğunu göstermiştir. Sonuçlar incelendiğinde, SNP belirteçleri veri setinin SilicoDArT belirteç sistemine kıyasla daha yüksek çeşitlilik değerlerine sahip olduğu anlaşılmıştır.

Bu tez çalışmasında, SNP markırlarıyla yapılan analizlerde, şeker pancarı ıslahı için ideal olan genetik olarak farklı genotipler olarak Ames 2644 ve Ames 8297 tanımlanmıştır. Islah çalışmaları için bitki genotip/hat/çeşitlerin genetik uzaklığı çok önemlidir. Şeker pancarı genetik kaynakları üç ana kümeye ayrılmıştır: SI, S-II ve S-III. SI kümesi, 35 genotip/hat/çeşit ve bunların %51'i ABD kökenli olan en küçük grubu oluşturmuştur. Avrupa genotip/hat/çeşitleri kümenin %14'ünü kapsamaktadır. S-III grubu, Orta Doğu, Güneydoğu ve Doğu Asya'dan 55 genotip/hat/çeşitle en büyük grubu oluşturmaktadır. Bu tez çalışmasında ayrıca, ABD'den hiçbir genotip/hat/çeşidin S-III-B'de gruplanmadığını, %80'den fazlasının Orta Doğu ülkelerinden oluştuğunu göstermiştir.

SilicoDArT belirteçlerinin kullanılmasıyla elde edilen sonuçlar, NSL 176303 (Sırbistan) ve PI 140355 (Karadağ) genotiplerinin en uzak genotipler olduğu ortaya çıkmıştır. Şeker pancarı çeşit/genotipleri D-I ve D-II olmak üzere iki gruba ayrılmıştır. D-I kümesi, ABD'den gelen genotiplerin %90'ını içermekte olup SNP tabanlı UPGMA kümesine benzemektedir. D-II kümesi ise çeşitlilik göstermekte ve Birleşik Krallık, Türkiye, İran ve Irak'tan gelen genitipleri içermektedir. SNP tabanlı kümeleme, D-I kümesini desteklerken, SilicoDArT belirteçlerine dayalı PCA kümeleme sonuçları UPGMA ve STRUCTURE sonuçlarıyla uyum göstermiştir.

AMOVA sonuçları, germplazmadaki genetik varyasyonların büyük ölçüde örnekler arasındaki veya kümeler içindeki farklılıklardan kaynaklandığını ve toplam varyasyonun %74.5-77.6'sını oluşturduğunu göstermiştir. Bu çalışma, genetik çeşitlilik değerlendirmesinde ve markır destekli ıslah çalışmalarında markır seçiminin önemini vurgulamaktadır. GBS türevi SNP'lerin ve SilicoDArT markırları bitkilerde genetik

çeşitlilik değerlendirmesinde ve markır destekli ıslah çalışmalarında başarıyla kullanılabilmektedir.

Genetik çeşitlilik analizi, büyük ölçüde coğrafi ve genetik faktörlerden etkilenen popülasyonlar arasında ve içinde önemli çeşitlilik olduğunu ortaya koymuştur. Hem SNP hem de SilicoDArT belirteçleri genetik çeşitliliği etkili bir şekilde göstermiştir. SNP belirteçleri 0,0069'luk bir varyans bileşeni ve SilicoDArT belirteçleri 0.0129'da biraz daha yüksek bir varyans bileşeni göstermiştir. Yüksek F istatistik değerleri (SNP için 0.189 ve SilicoDArT için 0.170), önceki çalışmalarla tutarlı olarak güçlü popülasyon farklılaşmasını doğrulamıştır.

Gen çeşitliliği (GD), polimorfik bilgi içeriği (PIC) ve minör alel frekansı (MAF) gibi çeşitlilik endeksleri her iki belirteç türü için de neredeyse aynı bulunmuştur. Ancak, gözlenen heterozigotluk (H_o) ve kendileme katsayılarındaki (F) farklılıklar, SilicoDArT belirteçlerinin heterozigotluğu daha iyi yakaladığını, SNP belirteçlerinin ise saflık tespiti için daha iyi çözünürlük sağladığını göstermiştir. SNP belirteçleri için daha yüksek etkili alel sayısı (Ne), genetik çeşitliliği yakalama yeteneklerini daha da desteklemiştir.

PCA ve kümeleme yöntemlerini kullanan popülasyon yapısı analizi, üreme geçmişi ve coğrafi izolasyondan etkilenen genetik farklılaşma kalıplarını ortaya koymuştur. SNP ve SilicoDArT belirteçleri, farklı özellikleri nedeniyle küçük farklılıklar gözlemlenmesine rağmen karşılaştırılabilir kümeleme sonuçları üretmiştir. Çalışma, birden fazla belirteç sisteminin entegre edilmesinin genetik çeşitlilik değerlendirmelerinin güvenilirliğini artırdığını doğrulamıştır.

Sonuçlar, ıslah ve bitki genetik kaynaklarının değerlendirilmesi için önemli çıktılar sunmuştur. Genetik olarak farklı şeker pancarı genotipleri biyotik/abiyotik stres faktörlerine karşı tolerans, şeker miktarı, kök kalitesi, bitki gelişimi, adaptasyon, kök rengi ve verimi iyileştirmek için değerli kaynaklar görevi görmektedir. Çalışma ayrıca, PCA, STRUCTURE ve kümeleme analizlerinin, genotipleri genetik geçmişlerine göre gruplandırmadaki etkinliğini de göstermiştir.

Sonuç olarak, bu araştırma gelecekteki markır destekli ıslah programları için değerli bir veri seti sağlamakta ve bitki ıslahı için genetik çeşitliliği belirlemenin önemini göstermektedir. Yüksek verimli genotipleme yöntemlerini güçlü istatistiksel

araçlarla bütünleştirerek, ıslahçılar üstün genotiplerin seçimini artırabilir ve yeni şeker pancarı çeşitlerinin geliştirilmesine destek sağlayabilir.

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