



Phenotypic and genetic characterization of soybean (*Glycine max*) genotypes for yield and drought stress tolerance

Khalid Mehmood^{1*}, Dilawar Khan¹, Muhammad Saeed¹, Malik Asad Khan Khajjak¹, Ata ur Rehman¹, Abdul Sattar Khetran¹, Ghulam Rasool² and Atta Ullah²

¹Agriculture Research institute Sariab Road Quetta, Pakistan

²Department of Plant Breeding and Genetics, Balochistan Agriculture College Quetta, Pakistan

*Corresponding authors: Khalid Mehmood (ubaidullahkhalid67@gmail.com)

Abstract

Soybean is the world's major oilseed crop which provides a significant amount of high-quality protein and edible oil. The Present research work was designed to assess the phenotypic variability, genetic characterization, and drought tolerance in soybean genotypes. A total of eight soybean genotypes were studied for field experiments during the year 2019 using Randomized Complete Block design (RCBD) at Balochistan Agriculture College, Quetta. Mean squares from Analysis of Variance (ANOVA) showed considerable phenotypic variation for most of the studied traits including plant height, maturity duration, pod length, number of seeds per pod, 100-seed weight, and seed yield plant⁻¹. The molecular genetic diversity among studied genotypes was confirmed through seven Simple Sequence Repeat (SSR) markers. Molecular data was scored qualitatively for the presence and absence of polymorphism using gel banding patterns. Out of seven SSRs, only four markers amplified and showed polymorphism. Molecular diversity displayed four major clusters at a 0.62 level of coefficient. Among the studied genotypes two lines namely, CH-NEsp-17 and Ertou # 2 showed high genetic diversity. Furthermore, the drought resistance of soybean genotypes was also determined through diaminobenzidine (DAB) staining process by using fresh leaves of each genotype with two treatments. Three genotypes, namely Ajmeri-2, CH-NEsp-17, Jhunghwang showed drought tolerance in both i.e., control as well as drought stress. However, genotype Ertou # 2 was found to be most susceptible. The results further demonstrated that the genotypes which showed high seed yield were also found drought tolerance. Thus, this study may facilitate future breeding programs in developing new soybean cultivars for high yield potential and drought resistance of soybean.

Keywords: Drought, Genetic diversity, Phenotypic, Soybean, SSR markers

To cite this article: Mehmood, K., Khan, D., Saeed, M., Khajjak, M. A. K., Rehman, A. U., Khetran, A. S., Rasool, G., & Ullah, A. (2023). Phenotypic and genetic characterization of soybean (*Glycine max*) genotypes for yield and drought stress tolerance. *Journal of Pure and Applied Agriculture*, 8(3), 22-33.

Introduction

Soybean (*Glycine max* (L.) Merrill) is the most important grain legume crop worldwide. It is a self-pollinated crop that belongs to Leguminosae family with diploid chromosome number $2n = 40$ and genome size is about 1.1-1.15 Gb (Arumuganathan & Earle, 1991). It is a highly nutritional crop for the human diet as well as for livestock as it contains 40-42% protein and 18 to 22% vegetable oil (Zhou et al., 2020). Soybean produces approximately two-thirds of the world's protein for cattle feeding and 60% of the world's edible oil. It is truly claiming the title of the meat that grows on plants (Khurshid et al., 2017). Its meal is an extremely rich source for aquaculture, livestock, and poultry feed (Agarwal et al., 2013). It has been cultivated in Northern China since prehistoric times, implying that domestication of soybean may occurred even earlier in this region (Dong et al., 2004). Soybean is regarded as a particularly important food crop from an economic standpoint (Liu, 2012). In East Asia, the wild soybean is found in Taiwan, Japan, China; the Korean Peninsula, and Russia (Boerma and Specht, 2004). There is substantial

disagreement on the location of origin of soybean domestication; and several regions, including southern and northeastern China; the Middle Yellow River basin in China; or many sites concurrently have been put forth (Hymowitz, 1970).

In Pakistan, soybean is cultivated on small acreage. Therefore, it is considered a minor oilseed crop of the country (Iqbal et al., 2010). It can be grown successfully in Balochistan considering both plains and hilly areas of the province. However, drought and low market value are the primary causes for the failure of this valuable crop (Kahair et al., 2003). The degree of phenotypic and genotypic variation in germplasm can be used to manage germplasm and provide an indicator of omissions in collections (Brown, 1978). It is essential to evaluate and conserve those elite germplasm lines which have high genetic variability (Popović et al., 2013). Quality and yield traits are negatively interrelated with each other. If one is improved, then the other is declined. So, it is essential to breakdown negative correlation among these characters (Maliket al., 2006). Any initiative for crop development needs genetic diversity to successful parental selection. The versatility of DNA-based molecular markers allowed

researchers to examine variation and variety in many plant species (Wang et al., 2006). By using DNA-based molecular markers, it is simple to compare the genetic makeup of two distinct plants without considering the effects of the environment on gene expression. Currently, a wide variety of DNA-based molecular markers, including RFLP, RAPD, and AFLP, are available to identify DNA polymorphism (Schulman, 2007).

Globally, there is concern over the low genetic diversity of farmed soybean germplasm (Hyten et al., 2006). Currently, SSRs were being employed successfully to identify genetic variation and connections between soybean varieties in various populations (Guan et al., 2010 and Mishra et al., 2022). Inadequate water availability quickly leads to a water scarcity in the plant body. Dehydration causes substantial changes in physiological systems that delay or even stop growth and jeopardise yield stability (Anjum et al., 2011; Rasheed et al., 2022). Drought-related yield losses in the field often vary between 30% and 90%, depending on crop species (Farooq et al., 2019). The major goal of this study was to examine the phenotypic and genotypic variability among soybean genotypes. Another goal was to identify the drought tolerant genotypes by using DAB staining procedure. Further, the identified genetically diverse genotypes accompanied with drought adaptive genotypes could be used to generate useful diversity.

Materials and Methods

Study area

Quetta district is located in the north of Balochistan province of Pakistan situated at 1,680 meters above the sea level and covering an area of 178 km². Here, the average annual temperature is 15.7 °C with average precipitation 159 mm.

Plant material and experimental site

The experiment was laid out during Kharif season with three replicates in a Randomized Complete Block Design (RCBD) at the field area of Department of Plant Breeding and Genetics Balochistan Agriculture College Quetta by evaluating eight soybean genotypes from diverse origins. The plant material was collected from Oilseed Research Program NARC, Islamabad. The experiment was performed at the experimental area of Balochistan Agriculture College, Quetta during Kharif 2019 (June-October). After deep ploughing ridges were made manually and seeds were planted with a 55centimetre row-row spacing and 5 centimeters plant-plant distance. Each genotype was seeded in three rows of each replication with 4 meter long. All genotypes were characterized following the standard procedures described by International Plant

Genetic Resources Institute [IPGRI], (1984). All morphological traits were analyzed using computer STATISTICA ver. 7.0. Data for various studied traits were recorded for five selected plants with the following procedure.

Days to flowering

Based on whole plot, days to flowering was observed visually by recording 50% of plant bloomed in each replication.

Days to maturity

Days to maturity were recorded based on whole plot and crop was harvested when 80% pods turned brown in each replication for each genotype.

Plant height (cm)

Data regarding plant height was measured with scale for each genotype from each replication by randomly selecting five competitive plants.

Number of pods per plant

The number of pods per plant was counted for each genotype from each replication by randomly selecting five plants and data was averaged.

100 seed-weight (g)

The seeds were harvested and dried at 30-35 °C temperatures. The dried seed lots were counted for each genotype separately and determined the 100-seed weight (g) using electric digital balance.

Grain yield plant⁻¹ (g)

Five plants were selected from each genotype was threshed manually. The grain weight of each genotype was recorded in “gram” and averaged.

DNA extraction

Sample collection

Genomic DNA was extracted from the young leaves of 8 plants of each genotype following the standard procedures of cetyltrimethylammonium bromide (CTAB) method (Saghai-Marooft, et al., 1984). The presence of extracted DNA was quantified on 0.8 percent agarose gel by comparing with 1Kb gene ruler. The final concentration for PCR amplification was adjusted at 50/μl nanogram.

Table 1 List of soybean genotypes and their passport information

Code	Genotypes name	Origin	Source	Status
Aj-2	Ajmeri-2	Pakistan	ORP	Commercial Variety
K-8	CH-Hsu-3	China	PGRP	Exotic Line
JH	Jhungwang	South Korea	AAUR	Exotic Line
K-0	CH-NEsp-17	China	PGRP	Exotic Line
KD	Daewonkong	S. Korea	AAUR	Exotic Line
Ajmeri	Ajmeri	Pakistan	ORP	Commercial Variety
NARC-II	NARC-II	Pakistan	ORP	Commercial Variety
K-98	Ertou#2	USA	PGRP	Exotic Line

Table 2 Details of 7 SSR primers used for the genotyping of soybean genotypes

Locus Name	Forward & reverse primer sequence (3'-5')	Fragment Size in bp	Modif
Satt440	TGAGAACGTTTGAAAAGAGAT GAAGAGATTAAGCATAAAGAATACTT	166–206	(ATT) ₁₄
Satt250	CGCCAGCTAGCTAGTCTCAT AATTTGCTCCAGTGTTTAAGTT	120–210	(ATT) ₁₉
Satt554	GCGATATGCTTTGTAAGAAAATTA GCGCAAGCCCAAATATTACAAATT	170–210	(ATT) ₃₃
Satt12	GCA ATT AGT TTT AAA ATG TTT C AGA ATA GAG CCT ACA TAT AAT CAT A	180–230	(ATT) ₁₉
Satt544	GCT ATG GGA AAA GGA TGT GTG GAG CTA CCC GAG ATG ATA CTC	220–230	(ATT) ₂₃
Satt264	CCTTTTGACAATTATGGCATATAGCATAGAAGGGCATC ATTCAGAT	160–210	(ATT) ₂₆
Satt294	GCGGGTCAAATGCAAATTATTTTT GCGCTCAGTGTGAAAGTTGTTTCTAT	250–280	(ATT) ₂₃

Electrophoretic analysis of SSR-PCR products

Images were captured using the Syngene multi-gene bio-imaging system under Jel-Dok conditions after the SSR PCR products were resolved by electrophoresis on 3.0% (w/v) agarose gels stained with ethidium-bromide (10mg/ml). Using a broad range 50 bp ladder (50-1000 bp) purchased from Ferment as Life Sciences, the molecular weight of bands was calculated and reported.

Scoring data

The study of the SSR markers data was qualitatively graded for presence or absence. To determine how genetically close the cultivars were, the similarity coefficient was applied. To determine the polymorphism information content (PIC) for SSRs, the following equation was used: according to Anderson et al.,1993, and results were documented.

$$PIC_j = 1 - \sum_i^R p_i^2$$

Where

i = the i th allele of the j th marker

r = the number of alleles at the j th marker

p = allele frequency

DAB staining

When hydrogen peroxide oxidizes DAB in the presence of some haem-containing proteins, such as peroxidases, a dark brown precipitate is produced. This precipitate is used as a stain to show where hydrogen peroxide is located in plant cells. The technique can be significantly altered to find hydrogen peroxide in various plant tissues.

Solution Preparation and DAB Staining

To create a 1 mg ml⁻¹ DAB solution, combine 50mg DAB and 45ml H₂O in a falcon tube. Add 0.2 M HCl to dissolve DAB, cover with aluminum foil, and add 2.5 ml Na₂HPO₄ and 25 l Tween 20. This creates a 10 mM Na₂HPO₄ staining solution, raising the pH.

Soybean plants with rosette leaves just beginning to pre-bolt were used for a study. Using a microbial elicitor solution, 0.5 M Flg22 was injected into the rosette leaves, eliciting innate immune responses. The treatment involved sampling three leaves from eight different plants and allowing the plants to develop. The leaves were preserved for three hours, and two milliliters of DAB staining solution was used to color the leaves. The leaves were vacuumed, and the DAB solution was replaced with bleaching solution. The plates were then boiled in a water bath at 90-95°C for 15 minutes, leaving behind brown precipitate. The samples were held at 4°C for up to four days. Direct visualization of the leaves was used for DAB staining.

Results and Discussion

In the current study, efforts were made to identify a few promising soybean genotypes using specific morphological traits, and molecular approach in order to create valid and practical varietal identification keys.

Analysis of variance for morphological traits

Analysis of variance (ANOVA) was computed for different morphological, and agronomic traits among

soybean genotypes for assessment of phenotypic variation. The results of ANOVA revealed highly significant differences among tested genotypes for all of the recorded traits including plant height at flowering stage, days to maturity, pod length, number of grains per pod, hundred grain weight and grain yield per plant. These findings regarding significant variation among soybean genotypes for various morphological traits was reported in several previous studies (Iqbal et al., 2008; Khan et al., 2014; Ghanbari et al., 2018).

Table 3 Analysis of variance for various morphological traits of soybean

Genotypes	(DF)	PHF (cm)	DM	PL (cm)	NGP	HGW (g)	GYP (g)
Replication	2	18.0517	7.0417	0.003	0.00007	0.2450	0.8045
Genotypes	7	74.994**	47.803**	0.4733**	0.1046**	21.581**	28.087**
Error	14	2.8898	17.9464	0.00326	0.00696	0.00696	2.4608
C.V	---	2.47	4.38	1.55	3.46	7.63	12.15

PHF = Plant height at flowering; DTM = days to maturity; PL = pod length, NGP = number of grains per pod, HGW = hundred grain weight; GWP = grain yield per plant

Significant variation for all eight traits among soybean genotypes was observed which showed the presence of adequate amount of genetic variability in studied soybean genotypes. The genetic potential of elite soybean genotypes for different morphological characters are defined by the mean values (Table 4). In a similar study, the results also showed significant variation between soybean genotypes for various morphological characters, like days to maturity, plant height, number of grains per pod, 100-seed weight and grain yield per plant (Ghanbari et al., 2018). Our results also showed that if the genotypes with high variability for different traits, there will be good chance for improvement in the existing genotypes by different conventional breeding techniques. Moreover, this variability observed can be used in different hybridization and selection programs for improving traits of interest. The result concerning mean values noticed during the present study are in accordance with the results found by Machado et al., 2017).

Mean Performance of agro-morphological traits

The use of plant diagnostic features has been the standard taxonomic method for both varietal purity testing and varietal identification. There were noticeable differences amongst the eight soybean types in terms of measured agro-morphological traits.

Plant height at flowering (cm)

Soybean genotypes showed differences for plant height. The genotypes' average plant height was 68.758 cm. NARC-II recorded the maximum plant height (73.333 cm), and Aj-2 had the lowest plant height (58.333 cm). These results are in line with the former scientist observation as they evaluated genetic diversity and interrelationships of agro-morphological characters in soybean including plant height (Iqbal et al., 2008). However, these findings showed contradiction in range of previously studies (Kumar et al., 2015; and Khatab et al., 2016).

Table 4 Mean performance of soybean genotypes for various morphological traits

S. No	Genotypes	PHF (cm)	DM	PL (cm)	NGP	HGW(g)	GYP(g)
1	Aj-2	58.333 A	99.67 A	3.1833A	2.3067A	8.050 A	10.220A
2	K8	64.733 A	100.33A	3.3867A	2.2333A	9.917A	9.687 AB
3	JH	68.80 AB	93.67A	3.9000B	2.2867B	12.560AB	13.333AB
4	K0	69.47 AB	89.00A	3.2867B	2.400BC	8.837B	10.853BC
5	KD	72.533 B	95.00A	4.1733C	2.680CD	14.163C	15.950CD
6	Ajmeri	71.200 B	97.33AB	3.9467D	2.473CD	13.947CD	15.853 D
7	NARC-II	73.333 C	100.0AB	4.1533D	2.6867D	14.550CD	17.187 D
8	K-98	71.667D	99.33B	3.5033E	2.2267D	9.170D	10.207 D
	G. Mean	68.758	96.792	3.6917	2.4117	11.399	12.808
	SE	0.9815	2.4458	0.0330	0.0482	0.5023	0.9057

CV%	2.47	4.38	1.55	3.46	7.63	12.15
LSD	2.9769	7.4187	0.1000	0.1461	1.5235	2.7471

PHF = plant height at flowering; DTM = days to maturity; PL = pod length, NGP = number of grains per pod, HGW = hundred grain weight; GWP = grain yield per plant

Days to maturity

Soybean genotypes exhibited a different range for maturity. Genotypes showed average days to maturity were 96.79 days. An exotic line named K8 took the maximum days to maturity (100.33 days), while K0 showed early (89 days) maturity. Our results were reliable with the previous studies in which similar range for days to maturity was recorded in soybean germplasm (Kumar et al., 2015).

Pod length (cm)

The studied pod length per plant among soybean genotypes varied greatly. Genotype KD and AJ-2 were observed

maximum to minimum 4.17 to 3.18 cm, respectively. This character was also measured by other scientists who worked on variability on morphological characters associated with pod shattering resistance in soybean (Krisnawati & Adie, 2017).

Number of grains per pod

The genotype namely K-98 and K8 had the lowest grains per pod 2.22 and 2.23, respectively, while the local variety exhibited highest grains (2.68) per pod. Our findings were consistent with previous studies in which similar range of seeds per pod in studied soybean germplasm (Kumar et al., 2015).



Fig.1 Pods of different size and colour indicating phenotypic variation.

100-seed weight(g)

The yield of all selected plants from each replication was combined and 100-seeds randomly picked to create a composite sample of well dried. Hundred seed weight ranged from 8.050-14.550 grams. The maximum test seed weight was found higher for two genotypes such as NARC-II and KD whereas lowest seed weight was recorded for AJ-2 and K0.

recorded in an exotic line K8 (9.687) grams. The average grain yield was (12.81 g). But these findings were contradicted in the range of previously studies (Li et al., 2020).

Grain yield per plant

The grain yield is important for both plant breeders and for farmers. In this study, high yield was observed in a local variety NARC-II (17.187 g), while low grain yield was

Flower colour

Flower colour based on visual field observation, were divided into two distinct categories, purple (3 genotypes) and white (5 genotypes). When flowers were in bloom in the field, varieties AJ-2, K8, K-D, Ajmeri, and genotype K-98 showed distinguished white flower colour. Variations in the genotypes' flower colours were caused by the action of the major genes. The development or inhibition of anthocyanin pigmentation by the genes determines the colour of the petal (Malik et al., 2011).



Fig.2 Flower colour variation among soybean genotypes.

Pearson correlation analysis

Among the studied traits concerned with soybean morphological attributes were found significantly positive correlation for plant height with several morphological traits like pod length, number of grains per pod, hundred grain weight and grain yield per plant. Similarly, most of the yield attributed traits showed highly significant positive relation with grain yield such as pod length, number of

grains and hundred grain weight. However, very weak but negative association was observed for days to maturity with plant height, grains per pod and grain yield per plant. The association of yield and yield attributed traits in soybean breeding is a very important in the selection process and identifying high yielding genotypes. Several previous studies performed correlation among soybean morphological traits and found similar association between yield components (Iqbal et al., 2008; Kumar et al., 2015; Ghanbari et al., 2018).

Table 5 Pearson’s correlation among soybean morphological traits

Traits	PHF	DTM	PL	NGP	HGW
DTM	-0.24				
PL	0.74**	0.02			
NGP	0.56**	-0.09	0.76**		
HGW	0.68**	0.03	0.98**	0.76**	
GYP	0.65**	-0.02	0.94**	0.88**	0.96**

Molecular diversity

Any initiative for crop development needs genetic diversity to successful parental selection. The versatility of DNA-based molecular markers allowed researchers to examine variation and variety in many plant species (Wang et al., 2006). By using DNA-based molecular markers, it is simple to compare the genetic makeup of two distinct plants without considering the effects of the environment on gene expression. Currently, a wide variety of DNA-

based molecular markers, including RFLP, RAPD, and AFLP, are available to identify DNA polymorphism (Schulman, 2007). The SSR approach was used in the current study to evaluate genetic polymorphism, and the primers generated a high level of polymorphism. The genotypes of two separate soybean gene pools were categorized in a genetic diversity study, and four SSR loci (Satt440, Satt012, Satt544, and Satt264) with high polymorphism were tested for genetic diversity estimation. Genetic diversity knowledge would assist plant breeders in broadening the genetic base of locally

adapted cultivars. As a result, estimating genetic diversity has become an important method for identifying genetically divergent parents with desirable traits (Oliveira et al. 2017). The genetic potential of the gene pool and the frequent recombination of diverse parents determine phenotypic variability (Oliveira et al., 2017). Due to their genetic potential, genotypes such as K-D, K0, K-98 and Aj-2 contributed the most overall variation and demonstrated stable performance for yield-related traits. High grain yield in soybean can be obtained by improving

several agro-morphological traits that directly contribute to high grain yield as a foundation for selecting superior genotypes (Kumar et al. 2015). Genetic diversity has been important in the collection, conservation, and exploitation of important genetic resources for soybean genetic improvement (Ramanatha Rao & Hodgkin 2002; Nawaz et al., 2020) As a result, the molecular markers system is a dependable method for identifying various soybean populations (Tasma et al. 2018).

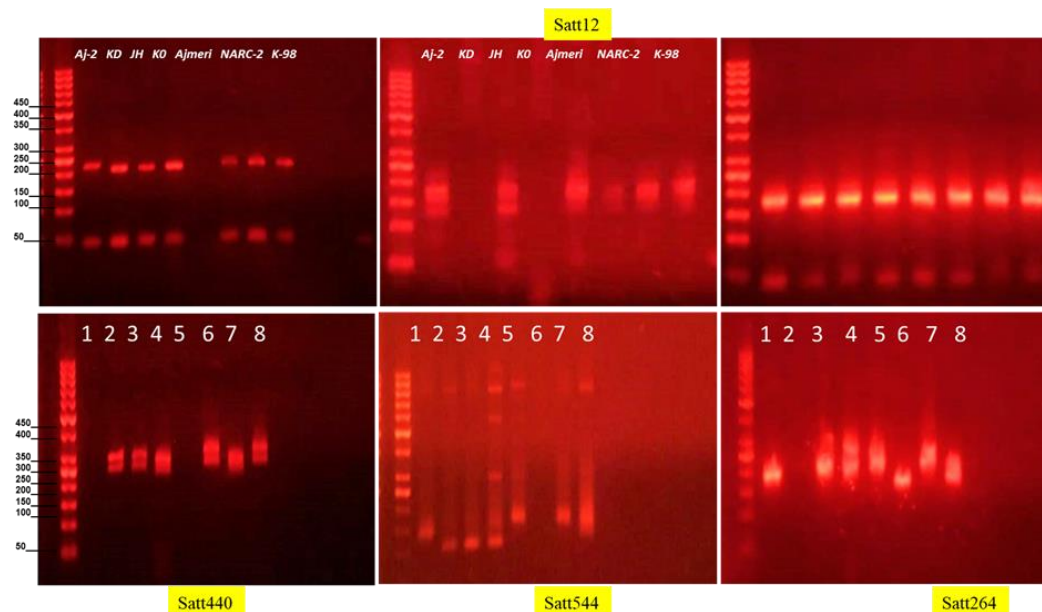


Fig. 3 Four informative loci Satt440, Satt012, Satt544 and Satt264

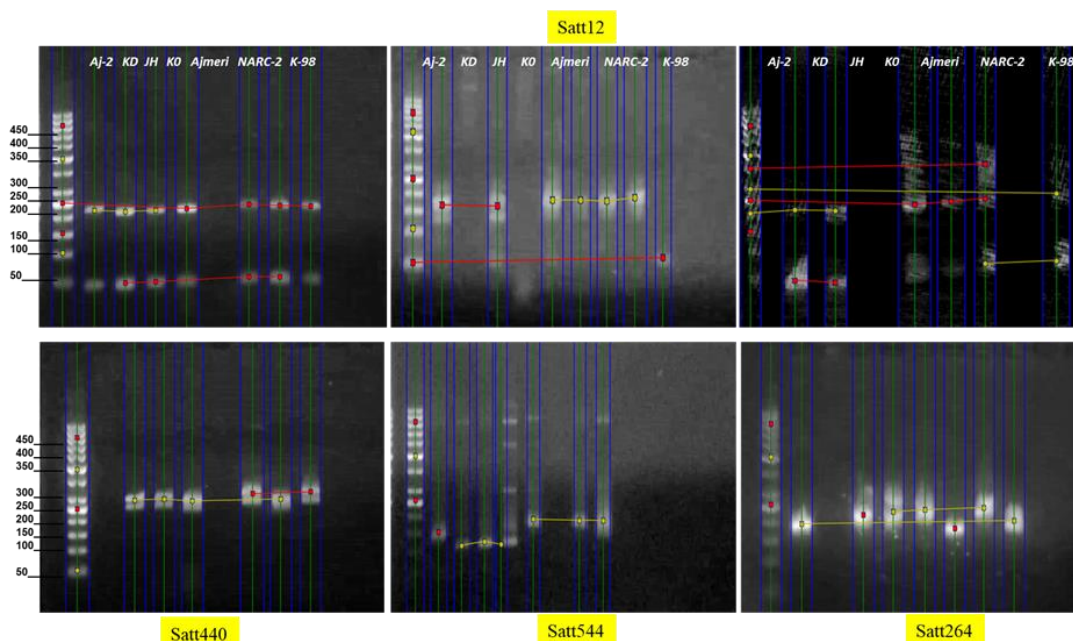


Fig. 4 Diverse genotypes:K-D, K0, K-98 and Aj-2

Cluster analysis based of SSR markers

Estimating genetic diversity among soybean genotypes is crucial for selecting divergent parents. SSR markers were used to separate the most diverse genotypes. Exotic lines 24600 and 24598 showed the highest genetic diversity,

while local genotypes NARC-II and Ajmeri-2 showed close resemblance. Various soybean breeders have used SSR markers for the assessment of genetic diversity in soybean germplasm by using cluster analysis (Koutu et al., 2019; Ullah et al., 2021; Kumar et al., 2022).

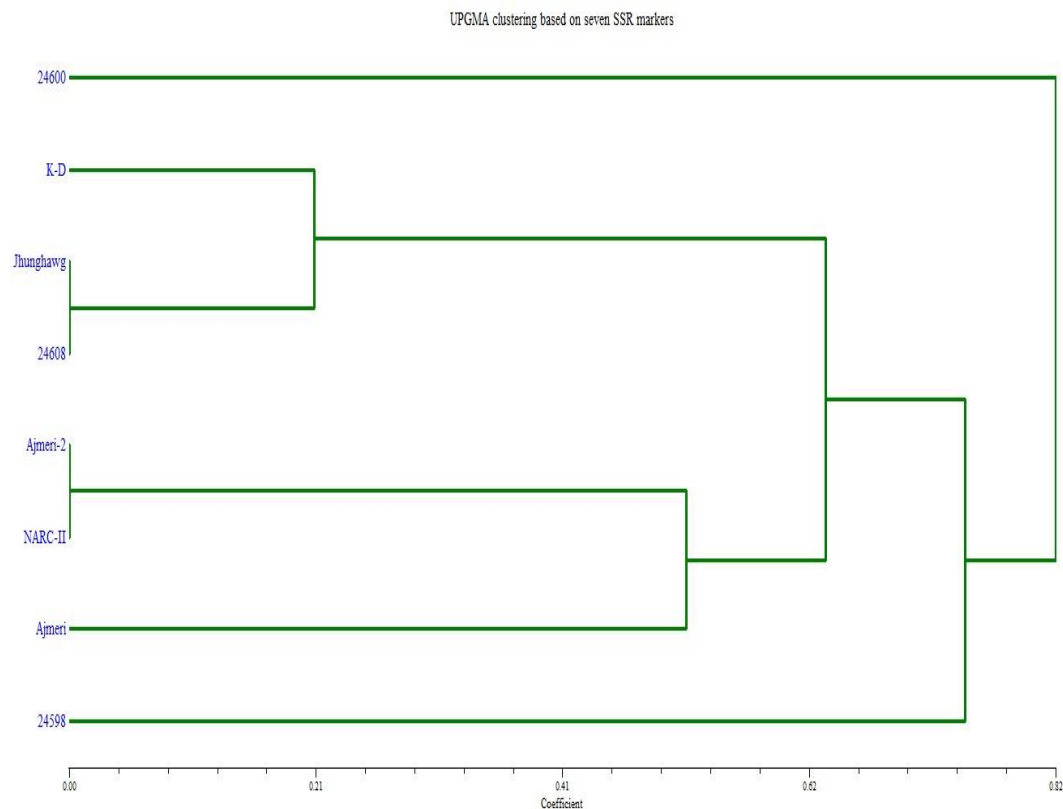


Fig. 5 Cluster analysis of eight soybean genotypes based on SSR markers

Characterization for drought tolerance

Compared with the control group; relative electric conductivity of leaves significantly increased in stressed group ($p < 0.05$), and it was 3.376% higher in stressed group.

Electrolyte leakage

Table 6 Effects of relative electric conductivity in Drought stressed and controlled

Electric Conductivity %	
Controlled	Stressed
11.5%	14.87%

To evaluate the integrity of the cell membrane during drought stress, electrolyte leakage was examined. We observed enhanced electrolyte leakage in AJ-2, K8, and JH; however, the impact of electrolyte leakage was

amplified following a two-minute immersion of the test tubes in boiling water. Changes in physiological traits in soybean with breeding advancements (Keepet al., 2016).

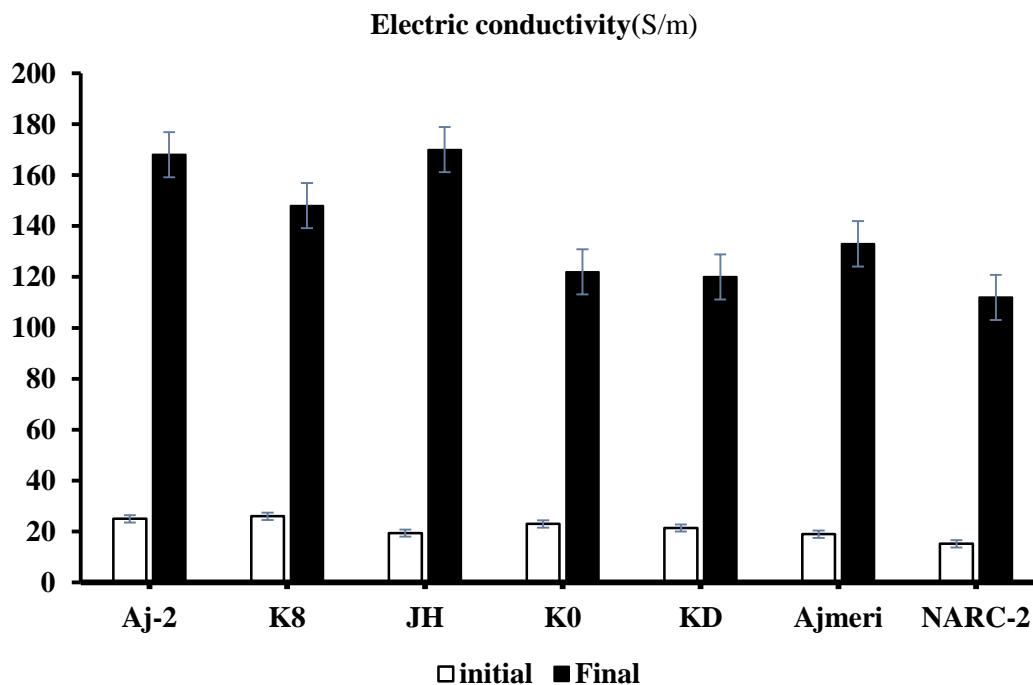
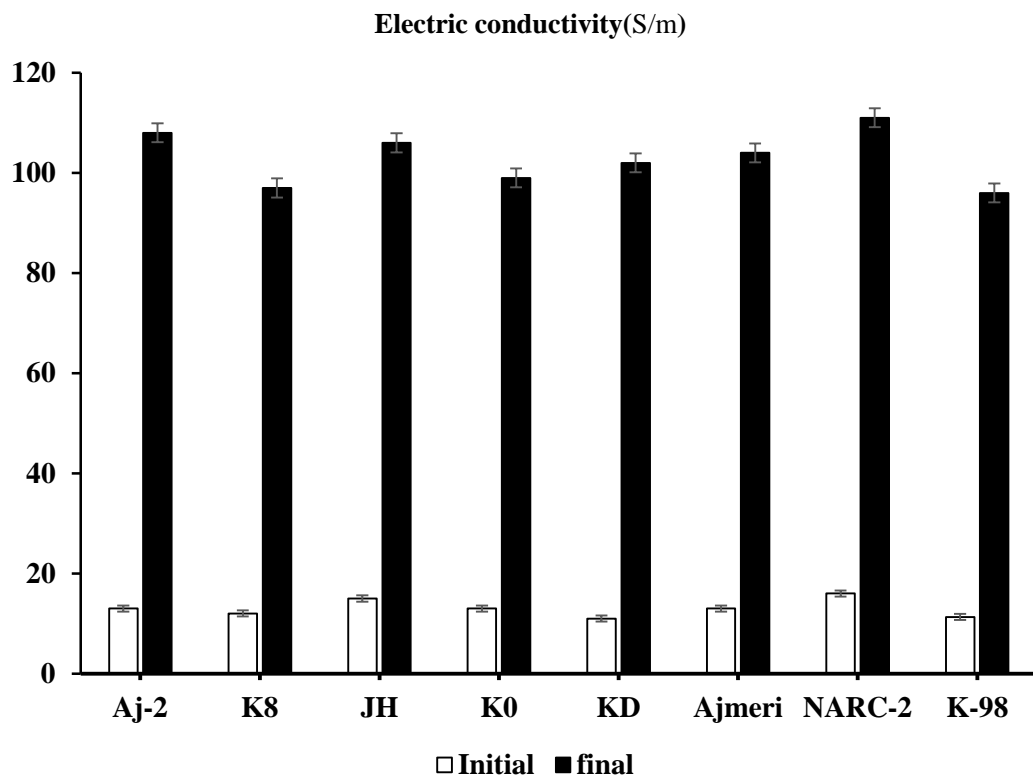


Fig. 6 (1) Stressed (2) Control

Diaminobenzidine (DAB) staining

DAB staining reveals severe H₂O₂ accumulation in susceptible genotypes, with reddish-brown patches present only at damaged areas, indicating higher tolerance

compared to resistant genotypes. Similar findings were also reported by (Mesquita et al., 2020) where the resistant genotypes showed lower accumulation of H₂O₂ and EC as compared to resistant genotypes, which mean our identified genotypes were also drought tolerance varieties.

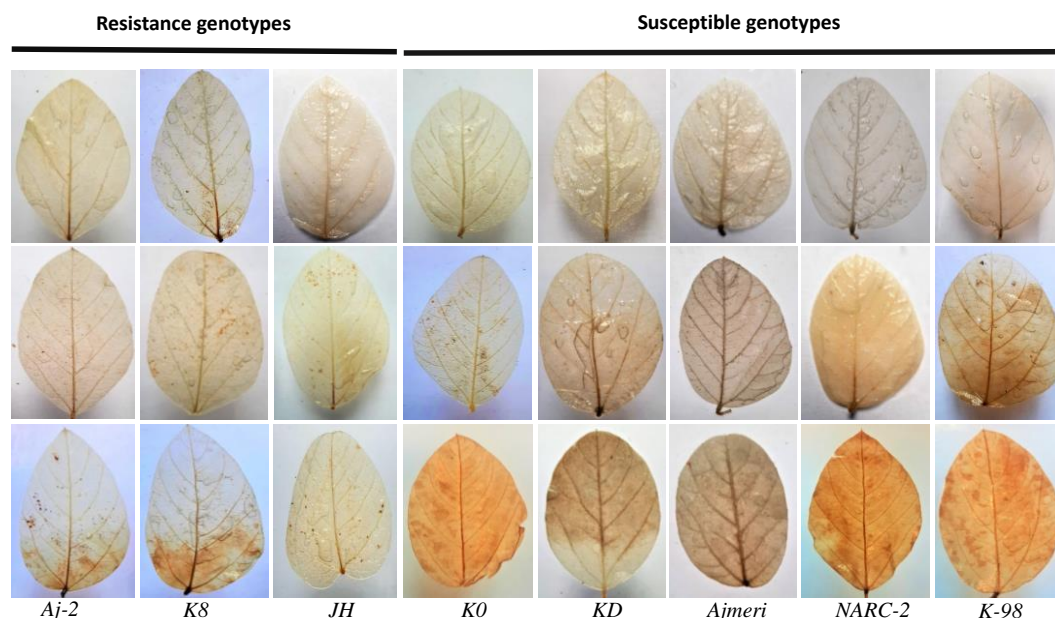


Fig. 7 On Left the varieties Aj-2, K8 and JH genotypes showed resistance against drought. (2) On Right the varieties K0, KD, Ajmeri, NARC-2, and K-98 genotypes showed reddish-brown patches indicating high amount of H₂O₂ accumulation.

Conclusion

The grain yield in soybean has a prime importance for both plant breeders and for farmer. In this study, high yield was observed in a local variety NARC-II (17.187 g), while low grain yield was recorded in an exotic line K8 (9.687) grams. The average grain yield for all genotypes was (12.81 g). Genetic diversity study categorized the soybean genotypes in two diverse gene pools (Korean Chinese and American Pakistani) and identified Four SSR loci (Satt440, Satt012, Satt544 and Satt264) with high polymorphism. An increase in electrolyte leakage was found in AJ-2, K8 and JH. While on DAB staining it was concluded that there are three genotypes resistance against drought stress (Aj-2, K8 and JH).

Acknowledgement: We are thankful to Dr. Ghulam Rasool, Associate Professor, Department of Plant Breeding and Genetics, Balochistan Agriculture College Quetta, and all lab members for practical facilitation in Molecular lab for this study.

Conflict of interest: The authors have no known contending financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Agarwal, D. K., Billore, S. D., Sharma, A. N., Dupare, B. U., & Srivastava, S. K. (2013). Soybean: introduction, improvement, and utilization in India—problems and prospects. *Agricultural Research*, 2(4), 293-300.
- Anderson, G. A., Churchill, G. A., Autrique, J. E., Tanksley, S. D., & Sorrells, M. E. (1993). Optimizing parental selection for genetic linkage

maps. *Genome*, 36(1), 181-186.

- Anjum, S. A., Xie, X., Wang, L. C., Saleem, M. F., Man, C., & Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research*, 6(9), 2026-2032.
- Arumuganathan, K., & Earle, E. D. (1991). Estimation of nuclear DNA content of plants by flow cytometry. *Plant Molecular Biology Reporter*, 9, 229-241.
- Boerma, H. R., & Specht, J. E. (2004). *Soybeans: improvement, production and uses* (No. Ed. 3). American Society of Agronomy.
- Brown, A. H. D. (1978). Isozymes, plant population genetic structure and genetic conservation. *Theoretical and Applied Genetics*, 52, 145-157.
- Dong, Y. S., Zhao, L. M., Liu, B., Wang, Z. W., Jin, Z. Q., & Sun, H. (2004). The genetic diversity of cultivated soybean grown in China. *Theoretical and Applied Genetics*, 108, 931-936.
- Farooq, M., Hussain, M., Ul-Allah, S., & Siddique, K. H. (2019). Physiological and agronomic approaches for improving water-use efficiency in crop plants. *Agricultural Water Management*, 219, 95-108.
- Ghanbari, S., Nooshkam, A., Fakheri, B. A., & Mahdinezhad, N. (2018). Assessment of yield and yield component of soybean genotypes (*Glycine max* L.) in north of Khuzestan. *Journal of Crop Science and Biotechnology*, 21, 435-441.
- Guan, R., Chang, R., Li, Y., Wang, L., Liu, Z., & Qiu, L. (2010). Genetic diversity comparison between Chinese and Japanese soybeans (*Glycine max* (L.) Merr.) revealed by nuclear SSRs. *Genetic Resources and Crop Evolution*, 57, 229-242.
- Hymowitz, T. (1970). On the domestication of the soybean. *Economic Botany*, 24(4), 408-421.
- Hyten, D. L., Song, Q., Zhu, Y., Choi, I. Y., Nelson, R. L.,

- Costa, J. M., ... & Cregan, P. B. (2006). Impacts of genetic bottlenecks on soybean genome diversity. *Proceedings of the National Academy of Sciences*, 103(45), 16666-16671.
- International Plant Genetic Resources Institute [IPGRI]. (1996). Descriptors for tomato (*Lycopersicon* spp). IPGRI, Rome, Italy.
- Iqbal, Z., Arshad, M., Ashraf, M., Mahmood, T., & Waheed, A. (2008). Evaluation of soybean [*Glycine max* (L.) Merrill] germplasm for some important morphological traits using multivariate analysis. *Pakistan Journal of Botany*, 40(6), 2323-2328.
- Iqbal, Z., Arshad, M., Ashraf, M., Naeem, R., Malik, M. F., & Waheed, A. (2010). Genetic divergence and correlation studies of soybean [*Glycine max* (L.) Merrill.] genotypes. *Pakistan Journal of Botany*, 42(2), 971-976.
- Kahair, S. M., Ali, A., & Salam, A. (2003). Production constraints of soybean in upland Balochistan. *Asian Journal of Plant Sciences*.
- Keep, N. R., Schapaugh Jr, W. T., Prasad, P. V. V., & Boyer Jr, J. E. (2016). Changes in physiological traits in soybean with breeding advancements. *Crop Science*, 56(1), 122-131.
- Khan, M. S. A., Karim, M. A., Haque, M. M., Karim, A. J. M. S., & Mian, M. A. K. (2014). Variations in agronomic traits of Soybean genotypes. *SAARC Journal of Agriculture*, 12(2), 90-100.
- Khatib, I. A., Morsy, A. R., Fares, W. M., & Kumamaru, T. (2016). Genetic diversity of soybean genotypes revealed by agro-morphological and SSR markers.
- Khurshid, H., Baig, D., Jan, S. A., Arshad, M., & Khan, M. A. (2017). Miracle crop: the present and future of soybean production in Pakistan. *MOJ Biology and Medicine*, 2(1), 189-191.
- Koutu, G. K., Shrivastava, A., Singh, Y., & Tiwari, S. (2019). Molecular characterization and genetic diversity assessment of soybean varieties using SSR markers. *International Journal of Current Microbiology and Applied Sciences*, 8(4), 173-182.
- Krisnawati, A., & Adie, M. M. (2017). Variability on morphological characters associated with pod shattering resistance in soybean. *Biodiversitas Journal of Biological Diversity*, 18(1), 73-77
- Kumar, A., Pandey, A., Aochen, C., & Pattanayak, A. (2015). Evaluation of genetic diversity and interrelationships of agro-morphological characters in soybean (*Glycine max*) genotypes. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 85, 397-405.
- Kumar, S. P. J., Susmita, C., Sripathy, K. V., Agarwal, D. K., Pal, G., Singh, A. N., ... & Simal-Gandara, J. (2022). Molecular characterization and genetic diversity studies of Indian soybean (*Glycine max* (L.) Merr.) cultivars using SSR markers. *Molecular Biology Reports*, 49(3), 2129-2140.
- Li, M., Liu, Y., Wang, C., Yang, X., Li, D., Zhang, X., ... & Zhao, L. (2020). Identification of traits contributing to high and stable yields in different soybean varieties across three Chinese latitudes. *Frontiers in Plant Science*, 10, 1642.
- Liu, K. (2012). *Soybeans: chemistry, technology, and utilization*. Springer.
- Machado, B. Q. V., Nogueira, A. P. O., Hamawaki, O. T., Rezende, G. F., Jorge, G. L., Silveira, I. C., ... & Hamawaki, C. D. L. (2017). Phenotypic and genotypic correlations between soybean agronomic traits and path analysis. *Genetics and Molecular Research*, 16(2), 1-11.
- Malik, M. F. A., Ashraf, M. U. H. A. M. M. A. D., Qureshi, A. S., & Khan, M. R. (2011). Investigation and comparison of some morphological traits of the soybean populations using cluster analysis. *Pakistan Journal of Botany*, 43(2), 1249-1255.
- Malik, M. F. A., Qureshi, A. S., Ashraf, M. U. H. A. M. M. A. D., & Ghafoor, A. B. D. U. L. (2006). Genetic variability of the main yield related characters in soybean. *International Journal of Agriculture and Biology*, 8(6), 815-819.
- Mesquita, R. O., Coutinho, F. S., Vital, C. E., Nepomuceno, A. L., Williams, T. C. R., de Oliveira Ramos, H. J., & Loureiro, M. E. (2020). Physiological approach to decipher the drought tolerance of a soybean genotype from Brazilian savana. *Plant Physiology and Biochemistry*, 151, 132-143.
- Mishra, N., Tripathi, M. K., Tiwari, S., Tripathi, N., Gupta, N., Sharma, A., ... & Tiwari, S. (2022). Characterization of soybean genotypes on the basis of yield attributing traits and SSR molecular markers. *book: Innovations in Science and Technology*, 3, 87-106.
- Nawaz, M. A., Lin, X., Chan, T. F., Ham, J., Shin, T. S., Ercisli, S., ... & Chung, G. (2020). Korean wild soybeans (*Glycine soja* Sieb & Zucc.): Geographic distribution and germplasm conservation. *Agronomy*, 10(2), 214.
- Oliveira, M. M., Sousa, L. B., Reis, M. C., Junior, E. S., Cardoso, D. B. O., Hamawaki, O. T., & Nogueira, A. P. O. (2017). Evaluation of genetic diversity among soybean (*Glycine max*) genotypes using univariate and multivariate analysis. *Genetics and Molecular Research*, 16(2), 1-10
- Popović, V., Miladinović, J., Tatić, M., Đekić, V., Dozet, G., Đukić, V., & Grahovac, N. (2013). Stability of soybean yield and quality components. *African Journal of Agricultural Research*, 8(45), 5651-5658.
- Ramanatha Rao, V., & Hodgkin, T. (2002). Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell, Tissue and Organ Culture*, 68, 1-19.
- Rasheed, A., Mahmood, A., Maqbool, R., Albaqami, M., Sher, A., Sattar, A., ... & Wu, Z. (2022). Key insights to develop drought-resilient soybean: A review. *Journal of King Saud University-Science*, 34(5), 102089.
- Saghai-Marouf, M. A., Soliman, K. M., Jorgensen, R. A., & Allard, R. (1984). Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance,

- chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences*, 81(24), 8014-8018.
- Schulman, A. H. (2007). Molecular markers to assess genetic diversity. *Euphytica*, 158(3), 313-321.
- Tasma, I. M., Yani, N. M. G., Purwaningdyah, R., Satyawan, D., Nugroho, K., Lestari, P., ... & Mastur, M. (2018). Genetic diversity analysis and F2 population development for breeding of long juvenile trait in soybean. *Jurnal AgroBiogen*, 14(1), 11-22.
- Ullah, A., Akram, Z., Malik, S. I., & Khan, K. S. U. (2021). Assessment of phenotypic and molecular diversity in soybean [*Glycine max* (L.) Merr.] germplasm using morpho-biochemical attributes and SSR markers. *Genetic Resources and Crop Evolution*, 68, 2827-2847.
- Wang, L., Guan, R., Zhangxiong, L., Chang, R., & Qiu, L. (2006). Genetic diversity of Chinese cultivated soybean revealed by SSR markers. *Crop Science*, 46(3), 1032-1038.
- Zhou, Y., Zhao, W., Lai, Y., Zhang, B., & Zhang, D. (2020). Edible plant oil: Global status, health issues, and perspectives. *Frontiers in Plant Science*, 11, 1315.