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MOLECULAR MARKER BASED COMPARISION OF DROUGHT TOLERANT AND SENSITIVE TURKISH SUNFLOWER (*HELIANTHUS ANNUS* L.) CULTIVARS

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Abstract
Helianthus annuus L. is the second most notable oilseed crop all around the world, after soybean, and comes from temperate North America. Sunflowers use for not only human consumption it uses also for many purposes. Morphological, geographical, molecular, and archaeological data indicate that sunflowers used as food, medicine, body painting in rituals, bioenergy and dye. Sunflower is a self-fertilize plant and it needs to pollen activity and honey bee for fertilization. There are some objectives in terms of sunflower breeding. These are drought resistance, resistance to disease and pets, breeding for self-fertile lines and branching shape. The pioneering sunflower breeding study was belonged to develop varieties with increased oil content. This was followed by the development of cytoplasmic male sterility (CMS) and haploid induction methods. In order to produce new cultivars with classical breeding methods is time consuming. To reduce this period uses double haploid technology and molecular marker-based technology. Molecular markers have been employed in a wide range of fields, including genetic mapping, paternity testing, identification of mutant genes related to hereditary diseases, cultivar identification, marker-assisted crop breeding, population history, epidemiology, food safety, and population studies. Among these, the start codon targeted (SCoT) marker has gained popularity for its ability to target a specific region around the ATG start codon, which is conserved across all plant species. SCoT markers are considered useful tools for studying genetic diversity in various plant species due to their simplicity, cost-effectiveness, high polymorphism, reproducibility, and time-saving attributes. Drought is defined as geographic location, amount, and time of precipitation. Also, it is defined as a shortage of water availability sufficient to cause a loss in yield or a period of no rainfall or irrigation that results in insufficient soil moisture leading to reduced crop growth and yield. Sunflower is one of the plants that needs a high amount of water during the development period. Studies of sunflower mainly focuses on drought response. This study aims to compare drought tolerant and sensitive Turkish cultivars using the SCoT marker system to find a linked DNA region that could be responsible for the tolerance. This research conducted with five common sunflower (Helianthus annus L.) genotypes that from three of them was drought tolerant, two of them was drought sensitive genotypes. The DNA of each sample was isolated from fresh leaves using the DNeasy Plant Mini Kit. Altogether 25 primers were tested and 13 SCoT primers gave enough polymorphism and PCR amplification was performed in a 2720 thermocycler. DNA bands were visualized by UV illumination using a gel document system (Bio-Rad). The approximate fragment size was compared with the GeneRuler™ 1 kb DNA ladder (Thermo Fisher Scientific). Banding profiles generated by SCoT primers were compiled into a data binary matrix based on the presence or absence of the selected band. The dendrogram and principal component analysis (PCA) were conducted using Past 4.04 software. Altogether 25 primers were tested and 13 SCoT primers successfully amplified 3 droughts tolerant and 2 drought sensitive genotypes of Helianthus annus L. and 13 SCoT primers gave 100% amplification with polymorphism. The total number of bands from 13 SCoT primers was 161, with 125 bands showing polymorphism and the average percentage of polymorphic bands was 76.79%. The size range of bands was from 250-8000 bp. The total number of bands range from 4 to 27 an average of 12.38 bands, while the number of polymorphic bands varied from 2 to 21 an average of 9.61 bands per primer. Based on the

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similarity and difference matrices highest value was belonged to drought tolerant cultivar with name 8129R. On the other hand, drought sensitive genotypes 9718A and 97251A have shown closely related values. The PCA result supports the results of similarity and distance matrices, which shows that 9718A and 97251A are closely related. In conclusion, the SCoT marker can be used as an effective molecular marker in case of sunflower genetic diversity analysis. Especially, drought tolerant cultivars TUNCA and P64LL62 showed similar characteristics in terms of genetical background of drought tolerant.

Keywords: Drought Tolerant, Drought Sensitive, Helianhus annus L., Molecular Markers, SCoT Marker.

1. INTRODUCTION

Helianthus annus L., known as sunflower, is belong to *Asteraceae* family. *Asteraceae* family, called as *Compositae*, is one of the biggest angiosperm plant families in between dicotyledons. In the world, 10% of all flowering plants have been constituted by the *Asteraceae* family which has 1620 genera and 23600 species (Funk et al., 2005; Funk et al., 2009). This family, which has 12 subfamilies, grows mostly in subtropical and temperate climates, especially in meadows, valleys, grassy plains, rolling plateaus, and mountain slopes (Funk et al., 2005; Bayer, 2007). The genus has been in existence for a considerable period, estimated to be from 4.75 to 22.7 million years ago, based on the analysis of chloroplast DNA (Schilling, 1997). The divergence of species within this genus occurred relatively recently, between 1.7 and 8.2 million years ago (Schilling, 1997).

The cultivation of sunflowers can be traced back to 4625 B.C., as evidenced by archaeological findings that suggest the American Indians were the first to engage in this practice (Crites,1993). Based on morphological, geographical, molecular, and archaeological evidence, it has been suggested that sunflowers were utilized by Indigenous peoples in North America for various purposes, such as food, medicine, and dye. According to this evidence, the cultivation of sunflowers occurred approximately 4,000 to 5,000 years ago in a region that spans from Mesoamerica through the United States and into southern Canada (Sala et al., 2012; Warburton et al., 2017).Sunflower cultivation as a field crop and for oil production truly began with the discovery of a method to extract oil from sunflower seeds by D.S. Bokarev from the Belgorod area in 1829 (Pustovoit, 1990). Scientific studies on sunflower breeding commenced in 1912 with the establishment of the Kruglik Plant Breeding and Experimental Station (Škorić, 1988).

Sunflower plants can grow up to 3 meters in height, with an inflorescence that can measure up to 30 cm in diameter. The inflorescence is a compressed raceme consisting of multiple sessile florets that all share the same receptacle, also known as the capitulum. The outside of the inflorescence is adorned with bright yellow ray florets, while the inside is filled with yellowish disc florets. Sunflowers exhibit a characteristic behaviour of turning their heads towards the sun during maturation, which is known as heliotropism. This movement ceases once the flowers begin to bloom (Harshavardan & Amendeep, 2021). The sunflower head is composed of multiple circles of florets. The outermost circle consists of five petals on each flower, which are typically golden yellow and sterile. The disc florets, which cover the large disc in the centre of the head, are composed of individual flowers that contain both pistils and stamens. The fibres in the disc are more common in the outer ring than the inner ring (Hu et al., 2010). The diameter of the sunflower head, or cup, typically falls within the range of 18 to 25 cm, but it can vary greatly between 5 and 50 cm across all genotypes. Furthermore, sunflowers have impressive yields, and head diameter is a parameter that can be greatly influenced by environmental factors, much like plant height (Kaya et

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al., 2012). The sunflower head is not a single flower, but instead comprises 1,000 to 2,000 individual flowers that are attached to the receptacle base. Sunflowers are self-fertile, relying on their own pollen for fertilization, but they also benefit from pollinators such as honey bees and other insects (Debaeke et al., 2017). Optimal growth of sunflowers occurs at warm temperatures of around 20-25°C during the day and 15-18°C at night. Inadequate sunlight may result in stunted growth and underdeveloped florets. To achieve optimal growth, sunflowers require sufficient water, particularly during germination and flowering stages. Sunflowers thrive best in nutrient-rich, well-drained soil, with a pH level between 6.0 and 7.5. Poor soil quality may lead to reduced growth and smaller buds (Karkanis et al., 2011; Laza et al., 2014). The root system of sunflowers is distinctive, as it extends both deeply and widely, allowing for efficient water and nutrient uptake. Sunflower roots grow faster than the leaves, and under favourable conditions, they can reach depths of more than 3 meters to access groundwater. The structure of the root system plays a significant role in the absorption of water and nutrients (Alberio et al., 2015). The blooming period for cultivated sunflowers usually lasts for 60-70 days, and they reach physiological maturity in 80-100 days. However, the total growth period can vary between 125-130 days depending on the genetic makeup of the sunflower and environmental factors (Schneiter et al., 1981). The seeds of the sunflower consist of both kernels and husks, and their oil content is 44% greater than that of canola and soybeans. Sunflower seeds contain 18% protein, 15% cellulose, 9% water, 14% minerals, and carbohydrates (Andrianasolo et al., 2016). The percentage of oil in sunflowers can vary depending on factors such as the variety of sunflower, the conditions in which it was grown, and the timing of the harvest. Generally, the weight of oil in sunflower seeds is between 35% and 50%. Scientists have reported the oil content of sunflowers ranging from 36.9% to 50.2% (Gómez et al., 2002).

There are multiple breeding objectives for sunflowers, including enhancing drought resistance, disease and pest resistance, and developing self-fertile and specific branching shape lines (Harshavardan & Amendeep, 2021). In subsequent years, researchers focused on developing cytoplasmic male sterility (CMS) through a cross between *Helianthus petiolaris* and cultivated sunflower (Leclercq, 1969). Traditional breeding methods have some limitations such as the requirement of significant space and resources for plant selection, leading to breeding programs taking up to a decade to develop a new sunflower line (Davey & Jan, 2010). To overcome the limitations associated with heterogeneity in lines, true breeding lines can be established. This can be achieved through repetitive backcrossing to the parental line with the desirable trait and progeny selection, or by developing haploids and doubling their chromosomes to form doubled haploid (DH) lines (Dwivedi et al., 2015). Haploid plants have a single set of chromosomes and are incapable of undergoing meiosis, and therefore are infertile (Murovec et al., 2012). However, their fertility can be restored through chemical or spontaneous chromosome doubling, resulting in 100% homozygosity in a single generation (Murovec et al., 2012; Brit, 2016). Doubled haploid (DH) lines eliminate the need for repeated backcrossing to a desirable parent line over multiple generations, thus greatly accelerating the generation of true-breeding lines (Karimi-Ashtiyani et al., 2015; Ishii, 2016). Doubled haploids (DHs) have several potential uses in plant breeding, such as accelerating the pyramiding of various mutants, facilitating forward mutagenesis screening, reducing ploidy levels (e.g., tetraploid to diploid), generating homozygotes for gametophyte-lethal mutations, and decreasing inbreeding depression associated with self pollination. These applications have been supported by various studies (Murovec et al., 2012; Karimi-Ashtiyani et al., 2015). Chromosome substitution lines can be rapidly generated using doubled haploids as a starting point (Ishii, 2016).

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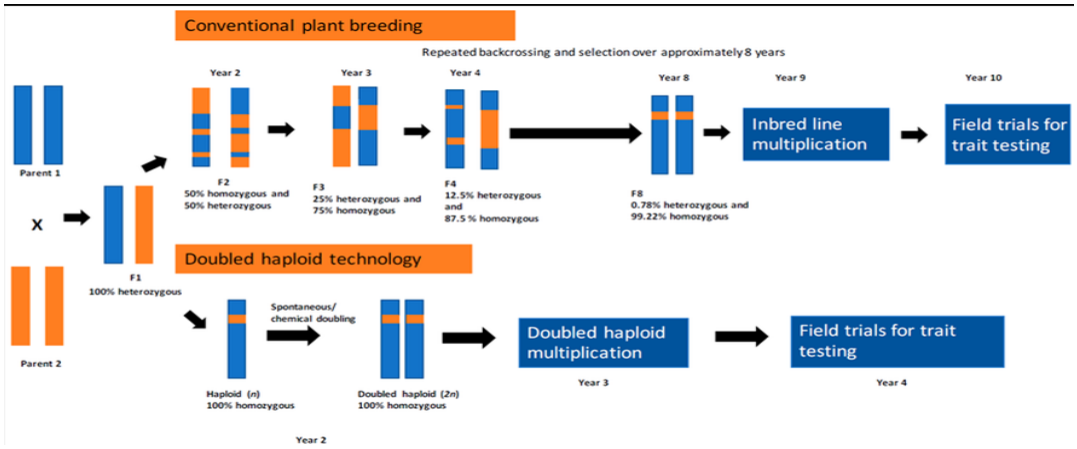

Figure 1. Comparison of conventional breeding and doubled haploid ('accelerated breeding') technology breeding methods (Eliby et al., 2022)

Sunflower breeding utilizes various haploid induction methods, including parthenogenesis. Experiments have been conducted to test the resistance of resulting haploid plants to broomrape, fungus, imidazoline, and downy mildew (Drumeva et al., 2014; Drumeva, 2017; Todorova, 1997). Another haploid induction method used in sunflower breeding is anther culture. This method has been applied for fertility restoration and has been studied for its effectiveness in improving resistance to broomrape, fungi, imidazoline, and downy mildew (Bohorova, 1985; Saji, 1998; Jonard, 1990). In 1987, Ishino made a discovery while studying genes that are associated with the conversion of alkaline phosphatase's isozyme in *E. coli*, which led to the development of CRISPR (Ishino et al., 1987; Ishino et al., 2018). For the past four decades, the transfer of sunflower plants via Agrobacterium has been on the rise (Bidney et al., 1992; Laparra et al., 1995; Rao et al., 1999; Weber et al., 2003; Ikeda et al., 2005; Mohamed et al., 2006). Studying molecular biology to develop transgenic sunflowers with traits such as pest resistance, herbicide resistance, and increased oil yield is essential. It is also crucial to investigate the ecological impact of these modifications (Mayrose et al., 2011; Malnoy et al., 2016; Wang et al., 2017). Moreover, a research study conducted a survey that resulted in the development of CAS-3 and CAS-5 mutants with high levels of stearic acid and palmitic acid contents, respectively (Osorio et al., 1995). CAS-14 mutants resulted in an increase of stearic acid content up to 37% (Fernández-Moya et al., 2002).

A molecular marker is a DNA sequence with a known position on the chromosome (Kumar, 1999). Agene that shows easily recognizable phenotypic traits can be used to identify individuals, or as a probe to label a chromosome or locus (King & Stansfield 1990; Schulmann 2007). Molecular markers are indicative of polymorphism, which may arise due to chance nucleotide variations or mutations at specific genomic loci, (Hartl & Clark 1997) markers enable the identification of genetic variations between individual organisms or species (Collard et al., 2005). Molecular markers have diverse applications, ranging from genetic mapping, paternity testing, and detecting mutant genes linked to hereditary diseases, to identifying cultivars, marker-assisted crop breeding, investigating population history and epidemiology, ensuring food safety, and conducting population studies (Hartl & Jones 2005). Sunflower is a model system for genomic studies in the *Asteraceae* family owing to its significance (Leclercq 1969; Paniego et al. 1999). The study of sunflower

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genetics is crucial due to the broad spectrum of traits present in their germplasm, including yield, plant height, seed number, earliness, and susceptibility to biotic and abiotic stresses (Thormann et al. 1994; Paniego et al. 1999). Further investigation into the speciation of this taxon is necessary due to the significant variation in species numbers. Sunflowers consist of diploids, tetraploids, and hexaploids with a total of 17 major chromosomes (Heiser, 1978). Traditionally, the relationship between sunflower species has been determined through morphological and hybridological analyses (Schillin & Heiser, 1981; Chandler et al., 1986). The diploid crop common sunflower has a chromosomal number of 2n=34 and a haploid genome size of 3000 Mb (Darvishzadeh et al. 2010). The initial composite genetic map for sunflowers, which included 278 single-locus SSR markers and 379 additional markers (public and proprietary), covered 1423 cM. This map has become a reference for sunflower genetic studies and has been augmented with more SSR markers using three new mapping populations (Yu et al., 2003). Chapman and Heesacker (2008) developed over 2000 SSR markers from genomic sequences (gSSR) and ESTs (EST-SSR), which are now applicable for mapping and genotyping. Sunflower maps have been constructed using gSSRs, EST-SSRs, INDELs, and TRAP markers. The development of several hundred microsatellite markers for sunflowers has significantly advanced the analysis of molecular genetic variation in this crop (Paniego et al. 2002; Yu et al. 2002).

SCoT (Start Codon Targeted) is a molecular marker that is dominant in nature and is used to study genetic polymorphism, diversity, and phylogenetic relationships. It targets the short-conserved region around the ATG start codon on both DNA strands. This marker employs a single 18-mer primer that anneals at a temperature of 50°C, as the region flanking the ATG start codon is highly conserved across plant species. Although a few SCoT markers can be codominant through insertion or deletion, they are rare. The amplification products of SCoT markers are distributed within gene regions, including genes, pseudogenes, and transposable elements that could also be primer binding sites. The spacing between the binding sites of the primer can be observed through agarose gel electrophoresis, making it a simple and cost-effective method (Davis et al. 1995; Collard & Mackill, 2009). The SCoT marker technology can detect the presence of both dominant and codominant markers (Nair et al., 2016). Zhang et al. (2015) noted that SCoT marker technology is simple to use and less expensive, making it widely applicable in laboratories with basic equipment. The technique's first validation was carried out in rice (*Oryza sativa*) (Collard & Mackill, 2009).

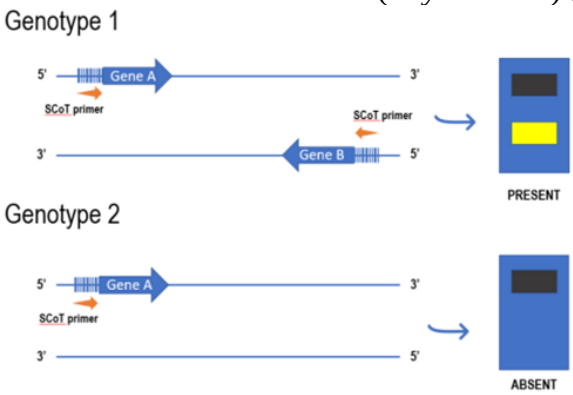

Figure 2. The principle of the SCoT primer in PCR amplification (Collard & Mackill, 2009)

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2. MATERIALS AND METHODS

Plant Materials

This research conducted with five common sunflower (*Helianthus annus* L.) genotypes that from three of them was drought tolerant, two of them was drought sensitive genotypes. Five of plant materials were provided from Trakya Agricultural Research Institute in Türkiye. Drought resistance genotypes name were "TUNCA, 8129R, P64LL62", drought sensitive genotypes names were "9718A, 97251A". P64LL62, a variety of sunflower, was developed in Turkey in 2009 with a grain yield of 348.8 kg/da, which was 10.1% higher than the average yield of 316.9 kg/da. It also exhibited an oil content of 50.0%, which was 7.4% higher than the standard varieties' average oil content of 46.5%. It reaches physiological maturity within 93-103 days. Pioneer company registered this variety in 2016. On the other hand, TUNCA was registered by Limagrain in 2008 and is known for its high yield potential, high oil content, and exceptional drought resistance (Çan E. 2019). In 2012, 8129R was registered at the Trakya Agricultural Research Institute in Türkiye. Later, in 2015, 9718A was also registered at the same institute. Finally, in 2019, 97251A was registered at the Trakya Agricultural Research Institute in Türkiye as well. Leaves samples were obtained from seeds. For each genotype, seeds were sowed, leaves were reached 3 or 4 cm length and then leaves collected for DNA extraction.

DNA Extraction

The DNeasy Plant Mini Kit (Qiagen 2016) was used to isolate DNA from fresh leaves in the study. To begin the process, approximately 100 mg of leaf samples were ground with liquid Nitrogen in a sterile mortar and pestle until a fine powder was obtained. The powder was transferred to a tube and 400 µl of AP1 and 4µl of RNase A were added. The mixture was then vortex homogenized and incubated at 65ºC for 10 minutes, with the tube being upturned 2-3 times during incubation. Following this, 130 µl of buffer P3 was added to the tube, mixed thoroughly, and then incubated on ice for 5 minutes. After centrifuging the lysate at 1400 rpm for 5 minutes to separate it from the tissue debris, it was transferred to a QIAshredder spin column and then placed in a 2 ml collection tube. Next, the tube containing the lysate and QIAshredder spin column was centrifuged at 1400 rpm for 2 minutes. The resulting flow-through was then carefully transferred to a new tube, and 675 µl of buffer AW1 was added. The mixture was mixed thoroughly by pipetting, and then 650 µl of this mixture was transferred to a DNeasy Mini Spin Column, which was then placed in a 2 ml collection tube. Afterward, the mixture was centrifuged at 8000 rpm for 1 minute and the resulting flow-through was discarded. This step was repeated with the remaining flow-through. First, the spin column was placed into a new 2 ml collection tube, and then 500 µl of buffer AW2 was added to it. After that, the mixture was centrifuged at 8000 rpm for 1 minute, and the resulting flow-through was discarded. Next, another 500 µl of buffer AW2 was added, and the tube was centrifuged at 1400 rpm for 2 minutes. To extract the DNA from the spin column, 100 µl of buffer AE was added and left at room temperature (15-25ºC) for 5 minutes. The spin column was then centrifuged at 8000 rpm for 1 minute, and another 100 µl of buffer AE was added. The resulting DNA solution was considered ready to use and was stored at -20ºC.

PCR Amplification and Gel Electrophoresis

A total of 25 primers were evaluated, out of which 13 SCoT primers were chosen for further data analysis due to sufficient polymorphism. PCR amplification was conducted with a reaction volume of 14 µl consisting of 5x DreamTaq red buffer at a final concentration of 1x, 0.4 µl dNTPs at a final concentration of 200 µM, 0.5 µM of the SCoT, 1 U Polymerase Phire, 0.6 µl DMSO at a final concentration of 3%, and 8.6 µl Milli-Q water. The PCR amplification was carried out using a 2720

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thermocycler with about 20-40 genomic DNAs. To amplify the SCoT primers, the PCR reaction was initiated with an initial denaturation step at 94ºC for 5 minutes. Subsequently, 35 cycles were performed with denaturation at 94ºC for 1 minute, annealing at 50ºC for 1 minute, and extension at 72ºC for 2 minutes. The final extension was carried out at 72ºC for 5 minutes. The SCoT PCR products were subjected to electrophoresis on a 1% TBE agarose gel and stained with ethidium bromide (EtBr) for 20-60 minutes at 90-100 voltage. The DNA bands were visualized under UV illumination using a gel documentation system (Bio-Rad). To determine the approximate fragment size, the GeneRuler™ 1 kb DNA ladder (Thermo Fisher Scientific) was used for comparison.

Data Analysis

A binary data matrix was created based on the presence (1) or absence (0) of clear, unambiguous, and reproducible bands generated by the selected SCoT primers. Smudged and poor bands were excluded from the analysis to ensure the reliability of the data. The resulting banding profiles were used to construct a dendrogram of primers and perform principal component analysis (PCA) using Past 4.04 software (Hammer et al., 2001).

3. RESULTS AND DISCUSSIONS

Characteristics of Polymorphic ScoT Markers in *Helianthus anuus* L.

Altogether 25 primers were tested and 13 SCoT primers successfully amplified 3 droughts tolerant and 2 drought sensitive genotypes of Helianthus annus L. The total number of bands from 13 SCoT primers was 161, with 125 bands showing polymorphism and the average percentage of polymorphic bands was 76.79%. The size range of bands was from 250-8000 bp. The total number of bands range from 4 to 27 an average of 12.38 bands, while the number of polymorphic bands varied from 2 to 21 an average of 9.61 bands per primer (Table 1).

Primer Name	Size Range (bp)	Total Number of Bands	Number of Polymorphic Bands	Percentage of Polymorphic Bands (%)
SCoT1	250-4000	8	6	75
SCoT2	250-8000	27	21	77.7
SCoT7	250-8000	8	5	62.5
SCoT8	250-8000	9	5	55.5
SCoT17	500-6000	8	7	87.5
SCoT18	250-5000	9	9	100
SCoT19	500-8000	18	16	88.8
SCoT20	500-4000	14	10	75.42
SCoT23	500-3000	4	2	50
SCoT24	250-5000	16	11	68.75
SCoT27	250-8000	15	13	86.6
SCoT29	250-5000	17	12	70.5
SCoT30	250-3000	8	8	100
Total		161	125	
Average		12.38	9.61	76.79

Table 1. Characteristics of SCoT amplification bands of Helianthus annus L.

SCoT2 gave the highest total number of bands (27), while SCoT23 gave the lowest total number of bands (4). The highest number of polymorphic bands observed SCoT18 and SCoT30 with 100%. The lowest number of polymorphic bands observed SCoT23 with 50%. On this study 25 primers were tested and 13 SCoT primers gave 100% amplification with polymorphism, while the other twelve SCoT primers were either monomorphic or did not give 100% amplification products. This consequence was also parallel with Mulpuri et al. (2013), who performed SCoT primers for *Jatropha curcas* accessions 26 out of 36 SCoT primers tested gave 100% amplicon with

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polymorphism. According to this study observed that SCoT primers could be utilized for diverse materials with genetic variation and give an informative and highly reproducible amplification product. Satya et al. (2015) used twenty-four start codons targeted (SCoT) markers to determine the genetic diversity and population structure of indigenous, introduced, and domesticated ramie (*Boehmeria nivea* L. Gaudich). The Indian ramie populations exhibited high SCoT polymorphism, high genetic differentiation, and moderate gene flow. This result put forwards that SCoT primers can be beneficial for DNA fingerprinting, population analysis, genetic diversity studies, parentage specification, and effective management of ramie genetic resources. In addition, the present study indicates the efficacy of employing SCoT markers in a cross-pollinated heterozygous species like *Boehmeria*, and would be helpful for further studies in population genetics, conservation genetics, and cultivar improvement.

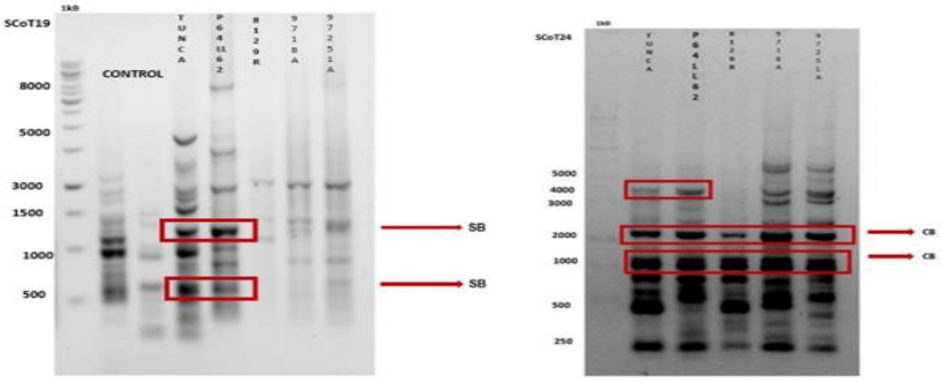

Figure 3. SCoT amplification profile with SCoT19 and SCoT24 primers

TUNCA and P64LL62 drought tolerant cultivars showed specific band (SB) characteristics, while the other drought tolerant cultivar did not show any specific band characteristic with SCoT19 primer. On the other hand, both drought tolerant and sensitive cultivars showed common band (CB) characteristics with SCoT24 primer (Figure 3). According to our results, this is a preliminary study to find DNA sequence linked to drought tolerant. Especially, drought tolerant cultivars TUNCA and P64LL62 showed similar characteristics in terms of genetical background of drought tolerant.

Figure 4 demonstrates the PCR banding pattern in case of all amplified fragments generated by 13 SCoT primer. Each colour can be matched to a given PCR product. It could be seen that the smallest number of DNA fragments was produced in TUNCA, while the most in P64LL6.

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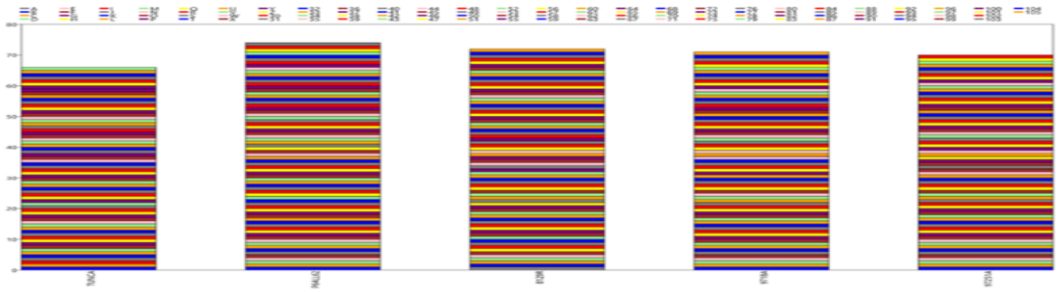

Figure 4. Stacked chart analysis of H.anuus cultivars using 13 SCoT primers (Past 4.04 software; Hammer et al. 2001)

Cluster Analysis Using ScoT Primers

A neighbour joining dendrogram (Past 4.04 software; Hammer et al. 2001) between 3 drought tolerant and 2 drought sensitive cultivars of *H.annus* using 13 SCoT primers similarity is shown Figure 5. The tolerant cultivars are marked by brown colour.

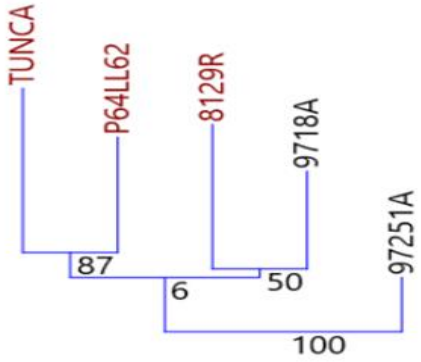

Figure 5. A neighbour-joining dendrogram with Euclidean distance between 3 drought tolerant and 2 drought sensitive cultivars of H.annus using 13 SCoT primers.

Table 2 has shown that the highest similarity value was belong to drought tolerant cultivars 8129R with 6,48 and the lowest value was belonged to drought sensitive 97251A with 4,123. In addition, drought sensitive genotypes 9718A and 97251A have shown closely related values. This highest similarity value could be due to the condition pedigree but no information is available on the origin of the varieties. We assume that the drought tolerance was introduced from the same gene source, and because of this, these genotypes have similar genetical background.

	TUNCA	P64LL62	8129R	9718A	97251A
TUNCA	0	5,2915026	6,4807407	5,5677644	5,6568542
P64LL62	5,2915026	0	5,6568542	4,5825757	4,6904158
8129R	6,4807407	5,6568542	0	4,5825757	4,8989795
9718A	5,5677644	4,5825757	4,5825757	0	4,1231056
97251A	5,6568542	4,6904158	4,8989795	4,1231056	0

Table 2. Similarity and distance matrices of H. annus genotypes

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Rayan and Osman (2019), examined phylogenetic relationships between Egyptian soybean cultivars using SCoT markers and protein patterns. This investigation indicates that the highest similarity value between two soybean varieties means that they are closely related, while the lowest similarity value means that they are genetically distant from each other. The result also shows that ScoT markers are potent markers for distinguishing and identifying different soybean cultivars.

Principle Component Analysis (PCA)

The polymorphic bands generated by 13 SCoT primers were analysed using principal component analysis (PCA) to determine the clustering pattern based on the characteristics of the bands obtained, thus making it easier to display the distribution of the data of each genotype in the form of a plot. Figure 6 shows the distribution of 3 drought tolerant and 2 drought sensitive genotypes of *H. annus*. The samples from drought tolerant named with TUNCA and 8129R contrast by coordinate 1. These two cultivars were found quite far from other genotypes and from each other. In coordinate 2, one of the droughts tolerant cultivars named as P64LL62 and the other drought sensitive cultivars named with 9718A and 97251A were located. In coordinate 2, one of the drought tolerant species P64LL62 and the drought sensitive species 9718A and 97251A are located. In addition, species of 9718A and 97251A were closely related to each other than the other accessions. This PCA result supports the results of similarity and distance matrices, which shows that 9718A and 97251A are closely related.

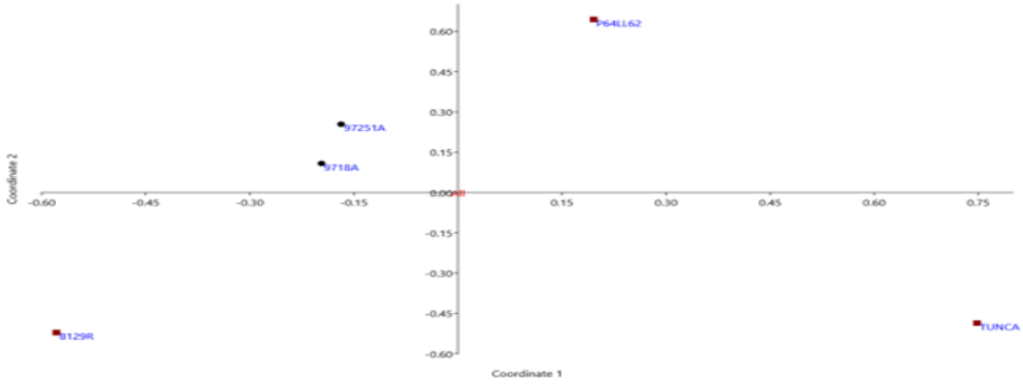

Figure 6. Principal component analysis (PCA) of H. annus genotypes using 13 SCoT primers. Tolerant cultivars are marked with a brown square, while sensitive ones are marked with a black circle.

Principal Component Analysis (PCA) is a statistical technique used to analyse a dataset consisting of inter-correlated variables describing observations. The aim of PCA is to extract meaningful information from the dataset and represent it as a set of new orthogonal variables known as principal components. By doing so, PCA allows for visualization of the similarities between observations and variables by plotting them as points on maps (Jolliffe, 2002; Saporta and Niang, 2009).

Igwe et al. (2017) utilized PCA with SCoT markers to investigate the genetic diversity in *Vigna unguiculata* L. (Walp). They amplified five SCoT markers, resulting in 52 alleles and 80 polymorphic loci. The study concluded that this method was effective in generating genetic fingerprints and could potentially be used for applications such as seed purity determination, efficient utilization, and management of genetic resources in cowpea.

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Axis	Eigenvalue	Percent
1	22.544	41.748
2	44998	24.629
3	9.9895	18.499
4	8.1669	15.124
5	-6.0279E-16	-1.1163E-15

Table 3. Data set of principle coordinate analysis of 5 H.annus genotypes

Principle coordinate analysis of *H.annus* generated with using 13 SCoT pirmers. The maximum eigenvalue was determined on the axis 2 with 44998, while the maximum data was belonged to axis 4 with 8.1669. In addition, maximum percentage was belonged to axis 1 with 41.748%, on the other hand minimum percentage was belonged to axis 4 with 15.124%.
The principal eigenvalue of an operator is a principal concept in modern analysis. The principle of eigenvalue is used to characterize the steadiness of the equilibrium of a reaction-diffusion equation enabling the description of perseverance criteria (Cantrell & Cosner 1989; Cantrell & Cosner 1998). Mandal et al. (2023) studied that genetic dissection, relationship and population structure of drumstick (*Moringa oleifera* Lam.) using agromorphological and with 36 SCoT markers. 20 primers showed polymorphic characterisation. Molecular variance using principal component analysis (PCA) showed 30.25% variations in the first three axes (axis 1-13.33%, axis 2- 9.33%, and axis 3-7.59%, respectively)

4. CONCLUSIONS

According to our results, we can state that SCoT marker system can be useful in case of sunflower genotype diversity analysis. This is a preliminary study to find DNA sequence linked to drought tolerance. Especially, drought tolerant cultivars TUNCA and P64LL62 showed similar characteristics according to our SCoT diversity analysis. We assume that the drought tolerance was introduced from the same gene source, and because of this, these genotypes have similar genetical background. We could identify specific DNA fragments appearing only in tolerant cultivars. Further experiments are needed, these PCR amplicons must be sequenced and BLAST searches can give direct answer about the character of these bands. However, further research is needed to confirm and ensure that the samples of sunflower genotypes by using more molecular markers analysis.

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