Investigation of Morpho-agronomic Performance, Genetic Diversity and Similarity Centers Exploration in International Safflower Panel

By

Fawad Ali

Department of Plant Sciences
Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad, Pakistan
2021

Investigation of Morpho-agronomic Performance, Genetic Diversity and Similarity Centers Exploration in International Safflower Panel

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

By

Fawad Ali

Department of Plant Sciences
Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad, Pakistan
2021

DEDICATED

TO

MY LOVING PARENTS

AND

ALL WELL-WISHERS WHO HELPED ME IN THIS JOURNEY

APPROVAL CERTIFICATE

This is to certify that the dissertation entitled "Investigation of Morpho-agronomic Performance, Genetic Diversity and Similarity Factors Exploration in International Safflower Panel" submitted by Mr. Farrukh Ali is accepted in its present form by the Department of Plant Sciences, Quaid-I-Azam University, Islamabad, Pakistan, as satisfying the dissertation requirement for the degree of Doctor of Philosophy (PhD) in Plant Sciences.

SUPERVISOR: _____

Dr. Tasawar Javed Chaudhary
Associate Professor
Department of Plant Sciences,
Quaid-I-Azam University,
Islamabad, Pakistan

DECLARATION

I **Mr. Fawad Ali** hereby state that my PhD thesis titled **"Investigation of Morpho-agronomic Performance, Genetic Diversity and Similarity Centers Exploration in International Safflower Panel"** is my own work and has not been submitted previously by me for taking any degree from Quaid-I-Azam University, Islamabad or anywhere else in the country/world.

At any time if my statement is found to be incorrect even after my graduation, the university has the right to withdraw my PhD degree.

Fawad Ali

Date:

APPROVAL CERTIFICATE

This is to certify that the research work presented in this thesis, entitled "Investigation of Morpho-agronomic Performance, Genetic Diversity and Suitability Studies Regarding to International Sunflower Trials" was conducted by Mr. Fawad Ali under the supervision of Dr. Hassan Javed Chaudhary.

No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Department of Plant Sciences, Quaid-i-Azam University Islamabad in fulfillment of the requirements for the degree of Doctor of Philosophy in the field of Plant Sciences. Department of Plant Sciences, Quaid-i-Azam University.

Student Name: Fawad Ali — Signature: _____

Examination Committee

a) External Examiner 1:
Dr. Muhammad Tariq Shahwani
Associate Professor
International Islamic University — Signature: _____

b) External Examiner 2:
Dr. Asma Jabeen
Assistant Professor
Fatima Jinnah Women University — Signature: _____

c) Internal Examiner:
Dr. Hassan Javed Chaudhary
Associate Professor
Quaid-i-Azam University — Signature: _____

Supervisor Name:
Dr. Hassan Javed Chaudhary — Signature: _____

Co-supervisor Name:
Dr. Mukhtar Ahmed Rabbani — Signature: _____
National Agricultural Research Centre, Islamabad

Name of HoD:
Dr. Abdul Samad Ahmad — Signature: _____

Plagiarism Certificate

It is certified that Mr. Rawan Ali (Registration No. 020116150004) has submitted his PhD dissertation entitled "Investigation of Morpho-agronomic Performance, Genetic Diversity and Similarity Centers Exploration in International Safflower Panel", has been checked on Turnitin for similarity index (plagiarism).

Overall plagiarism = 15%, that lies in the limit provided by HEC (19%).

Dr. Hassan Javed Chaudhary
Associate Professor
Department of Plant Sciences,
Quaid-i-Azam University,
Islamabad, Pakistan

Table of Contents

List of Tables

List of Figures

List of Appendices

ACKNOWLEDGMENTS

All praises to Almighty Allah Who created the universe. I would like to be grateful for the unconditional love and care of the Almighty Allah and for blessing me with patience and health to prepare this dissertation. I am obliged to show my affection and respect to Prophet Muhammad (SAW); a blessing for mankind.

I feel much pleasure in expressing my deepest gratitude to affectionate supervisor Dr. Hassan Javed Chaudhary, Associate Professor, Department of Plant Sciences, Quaid-I-Azam University, Islamabad for his dynamic and friendly supervision. He was so helpful and welcoming throughout the period of my research and dissertation writing.

I would like to thanks Dr. Malik Ashiq Rabbani (National Agricultural Research center, Islamabad-Pakistan), Dr. Faheem Shehzad Baloch and Dr. Muhammad Azhar Nadeem (Bolu abant Izzet Baysal University, Bolu-Turkey) for providing their support. I am also thankful to Dr. Abdul Samad Mumtaz, Chairman Department of Plant Sciences, Quaid-I-Azam University for his supportive attitude, humbleness, moral support and sincere guidelines during the study period.

I am thankful to Higher Education Commission (HEC) Pakistan for providing Indigenous Scholarship for Doctoral studies. I am also grateful to TUBITAK for providing me Pre-Doc Fellowship entitled “2216 - Research Fellowship Programme for International Researchers” at Bolu Abant Izzet Baysal University, Bolu-Turkey under the supervisor of Associate Professor Dr. Faheem Shehzad Baloch.

Special thanks to Abdurrahim Yilmaz (Turkey) and Sain Amjad Hussain (China) for their continuous support and encouragement in the process of learning and in the making of this dissertation must not go without acknowledging. Last but certainly not least, heartiest appreciations are goes to my beloved parent’s. My success is really due to their devoted prayers and encouragements.

FAWAD ALI

Abstract

Safflower has tremendous potential for various purposes; still area under safflower cultivation is limited. Safflower remains underutilized or neglected crop species due to low seed oil content, spininess and susceptibility to different diseases and pests attack. To reduce all such hampering factors and improve safflower productivity, we need an extensive investigation of the genetic diversity at different levels to identify germplasm containing novel alleles. During current Ph.D thesis, an international safflower panel was investigated for its morpho-agronomic performance conducting field experiments at two diverse locations (Pakistan and Turkey). Genetic diversity, population structure, and similarity centers were explored utilizing three molecular marker systems (iPBS-retrotransposon, ISSR and silicoDArT), while marker-trait associations were identified with silicoDArT markers. Safflower accessions provided by Plant Genetic Resources Institute, Pakistan (17 accessions) and Central Research Institute for Field Crop, Turkey (20 accessions) were also included along with international safflower panel (94 accessions) in iPBS-retrotransposon and ISSR studies. The planned aspects were analyzed in a systematic manner to achieve these goals.

The first study was conducted focusing the importance of the genetic diversity for crop improvement. The genetic variability that existed among and within populations for desirable agronomic traits can be used to develop elite cultivars. A total of 94 safflower accessions from 26 different countries were used in this study to evaluate morpho-agronomic performance, pattern of similarity centers and identification of best performing accessions by conducting two field experiments in two different geographical locations (Pakistan and Turkey) using augmented design. Genetic diversity for important yield and yield related traits was described including seed yield per plant (ranged from 4.86 to 51.02g), capitulum diameter (ranged from 17.30 to 28.30mm), branches per plant (ranged from 5.10 to 17.30) and capitula per plant (ranged from 8.70 to 80.40), and showed a good level of variation along with other studied traits in the current evaluation. Using the principal component analysis, it was observed that days to flower initiation, days to 50% flowering, days to flower completion, seed yield per plant, capitula per plant, branches per plant, seeds per capitulum and capitulum diameter were the major contributors to the observed genetic variability in the evaluated safflower panel. Seed yield per plant reflected a significant

and positive correlation with capitula per plant, branches per plant and capitulum diameter, and these traits can be suggested as a selection criterion in safflower breeding programs. The constellation plot and multivariate analysis was in agreement with the patterns of seven similarity centers based on seed yield per plant, capitula per plant, capitulum diameter, and branches per plant.

The second study was aimed to investigate the genetic diversity and population structure of 131 safflower accessions using 13 iPBS-retrotransposon markers. A total of 295 iPBS bands were observed among which 275 (93.22%) were found polymorphic. Mean Polymorphism information content (0.48) and diversity parameters including mean effective number of alleles (1.33), mean Shannon's information index (0.33), overall gene diversity (0.19), Fstatistic (0.21), and inbreeding coefficient (1.00) reflected the presence of sufficient amount of genetic diversity in the studied plant materials. Analysis of molecular variance (AMOVA) showed that more than 40% of genetic variation was derived from populations. Model-based structure, principal coordinate analysis (PCoA) and unweighted pair-group method with arithmetic means (UPGMA) algorithms clustered the 131 safflower accessions into four main populations A, B, C, D and an unclassified population, with no meaningful geographical origin. Most diverse accessions originated from Asian countries including; Afghanistan, Pakistan, China, Turkey, and India. Four accessions; Turkey3, Afghanistan4, Afghanistan2, and Pakistan24 were found most genetically distant. The findings of this study are most probably supported by the seven similarity centers hypothesis of safflower.

The third study was conducted to investigate genetic diversity, population structure and similarity centers pattern for 131 safflower accessions using 12 ISSR markers. A sum of 201 ISSR bands were obtained among which 188 (93.844%) were found polymorphic. Mean Polymorphism information content (0.448) and diversity parameters including mean effective number of alleles (1.655), mean Shannon's information index (0.557), mean expected heterozygosity (0.354), and mean overall gene diversity (0.377) showed a good level of genetic diversity in the studied safflower materials. Model-based structure, unweighted pair-group method with arithmetic means (UPGMA), and principal coordinate analysis (PCoA) clustered all accessions into three main populations; A, B, C and an unclassified population. Accessions originated from Asian countries like Pakistan and Israel were found most

diverse. Three accessions; Pakistan11, Israel1, and Pakistan26 were found most genetically distant. Analysis of molecular variance (AMOVA) revealed highly significant differentiation among the identified populations, and population × country combinations. The results presented in this work most probably supported the hypothesis of seven similarity centers of safflower.

The fourth study was performed to explore genetic diversity, similarity centers pattern, and marker trait associations of the 94 safflower accessions with DArTseq generated silicoDArT markers. Mean Diversity parameters including; observed number of alleles (1.99), effective number of alleles (1.54), Shannon's information index (0.48), expected heterozygosity (0.32), and unbiased expected heterozygosity (0.32) for the entire population reflected the presence of sufficient amount of genetic diversity in the international safflower panel using 12232 silicoDArT markers. Analysis of molecular variance (AMOVA) revealed that most of the variations (91%) in world safflower panel are due to differences within country groups. Model-based structure, Neighbor Joining algorithms, and principal coordinate analysis (PCoA) clustered the 94 safflower accessions into two populations representing meaningful heterotic groups for breeding purposes. Asian countries including; Egypt, India and Turkey exhibited the most diverse accessions in the available safflower panel. Three accessions; Egypt-5, Egypt-2, and India-2 were found most genetically distant. The 51.17% kinship coefficient ranged from -0.4 to 0, while 4.99% of the kinship coefficient ranged from 0.6 to 1, respectively in the international safflower panel. Current results supported the hypothesis of seven similarity centers for safflower throughout the world. Our study identified five significant marker-trait associations for traits *viz*., capitula per plant, 100-seed weight, plant height, seeds per capitulum, and seed yield per plant.

This is a pioneering study involving the comprehensive investigation of genetic diversity and similarity centers pattern of safflower at morpho-agronomic and molecular level. A new selection criteria was devised that can be implement to select best performing accessions in safflower breeding programs. Genetic diversity and population structure with iPBS-retrotransposon and marker-trait associations with silicoDArT markers were elucidated for the first time in safflower upto the best of our knowledge .We envisage that this study will be very helpful for global safflower

breeding community in order to develop cultivars with higher morpho-agronomic performance.

Chapter 1

General Introduction and Review of Literature

1.1. General Introduction

A steady increase in the production of safflower has been observed during the last two decades to meet the vegetable oil shortage. The safflower harvested area and production during the year 2017 was observed 840, 835 ha and 690, 846 tones, respectively (FAOSTAT, 2017). Total oil crops production worldwide during 2017-18 was recorded about 584.30 million tones (Anonymous, 2017a). It is interpreted by an FAO report predicted for 2018-2027 that global oilseeds production is expected to expand at around 1.50% per annum, well below the growth rates of the last decade. Vegetable oil has one of the highest trade shares (41.00%) of production of all agricultural commodities. This share is expected to remain stable throughout the outlook period, with global vegetable oil exports reaching 96 Mt by 2027 (Anonymous, 2017b). There is a dire need to focus on the breeding activities to cope with the oilseed shortage. The cultivated safflower varieties and breeding lines observed low genetic diversity; which reduced its utilization in the safflower breeding programs. Therefore, it is necessary to take into consideration the phenotypic and genotypic characterization of global safflower germplasm for the development of crop improvement strategies to enhance safflower production (Kumar *et al.,* 2015).

1.2. Underutilized/minor crop species and their role

Underutilized crops are considered all those plant species whose genetic capabilities are not fully explored. These (non-commodity) crops shared the larger biodiversity portfolio and remain underutilized by the farmer and consumer community for a number of factors including; agronomic, economic, and cultural (Padulosi and Hoeschle-Zeledon, 2004). Some of the major constraints of underutilized crops might include; poor shelf life, unrecognized nutritional value, poor consumption awareness, and reputational problems. Some of the crops are neglected up to the extent that severe genetic erosion of their gene pools often makes them as lost crops (Williams and Haq, 2002). Underutilized crops can overcome the constraints to higher production and better utilization as the demand for crop attributes changes. Many of the today world important oilseed crops i.e., oil palm and soybean were previously remained as underutilized crops (Kunkel, 1984). Besides the adaptation of underutilized crops to the marginal lands, these might also provide alternatives to the farmers to maximize the land usage in response to the climate

changes (Mayes *et al.*, 2011). Moreover, underutilized crop species may be helpful in the increasing demand for various types of natural and environment friendly products and also beneficial to the farmers and agricultural businesses in the form of diversified income (Thies, 2000).

1.3.1. General introduction of safflower

Safflower scientifically known as *Carthamus tinctorius* L. belongs to family *Compositae/Asteraceae*. Safflower is an annual, self-compatible, thistle-like, diploid (2n = 2x = 24) crop believed to have a single origin of domestication in the Fertile Crescent region dating to approximately 4,500 years ago (Van Zeist and Waterbolk-Van Rooijen, 1992). Its haploid genome size is approximately 1.4 Gb (Ali *et al.*, 2019b). Safflower has long taproots that facilitate water uptake even in the driest environments, enabling this crop to be grown on marginal lands where moisture would otherwise be limiting. The genus *Carthamus* comprised of 25 species (Ashri and Knowles, 1960; Hanelt, 1963) while, the reclassification (López, 1990; Vilatersana *et al.*, 2005) contract the number of species to 18 in the genus *Carthamus* which separated perennial subshurubs from annual herbs.

1.3.1 a. Origin and domestication of safflower

Safflower has been using since pre-historic time, while archeological remains of *Carthamus* species were found 7500 BC ago at sites of Syria (Marinova and Riehl, 2009). Distribution and cultivation of safflower from these sites to other areas like; Egypt, the Aegean, and southern Europe was occurred. Safflower is known as one of the oldest crop plant grown under dry and hot climatic conditions (Knowles and Ashri, 1995). It was observed from the archaeobotany that safflower has wide distribution in the areas including; Turkey, Syria, and the Levant that previously known as the Mesopotamian sub-region of the Irano-Turanian floristic region (Hanelt, 1963). Knowles and Ashri (1995) were the first who believe that safflower cultivation started in this region. Later on, Weiss (2000) identified central Syria, near the river Euphrates as place of safflower domestication.

1.3.1 b. Importance of safflower

Safflower, an underutilized crop is popular for its oil production and also used as an important medicinal and industrial plant from the Mediterranean region to the

Pacific Ocean, at latitudes between 45°N and 45°S. Safflower is well adapted to the dry lands due to its long root system that can penetrate up to the depth of about 220 cm. The presence of xerophytic spines attributes in safflower greatly contributes to tolerate the drought and heat stress (Ali *et al.*, 2019a). Safflower plant height ranged from 30 to 210 cm terminating in a globular flower heads, bright yellow, orange or red in color. Safflower capitulum/flower produces seeds ranged from 13 to 71 in number and takes 4 to 5 weeks to reach maturity after flowering. Safflower is cultivated in different parts of the world including United States of America, Canada, Mexico, Argentina, Eastern European Countries, Australia, Turkey, Egypt, Ethiopia, Kenya, China, Kazakhstan, Uzbekistan, India, Pakistan, and Afghanistan etc. (Ambreen et al., 2018). Traditionally, safflower was cultivated for its flowers (yellow-orange) that were utilized in making dyes, coloring, and food flavors (Ali *et al.*, 2019b). Safflower oil has better quality because it contain good amount of oleic acid and linoleic acid. Safflower florets have also been used for medicinal purposes in some parts of the world. For example, extracts from safflower flower florets have been shown to reduce hypertension and reduce blood cholesterol levels (Wang and Li, 1985). Safflower has popularized due to its huge potential as biofuel crop in the recent years (Dordas and Sioulas, 2009).

1.3.2. Safflower similarity centers

Early researchers observed the presence of similarity in safflower germplasm and proposed a number of similarity centers. The idea of safflower similarity centers was suggested by Knowles (1969). Accessions within the same center realized quite similarity to one another compared to accessions of the other similarity center. Knowles (1969) for the first time proposed seven similarity centers (1: Far East, 2: India-Pakistan, 3: Middle East, 4: Egypt, 5: Sudan, 6: Ethiopia, and 7: Europe) for safflower with respect to certain attributes including; plant height, branching, capitulum size, spines, and flower color. Ashri (1975) identified ten similarity centers (1: Near East, 2: Iran/Afghanistan, 3: Turkey, 4: Egypt, 5: Ethiopia, 6: Sudan, 7: Far East, 8: India/Pakistan, 9: Europe, and 10: Kenya) however, Chapman *et al.* (2010) proposed five safflower similarity centers for safflower (1: Near East, 2: Iran, Afghanistan, Turkey, 3: Egypt, Ethiopia, (Sudan), 4: Far East, India/Pakistan, (Sudan), 5: Europe) throughout the world. The presence of morpho-agronomic

differentiation among different safflower similarity centers suggested the availability of genetic diversity among the germplasm worldwide and vice versa.

1.3.3. Worldwide safflower germplasm resources and the idea of core collection

Germplasm are actually the gene pool for traits variability and play a vital role during the improvement of crop plants. Large population size of germplasm and its heterogeneous structure confines its easy availability and usage for different breeding programs (Noirot *et al.*, 1996; van Hintum *et al.*, 2000). Safflower germplasm have been conserved in different gene/seed banks of the world. National Bureau of Plant Genetic Resources in New Delhi (India) and Project Coordinating Unit for Safflower in Solapur (India) contain 2393 and 7525 safflower accessions, respectively. Western Regional Plant Introduction Station (WRPIS) (USA) contain more than 2400 accessions, while Iran and Turkey posses 200 and 125 accessions. Countries like, Iraq, Syria, Kazakhstan, Uzbekistan, and Tajikistan also contain some safflower germplasm traces. Frankel (1984) familiarized the idea of "core collection" for the most effective organization and application of the crop germplasm resources. A core collection is the minimum subset of that crop germplasm containing huge amount of variability wide spread in the whole germplasm. So, it is an easy job to characterize and evaluate the core collection as compared to the entire crop germplasm collection. Initial attempts were made to characterize and evaluate the core collection using agro-morphological traits and geographical distribution (Bhattacharjee *et al.*, 2007; Mahalakshmi *et al.*, 2007). With development of molecular markers, were used to elucidate genetic variability with greater efficacy. Usage of molecular markers greatly facilitated the development of robust germplasm core collections either alone (Zhang *et al.*, 2009) or in combination with phenotypic data (Díez *et al.*, 2012; Liu *et al.*, 2015).

1.4. Genetic diversity and its importance

Genetic characterization is considered the most important step for efficient securing and leveraging of the underutilized crop species resources (Padulosi *et al.,* 1999). The germplasm resources might include; cultivated plant materials, closely related, and wild species which are collected throughout the world. So, all these collections represent a potentially vital input parental resource for the safflower breeding improvement (Tanksley and McCouch, 1997). Wild germplasm and

unimproved landraces contain unexplored alleles important for the economical plant traits and adaptation to diverse environmental conditions might be particularly valued (McCouch *et al.*, 2013). Unfortunately, very few studies have been conducted regarding genetic diversity using safflower collections and its genetic potential remains untapped. The extent and distribution of variation at inter and intra species level is very critical to be known for its effective utilization especially in underutilized crops.

Dwivedi *et al.* (2005) developed safflower core subset of 570 accessions from 5522 accessions through recording data on 12 morphological traits and also using geographic information. The traits on which data was recorded revealed that the available genetic variation in the entire collection is preserved in the developed core subset. It was also observed that there is a strong phenotypic correlation among the studied traits both in the entire collection and core subset, predicting that the developed safflower core subset has preserved most of the co-adapted gene complexes controlling these correlations.

Jaradat and Shahid (2006) explored phenotypic diversity in the salt tolerant subset of safflower germplasm originated from 11 different countries of the three regions of the Middle East using important qualitative and quantitative traits. It was observed that the germplasm, among and within regions revealed high variability, especially for the traits related to yield and rosette period. A selection criteria was also applied based on high biological and seed yield, long rosette period and no or few spines. The combined applied selection criteria identified five best performing accessions (one each from Israel, Jordan, and Turkey, and two from Syria) and recommended to be introduced into the farming system as a multipurpose crop under saline agriculture.

Shivani *et al.* (2010) studied variability among 75 safflower lines and exhibited significant variation for all the studied traits. Safflower lines including; GMU-3327, GMU-3279, GMU 3325 and GMU-3313 were identified best performing for seed yield. Furthermore, seed yield revealed maximum contribution towards genetic variability followed by capitula per plant, seeds per capitulum, oil content, days to 50% flowering and days to maturity.

Shinwari *et al.* (2014) explored phenotypic diversity of 122 accessions obtained from different geographical regions of the world. Important qualitative and quantitative traits were considered for data recording and identified promising safflower accessions for the traits of economic significance. The traits like; capitula per plant, seeds per capitulum, seed yield, plant height, days to flowering initiation and days to maturity revealed significant variation. Important yield related traits; seed diameter, capitula per plant and seeds per capitulum observed highly significant (+ive) correlation with seed yield.

Kumar *et al.* (2016) characterized phenotypic diversity of the safflower collection comprised of 531 accessions for two consecutive seasons (2011-12 and 2012-13), recording data on 12 agronomically important traits. These materials were also first assessed using AFLP markers. All the studied traits observed significant variations in the safflower collection. It was the first study of developing safflower core collection utilizing both molecular marker and phenotypic data at the same time with geographical distribution.

1.5.1. Genetic diversity at molecular level

Genetic diversity assessment at the molecular level is vital for the crop improvement and germplasm resource conservation. Genetic diversity can be preferably detected with molecular markers as it overcomes most of the limitations occurring when using morphological and biochemical markers (Nadeem *et al.,* 2018b). It should be understand that various types of molecular markers have different characteristics and thus reflect diverse aspects of the genetic variation. Safflower germplasm characterization has been done using agro-morphological studies, biochemical analyses, and now day's molecular markers.

1.5.2. Inter simple sequence repeat (ISSR)

Zietkiewicz *et al.* (1994) developed the technique of inter simple sequence repeats. Usually long primers having a size of 15-30 bases are used in this technique. High annealing temperature usage is successfully allowed by the ISSR (about 45-60^{o}C); the amplified products are 200-2000 bp long and can be visualized with the help of agarose or PAGE (Fang and Roose, 1997). Segregating by simple Mendelian

laws of inheritance, ISSRs are categorized as dominant markers (Zietkiewicz *et al.*, 1994; Tsumura *et al.*, 1996).

1.5.3. Inter-primer binding site (iPBS)-Retrotransposons

Mobile genetic components changing its position throughout the genome are known as transposons. Transposons or transposable elements were first discovered in maize plant about 60 years ago (Finnegan, 1989; Grzebelus, 2006). Transposons were divided into two classes i.e., class I includes retro elements, such as retrotransposons. The class II comprised DNA transposons which changing their position in the genome by the cut-and-paste manner (Grzebelus, 2006). Primer binding sites (PBSs) of retrotransposons are utilized in this technique to overcome the problem of LTR sequence. Recently, inter-primer binding site (iPBS) markers have emerged as the most important and universal method for the determination of genetic diversity and relationships in various crops (Kalendar *et al.*, 2010; Kalendar *et al.*, 2011; Baloch *et al.*, 2015a).

1.5.4. Genotyping by sequencing (GBS) and Diversity Array Technology (DArTseq)

Genotyping by sequencing is known as a simple and multitudinous technology effectively using nowadays. GBS was for the first time developed in the Buckler lab under the Illumina next generation sequencing (NGS) platform. Sequencing cost has been lowered due to modernization in the NGS technology with the passage of time, thus assuring the GBS application for species having large genome with higher magnitude of diversity (Elshire *et al.*, 2011). Sequencing technologies resulted the development of sequence based markers i-e: Single-nucleotide polymorphism (SNP) and Diversity array technology (DArTSeq).

1.5.4 a. Diversity array technology (DArTSeq)

Polymorphic loci ranging from several hundreds to several thousands distributed over the whole genome are genotyped using this technology. This technology is considered as highly reproducible microarray hybridization. No previous sequencing information is needed to detect loci of desirable traits (Jaccoud *et al.*, 2001; Wenzl *et al.*, 2004). This technique is known to be most beneficial as it is high throughput and also very economical. A single-reaction assay can genotype

several thousand genomic loci with the discovery of polymorphic markers. As little as 50-100 ng genomic DNA is sufficient for the genotyping purpose. Utilization of an identical platform is occurred for the purpose of scoring and discovery of markers. After the discovery of a marker, there is no need of specific assays for genotyping, except starting polymorphic markers assembly into an array of a single genotype (Nadeem *et al.,* 2018a). High throughput assays of SNP have impressively increased for humans and model organisms and correspondingly the assay cost has also been decreased. As besides humans, the model organisms are limited in number, therefore it is very difficult to discover the sequence based polymorphism in these non-model organisms; including many crops with limited resources and often complex and polyploid genomes. Diversity Arrays Technology (DArT) has been developed for whole-genome sequencing of such non-model organisms without requiring the sequence information.

1.6. Molecular characterization in safflower

Utilizing molecular markers for the molecular characterization of the available genetic diversity in crop plants is more efficient. Sehgal *et al.* (2009) investigated genetic diversity and interrelationships among safflower similarity centers of 85 safflower accessions with 22 RAPD primers, 18 SSR primers, and 10 AFLP primers in combination. Mean polymorphism for RAPD, SSR, and AFLP primers was obtained 57.6, 68.0, and 71.2% among 111, 72, and 330 amplified loci, respectively. The parameters including; sum of the effective number of alleles (66.44), resolving power (59.16), and marker index (51.3) clearly declared AFLP marker system as most superior while exploring genetic variation in safflower accessions.

Chapman *et al.* (2010) tested safflower accessions from the previously proposed ten similarity centers along with individuals of the progenitor species using nuclear microsatellites. Five genetic clusters were obtained (1, Europe; 2, Turkey–Iran–Iraq–Afghanistan; 3, Israel–Jordan–Syria; 4, Egypt–Ethiopia; and 5, the Far East–India–Pakistan) proposing the presence of five similarity centers at molecular level during the current exploration. Information based on the presence of the genetic similarity between the progenitor and the Near Eastern safflower accessions confirmed a Near Eastern origin of safflower, which was according to the previous archaeological findings.

Lee *et al.* (2014) evaluated 100 safflower accessions derived from different geographical zones of the world using 30 highly polymorphic SSRs. The average number of alleles and expected heterozygosity was found 2.8 and 0.386, respectively. Investigation of population structure and phylogeny with 30 SSR primers exhibited genetic admixture between the geographical regions and genetic clustering.

Kumar *et al.* (2015) comprehensively investigate the presence of genetic diversity in a safflower collection of 531 accessions obtained from 43 different geographies; provide a more accurate representation of the crop genetic structure. The selected primer pairs generated a total of 381 fragments of which 157 were polymorphic among the analyzed accessions. High levels of genetic variability was resulted as indicated from genetic diversity indices obtained for the entire collection ($I = 0.4536$, $H = 0.2955$). STRUCTURE analysis obtained safflower clusters which could not be associated with their geographical origins. The BASP analysis showed geographical delineation with low admixture levels.

Ambreen *et al.* (2015) performed low throughput genome sequencing of safflower identifying 23,067 regions harboring perfect microsatellite loci. It was found that safflower genome was rich in di nucleotide repeats followed by tri-, tetra-, penta- and hexa-nucleotides. Out of 325 microsatellite loci, 294 loci produced robust amplification. A total of 23 safflower accessions from diversified agro-climatic zones of the world were assessed using the validated primers and resulted into the identification of 93 polymorphic primers (31.6%). Two to four alleles were observed at each locus, while mean polymorphism information content of 0.3075 was obtained.

1.7. Genetic mapping and maker-assisted selection (MAS) in safflower

Genetic mapping utilized techniques that help in the identification of genes locus and also estimate the distance between two genes. Genetic mapping worked on the principle of chromosomal recombination occurred during meiosis utilizing different molecular markers. There are two types of genetic mapping: (1) QTL mapping and (2) association mapping (Nadeem et al., 2018b). Marker assisted selection has greatly revolutionized the selection process dissecting complex traits by identifying the inherited markers linked to the trait of interest (Bernardo 2008). Research related to marker assisted breeding and linkage of the traits concern safflower is very limited (Hussain *et al.,* 2016). Research work has been conducted to

develop genetic maps and tag important morpho-agronomic traits in safflower (Hamdan *et al.,* 2008, 2012; Mayerhofer *et al.,* 2010; García-Moreno *et al.,* 2011; Pearl *et al.,* 2014; Mirzahashemi *et al.,* 2015; Ebrahimi *et al.,* 2017; Ambreen *et al.,* 2018) using RAPD, RFLP, SSR and SNP markers. Mayerhofer *et al.* (2010) for the first time developed genetic linkage map in safflower which serves as a foundation for its genetic studies.

Information related to genetic diversity and similarity centers is a key pillar to safflower breeders for better use of genetic resources and to handle genetic variation during different breeding programs. Dissecting the genetic mechanism of important morpho-agronomic traits is significant to breed elite cultivars with enhanced production. Research work involving the genome wide marker-trait association analysis is at the developmental stages in safflower. Safflower is an underutilized oilseed crop that received very little attention regarding the basic information of genetic diversity, similarity centers pattern and genome wide marker-trait associations, and need to paid more attention. Considering these research gaps in advancing the scientific knowledge, various studies have been designed conducting field experiments and utilizing different molecular markers exploring safflower accessions.

1.8. Objectives

The goals of this research work were therefore, to comprehensively investigate diversity at morpho-agronomic and molecular level, explore safflower similarity centers pattern, and to identify marker-trait associations for various morpho-agronomic traits in safflower panel collected from different countries.

To achieve these goals, the following studies were conducted in a systematic manner.

1. Investigation of morpho-agronomic performance and safflower similarity centers exploration by conducting field experiments at two diverse locations (Pakistan and Turkey).
2. Investigation of genetic diversity and safflower similarity centers exploration with different molecular marker systems; (1) iPBS-retrotransposon, (2) ISSR, and (3) silicoDArT markers.
3. Identification of marker-trait associations for important morpho-agronomic traits.

Chapter 2

Investigation of morpho-agronomic performance, similarity centers exploration and selection indices in international safflower panel for breeding perspectives

2.1. Introduction

Agronomic crops are grown on a large scale for consumption purposes because they provide food, feed, grain, oil and fiber. They also serve as a source of income to farmers and serve as an important source of raw materials for industries (Serce *et al.*, 2010; Cesur *et al.*, 2018; Galiana-Balaguer *et al.*, 2018).

About 75% of the global vegetable oil trade is derived from four main crops: soybean, oil palm, rapeseed, and sunflower. Such a huge share of these four crops led people to consider other oilseed crops as underutilized or neglected crops (Murphy, 1999). However, these underutilized oilseed crops represent a good source of genetic diversity and adaptation to diverse agro-ecological zones (Padulosi *et al.*, 1999; Thies, 2000; Özdemir *et al.*, 2018).

Safflower (*Carthamus tinctorius* L.) is one of the underutilized oilseed crops and belongs to family Asteraceae (Ali *et al.,* 2019b). It is known as one of the oldest crop plants grown under dry and hot climatic conditions of the Middle East, its domestication center (Knowles and Ashri, 1995). Safflower was first domesticated and grown due to its flowers for dyes, food coloring, and various medicinal uses but, it is also grown as an oilseed crop. Safflower is preferred over other oilseed crops due to its agronomic advantages such as drought resistance and adaptation to arid and semiarid conditions that represent important scenarios of the climate change (Weiss, 2000).

Safflower accessions belonging to specific geographical locations presents similarities on the basis of their morpho-agronomic traits and these geographical locations for safflower are known as its similarity centers. Various research studies have been conducted to explore the safflower similarity centers and different similarity centers have been proposed. Knowles (1969) proposed seven similarity centers (1: Far East, 2: India-5 Pakistan, 3: Middle East, 4: Egypt, 5: Sudan, 6: Ethiopia, and 7: Europe) for safflower while, Ashri (1975) identified ten similarity centers (1: Near East, 2: Iran/Afghanistan, 3: Turkey, 4: Egypt, 5: Ethiopia, 6: Sudan, 7: Far East, 8: India/Pakistan, 9: Europe, and 10: Kenya). Similarly, Chapman *et al.* (2010) proposed five similarity centers for safflower (1: Near East, 2: Iran, Afghanistan, Turkey, 3: Egypt, Ethiopia, (Sudan), 4: Far East, India/Pakistan, (Sudan), 5: Europe).

Safflower oil is rich in polyunsaturated fatty acids and this species is resistant to dry climates but, it shows unfavorable characteristics including low seed yield, low oil content, biotic stresses susceptibility, and spininess (Nimbkar, 2008). The cultivated safflower varieties and available breeding lines reflect a low level of genetic diversity which reduced its utilization in the safflower breeding programs. Therefore, it is highly needed to devise an extensive genetic and phenotypic characterization of the global safflower germplasm for the development of crop improvement strategies to enhance safflower productivity (Kumar *et al.*, 2015) and contribute to meet world oil demand. Characterization of the crop genetic resources provide an opportunity to find novel variations which can be helpful for the breeding activities (Baloch *et al.*, 2017; Nadeem *et al.*, 2018a; Yaldiz *et al.*, 2018). Plant phenotyping using easy-to-measure traits is particularly helpful for the preliminary evaluation of breeding nurseries (Asare *et al.*, 2011). Several studies have been conducted regarding safflower germplasm characterization using morpho-agronomic traits. Dwivedi *et al.* (2005) tested 570 safflower accessions in a core collection in search for plant characteristics including morpho-agronomic and quality traits, and the resistance to stresses. Jaradat and Shahid (2006) investigated 631 accessions of safflower from 11 countries using various morpho-agronomic traits, and revealed a good level of genetic variation. Kumar *et al.* (2016) evaluated 531 safflower accessions for 12 morpho-agronomic traits revealing significant variation; 85% of these accessions had plant height < 155cm and were more suitable for mechanical harvesting. Shivani *et al.* (2010) characterized 75 safflower accessions using morpho-agronomic traits and recommended four best performing accessions for different breeding objectives. They found maximum variability for seed yield and clustered all the accessions into eight groups. It has been suggested that phenotypic diversity in any crop plant is best estimated if morpho-agronomic traits evaluation is used along with proper multivariate analysis (Mohammadi and Prasanna, 2003; Vollmann *et al.*, 2005). Correlation analysis can be helpful to investigate the level of association between various traits and evaluated information can be effectively utilized as selection criteria for the improvement of crops (Iqbal *et al.*, 2006; Özer *et al.*, 2010; Baloch *et al.*, 2014).

2.2. Objectives

The current research work aimed:

- At evaluating the morpho-agronomic performance in the international panel of 94 safflower accessions across two diverse locations (Pakistan and Turkey).
- Devise selection criteria for the identification of superior safflower accessions.
- Exploration of similarity centers pattern.

2.3. Materials and methods

2.3.1. Plant material and phenotypic evaluation

Ninety four safflower accessions including one check cultivar named "Thori-78" from different geographical countries provided by United States Department of Agriculture (USDA) were used in the experiments **(Appendix I)**. Safflower field experiments were conducted at National Agricultural Research Center-Pakistan (2016-17) and Research Farm of Bolu Abant Izzet Baysal University-Turkey (2018), respectively. The experiments were arranged in augmented design at both locations with a single row having length of three meter for each safflower accession. Row to row and block to block distance of 50cm and 1m was maintained, respectively. Check cultivar named "Thori-78" used as control in this study, is the most commonly used cultivar in Pakistan due to its higher oil contents and resistance to various stresses and was repeated after every 16^{th} accessions in both experiments. Ten plants per each accession were maintained and used for data recording. Di-ammonium phosphate (DAP) and ammonium sulphate were used as source of fertilizer. All accessions were managed with the same agronomic practices and weeding was manually controlled.

Data was recorded on important qualitative and quantitative traits using International Board of Plant Genetic Resources (IBPGR) descriptors for safflower.

2.3.2. Qualitative traits

2.3.2.1. Growth habit

It was examined through visual observation that was either erect or bushy.

2.3.2.2. Early vigour

This parameter was estimated through visual observation to check out whether the vigor of the accessions is poor, intermediate or strong.

2.3.2.3. Leaf colour

Leaf colour was determined by visual inspection and was described as light green, green, and dark green.

2.3.2.4. Leaf shape

Leaf shape was determined by visual inspection. Various leaf shapes were described as ovate, oblong, and lanceolate.

2.3.2.5. Leaf margin

It was also determined by visual perception. Various leaf margins were described as entire, serrate, and parted.

2.3.2.6. Leaf spininess

Leaf spininess was observed by touching the plant leaves. Leaves of safflower were observed for no or few spines, intermediate or many spines. Leaf spininess was recorded at early flowering stage.

2.3.2.7. Leaf hairiness

This character was determined by touching the leaf surface and through visual sense. Leaf hairiness was categorized as non-hairy, intermediate, and many hairs.

2.3.2.8. Flower color

The flower color was observed by visual observation and was found as white, pale-yellow, yellow, yellow-orange, orange, orange-red, and red.

2.3.2.9. Capitulum shape

Capitulum shape was determined through visual observation. Different capitulum shapes were recorded like; conical, oval, and flattened.

2.3.2.10. Angle of branches

The branches angle was viewed through visual examination and were categorized as appressed (15-20^{o}), intermediated (20-60^{o}), and spreading (60-90^{o}).

2.3.2.11. Branching pattern

Three types of branching pattern were recorded like; basal, medium, and upper.

2.3.2.12. Seed shape

Randomly seed per each accession was selected and were categorized as oval, conical, and crescent.

2.3.3. Quantitative traits

2.3.3.1. Days to flowering initiation (no)

Days to flowering initiation was calculated from date of sowing till the days when at least 5% flowering initiation was observed in each accession.

2.3.3.2. Days to 50% flowering (no)

Days to 50% flowering was calculated from date of sowing till 50% flowering initiation was observed in each accession.

2.3.3.3. Days to flower completion (no)

It was calculated from the date of sowing till the days when at least 95% flowering completed in each accession.

2.3.3.4. Days to maturity (no)

Days to maturity was estimated by calculating the days from the date of sowing till the date when physiological maturity reached i.e. when 95% of the capitulum changed the color from green to yellow and the crop was ready to harvest.

2.3.3.5. Leaf length (cm)

Leaf length was calculated through actual measurement of the largest leaf started from the base to the apex of leaf blade excluding petiole. This data was noted at flowering stage.

2.3.3.6. Leaf width (cm)

Leaf width was examined through measurement across the widest portion of the same leaf used for leaf length. Similarly it was also noted at the flowering stage.

2.3.3.7. Plant height (cm)

Plant height was taken by meter rod in unit centimeter (cm). Plant height was measured on ten randomly selected plants of each accession. As the matter of fact, height is the length of space from ground to the peak of the plant where main capitulum is present.

2.3.3.8. Primary branches per plant

The primary branches of the ten randomly selected plants were counted through visual sense.

2.3.3.9. Capitula per plant

Total number of capitulum produced by the individual plant was counted manually. Same selected plants were used to calculate capitula per plant and the sum was then averaged.

2.3.3.10. Capitulum diameter (mm)

Diameter of each main capitulum of the selected plants was calculated through digital vernier caliper in millimeter (mm).

2.3.3.11. Seeds per capitulum

To determine number of seeds capitulum^{-1}**,** main capitulum of each selected plant was used from each accession. The selected capitulum was threshed separately and then number of seeds were counted and recorded.

2.3.3.12. 100-seed weight (g)

Randomly 100 seeds were taken and counted from each accession. These samples were weighed in gram with the help of an electronic balance.

2.3.3.13. Seed yield per plant (g)

The produce of each selected plant was threshed separately and dried up to uniform moisture content. The seeds were cleaned from impurities and dust and were weighed in gram with the help of an electronic balance.

2.3.4. Statistical tools

Augmented block design (Federer, 1956) with one standard check cultivar named "Thori-78" was used for this study and means were evaluated using the online software for augmented block design (Rathore *et al.*, 2004). Analysis of variance was computed for all the studied traits using the SAS statistical program (9.1.3 v.). The quantitative traits data from both location was averaged to calculate different parameters like mean, minimum, maximum, standard deviation, correlations, principal component analysis (PCA), and multivariate analysis using the statistical software XLSTAT (Addinsoft, 2018) (www.xlstat.com).

2.4. Results

2.4.1. Morpho-agronomic performance of safflower accessions

The studied plant traits revealed a wide range of variation in the evaluated safflower materials. Analysis of variance (ANOVA) was performed on 13 morpho-agronomic traits recorded across two different environments (Pakistan and Turkey) to understand the effects of accessions and locations (Table 2.1). Days to maturity, leaf length, capitula per plant, and seeds per capitulum has no effect on the accession. Mean data across two locations (Pakistan and Turkey) is presented in Table 2.2. The studied accessions reflected great variations for various traits at both locations (Pakistan and Turkey); all traits reflected greater performance in Pakistan except leaf length, seeds per capitulum, and 100-seed weight, which were more superior in the Turkey. Overall mean across two locations, minimum, maximum, and standard deviation is presented in Table 2.3. Days to flower initiation ranged from 113.5 to 131.5 with a mean of 120.95 days. Minimum days to flower initiation were recorded for accession India5, while the maximum was recorded in the accession Afghanistan2. Days to 50% flowering ranged from 117.5 to 137.5 with a mean of 126.48 days. Safflower accession India5 revealed minimum days to 50% flowering, while maximum days to 50% flowering were observed for accession Afghanistan2. Days to flower completion ranged from 121.5 to 143.5 with a mean of 133.09 days. Minimum and maximum days to flower completion were recorded for accessions India5 and Afghanistan2, respectively. Days to maturity ranged from 139.5 to 157.5 with a mean

of 148.50 days. Minimum days to maturity were recorded for accession India5, while highest number of days to maturity was recorded with Syria2 accession. Seed yield per plant ranged from 4.86 to 51.02 with a mean of 15.95g. Minimum seed yield per plant was obtained with accession France1, while maximum seed yield per plant was exhibited for accession China3. 100-seed weight ranged from 2.17 to 5.32g with a mean of 3.33g. Minimum and maximum 100-seed weight was revealed for accessions Afghanistan1and Egypt5, respectively.

Table 2.1: Analysis of variance for different traits of 94 safflower accessions across two locations

Traits	Source of Variation	Mean Squares
Days to Flower Initiation	Accessions	18.9516***
	Location	198803.4141***
Days to 50% Flowering	Accessions	34.9301***
	Location	189596.6111***
Days to Flower Completion	Accessions	38.8753***
	Location	171896.7475***
Days to Maturity	Accessions	30.2526
	Location	156410.2273***
Leaf Length	Accessions	9.2772996
	Location	94.0884854***
Leaf Width	Accessions	0.90938296*
	Location	10.18640455***
Plant Height	Accessions	212.16869***
	Location	65837.64985***
Branches Per Plant	Accessions	9.3901519*
	Location	15.5232000
Capitula Per Plant	Accessions	238.09251
	Location	12625.16336***
Seeds Per Capitulum	Accessions	54.357623
	Location	576.682667***
Capitulum Diameter	Accessions	11.320729***
	Location	165.477879***
Seed Yield Per Plant	Accessions	180.18912*
	Location	9472.65167***
100-Seed Weight	Accessions	0.71088189***
	Location	1.07804091

*Statistically significant, * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$)

Table 2.2 a: Mean data across two locations (Pakistan and Turkey) for various traits of 94 international safflower accessions panel

Traits	DFI		DFF		DFC		DM		LL		LW		PH	
Accessions	ISL	BOLU	ISL	BOLU	ISL	BOLU	ISL	BOLU	ISL	BOLU	ISL	BOLU	ISL	BOLU
Afghanistan-1	161	92	165	100	169	108	183	126	16±0.86	15.22±1.14	5.1±0.53	5.02±0.40	111±4.00	84.4±3.26
Afghanistan-2	163	100	169	106	174	113	188	125	14.7±0.37	9.68±0.66	4.55±0.18	3.6±0.61	134±6.00	81±2.07
Afghanistan-3	158	90	161	96	165	103	179	113	9.7±1.20	15.26±1.69	4.2±0.31	4.6±0.55	98±9.00	80.6±3.85
Argentina-1	163	83	150	86	152	92	166	113	14±1.40	13.48±0.76	4.7±0.41	4.28±0.30	93±3.00	65.2±3.25
Australia-1	156	88	160	95	164	103	178	113	19.85±0.95	12.56±0.57	6.3±0.62	3.82±0.24	108±3.00	64.2±3.92
Austria-1	154	88	159	93	164	106	178	125	15.25±1.33	15.44±1.72	4.8±0.69	4.04±0.40	108±3.00	77.2±4.28
Austria-2	151	94	158	100	162	105	176	125	14.4±1.73	12.42±0.74	3.2±0.58	4±0.16	110±1.00	57.8±2.33
Bangladesh-1	153	90	159	92	165	100	179	125	12.05±0.93	13.02±1.06	4.25±0.62	4.14±0.27	111±1.00	76.6±3.19
Bangladesh-2	154	87	159	96	165	103	179	113	17±0.70	12.94±0.98	4.9±0.42	4.16±0.24	102±5.00	71.2±4.68
Bangladesh-3	154	90	159	97	165	103	179	118	16.05±0.96	16.08±1.61	4.4±0.36	5.12±0.44	105±2.00	87.8±3.92
Bangladesh-4	141	87	151	92	153	100	167	124	10.002±1.13	12.62±0.82	2.5±0.18	4.5±0.25	78±2.00	52.2±3.07
China-1	155	88	162	94	166	100	180	125	14.15±0.78	16.14±0.47	4.5±0.26	5.8±0.41	110±4.00	86.4±2.54
China-2	158	94	164	100	168	106	182	118	19.75±1.06	11.92±0.25	5.15±0.37	3.68±0.15	114±5.00	74.8±3.20
China-3	151	83	158	88	164	99	178	113	8±1.13	13.88±0.08	3.2±0.56	4.1±0.16	96±2.00	74.4±3.96
China-4	151	89	157	97	172	105	186	115	17.2±1.82	16.6±1.05	4.8±0.80	4.54±0.29	109±1.00	81.4±9.22
China-5	152	87	157	98	162	109	176	125	12.9±1.57	24.9±1.60	4.2±0.61	5.32±0.46	96±2.00	95.4±7.45
China-6	155	97	160	107	168	115	182	125	22.4±1.86	14.94±0.90	8±1.01	4.28±0.21	121±1.00	69±4.29
China-7	158	93	162	105	166	115	180	125	11±1.08	19.64±1.48	2.6±0.56	4.34±0.49	81±3.00	78.6±2.04
Egypt-1	152	91	157	100	162	103	176	114	16.6±1.13	14.96±0.70	6.332±0.87	5.72±0.32	110±6.00	64.4±2.96
Egypt-2	161	91	166	100	170	106	184	117	20.276±0.76	14.62±0.27	5.626±0.23	3.62±0.23	133±2.00	63.6±1.47

Egypt-3	154	91	162	94	168	104	182	115	17.964±1.20	11.14±1.41	5.464±0.44	4.62±0.56	124±12.00	71.4±2.94
Egypt-4	153	90	159	93	167	100	181	125	20.732±1.47	15.78±1.49	5.7±0.52	4.36±0.34	107±3.00	75.2±2.22
Egypt-5	150	89	155	93	160	101	174	115	22.132±1.57	18.14±0.89	6.764±1.01	5.46±0.43	126±6.00	83.2±2.27
Egypt-6	150	90	155	96	160	104	174	127	13.032±0.79	14.46±1.20	4.064±0.33	4.3±0.53	106±4.00	79.8±4.68
France-1	154	97	159	105	163	110	177	125	13±1.55	14.72±1.02	3±0.72	3.94±0.33	96±1.00	68.4±5.08
Hungary-1	152	89	157	96	162	105	176	124	23.4±2.23	14.58±0.90	6.7±0.51	4.22±0.31	124±2.00	62.6±3.67
India-1	150	88	153	93	161	103	175	125	16.3±1.16	15.58±0.63	8.15±1.22	5.08±0.26	90±5.00	71.4±2.40
India-2	147	87	153	90	157	100	171	125	10.6±1.21	8.72±0.36	3.05±0.46	2.9±0.10	101±5.00	54.6±2.64
India-3	151	89	155	92	159	101	173	125	11.45±1.24	14.3±0.50	3.85±0.54	4.72±0.46	102±1.00	72.8±0.86
India-4	149	90	151	100	154	108	168	119	11.45±1.30	13.66±0.79	3.55±0.45	4.34±0.66	89±3.00	75.2±1.39
India-5	144	83	149	86	152	91	166	113	10.85±1.23	11.78±0.62	3.45±0.39	4.6±0.32	88±4.00	65.8±3.69
India-6	154	89	158	96	163	102	177	113	14.6±0.87	14.96±0.58	5.25±0.53	5.02±0.38	106±2.00	75.4±3.99
Iran-1	151	88	157	92	162	100	176	124	17.464±0.96	15.88±1.29	5.064±0.32	5.02±0.32	94±6.00	74.8±4.52
Iran-2	154	93	157	105	162	109	176	119	19.29±1.74	12.34±1.03	4.65±0.47	5.14±0.76	123±7.00	75±1.87
Iran-3	151	92	156	109	159	115	173	127	14.5±0.85	13.54±0.92	5.3±0.52	3.88±0.34	114±9.00	78.2±3.87
Iran-4	159	87	163	96	168	106	182	118	19.9±0.94	15.94±0.50	5.85±0.36	5.4±0.19	149±4.00	93.6±6.27
Iran-5	155	88	161	93	168	99	182	113	18.25±1.39	12.88±0.55	6.2±0.67	4.6±0.19	120±5.00	76±4.23
Iran-6	154	88	160	93	167	108	181	120	19.4±1.50	14.24±0.79	5.95±0.52	4.48±0.38	128±4.00	87±4.28
Iran-7	155	91	159	98	164	105	178	125	16.8±1.29	14.94±0.38	4.3±0.57	4.58±0.40	113±1.00	71.8±2.52
Israel-1	147	89	154	94	157	105	171	124	12.12±0.49	15.68±2.63	3.94±0.17	4.56±0.60	111±5.00	72.2±3.60
Israel-2	151	90	155	96	159	104	173	125	13.732±0.84	12.62±0.93	3.832±0.12	4.06±0.35	104±6.00	74.6±4.85
Israel-3	150	88	153	94	159	100	173	125	19.2±0.78	16.36±1.14	5.15±0.44	4.74±0.34	126±6.00	76.2±5.24
Israel-4	157	88	162	92	166	100	180	113	20.9±1.69	16.18±0.87	5.8±0.50	4.86±0.28	123±3.00	73.8±2.71

Iraq-1	152	97	157	103	167	110	181	125	16.6±0.89	13.46±1.21	5±0.42	3.72±0.31	131±5.00	81.2±3.92
Iraq-2	154	102	159	109	163	117	177	128	17.25±0.66	15.04±1.01	5.75±0.43	4.5±0.23	105±6.00	87.6±2.48
Jordan-1	155	88	159	90	163	100	177	114	16.7±1.81	16.54±1.45	5.664±0.32	4.98±0.45	109±4.00	82.4±3.78
Jordan-2	151	87	156	89	166	95	180	113	17.832±1.44	16.2±0.99	5.632±0.47	4.82±0.35	101±7.00	80.6±2.36
Jordan-3	151	87	158	92	164	100	178	113	13.55±1.38	12.1±0.61	4.85±0.63	4.54±0.32	103±4.00	77.8±0.97
Jordan-4	151	83	155	92	160	99	174	113	24.85±2.24	15.62±0.54	7.05±0.62	4.52±0.23	94±5.00	70±2.07
Jordan-5	153	88	159	92	167	100	181	113	16.3±1.31	14.76±0.83	5.67±0.76	4.56±0.33	102±5.00	71.2±2.63
Kazakhstan-1	150	85	152	89	154	97	168	113	13.95±1.08	14.24±1.13	4.25±0.38	5.28±0.45	110±1.00	65.6±3.49
Libya-1	155	89	160	98	165	107	179	124	11.5±1.24	13.96±0.79	4.5±0.46	4.74±0.30	115±2.00	74.8±2.65
Morocco-1	146	87	151	94	157	102	171	115	13.108±1.00	16.16±0.90	3.432±0.25	4.98±0.35	122±8.00	79.4±3.08
Morocco-2	153	91	157	99	163	103	177	124	12.3±0.78	15.14±1.01	4±0.32	5.04±0.34	114±4.00	88±3.24
Pakistan-1	148	88	153	92	157	105	171	124	17.5±0.85	16.56±1.01	5.564±0.36	4.9±0.17	120±6.00	78.6±2.68
Pakistan-2	147	85	151	89	155	93	169	118	17.832±1.46	16.42±1.37	5.864±0.31	4.68±0.57	89±3.00	61±1.70
Pakistan-3	147	83	150	86	155	92	169	118	14.264±0.84	13.28±0.57	4.8±0.34	4.32±0.33	82±3.00	56.4±1.21
Pakistan-4	150	84	151	87	156	92	170	118	12.8±1.23	12.02±0.29	3.932±0.18	4.2±0.23	76±1.00	44.2±1.88
Pakistan-5	146	86	152	91	157	99	171	118	12.8±0.53	12.14±0.83	4.3±0.25	3.72±0.21	111±2.00	71±3.81
Pakistan-6	153	90	157	94	163	106	177	124	14.664±1.16	13.68±0.45	4.832±0.14	4.56±0.16	108±2.00	67.8±1.96
Pakistan-7	152	88	155	93	160	102	174	125	18.032±1.54	16.48±0.86	5.3±0.24	4.98±0.39	103±5.00	73±3.78
Pakistan-8	153	89	156	95	159	100	173	118	17.2±1.20	12.72±0.68	6.35±0.64	4.1±0.24	96±7.00	58±3.94
Pakistan-9	153	90	157	96	160	107	174	118	16.95±1.27	13.82±1.17	5.29±0.59	4.5±0.50	99±7.00	64±4.79
Pakistan-10	152	89	158	97	171	108	185	125	15.6±1.69	14.72±0.59	4.5±0.65	4.68±0.18	113±3.00	73±6.14
Pakistan-11	150	87	155	91	161	100	175	115	15.38±1.15	8.72±0.61	4.7±0.22	3.28±0.22	121±2.00	66.4±3.75
Portugal-1	152	87	160	93	170	104	184	125	13.964±0.68	18.3±0.80	4.264±0.50	5.22±0.26	112±4.00	87.6±2.29

Portugal-2	152	96	159	104	168	107	182	125	14.964±0.47	15.92±0.66	4.8±0.26	4.08±0.06	124±2.00	89.6±2.16
Portugal-3	152	90	159	97	166	106	180	125	16.332±0.64	13.3±0.85	4.4±0.39	4.4±0.55	146±3.00	81.6±3.03
Portugal-4	151	92	156	101	161	106	175	125	18.932±1.25	14.12±0.29	7.032±0.34	4.56±0.27	126±8.00	66.8±3.72
Portugal-5	151	92	155	100	160	107	174	125	19.732±1.14	16.32±1.77	6±0.34	4.32±0.25	124±5.00	72.6±6.60
Portugal-6	157	96	166	103	171	112	185	125	17.3±0.70	13.38±2.31	5.09±0.63	4.7±0.48	120±5.00	81.4±3.26
Romania-1	153	92	155	101	159	107	173	124	15.36±1.99	15.24±1.22	4.48±0.61	4.74±0.22	123±4.00	80.2±3.58
Russia-1	150	90	155	100	159	112	173	125	14.5±0.72	13.44±1.28	4.95±0.43	3.94±0.56	131±3.00	74.8±5.51
Spain-1	151	88	155	93	159	101	173	125	14.264±0.77	15.66±0.57	5.532±0.30	5.42±0.13	111±6.00	71.2±2.73
Spain-2	154	88	158	92	162	100	176	125	12.032±0.56	15.44±0.95	4.364±0.29	4.98±0.41	118±3.00	85.4±1.66
Spain-3	151	89	159	95	160	106	174	125	15.732±0.69	15.1±0.43	5.3±0.31	5.06±0.25	113±5.00	89.6±1.21
Spain-4	155	88	161	93	164	100	178	115	14.532±1.18	13.2±0.74	5.8±0.46	5.34±0.54	108±3.00	81.8±3.38
Syria-1	154	89	160	96	164	103	178	125	15.792±1.24	15.9±0.94	4.05±0.35	5.84±0.94	119±4.00	83.6±4.71
Syria-2	157	93	169	100	174	109	188	127	15.35±0.82	15.56±0.85	4.4±0.33	4.7±0.20	121±4.00	82.8±4.18
Syria-3	151	89	155	96	160	104	174	118	10.9±0.84	14.56±0.68	4.6±0.59	4.76±0.36	95±2.00	82.2±3.04
Thailand-1	150	90	152	100	154	109	168	125	17.4±1.65	13.76±0.34	5.1±0.48	4.38±0.16	102±4.00	80.2±2.91
Turkey-1	150	86	154	90	160	99	174	125	13.8±0.91	14.16±0.80	4.55±0.40	4.62±0.44	118±6.00	83.2±5.07
Turkey-2	149	86	151	88	157	100	171	113	14.65±0.79	13.76±0.83	5.15±0.47	5.04±0.28	88±3.00	71.4±1.78
Turkey-3	155	83	159	90	164	102	178	118	13.7±0.79	11.98±0.99	3.95±0.37	3.72±0.39	99±5.00	59.8±3.67
Turkey-4	151	89	154	93	159	101	173	118	14.75±0.61	15.28±0.55	5±0.42	4.5±0.30	97±1.00	77±3.39
Turkey-5	150	87	152	91	154	108	168	125	13.5±1.03	11.94±0.74	4.6±0.43	3.92±0.44	136±2.00	73.2±2.87
Turkey-6	162	89	167	100	172	104	186	113	17.35±1.11	13.84±0.53	6.5±0.73	4.48±0.17	129±3.00	76.6±1.29
Turkey-7	154	89	159	94	169	108	183	124	14.55±1.84	12.94±0.96	5.55±0.62	4.66±0.45	137±4.00	85±2.14
Turkey-8	159	89	168	99	173	109	187	124	16.05±0.92	13.5±1.02	5.9±0.59	4.48±0.29	129±7.00	74.6±6.56

Turkey-9	152	90	157	99	162	106	176	125	13.7±1.77	12.92±0.71	4.2±0.71	4.52±0.20	122±2.00	79.2±2.42
Turkey-10	155	94	158	100	162	105	176	116	13.3±1.31	15.14±1.24	4.3±0.58	5.12±0.44	118±2.00	86.6±1.36
Uzbekistan-1	151	90	160	100	164	107	178	118	15.05±0.74	14.44±0.89	7.25±0.53	4.06±0.39	94±4.00	73.8±3.97
Uzbekistan-2	154	84	155	91	158	97	172	113	13.9±0.83	15.08±0.90	4.7±0.44	4.36±0.30	109±4.00	71±3.45
Uzbekistan-3	150	85	152	89	154	100	168	113	10.7±1.03	11.42±0.74	3.1±0.29	3.54±0.18	88±2.00	66.6±2.27

ISB: (National Agricultural Research Center) Islamabad, Pakistan; BOLU: Research Farm of Bolu Abant Izzet Baysal University, Bolu, Turkey; DFI: days to flower initiation; DFF: days to 50% flowering; DFC: days to flower completion; DM: days to maturity; LL: leaf length; LW: leaf width; PH: plant height; BPP: branches per plant; CPP: capitula per plant; SPC: seeds per capitulum; CD: capitulum diameter; SYP: seed yield per plant; 100-SW: 100-seed weight

Table 2.2 b: Mean data across two locations (Pakistan and Turkey) for various traits of 94 international safflower accessions panel

Traits	BPP		CPP		SPC		CD		SYP		100-SW	
Accessions	ISL	BOLU	ISL	BOLU	ISL	BOLU	ISL	BOLU	ISL	BOLU	ISL	BOLU
Afghanistan-1	7.00±0.45	10.40±2.5	26.80±2.92	22.60±4.06	29.5±2.02	23.8±7.84	23.62±1.85	23.58±0.66	8.16±1.56	3.63±1.17	2.41	1.92
Afghanistan-2	10.20±0.86	8.00±0.84	22.40±2.11	9.20±2.31	31.5±6.55	14.6±5.22	24.48±1.73	20.41±1.58	7.68±1.24	2.45±1.67	2.76	2.77
Afghanistan-3	21.20±0.66	13.40±1.75	73.20±2.52	21.60±7.12	18.3±1.87	28.4±8.25	18.92±2.02	22.12±1.29	25.61±2.92	6.93±2.28	1.88	3.14
Argentina-1	8.20±1.77	9.00±1.23	25.40±6.67	18.80±3.93	20.7±2.12	43.2±4.21	24.01±2.14	25.43±0.67	15.45±2.52	11.36±2.07	3.09	3.96
Australia-1	15.20±2.67	11.60±0.75	61.40±15.99	22.60±3.22	23.4±1.78	28.6±4.23	24.47±1.19	20.88±0.62	18.96±2.43	9.26±2.15	2.91	2.67
Austria-1	8.20±0.73	8.20±1.02	36.20±1.74	24.00±2.88	23.2±2.03	29.2±4.95	21.44±1.22	20.89±0.75	14.65±2.35	10.13±2.41	3.07	3.74
Austria-2	14.60±1.12	11.20±1.36	38.20±3.53	16.80±2.27	24.8±5.78	18.6±5.05	21.67±1.66	18.84±1.61	34.45±3.17	5.08±1.15	2.85	2.71
Bangladesh-1	10.80±1.24	7.40±1.12	19.40±4.02	15.80±1.66	14.1±2.06	23.2±4.31	22.48±1.85	22.07±0.74	21.2±3.19	5.94±0.63	2.99	2.75
Bangladesh-2	7.40±2.01	10.40±1.23	28.00±8.89	28.40±2.91	12.5±1.69	33.8±4.12	27.98±1.55	20.79±0.86	13.75±2.93	14.65±0.84	3.7	2.79
Bangladesh-3	8.80±1.5	8.40±0.51	26.00±5.22	16.20±1.36	15.5±3.41	36.2±6.42	20.65±2.4	24.18±1.43	15.65±1.98	6.01±2.05	3.13	2.5
Bangladesh-4	7.20±1.02	9.20±0.8	15.40±3.75	28.60±4.45	17.7±2.36	13.2±4.15	22.17±1.24	16.1±0.6	17.73±3.12	6.43±2.23	3.43	3.51
China-1	9.20±1.11	9.20±0.86	54.80±12.76	17.60±2.86	27.4±2.42	37.4±4.88	20.94±1.8	28.34±0.58	36.8±2.8	12.15±2.89	3.18	3.85
China-2	9.00±1.55	8.20±0.73	45.40±13.16	11.00±1.7	18.4±2.62	31.4±3.23	25.72±1.57	25.52±0.96	13.5±2.59	6.59±1.71	3.52	3.13
China-3	10.00±2.19	11.6 ±0.93	23.40±9.13	19.40±3.61	33±2.39	37.2±6.55	28.08±2.09	26.02±1.26	90.67±9.23	11.37±4.12	4.97	4.1
China-4	11.40±0.81	9.40±1.33	28.80±4.66	20.00±3.45	28.6±2.25	15.4±1.86	24.31±2.08	22.93±0.71	60.15±5.14	6.08±1.51	4.28	3.18
China-5	7.00±1.41	12.00±1.14	21.20±7.08	31.20±4.09	15.3±2.35	33.4±4.7	26.38±1.34	24.47±1.28	54.65±2.78	17.14±4.55	4.8	4.25
China-6	9.20±1.36	5.40±1.03	45.20±3.87	14.20±2.91	11.6±2.18	28.8±10.31	24.42±1.66	22.21±2.08	29.16±3.55	6.53±1.97	3.91	3.99
China-7	4.20±0.2	7.00±1.58	8.20±2.06	14.00±7.54	22.4±2.43	27.6±6.04	25.12±1.57	22.52±2.08	7.99±2.39	7.55±5.2	3.6	3.74
Egypt-1	8.80±1.39	9.20±1.39	24.40±4.57	13.20±1.8	16.7±1.15	26.4±6.28	27.1±1.57	23.39±1.28	8.99±1.23	4.43±1.57	2.73	4.15

Egypt-2	18.40±1.29	9.00±1.05	43.60±3.98	14.60±3.54	21.3±3.21	17±2.81	20.32±1.69	21.25±0.57	17.87±2.69	3.01±0.75	2.25	3.23
Egypt-3	12.20±3.65	8.80±1.39	50.40±6.8	22.40±3.75	15.8±4.23	28.4±4.06	30.96±1.31	25.64±0.76	62.73±7.71	8.99±1.95	4.35	3.23
Egypt-4	16.80±3.04	6.20±0.49	33.20±6.65	7.20±0.37	19.3±3.32	26.2±6.83	24.96±1.51	23.96±1.16	26.98±3.13	7.68±1.02	3.08	4.65
Egypt-5	13.60±2.98	8.40±1.08	36.60±9.1	15.40±3.7	27.2±4.52	12±2.39	29.02±1.31	24.28±1.67	59.66±4.75	5.98±1.33	5.29	5.35
Egypt-6	10.60±1.83	9.80±0.66	29.40±3.23	18.00±1.67	23±2.07	27±2.97	21.37±2.44	19.13±0.76	19.55±2.39	6.04±0.5	2.4	3.18
France-1	6.20±0.49	9.60±0.6	11.40±1.08	12.40±1.69	25.5±2.85	21.4±6.37	22.44±2.37	19.63 ±1.15	5.93±1.43	3.78 ±1.64	2.75	2.7
Hungary-1	16.20±1.16	10.00±1.58	67.80±25.85	21.80±2.52	22.8±2.12	19.8±3.1	25.41±1.33	22.75±1.06	49.48±3.63	13.63±1.29	2.8	4.14
India-1	8.80±1.83	8.00±0.32	32.40±9.92	15.80±0.8	29.7±3.45	20.8±1.93	24.37±2.14	21.48±0.99	18.45±2.02	9.55±1.64	3.95	4.34
India-2	12.00±2.43	12.40±1.08	33.20±6.37	35.00±11.3	21.2±3.64	16.2±6.51	19.21±1.84	18.83±1.42	7.93±1.39	9.9±5.56	2.86	3.97
India-3	8.80±1.98	5.40±0.51	16.60±5.46	18.60±3.43	18.5±1.92	21.2±3.34	23.19±1.31	21.79±0.47	9.73±1.85	3.72±1.1	3.96	3.49
India-4	8.40±2.06	12.00±1.52	17.20±5.04	19.00±3.69	22.9±2.68	9.4±2.6	22.34±2.02	20.08±0.65	9.78±2.35	5.21±1.61	3.72	2.92
India-5	10.60±2.93	8.40±1.89	22.80±7.98	17.60±1.69	19.8±1.82	10.2±2.69	19.89±1.57	18.51±0.78	7.27±1.63	3.36±0.65	4.38	4
India-6	7.80±0.92	12.40±0.81	40.20±9.88	21.20±1.66	12.3±2.05	24±3.77	25±1.71	22.61±0.62	13.99±2.59	4.87±1.29	2.98	3.41
Iran-1	15.00±2.76	11.00±0.55	53.80±12.64	18.40±3.08	32.5±5.03	28±6.24	28.82±1.38	22.4±2.1	49.72±5.23	9.19±3.93	3.27	4.45
Iran-2	15.00±3.7	8.00±0.55	62.00±20.43	19.00±3.85	30.9±5.32	41.6±8.08	28.27±1.51	26.95±0.79	20.52±2.34	6.64±2.31	3.2	2.9
Iran-3	18.40±3.01	9.60±0.93	59.40±17.2	20.00±2.3	19.2±1.78	29.4±5.5	30.28±1.38	24.2±1.29	33.47±3.7	2.18±0.59	3.16	2.24
Iran-4	9.80±0.73	10.40±1.13	31.40±1.21	24.60±4.51	24.4±2.77	28.2±4.59	23.69±1.8	21.95±1.4	3.87±1.14	11.56±3.04	2.01	3.06
Iran-5	14.40±1.69	9.00±1.24	35.60±3.78	18.60±1.69	33.1±3.77	23.8±4.77	25.26±1.55	23.85±0.34	5.49±1.53	4.28±0.66	2.06	3.2
Iran-6	11.40±3.57	10.80±0.73	53.80±18.05	21.00±2.07	27.1±1.85	40.4±9.48	26.75±1.33	25.07±1.2	26.29±3.42	9.75±1.45	3.94	3.7
Iran-7	11.20±0.8	8.40±1.03	47.80±6.31	18.60±2.56	41.1±0.99	43±5.58	26.67±1.18	26.12±1.74	21.38±3.69	7.25±0.42	2.48	2.76
Israel-1	6.40±0.51	8.60±1.21	17.20±0.8	9.60±2.5	16.3±1.83	23.8±7.15	14.7±1.38	23.02±0.67	6.25±1.22	9.85±1.06	3.27	3.34
Israel-2	5.00±2.32	8.60±1.17	12.60±1.63	18.20±5.23	21.9±2.33	26.8±4.31	26.59±1.81	21.24±1.24	9.11±2.61	10.42±4.08	3.98	2.96
Israel-3	9.80±1.46	11.80±0.66	35.00±5.02	28.00±4.95	25.4±5.84	24.4±2.25	27.32±1.38	23.06±0.41	26.7±2.84	6.08±1.55	4.5	2.99

Israel-4	6.60±1.12	9.60±1.63	40.80±9.33	23.20±3.61	17.6±2.36	22.8±7.12	24.74±1.36	20.24±1.1	28.35±4.07	17.01±2.29	3.83	4.46
Iraq-1	9.40±2.04	10.00±1.22	31.20±12.42	18.80±2.97	19.4±1.28	28.8±2.48	27.71±1.91	23.11±0.58	13.47±1.64	5.67±2.01	2.15	2.95
Iraq-2	17.50±4.17	11.00±0.71	47.75±12.02	8.80±1.77	30.5±4.67	24.2±6.76	27.65±1.67	22.42±1.33	25.65±2.36	3.56±1.3	3.75	2.78
Jordan-1	11.60±1.63	7.80±1.2	58.60±9.51	19.20±4.49	26±4.15	34.6±3.33	25.19±2.03	25.61±0.68	52.81±3.76	10.22±2	3.9	4.14
Jordan-2	16.00±3.08	8.60±1.12	73.20±27.39	19.00±1.58	16.4±1.44	20.6±2.8	26.14±1.38	21.9±1.8	71.1±3.69	7.27±1.42	2.32	4.56
Jordan-3	9.00±1.92	10.00±0.95	54.40±22.97	20.80±3.32	22.3±1.2	27.4±5.76	26.47±1.35	24.3±1.02	35.75±3.97	10.76±1.99	4.34	3.65
Jordan-4	9.60±1.75	8.20±0.49	65.80±16.75	23.20±3.07	21±2.61	28.8±6.91	24.27±1.39	21.62±1.27	30.65±3.52	10.12±1.73	3.57	4.48
Jordan-5	13.00±2.7	9.80±0.8	88.60±18.52	22.60±2.79	19.4±1.72	28±3.75	23.05±1.34	24.1±0.99	42.41±3.62	14.03±1.38	3.09	4.17
Kazakhstan-1	8.00±0.84	7.40±0.98	15.60±1.89	15.20±0.73	43.5±3.04	28±4.04	22.77±1.8	21.6±0.86	3.04±0.95	9.38±0.79	2.56	2.6
Libya-1	6.00±1	8.40±1.03	29.80±4.79	22.20±5.51	22.2±2.45	26.4±6.76	21.79±1.35	22.85±0.61	8.34±2.07	5.7±2.3	2.55	2.92
Morocco-1	14.60±2.2	7.00±1.52	34.20±3.07	31.60±8.7	26.8±3.19	24.6±5.58	21.38±1.55	25.28±3.46	10.14±1.42	11.35±3.99	2.55	3.41
Morocco-2	11.80±2.42	14.00±0.77	52.00±15.21	34.60±10.06	23.4±3.06	27±6.04	21.98±1.69	18.73±0.74	16.52±3.36	4.35±1.4	2.91	1.81
Pakistan-1	12.00±1.84	12.00±0.84	33.60±5.3	26.80±4.31	25.9±5.12	23±5.14	27.2±1.4	20.4±1.59	27±2.55	6.71±1.24	3.55	3.37
Pakistan-2	10.60±0.87	10.80±1.62	33.20±4.95	25.20±5.38	24.2±5.48	41.2±3.97	22.38±1.57	24.18±1.27	19.29±1.94	15.21±7.07	3.16	4.53
Pakistan-3	10.20±1.59	11.00±0.89	30.20±5.87	28.00±2.83	20.3±1.9	23.4±5.35	17.94±2.09	19.84±0.94	17.96±2.07	7.16±2.32	3.73	3.68
Pakistan-4	9.00±0.71	9.00±0.32	17.20±3.23	23.40±1.5	27±2.13	42.4±5.48	20.64±1.85	21.46±1.41	9.87±1.55	5.57±1.14	2.39	2.63
Pakistan-5	6.20±0.37	10.60±1.12	18.60±2.4	25.60±4.27	30.4±2.86	29±5.23	20.52±1.55	18.97±1.16	5.39±1.07	8.49±2.22	2.39	2.33
Pakistan-6	9.00±0.63	10.20±0.86	36.00±1	30.40±5.09	23.4±2.38	40.4±8.62	23.48±1.38	20.86±1.84	19.71±2.48	15.76±3.79	2.3	2.34
Pakistan-7	10.00±1.52	8.40±0.98	52.60±8.52	27.20±4.73	28.1±2.49	38.4±5.2	26.75±1.38	23.65±1.52	66.55±4.47	20.08±4.23	3.23	3.3
Pakistan-8	17.00±2.76	10.80±0.97	132.00±24.29	28.80±1.71	28.9±1.78	24.2±6.41	24.04±1.26	19.49±0.57	60.51±7.08	6.64±0.8	2.82	2.92
Pakistan-9	13.00±2.37	10.80±2.08	71.60±18.32	27.60±8.41	23.8±1.98	31.8±3.35	23.32±1.35	21.64±0.39	44.39±4.73	7.99±2.52	3.21	2.54
Pakistan-10	8.60±0.93	9.80±0.37	33.80±3.99	16.80±3.54	24±1.68	32.4±5.39	27.42±1.3	23.5±0.9	18.09±3.51	12.75±2.55	3.55	4.5
Pakistan-11	6.27±0.36	7.23±0.43	22.53±2.8	16.65±1.84	39.8±0.58	15.38±2.55	24.21±0.6	19.82±0.73	11.78±2.69	4.14±0.74	3.52	3.66

Portugal-1	8.60±1.12	12.60±0.93	41.20±9.91	29.00±4	15.5±2.39	31.8±7.87	27.85±1.31	24.9±1.08	18.61±2.4	13.56±2.13	3.79	3.94
Portugal-2	8.00±1.14	8.40±0.81	41.00±7.27	22.00±4.4	27.9±3.54	38.2±14.72	24.6±1.31	22.24±0.63	32.39±2.91	6.94±1.54	3.81	2.99
Portugal-3	6.20±0.49	9.00±0.55	21.60±3.5	26.00±4.37	18.8±1.42	29±3.54	29.79±1.31	24.12±1.25	24.84±3.9	9.17±2.57	3.93	4.16
Portugal-4	10.40±2.96	6.80±0.37	20.40±4.74	9.00±1.64	24.1±2.34	27.8±2.6	28.54±1.67	24.69±0.95	37±6.77	4.63±1.07	3.88	3.5
Portugal-5	9.00±1.14	12.00±1.22	33.00±2.07	17.00±3.35	25.4±2.35	43.4±7.08	26.87±1.82	24.26±1.45	16.03±1.81	9.65±2.3	3.28	3.12
Portugal-6	13.20±2.13	6.40±0.75	44.80±9.76	15.20±4.97	27.7±0.94	31.6±6.45	27.65±1.51	25.53±2.03	23.57±2.78	6.19±1.83	4.06	3.55
Romania-1	6.80±0.97	8.00±1.14	34.00±10.59	25.60±5.54	21.7±2.37	25±3.02	17.01±1.52	21.34±0.8	10.39±1.3	15.15±4.52	1.88	4.09
Russia-1	7.20±1.02	13.40±1.44	11.80±2.63	28.40±8.7	28±3.71	26.8±4.5	21.19±1.85	21.59±1	4.92±0.8	8.82±3.1	3.07	3.45
Spain-1	7.60±1.21	7.80±0.8	23.00±6.24	23.60±2.66	30.7±3.84	31.4±7.01	26.37±1.51	23.47±1.33	25.81±3.86	14.26±1.11	4.74	2.79
Spain-2	5.40±0.51	10.20±0.49	18.60±1.33	27.80±1.36	23.7±2.37	20.8±1.32	22.22±1.31	22.58±0.99	10.64±1.74	10.23±1.48	3.81	3.1
Spain-3	6.00±0.84	10.20±0.97	20.80±4.13	23.00±3.83	16.6±4.41	32.2±6.79	28.17±1.31	23.01±1.3	14.73±1.99	13.34±3.9	3.17	2.81
Spain-4	9.00±1.38	12.00±1	43.40±9.64	23.80±5.09	15.7±2.81	15.6±4.96	23.4±1.85	21.59±0.99	25.14±4.12	1.78±0.51	3.06	1.99
Syria-1	8.20±1.83	10.60±0.75	35.80±6.83	23.00±3.32	12.5±2.25	30.4±3.23	23.6±1.66	23.99±0.42	6.22±1	14.28±3.01	3.33	4.48
Syria-2	5.40±0.4	9.40±0.75	12.60±2.66	17.80±3.15	14.2±1.27	27.6±5.42	25.95±1.8	24.19±0.6	1.72±0.37	10.43±1.93	2.71	3.65
Syria-3	8.80±1.46	9.60±1.67	23.40±5.77	20.80±5.36	19.6±1.37	31±4.3	23.17±1.93	24.22±1.3	7.2±2.3	12.52±2.88	2.37	3.67
Thailand-1	7.60±1.17	8.80±1.83	21.50±5.58	18.40±3.84	12.5±1.41	34.2±2.56	24±1.66	25.72±0.7	7.32±1.79	8.19±2.19	2.77	4.12
Turkey-1	12.40±1.63	8.80±1.02	45.00±8.99	16.60±4.11	22.4±3.85	30.8±5.38	28.69±1.46	23.11±1.53	26.14±3.04	10.58±3.06	3.33	3.86
Turkey-2	9.00±1	10.20±1.11	33.00±3.39	19.20±3.25	11.3±1.6	20.8±5.59	25.27±1.5	22.95±0.74	23.07±3.09	8.35±1.48	4.22	4.26
Turkey-3	12.00±0.95	8.80±1.16	54.60±8.37	18.60±4.18	22.8±2.92	27.8± 9.6	22.83±1.97	21.99±1.67	27.35±3.74	5.99±3.9	3.35	3.1
Turkey-4	12.80±2.06	7.20±0.8	46.40±6.86	13.60±1.21	24.8±3.86	32±5.81	27.24±1.73	23.5±1.27	50.47±4.72	10.45±1.86	4.27	4.33
Turkey-5	5.60±0.81	11.00±1.25	31.60±19.97	31.80±4.5	20±3.08	33±6.27	20.45±1.57	23.85±1.67	6.41±1.51	16.92±3.23	2.41	3.88
Turkey-6	11.00±0.84	9.40±0.68	59.00±8.01	17.40±4.35	31±4.87	23±4.52	28.04±1.38	22.62±0.86	14.97±2.19	7.16±3.73	2.72	3.04
Turkey-7	12.20±2.6	10.00±1.14	74.00±22.69	23.80±3.65	13.8±1.08	27.8±2.69	24.58±1.28	24.68±1.41	24.29±3.19	10.68±3.29	3.02	3.35

Turkey-8	8.40±2.14	9.40±1.5	29.20±4.14	19.00±2.3	26.9±2.07	23.6±4.58	29.16±1.4	21.78±1.25	12.08±2.64	17.47±1.55	3.88	3.79
Turkey-9	8.00±0.32	9.20±1.16	46.20±10.92	17.80±4.21	18.8±1.43	43.2±7.55	26.42±1.18	25.56±1.26	30.02±4.42	13.07±3.99	3	3.1
Turkey-10	4.40±0.24	5.80±0.97	5.60±0.93	11.80±2.08	18.2±2.61	46.2±8.92	26.76±1.35	26.74±1.09	8.82±1.37	4.39±1.22	2.35	2.66
Uzbekistan-1	11.40±1.36	11.20±1.02	33.60±7.37	14.80±3.73	26.2±1.58	18.2±3.43	24.62±1.72	19.99±2.6	15.4±2.32	2.64±0.79	3.74	2.3
Uzbekistan-2	13.50±2.35	12.20±1.22	31.25±8.46	28.80±3.51	19.6±1.35	28.8±4.43	18.22±1.66	20.05±1.49	9±2.27	14.53±5.56	2.21	2.45
Uzbekistan-3	9.20±2.75	9.80±1.56	19.60±5.86	25.20±3.41	17.1±5.67	19.4±6	13.74±1.68	20.86±1.48	4.1±0.83	15.49±3.43	2.62	3.59

ISB: (National Agricultural Research Center) Islamabad, Pakistan; BOLU: Research Farm of Bolu Abant Izzet Baysal University, Bolu, Turkey; DFI: days to flower initiation; DFF: days to 50% flowering; DFC: days to flower completion; DM: days to maturity; LL: leaf length; LW: leaf width; PH: plant height; BPP: branches per plant; CPP: capitula per plant; SPC: seeds per capitulum; CD: capitulum diameter; SYP: seed yield per plant; 100-SW: 100-seed weight

Table 2.3: Mean, minimum, maximum, and standard deviation (StD) of the 13 morpho-agronomic traits in 94 international safflower accessions panel

Traits	Minimum	Maximum	Mean	Std. deviation
Days to flower initiation	113.5	131.5	120.946	3.033
Days to 50% flowering	117.5	137.5	126.478	4.1006
Days to flower completion	121.5	143.5	133.098	4.3712
Days to maturity	139.5	157.5	148.498	3.8143
Leaf length	9.66	20.235	14.9549	2.0515
Leaf width	2.975	6.615	4.7399	0.6531
Plant height	60.08	121.476	92.6249	10.3238
Branches per plant	5.1	17.3	9.8569	2.0503
Capitula per plant	8.7	80.4	28.9419	10.7033
Seeds per capitulum	15	42.05	25.2935	5.1874
Capitulum diameter	17.301	28.302	23.4978	2.3556
Seed yield per plant	4.855	51.021	15.9477	9.3188
100-seed weight	2.165	5.3195	3.3287	0.5933

Morpho-agronomic variations were also investigated at the countries level (Table 2.4) and Afghanistan revealed maximum days to flower initiation and days to 50% flowering, while Iraq exhibited maximum days to flower completion and days to maturity. Portugal showed maximum plant height and capitulum diameter. Hungary showed maximum leaf length, leaf width, capitulum per plant, and seed yield per plant, while maximum branches per plant, seeds per capitulum, and 100-seed weight were represented by Australia, Kazakhstan, and China, respectively.

Table 2.4: Country wise means of 94 international safflower accessions panel across two locations (Pakistan and Turkey)

Country	DFI	DFF	DFC	DM	LL	LW	PH	BPP	CPP	SPC	CD	SYP	100-SW
Afghanistan	127.33±3.81	132.83±4.50	138.66±4.75	152.33±5.57	13.42±1.89	4.51±0.50	98.01±9.04	11.70±4.85	29.30±16.29	24.35±1.99	22.18±1.55	9.07±6.24	2.48±0.30
Argentina	123±56.56	118±45.25	122±42.42	139.5±37.47	13.74±0.36	4.49±0.29	79.33±19.99	8.6±0.56	22.1±4.66	31.95±15.91	24.71±1.00	13.40±2.89	3.52±0.61
Australia	122±48.08	127.5±45.96	133.5±43.13	145.5±45.96	16.20±5.15	5.06±1.75	86.20±31.11	13.4±2.54	42±27.43	26±3.67	22.67±2.53	14.11±6.85	2.78±0.17
Austria	121.75±1.06	127.5±2.12	134.25±1.06	151±0.70	14.37±1.36	4.01±0.58	88.33±6.17	10.55±3.32	28.8±1.83	23.95±3.18	20.70±0.64	16.07±5.21	3.09±0.43
Bangladesh	119.50±3.71	125.62±2.95	131.75±3.57	148.00±2.97	13.72±2.18	4.24±0.54	85.54±14.27	8.70±0.39	22.22±4.41	20.77±4.62	22.05±2.17	12.67±1.51	3.09±0.31
China	122.21±3.63	129.21±3.92	136.78±3.71	150.71±2.64	15.95±2.68	4.60±0.90	91.94±6.73	8.77±1.81	25.31±7.80	26.27±5.44	24.78±1.33	25.73±15.43	3.89±0.47
Egypt	121.83±2.31	127.50±3.16	133.75±2.77	148.66±3.35	16.65±2.40	5.16±0.76	95.32±6.21	10.98±1.57	25.70±6.44	21.69±2.14	24.28±3.20	19.32±12.16	3.65±0.94
France	125.5±40.30	132±38.18	136.5±37.47	151±36.77	13.86±1.21	3.47±0.66	82.05±19.30	7.9±2.40	11.9±0.70	23.45±2.89	21.03±1.99	4.85±1.52	2.72±0.03
Hungary	120.5±44.54	126.5±43.13	133.5±40.30	150±36.77	18.99±6.23	5.46±1.75	93.34±43.48	13.1±4.38	44.8±32.52	21.3±2.12	24.08±1.88	31.55±25.34	3.46±0.94
India	118.41±2.81	123.00±3.31	129.25±4.05	145.83±3.95	12.85±2.27	4.49±1.24	82.57±5.38	9.58±1.73	24.13±6.88	18.85±3.59	21.44±1.99	8.64±3.01	3.66±0.42
Iran	121.85±1.40	128.50±2.75	135.14±2.34	149.57±1.51	16.09±1.21	5.02±0.42	99.92±11.78	11.60±1.49	34.57±5.36	31.62±6.17	25.73±1.64	15.11±7.99	3.03±0.57
Iraq	126.25±2.4	132±2.82	139.25±1.06	152.75±0.35	15.58±0.78	4.74±0.54	101.19±7.26	11.97±3.21	26.63±2.31	25.72±2.29	25.22±0.26	12.08±3.55	2.90±0.50
Israel	120.00±1.95	125.00±1.58	131.25±1.44	148.00±1.22	15.84±2.70	4.61±0.63	95.12±5.66	8.30±1.74	23.07±10.05	22.37±2.60	22.61±2.73	14.22±6.68	3.66±0.36
Jordan	119.40±1.71	124.20±1.20	131.40±1.51	145.60±1.34	16.44±2.67	5.22±0.39	89.14±5.10	10.36±1.42	44.54±7.15	24.45±4.19	24.26±1.09	28.51±7.36	3.82±0.27
Kazakhstan	117.5±45.96	120.5±44.54	125.5±40.30	140.5±38.89	14.09±0.20	4.76±0.72	87.66±31.20	7.7±0.42	15.4±0.28	35.75±10.96	22.18±0.83	6.20±4.48	2.57±0.02
Libya	122±46.66	129±43.84	136±41.01	151.5±38.89	12.73±1.73	4.62±0.17	94.98±28.54	7.2±1.69	26±5.37	24.3±2.97	22.32±0.74	7.01±1.86	2.73±0.26
Morocco	119.25±3.88	125.25±3.88	131.25±2.47	146.75±5.30	14.17±0.64	4.36±0.22	101.03±0.16	11.85±1.48	38.1±7.35	25.45±0.35	21.84±2.1	10.58±0.21	2.67±0.43
Pakistan	118.67±2.39	122.99±3.13	129.92±4.93	146.80±3.57	14.70±1.92	4.69±0.50	83.20±11.87	10.11±1.97	34.44±17.61	28.97±3.93	22.35±2.04	18.68±11.45	3.16±0.61
Portugal	122.33±2.50	129.41±3.00	136.50±2.98	152.50±2.30	16.04±1.14	4.90±0.52	102.75±6.42	9.21±1.25	26.68±7.20	28.43±4.64	25.92±1.31	16.88±2.97	3.66±0.31
Romania	122.5±43.13	128±38.18	133±36.77	148.5±34.64	15.3±0.08	4.61±0.18	101.82±30.57	7.4±0.84	29.8±5.94	23.35±2.33	19.17±3.05	12.76±3.36	2.98±1.56
Russia	120±42.42	127.5±38.89	135.5±33.23	149±33.94	13.97±0.75	4.44±0.71	102.72±39.49	10.3±4.38	20.1±11.73	27.4±0.84	21.39±0.28	6.87±2.75	3.26±0.27
Spain	120.50±0.91	125.75±1.50	131.50±1.29	148.87±1.70	14.49±0.82	5.22±0.40	97.10±5.14	8.52±1.32	25.50±5.43	23.33±6.34	23.85±1.64	14.49±4.01	3.18±0.54
Syria	122.16±2.56	129.33±4.64	135.66±5.10	151.66±5.75	14.67±1.69	4.72±0.20	97.40±7.58	8.66±1.10	22.23±7.10	22.55±2.39	24.18±0.76	8.72±2.30	3.36±0.47
Thailand	120±42.42	126±36.77	131.5±31.82	146.5±30.40	15.58±2.57	4.74±0.50	90.88±15.11	8.2±0.84	19.95±2.19	23.35±15.34	24.86±1.21	7.75±0.61	3.44±0.95
Turkey	120.95±2.87	126.15±4.85	133.70±4.23	148.65±3.96	14.04±0.92	4.73±0.49	96.98±10.91	9.28±1.72	30.71±10.40	25.91±4.68	24.81±1.51	16.43±6.49	3.39±0.58
Uzbekistan	119.00±1.50	124.50±4.92	130.00±4.76	143.66±3.88	13.43±2.05	4.50±1.16	83.79±6.35	11.21±1.67	25.54±3.98	21.55±3.02	19.58±2.53	10.19±1.41	2.81±0.42

To investigate genetic diversity more comprehensively in an international safflower panel of 94 accessions, various qualitative traits were recorded at the proper time (Table 2.5). Leaf colour was observed as light green (25.53% of total accessions) and dark green (74.47% of total accessions). Most of the safflower accessions (84.04% of total accessions) showed strong early vigor, while intermediate early vigor (15.96% of total accessions) was also observed. Growth habit was revealed as erect (75.53% of total accessions) and bushy (24.47% of total accessions) type. Leaf shape was classified as ovate (84.04% of total accessions), lanceolate (2.13% of total accessions), and oblong (13.83% of total accessions). Leaf margins revealed three categories; entire (9.57% of total accessions), serrate or dentate (78.72% of total accessions), and parted (11.70% of total accessions). All the safflower accessions (100%) showed non-hairy leaf trait. For the Leaf spininess, 31.91% of total accessions contained no spines, few spines in 23.40% of total accessions, intermediate spines in 22.34% of total accessions, and many spines in 22.34% of total accessions. Branching pattern was observed as basal (3.19% of total accessions), medium (84.04% of total accessions), and upper (12.77% of total accessions). Angle of branches was classified as appressed with angle of 15-20^{o} (7.45% of total accessions), intermediate with angle of 20-60^{o} (86.17% of total accessions), and spreading type with angle of 60-90^{o} (6.38% of total accessions). Flower colour, an important trait for the genetic diversity classification in safflower was categorized as pale-yellow (1.06% of total accession), yellow (38.30% of total accessions), yellow-orange (55.32% of total accessions), orange (2.13% of total accessions), orange-red (1.06% of total accession), and red (2.13% of total accessions). Head/capitulum shape was recorded as conical (95.75% of total accessions), oval (1.06% of total accession), and flattened (3.19% of total accessions). Seed shape was observed as oval (24.47% of total accessions), conical (71.28% of total accessions), and crescent (4.26% of total accessions).

Table 2.5: List of qualitative parameters recorded across two locations (Pakistan and Turkey) of 94 international safflower accessions panel

Accessions	Leaf Colour	Early Vigor	Growth Habit	Leaf Shape	Leaf Margins	Leaf Hairiness	Leaf Spininess	Branching Pattern	Angle of Branches	Flower Color	Head Shape	Seed Shape
Afghanistan1	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Intermediate	Medium	Intermediate (20-60)	Yellow	Conical	Oval
Afghanistan2	Dark Green	Intermediate	Erect	Ovate	Entire	Non-hairy	No spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Afghanistan3	Dark Green	Strong	Bushy	Ovate	Entire	Non-hairy	No spines	Medium	Intermediate (20-60)	yellow Orange	Flattened	Conical
Argentina1	Light Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Intermediate	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Australia1	Dark Green	Strong	Bushy	Ovate	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	red	Conical	Conical
Austria1	Light Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Austria2	Dark Green	Intermediate	Bushy	Ovate	Parted	Non-hairy	Many spines	Basal	Intermediate (20-60)	yellow Orange	Conical	Oval
Bangladesh1	Dark Green	Strong	Erect	Ovate	Entire	Non-hairy	No spines	Upper	Intermediate (20-60)	Yellow	Conical	Conical
Bangladesh2	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	pale-yellow	Conical	Conical
Bangladesh3	Dark Green	Strong	Erect	Oblong	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Bangladesh4	Light Green	Strong	Bushy	Ovate	Entire	Non-hairy	Few spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
China1	Light Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Many spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
China2	Light Green	Intermediate	Erect	Ovate	Serrate or dentate	Non-hairy	Intermediate	Medium	Intermediate (20-60)	yellow Orange	Flattened	Oval
China3	Dark Green	Strong	Erect	Ovate	Parted	Non-hairy	Many spines	Medium	Intermediate (20-60)	orange red	Conical	Conical
China4	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
China5	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
China6	Light Green	Intermediate	Bushy	Ovate	Serrate or dentate	Non-hairy	Many spines	Medium	Spreading (60-90)	Yellow	Conical	Conical
China7	Light Green	Intermediate	Erect	Ovate	Serrate or dentate	Non-hairy	Intermediate	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Egypt1	Dark Green	Intermediate	Bushy	Ovate	Parted	Non-hairy	Few spines	Medium	Spreading (60-90)	yellow Orange	Conical	Conical
Egypt2	Light Green	Intermediate	Bushy	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Oval
Egypt3	Dark Green	Strong	Bushy	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	Yellow	Conical	Crescent
Egypt4	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Upper	Intermediate (20-60)	yellow Orange	Conical	Oval
Egypt5	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Intermediate	Upper	Appressed (15-20)	yellow Orange	Conical	Crescent
Egypt6	Light Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Intermediate	Upper	Intermediate (20-60)	yellow Orange	Conical	Oval
France1	Light Green	Intermediate	Erect	Ovate	Entire	Non-hairy	No spines	Medium	Intermediate (20-60)	Yellow	Conical	Oval

Hungary1	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	Yellow	Conical	Oval
India1	Light Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Intermediate	Upper	Intermediate (20-60)	Yellow	Oval	Crescent
India2	Light Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Intermediate	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
India3	Light Green	Strong	Erect	Ovate	Parted	Non-hairy	Many spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
India4	Dark Green	Strong	Bushy	Ovate	Serrate or dentate	Non-hairy	Intermediate	Medium	Intermediate (20-60)	Yellow	Conical	Conical
India5	Light Green	Strong	Erect	Ovate	Parted	Non-hairy	Many spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
India6	Dark Green	Strong	Bushy	Oblong	Serrate or dentate	Non-hairy	Many spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Iran1	Dark Green	Strong	Erect	Ovate	Parted	Non-hairy	Many spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Iran2	Light Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Many spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Oval
Iran3	Light Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Oval
Iran4	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Iran5	Dark Green	Strong	Erect	Ovate	Entire	Non-hairy	No spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Oval
Iran6	Dark Green	Strong	Erect	Oblong	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Iran7	Dark Green	Strong	Erect	Oblong	Serrate or dentate	Non-hairy	Intermediate	Medium	Intermediate (20-60)	Yellow	Flattened	Oval
Israel1	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Many spines	Medium	Spreading (60-90)	Yellow	Conical	Conical
Israel2	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Israel3	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Israel4	Dark Green	Strong	Bushy	Ovate	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Iraq1	Dark Green	Intermediate	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	Yellow	Conical	Oval
Iraq2	Dark Green	Intermediate	Erect	Ovate	Serrate or dentate	Non-hairy	Many spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Jordan1	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Few spines	Upper	Intermediate (20-60)	yellow Orange	Conical	Conical
Jordan2	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	red	Conical	Conical
Jordan3	Dark Green	Strong	Erect	Oblong	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Jordan4	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Jordan5	Dark Green	Strong	Bushy	Ovate	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Kazakhstan1	Light Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Intermediate	Medium	Appressed (15-20)	yellow Orange	Conical	Conical
Libya1	Dark Green	Strong	Erect	Oblong	Serrate or dentate	Non-hairy	Intermediate	Medium	Intermediate (20-60)	Yellow	Conical	Oval
Morocco1	Dark Green	Strong	Bushy	Ovate	Parted	Non-hairy	No spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Morocco2	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical

Pakistan1	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Pakistan2	Dark Green	Strong	Bushy	Ovate	Parted	Non-hairy	Intermediate	Medium	Spreading (60-90)	yellow Orange	Conical	Oval
Pakistan3	Light Green	Strong	Bushy	Ovate	Parted	Non-hairy	Many spines	Medium	Spreading (60-90)	yellow Orange	Conical	Oval
Pakistan4	Light Green	Intermediate	Bushy	Ovate	Serrate or dentate	Non-hairy	Many spines	Basal	Intermediate (20-60)	yellow Orange	Conical	Oval
Pakistan5	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Oval
Pakistan6	Dark Green	Strong	Bushy	Ovate	Serrate or dentate	Non-hairy	Intermediate	Medium	Spreading (60-90)	Yellow	Conical	Oval
Pakistan7	Dark Green	Strong	Bushy	Ovate	Serrate or dentate	Non-hairy	Many spines	Upper	Intermediate (20-60)	yellow Orange	Conical	Conical
Pakistan8	Dark Green	Intermediate	Erect	Ovate	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	Yellow	Conical	Oval
Pakistan9	Dark Green	Strong	Bushy	Oblong	Parted	Non-hairy	Many spines	Basal	Intermediate (20-60)	Yellow	Conical	Conical
Pakistan10	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Intermediate	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Pakistan11	Light Green	Strong	Erect	Lanceolate	Serrate or dentate	Non-hairy	No spines	Upper	Appressed (15-20)	yellow Orange	Conical	Conical
Portugal1	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Many spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Portugal2	Light Green	Intermediate	Erect	Ovate	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Portugal3	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Portugal4	Dark Green	Intermediate	Erect	Ovate	Serrate or dentate	Non-hairy	Few spines	Upper	Intermediate (20-60)	yellow Orange	Conical	Conical
Portugal5	Dark Green	Intermediate	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Upper	Intermediate (20-60)	Yellow	Conical	Conical
Portugal6	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Few spines	Upper	Intermediate (20-60)	yellow Orange	Conical	Conical
Romania1	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Many spines	Medium	Intermediate (20-60)	Yellow	Conical	Oval
Russia1	Dark Green	Strong	Bushy	Ovate	Serrate or dentate	Non-hairy	Many spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Spain1	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Intermediate	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Spain2	Dark Green	Strong	Erect	Lanceolate	Serrate or dentate	Non-hairy	Intermediate	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Spain3	Dark Green	Strong	Erect	Oblong	Serrate or dentate	Non-hairy	Many spines	Medium	Appressed (15-20)	Yellow	Conical	Conical
Spain4	Dark Green	Strong	Erect	Oblong	Serrate or dentate	Non-hairy	Few spines	Medium	Intermediate (20-60)	orange	Conical	Conical
Syria1	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Syria2	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Syria3	Dark Green	Strong	Erect	Oblong	Serrate or dentate	Non-hairy	Intermediate	Upper	Intermediate (20-60)	yellow Orange	Conical	Conical
Thailand1	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Oval
Turkey1	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	Intermediate	Medium	Intermediate (20-60)	yellow Orange	Conical	Crescent
Turkey2	Light Green	Strong	Bushy	Ovate	Serrate or dentate	Non-hairy	Many spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical

Turkey3	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	orange	Conical	Conical
Turkey4	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	yellow Orange	Conical	Conical
Turkey5	Dark Green	Strong	Erect	Oblong	Serrate or dentate	Non-hairy	No spines	Medium	Appressed (15-20)	Yellow	Conical	Conical
Turkey6	Dark Green	Strong	Erect	Oblong	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Turkey7	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Turkey8	Dark Green	Strong	Erect	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Turkey9	Dark Green	Strong	Erect	Oblong	Entire	Non-hairy	Few spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Turkey10	Dark Green	Strong	Erect	Ovate	Entire	Non-hairy	Intermediate	Medium	Intermediate (20-60)	yellow Orange	Conical	Oval
Uzbekistan1	Light Green	Strong	Erect	Ovate	Parted	Non-hairy	Many spines	Medium	Intermediate (20-60)	Yellow	Conical	Conical
Uzbekistan2	Light Green	Strong	Bushy	Ovate	Serrate or dentate	Non-hairy	No spines	Medium	Appressed (15-20)	yellow Orange	Conical	Conical
Uzbekistan3	Dark Green	Strong	Bushy	Ovate	Entire	Non-hairy	Intermediate	Medium	Appressed (15-20)	yellow Orange	Conical	Conical

2.4.2. Correlation, principal component analysis, constellation plot, and multivariate analysis

Correlation coefficient among 94 international safflower accessions is presented in Table 2.6. Days to flower initiation, days to 50% flowering, days to flower completion, and days to maturity were significantly correlated (+ve) with leaf length, plant height, and capitulum diameter. Days to flower initiation and days to 50% flowering revealed significant correlation (-ve) with the trait 100-seed-weight. Plant height showed significant relationship (+ve) with leaf length, leaf width, and capitulum diameter. Capitula per plant exhibited significant correlation (+ve) with leaf length, leaf width, branches per plant, and seed yield per plant. There was significant correlation (+ve) between seeds per capitulum and capitulum diameter. Capitulum diameter revealed significant correlation (+ve) with seed yield per plant. Seed yield per plant was significantly correlated (+ve) with leaf length, leaf width, branches per plant, capitulum diameter, and 100-seed weight. 100-seed weight showed significant relationship (+ve) with leaf length, leaf width, and capitulum diameter.

Table 2.6: Correlation coefficients among 13morpho-agronomic traits in 94 international safflower accessions panel

Traits	DFI	DFF	DFC	DM	LL	LW	PH	BPP	CPP	SPC	CD	SYP
DFI	1											
DFF	0.8862*	1										
DFC	0.7913*	0.9157*	1									
DM	0.6263*	0.7330*	0.8301*	1								
LL	0.2220*	0.2464*	0.2673*	0.2125*	1							
LW	0.1408	0.1375	0.1449	0.0666	0.7138*	1						
PH	0.4728*	0.5128*	0.5930*	0.5354*	0.3200*	0.2753*	1					
BPP	-0.0050	0.0064	-0.0108	-0.0917	0.1196	0.1209	-0.0600	1				
CPP	-0.0258	-0.0334	0.0027	-0.0595	0.2191*	0.2839*	-0.0022	**0.6219***	1			
SPC	0.1024	0.0274	0.0160	0.0671	0.1179	0.0650	0.0960	-0.0600	0.0772	1		
CD	0.2945*	0.3231*	0.3839*	0.3233*	0.4165*	0.4229*	0.4284*	-0.0411	0.0689	0.3853*	1	
SYP	-0.1499	-0.1411	-0.0274	-0.0229	0.3372*	0.2517*	-0.0304	**0.3071***	**0.4985***	0.1585	**0.3918***	1
100-SW	-0.2856*	-0.2397*	-0.1017	-0.0482	0.3024*	0.2313*	-0.0581	-0.1415	-0.0426	-0.1522	0.3513*	**0.4784***

*Statistically significant at $P \leq 0.05$, DFI: days to flower initiation; DFF: days to 50% flowering; DFC: days to flower completion; DM: days to maturity; LL: leaf length; LW: leaf width; PH: plant height; BPP: branches per plant; CPP: capitula per plant; SPC: seeds per capitulum; CD: capitulum diameter; SYP: seed yield per plant; 100-SW: 100-seed weight

When applying principal component analysis on 13 morpho-agronomic traits together, the first four principal components were selected which accounted for 75.16% of the total variation (Table 2.7). The first principal component (PC1) contributed a total of 32.83% of the variation, showing highest contribution from days to flower completion (0.44). PC2 explained a total of 20.71% of the variation with highest contribution from seed yield per plant (0.48). In the same way, PC3 and PC4 revealed a total of 12.71 and 8.91% variation having highest contribution from branches per plant (0.61) and seeds per capitulum (0.86), respectively.

Table 2.7: Eigen-values of the first four principal component axes (PC) in94 international safflower accessions panel

Traits	PC1	PC2	PC3	PC4
Days to flower initiation	**0.4027**	-0.1782	**0.1300**	0.0159
Days to 50% flowering	**0.4308**	-0.1793	0.1157	-0.0760
Days to flower completion	**0.4430**	-0.1257	0.0582	-0.1123
Days to maturity	0.3920	-0.1335	-0.0218	-0.0652
Leaf length	0.2382	**0.3666**	-0.1110	-0.1523
Leaf width	0.1859	**0.3765**	-0.0804	-0.1461
Plant height	0.3491	-0.0132	-0.0763	0.0066
Branches per plant	0.0018	0.2426	**0.6136**	-0.0847
Capitula per plant	0.0309	**0.3606**	**0.5255**	0.0294
Seeds per capitulum	0.0856	0.1048	-0.0564	**0.8644**
Capitulum diameter	0.2815	0.2769	-0.2750	**0.2763**
Seed yield per plant	0.0309	**0.4840**	0.0353	**0.0548**
100-seed weight	-0.0271	0.3397	-0.4567	-0.3131
Eigen value	4.2675	2.6925	1.6520	1.1582
Variability (%)	32.8269	20.7116	12.7076	8.9090
Cumulative %	32.8269	53.5385	66.2461	75.1552

Constellation plot implemented in JMP 14.1.0 statistical software (2018, SAS Institute Inc., Cary, NC, USA) divided the evaluated safflower accessions into three main groups: 26 accessions (27.66% of the total accessions) in the group A (blue), 31 accessions (32.98% of the total accessions) in the group B (green), and 37 accessions (39.36% of the total accessions) in the group C (red) (Figure 2.1). Multivariate analysis was performed which also revealed three groups and supported the constellation plot clustering of 94 safflower accessions (Figure 2.2).

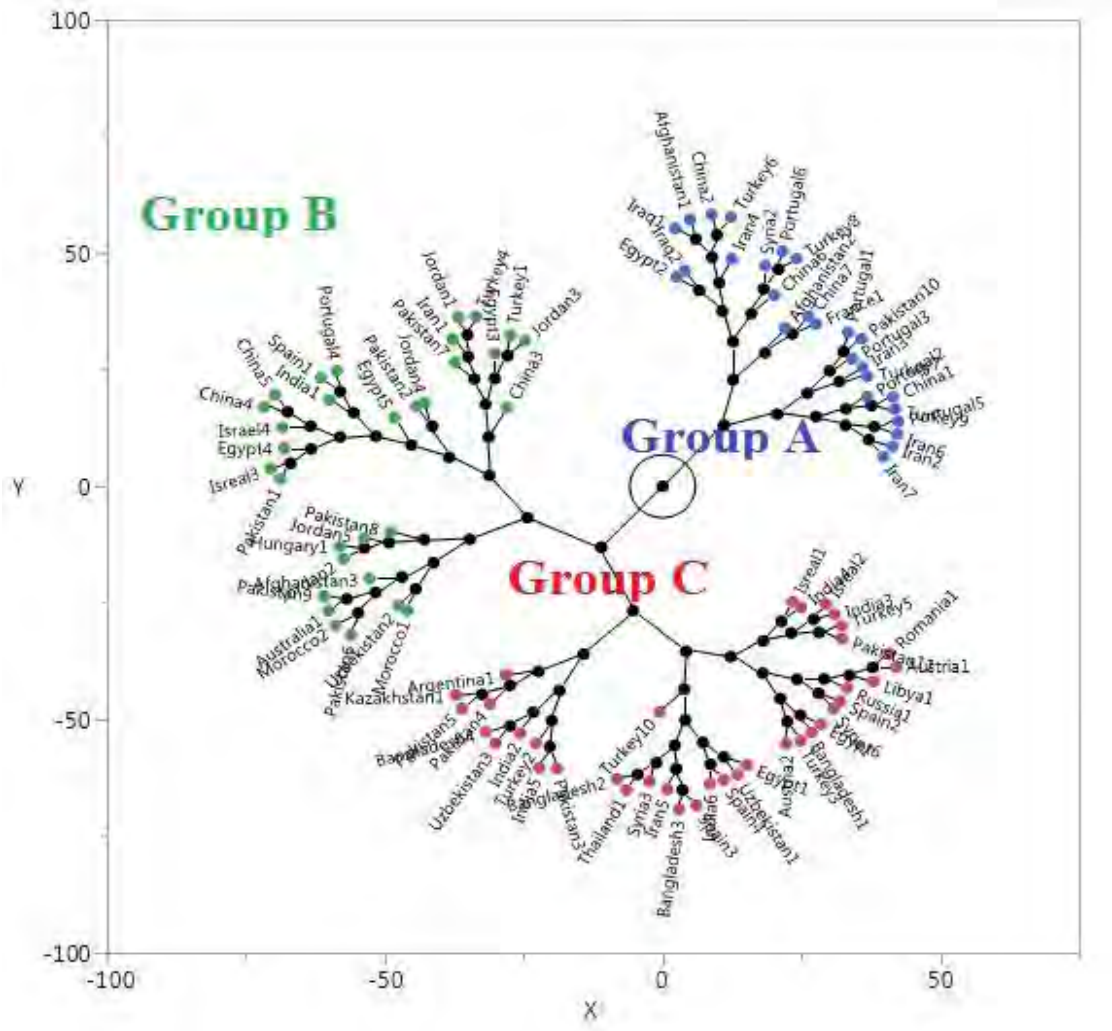


Figure 2.1: Constellation plot analysis divided the evaluated 94 international safflower accessions panel into three groups

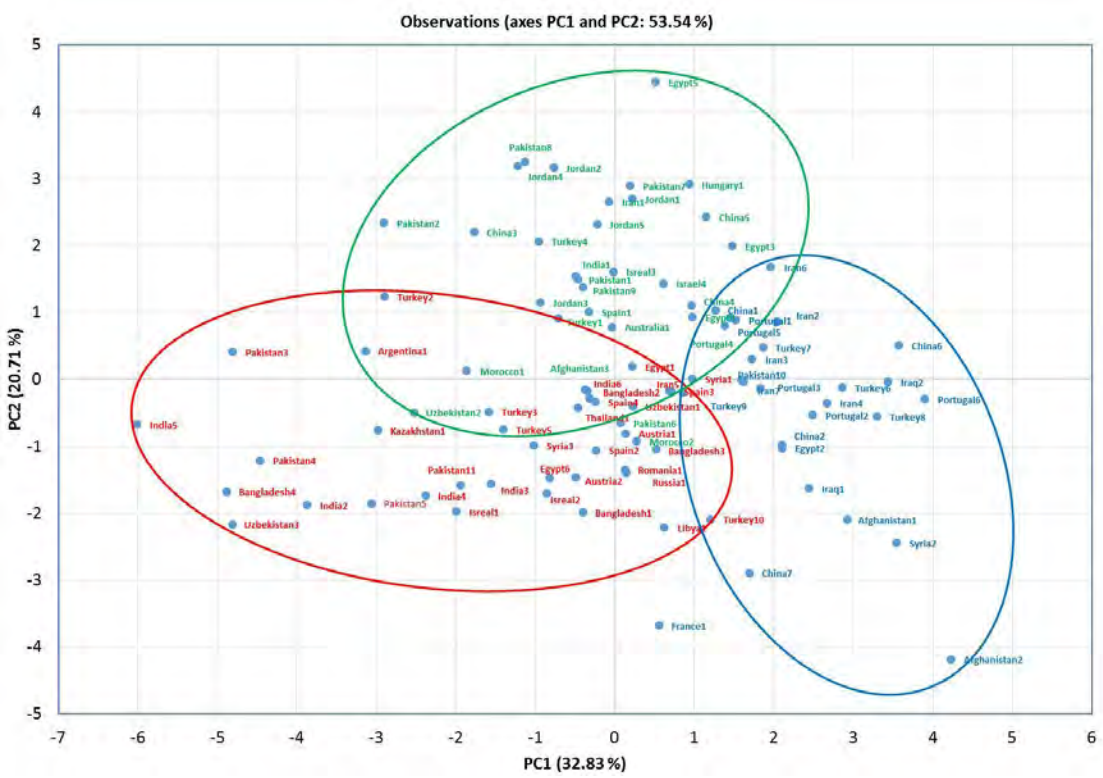


Figure 2.2: Multivariate analysis revealed three groups in 94 international safflower accessions panel

2.5. Discussion

Analysis of variance (ANOVA) was performed which revealed significant variations due to accessions as well as locations. Accessions were found highly significant for days to flower initiation, days to 50% flowering, days to flower completion, leaf width, plant height, branches per plant, capitulum diameter, 100-seed weight, and seed yield per plant among safflower accessions, while Location revealed significant differences for all the studied traits except branches per plant and 100-seed weight (Table 2).The finding of the presence of significant variation in the studied safflower accessions and the environmental factors strongly affecting the various attributes of safflower are supported by Ashri *et al.* (1975). Table 3 reflected the performance of safflower accessions for various traits at both locations and it is cleared that overall performance of safflower accessions was found superior in Pakistan as compared to Turkey. However, few traits like leaf length, seeds per capitulum, and 100-seed weight showed better performance in the Turkey as well. These differences may due to environmental conditions and soil properties of location. Variations in the locations are confirmed by the ANOVA as well. The presence of morpho-agronomic variability in the current safflower accessions reflected their long term response to the selective pressure (both spatial and temporal) and to deliberate selection of the farmers for preferred phenotypes which ultimately lead to their morpho-agronomic changes (Abede and Bjornstad, 1996; Vom Brocke *et al.*, 2003). Breeding methods based on different morpho-agronomic traits has a significant role in the development of high-yielding genotypes. Morphological markers are the visually characterized phenotypic traits such as flower color and leaf spininess in safflower and serve usefully the purposes of plant breeders (Golkar *et al.*, 2010). The present study revealed sufficient variability for qualitative traits especially flower color and leaf spininess. Generally speaking, safflower is a spiny crop plant with most of its genotypes containing many sharp spines on its leaves and bracts (Bradley *et al.*, 1999). Therefore, one of the major goals during safflower breeding programs is to develop cultivars which are spineless and also exhibiting high yield (Golkar *et al.,* 2010). Also, safflower spininess and flower color are more expected to be used as valuable morphological markers in marker assisted selection during breeding programs (Golkar *et al.,* 2010). Safflower leaf spininess is considered as a handicap in the areas where this crop is manually harvested (Chaudhry, 1986). Good range of variations for the studied traits were observed among the 94 safflower

accessions collected from 26 countries of the world (Table 4). Ramachandram (1985) reported the existence of great level of variations for seed yield in this crop and revealed its great potential as a major oilseed crop. Early and late plant maturing are important characteristics in safflower breeding programs as they enable us to develop cultivars for various agro-ecological zones with different photoperiod and thermo-sensitivity (Suddihiyam *et al.*, 1992; ur Rehman *et al.*, 2009). Early maturing safflower cultivars can be used an alternative strategy to escape damages from insects and disease infections (Golkar, 2011). Early maturing safflower accessions would compete with crops like wheat and also encouraged for cultivation on marginal lands. Instead of direct selection for seed yield, it would be better to focus on various yield contributing traits for the efficient improvement of safflower yield due to pleiotropic effects. The plant height variability obtained in this study was supported by the findings obtained in Esendal (1990); short-statured safflower accessions are better suited for mechanical harvesting (Weiss, 2000). (Shinwari *et al.*, 2014) obtained capitulum diameter and seed yield per plant in the range of 15.5 to 30.4cm and 3.0 to 38.1g which were in line with our results. Branches per plant were observed as one of the important yield trait showing a strong relationship with yield in safflower (Golkar *et al.*, 2012). Golkar *et al.* (2011) found branches per plant with a mean of 8.5 which were in the same range as our results. Either accessions kinship or similar environmental conditions can explain the similarity of these findings. Zheng *et al.* (1993) emphasized on the indirect selection of higher capitula per plant and 1000-seed weight with lower number of branches per plant for the improvement of safflower. Further, capitula per plant and capitulum weight were suggested as important traits for the improvement of safflower yield (Corleto *et al.*, 1997; Rao and Ramachandram, 1997; Mozaffari and Asadi, 2006). Capitula per plant is supposed to be an important seed yield determinant and revealed highest relationship (+ve) with seed yield. Yield attributes revealed the presence of a good level of variability during the current exploration and indicated that an efficient selection could be employed on these yield components for the improvement of safflower. The yield attributes like; branches per plant, capitula per plant, and capitulum diameter were found more diverse and had significant correlation (+ve) with seed yield per plant and could be used as selection criteria for breeding purposes. Correlation analysis is mainly applied to understand the association among the various traits and the evaluated information can be best used for crop improvement by indirect selection of the components

effecting crop yield (Sharaan and Ghallab, 1997; Karaköy *et al.*, 2014). Crop improvement depends upon the success of the selection criteria. Importance of the traits can be judged from its direct or indirect effects upon yield components, especially seed yield. It is therefore very important to know about the relative effects of the traits influencing the economic traits in a desirable direction and to be selected in the crop improvement programs (Vrijendra *et al.*, 2004). Traits like; Plant height, branches per plant, capitula per plant, seeds per capitulum, capitulum diameter, and 1000-seed weight are the most important traits in safflower improvement for increasing seed yield (Hamadi *et al.*, 2001; Rudra Naik *et al.*, 2001) as it revealed either direct or indirect correlation with seed yield (Çamaş and Esendal, 2006; Mahasi *et al.*, 2006). Days to flower initiation, days to 50% flowering, days to flower completion, and days to maturity were significantly correlated (+ve) with plant height. Zheng *et al.* (1993) stated that the taller safflower accessions have longer flowering time which was in line to our current study. Bidgoli *et al.* (2006) studied correlation in various safflower accessions and exhibited significant correlation (-ve) of days to flower initiation and days to 50% flowering with 1000-seed weight. They obtained significant correlation (+ve) between seeds per capitulum and capitulum diameter. Similarly, 1000-seed weight showed significant relationship (+ve) with capitulum diameter. Arslan (2007) exhibited significant correlation (+ve) between plant height and capitulum diameter and supported our results. Significant correlation (+ve) between capitula per plant and branches per plant were also reported by Mahasi *et al.* (2006) and strengthen our results. Our current results confirmed the findings of Omidi (2002) and Bagheri *et al.* (2001) as they reported significant correlation (+ve) of capitulum diameter with seed yield per plant. Our results about significant correlation (+ve) of seed yield per plant with branches per plant and 100-seed weight was strongly supported by Tuncturk and Ciftci (2004) as they reported same findings, while studying safflower under different fertilizer and row spacing levels. It clearly suggested that an increase in any of the traits having positive correlation with seed yield per plant will ultimately boost the safflower yield.

Principal component analysis (PCA) helps to recognize important plant traits that are used to characterize the variations among experimental materials (Chakravorty *et al.*, 2013). Principal component analysis precisely classified the 13 morpho-agronomic traits into thirteen principal components among which the first

four principal components; PC1, PC2, PC3, and PC4 were selected based on the magnitude of respective Eigen values. These four components explained nearly 75.16% of the total genetic variation (Table 7).The first principal component (PC1) contributed about 32.83% of the variation, showing highest contributions from days to flower completion (0.44) followed by days to 50% flowering (0.43) and days to flower initiation (0.40). Owing to the high amount of maturity traits contribution, the PC1 was named as maturity component. PC2 explained 20.71% of the variation with highest contributions from seed yield per plant (0.48) followed by leaf length (0.37) and capitula per plant (0.36). PC3 revealed 12.71% of the variation having highest contributions from branches per plant (0.61) followed by capitula per plant (0.53) and days to flower initiation (0.13). PC4 revealed 8.91% of the variation having highest contributions from seeds per capitulum (0.86) followed by capitulum diameter (0.28) and seed yield per plant (0.05).

The results suggested that traits; days to flower initiation, days to 50% flowering, days to flower completion, seed yield per plant, capitula per plant, branches per plant, seeds per capitulum, and capitulum diameter were responsible for the genetic variation in the current international safflower panel. It is interpreted from the above that the traits consistently contributing variation in each PC may be governed by genes that should be useful during selection to develop desirable cultivars in safflower breeding programs. These morpho-agronomic traits are the drivers of the observed genetic variability and should be considered in the process of genetic combinations during crossing and screening elite safflower accessions. It is concluded that principal component analysis is very helpful to identify relationship between different traits and in this study it revealed that maximum variations are due to seed yield per plant and also to predict the best selection indices for the yield improvement in various safflower breeding programs.

International safflower panel comprised of 94 accessions were clustered into three groups (A, B, and C) on the basis of important yield traits like; seed yield per plant, capitula per plant, capitulum diameter, and branches per plant. Knowles (1969) proposed seven similarity centers (1: Far East, 2: India-Pakistan, 3: Middle East, 4: Egypt, 5: Sudan, 6: Ethiopia, and 7: Europe) for safflower using various plant traits as a standard (Table 2.8). Our current constellation plot analysis revealed that safflower accessions from Iran, Syria, Turkey, Afghanistan, and Iraq were clustered in group A

(blue) showing the Middle East similarity center. Group A (blue) also comprised of safflower accessions from Portugal and France which constituted the Europe similarity center. Group B (green) comprised of the Middle East, India-Pakistan, Europe, and Egypt similarity centers as this group exhibited safflower accessions from Jordan, Turkey, Iran, Israel, and Afghanistan (the Middle East center), India and Pakistan (India-Pakistan center), Spain, Hungary, Portugal, Australia, and Morocco (Europe center) and Egypt (Egypt center). In very similar way, group C comprised of safflower accessions belonging to three different similarity centers; Middle East (Syria, Iran, Israel, Turkey), India-Pakistan (India, Pakistan, Bangladesh), and Europe (Argentina, Spain, Austria, Romania). Overall, our current constellation plot analysis exhibited the seven similarity centers pattern based on the yield traits (seed yield per plant, capitula per plant, branches per plant, and capitulum diameter) other than the standard traits proposed by Knowles (1969). So, it is needed to be further tested and after confirmation, these yield traits should also be used along with other standard traits to consolidate the number of safflower similarity centers more comprehensively. Multivariate analysis clustered the 94 safflower accessions into three different groups in the same pattern as revealed from constellation plot analysis (Figure 2). The basic grouping factors were seed yield per plant, capitula per plant, branches per plant, and capitulum diameter.

Table 2.8: List of the seven safflower similarity centers based on various morpho-agronomic traits proposed by Knowles (1969)

Center	Height	Branching	Spines	Head size	Flower color
Far East	Tall	Intermediate	Spines, Spineless	Intermediate	Orange
India-Pakistan	Short	Many	Spines	Small, intermediate	Orange, white, red
Middle East	Tall	Few	Spineless	Intermediate, Large	Red, orange, yellow, white
Egypt	Intermediate	Few	Spines, Spineless	Large, Intermediate	Orange, yellow, white, red
Sudan	Short, Intermediate	Intermediate	Spines	Small, Intermediate	Yellow, orange
Ethiopia	Tall	Many	Spines	Small	Red
Europe	Intermediate	Intermediate	Spines, Spineless	Intermediate	Orange, red, yellow, white

Mean performance of selected four morpho-agronomic traits along with plant height across two locations (Pakistan and Turkey) of the safflower accessions with respect to its similarity centers is provided in Table 2.9. Our current results of capitulum diameter and plant height observed the same pattern as proposed by Knowles (1969). We recorded highest capitulum diameter for Middle East (24.40mm) and Egypt (24.28mm) similarity centers that is in accordance with Knowles hypothesis as he observed large and intermediate capitulum/head size. We reported intermediated capitulum diameter for Europe (23.56mm) and Far East (23.07mm) similarity centers that obey the Knowles hypothesis as he also obtained intermediate head size for these two similarity centers. Furthermore, we recorded small capitulum diameter (22.03mm) for India-Pakistan similarity center which made agreement to the Knowles hypothesis as he also reported small head size for the said similarity center. Similarly, we recorded maximum, minimum and intermediate plant height for Middle East (96cm), India-Pakistan (83cm) and Europe (92cm) similarity centers which is exactly according to the Knowles hypothesis. We obtained intermediate and tall plant height for Far East (92cm) and Egypt (95cm) similarity centers which is contradictory to the Knowles hypothesis. Occurrence of mismatches to Knowles hypothesis might be due to presence of some admixture in the international safflower panel. The key factors responsible for such admixture are mutation, migration and selection by humans (Pearl and Burke, 2014).

Table 2.9: Mean performance of selected morpho-agronomic traits along with plant height of the safflower accessions across two locations (Pakistan and Turkey) with respect to similarity center

Similarity Center	Branches per plant	Capitula per plant	Capitulum diameter (mm)	Seed yield per plant (g)	Plant height (cm)
Middle East	10.12	31.53	24.40	16.09	96.83
India-Pakistan	9.69	29.17	22.03	14.67	83.77
Far East	9.18	23.95	23.07	17.04	92.00
Europe	9.72	28.76	23.56	15.23	92.44
Egypt	10.98	25.70	24.28	19.33	95.32

2.6. Selection of best performing accessions

Various statistical analysis like correlation, principal component analysis, constellation plot, and multivariate studies have been previously used to explore the morpho-agronomic traits and to select the best performing accessions (Kotecha, 1979; Pascual-Villalobos and Alburquerque, 1996). Yield traits like; capitula per plant, seeds per capitulum, seed weight, and capitulum diameter were known as important yield determinants (Chaudhary, 1990; Pascual-Villalobos and Alburquerque, 1996; Omidi, 2000). Golkar *et al.* (2011) suggested seeds per capitulum and capitula per plant as important selection criteria for improvement of safflower seed yield. Similarly, Chaudhary (1990) pointed out that safflower agronomic traits like plant height, leaf number, primary branches per plant, seeds per capitulum, and 1000-seed weight had positive effects on seed yield. Further, he suggested selection criteria combining seeds per capitulum, capitula per plant, and 1000-seed weight to be efficiently used in selecting high yielding genotypes during selection process. Therefore, this study was also aimed to investigate the accessions superior in various traits under both locations. On the bases of the principal component analysis, it was observed that days to flower initiation, days to 50% flowering, days to flower completion, seed yield per plant, capitula per plant, branches per plant, seeds per capitulum, and capitulum diameter were the major variability contributing

components. But as revealed from the correlation analysis, it was suggested that seed yield per plant had significant (+ve) relationship with capitula per plant, branches per plant, and capitulum diameter. Therefore, above mentioned four traits (seed yield per plant, capitula per plant, branches per plant, and capitulum diameter) can be used to select the best performing accessions. After applying 20% selection response to yield traits, 20 safflower accessions were separated and recommended for future safflower breeding programs for various important morpho-agronomic traits to improve production (Table 2.9).

Table 2.10: List of promising safflower accessions with mean data values evaluated at two diverse environments of Pakistan and Turkey during 2016-17 and 2018

Accession	DFI	DFF	DFC	DM	LL	LW	PH	BPP	CPP	SPC	CD	SY	100-SW
Pakistan-7	120	124	131	149.5	17.256	5.14	88.062	9.2	39.9	33.25	25.199	43.313	3.261
Egypt-3	122.5	128	136	148.5	14.552	5.042	97.93	10.5	36.4	22.1	28.302	35.859	3.7875
Egypt-5	119.5	124	130.5	144.5	20.136	6.112	104.592	11	26	19.6	26.652	32.82	5.3195
Iran-1	119.5	124.5	131	150	16.672	5.042	84.454	13	36.1	30.25	25.611	29.457	3.8585
Jordan-1	121.5	124.5	131.5	145.5	16.62	5.322	95.796	9.7	38.9	30.3	25.4	31.516	4.0205
Jordan-2	119	122.5	130.5	146.5	17.016	5.226	90.696	12.3	46.1	18.5	24.02	39.187	3.4405
Portugal-4	121.5	128.5	133.5	150	16.526	5.796	96.646	8.6	14.7	25.95	26.616	20.814	3.688
China-1	121.5	128	133	152.5	15.145	5.15	98.318	9.2	36.2	32.4	24.643	24.476	3.5155
Turkey-4	120	123.5	130	145.5	15.015	4.75	87.204	10	30	28.4	25.372	30.462	4.301
Pakistan-8	121	125.5	129.5	145.5	14.96	5.225	76.752	13.9	80.4	26.55	21.767	33.576	2.868
Pakistan-9	121.5	126.5	133.5	146	15.385	4.895	81.276	11.9	49.6	27.8	22.482	26.19	2.874
Jordan-3	119	125	132	145.5	12.825	4.695	90.502	9.5	37.6	24.85	25.387	23.253	3.994
Jordan-4	117	123.5	129.5	143.5	20.235	5.785	82.1	8.9	44.5	24.9	22.944	20.385	4.026
Jordan-5	120.5	125.5	133.5	147	15.53	5.115	86.654	11.4	55.6	23.7	23.577	28.219	3.6305
Israel-4	122.5	127	133	146.5	18.54	5.33	98.368	8.1	32	20.2	22.493	22.681	4.1455
Hungary-1	120.5	126.5	133.5	150	18.99	5.46	93.346	13.1	44.8	21.3	24.08	31.556	3.469
Turkey-9	121	128	134	150.5	13.31	4.36	100.392	8.6	32	31	25.993	21.545	3.0485
China-3	117	123	131.5	145.5	10.94	3.65	85.252	10.8	21.4	35.1	27.052	51.021	4.5325
China-4	120	127	138.5	150.5	16.9	4.67	95.122	10.4	24.4	22	23.623	33.114	3.7275
China-5	119.5	127.5	135.5	150.5	18.9	4.76	95.652	9.5	26.2	24.35	25.427	35.896	4.5225

2.7. Conclusion

The data obtained from this study could be useful for safflower breeders and seed producers concerned with increasing seed yield. Genetic diversity for important yield and its related traits was described including capitulum diameter (17.30 to 28.30mm), branches per plant (5.10 to 17.30), capitula per plant (8.70 to 80.40), and seed yield per plant (4.86 to 51.02g), and showed a good level of variation in the studied germplasm. Using the principal component analysis, it was observed that days to flower initiation, days to 50% flowering, days to flower completion, seed yield per plant, capitula per plant, branches per plant, seeds per capitulum, and capitulum diameter were the major contributors to the observed genetic variability in the evaluated safflower panel. Seed yield per plant reflected a significant and positive correlation with capitula per plant, branches per plant and capitulum diameter, and these traits can be suggested as a selection criterion in safflower breeding programs. The constellation plot and multivariate analysis were in agreement with the patterns of seven similarity centers based on seed yield per plant, capitula per plant, capitulum diameter, and branches per plant.

Chapter 3

Mobile genomic element diversity and similarity centers exploration in world collection of safflower (*Carthamus tinctorius* L.) panel using iPBS-retrotransposon markers

3.1. Introduction

Crucial challenges that confront food production in the 21th century includes; world climate change, human activities, population expansion, plant colonization, and raising competition for land, water, and energy (Godfray *et al.,* 2010). Higher environmental effects like; changing drought and salinity patterns and the emerging attacks of new pests and diseases are expected due to increasing global temperatures, ultimately effect plant growth and yield (Tester and Langridge, 2010). Changes in the world atmospheric CO_2 had negative effects to biodiversity and endanger crop production by the stimulation of invasive weeds growth (Raizada, *et al.,* 2009). The fast-growing world population enhanced the pressure upon agricultural crop production (Kastner *et al.,* 2012; Dempewolf *et al.,* 2014; Khoury *et al.,* 2014). It is therefore important to practice the underutilized crops and also develop new cultivars to meet the present and future world food challenges.

Various hypotheses about the number of safflower similarity centers throughout the world were proposed by Knowles (1969), Ashri (1975), and Chapman *et al.* (2010). It is estimated that safflower is cultivated in nearly 20 countries with a total cultivated area of 1,140,002 hectares and the production of 948,516 tons (FAOSTAT, 2015). Safflower major producer countries include, Russian Federation (286,351 tons), Kazakhstan (167,243 tons), Mexico (121,767 tons), USA (99,830 tons), Turkey (58,000 tons), and India (53,000 tons) which account for about 71% of the total world production (FAOSTAT, 2015). In spite of containing good amount of polyunsaturated fatty acids and being resistant to dry conditions, still safflower did not gain the status of major oilseed crop. The primary factors which prevented its cultivation on large scale are low seed yield, low oil content, biotic stresses susceptibility, and spininess (Nimbkar, 2008). Therefore, the enhanced acceptability and utilization of safflower as an oilseed crop will require genetic improvement for the traits of interest. To this end, genetic diversity can be an effective approach by providing a good source of variations upon which breeding programs can build (Nadeem *et al.,* 2018a). However, it is unfortunate that current safflower germplasm and breeding lines displayed low levels of genetic diversity, and were therefore of reduced usefulness in breeding programs. An extensive diversity characterization (both at genotypic and phenotypic levels) of the global safflower genetic resources can help broaden the genetic base and diversity in the safflower crop, and identify

elite accessions (Collard *et al.*, 2005; Kumar *et al.*, 2015). Safflower genetic diversity was investigated using different molecular markers; Random Amplified Polymorphic DNA, Inter Simple Sequence Repeat, Amplified Fragment Length Polymorphism, Simple Sequence Repeats, and Single Nucleotide Polymorphism (Johnson *et al.*, 2007; Yang *et al.*, 2007; Amini *et al.*, 2008; Khan *et al.*, 2009; Sehgal *et al.*, 2009; Chapman *et al.*, 2010; Lee *et al.*, 2014; Pearl and Burke, 2014; Ambreen *et al.*, 2015; Kumar *et al.*, 2015; Ambreen *et al.*, 2018), but so far, iPBS-retrotransposon markers have not been used to investigate the genetic diversity in safflower.

Retrotransposons are known as an important component of the plant genome in terms of structural evolution and have great potential of changing its position and copy number across plant genome (Finnegan, 1989). Retrotransposons are genetic elements ranging from 50 to 90% in various plant genomes depending upon the plant species (SanMiguel *et al.*, 1996). Long terminal repeat (LTR) and non- long terminal repeat (non-LTR) are the two classes of retrotransposons, and plant genome reveal higher proportions of LTR retrotransposons as compare to non-LTR (Nadeem *et al.*, 2018b). Limitations in the retrotransposon marker systems resulted in the development of a new marker system named Inter-primer binding site (iPBS) retrotransposons having universal applicability (Arystanbekkyzy *et al.*, 2018; Nadeem *et al.*, 2018b). iPBS is a PCR-based, universal marker system and depends upon the presence of tRNA as a reverse transcriptase primer binding site (Kalendar *et al.*, 2010). Minimum cost and high efficiency of iPBS-retrotransposons make them good marker system (Nadeem *et al.,* 2018b). Various crops like, pea, chickpea, Lens, Turkish okra, Tobacco, and common bean have been studied efficiently using iPBS-retrotransposon markers system (Andeden *et al.*, 2013; Baloch *et al.*, 2015a; Baloch *et al.*, 2015b; Yıldız *et al.*, 2015; Aydin and Baloch, 2019).

Several crop species have been improved utilizing molecular markers in various crop breeding programs (Varshney *et al.*, 2007). However, for safflower, its genetics and genomics were less studied, which can explain the lack of reliable marker systems for use in the process of developing superior safflower cultivars (García-Moreno *et al.*, 2010; Hamdan *et al.*, 2011).

3.2. Objectives

This study was conducted to evaluate:

- The genetic diversity and population structure of safflower accessions using iPBS-retrotransposons as a start for further scientific investigations and practical breeding use cases.
- Explore safflower similarity centers pattern using iPBS-retrotransposons.

3.3. Materials and Methods

3.3.1. Plant materials and DNA isolation

Experimental materials comprising 131 safflower accessions collected from 28 different countries were evaluated in this study. Among these accessions, 94, 17, and 20 originated from the United States Department of Agriculture (USDA), Plant Genetic Resources Institute Pakistan, and from the Turkish Central Research Institute for Field Crops (Appendix II). A total of 94 accessions from USDA and 17 from Pakistan used in this study were landraces. The 20 Turkish accessions were single plant selection among international germplasm from USDA and are candidate cultivars. Seeds of each accession were sown at the research and experimental area of Bolu Abant Izzet Baysal University. Fresh, young and healthy leaves were harvested at proper time for the isolation of DNA, brought to laboratory and frozen at -80°C for later use. DNA extraction was performed using the bulk leaves of each accession, and followed CTAB protocol (Doyle and Doyle, 1990) with slight modifications (Baloch *et al.*, 2016). DNA concentration of each accession was measured using agarose gel (0.8%) and was also confirmed with the help of NanoDrop (DeNovix DS-11 FX, USA). Final DNA concentration for the 131 accession samples to be used in polymerase chain reactions (PCR) was adjusted to 5 ng/µL; the samples were stored at -25 ^{o}C till the start of PCR amplifications.

3.3.2. iPBS-retrotransposon PCR amplifications

Seventy iPBS-retrotransposon primers were initially screened using eight randomly selected accessions of safflower for PCR amplifications (Kalendar *et al.,* 2010). Out of the 70 iPBS-retrotransposon primers, 13 were found polymorphic and selected for PCR amplification, and produced strong bands (Table 3.1). A total reaction volume of 20 µL for PCR amplifications were comprised of 3 ng/ul template DNA, 2 µLdNTPs (Thermo Scientific), 0.2 µL U Taq DNA polymerase (Thermo Scientific), 3.2 µL primer, 2 µL 1x PCR buffer (Thermo Scientific), 2 µL MgCl2 and 7.6 µL distilled water. Reactions were performed in the sequence of denaturation at 95 ^{o}C for 3 min, subsequently followed by 30 denaturation cycles at 95 ^{o}C for 15 sec, annealing temperature 50–65 ^{o}C for one minute depending upon the primer, and a final extension for five minute at 72 ^{o}C (Kalendar *et al.,* 2010). The amplified fragments were electrophoresed on agarose gel 1.8% (w/v) using 0.5x TBE buffer at a

constant voltage of 120 V for 230 minute. Staining of the gel was performed with ethidium bromide and visualized using UV Imager Gel Doc XR+ system (Bio-Rad, USA) light and photographed. A 100 bp+ DNA ladder was used as molecular weight marker.

Table 3.1: List of 13 iPBS-retrotransposon primers with their sequence and annealing temperature used to determine genetic diversity among 131 safflower accessions

Primer name	Sequence	Annealing temperature (^{o}C)
iPBS2252	TCATGGCTCATGATACCA	52
iPBS2376	TAGATGGCACCA	52
iPBS2377	ACGAAGGGACCA	53
iPBS2391	ATCTGTCAGCCA	52
iPBS2398	GAACCCTTGCCGATACCA	51
iPBS2228	CATTGGCTCTTGATACCA	53
iPBS2374	CCCAGCAAACCA	53
iPBS2399	AAACTGGCAACGGCGCCA	52
iPBS2401	AGTTAAGCTTTGATACCA	53
iPBS2239	ACCTAGGCTCGGATGCCA	52
iPBS2375	TCGCATCAACCA	52
iPBS2383	GCATGGCCTCCA	53
iPBS2392	TAGATGGTGCCA	52

3.3.3. Data analysis

Strong, clear, and unambiguous bands were selected for scoring. iPBS-retrotransposon markers are dominantly inherited markers and were therefore scored using the binary system: 0 or 1, respectively, for the absence and presence of specific bands with respect to 100 bp+ DNA ladder **(Appendix III)**. For individual iPBS-retrotransposon markers, PopGene ver. 1.32 (Yeh *et al.,* 2000) was implemented to calculate various important genetic diversity parameters including effective alleles number (Ne), Shannon's Information Index (I), and gene diversity (He) (Table 3.2). Polymorphism information content (PIC) was computed for each iPBS-retrotransposon marker following Baloch *et al.* (2015a) criteria. At the safflower samples level, the diversity metrics evaluated included the overall gene diversity (Ht), inbreeding coefficient (Fis) and the pair-wise FST (measure of genetic structure), all of which were determined using hierfstat R package (Team, 2013) following the algorithms of Goudet *et al.* (1996) and Yang (1998). R statistical software was used to compute pairwise genetic distance (GDj) as measured by Jaccard's coefficient (Jaccard, 1908). The population structure was assessed using the Bayesian clustering model-based STRUCTURE software, while unweighted pair group method with arithmetic mean (UPGMA), and Principle coordinate analysis (PCoA) were determined using R package. The most suitable number of clusters (K subpopulations) was determined following the protocol of Evanno *et al.* (2005) using STRUCTURE software. A total of ten independent runs were set for each K value, and for each run, the initial burn-in period was set to 500 with 500,000 MCMC (Markov chain Monte Carlo) iterations with no prior information on the origin of individuals. We plotted the clusters number (K) against logarithm probability relative to standard deviation (ΔK). Final assignment of individual accessions was based on the magnitude of the membership coefficient being greater than or equal to 50% as suggested by Habyarimana (2016) and Nadeem *et al.* (2018a). R statistical software was used to analysis of molecular variance (AMOVA) for considering two main population strata: the model based structure and the country of origin of the accessions.

3.4. Results

3.4.1. iPBS-retrotransposon marker analysis and genetic diversity

Thirteen most polymorphic iPBS-retrotransposon primers produced a total of 295 clear and strong scorable bands with an average of 22.69 bands per primer across 131 safflower accessions. Out of the 295 scorable bands, 275 (93.22%) were polymorphic with an average of 19.77 bands per primer (Table 3.2). The highest (36) and lowest (10) number of scorable bands were observed for primers iPBS2377 and iPBS2391, respectively. The primers iPBS2376 and iPBS2377 revealed highest number of polymorphic bands (29) each and exhibited highest information content (PIC), while primer iPBS2391 revealed least number of polymorphic bands (8) and was least informative. The PIC value ranged from 0.23 (iPBS2401 primer) to 0.78 (iPBS2377 primer) with a mean of 0.48. Highest (1.51) and lowest (1.16) number of effective alleles were observed for primers iPBS2392 and iPBS2401, respectively with an average of 1.33 effective number of alleles. Similarly, maximum (0.46) and minimum (0.20) Shannon's information index was reported for primers iPBS2377 and iPBS2401 and iPBS2228 respectively, having an average value of 0.33. Highest (0.30) level of gene diversity was recorded for primer iPBS2377 while, lowest (0.11) level of gene diversity was observed for primer iPBS2401 with an average of 0.21. At the safflower accession samples level, the overall gene diversity (Ht), Fstatistic (Fst) and inbreeding coefficient (Fis) were 0.19, 0.21, and 1, respectively. The mean genetic diversity indices; observed number of alleles (1.86), effective number of alleles (1.34), Nei's gene diversity (0.21), Shannon's information index (0.33), and overall gene diversity (0.20) across four populations and one unclassified population were also determined (Table 3.3). Population A revealed observed number of alleles (1.68), effective number of alleles (1.28), Nei's gene diversity (0.17), Shannon's information index (0.28), and overall gene diversity (0.15). Population B revealed observed number of alleles (1.70), effective number of alleles (1.33), Nei's gene diversity (0.20), Shannon's information index (0.31), and overall gene diversity (0.19). Population C revealed observed number of alleles (1.63), effective number of alleles (1.26), Nei's gene diversity (0.16), Shannon's information index (0.26), and overall gene diversity (0.15). Population D revealed observed number of alleles (1.65), effective number of alleles (1.29), Nei's gene diversity (0.18), Shannon's information index (0.28), and overall gene diversity (0.17). Unclassified population

revealed observed number of alleles (1.51), effective number of alleles (1.22), Nei's gene diversity (0.14), Shannon's information index (0.22), and overall gene diversity (0.13).

Table 3.2: List of various diversity parameters computed to evaluate genetic diversity among 131 safflower accessions using 13 iPBS- retrotransposon primers

Primers	Total Bands	Polymorphic Bands	Polymorphism (%)	PIC	Ne	I	He	Ht
iPBS2252	20	15	75	0.432	1.2399	0.2666	0.1609	0.16092
iPBS2376	32	29	90.6	0.531	1.4461	0.4171	0.2746	0.26511
iPBS2377	36	29	80.6	0.781	1.4935	0.4578	0.3011	0.28914
iPBS2391	10	8	80	0.663	1.4672	0.4143	0.2745	0.27452
iPBS2398	22	20	90.9	0.316	1.3023	0.2901	0.1835	0.18353
iPBS2228	16	14	87.5	0.323	1.1813	0.1999	0.12	0.12005
iPBS2374	27	26	96.3	0.374	1.2904	0.313	0.1939	0.19393
iPBS2399	28	26	92.9	0.271	1.2293	0.2248	0.14	0.12747
iPBS2401	22	19	86.4	0.231	1.1578	0.1998	0.1117	0.07055
iPBS2239	28	26	92.9	0.623	1.324	0.3353	0.2084	0.18431
iPBS2375	22	20	90.9	0.587	1.4451	0.4055	0.2655	0.25677
iPBS2383	15	11	73.3	0.488	1.2603	0.2787	0.1693	0.12818
iPBS2392	17	14	82.4	0.582	1.5053	0.4372	0.2909	0.27281
Mean	22.69	19.77	86.1	0.477	1.334	0.3261	0.2073	0.19441
Total	295	275	-	-	-	-	-	-

PIC: Polymorphism information content, Ne: effective alleles number, I: Shannon's Information Index, He: gene diversity, Ht: overall gene diversity

Table 3.3: Various diversity parameters computed to evaluate genetic diversity among 131 safflower accessions across populations using 13 iPBS-retrotransposon primers

Populations	Na	Ne	H	I	Ht	Mean Jaccard Genetic distance (GD)	GD Range
Population A	1.6814	1.2831	0.1748	0.2754	0.1498	0.222	0.05-0.339
Population B	1.6983	1.3255	0.1992	0.3096	0.1944	0.242	0.057-0.33
Population C	1.6305	1.2572	0.1616	0.2553	0.1459	0.238	0.126-0.357
Population D	1.6542	1.2931	0.1816	0.2840	0.1685	0.309	0.148-0.455
UP	1.5085	1.2150	0.1373	0.2176	0.1311	0.277	0.134-0.372
Overall	1.8644	1.3399	0.2106	0.3312	0.1971	0.288	0.05-0.507

Na: observed number of alleles, Ne: effective alleles number, I: Shannon's Information Index, h: gene diversity, Ht: overall gene diversity, UP: unclassified population

To clearly understand the broader picture of genetic diversity, pairwise genetic distance among 131 safflower accessions was measured with the Jaccard coefficient. The mean Jaccard genetic distance across the evaluated accessions was 0.288. The highest genetic distance (0.51) was observed between Turkey3 and Afghanistan4 accessions. Similarly, lowest genetic distance (0.05) was present between Afghanistan4 and Afghanistan5 accessions. Genetic distance was calculated across the populations and mean genetic distance for population A (0.22), population B (0.24), population C (0.24), population D (0.31), and unclassified population (0.28) was also obtained.

Analysis of molecular variance (AMOVA) was computed considering two main population strata: the model based structure and the country of origin of the accessions (Table 3.4). AMOVA revealed that the country of origin was not significant, while the model statistically significant effects on the molecular genotypic variability resulted from model-based structure (P = 0.005), country within model-based populations (P = 0.02), and model-based populations within country (P = 0.047). Variations between countries were not significant (P = 0.07), whereas variations within countries (P = 0.037) and between populations (P = 0.046) were significant (Table 3.5). The variations within and between populations explained 43 and 5 percent, respectively, of the genetic structure (Table 3.6). The country within population and the population within country explained 35 and 52 percent of the observed structure.

Table 3.4: Analysis of molecular variance (AMOVA) revealing genetic diversity in; (A) country within STRUCTURE populations, (B) populations within country

			A			
Source	Df	SS	MS	F.Model	R2	Pr(>F)
Country	27	9417	348.78	1.4789	0.22364	0.152
country: group	26	14531	558.89	2.3698	0.34509	0.02*
Residuals	77	18160	235.84	0.43126		
Total	130	42108	1			
			B			
Source	Df	SS	MS	F.Model	R2	Pr(>F)
Structure	4	2177	544.35	2.3081	0.05171	0.005 **
group: country	49	21771	444.3	1.8839	0.51703	0.047 *
Residuals	77	18160	235.84		0.43126	
Total	130	42108			1	

"**" significance at the 0.1% nominal level and "*" significance at the 1% nominal level; Country:group = country within STRUCTURE populations; Group:country = populations within country

Table 3.5: Analysis of molecular variance (AMOVA) revealing genetic diversity within the studied 131safflower accessions

Test	Obs	Std.Obs	Alter	Pvalue
Variations within samples	235.839	-1.9713	Less	0.037
Variations between samples	92.2606	1.69048	Greater	0.07
Variations between group	-2.3109	2.06289	Greater	0.046

Table 3.6: Analysis of molecular variance (AMOVA) revealing intra-genetic diversity within different Structure populations

Source	Df	SS	MS	F.Model	R2	Pr(>F)
Populations	4	278.63	69.658	8.3981	0.21049	0.001 ***
Within populations	126	1045.11	8.295		0.78951	
Total	130	1323.74			1	

"***" corresponds to significance at the 0.05% nominal level

In accordance with the observed most suitable goodness of fit (K = 4)(Figure 3.1), the Bayesian clustering model implemented in STRUCTURE software divided the evaluated safflower accessions into four main populations; 31 accessions (23.66% of the total accessions) in the population A (black), 22 accessions (16.79% of the total accessions) in the population B (red), 33 accessions (25.19% of the total accessions) in the population C (blue), 27 accessions (20.61% of the total accessions) in the population D (pink) (Figure 3.2). Eighteen accessions (on the right-most end of the structure graph) did not reach the membership threshold (50%) and were named unclassified population. The UPGMA based clustering divided 131 safflower accessions into four main clusters corresponding to the four populations (populations A, B, C, D) identified using the model-based structure (Figure 3.3). The unclassified accessions were dispersed throughout the four populations, particularly in population D where 9 (50%) of the unclassified accessions clustered. PCoA divided all accessions into four populations; A, B, C, and D similar to structure based clustering, with the unclassified accessions being dispersed particularly throughout populations B, C, and D (Figure 3.4).

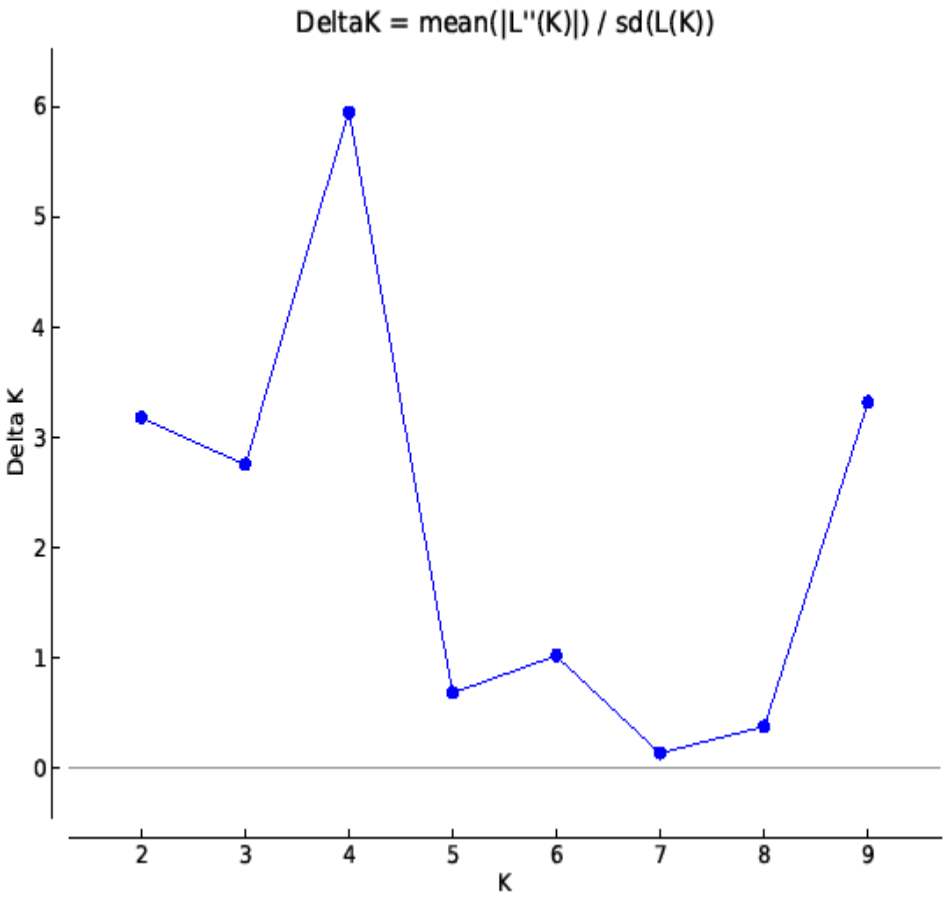


Figure 3.1: DeltaK value revealed the presence of K=4 in 131 safflower accessions using 13 iPBS-retrotransposon markers

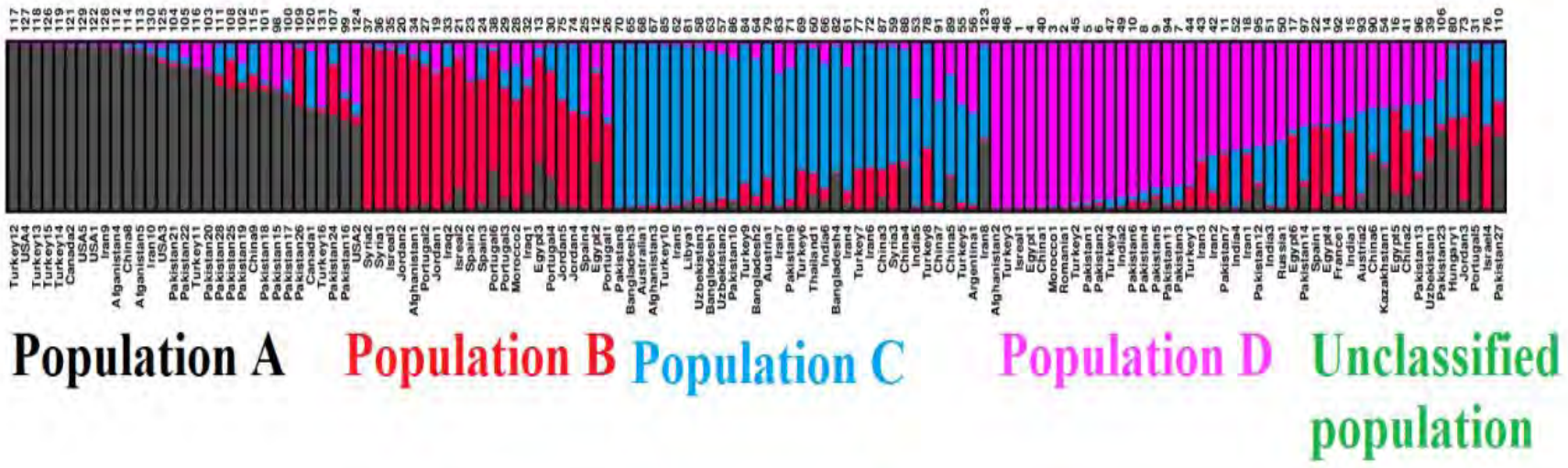


Figure 3.2: Structure-based clustering among 131 safflower accessions using iPBS-retrotransposon markers

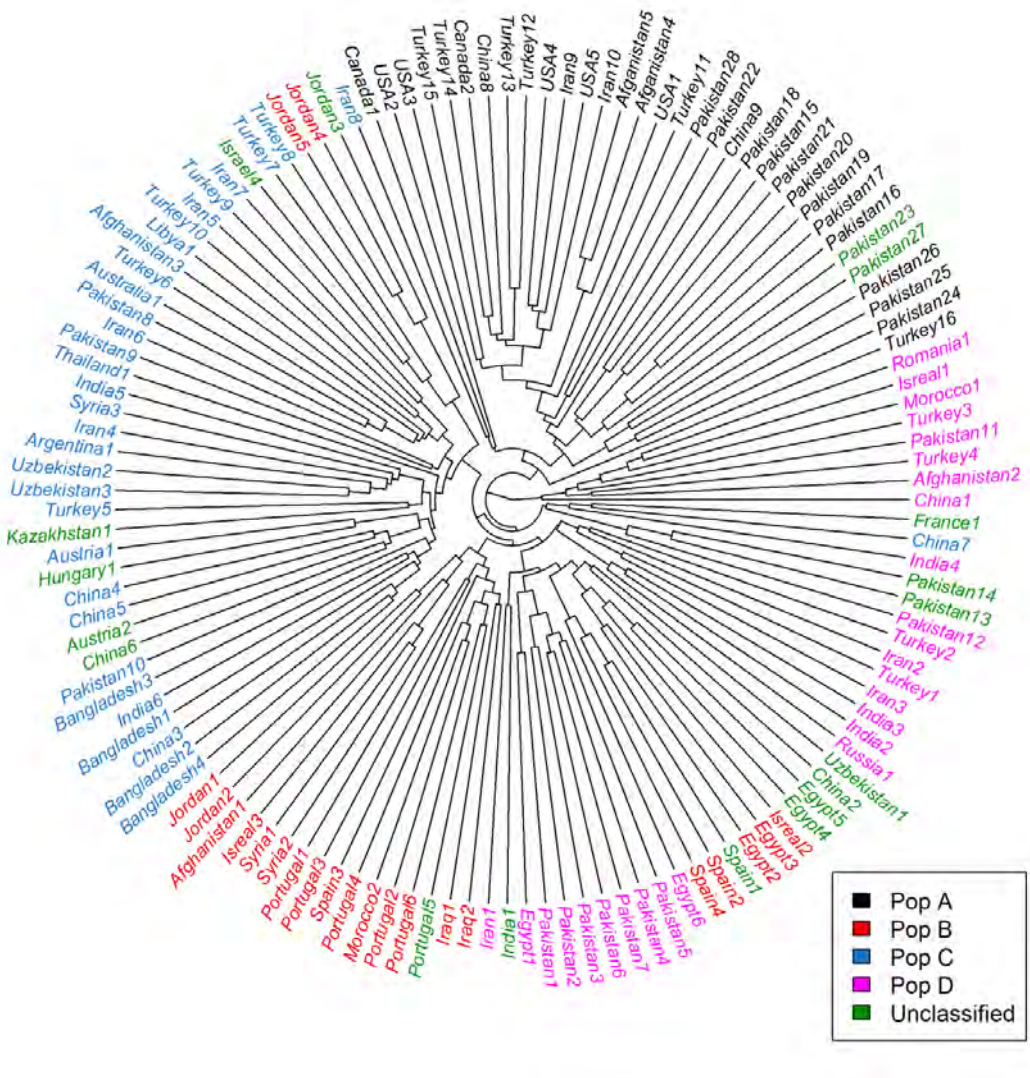


Figure 3.3: UPGMA based clustering among 131 safflower accessions using 13 iPBS-retrotransposon markers

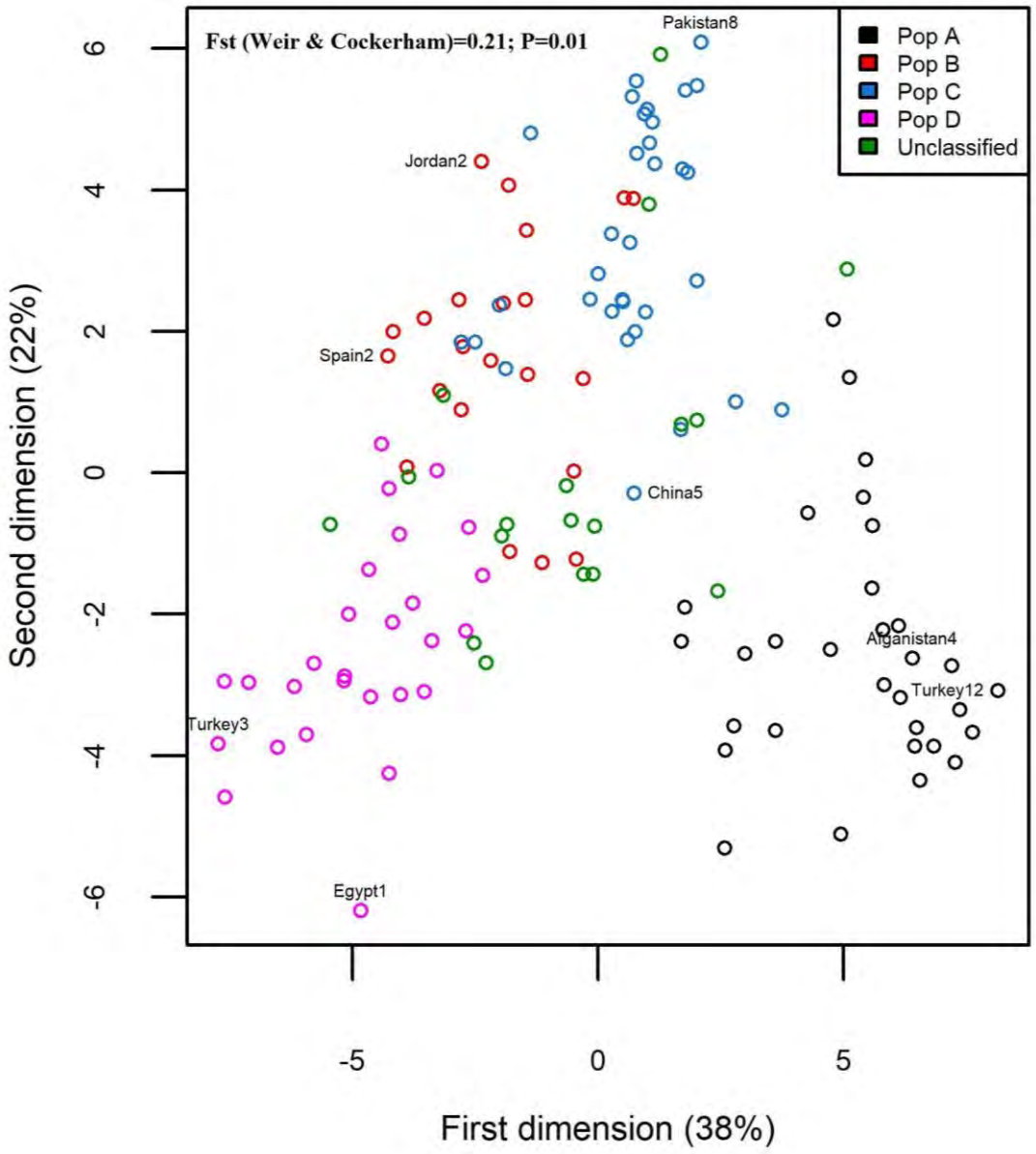


Figure 3.4: Principal coordinate analysis (PCoA) among 131 safflower accessions using 13 iPBS-retrotransposon markers

3.5. Discussion

3.5.1. iPBS-retrotransposons in assessing genetic diversity of safflower panel

To the best of our knowledge, the present investigation represents the first attempt to elucidate the genetic diversity and population structure of safflower accessions at DNA level using iPBS-retrotransposons. It was observed that retrotransposons are abundant and widely distributed throughout plant genome (Kumar and Bennetzen, 1999) and huge amount of error-prone retroviral replications lead to the accumulation of these genetic variations (Casacuberta *et al.*, 1997; Cordaux and Batzer, 2009). iPBS based markers have been utilized greatly for fingerprinting and genetic diversity investigation in plants (Andeden *et al.*, 2013; Guo *et al.*, 2014; Baloch *et al.*, 2016; Jing-Yuan *et al.*, 2018). A total of 13 polymorphic iPBS- retrotransposon markers were used in this study to carry out genetic diversity in safflower panel comprised of 131 accessions from 28 different countries, and 295 clear and strong bands were recorded. The average number of bands per primer was 22.69 while, 275 (93.22%) out of 295 bands were polymorphic. Mean polymorphism found in this study was higher than that of Yang *et al.* (2007), as they reported 82.7% polymorphism using ISSRs in 48 safflower accessions. Furthermore, Sehgal *et al.* (2009) obtained even lower polymorphism levels of 57.6, 68.0, and 71.2% using RAPD, SSR and AFLP markers, respectively. Polymorphism is one of the key requirements to determine good quality genetic markers; therefore, iPBS markers satisfy this requirement in safflower.

Polymorphism information content (PIC) is a widely used metric of the usefulness of molecular markers (Anderson *et al.*, 1993). The PIC was found higher (0.48) in this work than in the findings by Hamdan *et al.* (2011), Barati and Arzani (2012), Derakhshan *et al.* (2014), Lee *et al.* (2014), Ambreen *et al.* (2015), and Ambreen *et al.* (2018), all of whom used SSR markers to evaluate the genetic diversity in safflower. In their works, Houmanat *et al.* (2016) found lower PIC value of 0.23 relative to this study, using ISSRs markers in safflower. These results clearly suggest that more diverse iPBS-retrotransposon markers loci can be identified and effectively used as a tool for assessing genetic diversity and other investigations relying on genetic variants. Maximum number of effective alleles is desirable because it represent the presence of higher level of genetic variations. Number of effective

alleles (1.16 to 1.51) found in this work was in the similar range (1.29 to 1.72) to that of Panahi and Neghab (2013) using ISSR markers to assess the genetic diversity in Iranian safflower germplasm. Similarly, Sung *et al.* (2010) obtained lower range of effective number of alleles (1.02 to 1.09) than us using RAPD markers. Possible reason behind the presence of higher number of effective alleles in this study might be the differences of experimental materials used during evaluation and also the different molecular marker system. Shannon's information index usually distinguishes the level of available genetic diversity in a population, combining abundance and evenness. Kumar *et al.* (2015) reported lower range of Shannon's information index (0.24 to 0.44) than this study using AFLP markers, highlighting the safflower accessions evaluated in this work were more diverse with genetic variants being more evenly distributed throughout the population. This was confirmed also by the level of gene diversity which was found higher than that of Pearl and Burke (2014) and Ambreen *et al.* (2018).

To know the genetic diversity more clearly, diversity metrics like; overall gene diversity (0.24), Fst (0.21), and Fis (1) were also computed. The Fst (a measure of genetic differentiation) obtained in this work was comparable with the findings of Ambreen *et al.* (2018) as they obtained Fst in the range 0.08 to 0.29. On the other hand, Mokhtari *et al.* (2018) obtained mean Fis value of 0.01 which is lower than that (1.00) presented in this work. Safflower is a self-pollinated crop; higher Fis values are therefore expected. In this study, the estimated Fst value (0.21) was higher than the variation explained by the genetic population as evaluated by the analysis of molecular variance (AMOVA). The difference of magnitude between the two metrics was expected as Fst accounted only for genetic populations as a source of variation, while AMOVA accounted for genetic populations and the provenance of the accessions. To understand the variations level more clearly, various diversity indices were calculated at the population's level and population B was found superior by representing higher values for these diversity indices. On the other hand, unclassified population reflected lesser level of diversity by accounting lower values for these diversity indices.

The evaluation of pairwise genetic distance showed a mean of 0.288, with the highest genetic distance between accessions Turkey3 and Afghanistan4, followed by Afghanistan2 and Pakistan24 with respective distance values of 0.51 and 0.49.

Greater similarity was found between Afghanistan4 and Afghanistan5 accessions showing least genetic distance of 0.05. One understandable reason behind the presence of maximum genetic similarity might be due to their origin from the common parents. To explore the genetic diversity more clearly, genetic distances were also calculated at the population level and mean maximum genetic distance was reflected by the population D and minimum was resulted by population A. Within populations, Turkey16 and China9 reflected maximum genetic distance and minimum was present between Afghanistan4 and Afghanistan5 accessions belonging to population A. Within population B, maximum genetic distance was observed between accessions Iraq1 and Jordan4, while minimum genetic distance was shown between accessions Jordan4 and Jordan5. Argentina1 and Iran8 were the two most distinct accessions reflecting maximum genetic distance in the population C and Australia1 and Turkey6 were found two most genetically similar accession of population C representing minimum genetic distance. Within population D, Turkey3 and Iran9 were most diverse accessions and Kazakhstan1 and Pakistan14 were two genetically distinct accessions belonging to unclassified population. Germplasm containing desirable plant traits can be usefully integrated in breeding programs to develop superior cultivars (Arystanbekkyzy *et al.,* 2018), particularly through controlled hybridizations involving genetically distant parental lines. The above four most diverse accessions identified in this work can be recommended as a candidate parents for future safflower breeding programs.

The analysis of molecular variance (AMOVA) was used to determine the pattern of the partition of the total gene diversity among and within populations, and the countries of origin (Lynch and Milligan, 1994). AMOVA showed that most of genetic structure was explained by variations from individuals within populations, the genetic populations within countries and the countries within genetic populations. These findings are in agreement with Wodajo *et al.* (2015), as they reported more within-population (98.9%) importance on genetic structure than among populations (1.1%) using ISSR markers to evaluate 70 safflower accessions from Ethiopia. The discrepancy in terms of the magnitude of variance components explained by the differing sources of variation included in the AMOVA model. The authors included in their model only the population as a source of variation, while in this work two

sources of variation were considered including the population and the country of origin.

The model-based structure application proved more robust and informative in previous investigations (Bouchet *et al.*, 2012; Newell *et al.*, 2013). Structure was therefore used in this work as a benchmark for clustering algorithms. Using structure, the 131 safflower accessions were partitioned into four main populations (A, B, C, and D), and 18 individuals with poor membership coefficients across clusters were considered unclassified population (Figure 3.2). A total of 31, 22, 33, 27, and 18 safflower accessions were found in populations A, B, C, D, and unclassified population, respectively. Population A comprised of 31 safflower accessions from Turkey, USA, Canada, Iran, Afghanistan, China, and Pakistan. This population represents the accessions from the Asian and North American regions. Population B consisted of 22 safflower accessions from different countries including Syria, Israel, Jordan, Afghanistan, Portugal, Spain, Morocco, Iraq, and Egypt. Population B contained the accessions from the Mediterranean countries and all clustering of these accessions together represents their genetic similarity. The 33 safflower accessions found in population C were collected from Pakistan, Bangladesh, Australia, Afghanistan, Turkey, Iran, Libya, Uzbekistan, Thailand, India, China, Syria, and Argentina. Population C comprised of accessions from the Asian and Mediterranean countries and clustering of accessions from both regions proposed the distribution of safflower from Mediterranean region to Asia through Turkey. Population D comprised of 27 safflower accessions from Afghanistan, Turkey, Israel, Egypt, China, Morocco, Romania, Pakistan, India, Iran, and Russia. The unclassified population composed of 18 safflower accessions from Pakistan, Spain, Egypt, France, India, Austria, China, Kazakhstan, Uzbekistan, Hungry, Jordan, Portugal, and Israel. Clustering of accessions from Mediterranean countries confirmed this region as center of origin/domestication for safflower especially Syria (Marinova and Riehl, 2009) and from this region, it is distributed to other parts of the world. Turkey, represents a great level of biodiversity, differentiation center among the continents, and played a vital role to connect the continents with each other (Arystanbekkyzy *et al.,* 2018).

On continents basis, population A clustered a total of 7 and 24 accessions belonging to American and Asian continents respectively. In population B, 3, 11 and 8 accessions originated from Africa, Asia and Europe, respectively. Population C

comprised accessions from America (2), Asia (29), Europe (1), and Oceania (1). In population D most of the accessions originated from Asia (23), while a few accessions came from Africa (3) and Europe (1). The unclassified population contained genotypes mostly from Asia (11), while the other few came from Africa (2) and Europe (5) accessions also made divergence from above four populations by making their separate group. Clearly, the clustering based on molecular markers did not discriminate the origins of the safflower accessions evaluated in this work, which was also confirmed by the AMOVA inferences. Accessions from different countries clustered together, implying that kinship was more determinant for the population structure than the geographical provenance. In addition to sharing common parentage, similarities of accessions in same group during clustering might also be due to convergent evolution and selection (Golkar *et al.*, 2011). It can therefore be inferred that populations from different geographical regions shared a great proportion of genetic diversity. The design of the experiment in this work cannot provide explanation of the observed predominance of Asian safflower accessions. However, the above countries of origin are part of the seven "centers of similarity" (the Far East, India-Pakistan, the Middle East, Egypt, Sudan, Ethiopia and Europe) as recognized by Knowles (1969). Safflower accessions from Afghanistan, Pakistan, Turkey, India, and particularly from China were found more diverse as they were present in all populations. The higher diversity observed in the Asian safflower accessions is a strong evidence of their wider adaptability, which is supported by the findings of Zhang (2001) and Yang *et al.* (2007).

In 1969, Knowles recognized the existence of seven safflower similarity centers across the world. Overall, the centers of similarity were represented by several accessions in this study. However, the molecular marker data used in this study did not provide fully alignment to the above Knowles's hypothesis on the similarity centers. Indeed accessions belonging to different similarity centers were clustered together. This lack of importance of similarity centers in defining molecular-based populations was reported in scientific literature (Chapman and Burke, 2007). In population A, the safflower accessions locally collected from Pakistan were mostly (12 accessions) part of the India-Pakistan similarity center. Also, six accessions from Turkey, two from Afghanistan, and two from Iran were present in this population and can be assigned to the Middle East similarity center. Population B comprised of

safflower accessions from Syria (2), Israel (2), Jordan (4), Afghanistan (1), and Iraq (2) belonging to the Middle East similarity center. Similarly, population B contains safflower accessions from Spain (3), Portugal (5), and Morocco (1) which are part of the Europe similarity center. Population C exhibited safflower accessions from Afghanistan (1), Turkey (6), Iran (5), and Syria (1) revealing the Middle East similarity center. Also, population C contains accessions from Pakistan (3), Bangladesh (4), and India (2) showing the India-Pakistan similarity center. Population D revealed the India-Pakistan similarity center by containing accessions from India (3) and Pakistan (9). Population D also exhibits the Middle East similarity center because it contains accessions from Afghanistan (1), Turkey (4), Israel (1), and Iran (3). The unclassified population revealed the presence of Europe similarity center as it contains one accession from each country; Spain, France, Austria, Hungry, and Portugal. In the same way, India-Pakistan similarity center was also available in the unclassified population due to the presence of safflower accessions from Pakistan (4) and India (1). There is a still need for more research in order to shed more light on the safflower similarity centers at molecular level by collecting and evaluating accessions from all known similarity centers.

The investigation of genetic relationships between the 131 accessions using UPGMA clustering algorithm resulted in a clustering pattern comparable with the model-based algorithm with a few exceptions as two (Jordan4, Jordan5) and five (Israel2, Egypt2, Egypt3, Spain2, Spain4) population B accessions clustered with population D and population C, respectively, and UPGMA discrepantly clustered the accession Iran8 (population C) in population A (Figure 3.3). Since these accessions displayed mostly full membership coefficients in model-based Structure, the discrepancy observed in UPGMA clustering approaches can be explained by its reduced resolution power relative to the model-based Structure (Bouchet *et al.,* 2012; Newell *et al.,* 2013).

Principal coordinate analysis (PCoA) greatly supported the structure based clustering of 131 safflower accessions using 13 iPBS-retrotransposon primers (Figure 3.4). The four populations were clearly distinguishable, and the unclassified population was disseminated throughout the other populations, particularly throughout populations B, C, and D. These light discrepancies between PCoA and model-based structure can derive from differing clustering resolution, with model-

based structure exhibiting more resolution. Indeed, 40% of the variation in the overall genetic structure was not accounted for by the first two PCoA dimensions presented in this work. The above-mentioned misclassifications of accessions in the principal coordinate space can be explained by the existence of genomic admixture. PCoA analysis revealed the same pattern of distribution of similarity centers as identified by structure based analysis. Population A, B, and D exhibited the Middle East similarity centers as they contain safflower accessions from Turkey, Afghanistan, Iran, Syria, Israel, Jordan, and Iraq. Population C comprised of India-Pakistan similarity center by containing safflower accession from India, Pakistan, and Bangladesh. Europe similarity center is present in population B and in the unclassified population of PCoA based analysis. It suggests more research work regarding the confirmation of safflower similarity centers at molecular level. Overall, iPBS-retrotransposons revealed a good spectrum of genome diversity in safflower and the explored genetic diversity can be used in future safflower breeding programs. As iPBS-retrotransposon marker system demonstrated competitive results in this work and in previous investigations, it is warranted to focus further attention on collecting and evaluating safflower germplasm at molecular level using iPBS-retrotransposons as an important tool for enhancing productivity. To contribute to the yet unending discussion on the safflower similarity centers, a robust sampling techniques including random sampling without replacement can be implemented on the accessions in major world safflower seed repositories; the sampled materials can be evaluated using clustering algorithms such as those implemented in this work.

3.6. Conclusion

A good level of genetic diversity was identified among 131 safflower accessions. The importance of genetic populations on the genetic structure was significant, but its magnitude was lesser than the importance the variations of individuals within genetic populations. The provenance of the samples showed no effects on the genetic structures in the 131 accessions. Our results most probably obey the seven similarity centers hypothesis of safflower but still there is need to conduct further research works to confirm these similarity centers at the molecular level. Generally, safflower accessions from Asian countries like Afghanistan, Pakistan, China, Turkey, and India were found diverse. Specifically, among 131 safflower germplasm, accessions Turkey3, Afghanistan4, Afghanistan2, and Pakistan24 were found most diverse at molecular level and might be recommended as a candidate parents for future safflower breeding programs. This is a first study to explore the genetic diversity and population structure in safflower accessions using the iPBS-retrotransposon markers. The information provided in this work will therefore be helpful for scientists interested in safflower breeding.

Chapter 4

Molecular characterization of genetic diversity and similarity centers of safflower accessions with ISSR markers

4.1. Introduction

The fast growth of the world's population requires sufficient availability of food in terms of calories and other nutrients (Long *et al.*, 2015). Daily average of per capita available calories across the world was 2789 kcal in the year 2000, and it is believed that it will become 3130 kcal by year 2050 (FAOSTAT, 2012), highlighting a steady increase in food demand paralleling the growth of world's population. A steady increase in the production of safflower has been observed during the last two decades to meet the vegetable oil shortage. Scientists agree on the importance of increasing the crop yield instead of increasing area under the cultivation in the process of sustainable agriculture (Godfray *et al.*, 2010). Therefore in order to mitigate the vegetable oilseed shortage and avoid future demand problems, there is a need to focus on the breeding activities.

Different scientists suggested various number of safflower similarity centers throughout the world (Knowles, 1969; Ashri, 1975; Chapman et al., 2010). Need of the safflower genome molecular characterization and development of the efficient molecular markers has been recognized by a number of research groups (Amiri *et al.*, 2001; Johnson *et al.*, 2007). Molecular markers overcome limitations present in the morphological and biochemical markers by detecting diversity at the DNA level (Nadeem *et al.,* 2018b). It should be understood that different molecular markers have different characteristics, thus reflecting the different genetic diversity aspects (Talebi *et al.*, 2012; Nadeem *et al.*, 2018b). Safflower genetic diversity using several molecular markers like; Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSRs), iPBS-retrotransposon, and Single Nucleotide Polymorphism (SNPs) have been estimated (Johnson *et al.,* 2007; Yang *et al.,* 2007; Amini *et al.,* 2008; Khan *et al.,* 2009; Chapman *et al.,* 2010; Lee *et al.,* 2014; Pearl and Burke, 2014; Ambreen *et al.,* 2015; Kumar *et al.,* 2015; Ambreen *et al.,* 2018; Ali *et al.,* 2019b). These researchers suggested the presence of good level of genetic diversity among different global safflower germplasm and also validate some of the similarity centers that were initially based on various morpho-agronomic traits (Johnson *et al.,* 2007; Chapman *et al.,* 2010; Pearl and Burke, 2014; Kumar *et al.,* 2015). Inter-simple sequence repeats (ISSR) primers based on di, tetra, or penta-nucleotide repeats are routinely used for various purposes (Zietkiewicz *et al.*, 1994).

The advantages of simple procedure, low cost, high reproducibility, and excellent stability made ISSR primers suitable for the determination of genetic diversity analysis (Rawat *et al.*, 2016; Hadian *et al.*, 2017), mapping studies (Casasoli *et al.*, 2001; Cekic *et al.*, 2001; Tanyolac, 2003), and germplasm identification (Nagaoka and Ogihara, 1997; Potter *et al.*, 2002). ISSR markers has been effectively employed for the determination of genetic diversity and molecular characterization of different crops including; cluster bean (Ansari *et al.,* 2016), Chickpea (Gautam *et al.*, 2016), and Brassica (Khalil and zayat, 2019) etc. Besides these crops, ISSR markers were also used for the genetic diversity and molecular characterization of safflower by various researchers (Yang *et al.*, 2007; Sabzalian *et al.*, 2009; Golkar *et al.*, 2011; Bagmohammadi *et al.*, 2012; Majidi and Zadhoush, 2014; Houmanat *et al.*, 2016) revealing its efficient potential for the germplasm characterization.

4.2. Objectives

The current study was aimed to determine the genetic diversity, population structure and safflower similarity centers at molecular level using ISSR markers and the useful information will be then used for the future safflower breeding programs by the scientific community.

4.3. Material and methods

4.3.1. Plant materials and DNA isolation

One hundred and thirty one safflower accessions collected from 28 different countries were tested during the current study. The safflower accessions; 94, 17, and 20 were provided by the United States Department of Agriculture (USDA), Plant Genetic Resources Institute (PGRI) Pakistan, and the Turkish Central Research Institute for Field Crops, respectively (Appendix II). The safflower accessions provided by USDA (94 accessions) and PGRI (17 accessions) were landraces. The 20 Turkish safflower accessions were single plant selection among the international safflower germplasm obtained from the USDA and are candidate cultivars. Safflower seeds of each accession were sown at the research and experimental area of Bolu Abant Izzet Baysal University. The fresh, healthy and young leaves from each accession were harvested for the DNA isolation and frozen at the temperature of -80°C in laboratory. Bulk of leaves from each accession was used for the DNA extraction following the CTAB protocol (Doyle and Doyle, 1990) with slight modifications (Baloch *et al.,* 2016). DNA concentration was estimated using agarose gel (0.8%) and was then confirmed with NanoDrop (DeNovix DS-11 FX, USA). Final DNA concentration of the 131 safflower accessions was adjusted to5 ngμL^{-1} for the purpose of polymerase chain reactions. All samples were stored at the temperature of -25 ^{o}C until PCR amplification.

4.3.2. ISSR PCR amplifications

Ninety ISSR primers were initially screened using eight randomly selected accessions of safflower for PCR amplifications. Twelve ISSR primers were found most polymorphic by producing strong bands and were used for the amplification of PCR (Table 4.1). A total reaction volume of 25 µL for PCR amplifications were comprised of 4 ngul^{-1} template DNA, 4 µL dNTPs (Thermo Scientific), 0.2 µL U Taq DNA polymerase (Thermo Scientific), 1 µL primer, 2.5 µL 1$_X$PCR buffer (Thermo Scientific), 2 µL MgCl2 and 11.3 µL distilled water. Reactions were performed in the sequence of denaturation at 94^{o}C for 3 min, subsequently followed by 30 denaturation cycles at 94^{o}C for 1 min, annealing temperature of 48-54^{o}C for one minute depending upon the primer, and a final extension for 10 min at 72 ^{o}C. Agarose gel 1.8% (w/v) containing 0.5$_X$TBE buffer was used for the electrophoreses of the amplified DNA

fragments at a constant voltage of 120 V for 240 min. Ethidium bromide was used to perform the staining of the gel and then visualized using UV Imager Gel Doc XR+ system (Bio-Rad, USA) light and photographed. A 100 bp+ DNA ladder was used as molecular weight marker.

Table 4.1: Sequence and annealing temperature of 12 ISSR primers used to determine genetic diversity among 131 safflower accessions

Primer name	Sequence	Annealing temperature (^{o}C)
ISSR809	AGAGAGAGAGAGAGAGG	52
ISSR810	GAGAGAGAGAGAGAGAT	52
ISSR811	GAGAGAGAGAGAGAGAC	53
ISSR812	GAGAGAGAGAGAGAGAA	52
ISSR817	CACACACACACACACAA	51
ISSR818	CACACACACACACACAG	53
ISSR819	GTGTGTGTGTGTGTGTA	53
ISSR827	ACACACACACACACACG	52
ISSR830	TGTGTGTGTGTGTGTGG	53
ISSR834	AGAGAGAGAGAGAGAGYT	52
ISSR840	GAGAGAGAGAGAGAGAYT	52
ISSR868	GAAGAAGAAGAAGAAGAA	53
ISSR809	AGAGAGAGAGAGAGAGG	52

4.3.3. Data analysis

Strong, unambiguous, and clear bands were used for the purpose of scoring, while vague bands were not selected as they could not be easily detected. ISSR markers are dominant markers and scored in a binary matrix as 1 for present or 0 for absent respectively, of all the bands with relative to 100 bp+ DNA ladder **(Appendix IV)**. PopGene ver. 1.32 (Yeh *et al.*, 2000) was applied to compute genetic diversity indices like; effective alleles number (Ne), Shannon's Information Index (I), and gene diversity (He) for individual ISSR markers (Table 4.2). Baloch *et al.* (2015a) criteria was used to determine the Polymorphism information content (PIC) for each ISSR marker. Pairwise genetic distance (GDj) was determined using R statistical software as measured by Jaccard's coefficient (Jaccard, 1908). Analysis of molecular variance (AMOVA) was investigated using R statistical software considering variation among structure populations and structure populations within country (Table 4.3).The population structure was assessed using the Bayesian clustering model-based STRUCTURE software. The UPGMA and Principle coordinate analysis (PCoA) was performed using R software to explore the level of diversity among 131 safflower accessions (Team, 2013). Evanno *et al.* (2005) protocol was used through STRUCTURE software to determine the most suitable number of clusters (K subpopulations). We plotted the clusters number (K) against logarithm probability relative to standard deviation (ΔK). Assignment of the individual safflower accessions to the separate population was based on the membership coefficient magnitude being greater than or equal to 50% as outlined by Habyarimana (2016).

4.4. Results

4.4.1. ISSR marker analysis and genetic diversity

Twelve most polymorphic ISSR primers produced a total of 201scorable bands having average of 16.75 bands per primer using 131 safflower accessions. Among 201 ISSR bands, 188 (93.844%) were identified polymorphic having average of 15.67 bands per primer (Table 4.2). Primer ISSR809 displayed the highest number of total (22) and polymorphic (21) bands, while lowest number of total (11) and polymorphic (10) bands were found with primer ISSR868. Diversity parameters like mean polymorphism information content, mean effective number of alleles, mean Nei's gene diversity, mean Shannon's Information index, and mean expected

heterozygosity were, respectively, 0.448, 1.655, 0.377, 0.557, and 0.354 among the 12 ISSR primers using 131 safflower accessions (Table 4.2). The primer ISSR868 was the most informative by revealing a good amount of polymorphism information content (0.592), effective number of alleles (1.849), Nei's gene diversity (0.454), Shannon's Information index (0.645), and expected heterozygosity (0.441), while the primer ISSR810 was least informative by exhibiting low values of polymorphism information content (0.274), effective number of alleles (1.458), Nei's gene diversity (0.282), Shannon's Information index (0.436), and expected heterozygosity (0.253).

Table 4.2: Diversity parameters computed to evaluate genetic diversity among 131 safflower accessions using 12 ISSR primers

Primer	Total bands	Polymorphic bands	Polymorphism (%)	PIC	ne*	h*	I*	Ht
ISSR809	22	21	95.455	0.426	1.633	0.371	0.563	0.340
ISSR810	20	16	80.000	0.274	1.458	0.282	0.436	0.253
ISSR811	15	13	86.667	0.334	1.555	0.338	0.513	0.328
ISSR812	19	18	94.737	0.454	1.651	0.388	0.574	0.386
ISSR817	17	17	100.000	0.505	1.780	0.420	0.605	0.398
ISSR818	12	12	100.000	0.445	1.565	0.341	0.516	0.291
ISSR819	18	16	88.889	0.360	1.626	0.358	0.531	0.338
ISSR827	19	19	100.000	0.489	1.696	0.396	0.580	0.374
ISSR830	14	14	100.000	0.334	1.563	0.341	0.514	0.327
ISSR834	19	17	89.474	0.585	1.716	0.408	0.596	0.370
ISSR840	15	15	100.000	0.577	1.773	0.428	0.617	0.403
ISSR868	11	10	90.909	0.592	1.849	0.454	0.645	0.441
Mean	201	188	93.844	0.448	1.655	0.377	0.557	0.354

* ne = Effective number of alleles; * h = Nei's (1973) gene diversity; * I = Shannon's Information index ; Ht = Expected hetrozygosit

Pairwise genetic distance with the Jaccard coefficient was computed among the 131 safflower accessions in order to understand the picture of genetic diversity more clearly. The mean genetic distance among 131 accessions was found 0.336. Accessions Pakistan11 and Israel1 revealed highest genetic distance (0.816), while accessions USA5 and Iran10 showed lowest genetic distance (0.063). Analysis of molecular variance (AMOVA) resulted in highly significant effects of model-based structure (P=0.001) and model-based structure × country combination (P=0.003) on genetic differentiation (Table 4.3).

In accordance with the observed most suitable goodness of fit (K=3) (Figure 4.1), the Bayesian clustering model implemented in STRUCTURE software divided the evaluated safflower accessions into three main populations; 47 accessions (35.88%) in the population A (green), 19 accessions (14.50%) in the population B (red), 64 accessions (48.86%) in the population C (blue), and 1 accession (0.76%) in an unclassified population (Figure 4.2). The UPGMA clustering divided 131 safflower accessions into three main populations and an unclassified population corresponding to the populations identified using the model-based structure (Figure 4.3). PCoA divided all accessions into three populations; A, B, and C and an unclassified population which were similar to structure based clustering (Figure 4.4).

Table 4.3: Analysis of molecular variance (AMOVA) revealing genetic diversity among structure populations and STRUCTURE populations within country

Source of variation	Df	SS	MS	F Model	RSq	P value
Clusters (Populations)	3	436.8	145.592	6.4063	0.12616	0.001 ***
Cluster:country	44	1139.1	25.889	1.1392	0.32902	0.003 **
Residuals	83	1886.3	22.726	NA	0.54482	NA
Total	130	3462.2	NA	NA	1	NA

"**" significance at the 0.1% nominal level and "***" corresponds to significance at the 0.05% nominal level

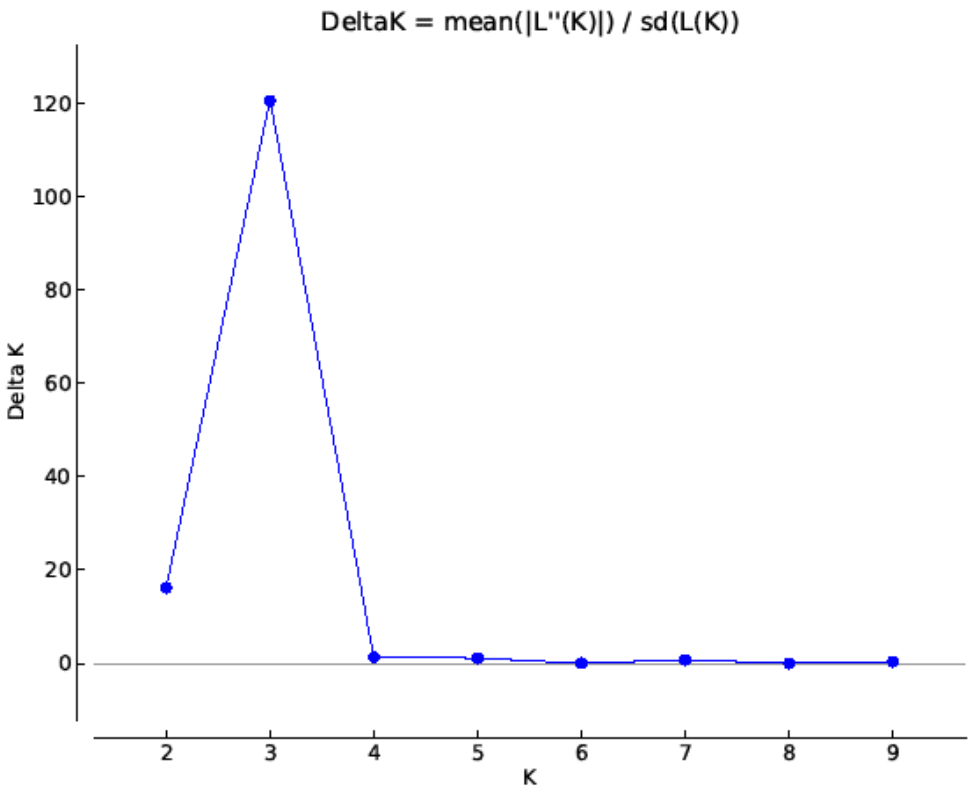


Figure 4.1: DeltaK value revealed the presence of K=3 in 131 safflower accessions using 12 ISSR markers

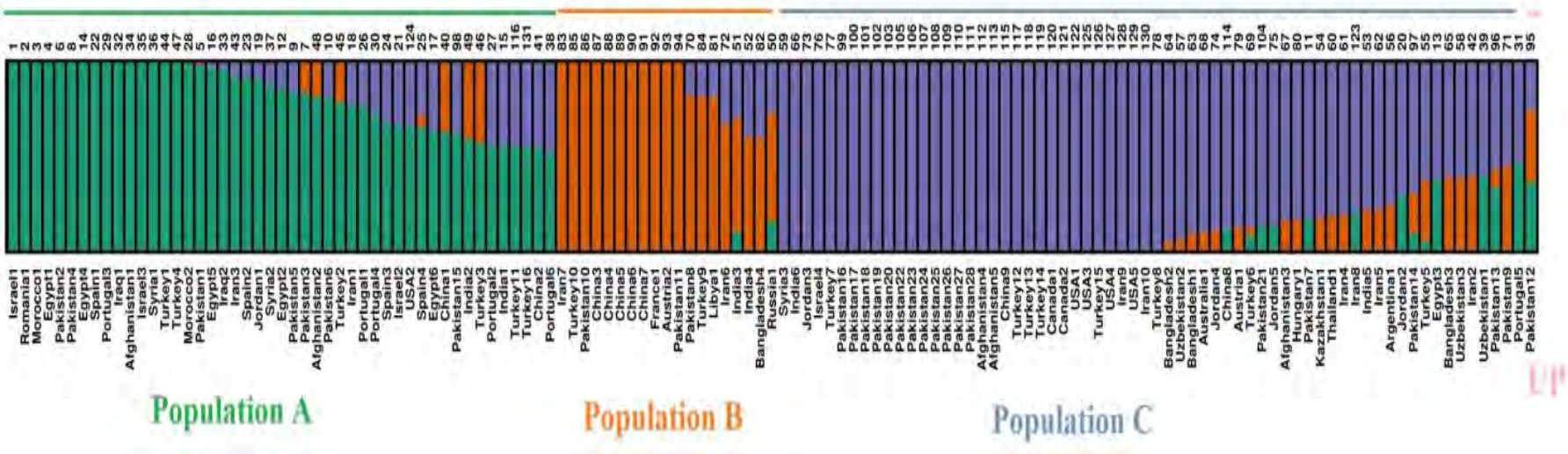


Figure 4.2: Structure-based clustering among 131 safflower accessions using 12 ISSR primers

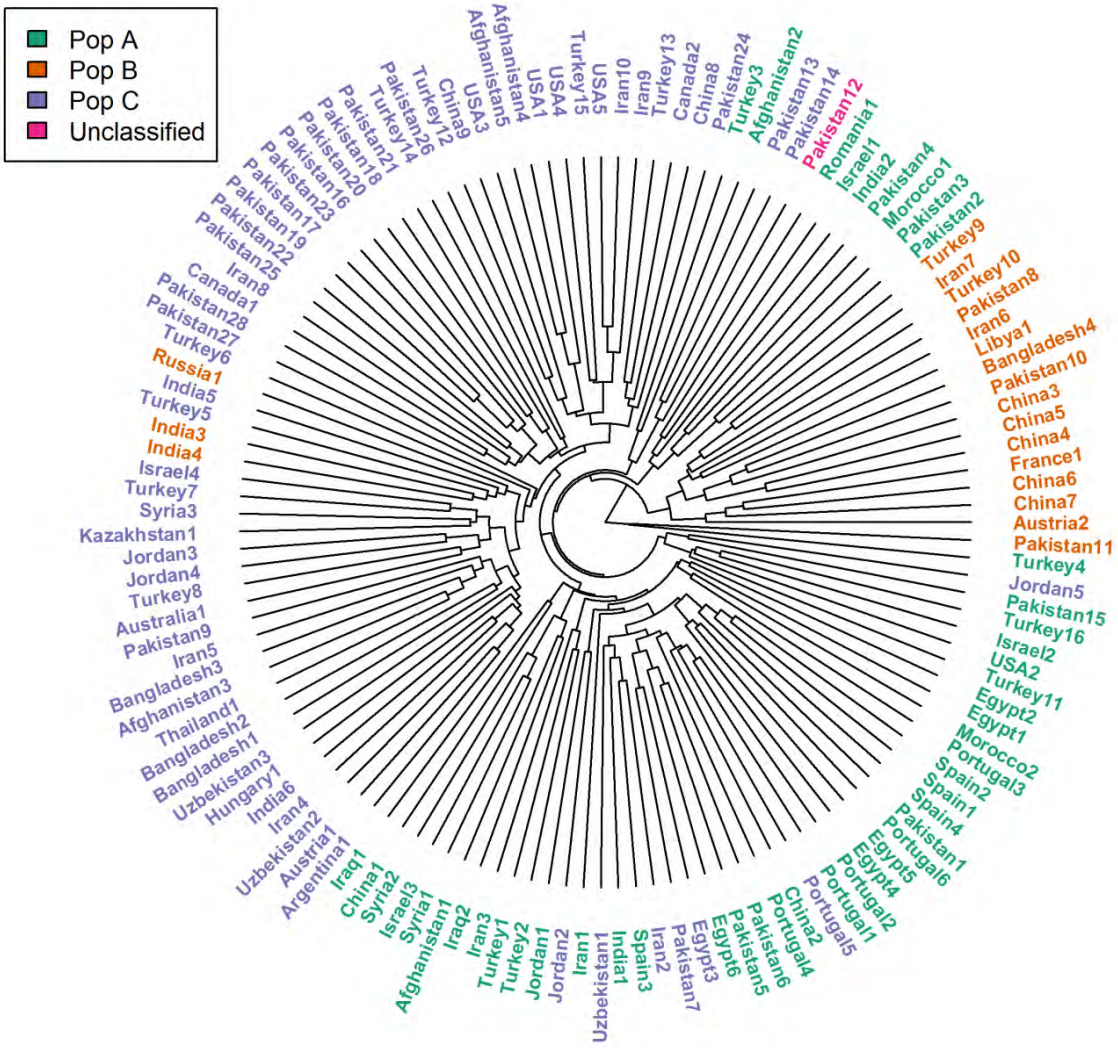


Figure 4.3: UPGMA based clustering among 131 safflower accessions using 12 ISSR primers

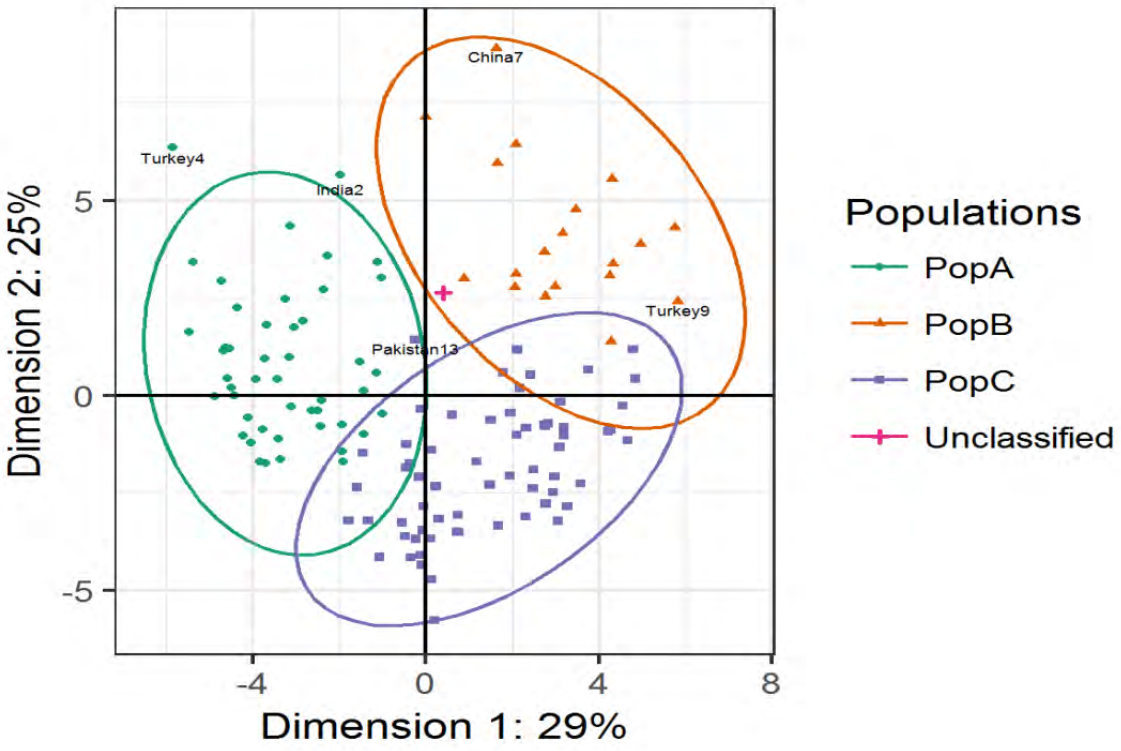


Figure 4.4: Principal Coordinate analysis (PCoA) among 131 safflower accessions using 12 ISSR primers

4.5. Discussion

The knowledge on the partition of the genetic variation that existed in crop gene pools is helpful to describe the evolution of crop lineages and also disclose the unexplored sources of variation that enhance future crop improvement efforts (Tanksley and McCouch, 1997; Yamasaki *et al.*, 2005). Until now, population genetic analysis regarding safflower gene pool has not been fully exploited, and also the hypothesis of Knowles (1969) and Ashri (1975) about the safflower similarity centers is still unclear at the genetic level. Our data presented herein strongly supported the Knowles (1969) hypothesis proposing seven similarity centers. Very few attempts have been done to investigate the total spectrum of variation in global safflower germplasm at the DNA level. Genetic diversity characterization within safflower gene pools is vital for its development and improvement. Our results about mean polymorphism (93.844%) was higher to that of Houmanat *et al.* (2016), as they found mean polymorphism of 63.38% using ISSR markers evaluating a safflower set of 55 accessions. Similarly, Golkar *et al.* (2011) reported lower polymorphism (70%) than ours using ISSR markers. Polymorphism information content (PIC) is a widely used metric of the usefulness of molecular markers (Anderson *et al.*, 1993). Higher PIC (0.448) was obtained in the current study in comparison to Talebi and Abhari (2016). They evaluated 25 safflower accessions using 13 ISSR markers. Moreover, Houmanat *et al.* (2016) revealed lower PIC value (0.23) than us using ISSR markers in safflower. The presence of higher number of effective alleles revealed the availability of maximum level of genetic diversity and is always desirable. We obtained higher effective number of alleles (1.458 to 1.849) than that of Sung *et al.* (2010) (1.02 to 1.09). Obtaining superiority of various diversity parameters in this study than the previous results might be due to the difference of the experimental materials used in the current assessment and also the difference of the ISSR markers used. Shannon's information index usually distinguishes the level of available genetic diversity in a population, combining abundance and evenness. Kumar *et al.* (2015) observed lower Shannon's information index (0.24 to 0.44) contrary to our observation (0.436 to 0.645) using AFLP markers. It is a clear indication of the presence of higher level of genetic diversity in the studied safflower accessions with genetic variants evenly distributed throughout the population. Wodajo *et al.* (2015) reported lower mean Shannon's information index (0.46) than us (0.557) using ISSR markers. Our results

about expected heterozygosity (0.354) are supported by Lee *et al.* (2014) as they revealed similar expected heterozygosity (0.386). Wodajo *et al.* (2015) studied 70 safflower accessions using ISSR markers and found Nei's gene diversity of 0.30, which is lesser than the value (0.377) obtained in this study. Diversity parameters revealed the presence of higher genetic variability in the studied materials suggesting the studied safflower accessions can provide useful building blocks for future breeding programs to enhance safflower productivity. Also, the ISSR markers used in this evaluation should be used for the genetic diversity investigation as these markers exhibited higher diversity levels.

The evaluation of pairwise genetic distance showed a mean of 0.336, with the highest genetic distance between accessions Pakistan11 and Israel1, followed by Pakistan26 and Israel1 with respective distance values of 0.816 and 0.808. Greater similarity was found between USA5 and Iran10 accessions showing least genetic distance of 0.063. One understandable reason behind the presence of maximum genetic similarity might be due to their origin from the common parents. The three most diverse safflower accessions (Pakistan11, Israel1, and Pakistan26) identified during the current study can be recommended as a candidate parents for future breeding programs. The analysis of molecular variance (AMOVA) was used to determine the pattern of the partition of the total gene diversity among and within populations, and to assess genetic differentiation. AMOVA showed that most of genetic structure was explained by variations among populations and the genetic populations within countries (Table 4.3).

The model-based structure application proved more robust and informative in previous investigations (Bouchet *et al.,* 2012; Nadeem *et al.,* 2018a; Ali *et al.,* 2019b). Structure was therefore used in this work as a benchmark for clustering algorithms. The studied 131 safflower accessions were clearly separated into three main populations; A, B, and C, and an unclassified population using structure (Figure 4.2). Population A consists of 47 accessions originated from Israel (3 accessions), Romania (1 accession), Morocco (2 accessions), Egypt (5 accessions), Pakistan (7 accessions), Spain (4 accessions), Portugal (5 accessions), Iraq (2 accessions), Syria (2 accession), Turkey (6 accessions), Iran (2 accessions), Jordan (1accession), Afghanistan (2 accession), USA (1 accession), China(2 accessions), and India (2 accessions). Population B comprised of 19 safflower accessions including; Iran (2 accessions),

Turkey (2 accessions), Pakistan (3 accessions), China (5 accessions), France (1accession), Austria (1accession), Libya (1accession), India (2 accessions), Bangladesh (1 accession), and Russia (1accession). Clustering of safflower accessions from Mediterranean region with Europe and Asian countries identify its origin/domestication from Mediterranean region and distribution to other parts of the world. The 64 safflower accessions clustered in population C were originated from Syria (1accession), India (2 accessions), Jordan (4 accessions), Israel (1accession), Turkey (8 accessions), Afghanistan (3 accessions), China (2 accessions), Canada (2 accession), USA (4 accessions), Iran (6 accession), Bangladesh (3 accessions), Uzbekistan (3 accessions), Australia (1accession), Austria (1accession), Pakistan (17 accessions), Hungary (1accession), Kazakhstan (1accession), Thailand (1accession), Argentina (1accession), Egypt (1accession), and Portugal(1 accession). Clustering pattern of accessions and their distribution in population C was found similar to populations A and B. Distribution of safflower accessions from Mediterranean region to Asia took place through Turkey, being used as a bridge. According to Nadeem *et al.* (2018a), Turkey acts as bridge for the diffusion of various crops among the continents. One safflower accession originated from Pakistan (Pakistan12) made up the unclassified population as its membership coefficient magnitude was less than 50% as proposed by Habyarimana (2016).

Population A included accessions from Asia (29 accessions), Europe (10 accessions), Africa (7 accessions), and American (1 accession) continents. Population B exhibited accessions from Asia (16 accessions), Europe (2 accessions), and Africa (1 accession). Population C revealed accessions from Asia (52 accessions), America (7 accessions), Oceania (1 accession), Europe (3 accessions), and Africa (1 accession). The unclassified population exhibited only one accession that is originated from Asian (Pakistan) continent. Besides sharing common parentage, accessions similarity in the same population during the clustering might also be due to convergent evolution and selection (Golkar *et al.,* 2011). Population C stood the most diverse population as it comprised accessions from all the available continents.

Knowles (1969) suggested the presence of seven similarity centers for safflower throughout the world using various morpho-agronomic traits. Most of the accessions evaluated in this study follow the hypothesis of seven similarity centers at molecular level. But the data obtained from the ISSR markers in this study did not

fully support the Knowles's hypothesis of similarity centers. Safflower accessions from different similarity centers clustered together and highlighted the lack of importance of similarity centers at molecular level which was previously reported in the scientific literature (Chapman and Burke, 2007). Safflower accessions from Israel, Iraq, Syria, Turkey, Iran, and Jordan were present in population A and can be assigned to the Middle East similarity center. Similarly, accessions from India and Pakistan were also present in population A comprising the India-Pakistan similarity center. Accessions from Pakistan, India, and Bangladesh were clustered in population B and made the India-Pakistan similarity center. Population C revealed the Middle East similarity center as it exhibited safflower accessions from Syria, Jordan, Israel, Turkey, Afghanistan, and Iran. Population C also exhibited safflower accessions from the India-Pakistan (India, Bangladesh, and Pakistan) and Europe (Australia, Austria, Hungary, Argentina, and Portugal) similarity centers. Very recently Ali *et al.* (2019b) aimed to evaluate the similar centers pattern at molecular level using 13 iPBS-retrotransposon markers and supported the Knowles (1969) hypothesis proposing seven similar centers. Our results are supported by their findings revealing similar safflower similarity centers patterns. Besides obtaining supportive results to the Knowles's hypothesis of seven similarity centers, still there is a need to conduct more research at the molecular level by collecting and testing safflower accessions from the all proposed similarity centers.

The exploration of genetic relationships between the studied 131 safflower accessions using UPGMA resulted in a comparable clustering pattern to that of model-based algorithm with few exceptions as three accessions belonging to population B (Russia1, India3, and India4) clustered with population C (Figure 4.3). Seven accessions belonging to population C (Jordan5, Portugal5, Egypt3, Pakistan7, Iran2, Uzbekistan1, and Jordan2) clustered with population A. Similarly, two accessions from population A (Turkay3 and Afghanistan2) clustered with population C. Unclassified safflower accession (Pakistan12) clustered with population A. Accessions present in the same population revealed full membership coefficients in model-based Structure. The discrepancies displayed in UPGMA clustering might be described by its reduced resolution power relative to the model-based Structure (Bouchet *et al.,* 2012).

Principal coordinate analysis (PCoA) confirmed the clustering based on structure algorithm of 131 safflower accessions into clearly distinguishable three main populations and an unclassified population using 12 ISSR primers (Figure 4.4). The occurrence of some light differences between model-based structure and PCoA can derive from its differing clustering resolution, with more resolution revealed by the model-based structure analysis. Existence of the genomic admixture might be the reason for the misclassification in the principal coordinate space of the 131 safflower accessions. Also, same pattern of the similarity centers as obtained through structure based analysis, was exhibited by PCoA analysis.

4.6. Conclusion

The presence of good level of genome diversity was observed among the studied materials. Model-based structure, unweighted pair-group method with arithmetic means (UPGMA), and principal coordinate analysis (PCoA) clustered all accessions into three main populations; A, B, and C and an unclassified population. Accessions originated from Asian countries like Pakistan and Israel were found most diverse. Three accessions, Pakistan11, Israel1, and Pakistan26 were found most genetically distant and might be used as parental sources for genetic combinations in safflower breeding activities. Analysis of molecular variance (AMOVA) revealed highly significant differentiation among the identified populations, and population × country combinations. The results presented in this work most probably supported the hypothesis of seven similarity centers of safflower but need to be validated with further confirmed investigations with advanced molecular marker system like DArTseq. The information provided herein is expected to be helpful for the scientific community interested in safflower breeding.

Chapter 5

Molecular characterization of genetic diversity, similarity centers exploration, and marker-trait associations in international safflower panel using whole-genome DArTseq-generated silicoDArT marker information

5.1. Introduction

Earlier plant selection was focused upon the traits to overall yield, its harvesting period, and edibility (Konishi *et al.,* 2006; Hua *et al.,* 2015). The adapted selection criteria lead to the genetic bottlenecks with different degrees in various crop species (Buckler *et al.,* 2001; Miller and Gross, 2011; Meyer *et al.,* 2012). The knowledge concern to genetic diversity and their association to the crop gene pool is a prerequisite to the safflower improvement. Novel genetic variation present in the crop gene pool can be characterized for the efficient utilization in the future breeding programs (Baloch *et al.,* 2017; Nadeem *et al.,* 2018a; Yaldiz *et al.,* 2018; Ali *et al.,* 2019b). The identified diverse germplasm accessions based on genetic diversity analysis aid in the introgression of novel alleles to the high yielding cultivars and utilized in planning different crosses combinations to develop various segregating populations (Barrett and Kidwell, 1998; Thompson *et al.,* 1998). Genetic diversity available in the characterized germplasm remains as an important source of the novel alleles to plan efficient crop improvement strategies and to develop sustainable farming systems (Jing *et al.,* 2010).

Continuous gene flow in the form of improved cultivars takes place to the farmer fields especially in developed countries that lead to crop improvement. Implementation of the advanced biotechnological tools for crop improvement in safflower is very limited. Very few research groups are working on the different aspects of safflower and access to the advanced biotechnological tools in terms of information, equipment, and various techniques are insufficient. Keen interest has been witnessed from the industrial sector in the safflower production for different purposes. But the safflower breeding programs experience inadequate complementation with advance biotechnological tools. Regular framework genetic map for safflower is not developed up till now. It was also suggested that safflower germplasm presenting a good amount of genetic variability and utilizing different molecular marker systems could better genotype several important traits (Sujatha *et al.,* 2008).

Next generation sequencing technologies, such as genotyping by sequencing (GBS) and multiplex sequencing, aid in the generation of massive genetic data for various applications (Raman *et al.,* 2011). Application of the current polymerase

chain reaction (PCR)-based marker technologies aiming at whole genome analysis for association studies, construction of genetic maps, assessment of the collected germplasm for large scale molecular evaluation and genome wide selection of the desirable alleles are not attainable due to consumable and labor costs (Raman *et al.,* 2011). The application of DNA hybridization-based technologies like some SNP technologies and Diversity Arrays Technology are more suitable for such purposes. Hassani *et al.* (2020) implemented DArTseq technology to assess genetic diversity in 89 safflower accessions originating from different countries of the world. They applied 1136 silicoDArT markers along with 2295 SNPs in their investigation.

Linkage analysis, also known as QTL mapping, helps in the identification of genomic regions controlling complex plant traits. QTL mapping is a time-consuming technique that needs mapping populations to be developed from bi-parents. QTL mapping captures less allelic variation utilizing bi-parental populations due to the very low rate of occurrence of recombination events and low mapping resolution (Flint-Garcia et al., 2005). Association mapping is a more efficient and faster technique, which provides higher resolution of complex plant traits in comparison to QTL mapping. Association mapping emerged as a promising technique to avoid limitations present in QTL mapping (Yu and Buckler, 2006; Abdurakhmonov and Abdukarimov, 2008). Relationships between plant traits and genetic polymorphisms observed in a heterogeneous assembly of distinct individuals, utilizing naturally occurring recombination events, aid in fine scale mapping of traits. Ebrahimi *et al.* (2017) and Ambreen *et al.* (2018) identified marker-trait associations in safflower, utilizing SSR and AFLP marker systems, respectively. We implemented a total of 12232 silicoDArT markers detected by a DArTseq approach of genotyping by sequencing in a safflower panel collected from 26 countries.

5.2. Aims and objectives

This study aimed on the establishment of the usefulness of silicoDArT markers to:

- Investigate genetic diversity and population structure of safflower accessions.
- Explore the pattern of safflower similarity centers.
- Identify marker-trait associations in international safflower panel.

5.3. Materials and methods

5.3.1. Plant materials and phenotypic evaluation

A total of 94 safflower accessions originating from 26 countries were used as plant materials in this study. Seeds of the evaluated germplasm were provided by the United States Department of Agriculture (USDA) (**Appendix I**). The experimental materials were sown at two diverse locations, i.e., Pakistan and Turkey. The First experiment was conducted at the National Agricultural Research Center (Pakistan), whereas the second experiment was conducted at the research and experimental area of Bolu Abant Izzet Baysal University (Turkey) during 2016-2017 and 2018, respectively. Field experiments were performed by implementing an augmented block design. Seeds of each safflower accession were planted in elementary plots with a row length, inter-row and intra-row spacing of 3m, 50cm, and 10cm respectively. A total of 10 plants for each accession were maintained for the phenotypic characterization. Thori-78 was included as check cultivar. Di-ammonium phosphate (DAP) and ammonium sulfate were applied as a source of fertilizer, while standard cultural practices were performed at both locations.

5.3.2. Genomic DNA isolation

To extract the genomic DNA from each accession, fresh, healthy and young leaves were harvested and kept frozen in the laboratory at -80 °C. DNA isolation of each safflower accession was performed utilizing the bulk of leaves from 10 individuals. The individuals used for the purpose of DNA isolation were from plants of the original seeds from the gene bank. DNA isolation was performed according to CTAB protocol (Doyle and Doyle, 1990) and a specific protocol suggested by Diversity Arrays Technology. DNA concentration was estimated with agarose gel (0.80%) and was then confirmed with NanoDrop (DeNovix DS-11 FX, USA). For the genotyping by sequencing (GBS) analysis, DNA was diluted and a 50 $ng.\mu l^{-1}$ DNA concentration was maintained. The prepared DNA samples were sent to Diversity Array Technology Pty, Ltd., Bruce, Australia, for DArTseq analyses of GBS (http://www.diversityarrays.com/).

5.3.3. DArTseq-generated silicoDArT marker analysis

DArTseq technology is a complexity reduction method and next generation sequencing platform (Elshire *et al.,* 2011). DArTseq facilitated the selection of the genome fractions containing active genes associated with agronomically important plant traits (Raman *et al.,* 2014). Digestion/ligation reactions were used for the processing of DNA samples following the method described by Kilian *et al.* (2012). Mixed fragments (PstI–MseI) were amplified by performing 30 rounds of PCR cycles. Details of silicoDArT markers analysis can be found in earlier studies (Kilian *et al.,* 2012; Li *et al.,* 2015).

5.3.4. Statistical analysis

5.3.4.1. Phenotypic data analysis

Online software developed by Rathore *et al.* (2004) for statistical inferences of augmented block design was used. Analysis of variance (ANOVA) for mean data of both locations was calculated through SAS 9.3 version. Data recorded on important morpho-agronomic traits of both field experiments was averaged and used to calculate parameters like minimum, maximum, mean and standard deviation utilizing statistical software XLSTAT (Addinsoft, 2018) (www.xlstat.com).

5.3.4.2. DArTseq markers analysis

All images were analyzed from the DArTseq platform using DArTsoft v.7.4.7 (DArT P/L, Canberra, Australia). SilicoDArT are dominant markers that were detected through DArTseq and scored using the binary fashion, as 1 for presence and 0 for absence, of the restriction fragment in the genomic representation of each sample (Cömertpay *et al.*, 2012; Baloch *et al.*, 2017). Screening of the markers was done with various parameters including call rate, polymorphism information content (PIC) and reproducibility being considered. Markers with PIC, reproducibility and call rate lower than 0.10, 100% and 0.80% were ignored during bioinformatics analyses to avoid false inferences.

5.3.4.3. Genetic diversity analyses

The proportion of shared alleles that were obtained from silicoDArT markers, were used to compute the genetic distances among the safflower accessions using

Jaccard's coefficients of genetic distance. Important diversity metrics; observed number of alleles (Na), effective number of alleles (Ne), Shannon's Information Index (I), expected heterozygosity (He), and unbiased expected heterozygosity (uHe) were estimated for the entire population following GenAlEx 6.5 (Peakall and Smouse, 2006). The kinship coefficients between safflower accessions were calculated with hierfstat R package to investigate the pairwise relationships of the 94 safflower accessions. Analysis of molecular variance was computed with GenALEx software considering total variation into two strata, i-e., among countries and within country group.

Population structure of the studied safflower accessions was evaluated with model-based Bayesian clustering algorithms, Neighbor Joining, and principal coordinate analysis (PCoA). STRUCTURE software (version 2.3.4; Pritchard *et al.*, 2000) was used for the implementation of Bayesian model-based clustering. The most suitable number of clusters (K subpopulations) ranging from 1 to 10 was determined applying STRUCTURE software following the protocol of Evanno *et al.* (2005). For each K value and for each run, ten independent runs were set. The initial burn-in period was set to 500 with 500,000 MCMC (Markov chain Monte Carlo) iterations with no prior information on the origin of individuals. The posterior probability of the data for a given K (Pritchard *et al.*, 2000) and the Evanno *et al.* (2005) method was used for the estimation of the true value of K. We plotted the number of clusters against logarithm probability relative to standard deviation (ΔK) to determine the suitable number of clusters (number of K; number of subpopulations) as explained by Evanno *et al.* (2005). The number of clusters (K value) was further confirmed with scree plot analysis. The PCoA was performed following the GenALEx analysis, while Neighbor Joining tree was constructed with hierfstat R package. The populations obtained from the Neighbor Joining and PCoA were named and colored with the same clusters pattern identified with model based Structure algorithm for the coherence purposes.

5.3.4.4. Genome-wide association mapping

As safflower genome is yet to be sequence, therefore DArTseq Pty Ltd did not provide us chromosomes number and chromosomal position of resulted markers. Therefore, during marker-trait analysis, we considered 12 chromosomes of safflower

as pseudo chromosome and supposedly distributed resulted markers from GBS analysis on these pseudo chromosome and performed GWAS analysis. A Mixed linear model (MLM, Q + K) approach was applied to inspect marker-trait associations (MTAs) via TASSEL 5.0.5 (Bradbury *et al.,* 2007). The population and family structure were corrected utilizing Q-metrics (Q) and kinship (K) during association analysis, as suggested by Nadeem *et al.* (2020). Scaled identity was utilized to detect kinship matrix by the descent method applied in TASSEL 5.0.5 (Bradbury *et al.,* 2007). In the results of association analysis, the *p* value signifies the relatedness of a marker with the associated trait, and R^2 reflects the proportion of phenotypic variation resulting from a significant marker (Jin *et al.,* 2011). SilicoDArT markers with Bonferroni and FDR thresholds $p = 0.01$ were taken as significantly associated with the 100-seed weight. A Pseudo-Manhattan plot was developed using the qq-man R Package in the R 4.0.0 statistical software (Turner, 2014).

5.4. Results

5.4.1. Phenotypic data evaluation

During this study, important safflower morpho-agronomic traits were recorded at its proper time and revealed a wide range of variation. Analysis of variance (ANOVA) for mean data across both locations revealed significant differences among the studied safflower accessions for most of the studied traits (Table 5.1). Minimum, maximum, mean and standard deviation values for morpho-agronomic traits also reflected sufficient phenotypic variation in the studied safflower panel (Table 5.2). This reflects the presence of genetic variability and suggests that the safflower accessions studied here are suitable for association analysis.

Table 5.1: Analysis of variance for different traits of 94 safflower accessions across two locations

Traits	Source of Variation	Mean Squares
Days to Flower Initiation	Accessions	18.9516***
	Location	198803.4141***
Days to 50% Flowering	Accessions	34.9301***
	Location	189596.6111***
Days to Flower Completion	Accessions	38.8753***
	Location	171896.7475***
Days to Maturity	Accessions	30.2526
	Location	156410.2273***
Leaf Length	Accessions	9.2772996
	Location	94.0884854***
Leaf Width	Accessions	0.90938296*
	Location	10.18640455***
Plant Height	Accessions	212.16869***
	Location	65837.64985***
Branches Per Plant	Accessions	9.3901519*
	Location	15.5232000
Capitula Per Plant	Accessions	238.09251
	Location	12625.16336***
Seeds Per Capitulum	Accessions	54.357623
	Location	576.682667***
Capitulum Diameter	Accessions	11.320729***
	Location	165.477879***
Seed Yield Per Plant	Accessions	180.18912*
	Location	9472.65167***
100-Seed Weight	Accessions	0.71088189***
	Location	1.07804091

*Statistically significant, * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$)

Table 5.2: Mean, minimum, maximum, and standard deviation (StD) of the 13 morpho-agronomic traits in 94 international safflower accessions panel

Traits	Minimum	Maximum	Mean	Std. deviation
Days to flower initiation	113.5	131.5	120.946	3.033
Days to 50% flowering	117.5	137.5	126.478	4.1006
Days to flower completion	121.5	143.5	133.098	4.3712
Days to maturity	139.5	157.5	148.498	3.8143
Leaf length	9.66	20.235	14.9549	2.0515
Leaf width	2.975	6.615	4.7399	0.6531
Plant height	60.08	121.476	92.6249	10.3238
Branches per plant	5.1	17.3	9.8569	2.0503
Capitula per plant	8.7	80.4	28.9419	10.7033
Seeds per capitulum	15	42.05	25.2935	5.1874
Capitulum diameter	17.301	28.302	23.4978	2.3556
Seed yield per plant	4.855	51.021	15.9477	9.3188
100-seed weight	2.165	5.3195	3.3287	0.5933

5.4.2. SilicoDArT profiling by GBS

DArTseq profiling of 94 safflower accessions resulted in a total of 29,048 silicoDArT markers. This dataset was filtered by accounting markers having less than 5% missing data, polymorphism information content (PIC) value of 0.10 to 0.50, call rate greater than 0.81, and 100% reproducibility, to retain 12,232 high quality markers for further analysis. Figure 5.1 shows the distribution of PIC values of the filtered silicoDArT marker dataset. The whole safflower panel revealed maximum and minimum PIC values of 0.50 and 0.10 respectively, with an average of 0.31. Highest and lowest call rate values of 1.00% and 0.81%, with an average of 0.93%, were obtained through the whole safflower panel.

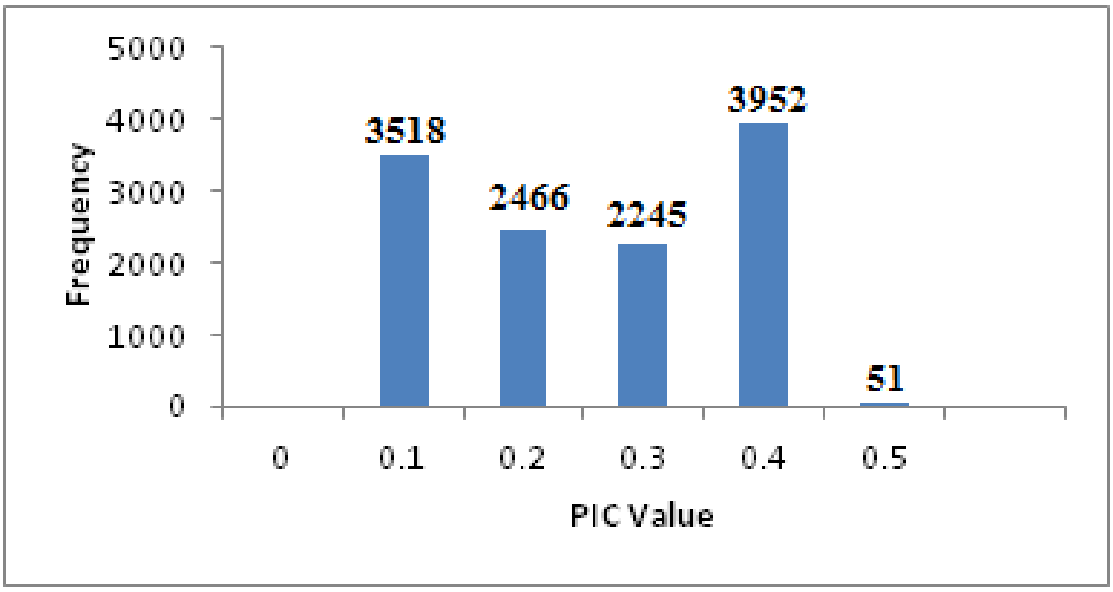


Figure 5.1: The frequency distribution of polymorphism information contents of 12232 silicoDArT markers

5.4.3. Genetic diversity and population structure analysis in safflower panel

Various diversity parameters such as observed number of alleles (1.99), effective number of alleles (1.54), Shannon's information index (0.48), expected heterozygosity (0.32), and unbiased expected heterozygosity (0.32) reflected a good level of genetic variation in the studied germplasm (Table 5.3). Maximum genetic distance (0.76) was found between Egypt-2 and India-2 accessions, while mean genetic distance for the entire safflower population was 0.50. Diversity indices were investigated on country basis, and Pakistan and Turkey revealed the existence of maximum percentage of polymorphic loci and high diversity parameters from rest of the countries (Table 5.3).

Table 5.3: Diversity indices calculated to investigate genetic diversity for whole safflower panel and accessions grouped according to country of origin with silicoDArT markers

Population/Country	Polymorphic Loci (%)	Na	Ne	I	He	uHe	Mean GD	GD Range
Overall population	-	1.99	1.54	0.48	0.32	0.32	0.5	0.14–0.76
Afghanistan	74.97	1.53	1.45	0.41	0.28	0.34	0.46	-
Austria	49.96	1.25	1.35	0.3	0.21	0.28	0.48	-
Bangladesh	87.37	1.74	1.57	0.48	0.33	0.37	0.44	-
China	98.44	1.98	1.66	0.56	0.38	0.41	0.46	-
Egypt	96.73	1.94	1.63	0.54	0.36	0.4	0.41	-
India	96.73	1.95	1.65	0.55	0.37	0.41	0.48	-
Iran	98.44	1.96	1.65	0.55	0.37	0.4	0.45	-
Iraq	49.9	1.24	1.35	0.3	0.21	0.28	0.42	-
Israel	87.37	1.73	1.57	0.48	0.33	0.38	0.44	-
Jordan	93.53	1.9	1.63	0.53	0.36	0.4	0.27	-
Morocco	49.9	1.23	1.34	0.3	0.2	0.26	0.42	-
Pakistan	99.81	1.98	1.69	0.58	0.39	0.42	0.44	-

Portugal	96.73	1.93	1.63	0.51	0.36	0.4	0.42	-
Spain	87.37	1.81	1.59	0.49	0.34	0.39	0.38	-
Syria	74.97	1.61	1.5	0.42	0.28	0.34	0.38	-
Turkey	99.82	1.99	1.68	0.58	0.39	0.42	0.53	-
Uzbekistan	74.97	1.62	1.52	0.43	0.29	0.35	0.48	-

Na: Observed number of alleles, Ne: Number of effective alleles, I: Shannon's information index, He: Expected heterozygosity, uHe: Unbiased expected heterozygosity, GD: Jaccard Genetic distance

Pairwise kinship coefficients ranged from -1.45 to 1.24 for the entire safflower panel. A total of 51.17% kinship values ranged from -0.40 to 0. 4.99% of the kinship coefficient values, which ranged from 0.60 to 1.00; however, 0.21% of the kinship coefficients ranged from 1.10 to 1.30, respectively (Figure 5.2). Analysis of molecular variance (AMOVA) revealed the division of the total variation into two stratum; i.e., among countries (9%) and within country group (91%) (Table 5.4). The ΔK peak at K = two in the structure analysis revealed that the genetic structure of the 94 safflower accessions is divided into two groups (Figure 5.3).

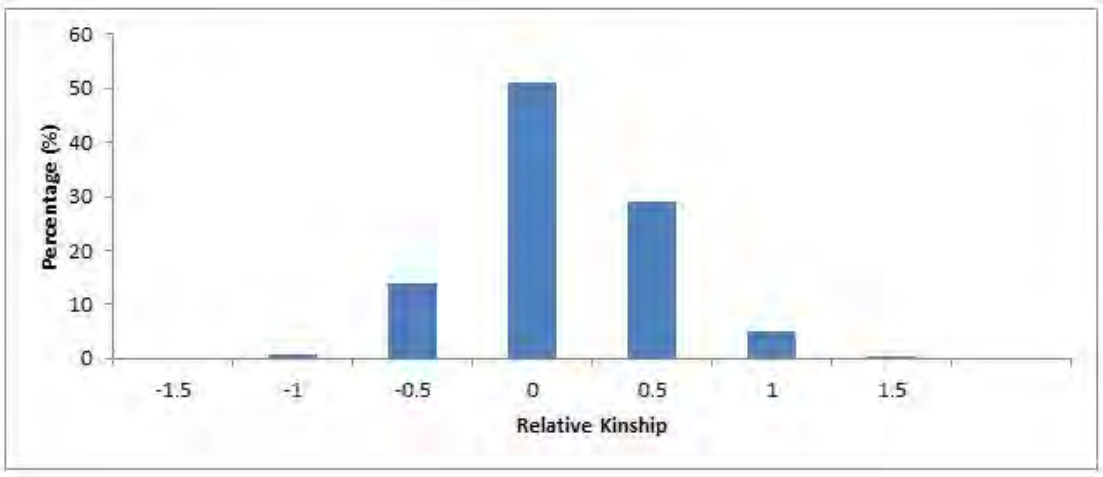


Figure 5.2: The proportion of pairwise kinship coefficients in international safflower panel

Table 5.4: Analysis of molecular variance among countries and within country groups of safflower germplasm

Source of Variation	Df	SS	MS	Est. Var.	% Variations
Among Countries	25	56719.33	3336.43	225.00	9
Within Country	68	165646.96	2179.56	2179.56	91
Total	93	222366.29	-	2404.57	100

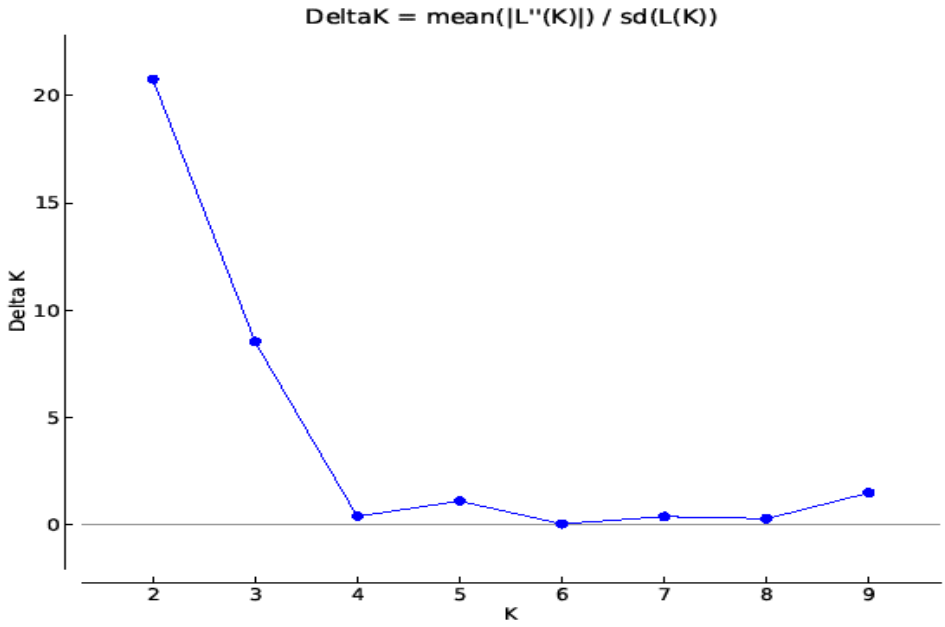


Figure 5.3: Delta K for the entire safflower population indicating the presence of two subpopulations at K = 2

The Bayesian clustering model grouped the international safflower panel into two populations implemented in STRUCTURE software on the basis of membership coefficient: 47 accessions (50% of the total accessions) in population A (blue) and 47 accessions (50% of the total accessions) in population B (orange) (Figure 5.4). Clustering of the safflower accessions within the same population revealed membership coefficients of either 50% or greater than 50% as proposed by Habyarimana (2016). The Neighbor Joining analysis divided the 94 safflower accessions into two populations (A and B), each containing 47 accessions (Figure 5.5). PCoA was also performed and results showed a clustering pattern comparable with Neighbor Joining analysis and model-based structure (Figure 5.6).

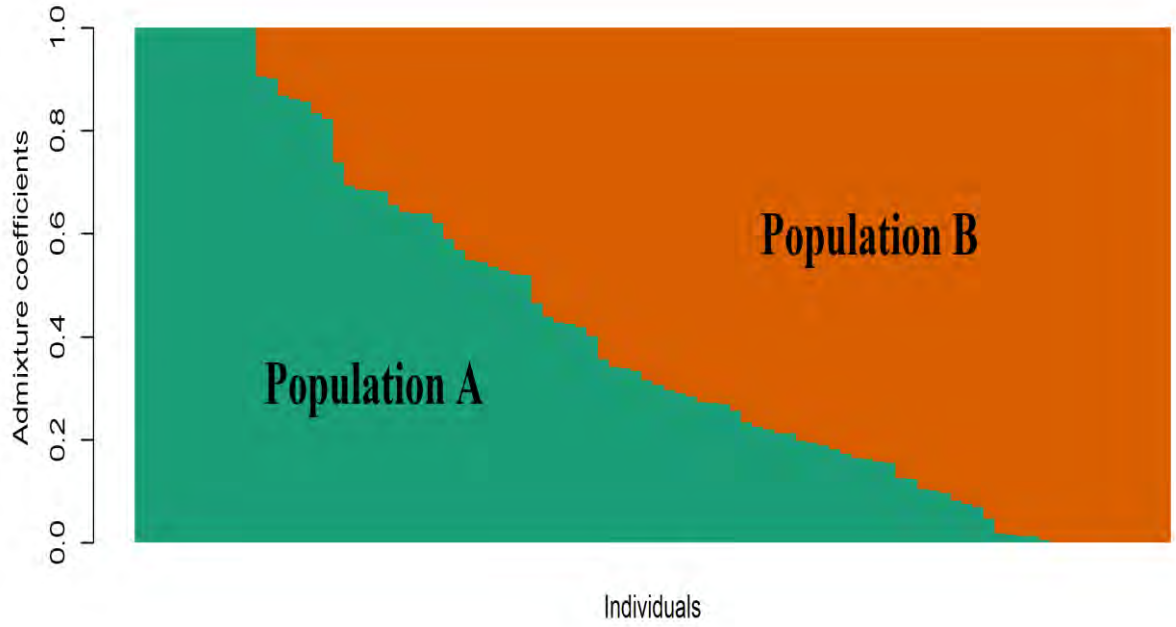


Figure 5.4: Structure-based clustering of the 94 safflower accessions using silicoDArT molecular markers

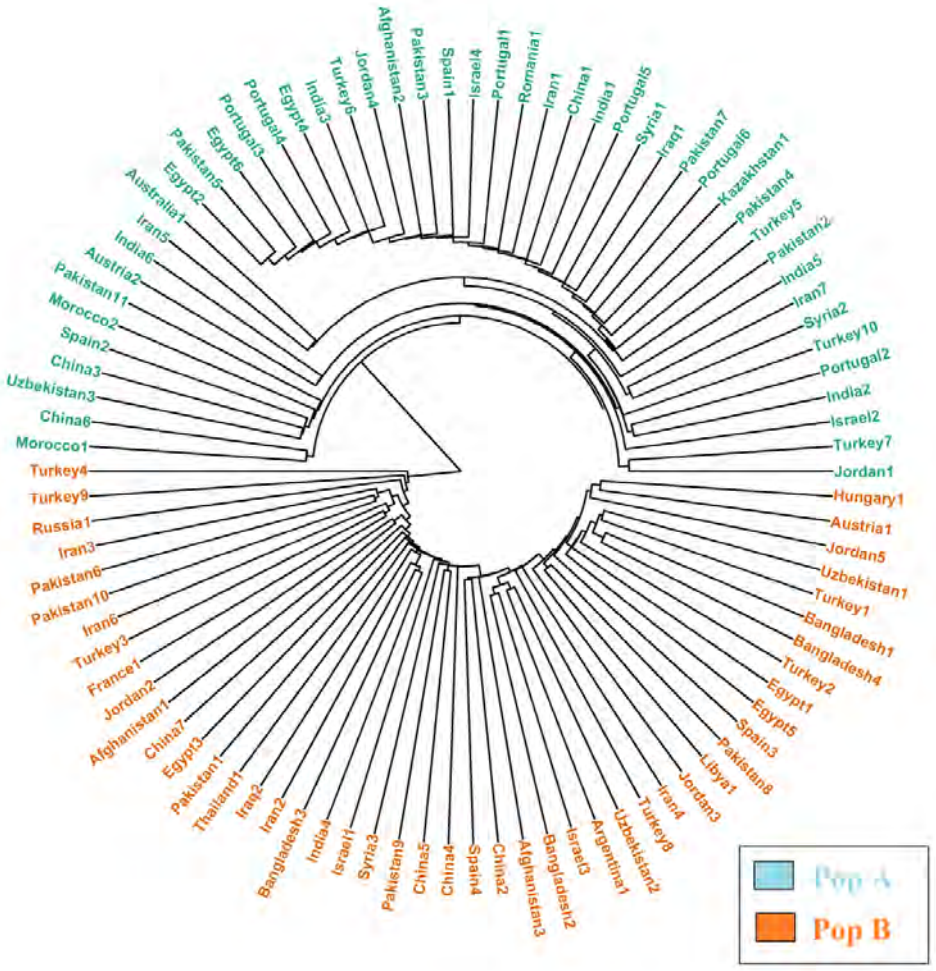


Figure 5.5: Neighbor joining-based clustering of the 94 safflower accessions using silicoDArT molecular markers

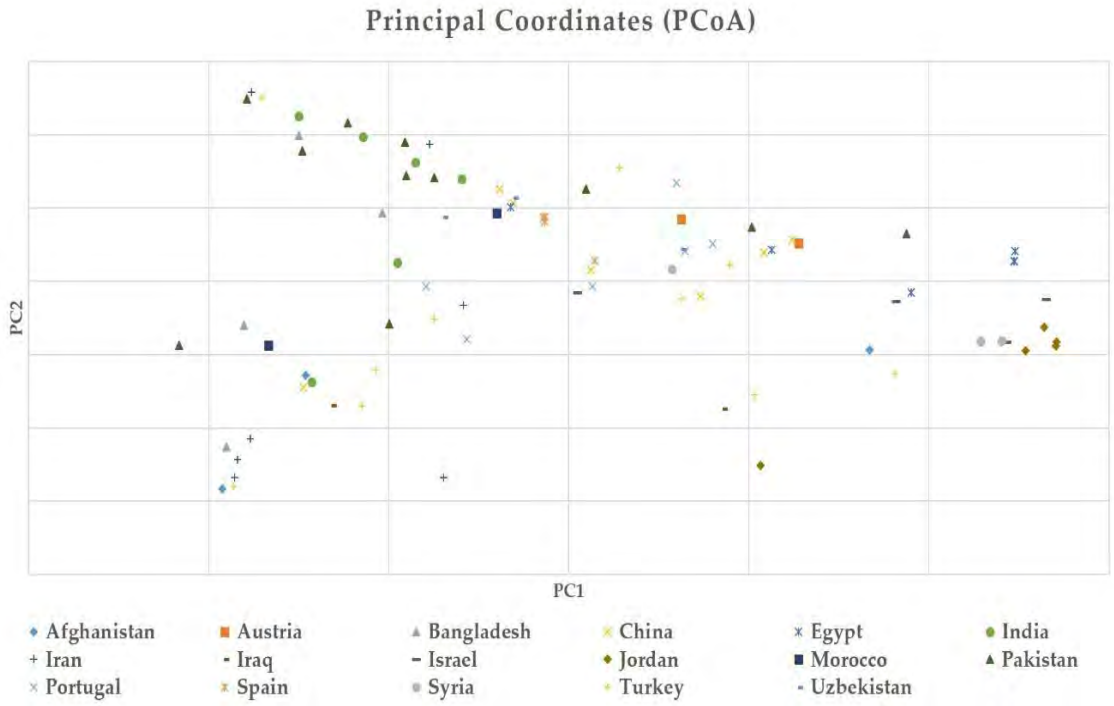


Figure 5.6: Principal coordinate analysis (PCoA) of the 94 safflower accessions using silicoDArT molecular markers

5.4.4. Marker-trait associations for important morpho-agronomic traits

The MLM (Q + K) model was performed to assess marker-trait associations for important morpho-agronomic traits in the international safflower panel. Out of the 13 morpho-agronomic traits of safflower, five traits exposed significant marker-trait associations (Table 5.5). Only one marker named DArT-38077549 made a significant association (p-value; 2.56E-04) for capitulum per plant (Figure 5.7). This marker explained 15.7% of the variation for capitulum per plant. DArT-38077549 marker was present on supposedly chromosome nine. 100-seed weight in safflower proposed two markers i-e: DArT-45483051 and DArT-15672391 revealing significant association (p-value; 1.17E-04 and 1.15E-04), respectively (Figure 5.8). The two identified markers exposed 17.4 and 18.6% variation for 100-seed weight. DArT-45483051 and DArT-15672391 markers were present on supposedly chromosome two and three, respectively. Plant height in safflower proposed two markers i-e: DArT-22763576 and DArT-22763253 revealing significant association (p-value;

1.94E-04 and 1.44E-04), respectively (Figure 5.9). The two identified markers exposed 18.5 and 17.5% variation for plant height. DArT-22763576 and DArT-22763253 markers were present on supposedly chromosome three and eight, respectively. Seeds per capitulum in safflower proposed two markers i-e: DArT-38079422 and DArT-100043360 revealing significant association (p-value; 2.00E-04 and 3.35E-04), respectively (Figure 5.10). The two identified markers exposed 18.1 and 15% variation for seeds per capitulum. DArT-38079422 and DArT-100043360 markers were present on supposedly chromosome 10 and 12, respectively. Seed yield per plant in safflower proposed five markers i-e: DArT-100004992, DArT-100004976, DArT-100004975, DArT-100039734 and DArT-100045083 revealing significant association (p-value; 3.99E-05, 4.99E-05, 9.21E-05, 1.07E-04, and 1.23E-04), respectively (Figure 5.11). The five identified markers exposed 20.5, 12.7, 18.3, 17.7, and 17.3% variation for seed yield per plant. DArT-100004992, DArT-100004976 markers were present on supposedly chromosome eight, while DArT-100039734 and DArT-100045083 markers were present on supposedly chromosome 10. Similarly, DArT-100004975 marker was present on chromosome nine.

Table 5.5: Marker-trait associations of the five morpho-agronomic traits with its associated markers in 94 international safflower accessions panel

Trait	Marker	***p*-value**	R^2
Capitula per plant	DArT-38077549	2.56E-04	15.70%
100-seed weight	DArT-45483051	1.17E-04	17.4
	DArT-15672391	1.15E-04	18.60%
Plant height	DArT-22763576	1.94E-04	18.5
	DArT-22763253	1.44E-04	17.5
Seeds per capitulum	DArT-38079422	2.00E-04	18.1
	DArT-100043360	3.35E-04	15%
Seed yield per plant	DArT-100004992	3.99E-05	20.50%
	DArT-100004976	4.99E-05	12.70%
	DArT-100004975	9.21E-05	18.30%
	DArT-100039734	1.07E-04	17.70%
	DArT-100045083	1.23E-04	17.30%

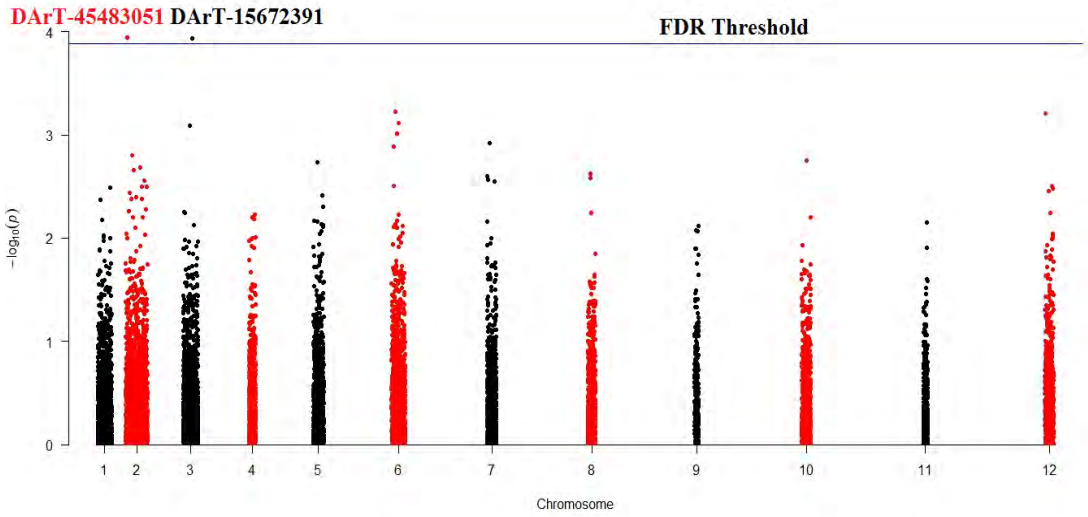


Figure 5.7: Pseudo manhattan plot for capitula per plant in world safflower panel. DArT-38077549 was considered statistically (FDR thresholds $p = 0.01$) associated with this trait

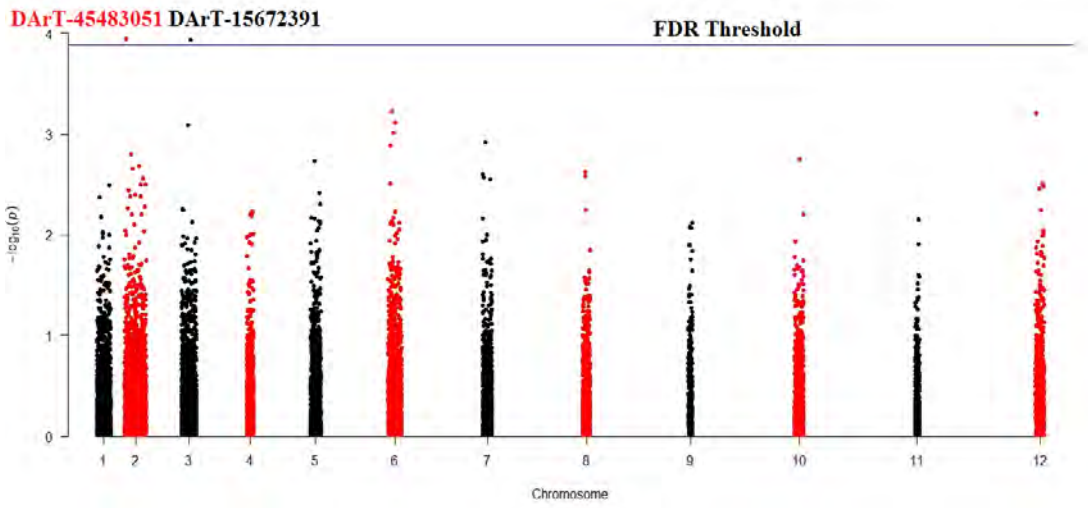


Figure 5.8: Pseudo manhattan plot for 100-seed weight in world safflower panel. DArT-45483051 and DArT-15672391 were considered statistically (FDR thresholds $p = 0.01$) associated with this trait

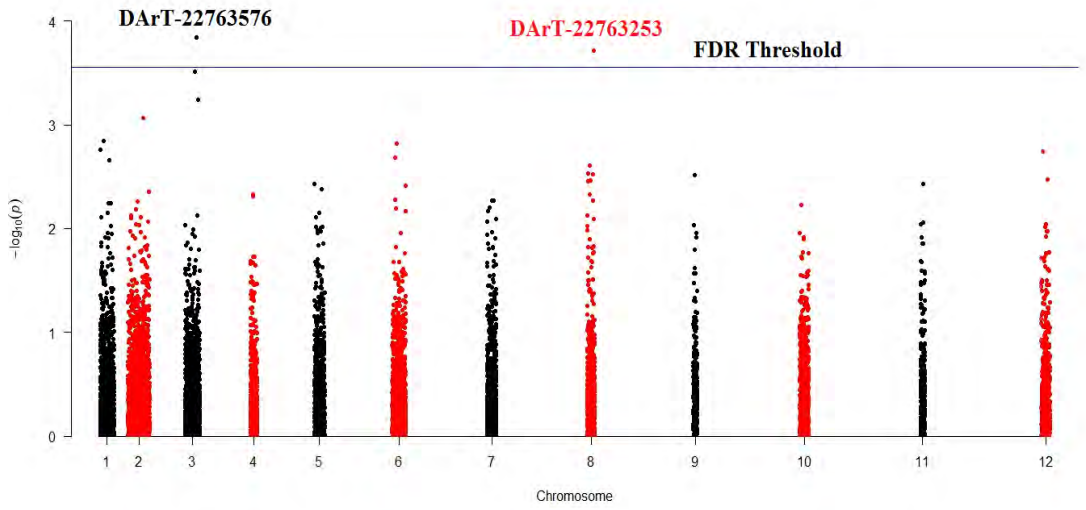


Figure 5.9: Pseudo manhattan plot for plant height in world safflower panel. DArT-22763576 and DArT-22763253 were considered statistically (FDR thresholds $p = 0.01$) associated with this trait

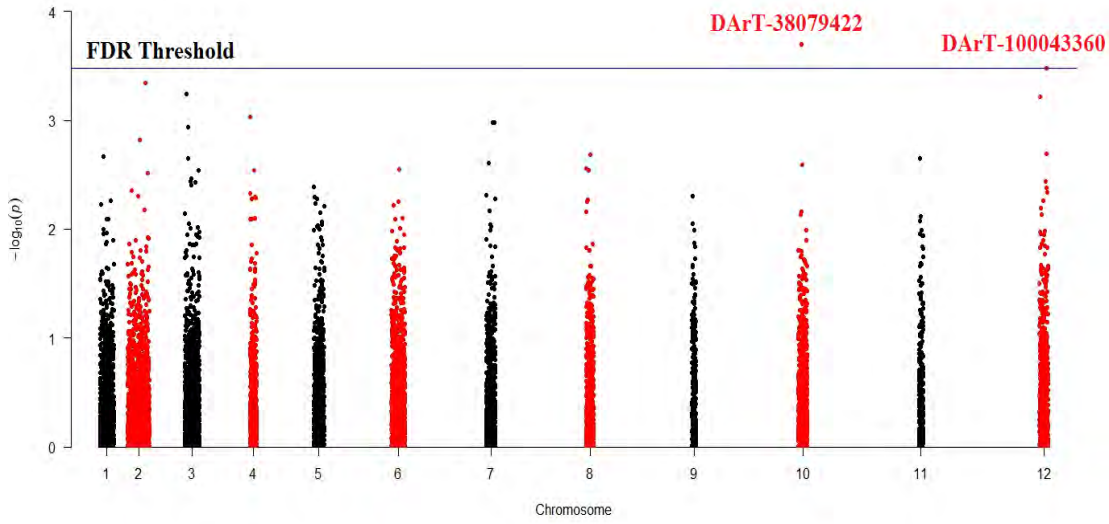


Figure 5.10: Pseudo manhattan plot for seeds per capitulum in world safflower panel. DArT-38079422 and DArT-100043360 were considered statistically (FDR thresholds $p = 0.01$) associated with this trait

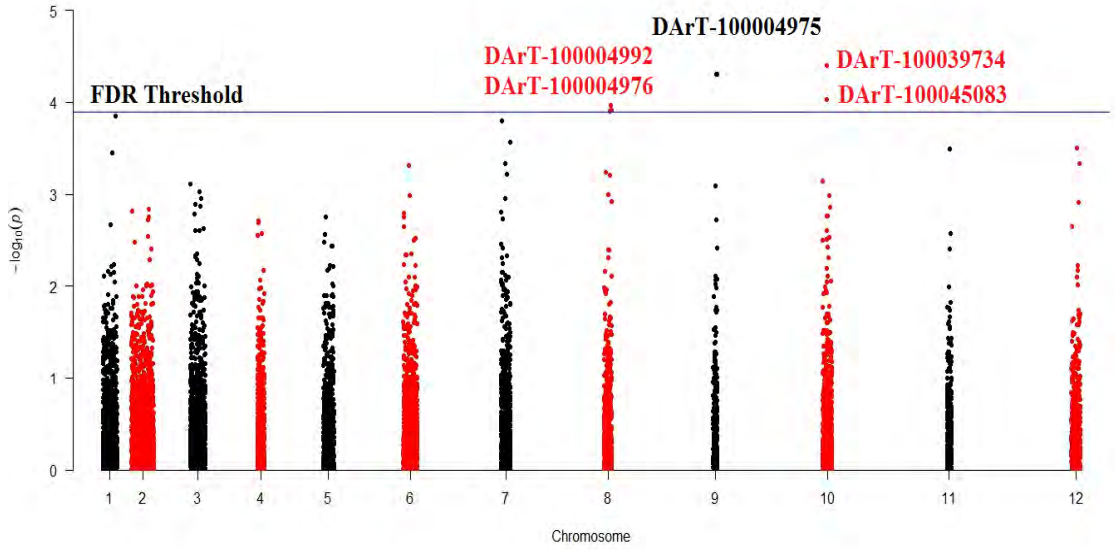


Figure 5.11: Pseudo manhattan plot for seed yield per plant in world safflower panel. DArT-100004992, DArT-100004976, DArT-100004975, DArT-100039734 and DArT-100045083 were considered statistically (FDR thresholds $p = 0.01$) associated with this trait

5.5. Discussion

Analysis of variance (ANOVA) was performed on 13 morpho-agronomic traits recorded across two different locations (Pakistan and Turkey) to understand the effect of accessions and locations (Table 5.1). The studied safflower panel revealed statistically significant differences for most of the traits. These results were found to be in line with El-Lattief (2012) as they also found statistically significant genotypic effects for various agronomic traits of safflower. It was observed that days to maturity, leaf length, capitula per plant and seeds per capitulum has no significant effect on the accession. The studied accessions reflected greater variations for various traits at both locations (Pakistan and Turkey), all traits reflected greater performance in Pakistan except leaf length, seeds per capitulum and 100-seed weight, which were more superior in the Turkey. Overall mean across two locations, maximum, minimum and standard deviation is presented in Table 5.2.

DArTseq technology gained the attention of scientists globally due to low cost and high throughput nature. DArTseq technology has been used to explore the genetic diversity and population structure of different crops with a large number of entries and complex genomes (Chen *et al.,* 2018; Nadeem *et al.,* 2018). Hassani *et al.* (2020) used DArTseq technology to explore genetic variations in a world panel of 89 safflower genotypes of diverse origin. The safflower panel utilized in their investigation is different from our panel except one accession, i.e., Afghanistan-1. During this study we also aimed to explore the genetic diversity, population structure and marker-trait association in an international safflower panel using silicoDArT markers. Hassani *et al.* (2020) used 1136 silicoDArT and 2295 SNP markers, while we used a higher number of markers (12232) for the molecular characterization. Moreover, Hassani *et al.* (2020) used germplasm from 12 countries, while we included germplasm from 26 countries to explore population structure more extensively.

Polymorphism information content (PIC) value is a measure of polymorphism which provides information regarding the genetic diversity or DNA segment in a studied population, and indicates the allele's evolutionary pressure and mutations that occurred at a locus over a time period. The range of the PIC value (0.10 to 0.50) obtained in this study suggests the existence of a high level of genetic variation that

might be derived utilizing a large number of good quality markers in a diversified safflower panel. An average PIC value of 0.31 across all the silicoDArT markers was obtained during this study. PIC values were distributed asymmetrically and were skewed towards the lower values. More than 50% of the implemented silicoDArT markers revealed a PIC value of more than 0.30, which indicates the informativeness and usefulness of these markers for genetic diversity, population structure, and marker-trait association in safflower (Figure 5.1).

Diversity parameters including observed number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I), expected heterozygosity (He), and unbiased expected heterozygosity (uHe) for the entire population of 94 safflower accessions, which were 1.99, 1.54, 0.48, 0.32, and 0.32, respectively. Previous use of different gel-based marker systems obtained lower diversity metrics values than our current results from the silicoDArT marker system (Johnson *et al.,* 2007; Sung *et al.,* 2010; Panahi and Neghab, 2013; Ali *et al.,* 2019b). The most prominent reason for getting good diversity results is likely due to higher capability of the silicoDArT marker system in comparison with other gel-based marker systems. Differences in the experimental materials might also be another reason of revealing higher polymorphism in this study. Furthermore, results of diversity indices on the basis of collection countries revealed the highest polymorphism and genetic diversity for safflower genotypes from Pakistan and Turkey, while the lowest polymorphism and genetic diversity was obtained for safflower accessions originating from Iraq and Morocco. In a similar way, the highest mean genetic distance was observed for accessions originating from Turkey, and followed by India, Austria and Uzbekistan. The lowest mean genetic distance was observed for accessions originating from Jordan, followed by Spain (Table 5.3).

The Jaccard coefficients of genetic distance resulted in a mean value of 0.50 for the entire population of 94 safflower accessions. A maximum genetic distance was proposed between safflower accessions Egypt-2 and India-2, followed by Egypt-5 and India-2 with genetic distance values of 0.76 and 0.76, respectively. The highest genetic similarity was recorded between safflower accessions Spain-1 and Spain-2, with a genetic distance value of 0.14. The presence of higher genetic similarity between safflower accessions is possibly because of their origin from common parents. The most diverse safflower accessions identified (Egypt-2, India-2, and

Egypt-5) during the current evaluation can be recommended as candidate parental lines for future safflower breeding activities. The inferences obtained from kinship coefficient estimations with silicoDArT markers are robust to population structure. Negative kinship coefficients were also observed, suggesting an unrelated relationship between the safflower accessions. The close relatives can be inferred fairly reliably based on the estimated kinship coefficients. Thus, it is suggested that most of the safflower accessions were less related, having kinship coefficients of either 0 or below 0 (Figure 5.2). Analysis of molecular variance (AMOVA) revealed the division of the total variation into two strata, i.e., among countries and within country. A total of 91% of the genetic diversity was present within country group (Table 5.4). This is supported by Hassani *et al.* (2020), where the majority of genetic variation among accessions within populations was obtained. The presence of a higher level of genetic variation within populations can be attributed to gene flow, which depends on the informal seed exchanges between farmers of different ecological zones (Hirano *et al.,* 2008).

5.5.1. Genetic structure and diversity in safflower panel

The three clustering algorithms important to genetic diversity and population structure analysis (model-based structure, Neighbor Joining, and PCoA) were implemented and revealed that the safflower accessions were successfully grouped by the silicoDArT markers based on geographical regions. Among the three clustering algorithms, more preference was given to the model-based structure algorithms. The reason for giving such a high preference to the structure is that this algorithm revealed more robustness in the previous works (Bouchet *et al.,* 2012; Newell *et al.,* 2013). Structure algorithm divided the whole germplasm panel into two genetic populations: population A and population B. These populations will aid in the selection of the parental accessions, which can used to design and conduct various crossing combinations for safflower genetic improvement (Figure 5.4).

Clustering of the safflower accessions was observed proposing various similarity centers based on model-based structure. Safflower accessions in population A mainly belong from the Middle East center: Iran, Afghanistan, Israel, Jordan, Iraq, Syria, Turkey; Europe center: Portugal, Romania, Spain, Austria, Morocco; India-Pakistan center: India, Pakistan and Egypt center: Egypt. Population B includes

safflower accessions from the Middle East center: Iran, Afghanistan, Israel, Jordan, Iraq, Syria, Turkey; India-Pakistan center: India, Pakistan, Bangladesh; Europe center: France, Spain, Austria, Hungary; Egypt center: Egypt, and Far East center: China, Thailand. Safflower accessions from the Mediterranean region clustered together in both populations (A and B) reveal their genetic similarity and share same parentage. Clustering of the safflower accessions from Mediterranean countries proposed this region as their center of domestication especially Syria (Marinova and Riehl, 2009). Clustering of the safflower accessions originating from Mediterranean region to other continents suggested the distribution of safflower accessions from the Mediterranean region to other geographies. Turkey signifies high level of biodiversity and differentiation center among the continents, thus reflected key role in the connection of different continents with each other (Arystanbekkyzy *et al.,* 2018). Some safflower accessions from different similarity centers were also clustered together and highlighted the lack of importance of similarity centers at molecular level which was previously reported in the scientific literature (Chapman and Burke, 2007). Ali *et al.* (2019, 2020) evaluated the same panel along with some other accessions for the identification of genetic diversity and similarity centers exploration with iPBS-retrotransposon and ISSR markers and consolidated the Knowles hypothesis of the presence of seven similarity centers among the previously suggested hypothesis about similarity centers for safflower.

Neighbor joining analysis divided the studied germplasm into two populations based on their geographical origin (Figure 5.5). Structure-based clustering of the 94 safflower accessions was also greatly supported by the principal coordinate analysis (PCoA) with silicoDArT markers information. PCoA resulted in clustering of safflower accessions on the basis of their geographical origins (Figure 5.6). The occurrence of slight discrepancies between model-based structure and PCoA can derive from differing clustering resolution, with model-based structure exhibiting more resolution.

5.5.2. Marker-trait associations for important morpho-agronomic traits

Identification of loci influencing important plant morpho-agronomic traits is a prerequisite to marker assisted breeding for enhancement of crop productivity. Very few studies have been conducted to identify markers/loci associated with morpho-

agronomic traits in safflower (Hamdan *et al.,* 2008, 2012; Mayerhofer *et al.,* 2010; García-Moreno *et al.,* 2011; Pearl *et al.,* 2014; Ebrahimi *et al.,* 2017, Ambreen et al., 2018). The current investigation involved association analysis that resulted in identification of silicoDArT markers associated with five morpho-agronomic traits including; capitula per plant, 100-seed weight, plant height, seeds per capitulum and seed yield per plant (Table 5.5). The identified linked traits were also previously reported to influence crop yield (Patil, 1998). Plant height determines plant architecture and also influences crop yield. It is quantitatively inherited trait and a large number of QTLs associated with plant height have been reported in different crop systems (Wu *et al.,* 2010; Morris *et al.,* 2013; Zanke *et al.,* 2014). Our current investigation involved the identification of two silicoDArT markers (DArT-22763576 and DArT-22763253) associated with plant height. Earlier studies reported different loci/markers linked with plant height. Ambreen *et al.* (2018) reported two loci (NGSaf_156 and NGSaf_296) associated with plant height utilizing SSR markers. Mirzahashemi *et al.* (2015) identified two markers (qPh6_1 and qPh6_2) associated with plant height. Safflower seed comprised 33-60% hull and 40-67% kernel. It was also reported that falling hull content in safflower significantly increase seed oil content (Dajue and Mündel, 1996). Our current investigation involved the identification of two silicoDArT markers (DArT-45483051 and DArT-15672391) associated with 100-seed weight. Earlier studies reported different loci/markers linked with 100-seed weight. Ambreen *et al.* (2018) reported two loci (NGSaf_306 and NGSaf_309) associated with 100-seed weight utilizing SSR markers. Mirzahashemi *et al.* (2015) identified one marker (qThsw5) associated with 100-seed weight. The so far reported loci/markers linked to 100-seed weight may play key role in translating the genetic relationship between hull thickness and oil content. Capitula per plant is known as one of the important yield related traits in safflower. Our current investigation involved the identification of one silicoDArT marker (DArT-38077549) associated with capitula per plant. Ambreen *et al.* (2018) reported one locus (NGSaf_279) associated with capitula per plant utilizing SSR markers. Mirzahashemi *et al.* (2015) identified one marker (qCpno2) associated with capitula per plant. Pearl *et al.* (2014) proposed one marker (H76) linked with capitula per plant using ESTs. Our current investigation involved the identification of two silicoDArT markers (DArT-38079422 and DArT-100043360) associated with seeds per capitulum. No molecular markers/loci were reported that linked with seeds per capitulum in

safflower. Association analysis for seed yield per plant exhibited five marker-trait associations in safflower. Five molecular markers associated with seed yield per plant includes; DArT-100004992, DArT-100004976, DArT-100004975, DArT-100039734, and DArT-100045083. Mirzahashemi *et al.* (2015) obtained two molecular markers (qSyp2 and qSyp9) associated with seed yield per plant in safflower.

The evaluated germplasm reflected a wide range of phenotypic variations for most of the studied traits. Moreover, various genetic diversity indices also confirmed the existence of higher polymorphism in the evaluated germplasm at molecular level. Characterization of germplasm provides us with an opportunity to unlock the novel genetic variations that can be utilized for breeding purposes (Nadeem *et al.,* 2020). This is a pioneer study concerning the investigation of marker-trait association for morpho-agronomic traits in safflower using GBS analysis. We believe that these identified markers can be helpful in safflower marker-assisted breeding in order to develop improved cultivars.

5.6. Conclusion

The current evaluation revealed a good level of genetic diversity in the studied safflower panel from the silicoDArT markers information. Analysis of variance (ANOVA) revealed significant genotypic effect for all the studied traits except days to maturity, leaf length, capitula per plant, and seeds per capitulum. Analysis of molecular variance (AMOVA) revealed the division of total variations into two stratum i.e., among countries and within country. A total of 91% of the genetic variation was present within country and low variation (9%) was observed among the countries. Findings of genetic distance calculated at countries basis confirmed that mostly variations resulted in this study are because of diverse individuals within countries. Safflower accessions Egypt-5, Egypt-2, and India-2 showed the highest genetic distance among the studied panel and hence might be recommended as candidate parental lines for safflower breeding programs. Model-based structure analysis, Neighbor joining analysis and Principal coordinate analysis (PCoA) clustered the safflower accessions on the basis of their geographical origin. Current results most probably supported the hypothesis of seven similarity centers for safflower throughout the world. This is a pioneer study uncovering the marker-trait association analysis for important morpho-agronomic traits in safflower. Our study identified five significant marker-trait associations for traits *viz*., capitula per plant, 100-seed weight, plant height, seeds per capitulum, and seed yield per plant. These markers can be used in marker-assisted breeding to develop safflower cultivars with improved yield.

Summary and Conclusion

Summary and Conclusion

Underutilized or neglected oilseed crop species play a wide range of roles to the improvement of oilseed production. These crop species are the part of a focused effort to help the poor for subsistence and income, reduce the risk of the over-reliance on the limited number of major oilseed crops, and promote sustainable agriculture. Safflower should be one of the options to be grown even in dry lands. Study of the genetic diversity through morpho-agronomic traits and utilizing different molecular markers contribute to the improvement of economically important plant traits. The availability of diverse germplasm is the first and most important step for running an efficient breeding program. An international safflower panel was explored during this study for its morpho-agronomic performance in field conditions at two diverse locations (Pakistan and Turkey). Genetic diversity and similarity centers of the safflower accessions were investigated with three marker systems (iPBS-retrotransposons, ISSR and silicoDArT). Marker-trait associations of the 13 morpho-agronomic traits were evaluated with silicoDArT markers. Safflower accessions provided by Plant Genetic Resources Institute, Pakistan (17 accessions) and Central Research Institute for Field Crop, Turkey (20 accessions) were also included along with international safflower panel (94 accessions) in iPBS-retrotransposon and ISSR studies.

Investigation of the morpho-agronomic performance showed genetic diversity for important yield and yield related traits including; capitulum diameter (17.30 to 28.30mm), branches per plant (5.10 to 17.30), capitula per plant (8.70 to 80.40), and seed yield per plant (4.86 to 51.02g). Important plant traits including; seed yield per plant, capitula per plant, branches per plant and capitulum diameter were utilized for the identification of best performing safflower accessions implementing the principal component analysis and correlation analysis. The constellation plot and multivariate analysis aligned with the Knowles hypothesis of seven similarity centers for safflower worldwide. It was also recommended to add the currently identified safflower traits (seed yield per plant, capitula per plant, branches per plant, and capitulum diameter) to the previously utilized standard traits for consolidation of the actual number of safflower similarity centers. Furthermore, safflower similarity centers were observed with iPBS-retrotransposon, ISSR and silicoDArT marker systems and supported the Knowles hypothesis of seven similarity centers among the previously proposed

hypotheses worldwide. The clustering algorithms important to genetic diversity and population structure analysis (model-based structure, Neighbor Joining, UPGMA, and PCoA) were implemented and revealed that the safflower accessions were successfully grouped showing full membership coefficients of either 50% or greater than 50%. We identified significant five marker-trait associations for important morpho-agronomic traits; capitula per plant, 100-seed weight, plant height, seeds per capitulum, and seed yield per plant. The identified marker-trait associations should be used in marker assisted breeding programs. Information provided herein comprehensively explored the presence of phenotypic and genotypic variation, supported the Knowles hypothesis of seven similarity centers and identified significant five marker-trait associations which would be helpful for the development of candidate varieties responding to breeders, farmers and consumer preferences.

Future Recommendations

- The newly devised selection criteria based on seed yield per plant, capitula per plant, branches per plant and capitulum diameter can be used for the identification and selection of elite safflower accessions in breeding programs.
- A total of 20 safflower accessions has been identified on the basis of their superior phenotypic performance in two different geographical locations (Pakistan and Turkey) and can be used as candidate parents for various breeding activities in safflower.
- The identified linked markers for important morpho-agronomic traits during current investigation can be utilized in safflower marker assisted breeding programs.
- The current genome-wide association study will attract the scientific community to think about safflower whole genome sequencing and identify markers along with its chromosome number and position for traits of interest like agronomic traits, biotic and a-biotic traits.

Literature Cited

Literature cited

Abdurakhmonov, I. Y. and A. Abdukarimov. 2008. Application of association mapping to understanding the genetic diversity of plant germplasm resources. International journal of plant genomics, 2008.

Abede, D. and A. Bjornstad. 1996. Genetic diversity of Ethiopian barleys in relation to geographical regions, altitude range and agro-ecological zones as an aid to germplasm collection and conservation strategy. Hereditas, 124: 17-29.

Ali, F., A. Yilmaz, H. J. Chaudhary, M. A. Nadeem, M. A. Rabbani, Y. Arslan, M. A. Nawaz, E. Habyarimana and F. S. Baloch. 2019a. Investigation of morpho-agronomic performance and selection indices in the international safflower panel for breeding perspectives. Turkish Journal of Agriculture and Forestry: 43. doi:10.3906/tar-1902-49.

Ali, F., A. Yılmaz, M. A. Nadeem, E. Habyarimana, I. Subaşı, M. A. Nawaz, H. J. Chaudhary, M. Q. Shahid, S. Ercişli and M. A. B. Zia. 2019b. Mobile genomic element diversity in world collection of safflower (*Carthamus tinctorius* L.) panel using ipbs-retrotransposon markers. PloS one, 14(2): e0211985.

Ali, F., M. A. Nadeem, E. Habyarimana, A. Yılmaz, M. A. Nawaz, I. H. Khalil, S. Ercişli, G. Chung, H. J. Chaudhary and F. S. Baloch. 2020. Molecular characterization of genetic diversity and similarity centers of safflower accessions with ISSR markers. Brazilian Journal of Botany, pp: 1-13. https://doi.org/10.1007/s40415-019-00574-7

Ambreen, H., S. Kumar, A. Kumar, M. Agarwal, A. Jagannath and S. Goel. 2018. Association mapping for important agronomic traits in safflower (*Carthamus tinctorius* L.) core collection using microsatellite markers. Frontiers in Plant Science, 9: 402.

Ambreen, H., S. Kumar, M. T. Variath, G. Joshi, S. Bali, M. Agarwal, A. Kumar, A. Jagannath and S. Goel. 2015. Development of genomic microsatellite markers in *Carthamus tinctorius* L. (safflower) using next generation sequencing and assessment of their cross-species transferability and utility for diversity analysis. PloS one, 10(8): e0135443.

Amini, F., G. Saeidi and A. Arzani. 2008. Study of genetic diversity in safflower genotypes using agro-morphological traits and RAPD markers. Euphytica, 163(1): 21-30.

Amiri, R., S. Azdi, M. Ghanadha and M. Abd. 2001. Detection of DNA polymorphism in landrace populations of safflower in Iran using RAPD-PCR technique. Iranian Journal of Agricultural Sciences, 32(4): 737-745.

Andeden, E. E., F. S. Baloch, M. Derya, B. Kilian and H. Özkan. 2013. Ipbs-retrotransposons-based genetic diversity and relationship among wild annual cicer species. Journal of Plant Biochemistry and Biotechnology, 22(4): 453-466.

Anderson, J. A., G. Churchill, J. Autrique, S. Tanksley and M. Sorrells. 1993. Optimizing parental selection for genetic linkage maps. Genome, 36(1): 181-186.

Anonymous, 2017a. [Available Online] http://www.fao.org/3/CA0239EN/ca0239en.pdf

Anonymous, 2017b. [Available Online] http://www.fao.org/docrep/i9166e/i9166e_Chapter4_Oilseeds.pdf

Anonymous. 1985. Safflower Improvement. Thirteenth Research Report, Nimbkar Agricultural Research Institute, Phaltan, District, Satara, Maharashtra, India. pp: 69.

Ansari, A., P. Sikarwar, S. Lade, H. Yadav and S. Ranade. 2016. Genetic diversity clusters in germplasm of cluster bean (*Cyamopsis tetragonoloba* L., Taub), an important food and an industrial legume crop. Journal of Agricultural Science and Technology, 18:1407-1418.

Arslan, B. 2007. The path analysis of yield and its components in safflower (*Carthamus tinctorius* L.). Journal of Biological Sciences, 7(4): 668-672.

Arystanbekkyzy, M., M. A. Nadeem, H. Aktas, M. Z. Yeken, N. Zencirci, M. A. Nawaz, F. Ali, M. S. Haider, K. Tunc and G. Chung. 2018. Phylogenetic and taxonomic relationship of turkish wild and cultivated emmer (*Triticum turgidum ssp. dicoccoides*) revealed by ipbsretrotransposons markers.

International Journal of Agriculture and Biology, 10. DOI: 10.17957/IJAB/15.0876.

Asare, P., I. Galyuon, J. Sarfo and J. Tetteh. 2011. Morphological and molecular based diversity studies of some cassava (*Manihot esculenta* crantz) germplasm in ghana. African Journal of Biotechnology, 10(63): 13900-13908. DOI: 10.5897/AJB11.929.

Ashri, A. 1975. Evaluation of the germ plasm collection of safflower, *Carthamus tinctorius* LV distribution and regional divergence for morphological characters. Euphytica, 24(3): 651-659.

Ashri, A. and P. Knowles. 1960. Cytogenetics of safflower (*Carthamus* L.) species and their hybrids. Agronomy Journal, 52: 11-17.

Aydin, M. F. and F. S. Baloch. 2019. Exploring the genetic diversity and population structure of turkish common bean germplasm by the ipbs-retrotransposons markers. Legume Research-An International Journal, 42(1): 18-24.

Bagheri, A., B. Yazdi-Samadi, M. Taeb and M. Ahmadi. 2001. Study of correlations and relations between plant yield and quantitative and qualitative other traits in safflower. Iranian Journal of Agricultural Sciences, 32(2): 295-307.

Bagmohammadi, H., M. Pahlevani, A. Ahmadikhah and S. E. Razavi. 2012. Genetic variation of safflower (*Carthamus tinctorius* L.) and related species revealed by issr analysis. Plant Breeding and Seed Science, 66(1): 139-150.

Baloch, F. S., A. Alsaleh, E. E. Andeden, R. Hatipoğlu, M. Nachit and H. Özkan. 2016. High levels of segregation distortion in the molecular linkage map of bread wheat representing the west asia and north africa region. Turkish Journal of Agriculture and Forestry, 40(3): 352-364.

Baloch, F. S., A. Alsaleh, L. E. S. de Miera, R. Hatipoğlu, V. Çiftçi, T. Karaköy, M. Yıldız and H. Özkan. 2015a. DNA based ipbs-retrotransposon markers for investigating the population structure of pea (*Pisum sativum*) germplasm from turkey. Biochemical Systematics and Ecology, 61: 244-252.

Baloch, F. S., A. Alsaleh, M. Q. Shahid, V. Çiftçi, L. E. S. de Miera, M. Aasim, M. A. Nadeem, H. Aktaş, H. Özkan and R. Hatipoğlu. 2017. A whole genome

dartseq and snp analysis for genetic diversity assessment in durum wheat from central fertile crescent. PloS one, 12(1): e0167821.

Baloch, F. S., M. Derya, E. E. Andeden, A. Alsaleh, G. Cömertpay, B. Kilian and H. Özkan. 2015b. Inter-primer binding site retrotransposon and inter-simple sequence repeat diversity among wild lens species. Biochemical Systematics and Ecology, 58: 162-168.

Baloch, F. S., T. Karaköy, A. Demirbaş, F. Toklu, H. Özkan and R. Hatipoğlu. 2014. Variation of some seed mineral contents in open pollinated faba bean (*Vicia faba* L.) landraces from turkey. Turkish Journal of Agriculture and Forestry, 38(5): 591-602.

Barati, M. and A. Arzani. 2012. Genetic diversity revealed by EST-SSR markers in cultivated and wild safflower. Biochemical Systematics and Ecology, 44: 117-123.

Barrett, B. and K. K. Kidwell. 1998. Aflp-based genetic diversity assessment among wheat cultivars from the pacific northwest. Crop Science, 38(5): 1261-1271.

Bernardo, R. 2008. Molecular markers and selection for complex traits in plants: Learning from the last 20 years. Crop Science, 48(5): 1649-1664.

Bhattacharjee, R., I. Khairwal, P. J. Bramel and K. Reddy. 2007. Establishment of a pearl millet [*Pennisetum glaucum* (L.) R. Br.] core collection based on geographical distribution and quantitative traits. Euphytica, 155(1-2): 35-45.

Bidgoli, A. M., G. A. Akbari, M. J. Mirhadi, E. Z and S. Soufizadeh. 2006. Path analysis of the relationships between seed yield and some morphological and phenological traits in safflower (*Carthamus tinctorius* L.). Euphytica, 148(3): 261-268.

Bouchet, S., D. Pot, M. Deu, J. F. Rami, C. Billot, X. Perrier, R. Rivallan, L. Gardes, L. Xia and P. Wenzl. 2012. Genetic structure, linkage disequilibrium and signature of selection in sorghum: Lessons from physically anchored DArT markers. PloS one, 7(3): e33470.

Bradbury, P. J., Z. Zhang, D. E. Kroon, T. M. Casstevens, Y. Ramdoss and E. S. Buckler. 2007. Tassel: Software for association mapping of complex traits in diverse samples. Bioinformatics, 23(19): 2633-2635.

Bradley, V. L., R. L. Guenthner, R. C. Johnson and R. M. Hannan. 1999. Evaluation of safflower germplasm for ornamental use. Perspectives on new crops and new uses. Alexandria: ASHS Press: 433-435.

Buckler, E. S., J. M. Thornsberry and S. Kresovich. 2001. Molecular diversity, structure and domestication of grasses. Genetics Research, 77(3): 213-218.

Çamaş, N. and E. Esendal. 2006. Estimates of broad-sense heritability for seed yield and yield components of safflower (*Carthamus tinctorius* L.). Hereditas, 143(2006): 55-57.

Casacuberta, J. M., S. Vernhettes, C. Audeon and M. A. Grandbastien. 1997. Quasispecies in retrotransposons: A role for sequence variability in tnt1 evolution. In: Evolution and impact of transposable elements. Springer: pp: 109-117.

Casasoli, M., C. Mattioni, M. Cherubini and F. Villani. 2001. A genetic linkage map of European chestnut (*Castanea sativa* Mill.) based on RAPD, ISSR and isozyme markers. Theoretical and Applied Genetics, 102(8): 1190-1199.

Cekic, C., N. Battey and M. Wilkinson. 2001. The potential of ISSR-PCR primer-pair combinations for genetic linkage analysis using the seasonal flowering locus in Fragaria as a model. Theoretical and Applied Genetics, 103(4): 540-546.

Cesur, C., T. Eryilmaz, T. Uskutoğlu, H. Doğan and B. C. Şenkal. 2018. Cocklebur (*Xanthium strumarium* L.) seed oil and its properties as an alternative biodiesel source. Turkish Journal of Agriculture and Forestry, 42(1): 29-37.

Chakravorty, A., P. Ghosh and P. Sahu. 2013. Multivariate analysis of phenotypic diversity of landraces of rice of west bengal. American Journal of Experimental Agriculture, 3(1): 110-123.

Chapman, M. A. and J. M. Burke. 2007. DNA sequence diversity and the origin of cultivated safflower (*Carthamus tinctorius* L.; Asteraceae). BMC Plant Biology, 7(1): 60.

Chapman, M. A., J. Hvala, J. Strever and J. M. Burke. 2010. Population genetic analysis of safflower (*Carthamus tinctorius*; Asteraceae) reveals a near eastern origin and five centers of diversity. American Journal of Botany, 97(5): 831-840.

Chaudhary, S. K. 1990. Path analysis for seed yield in safflower (*Carthamus tinctorius* L.) in acid soil under mid altitude conditions. International Journal of Tropical Agriculture, 8(2): 129-132.

Chaudhry, A. 1986. Evaluation and culture of sunflower and safflower in dobari lands of sind. 1st Annual Report, PL480 Program of USAID, Project No. PK-ARS-226, Grant No. FG. PA, 395: 25.

Chen, T., P. A. Tantasawat, W. Wang, X. Gao and L. Zhang. 2018. Population structure of chinese southwest wheat germplasms resistant to stripe rust and powdery mildew using the DArT-seq technique. Rural Science, 48(4). http://dx.doi.org/10.1590/0103-8478cr20160066.

Collard, B. C., M. Jahufer, J. Brouwer and E. Pang. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica, 142(1-2): 169-196.

Cömertpay, G., F. S. Baloch, B. Kilian, A. C. Ülger and H. Özkan. 2012. Diversity assessment of Turkish maize landraces based on fluorescent labelled SSR markers. Plant Molecular Biology Reporter, 30(2): 261-274.

Cordaux, R. and M. A. Batzer. 2009. The impact of retrotransposons on human genome evolution. Nature Reviews Genetics, 10(10): 691.

Corleto, A., E. Cazzato and P. Ventricelli. 1997. Performance of hybrid and open pollinated safflower in two different Mediterranean environments. In: Proceeding of the Fourth International Safflower Conference, Bari. pp: 276-278.

Dajue, L. and H. H. Mündel. 1996. Safflower. *Carthamus tinctorius* L. promoting the conservation and use of underutilized and neglected crops. 7th Edition, Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic, Rome.

Dempewolf, H., R. J. Eastwood, L. Guarino, C. K. Khoury, J. V. Müller and J. Toll. 2014. Adapting agriculture to climate change: A global initiative to collect, conserve, and use crop wild relatives. Agroecology and Sustainable Food Systems, 38(4): 369-377.

Derakhshan, E., M. Majidi, Y. Sharafi and A. Mirlohi. 2014. Discrimination and genetic diversity of cultivated and wild safflowers (*Carthamus* spp.) using EST-microsatellites markers. Biochemical Systematics and Ecology, 54: 130-136.

Díez, C. M., A. Imperato, L. Rallo, D. Barranco and I. Trujillo. 2012. Worldwide core collection of olive cultivars based on simple sequence repeat and morphological markers. Crop Science, 52(1): 211-221.

Dordas, C. A. and C. Sioulas. 2009. Dry matter and nitrogen accumulation, partitioning, and retranslocation in safflower (*Carthamus tinctorius* L.) as affected by nitrogen fertilization. Field Crops Research, 110(1): 35-43.

Doyle, J. J. and J. L. Doyle. 1990. Isolation ofplant DNA from fresh tissue. Focus, 12(13): 39-40.

Dwivedi, S. L., H. D. Upadhyaya and D. M. Hegde. 2005. Development of core collection using geographic information and morphological descriptors in safflower (*Carthamus tinctorius* L.) germplasm. Genetic Resources and Crop Evolution, 52:(7) 821-830.

Ebrahimi, F., M. M. Majidi, A. Arzani and G. Mohammadi-Nejad. 2017. Association analysis of molecular markers with traits under drought stress in safflower. Crop and Pasture Science, 68(2): 167-175.

El-Lattief, E. A. 2012. Evaluation of 25 safflower genotypes for seed and oil yields under arid environment in upper Egypt. Asian Journal of Crop Science, 4: 72-79..

Elshire, R. J., J. C. Glaubitz, Q. Sun, J. A. Poland, K. Kawamoto, E. S. Buckler and S. E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PloS one, 6(5): e19379.

Esendal, E. 1990. Samsun ekolojik şartlarında kışlık ve yazlık olarak ekilen aspir çeşitlerinin verim ve bazı özellikleri üzerine bir araştırma. Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi, 5(1-2): 49-66.

Evanno, G., S. Regnaut and J. Goudet. 2005. Detecting the number of clusters of individuals using the software structure: A simulation study. Molecular Ecology, 14(8): 2611-2620.

Fang, D. and M. Roose. 1997. Identification of closely related citrus cultivars with inter-simple sequence repeat markers. Theoretical and Applied Genetics, 95(3): 408-417.

FAOSTAT. 2012. [Available Online] http://www.fao.org/faostat/en/#data/QD

FAOSTAT. 2015. [Available Online] http://www.fao.org/faostat/en/#data/QD

FAOSTAT. 2017. [Available Online] http://www.fao.org/faostat/en/#data/QD

Federer, W. 1956. Augmented (or hoonuiaku) designs hawaiian planters record. Honolulu.

Finnegan, D. J. 1989. Eukaryotic transposable elements and genome evolution. Trends in Genetics, 5: 103-107.

Flint-Garcia, S. A., A. C. Thuillet, J. Yu, G. Pressoir, S. M. Romero, S. E. Mitchell, J. Doebley, S. Kresovich, M. M. Goodman and E. S. Buckler. 2005. Maize association population: A high-resolution platform for quantitative trait locus dissection. The Plant Journal, 44(6): 1054-1064.

Frankel, O. 1984. Genetic perspectives of germplasm conservation. Genetic manipulation: impact on man and society. Cambridge University Press, Cambridge, 61(3): 161-170.

Galiana-Balaguer, L., G. Ibáñez, J. Cebolla-Cornejo and S. Rosello. 2018. Evaluation of germplasm in solanum section lycopersicon for tomato taste improvement. Turkish Journal of Agriculture and Forestry, 42(5): 309-321.

García-Moreno, M. J., J. M. Fernández-Martínez, L. Velasco and B. Pérez-Vich. 2011. Molecular tagging and candidate gene analysis of the high gamma-tocopherol trait in safflower (*Carthamus tinctorius* l.). Molecular breeding, 28(3): 367-379.

García-Moreno, M. J., L. Velasco and B. Pérez-Vich. 2010. Transferability of non-genic microsatellite and gene-based sunflower markers to safflower. Euphytica, 175(2): 145-150.

Gautam, A., N. Gupta, R. Bhadkariya, N. Srivastava and S. Bhagyawant. 2016. Genetic diversity analysis in chickpea employing ISSR markers. Agrotechnology, 5(152): 2.

Godfray, H. C. J., J. R. Beddington, I. R. Crute, L. Haddad, D. Lawrence, J. F. Muir, J. Pretty, S. Robinson, S. M. Thomas and C. Toulmin. 2010. Food security: The challenge of feeding 9 billion people. Science, 327(5967): 812-818.

Golkar, P. 2011. Genetic analysis of earliness and its components in safflower (*Carthamus tinctorious* L.). African Journal of Agricultural Research, 6(14): 3264-3271.

Golkar, P., A. Arzani and A. Rezaei. 2010. Inheritance of flower colour and spinelessness in safflower (*Carthamus tinctorius* L.). Journal of Genetics, 89(2): 259-262.

Golkar, P., A. Arzani and A. Rezaei. 2012. Genetic analysis of agronomic traits in safflower (*Carthamus tinctorious* L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 40(1): 276-281.

Golkar, P., A. Arzani and A.M. Rezaei. 2011. Genetic variation in safflower (*Carthamus tinctorious* L.) for seed quality-related traits and inter-simple sequence repeat (issr) markers. International Journal of Molecular Sciences, 12(4): 2664-2677.

Goudet, J., M. Raymond, T. de Meeüs and F. Rousset. 1996. Testing differentiation in diploid populations. Genetics, 144(4): 1933-1940.

Grzebelus, D. 2006. Transposon insertion polymorphism as a new source of molecular markers. Journal of Fruit and Ornamental Plant Research, 14: 21.

Guo, D. L., M. X. Guo, X. G. Hou and G. H. Zhang. 2014. Molecular diversity analysis of grape varieties based on iPBS markers. Biochemical Systematics and Ecology, 52: 27-32.

Habyarimana, E. 2016. Genomic prediction for yield improvement and safeguarding of genetic diversity in CIMMYT spring wheat (*Triticum aestivum* L.). Australian Journal of Crop Science, 10(1): 127.

Hadian, J., S. Raeisi, A. Azizi, M. Pezhmanmehr and A. Sarkhosh. 2017. Genetic diversity of natural populations of medicinally valuable plant Satureja khuzistanica jamzad based on ISSR markers. Brazilian Journal of Botany, 40(3): 771-781.

Hamadi, B., I. Hamrouni and B. Marzouk. 2001. Comparison of yield components and oil content of selected safflower (*Carthamus tinctorius* L.) accessions in Tunisia. In: International Safflower Conference.

Hamdan, Y. A., M. J. García-Moreno, J. M. Fernández-Martínez, L. Velasco and B. Pérez-Vich. 2012. Mapping of major and modifying genes for high oleic acid content in safflower. Molecular breeding, 30(3): 1279-1293.

Hamdan, Y., B. Pérez-Vich, J. Fernández-Martínez and L. Velasco. 2008. Inheritance of very high linoleic acid content and its relationship with nuclear male sterility in safflower. Plant Breeding, 127(5): 507-509.

Hamdan, Y., M. J. García-Moreno, J. Redondo-Nevado, L. Velasco and B. Pérez-Vich. 2011. Development and characterization of genomic microsatellite markers in safflower (*Carthamus tinctorius* L.). Plant Breeding, 130(2): 237-241.

Hanelt, P. 1963. Monographische ubersicht der gattung *Carthamus* L. (Compositae). Feddes Repertorium, 67: 41-180.

Hassani, S. M. R., R. Talebi, S. S. Pourdad, A. M. Naji and F. Fayaz. 2020. In‐depth genome diversity, population structure and linkage disequilibrium analysis of worldwide diverse safflower (*Carthamus tinctorius* L.) accessions using NGS data generated by DArTseq technology. Molecular Biology Reports, 47: 2123-2135. doi:10.1007/s11033-020-05312-x

Hirano, R., A. Kikuchi, M. Kawase and K. N. Watanabe. 2008. Evaluation of genetic diversity of bread wheat landraces from pakistan by AFLP and implications for a future collection strategy. Genetic Resources and Crop Evolution, 55(7): 1007-1015.

Houmanat, K., J. Charafi, H. Mazouz, M. El Fechtali and A. Nabloussi. 2016. Genetic diversity analysis of safflower (*Carthamus tinctorius*) accessions from different geographic origins using ISSR markers. International Journal of Agriculture and Biology, 18(6): 881-887.

Hua, L., D. R. Wang, L. Tan, Y. Fu, F. Liu, L. Xiao, Z. Zhu, Q. Fu, X. Sun and P. Gu. 2015. Laba1, a domestication gene associated with long, barbed awns in wild rice. The Plant Cell, 27(7): 1875-1888.

Hussain, M. I., D. A. Lyra, M. Farooq, N. Nikoloudakis and N. Khalid. 2016. Salt and drought stresses in safflower: A review. Agronomy for sustainable development, 36(1): 4.

Iqbal, M., K. Hayat, R. S. A. Khan and A. Sadiq. 2006. Correlation and path coefficient analysis for earliness and yield traits in cotton (*G. hirsutum* L.). Asian Journal of Plant Sciences, 5: 341-344.

Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. Bulletin de la Societe Vaudoise de la science naturelle 44:223-276.

Jaccoud, D., K. Peng, D. Feinstein and A. Kilian. 2001. Diversity arrays: A solid state technology for sequence information independent genotyping. Nucleic Acids Research, 29(4): e25-e25.

Jaradat, A. and M. Shahid. 2006. Patterns of phenotypic variation in a germplasm collection of *Carthamus tinctorius* L. From the middle east. Genetic Resources and Crop Evolution, 53(2): 225-244.

Jin, F. X., S. D. Ji, X. B. Xie, J. W. Kang, H. G. Ju and S. N. Ahn. 2011. Detection of epistatic interaction of two qtls, gw8. 1 and gw9. 1, underlying grain weight using nearly isogenic lines in rice. Breeding science, 61(1): 69-75.

Jing, R., A. Vershinin, J. Grzebyta, P. Shaw, P. Smýkal, D. Marshall, M. J. Ambrose, T. N. Ellis and A. J. Flavell. 2010. The genetic diversity and evolution of field pea (*pisum*) studied by high throughput retrotransposon based insertion polymorphism (rbip) marker analysis. BMC Evolutionary Biology, 10(1): 44.

Jing-Yuan, X., Z. Yan, Y. Ze, W. Gang, X. Guo-Yong and Q. Min-Jian. 2018. Molecular diversity analysis of Tetradium ruticarpum (wuzhuyu) in china based on inter-primer binding site (iPBS) markers and inter-simple sequence repeat (ISSR) markers. Chinese Journal of Natural Medicines, 16(1): 1-9.

Johnson, R. C., T. Kisha and M. Evans. 2007. Characterizing safflower germplasm with AFLP molecular markers. Crop Science, 47(4): 1728-1736.

Johnson, R., V. Bradley and T. Kisha. 2008. Safflower germplasm. Past, present, and future. In: safflower: unexploited potential and world adaptability. 7th International Safflower Conference, Wagga Wagga, New South Wales, Australia, 3-6 November. Agri-MC Marketing and Communication. pp: 1-7.

Kalendar, R., A. Flavell, T. Ellis, T. Sjakste, C. Moisy and A. H. Schulman. 2011. Analysis of plant diversity with retrotransposon-based molecular markers. Heredity, 106(4): 520.

Kalendar, R., K. Antonius, P. Smýkal and A. H. Schulman. 2010. Ipbs: A universal method for DNA fingerprinting and retrotransposon isolation. Theoretical and Applied Genetics, 121(8): 1419-1430.

Karaköy, T., F. S. Baloch, F. Toklu and H. Özkan. 2014. Variation for selected morphological and quality-related traits among 178 faba bean landraces collected from Turkey. Plant Genetic Resources, 12(1): 5-13.

Kastner, T., M. J. I. Rivas, W. Koch and S. Nonhebel. 2012. Global changes in diets and the consequences for land requirements for food. Proceedings of the National Academy of Sciences, 109(18): 6868-6872.

Khalil, R. M. A. and M. A. S. El zayat. 2019. Molecular characterization of some Brassica species. Advances in Plants and Agriculture Research, 9:112-119.

Khan, M. A., S. von Witzke-Ehbrecht, B. L. Maass and H. C. Becker. 2009. Relationships among different geographical groups, agro-morphology, fatty acid composition and RAPD marker diversity in safflower (*Carthamus tinctorius*). Genetic Resources and Crop Evolution, 56(1): 19-30.

Khoury, C. K., A. D. Bjorkman, H. Dempewolf, J. Ramirez-Villegas, L. Guarino, A. Jarvis, L. H. Rieseberg and P. C. Struik. 2014. Increasing homogeneity in global food supplies and the implications for food security. Proceedings of the National Academy of Sciences, 111(11): 4001-4006.

Kilian, A., P. Wenzl, E. Huttner, J. Carling, L. Xia, H. Blois, V. Caig, K. Heller-Uszynska, D. Jaccoud and C. Hopper. 2012. Diversity arrays technology: A generic genome profiling technology on open platforms. In: Data production and analysis in population genomics. Springer: pp: 67-89.

Knowles, P. 1969. Centers of plant diversity and conservation of crop germplasm: Safflower. Economic Botany, 23(4): 324-329.

Knowles, P. and A. Ashri. 1995. Evolution of crop plants. Longman, Harlow, 31: 47-50.

Konishi, S., T. Izawa, S. Y. Lin, K. Ebana, Y. Fukuta, T. Sasaki and M. Yano. 2006. An SNP caused loss of seed shattering during rice domestication. Science, 312(5778): 1392-1396.

Kotecha, A. 1979. Inheritance and association of six traits in safflower. Crop Science, 19(4): 523-527.

Kumar, A. and J. L. Bennetzen. 1999. Plant retrotransposons. Annual Review of Genetics, 33(1): 479-532.

Kumar, S., H. Ambreen, M. T. Variath, A. R. Rao, M. Agarwal, A. Kumar, S. Goel and A. Jagannath. 2016. Utilization of molecular, phenotypic, and geographical diversity to develop compact composite core collection in the oilseed crop, safflower (*Carthamus tinctorius* L.) through maximization strategy. Frontiers in Plant Science, 7: 1554.

Kumar, S., H. Ambreen, T. Murali, S. Bali, M. Agarwal, A. Kumar, S. Goel and A. Jagannath. 2015. Assessment of genetic diversity and population structure in a global reference collection of 531 accessions of *Carthamus tinctorius* L. (safflower) using AFLP markers. Plant Molecular Biology Reporter, 33(5): 1299-1313.

Kunkel, G. 1984. Plants for human consumption. Koeltz Scientific Books.

Lee, G. A., J. S. Sung, S. Y. Lee, J. W. Chung, J. Y. Yi, Y. G. Kim and M. C. Lee. 2014. Genetic assessment of safflower (*Carthamus tinctorius* L.) collection with microsatellite markers acquired via pyrosequencing method. Molecular Ecology Resources, 14(1): 69-78.

Li, D., M. Zhou and V. Ramanatha Rao. 1993. Characterization and evaluation of safflower germplasm. Geological Publishers House.

Li, H., P. Vikram, R. P. Singh, A. Kilian, J. Carling, J. Song, J. A. Burgueno-Ferreira, S. Bhavani, J. Huerta-Espino and T. Payne. 2015. A high density GBS map of bread wheat and its application for dissecting complex disease resistance traits. BMC Genomics, 16(1): 216.

Liu, W., M. Q. Shahid, L. Bai, Z. Lu, Y. Chen, L. Jiang, M. Diao, X. Liu and Y. Lu. 2015. Evaluation of genetic diversity and development of a core collection of

wild rice (*Oryza rufipogon* Griff.) populations in China. PloS one, 10(12): e0145990.

Long, S. P., A. Marshall-Colon and X.G. Zhu. 2015. Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. Cell, 161(1): 56-66.

López, G. 1990. Acerca de la clasificación natural del género Carthamus L. sl Anales del Jardın Botánico de Madrid, 47: 11-34.

Lynch, M. and B. G. Milligan. 1994. Analysis of population genetic structure with RAPD markers. Molecular Ecology, 3(2): 91-99.

Mahalakshmi, V., Q. Ng, M. Lawson and R. Ortiz. 2007. Cowpea [*Vigna unguiculata* (L.) Walp.] core collection defined by geographical, agronomical and botanical descriptors. Plant Genetic Resources, 5(3): 113-119.

Mahasi, M., R. Pathak, F. Wachira, T. Riungu, M. Kinyua and J. Kamundia. 2006. Correlations and path coefficient analysis in exotic safflower (*Carthamus tinctorious* L.) genotypes tested in the arid and semi arid lands (Asals) of Kenya. Asian Journal of Plant Sciences, 5(6): 1035-1038.

Majidi, M. M. and S. Zadhoush. 2014. Molecular and morphological variation in a world-wide collection of safflower. Crop Science, 54(5): 2109-2119.

Marinova, E. and S. Riehl. 2009. Carthamus species in the ancient near east and south-eastern europe: Archaeobotanical evidence for their distribution and use as a source of oil. Vegetation History and Archaeobotany, 18(4): 341-349.

Mayerhofer, R., C. Archibald, V. Bowles and A. G. Good. 2010. Development of molecular markers and linkage maps for the *carthamus* species *c. Tinctorius* and *c. Oxyacanthus*. Genome, 53(4): 266-276.

Mayes, S., F. Massawe, P. Alderson, J. Roberts, S. A. Ali and M. Hermann. 2011. The potential for underutilized crops to improve security of food production. Journal of Experimental Botany, 63(3): 1075-1079.

McCouch, S., G. J. Baute, J. Bradeen, P. Bramel, P. K. Bretting, E. Buckler, J. M. Burke, D. Charest, S. Cloutier and G. Cole. 2013. Agriculture: Feeding the future. Nature, 499(7456): 23.

Meyer, R. S., A. E. DuVal and H. R. Jensen. 2012. Patterns and processes in crop domestication: An historical review and quantitative analysis of 203 global food crops. New Phytologist, 196(1): 29-48.

Miller, A. J. and B. L. Gross. 2011. From forest to field: Perennial fruit crop domestication. American journal of botany, 98(9): 1389-1414.

Mirzahashemi, M., G. Mohammadi-Nejad and P. Golkar. 2015. A qtl linkage map of safflower for yield under drought stress at reproductive stage. Iranian Journal of Genetics and Plant Breeding, 4(2): 20-27.

Mohammadi, S. and B. Prasanna. 2003. Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Science, 43(4): 1235-1248.

Mokhtari, N., B. Sayed-Tabatabaei, M. Bahar and H. Arabnezhad. 2018. Assessment of genetic diversity and population genetic structure of *Carthamus* species and Iranian cultivar collection using developed SSR markers. Journal of Genetics, 97(1): 67-78.

Morris, G. P., P. Ramu, S. P. Deshpande, C. T. Hash, T. Shah, H. D. Upadhyaya, O. Riera-Lizarazu, P. J. Brown, C. B. Acharya and S. E. Mitchell. 2013. Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proceedings of the National Academy of Sciences, 110(2): 453-458.

Mozaffari, K. and A.A. Asadi. 2006. Relationships among traits using correlation, principal components and path analysis in safflower mutants sown in irrigated and drought stress condition. Asian Journal of Plant Sciences, 5(6): 977-983.

Murphy, D.J. 1999. The future of new and genetically modified oil crops. Perspectives on new crops and new uses. ASHS Press, Alexandria, Virginia: 216-219.

Nadeem, M. A., E. Habyarimana, V. Çiftçi, M. A. Nawaz, T. Karaköy, G. Comertpay, M. Q. Shahid, R. Hatipoğlu, M. Z. Yeken and F. Ali. 2018a. Characterization of genetic diversity in turkish common bean gene pool using phenotypic and whole-genome DArTseq-generated silicoDArT marker information. PloS one, 13(10): e0205363.

Nadeem, M. A., M. A. Nawaz, M. Q. Shahid, Y. Doğan, G. Comertpay, M. Yıldız, R. Hatipoğlu, F. Ahmad, A. Alsaleh and N. Labhane. 2018b. DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. Biotechnology and Biotechnological Equipment, 32(2): 261-285.

Nadeem, M. A., M. Gündogdu, S. Ercisli, T. Karaköy, O. Saracoglu, E. Habyarimana, X. Lin, R. Hatipoglu, M. A. Nawaz, M. Sameeullah, F. Ahmad, B. Jung, G. Chung and F. S. Baloch. 2020. Uncovering phenotypic diversity and DArTseq marker loci associated with antioxidant activity in common bean. Genes, 11: 36. doi:10.3390/genes11010036.

Nagaoka, T. and Y. Ogihara. 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theoretical and Applied Genetics, 94(5): 597-602.

Newell, M. A., D. Cook, H. Hofmann and J. L. Jannink. 2013. An algorithm for deciding the number of clusters and validation using simulated data with application to exploring crop population structure. The Annals of Applied Statistics: 1898-1916.

Nimbkar, N. 2008. Issues in safflower production in india. In: Safflower: Unexploited potential and world adaptability. Proceedings of the Seventh International Safflower Conference, Wagga Wagga, New South Wales, Australia.

Noirot, M., S. Hamon and F. Anthony. 1996. The principal component scoring: A new method of constituting a core collection using quantitative data. Genetic Resources and Crop Evolution, 43(1): 1-6.

Omidi, T. A. H. 2000. Correlation between traits and path analysis for grain and oil yield in spring safflower. Sesame Safflower Newsletter, 15: 78-82.

Omidi, T. A. H. 2002. Correlation between traits and path analysis for grain and oil yield in spring safflower. Journal of Plant Seed, 18(2): 229-240.

Özdemir, İ. S., Ö. Karaoğlu, Ç. Dağ and S. Bekiroğlu. 2018. Assessment of sesame oil fatty acid and sterol composition with FT-NIR spectroscopy and chemometrics. Turkish Journal of Agriculture and Forestry, 42(6): 444-452.

Özer, S., T. Karaköy, F. Toklu, F.S. Baloch, B. Kilian and H. Özkan. 2010. Nutritional and physicochemical variation in Turkish kabuli chickpea (*Cicer arietinum* L.) landraces. Euphytica, 175(2): 237-249.

Padulosi, S. and I. Hoeschle-Zeledon. 2004. Underutilized plant species: What are they? LEISA-LEUSDEN-, 20: 5-6.

Padulosi, S., P. Eyzaquirre and T. Hodgkin. 1999. Challenges and strategies in promoting conservation and use of neglected and underutilized crop species. Perspectives on new crops and new uses: 140-145.

Panahi, B. and M. G. Neghab. 2013. Genetic characterization of iranian safflower (*Carthamus tinctorius*) using inter simple sequence repeats (ISSR) markers. Physiology and Molecular Biology of Plants, 19(2): 239-243.

Pascual-Villalobos, M. J. and N. Alburquerque. 1996. Genetic variation of a safflower germplasm collection grown as a winter crop in southern Spain. Euphytica, 92(3): 327-332.

Patil, H. 1998. Genetic variability, association and path analysis in safflower. Indian Journal of Agricultural Research, 32(1): 46-50.

Peakall, R. and P.E. Smouse. 2006. Genalex 6: Genetic analysis in excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6(1): 288-295.

Pearl, S. A. and J. M. Burke. 2014. Genetic diversity in *Carthamus tinctorius* (Asteraceae; safflower), an underutilized oilseed crop. American Journal of Botany, 101(10): 1640-1650.

Pearl, S. A., J. E. Bowers, S. Reyes-Chin-Wo, R. W. Michelmore and J. M. Burke. 2014. Genetic analysis of safflower domestication. BMC plant Biology, 14(1): 43.

Potter, D., F. Gao, G. Aiello, C. Leslie and G. McGranahan. 2002. Intersimple sequence repeat markers for fingerprinting and determining genetic relationships of walnut (*Juglans regia*) cultivars. Journal of the American Society for Horticultural Science, 127(1): 75-81.

Pritchard, J. K., M. Stephens and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics, 155(2): 945-959.

Raizada, P., A. Singh and A. Raghubanshi. 2009. Comparative response of seedlings of selected native dry tropical and alien invasive species to CO_2 enrichment. Journal of Plant Ecology, 2(2): 69-75.

Ramachandram, M. 1985. Genetic improvement of oil yield in safflower, problems and prospects. Journal of Oilseeds Research, 2: 1-9.

Raman, H., R. Raman, A. Kilian, F. Detering, J. Carling, N. Coombes, S. Diffey, G. Kadkol, D. Edwards and M. McCully. 2014. Genome-wide delineation of natural variation for pod shatter resistance in Brassica napus. PloS one, 9(7): e101673.

Raman, H., R. Raman, M. N. Nelson, M. Aslam, R. Rajasekaran, N. Wratten, W. A. Cowling, A. Kilian, A. G. Sharpe and J. Schondelmaier. 2011. Diversity array technology markers: Genetic diversity analyses and linkage map construction in rapeseed (*Brassica napus* L.). DNA research, 19(1): 51-65.

Rao, V. and M. Ramachandram. 1997. An analysis of association of yield and oil in safflower. In: Fourth International Safflower Conference. Italy, Bari. pp: 2-7.

Rathore, A., R. Parsad and V. Gupta. 2004. Computer aided construction and analysis of augmented designs.

Rawat, S., A. K. Jugran, I. D. Bhatt, R. S. Rawal and S. K. Nandi. 2016. Genetic diversity analysis in natural populations of Roscoea procera Wall. From West Himalaya, India. Brazilian Journal of Botany, 39(2): 621-630.

Rudra Naik, V., G. Gulganji, C. Mallapur and S. Raju. 2001. Association analysis in safflower under rainfed conditions. In: 5th international safflower conference, Montana, Usa. July. pp: 23-27.

Sabzalian, M. R., A. Mirlohi, G. Saeidi and M. T. Rabbani. 2009. Genetic variation among populations of wild safflower, *Carthamus oxyacanthus* analyzed by agro-morphological traits and ISSR markers. Genetic Resources and Crop Evolution, 56(8): 1057-1064.

SanMiguel, P., A. Tikhonov, Y. K. Jin, N. Motchoulskaia, D. Zakharov, A. Melake-Berhan, P. S. Springer, K. J. Edwards, M. Lee and Z. Avramova. 1996. Nested retrotransposons in the intergenic regions of the maize genome. Science, 274(5288): 765-768.

Saxena, M., J. Singh, S. Deshpande and R. Choudhari. 2008. Two decades of safflower in madhya pradesh from 1984-2004. In: Safflower: unexplored potential and world adaptability. Proceedings of the 7th International Safflower Conference, New South Wales, Australia, Wagga Wagga.

Sehgal, D., V. R. Rajpal, S. N. Raina, T. Sasanuma and T. Sasakuma. 2009. Assaying polymorphism at DNA level for genetic diversity diagnostics of the safflower (*Carthamus tinctorius* L.) world germplasm resources. Genetica, 135(3): 457-470.

Serce, S., S. Ercisli, M. Sengul, K. Gunduz and E. Orhan. 2010. Antioxidant activities and fatty acid composition of wild grown myrtle (*Myrtus communis* L.) fruits. Pharmacognosy magazine, 6(21): 9.

Sharaan, A. and K. Ghallab. 1997. Character association at different location in sesame. Sesame and Safflower Newsletter, 12: 66-75.

Shinwari, Z. K., H. Rehman and M. A. Rabbani. 2014. Morphological traits based genetic diversity in safflower (*Carthamus tinctorius* L.). Pakistan Journal of Botany, 46(4): 1389-1395.

Shivani, D., C. Sreelakshmi and C. S. Kumar. 2010. Genetic divergence studies in safflower, *Carthamus tinctorius* L. Electronic Journal of Plant Breeding, 1(5): 1354-1357.

Suddihiyam, P., B. T. Steer and D. W. Turner. 1992. The flowering of sesame (*Sesamum indicum* L.) in response to temperature and photoperiod. Australian Journal of Agricultural Research, 43(5): 1101-1116.

Sujatha, M., A. Geetha, P. Sivakumar and N. Palanisamy. 2008. Biotechnological interventions for genetic improvement of safflower. In: Proceedings of the 7th international safflower conference. pp: 3-6.

Sung, J. S., G. T. Cho, G. A. Lee, H. J. Baek and M. K. Huh. 2010. Phylogenetic relationships and genetic diversity in collected resources of *Carthamus tinctorius* by random amplified polymorphic DNA markers. Journal of Life Science, 20(12): 1764-1771.

Talebi, R. and S. A. Abhari. 2016. Evaluation of genetic diversity in safflower (*Carthamus tinctorius* L.) using agro-morphological, fatty acid composition and ISSR molecular markers. Research Journal of Biotechnology Vol, 11: 7.

Talebi, R., F. Fayaz and E. Karami. 2012. Morphometric and amplified fragment length polymorphism marker analysis in some landrace wheat (*Triticum aestivum*) genotypes collected from north-west Iran. Environmental and Experimental Biology, 10: 49-56.

Tanksley, S. D. and S. R. McCouch. 1997. Seed banks and molecular maps: Unlocking genetic potential from the wild. Science, 277(5329): 1063-1066.

Tanyolac, B. 2003. Inter-simple sequence repeat (ISSR) and RAPD variation among wild barley (*Hordeum. vulgare* subsp. spontaneum) populations from west Turkey. Genetic Resources and Crop Evolution, 50(6): 611-614.

Team, R.C. 2013. R: A language and environment for statistical computing.

Tester, M. and P. Langridge. 2010. Breeding technologies to increase crop production in a changing world. Science, 327(5967): 818-822.

Thies, E. 2000. Promising and underutilized species, crops and breeds. GTZ.

Thompson, J. A., R. L. Nelson and L. O. Vodkin. 1998. Identification of diverse soybean germplasm using rapd markers. Crop Science, 38(5): 1348-1355.

Tsumura, Y., K. Ohba and S. Strauss. 1996. Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas-fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). Theoretical and Applied Genetics, 92(1): 40-45.

Tuncturk, M. and V. Ciftci. 2004. Relationships among traits using correlation and path coefficient analysis in safflower (*Carthamus tinctorius* L.) sown different fertilization levels and row spacing. Asian Journal of Plant Sciences, 3(6): 683-686.

Turner, S. D. 2014. qqman: An R package for visualizing GWAS results using QQ and Manhattan plots. Biorxiv, 005165, 1-2.

ur Rehman, A., I. Habib, N. Ahmad, M. Hussain, M. A. Khan, J. Farooq and M. A. Ali. 2009. Screening wheat germplasm for heat tolerance at terminal growth stage. Plant Omics, 2(1): 9.

van Hintum, T. J., A. H. D. Brown and C. Spillane. 2000. Core collections of plant genetic resources. Bioversity International.

Van Zeist, W. and W. Waterbolk-Van Rooijen. 1992. Two interesting floral finds from third millennium BC Tell Hammam et-Turkman, northern Syria. Vegetation History and Archaeobotany, 1(3): 157-161.

Varshney, R. K., T. Mahendar, R. K. Aggarwal and A. Börner. 2007. Genic molecular markers in plants: Development and applications. In: Genomics-assisted crop improvement. Springer: pp: 13-29.

Vilatersana, R., T. Garnatje, A. Susanna and N. Garcia-Jacas. 2005. Taxonomic problems in *Carthamus* (Asteraceae): RAPD markers and sectional classification. Botanical Journal of the Linnean Society, 147(3): 375-383.

Vollmann, J., H. Grausgruber, G. Stift, V. Dryzhyruk and T. Lelley. 2005. Genetic diversity in camelina germplasm as revealed by seed quality characteristics and RAPD polymorphism. Plant Breeding, 124(5): 446-453.

Vom Brocke, K., A. Christinck, R. Weltzien, T. Presterl and H. H. Geiger. 2003. Farmers' seed systems and management practices determine pearl millet genetic diversity patterns in semiarid regions of India. Crop Science, 43(5): 1680-1689.

Vrijendra, S., M. B. Deshpande, S. V. Choudhari and N. Nimbkar. 2004. Correlation and path coefficient analysis in safflower (*Carthamus tinctorius* L.). Sesame and Safflower Newsletter, (19).

Wang, G. and Y. Li, 1985. Clinical application of safflower (*Carthamus tinctorius*). Zhejiang Journal of Traditional Chinese Medicine, 20: 42-43.

Weiss, E. 2000. Oil seed crops blackwell Sci Led. Plant Path, 15(14): 42-47.

Wenzl, P., J. Carling, D. Kudrna, D. Jaccoud, E. Huttner, A. Kleinhofs and A. Kilian. 2004. Diversity arrays technology (DArT) for whole-genome profiling of barley. Proceedings of the National Academy of Sciences, 101(26): 9915-9920.

Williams, J. and N. Haq. 2002. Global research on underutilised crops; an assessment of current activities and proposals for enhanced cooperation. Southampton, UK: ICUC.

Wodajo, B., F. B. Mustefa and K. Tesfaye. 2015. Clustering analysis of Ethiopian safflower (*Carthamus tinctorius*) using ISSR markers. International Journal of Scientific and Research Publications, 5.

Wu, X., Z. Wang, X. Chang and R. Jing. 2010. Genetic dissection of the developmental behaviours of plant height in wheat under diverse water regimes. Journal of experimental botany, 61(11): 2923-2937.

Yaldiz, G., M. Camlica, M. A. Nadeem, M. A. Nawaz and F.S. Baloch. 2018. Genetic diversity assessment in Nicotiana tabacum L. With iPBS-retrotransposons. Turkish Journal of Agriculture and Forestry, 42(3): 154-164.

Yamasaki, M., M. I. Tenaillon, I. V. Bi, S. G. Schroeder, H. Sanchez-Villeda, J. F. Doebley, B. S. Gaut and M. D. McMullen. 2005. A large-scale screen for artificial selection in maize identifies candidate agronomic loci for domestication and crop improvement. The Plant Cell, 17(11): 2859-2872.

Yang, R. C. 1998. Estimating hierarchical f-statistics. Evolution, 52(4): 950-956.

Yang, Y. X., W. Wu, Y. L. Zheng, L. Chen, R. J. Liu and C. Y. Huang. 2007. Genetic diversity and relationships among safflower (*Carthamus tinctorius* L.) analyzed by inter-simple sequence repeats (ISSRs). Genetic Resources and Crop Evolution, 54(5): 1043-1051.

Yeh, F., R. Yang, T. Boyle, Z. Ye and J. Xiyan. 2000. Popgene32, microsoft windows-based freeware for population genetic analysis, version 1.32. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada.

Yıldız, M., M. Koçak and F. Baloch. 2015. Genetic bottlenecks in turkish okra germplasm and utility of iPBS retrotransposon markers for genetic diversity assessment. Genetics and Molecular Research, 14(3): 10588-10602.

Yu, J. and E. S. Buckler. 2006. Genetic association mapping and genome organization of maize. Current opinion in biotechnology, 17(2): 155-160.

Zanke, C. D., J. Ling, J. Plieske, S. Kollers, E. Ebmeyer, V. Korzun, O. Argillier, G. Stiewe, M. Hinze and K. Neumann. 2014. Whole genome association mapping of plant height in winter wheat (*Triticum aestivum* l.). PloS one, 9(11): e113287.

Zhang, C., X. Chen, Y. Zhang, Z. Yuan, Z. Liu, Y. Wang and Q. Lin. 2009. A method for constructing core collection of Malus sieversii using molecular markers. Scientia Agricultura Sinica, 42(2): 597-604.

Zhang, Z. 2001. Genetic diversity and classification of safflower (*Carthamus tinctorius* L.) germplasm by isozyme techniques. In: Proceedings of the 5th International Safflower Conference, Williston, North Dakota and Sidney, Montana, USA, 23-27 July. Safflower: a multipurpose species with unexploited potential and world adaptability. Department of Plant Pathology, North Dakota State University: pp: 157-162.

Zheng, N., C. Futang, S. Xinchun and W. Yancai. 1993. Path analysis of correlated characters on flower yield of safflower individuals. In: Third International Safflower Conference, Beijing, China. pp: 582-588.

Zietkiewicz, E., A. Rafalski and D. Labuda. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20(2): 176-183.

Appendices

Appendix I: List of 94 international safflower accessions panel evaluated using 13 morpho-agronomic traits across two locations (Pakistan and Turkey) and DArT molecular markers

S.No	Accession Name	Accession No	Donor Organization	Country Origin	Plant ID	Continent
1	Afghanistan-1	30614	USDA	Afghanistan	P1-253764	Asia
2	Afghanistan-2	30653	USDA	Afghanistan	P1-304592	Asia
3	Afghanistan-3	33541	USDA	Afghanistan	PI 220647	Asia
4	Argentina-1	30695	USDA	Argentina	P1-367833	America
5	Australia-1	33542	USDA	Australia	PI 235660	Oceania
6	Austria-1	33568	USDA	Austria	PI 253519	Europe
7	Austria-2	33670	USDA	Austria	BVAL-901352	Europe
8	Bangladesh-1	31509	USDA	Bangladesh	PI-401472	Asia
9	Bangladesh-2	31510	USDA	Bangladesh	PI-401478	Asia
10	Bangladesh-3	31511	USDA	Bangladesh	PI-401480	Asia
11	Bangladesh-4	33609	USDA	Bangladesh	PI 401470	Asia
12	China-1	30624	USDA	China	P1-262452	Asia
13	China-2	30625	USDA	China	P1-262453	Asia
14	China-3	33638	USDA	China	PI 543979	Asia
15	China-4	33639	USDA	China	PI 543982	Asia
16	China-5	33642	USDA	China	PI 544001	Asia
17	China-6	33651	USDA	China	PI 568809	Asia

18	China-7	33661	USDA	China	PI 568874	Asia
19	Egypt-1	30563	USDA	Egypt	P1-250082	Africa
20	Egypt-2	30574	USDA	Egypt	P1-250528	Africa
21	Egypt-3	30577	USDA	Egypt	P1-250532	Africa
22	Egypt-4	30578	USDA	Egypt	P1-250540	Africa
23	Egypt-5	30580	USDA	Egypt	P1-250605	Africa
24	Egypt-6	30581	USDA	Egypt	P1-250608	Africa
25	France-1	33662	USDA	France	PI 576985	Europe
26	Hungary-1	33575	USDA	Hungary	PI 288983	Europe
27	India-1	30579	USDA	India	P1-250601	Asia
28	India-2	30662	USDA	India	P1-305195	Asia
29	India-3	30673	USDA	India	P1-306926	Asia
30	India-4	30674	USDA	India	P1-306941	Asia
31	India-5	30677	USDA	India	P1-306976	Asia
32	India-6	33538	USDA	India	PI 199878	Asia
33	Iran-1	30588	USDA	Iran	P1-250720	Asia
34	Iran-2	30631	USDA	Iran	P1-304444	Asia
35	Iran-3	30633	USDA	Iran	P1-304448	Asia
36	Iran-4	30713	USDA	Iran	P1-405958	Asia
37	Iran-5	30718	USDA	Iran	P1-405967	Asia
38	Iran-6	33556	USDA	Iran	PI 250840	Asia

39	Iran-7	33621	USDA	Iran	PI 406010	Asia
40	Israel-1	30548	USDA	Israel	P1-198990	Asia
41	Israel-2	30594	USDA	Israel	P1-253386	Asia
42	Israel-3	3015	USDA	Israel	P1-253892	Asia
43	Israel-4	33564	USDA	Israel	PI 251290	Asia
44	Iraq-1	30612	USDA	Iraq	P1-253761	Asia
45	Iraq-2	30613	USDA	Iraq	P1-253762	Asia
46	Jordan-1	30589	USDA	Jordan	P1-251284	Asia
47	Jordan-2	30590	USDA	Jordan	P1-251285	Asia
48	Jordan-3	33559	USDA	Jordan	PI 251265	Asia
49	Jordan-4	33560	USDA	Jordan	PI 251267	Asia
50	Jordan-5	33561	USDA	Jordan	PI 251268	Asia
51	Kazakhstan-1	30681	USDA	Kazakhstan	P1-314650	Asia
52	Libya-1	33608	USDA	Libya	PI 393499	Africa
53	Morocco-1	30552	USDA	Morocco	P1-239042	Africa
54	Morocco-2	30606	USDA	Morocco	P1-253560	Africa
55	Pakistan-1	30564	USDA	Pakistan	P1-250194	Asia
56	Pakistan-2	30565	USDA	Pakistan	P1-250201	Asia
57	Pakistan-3	30567	USDA	Pakistan	P1-250345	Asia
58	Pakistan-4	30568	USDA	Pakistan	P1-250346	Asia
59	Pakistan-5	30569	USDA	Pakistan	P1-250351	Asia

60	Pakistan-6	30570	USDA	Pakistan	P1-250353	Asia
61	Pakistan-7	30573	USDA	Pakistan	P1-250481	Asia
62	Pakistan-8	33547	USDA	Pakistan	PI 250474	Asia
63	Pakistan-9	33548	USDA	Pakistan	PI 250478	Asia
64	Pakistan-10	33635	USDA	Pakistan	PI 426521	Asia
65	Pakistan-11	Check	PGRI-Pakistan	Pakistan	Thori-78	Asia
66	Portugal-1	30604	USDA	Portugal	P1-253553	Europe
67	Portugal-2	30605	USDA	Portugal	P1-253556	Europe
68	Portugal-3	30608	USDA	Portugal	P1-253564	Europe
69	Portugal-4	30610	USDA	Portugal	P1-253569	Europe
70	Portugal-5	30611	USDA	Portugal	P1-253571	Europe
71	Portugal-6	30620	USDA	Portugal	P1-258412	Europe
72	Romania-1	30549	USDA	Romania	P1-209287	Europe
73	Russia-1	30663	USDA	Russia	P1-305535	Asia
74	Spain-1	30595	USDA	Spain	P1-253388	Europe
75	Spain-2	30596	USDA	Spain	P1-253391	Europe
76	Spain-3	30597	USDA	Spain	P1-253394	Europe
77	Spain-4	30598	USDA	Spain	P1-253395	Europe
78	Syria-1	30616	USDA	Syria	P1-253898	Asia
79	Syria-2	30617	USDA	Syria	P1-253900	Asia
80	Syria-3	30700	USDA	Syria	P1-386174	Asia

81	Thailand-1	30701	USDA	Thailand	P1-387821	Asia
82	Turkey-1	30646	USDA	Turkey	P1-304498	Asia
83	Turkey-2	30648	USDA	Turkey	P1-304502	Asia
84	Turkey-3	30650	USDA	Turkey	P1-304504	Asia
85	Turkey-4	30651	USDA	Turkey	P1-304505	Asia
86	Turkey-5	30688	USDA	Turkey	P1-340086	Asia
87	Turkey-6	33543	USDA	Turkey	PI 237538	Asia
88	Turkey-7	33565	USDA	Turkey	PI 251978	Asia
89	Turkey-8	33567	USDA	Turkey	PI 251984	Asia
90	Turkey-9	33627	USDA	Turkey	PI 406701	Asia
91	Turkey-10	33628	USDA	Turkey	PI 406702	Asia
92	Uzbekistan-1	30623	USDA	Uzbekistan	P1-262435	Asia
93	Uzbekistan-2	30696	USDA	Uzbekistan	P1-369846	Asia
94	Uzbekistan-3	30697	USDA	Uzbekistan	P1-369853	Asia

Appendix II: List of 131 safflower accessions evaluated for molecular characterization and population structure analysis using 13 iPBS-retrotransposon and 12 ISSR markers

S.No	Accession Name	Accession No	Donor Organization	Location	Province/Distt	Origin	Plant ID	Continent
1	Afghanistan-1	30614	USDA	-	-	Afghanistan	P1-253764	Asia
2	Afghanistan-2	30653	USDA	-	-	Afghanistan	P1-304592	Asia
3	Afghanistan-3	33541	USDA	-	-	Afghanistan	PI 220647	Asia
4	Afghanistan-4	7-T	CRIFC-Turkey	-	-	Afghanistan	-	Asia
5	Afghanistan-5	9-T	CRIFC-Turkey	-	-	Afghanistan	-	Asia
6	Argentina-1	30695	USDA	-	-	Argentina	P1-367833	America
7	Australia-1	33542	USDA	-	-	Australia	PI 235660	Oceania
8	Austria-1	33568	USDA	-	-	Austria	PI 253519	Europe
9	Austria-2	33670	USDA	-	-	Austria	BVAL-901352	Europe
10	Bangladesh-1	31509	USDA	-	-	Bangladesh	PI-401472	Asia
11	Bangladesh-2	31510	USDA	-	-	Bangladesh	PI-401478	Asia
12	Bangladesh-3	31511	USDA	-	-	Bangladesh	PI-401480	Asia
13	Bangladesh-4	33609	USDA	-	-	Bangladesh	PI 401470	Asia
14	Canada-1	74-T	CRIFC-Turkey	-	-	Canada	-	America
15	Canada-2	75-T	CRIFC-Turkey	-	-	Canada	-	America
16	China-1	30624	USDA	-	-	China	P1-262452	Asia
17	China-2	30625	USDA	-	-	China	P1-262453	Asia

18	China-3	33638	USDA	-	-	China	PI 543979	Asia
19	China-4	33639	USDA	-	-	China	PI 543982	Asia
20	China-5	33642	USDA	-	-	China	PI 544001	Asia
21	China-6	33651	USDA	-	-	China	PI 568809	Asia
22	China-7	33661	USDA	-	-	China	PI 568874	Asia
23	China-8	27-T	CRIFC-Turkey	-	-	China	-	Asia
24	China-9	29-T	CRIFC-Turkey	-	-	China	-	Asia
25	Egypt-1	30563	USDA	-	-	Egypt	P1-250082	Africa
26	Egypt-2	30574	USDA	-	-	Egypt	P1-250528	Africa
27	Egypt-3	30577	USDA	-	-	Egypt	P1-250532	Africa
28	Egypt-4	30578	USDA	-	-	Egypt	P1-250540	Africa
29	Egypt-5	30580	USDA	-	-	Egypt	P1-250605	Africa
30	Egypt-6	30581	USDA	-	-	Egypt	P1-250608	Africa
31	France-1	33662	USDA	-	-	France	PI 576985	Europe
32	Hungary-1	33575	USDA	-	-	Hungary	PI 288983	Europe
33	India-1	30579	USDA	-	-	India	P1-250601	Asia
34	India-2	30662	USDA	-	-	India	P1-305195	Asia
35	India-3	30673	USDA	-	-	India	P1-306926	Asia
36	India-4	30674	USDA	-	-	India	P1-306941	Asia
37	India-5	30677	USDA	-	-	India	P1-306976	Asia
38	India-6	33538	USDA	-	-	India	PI 199878	Asia

39	Iran-1	30588	USDA	-	-	Iran	P1-250720	Asia
40	Iran-2	30631	USDA	-	-	Iran	P1-304444	Asia
41	Iran-3	30633	USDA	-	-	Iran	P1-304448	Asia
42	Iran-4	30713	USDA	-	-	Iran	P1-405958	Asia
43	Iran-5	30718	USDA	-	-	Iran	P1-405967	Asia
44	Iran-6	33556	USDA	-	-	Iran	PI 250840	Asia
45	Iran-7	33621	USDA	-	-	Iran	PI 406010	Asia
46	Iran-8	116-T	CRIFC-Turkey	-	-	Iran	-	Asia
47	Iran-9	152-T	CRIFC-Turkey	-	-	Iran	-	Asia
48	Iran-10	177-T	CRIFC-Turkey	-	-	Iran	-	Asia
49	Israel-1	30548	USDA	-	-	Israel	P1-198990	Asia
50	Israel-2	30594	USDA	-	-	Israel	P1-253386	Asia
51	Israel-3	3015	USDA	-	-	Israel	P1-253892	Asia
52	Israel-4	33564	USDA	-	-	Israel	PI 251290	Asia
53	Iraq-1	30612	USDA	-	-	Iraq	P1-253761	Asia
54	Iraq-2	30613	USDA	-	-	Iraq	P1-253762	Asia
55	Jordan-1	30589	USDA	-	-	Jordan	P1-251284	Asia
56	Jordan-2	30590	USDA	-	-	Jordan	P1-251285	Asia
57	Jordan-3	33559	USDA	-	-	Jordan	PI 251265	Asia
58	Jordan-4	33560	USDA	-	-	Jordan	PI 251267	Asia
59	Jordan-5	33561	USDA	-	-	Jordan	PI 251268	Asia

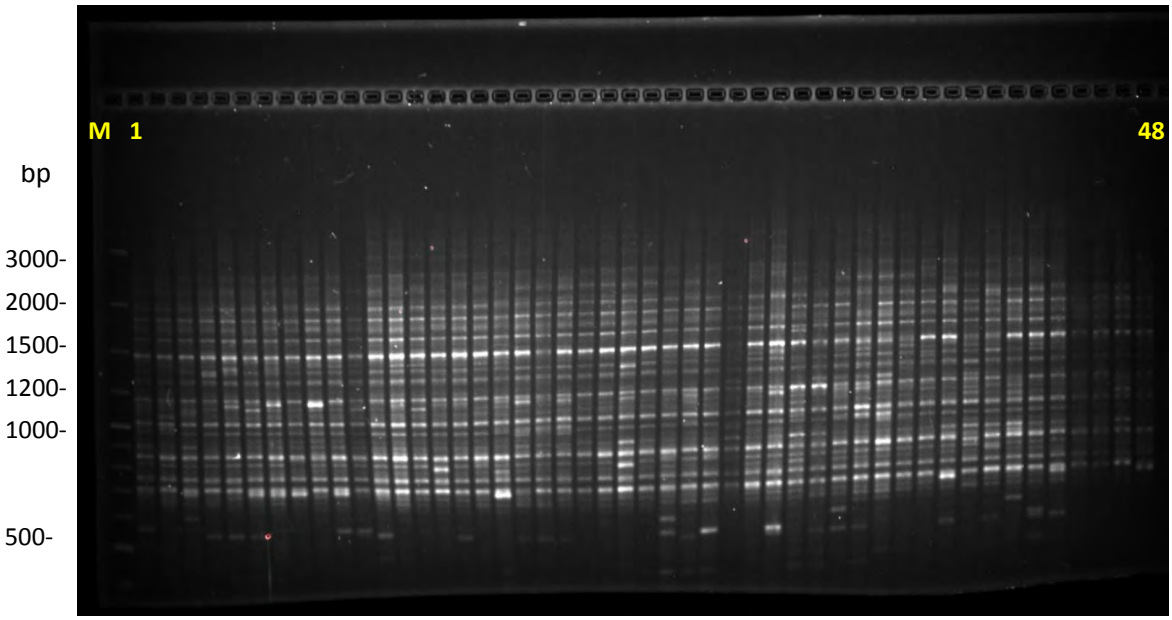
60	Kazakhstan-1	30681	USDA	-	-	Kazakhstan	P1-314650	Asia
61	Libya-1	33608	USDA	-	-	Libya	PI 393499	Africa
62	Morocco-1	30552	USDA	-	-	Morocco	P1-239042	Africa
63	Morocco-2	30606	USDA	-	-	Morocco	P1-253560	Africa
64	Pakistan-1	30564	USDA	-	-	Pakistan	P1-250194	Asia
65	Pakistan-2	30565	USDA	-	-	Pakistan	P1-250201	Asia
66	Pakistan-3	30567	USDA	-	-	Pakistan	P1-250345	Asia
67	Pakistan-4	30568	USDA	-	-	Pakistan	P1-250346	Asia
68	Pakistan-5	30569	USDA	-	-	Pakistan	P1-250351	Asia
69	Pakistan-6	30570	USDA	-	-	Pakistan	P1-250353	Asia
70	Pakistan-7	30573	USDA	-	-	Pakistan	P1-250481	Asia
71	Pakistan-8	33547	USDA	-	-	Pakistan	PI 250474	Asia
72	Pakistan-9	33548	USDA	-	-	Pakistan	PI 250478	Asia
73	Pakistan-10	33635	USDA	-	-	Pakistan	PI 426521	Asia
74	Pakistan-11	Check	PGRI-Pakistan	-	-	Pakistan	Thori-78	Asia
75	Pakistan-12	16266	PGRI-Pakistan	Jacobabad	Sindh	Pakistan	-	Asia
76	Pakistan-13	16267	PGRI-Pakistan	Shikarpur	Sindh	Pakistan	-	Asia
77	Pakistan-14	16268	PGRI-Pakistan	Shikarpur	Sindh	Pakistan	-	Asia
78	Pakistan-15	16269	PGRI-Pakistan	Larkana	Sindh	Pakistan	-	Asia
79	Pakistan-16	16270	PGRI-Pakistan	Larkana	Sindh	Pakistan	-	Asia
80	Pakistan-17	16355	PGRI-Pakistan	Dadu	Sindh	Pakistan	-	Asia

81	Pakistan-18	16356	PGRI-Pakistan	Dadu	Sindh	Pakistan	-	Asia
82	Pakistan-19	16357	PGRI-Pakistan	Karachi	Sindh	Pakistan	-	Asia
83	Pakistan-20	16358	PGRI-Pakistan	Karachi	Sindh	Pakistan	-	Asia
84	Pakistan-21	16359	PGRI-Pakistan	Gilgit	GB	Pakistan	-	Asia
85	Pakistan-22	19233	PGRI-Pakistan	Gilgit	GB	Pakistan	-	Asia
86	Pakistan-23	20920	PGRI-Pakistan	Islamabad	Federal Areas	Pakistan	-	Asia
87	Pakistan-24	21933	PGRI-Pakistan	Karachi	Sindh	Pakistan	-	Asia
88	Pakistan-25	24779	PGRI-Pakistan	Quetta	Balochistan	Pakistan	-	Asia
89	Pakistan-26	27549	PGRI-Pakistan	Hyderabad	Sindh	Pakistan	-	Asia
90	Pakistan-27	30698	PGRI-Pakistan	Hyderabad	Shindh	Pakistan	-	Asia
91	Pakistan-28	35803	PGRI-Pakistan	Gakooch	Gilgit/Balistan	Pakistan	-	Asia
92	Portugal-1	30604	USDA	-	-	Portugal	P1-253553	Europe
93	Portugal-2	30605	USDA	-	-	Portugal	P1-253556	Europe
94	Portugal-3	30608	USDA	-	-	Portugal	P1-253564	Europe
95	Portugal-4	30610	USDA	-	-	Portugal	P1-253569	Europe
96	Portugal-5	30611	USDA	-	-	Portugal	P1-253571	Europe
97	Portugal-6	30620	USDA	-	-	Portugal	P1-258412	Europe
98	Romania-1	30549	USDA	-	-	Romania	P1-209287	Europe
99	Russia-1	30663	USDA	-	-	Russia	P1-305535	Asia
100	Spain-1	30595	USDA	-	-	Spain	P1-253388	Europe
101	Spain-2	30596	USDA	-	-	Spain	P1-253391	Europe

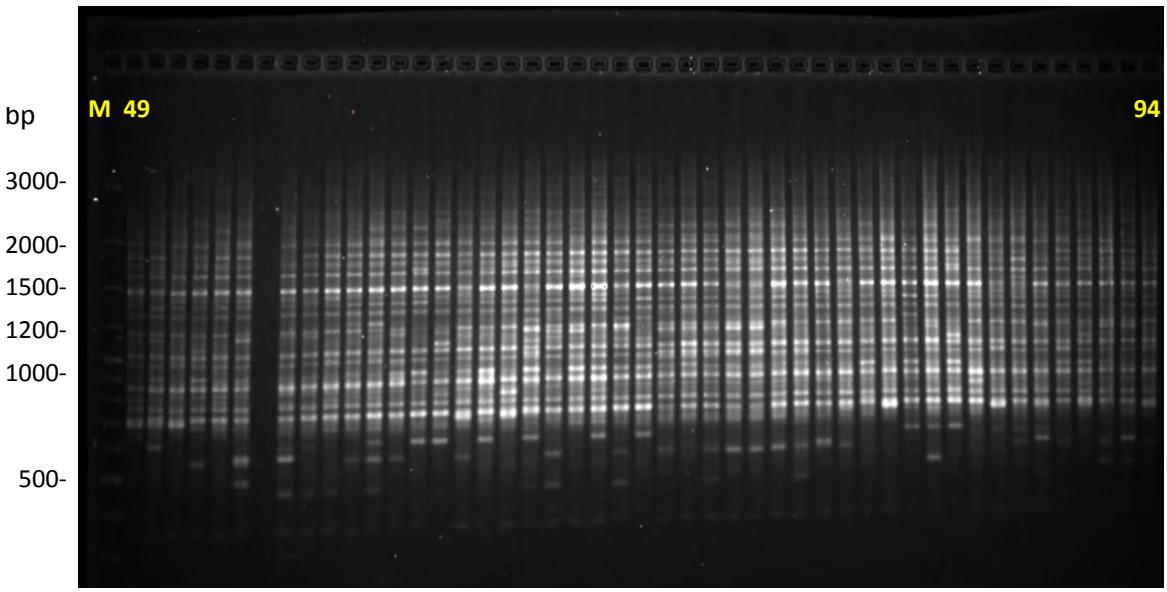
102	Spain-3	30597	USDA	-	-	Spain	P1-253394	Europe
103	Spain-4	30598	USDA	-	-	Spain	P1-253395	Europe
104	Syria-1	30616	USDA	-	-	Syria	P1-253898	Asia
105	Syria-2	30617	USDA	-	-	Syria	P1-253900	Asia
106	Syria-3	30700	USDA	-	-	Syria	P1-386174	Asia
107	Thailand-1	30701	USDA	-	-	Thailand	P1-387821	Asia
108	Turkey-1	30646	USDA	-	-	Turkey	P1-304498	Asia
109	Turkey-2	30648	USDA	-	-	Turkey	P1-304502	Asia
110	Turkey-3	30650	USDA	-	-	Turkey	P1-304504	Asia
111	Turkey-4	30651	USDA	-	-	Turkey	P1-304505	Asia
112	Turkey-5	30688	USDA	-	-	Turkey	P1-340086	Asia
113	Turkey-6	33543	USDA	-	-	Turkey	PI 237538	Asia
114	Turkey-7	33565	USDA	-	-	Turkey	PI 251978	Asia
115	Turkey-8	33567	USDA	-	-	Turkey	PI 251984	Asia
116	Turkey-9	33627	USDA	-	-	Turkey	PI 406701	Asia
117	Turkey-10	33628	USDA	-	-	Turkey	PI 406702	Asia
118	Turkey-11	36-T	CRIFC-Turkey	-	Tarme	Turkey	-	Asia
119	Turkey-12	37-T	CRIFC-Turkey	-	Tarme	Turkey	-	Asia
120	Turkey-13	57-T	CRIFC-Turkey	-	Elbistan	Turkey	-	Asia
121	Turkey-14	58-T	CRIFC-Turkey	-	Elbistan	Turkey	-	Asia
122	Turkey-15	134-T	CRIFC-Turkey	-	Tarme	Turkey	-	Asia

123	Turkey-16	277-T	CRIFC-Turkey	-	Tarme	Turkey	-	Asia
124	USA-1	80-T	CRIFC-Turkey	-	Montana	USA	-	America
125	USA-2	130-T	CRIFC-Turkey	-	-	USA	-	America
126	USA-3	132-T	CRIFC-Turkey	-	-	USA	-	America
127	USA-4	149-T	CRIFC-Turkey	-	İdoha	USA	-	America
128	USA-5	153-T	CRIFC-Turkey	-	İdoha	USA	-	America
129	Uzbekistan-1	30623	USDA	-	-	Uzbekistan	P1-262435	Asia
130	Uzbekistan-2	30696	USDA	-	-	Uzbekistan	P1-369846	Asia
131	Uzbekistan-3	30697	USDA	-	-	Uzbekistan	P1-369853	Asia

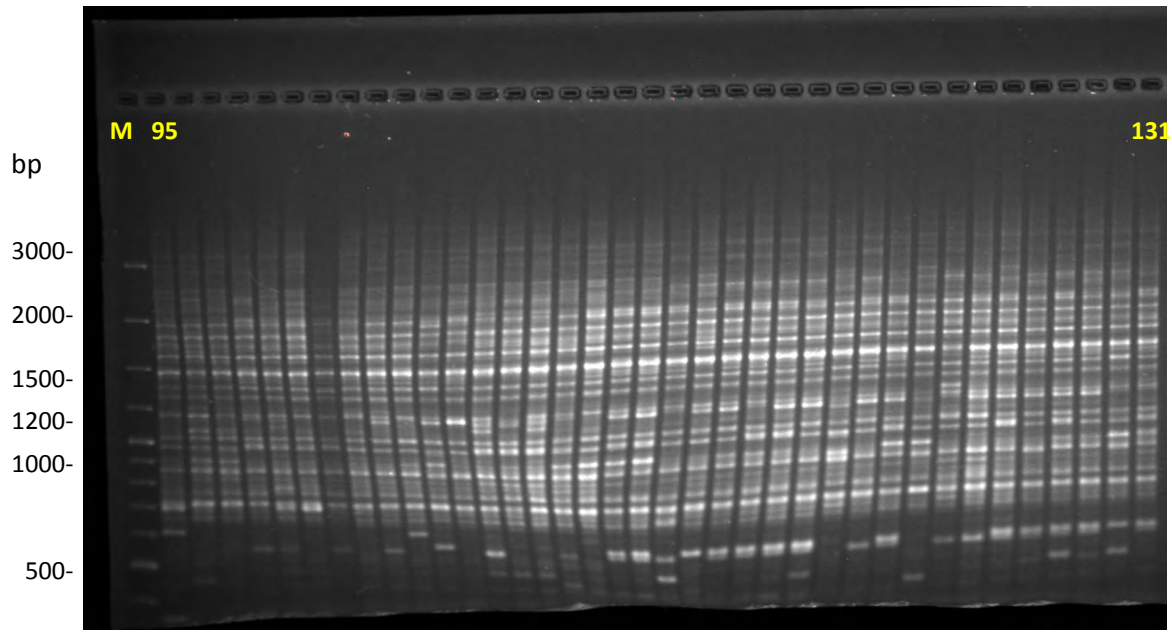
USDA: United States Department of Agriculture; PGRI: Plant Genetic Resources Institute; CRIFC: Central Research Institute for Field Crop; - Not know



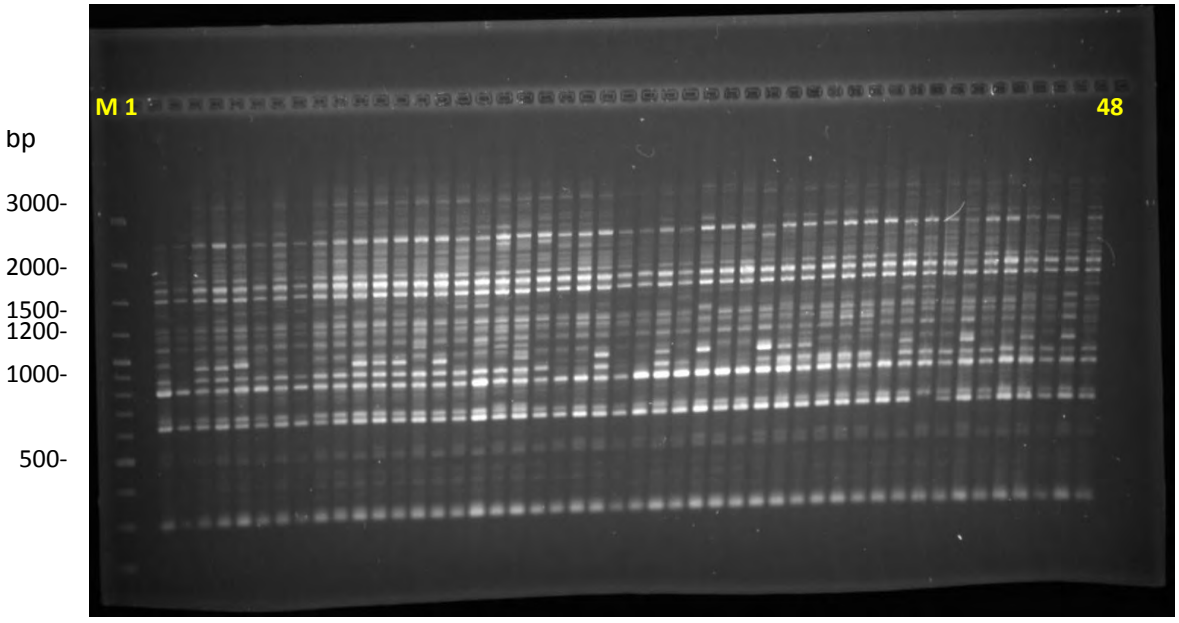
Appendix III Picture 1a: Gel imaging picture of the primer iPBS2228 revealing genetic diversity among 131 safflower accessions



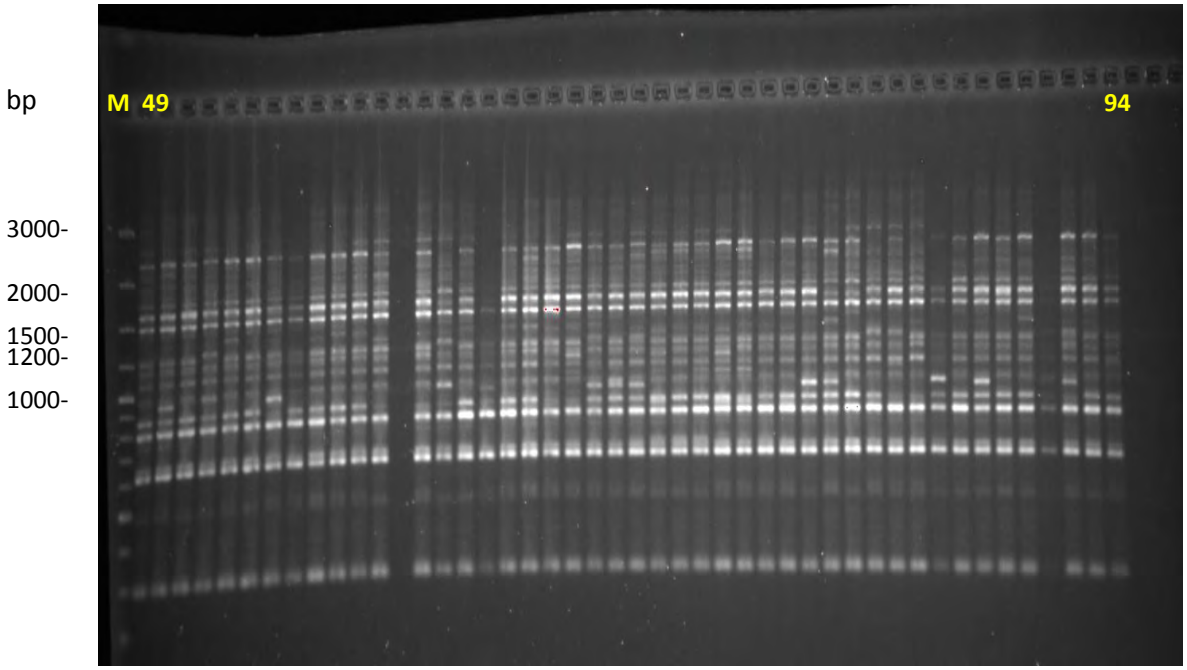
Appendix III Picture 1b: Gel imaging picture of the primer iPBS2228 revealing genetic diversity among 131 safflower accessions



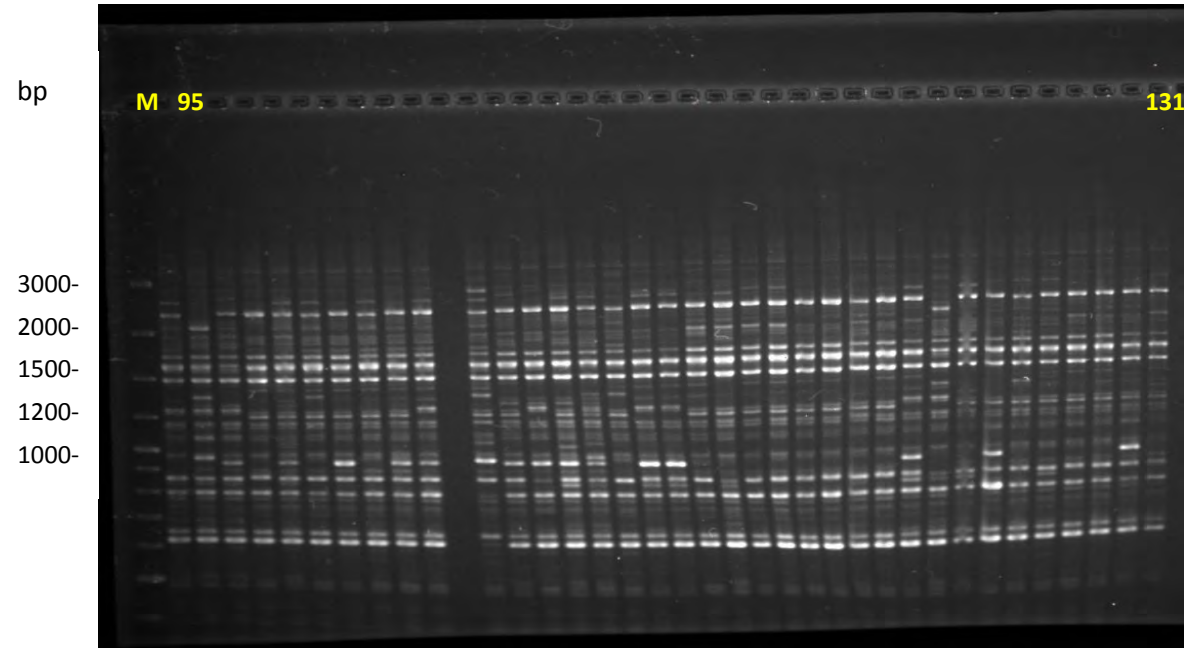
Appendix III Picture 1c: Gel imaging picture of the primer iPBS2228 revealing genetic diversity among 131 safflower accessions



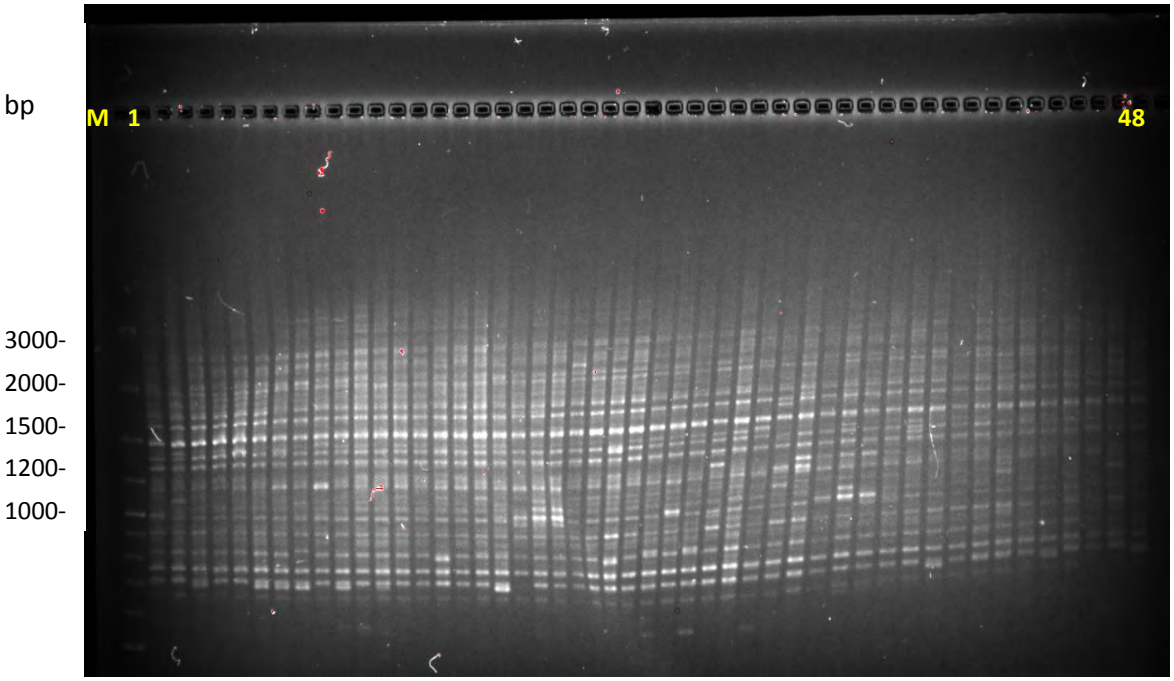
Appendix III Picture 2a: Gel imaging picture of the primer iPBS2239 revealing genetic diversity among 131 safflower accessions



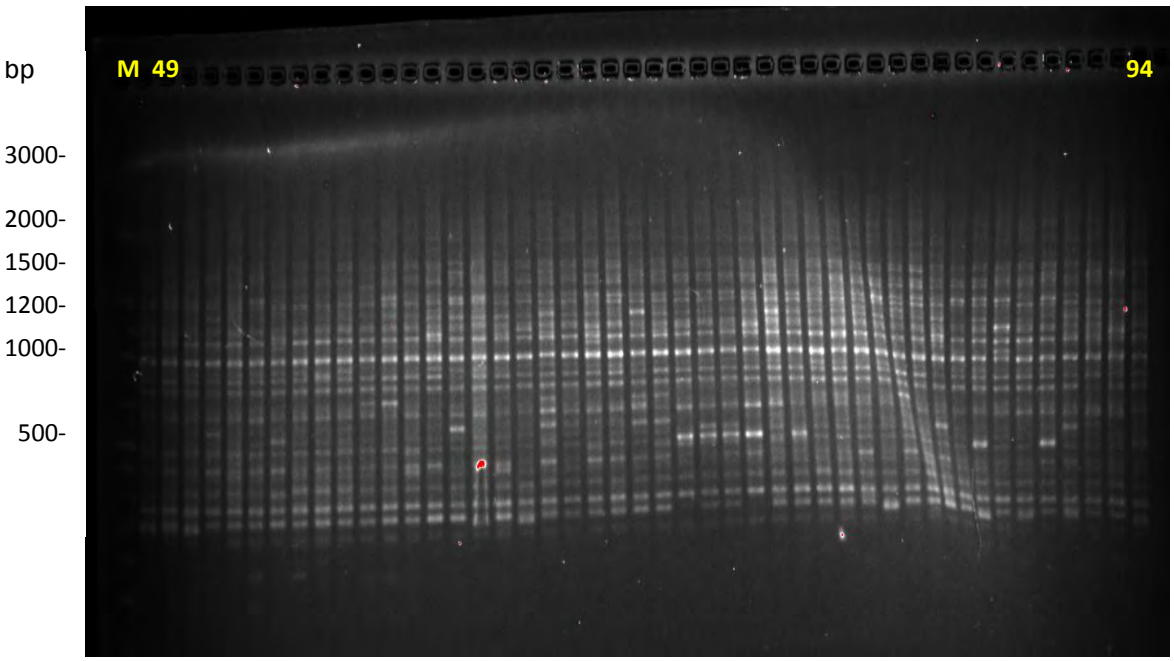
Appendix III Picture 2b: Gel imaging picture of the primer iPBS2239 revealing genetic diversity among 131 safflower accessions



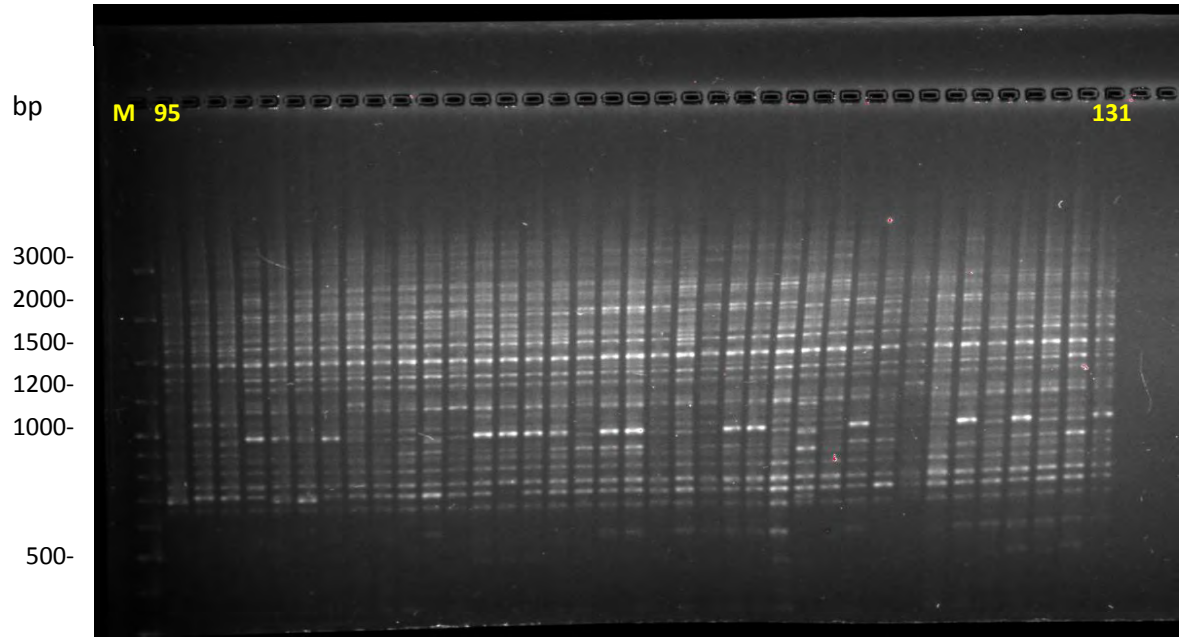
Appendix III Picture 2c: Gel imaging picture of the primer iPBS2239 revealing genetic diversity among 131 safflower accessions



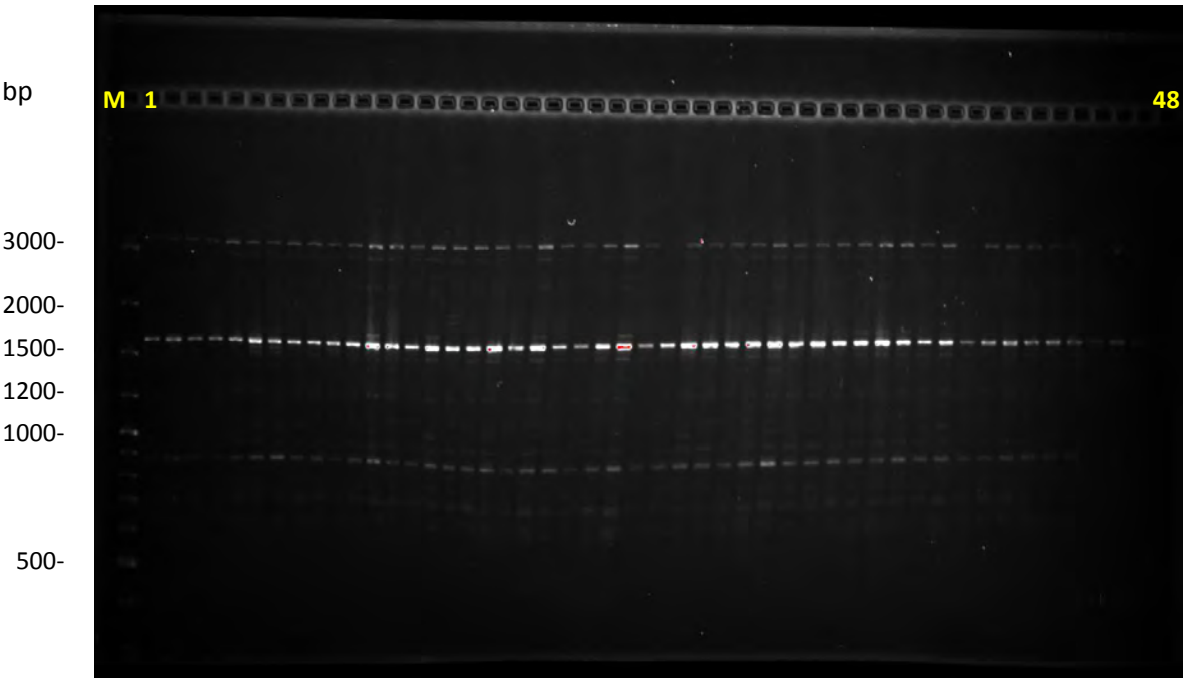
Appendix III Picture 3a: Gel imaging picture of the primer iPBS2252 revealing genetic diversity among 131 safflower accessions



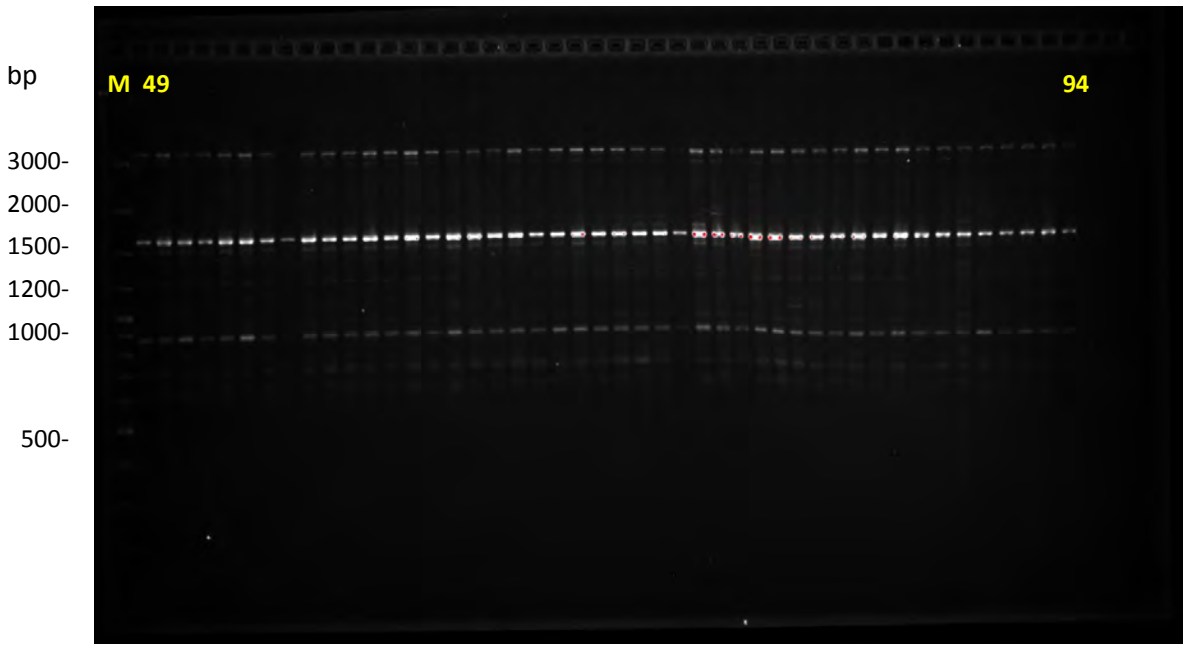
Appendix III Picture 3b: Gel imaging picture of the primer iPBS2252 revealing genetic diversity among 131 safflower accessions



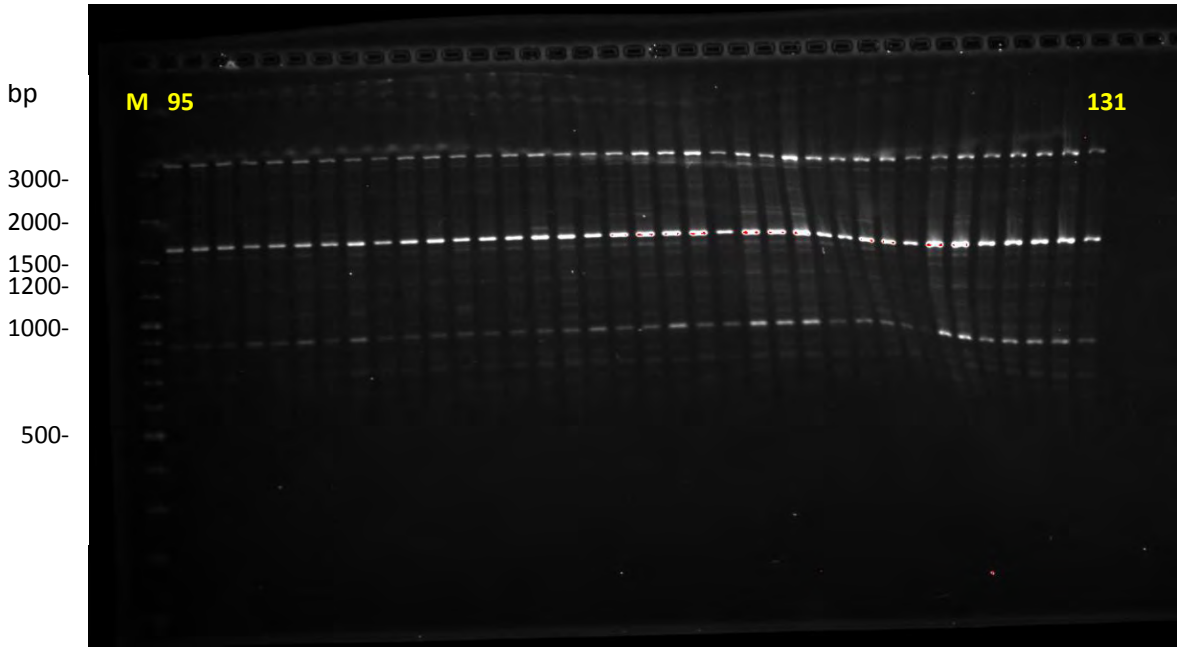
Appendix III Picture 3c: Gel imaging picture of the primer iPBS2252 revealing genetic diversity among 131 safflower accessions



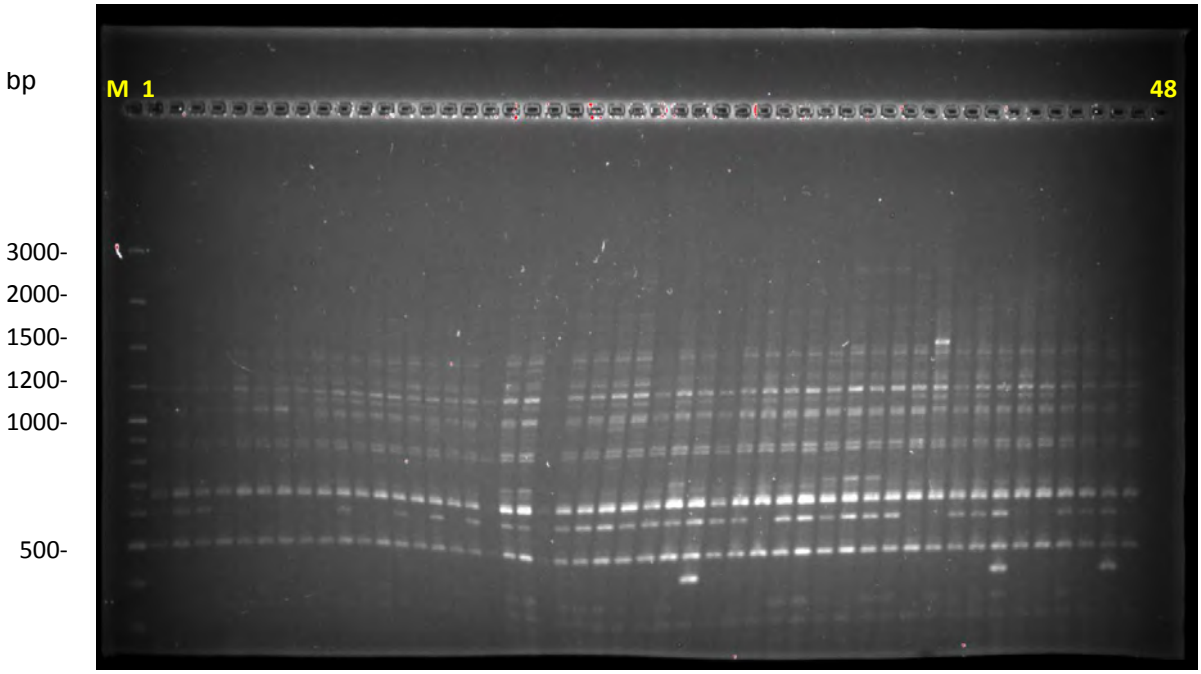
Appendix III Picture 4a: Gel imaging picture of the primer iPBS 2374 revealing genetic diversity among 131 safflower accessions



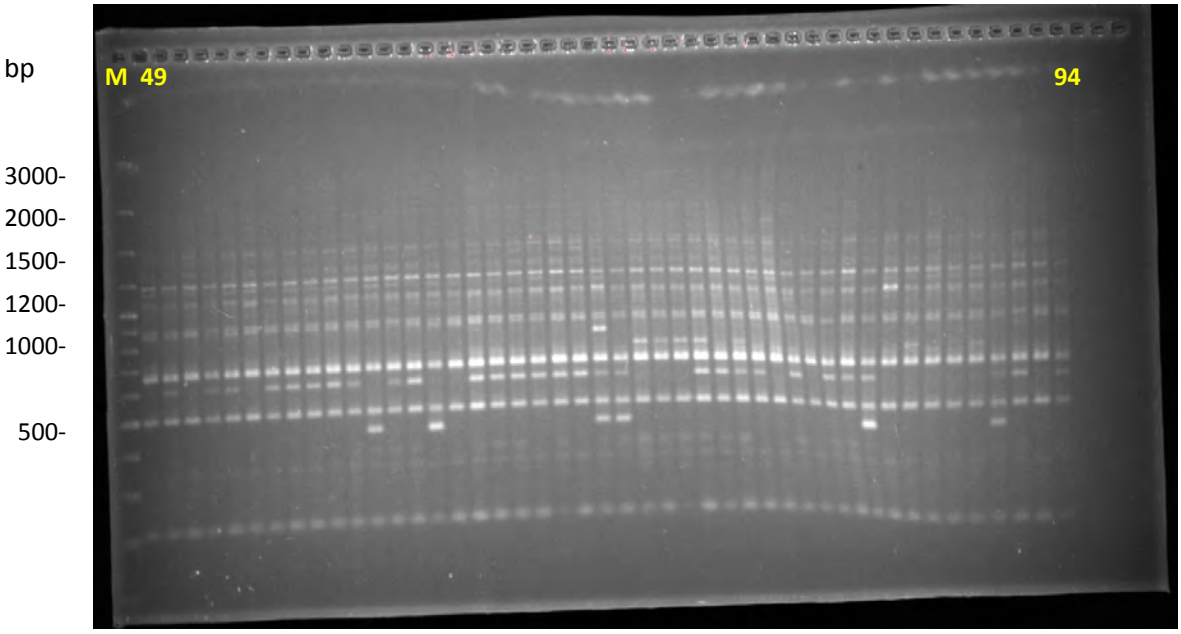
Appendix III Picture 4b: Gel imaging picture of the primer iPBS2374 revealing genetic diversity among 131 safflower accessions



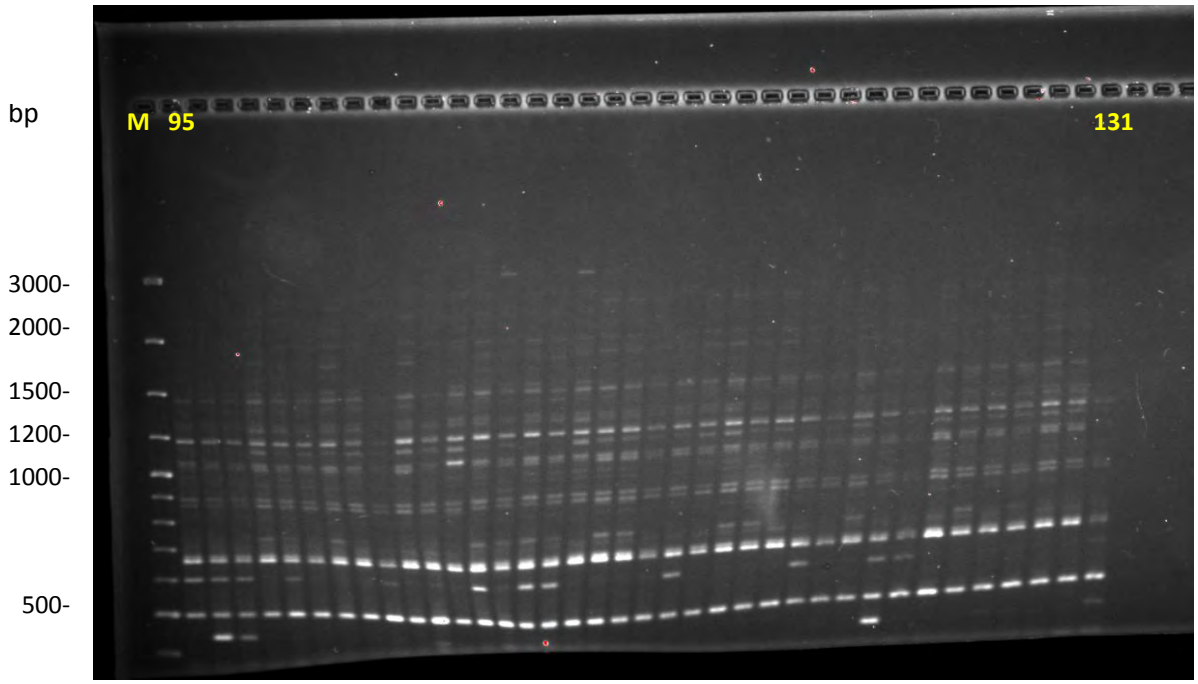
Appendix III Picture 4c: Gel imaging picture of the primer iPBS2374 revealing genetic diversity among 131 safflower accessions



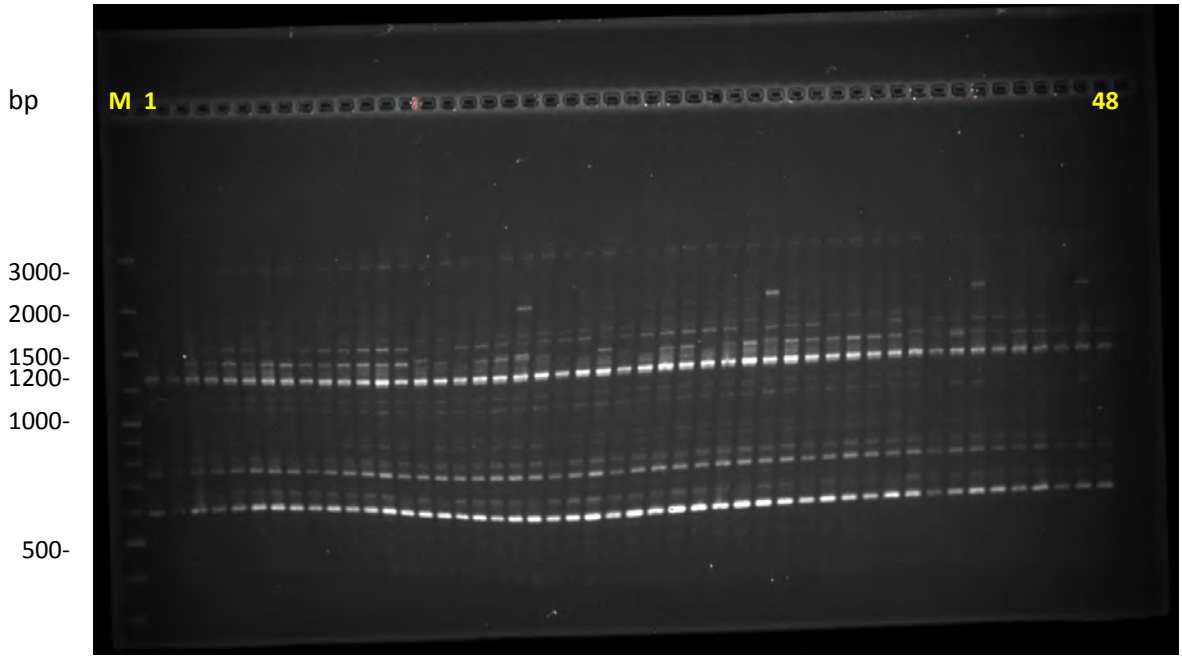
Appendix III Picture 5a: Gel imaging picture of the primer iPBS2375revealing genetic diversity among 131 safflower accessions



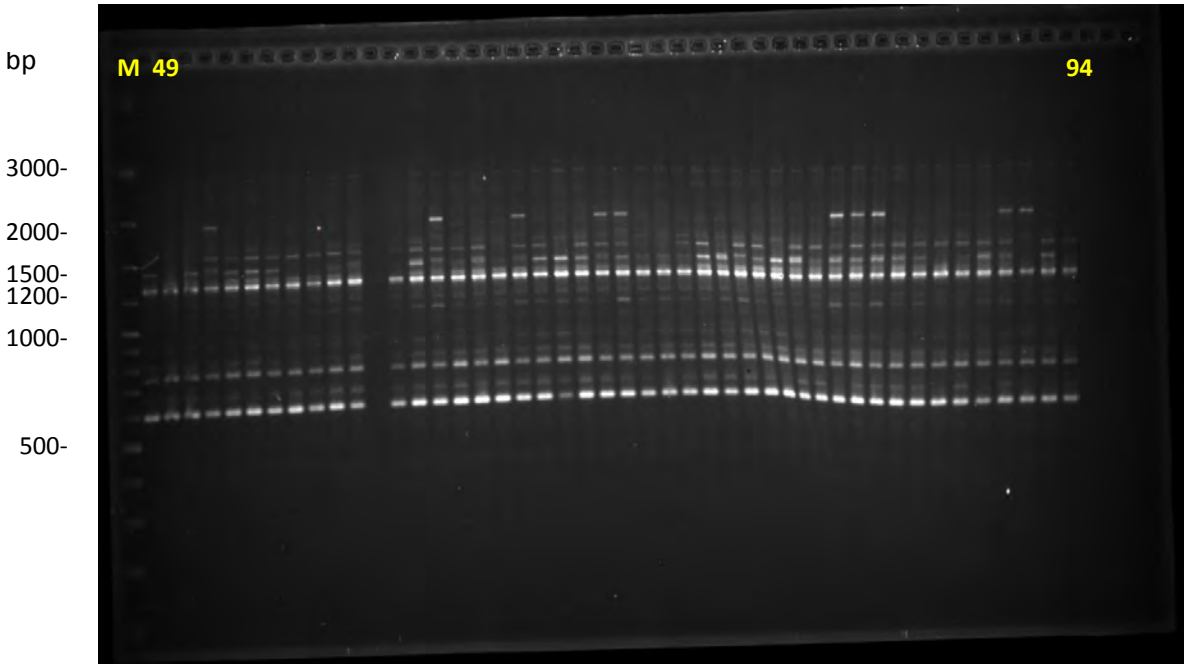
Appendix III Picture 5b: Gel imaging picture of the primer iPBS2375 revealing genetic diversity among 131 safflower accessions



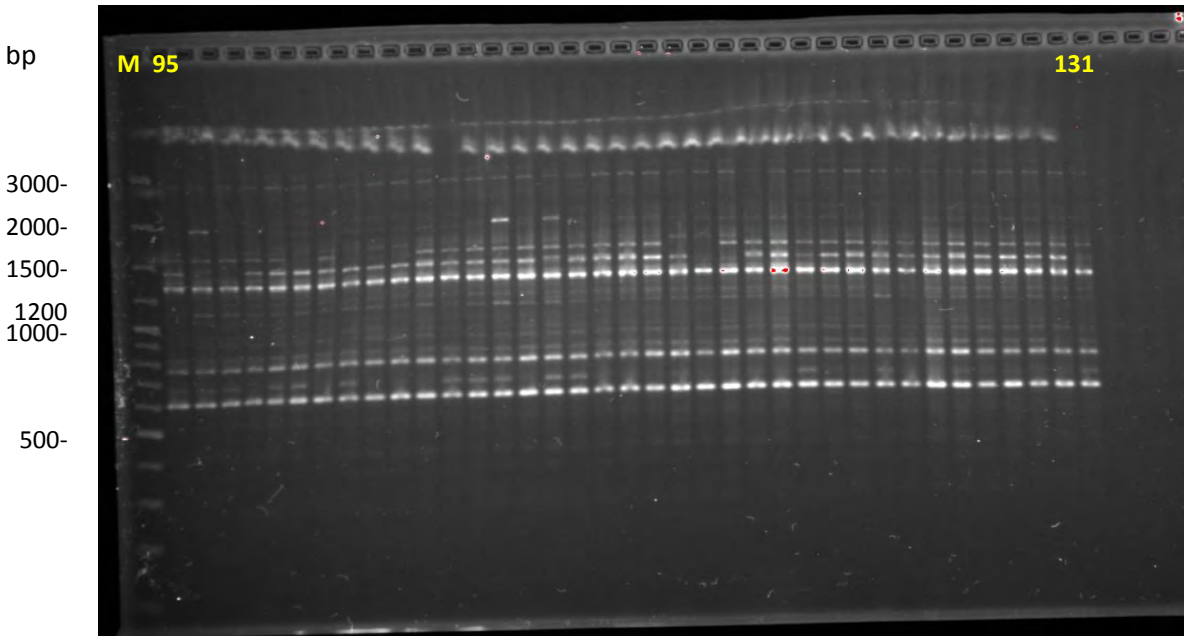
Appendix III Picture 5c: Gel imaging picture of the primer iPBS2375 revealing genetic diversity among 131 safflower accessions



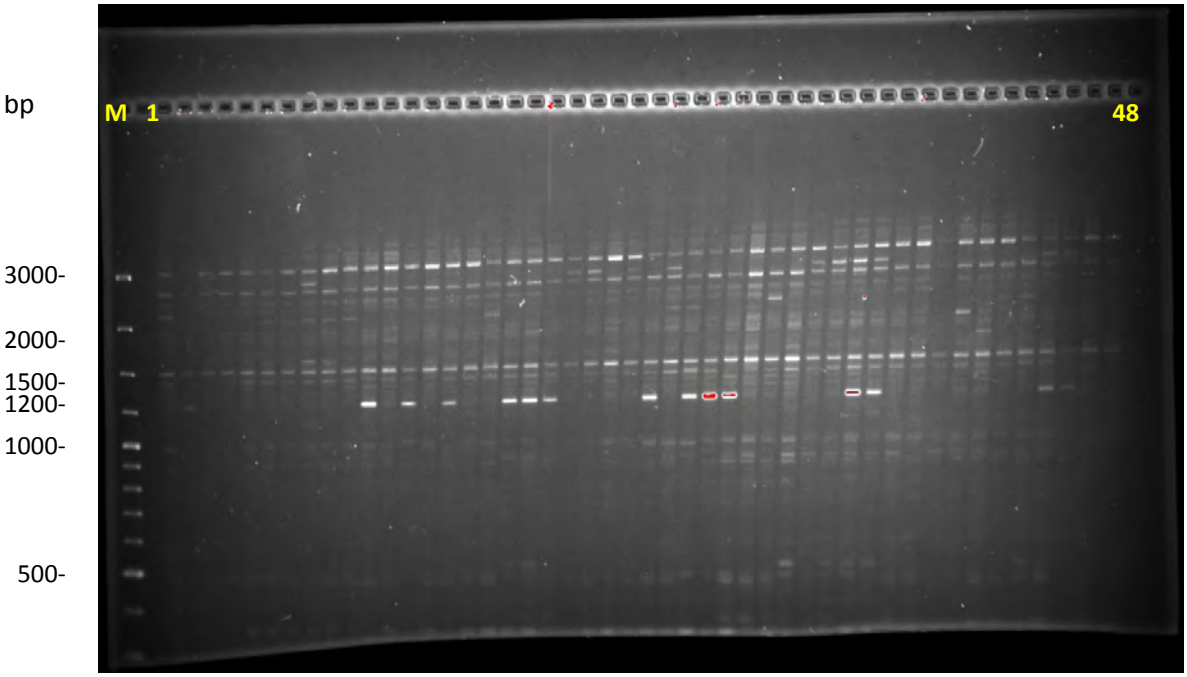
Appendix III Picture 6a: Gel imaging picture of the primer iPBS2376 revealing genetic diversity among 131 safflower accessions



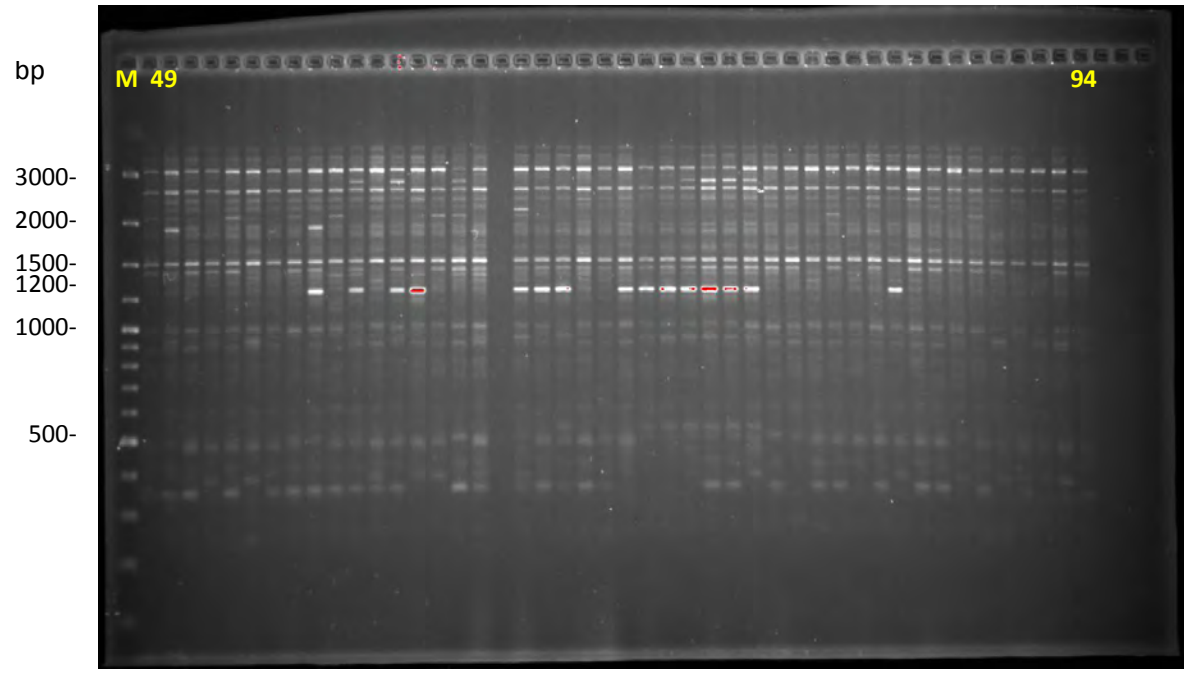
Appendix III Picture 6b: Gel imaging picture of the primer iPBS2376 revealing genetic diversity among 131 safflower accessions



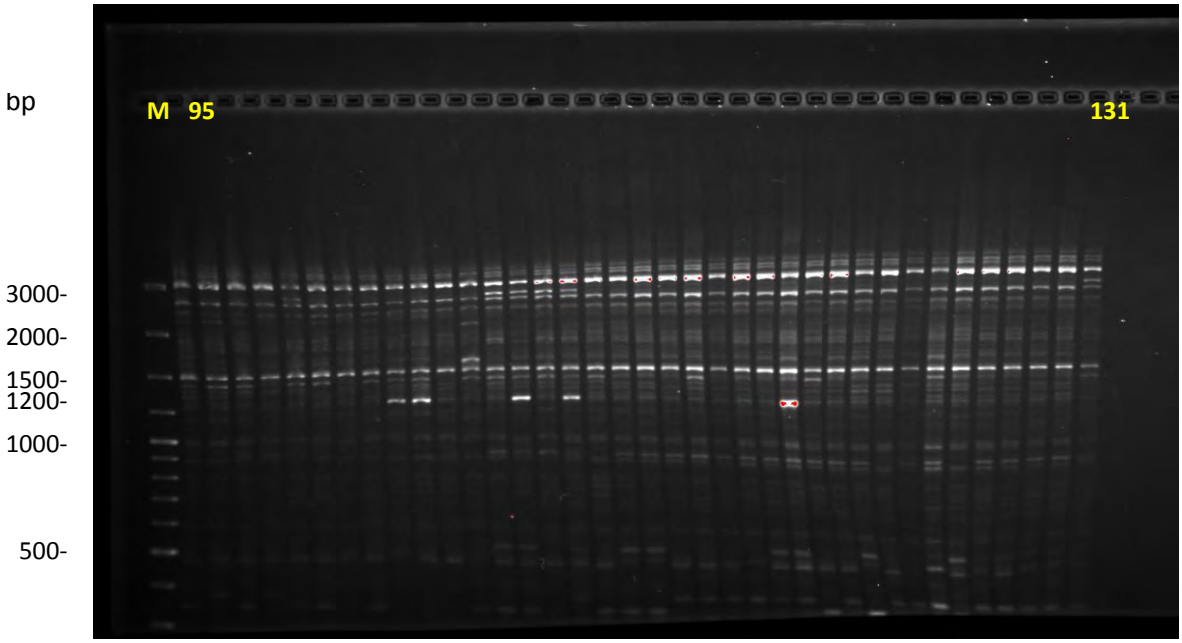
Appendix III Picture 6c: Gel imaging picture of the primer iPBS2376 revealing genetic diversity among 131 safflower accessions



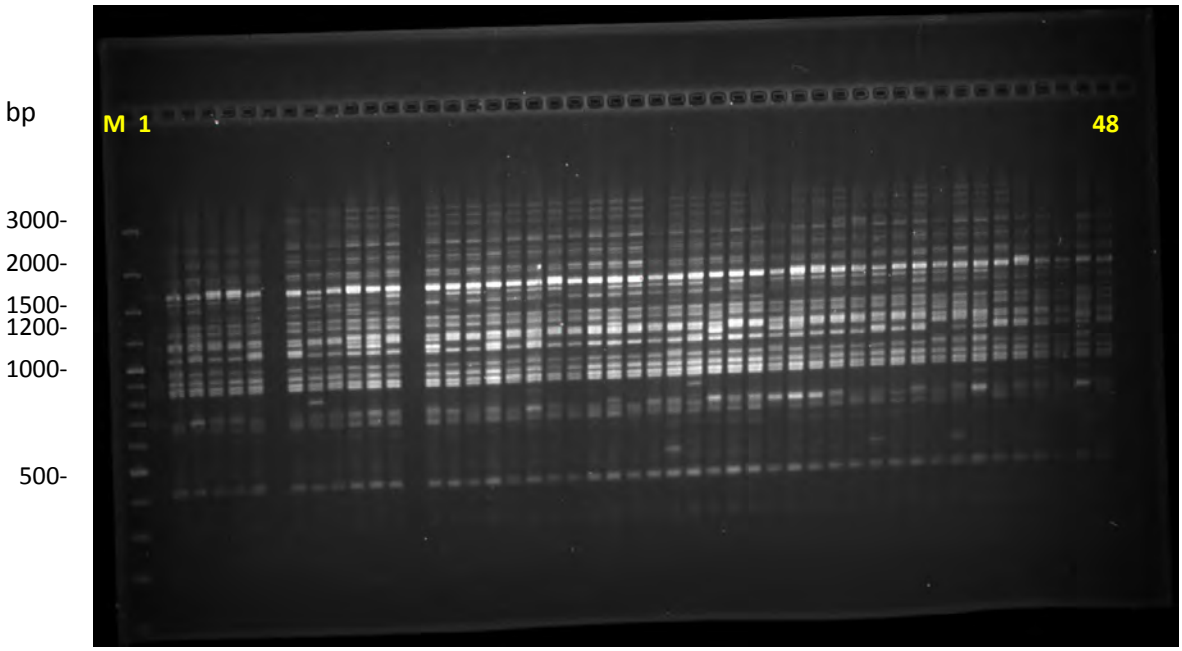
Appendix III Picture 7a: Gel imaging picture of the primer iPBS2377 revealing genetic diversity among 131 safflower accessions



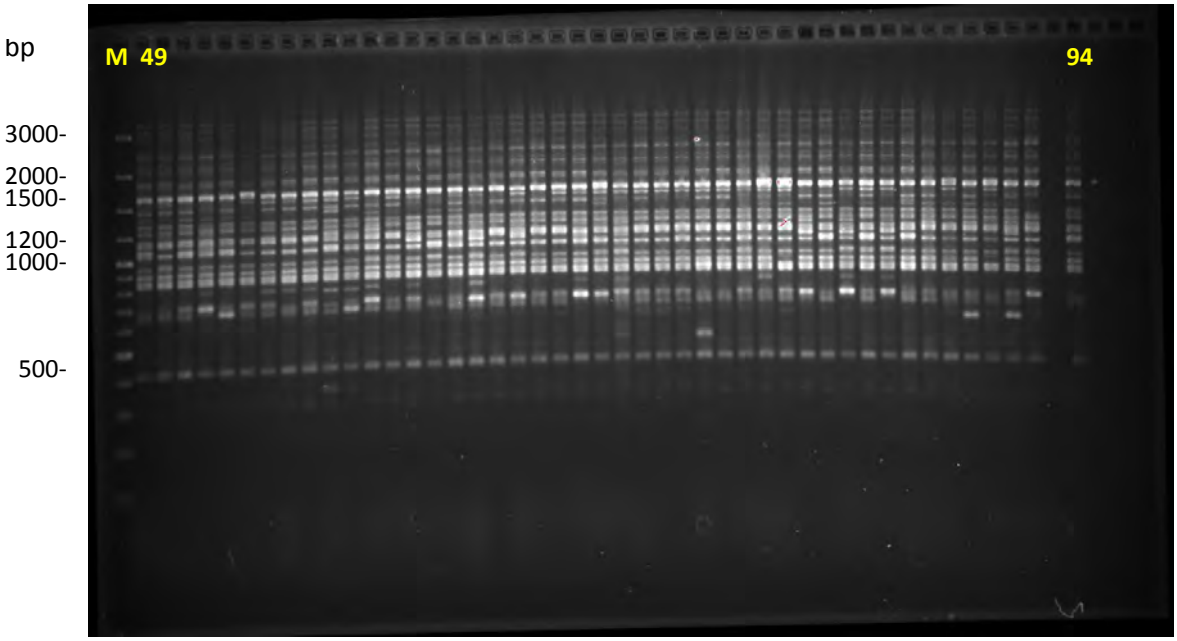
Appendix III Picture 7b: Gel imaging picture of the primer iPBS2377 revealing genetic diversity among 131 safflower accessions



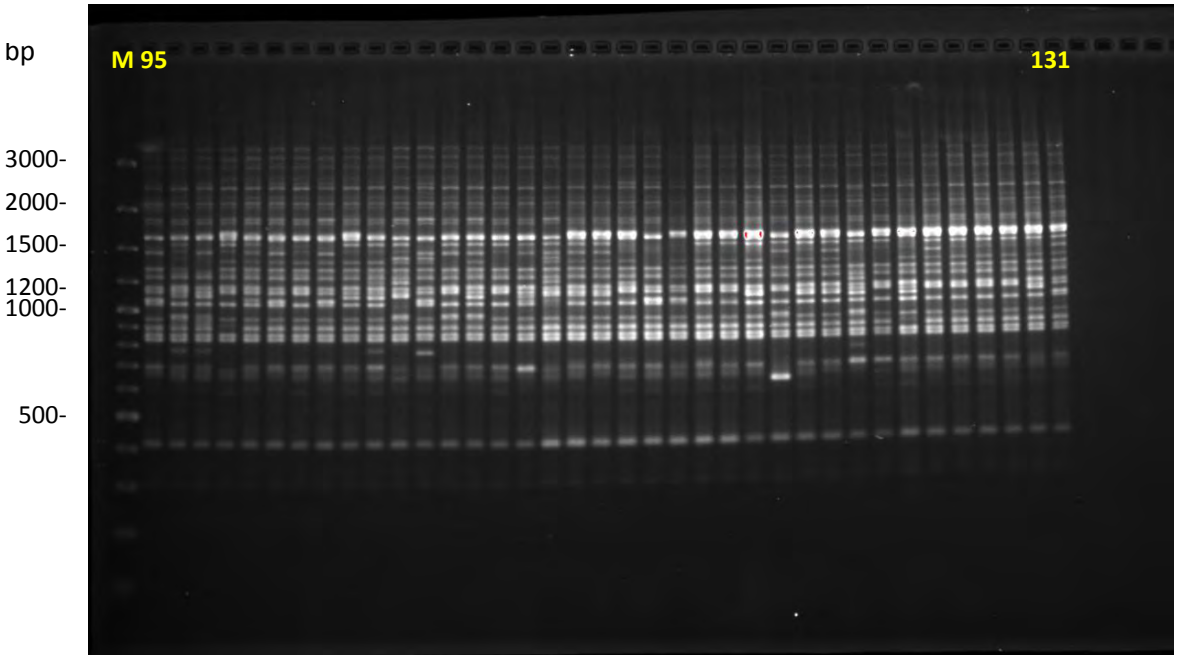
Appendix III Picture 7c: Gel imaging picture of the primer iPBS2377 revealing genetic diversity among 131 safflower accessions



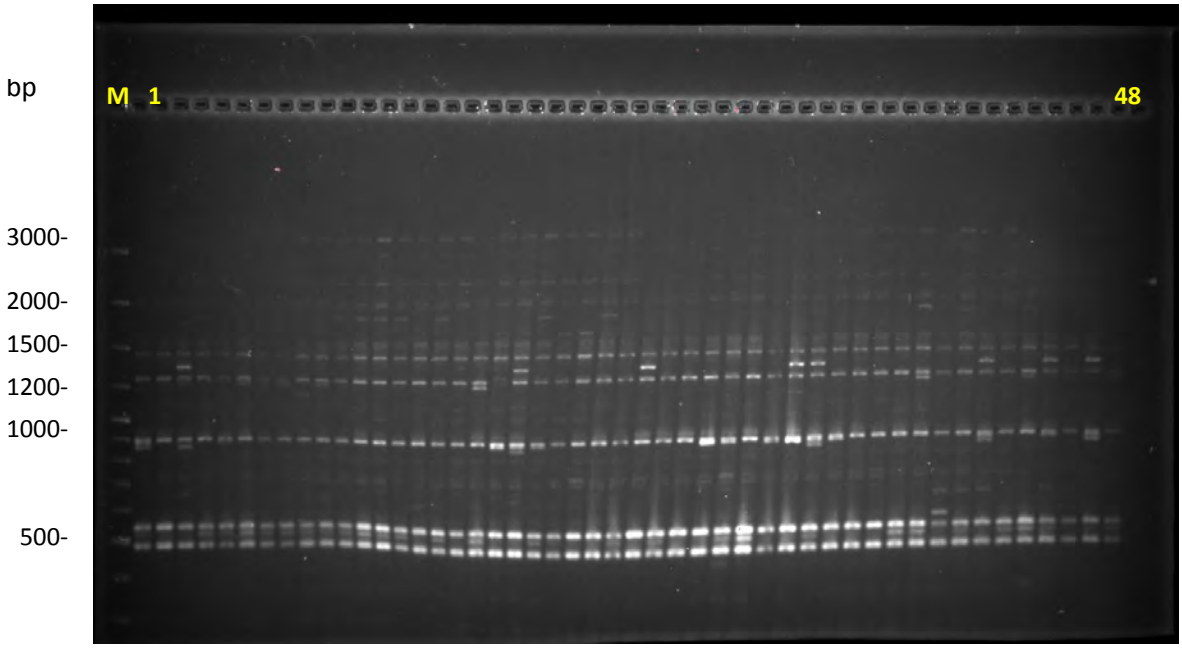
Appendix III Picture 8a: Gel imaging picture of the primer iPBS2383 revealing genetic diversity among 131 safflower accessions



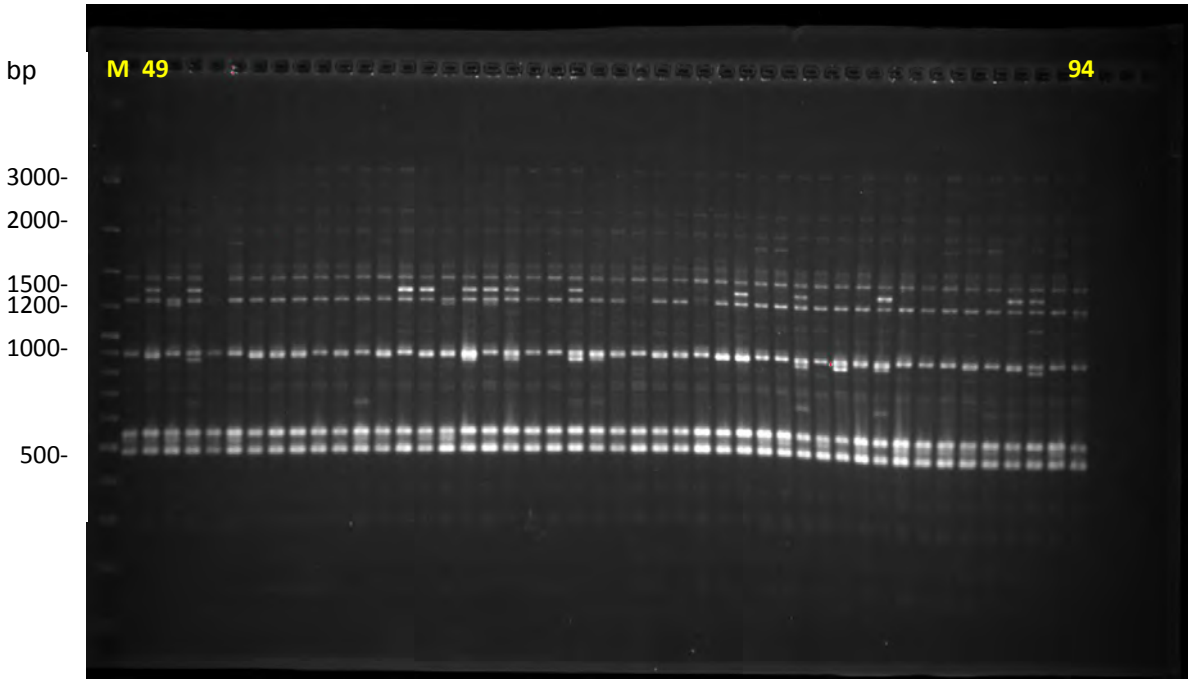
Appendix III Picture 8b: Gel imaging picture of the primer iPBS2383 revealing genetic diversity among 131 safflower accessions



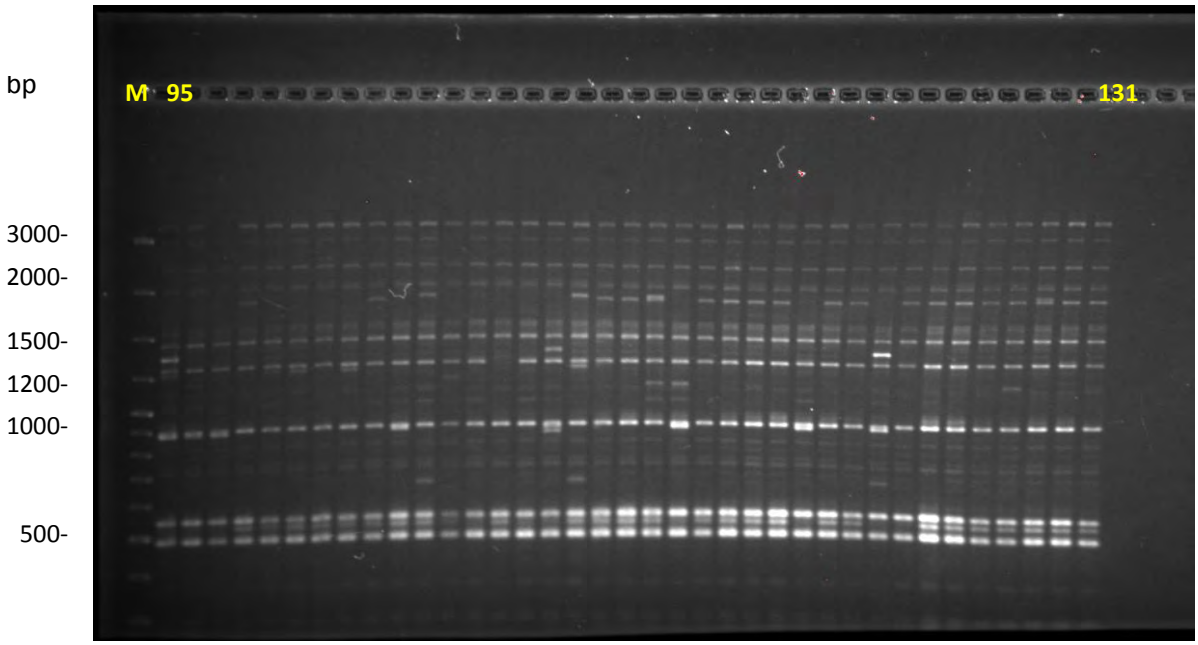
Appendix III Figure 8c: Gel imaging picture of the primer iPBS2383 revealing genetic diversity among 131 safflower accessions



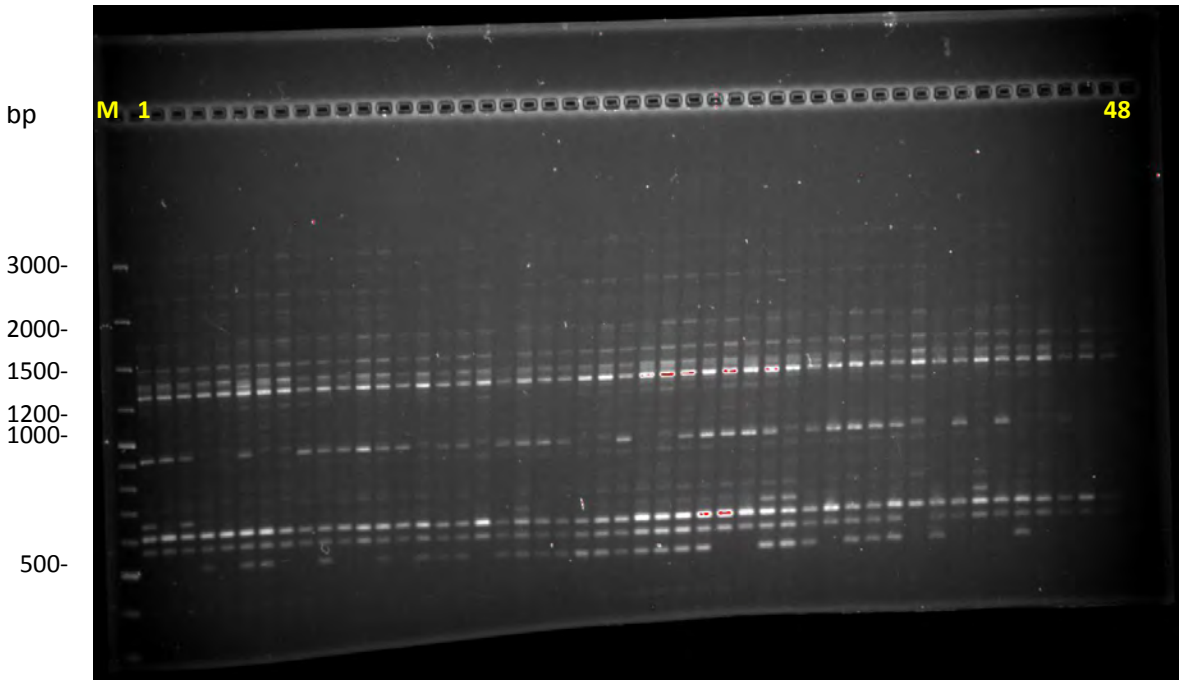
Appendix III Picture 9a: Gel imaging picture of the primer iPBS2391 revealing genetic diversity among 131 safflower accessions



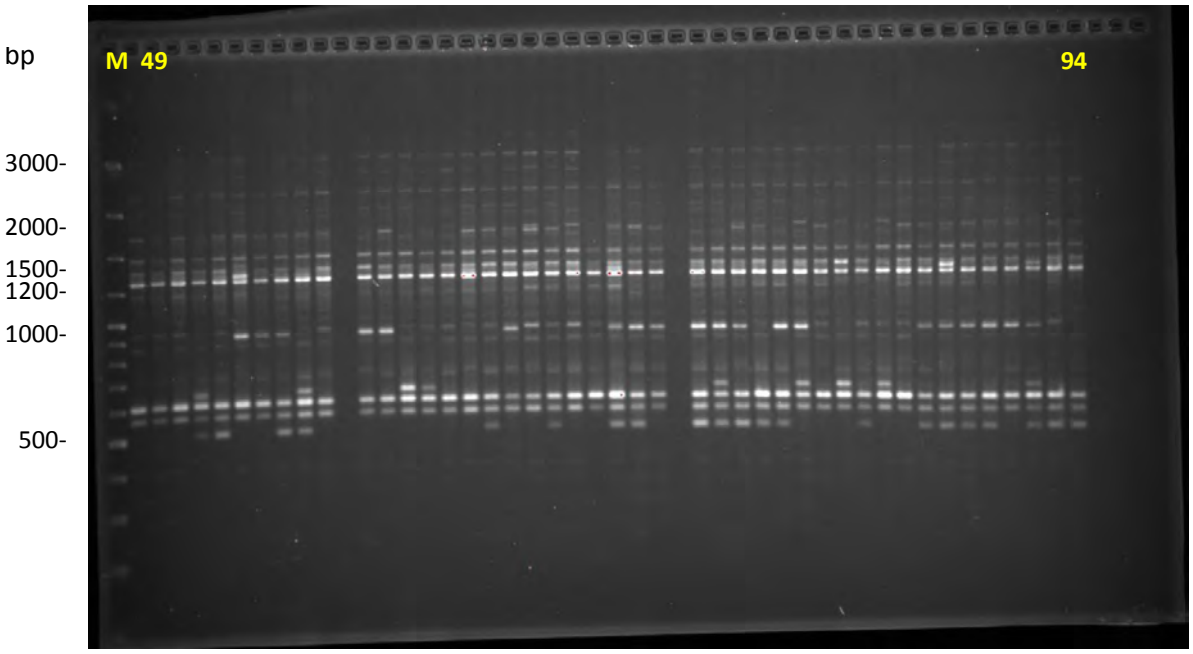
Appendix III Picture 9b: Gel imaging picture of the primer iPBS2391 revealing genetic diversity among 131 safflower accessions



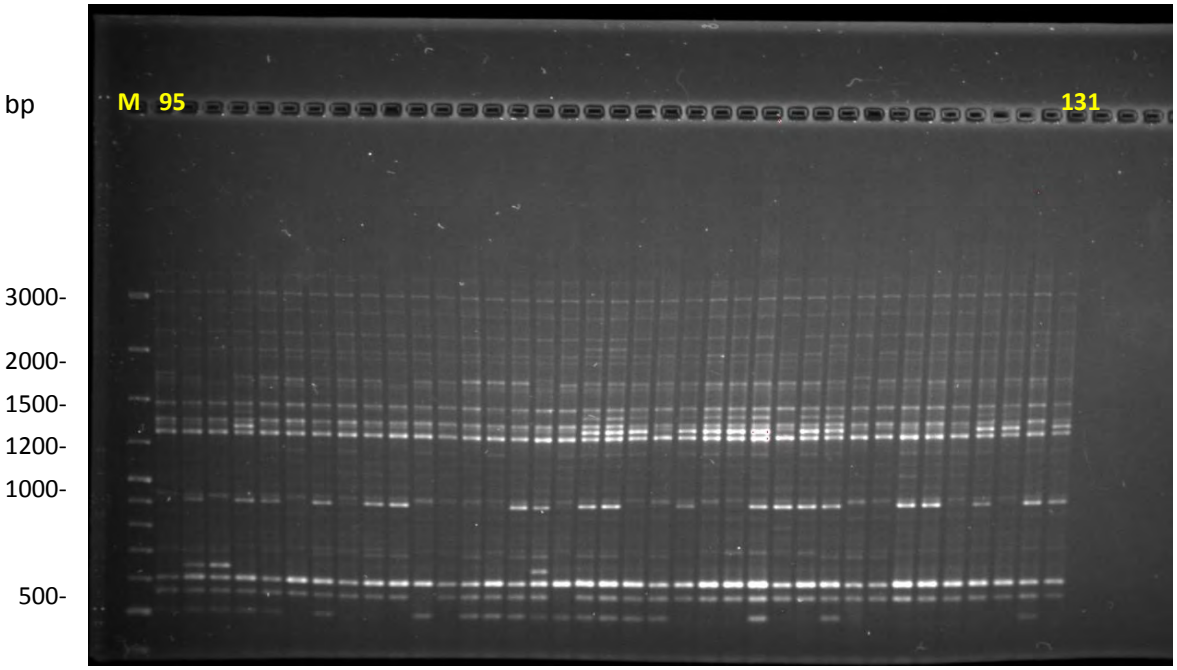
Appendix III Picture 9c: Gel imaging picture of the primer iPBS2391 revealing genetic diversity among 131 safflower accessions



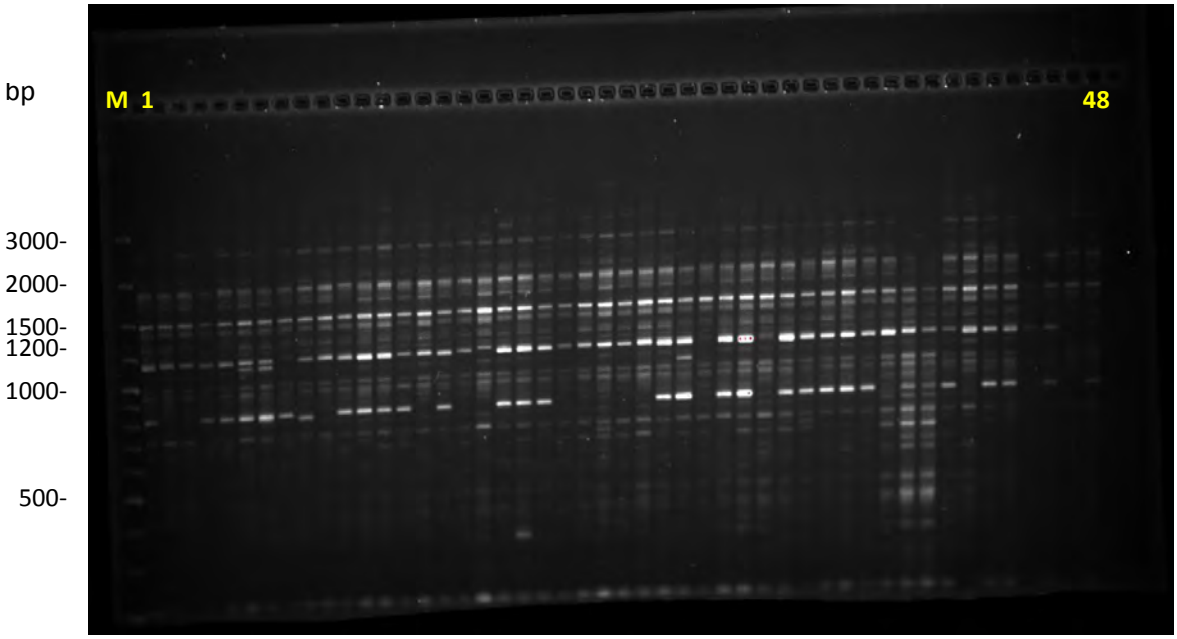
Appendix III Picture 10a: Gel imaging picture of the primer iPBS2392 revealing genetic diversity among 131 safflower accessions



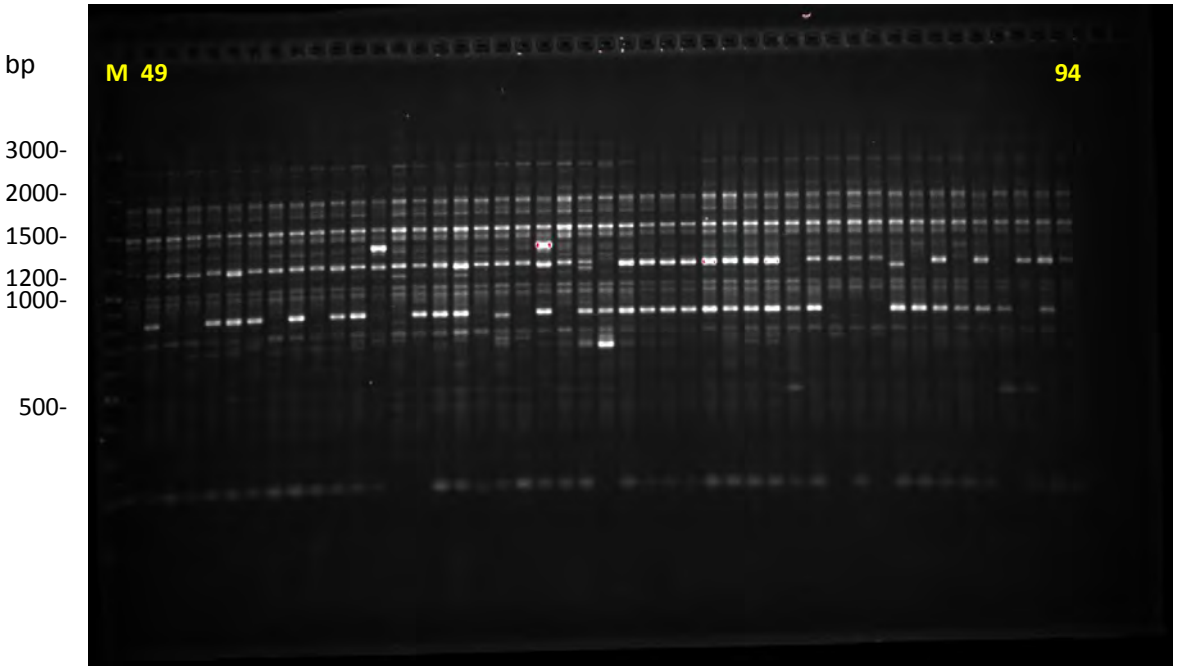
Appendix III Picture 10b: Gel imaging picture of the primer iPBS2392 revealing genetic diversity among 131 safflower accessions



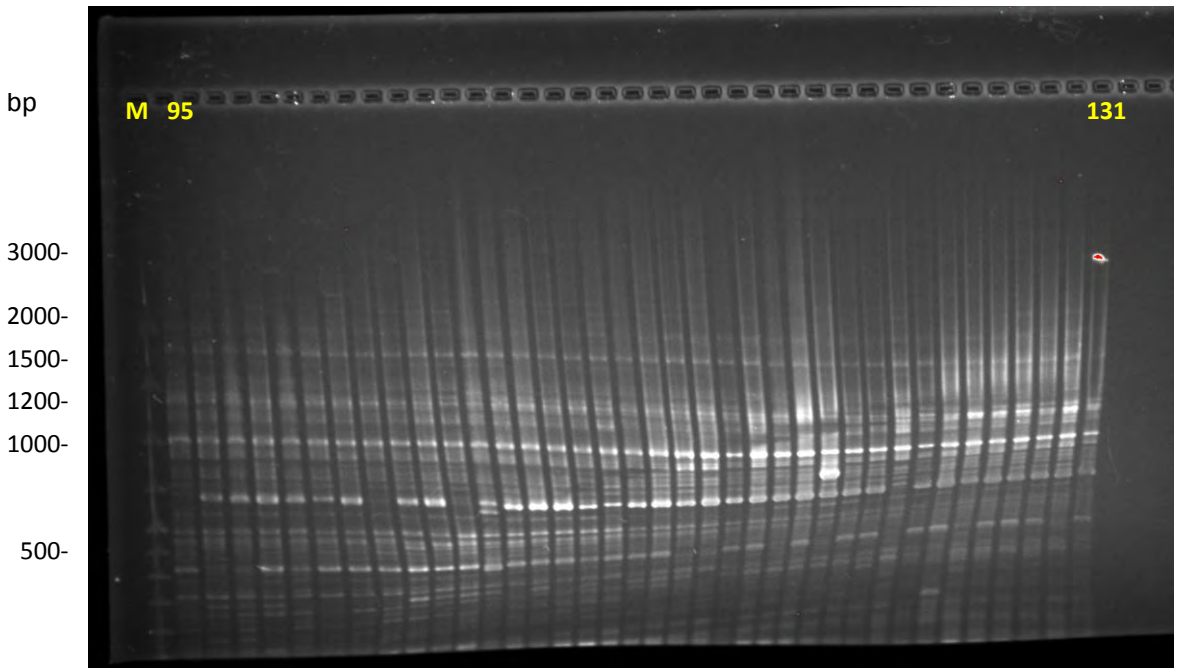
Appendix III Picture 10c: Gel imaging picture of the primer iPBS2392 revealing genetic diversity among 131 safflower accessions



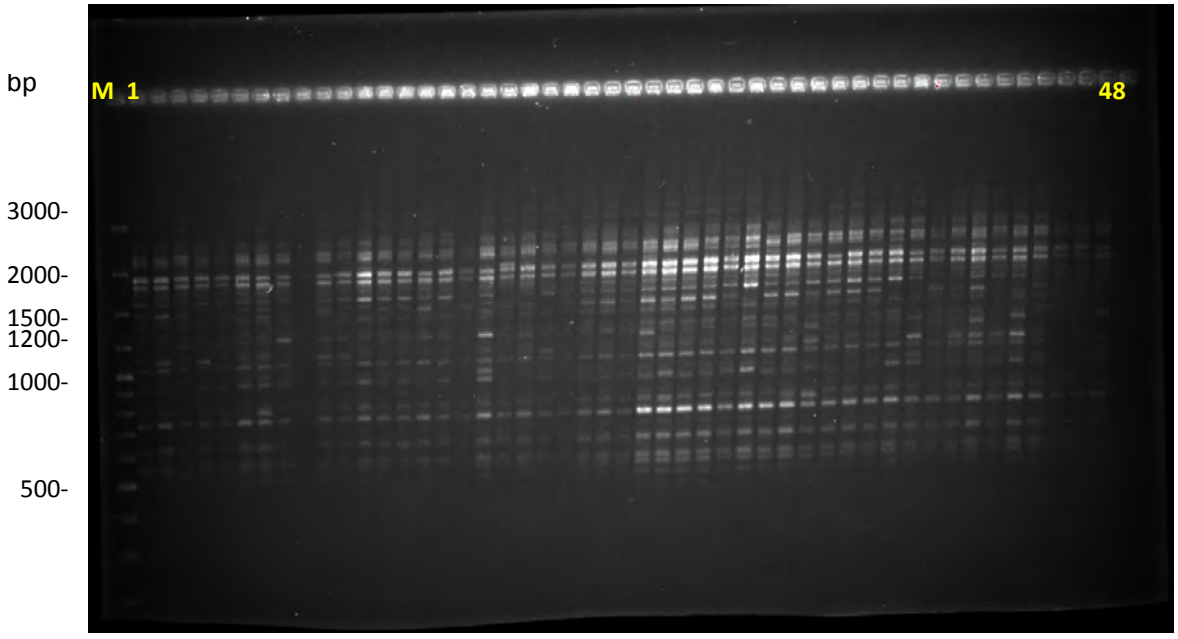
Appendix III Picture 11a: Gel imaging picture of the primer iPBS2398 revealing genetic diversity among 131 safflower accessions



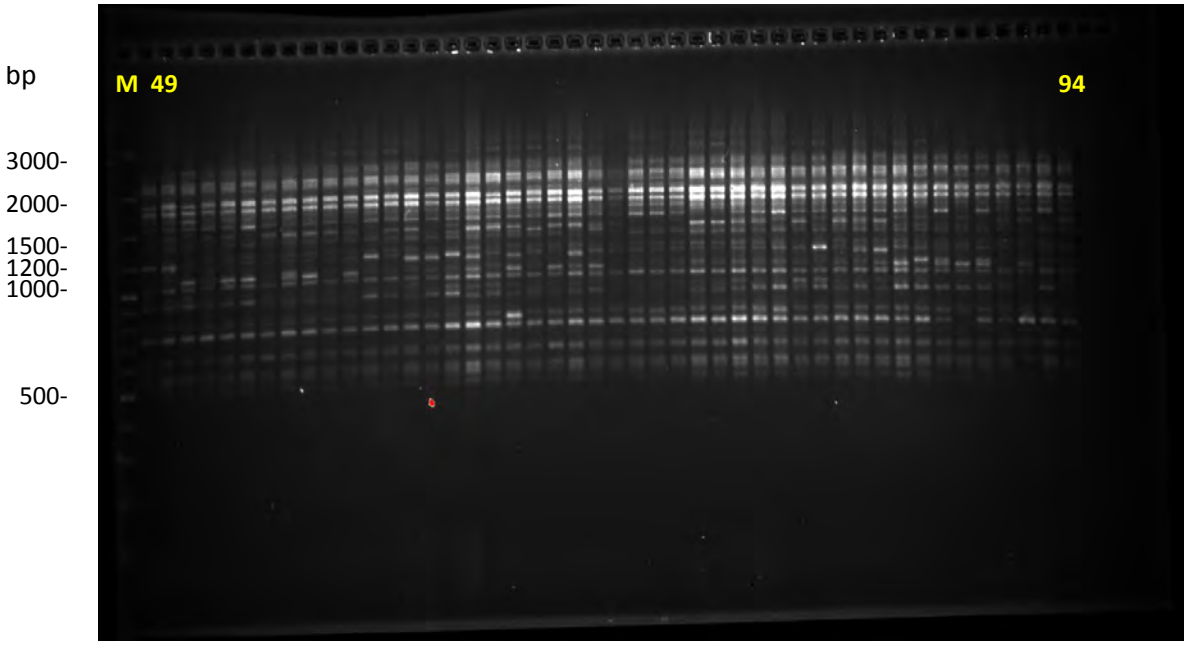
Appendix III Picture 11b: Gel imaging picture of the primer iPBS2398 revealing genetic diversity among 131 safflower accessions



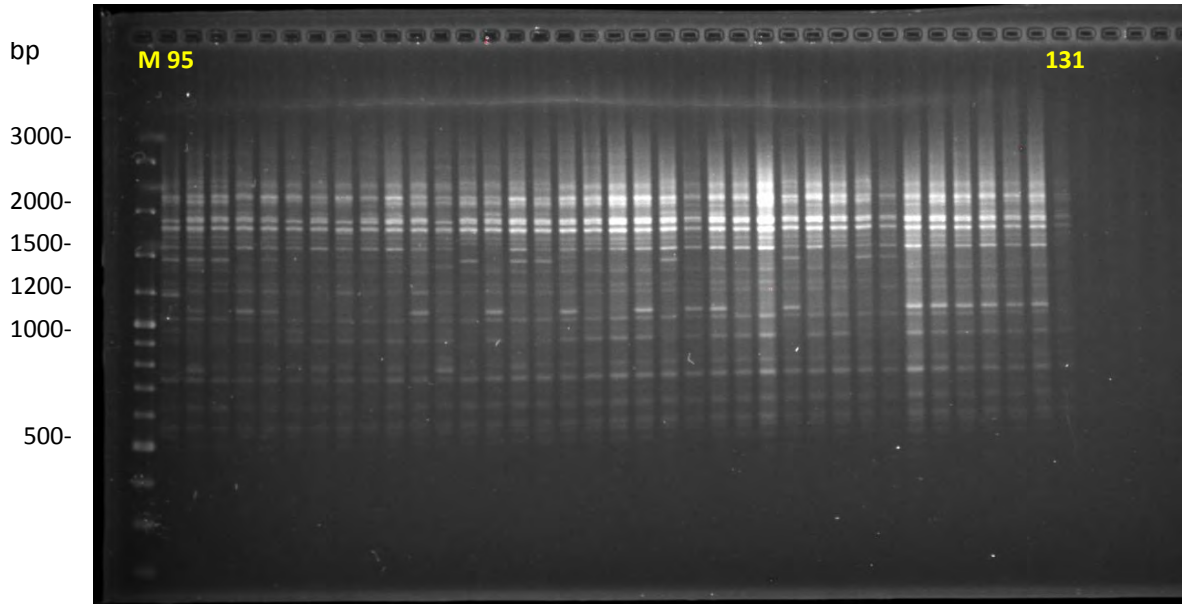
Appendix III Picture 11c: Gel imaging picture of the primer iPBS2398 revealing genetic diversity among 131 safflower accessions



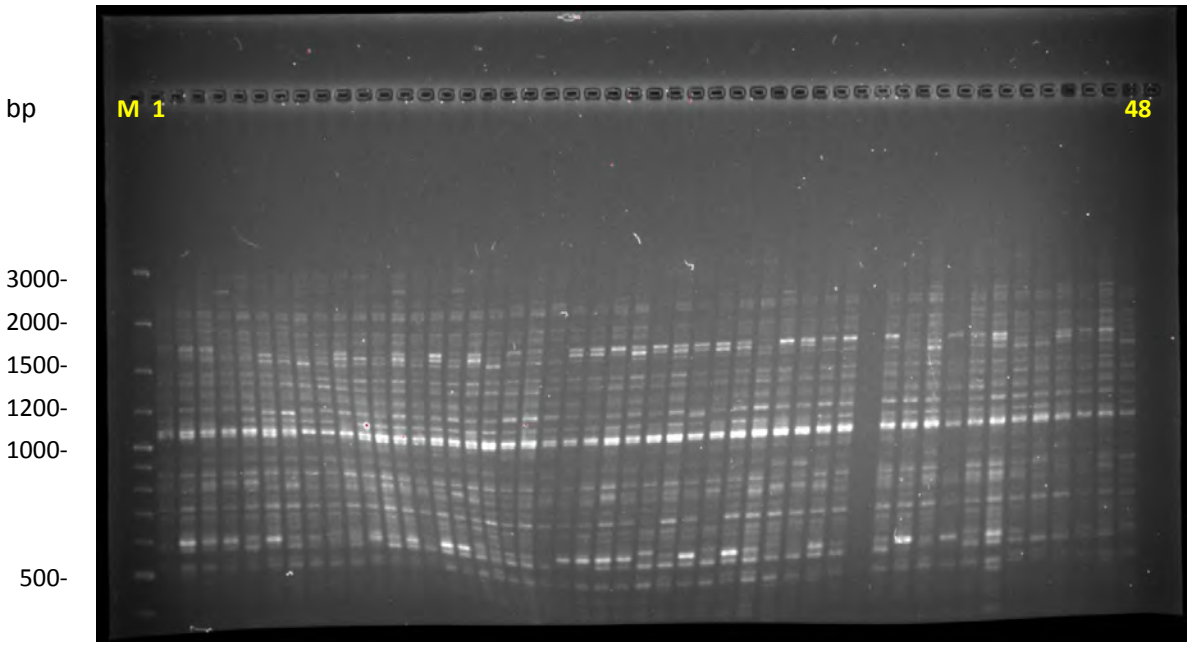
Appendix III Picture 12a: Gel imaging picture of the primer iPBS2399 revealing genetic diversity among 131 safflower accessions



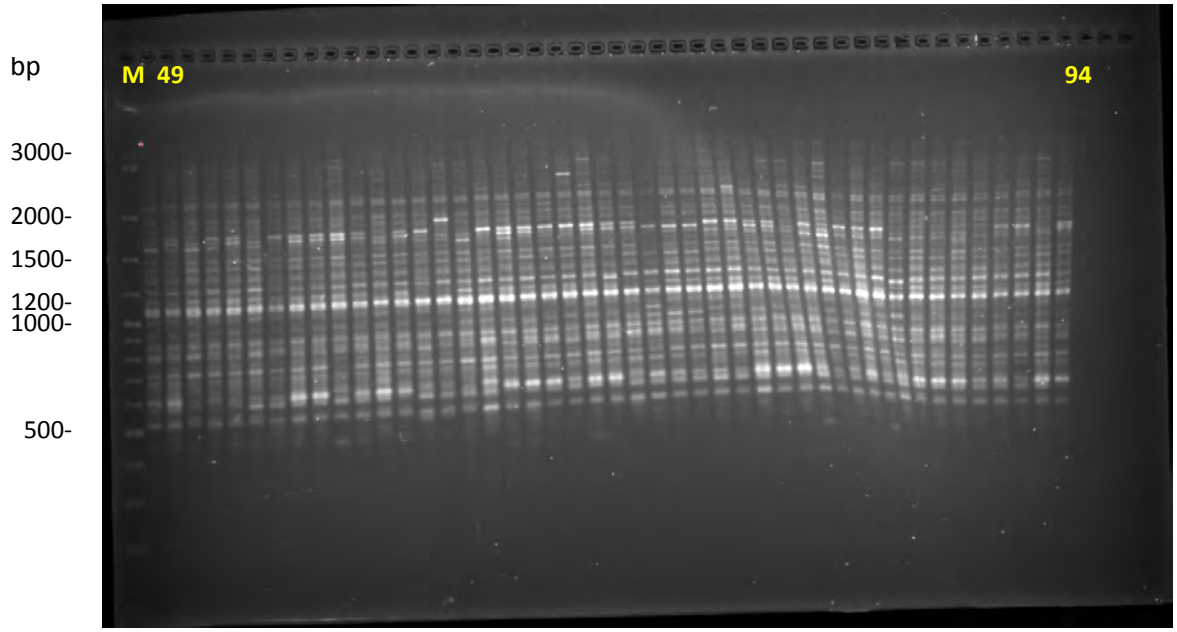
Appendix III Picture 12b: Gel imaging picture of the primer iPBS2399 revealing genetic diversity among 131 safflower accessions



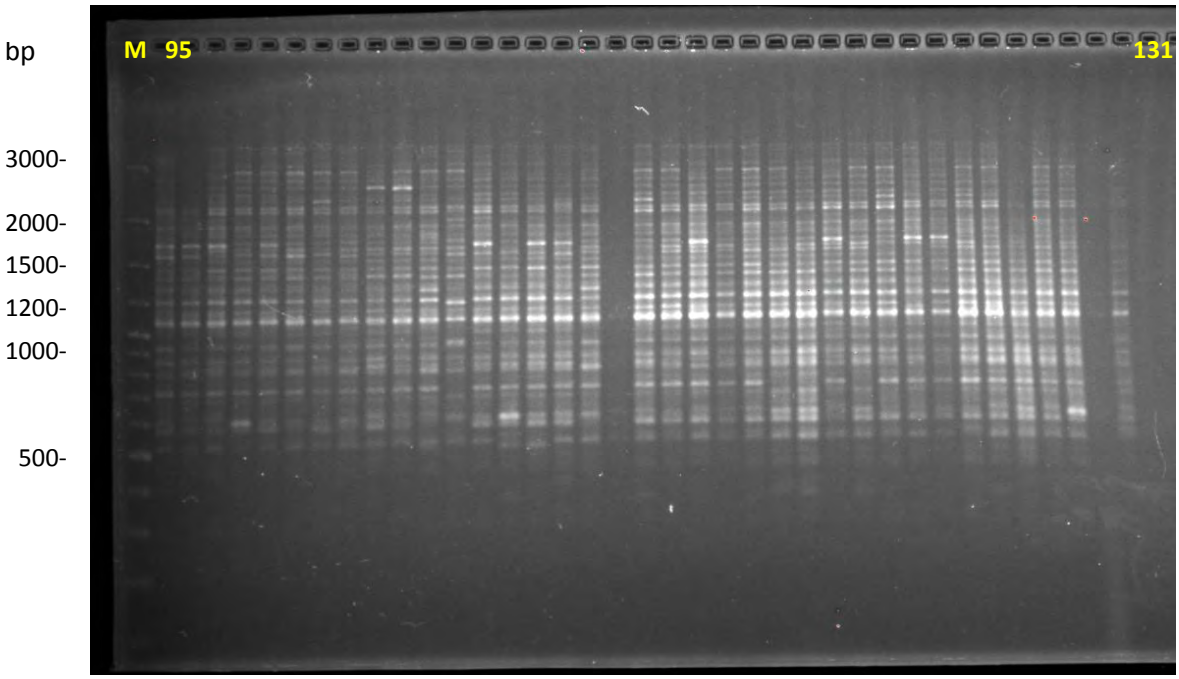
Appendix III Picture 12c: Gel imaging picture of the primer iPBS2399 revealing genetic diversity among 131 safflower accessions



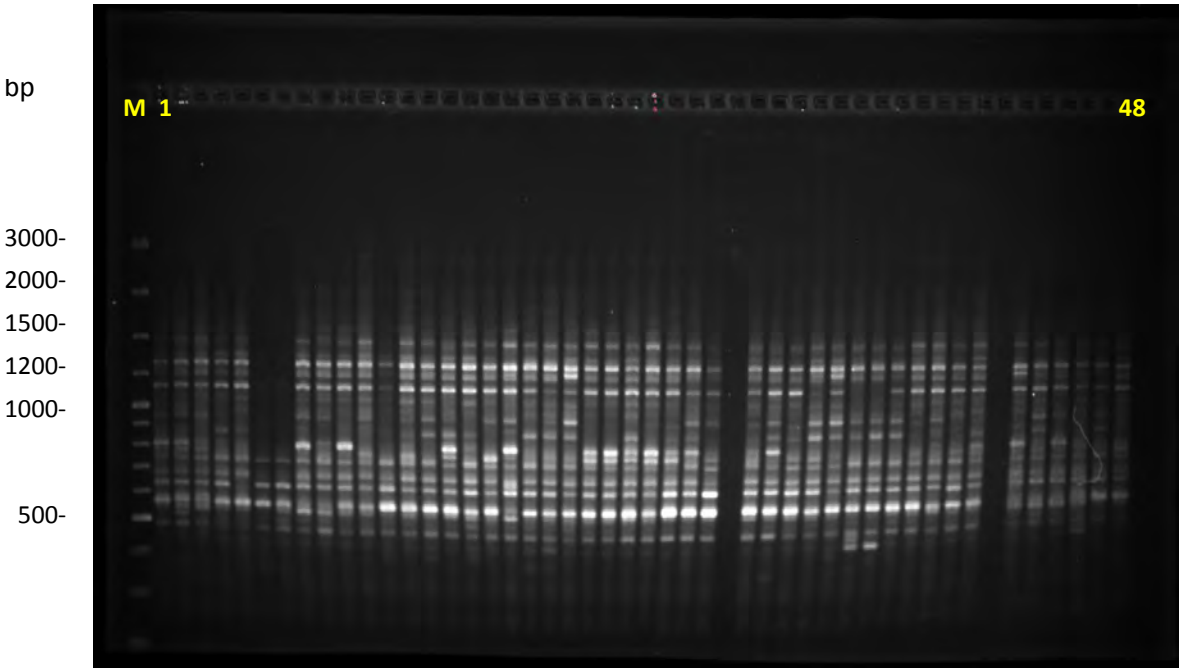
Appendix III Picture 13a: Gel imaging picture of the primer iPBS2401 revealing genetic diversity among 131 safflower accessions



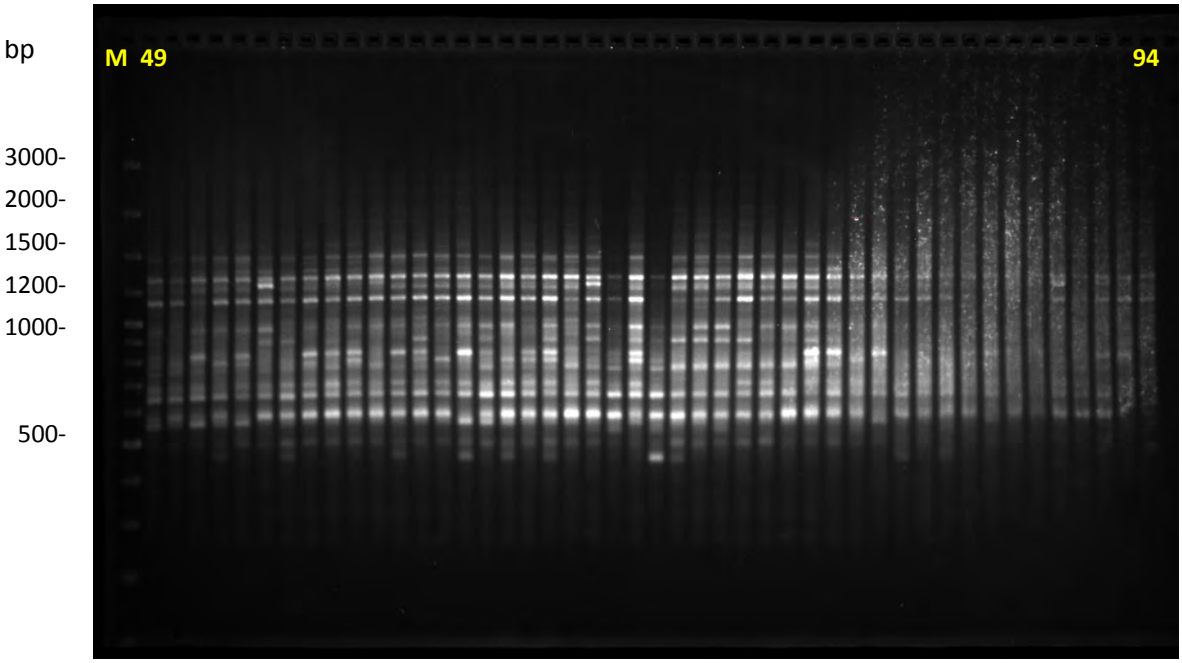
Appendix III Picture 13b: Gel imaging picture of the primer iPBS2401 revealing genetic diversity among 131 safflower accessions



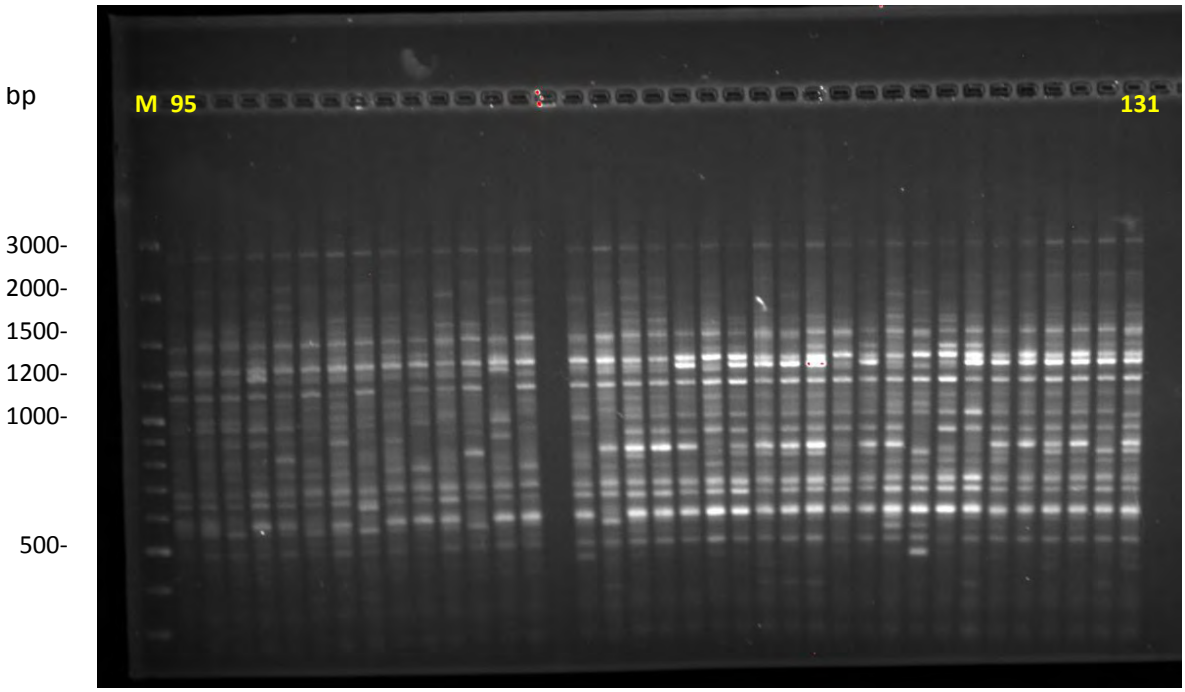
Appendix III Picture 13c: Gel imaging picture of the primer iPBS2401 revealing genetic diversity among 131 safflower accessions



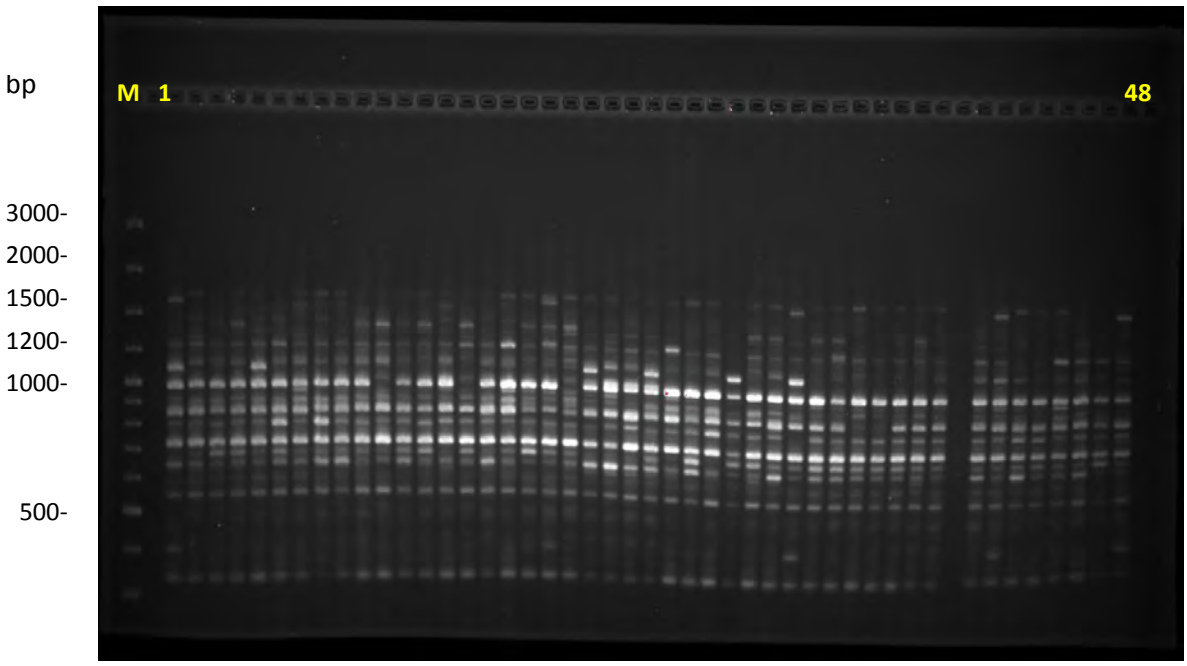
Appendix III Picture 1a: Gel imaging picture of the primer ISSR809 revealing genetic diversity among 131 safflower accessions



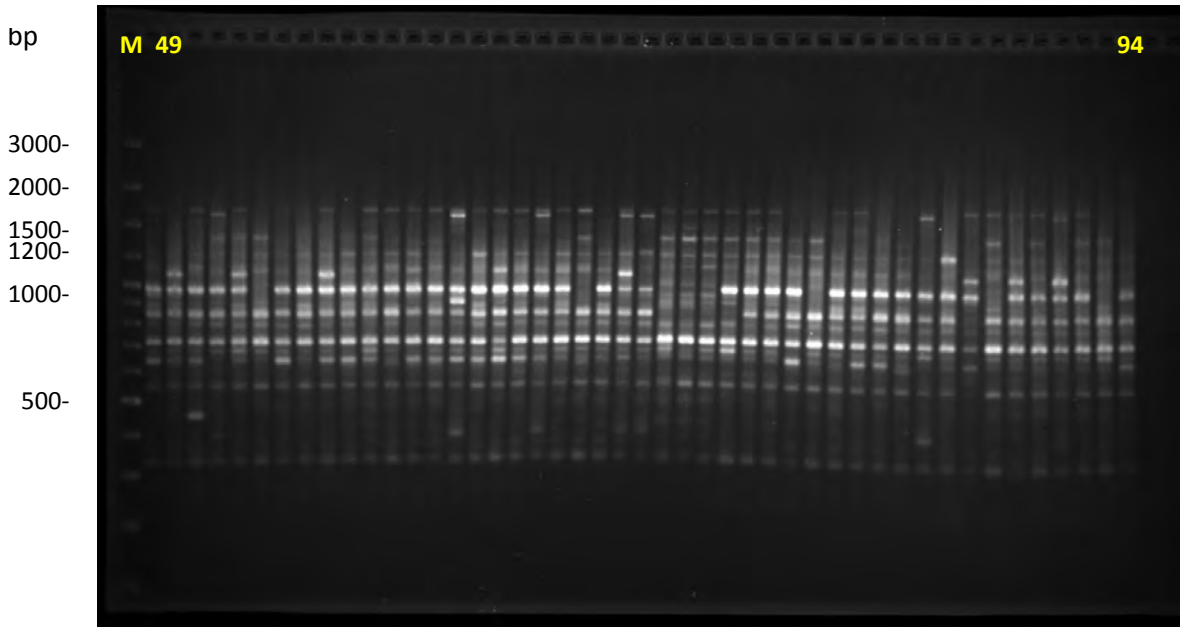
Appendix IV Picture 1b: Gel imaging picture of the primer ISSR809 revealing genetic diversity among 131 safflower accessions



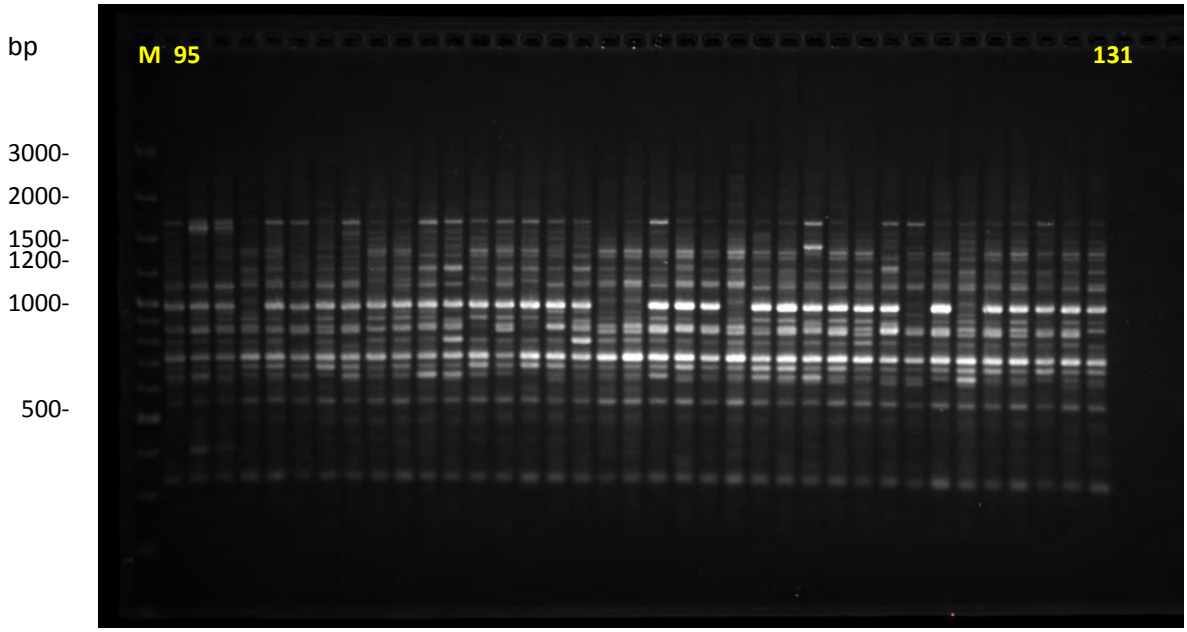
Appendix IV Picture 1c: Gel imaging picture of the primer ISSR809 revealing genetic diversity among 131 safflower accessions



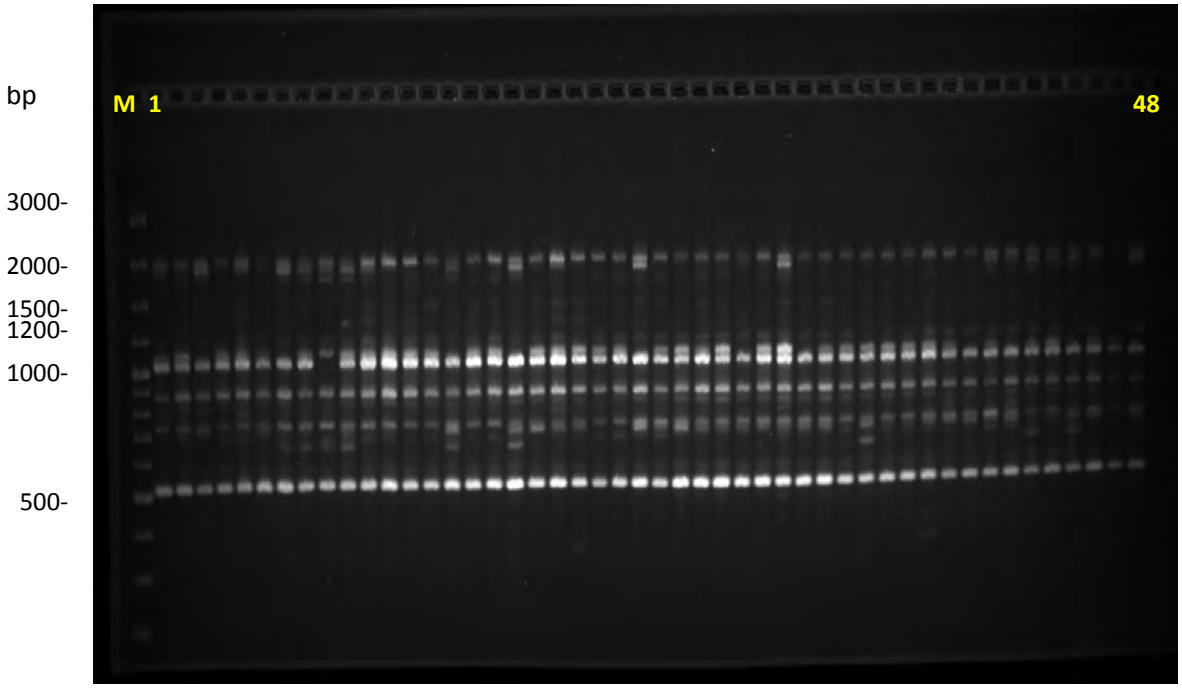
Appendix IV Picture 2a: Gel imaging picture of the primer ISSR810 revealing genetic diversity among 131 safflower accessions



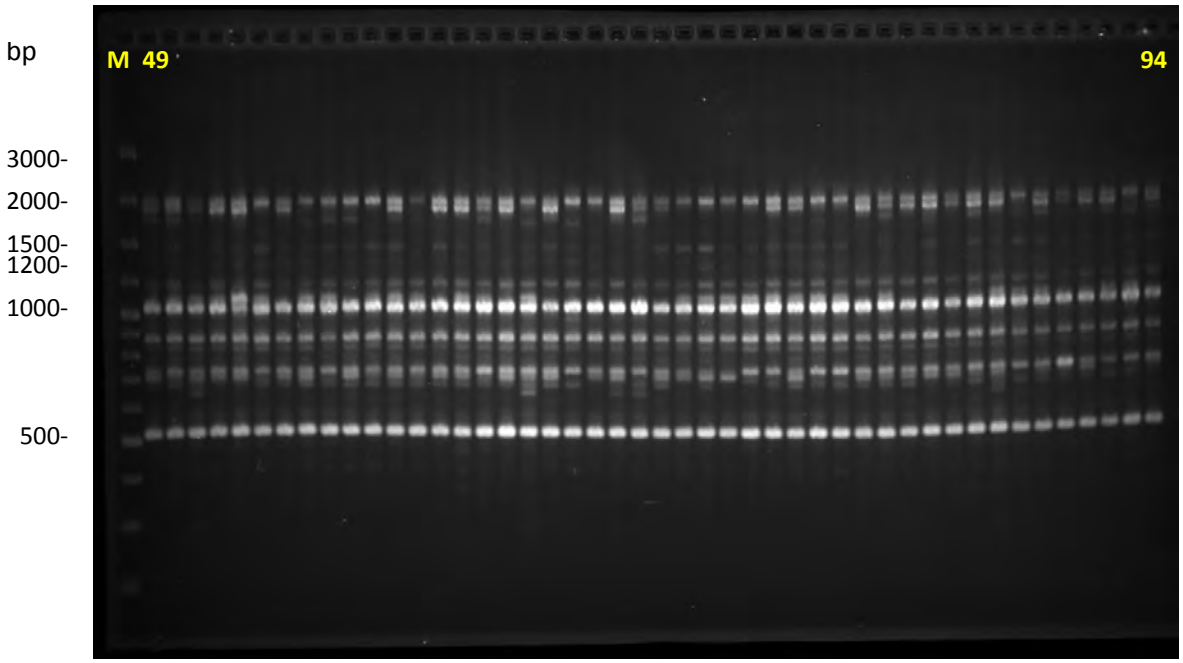
Appendix IV Picture 2b: Gel imaging picture of the primer ISSR810 revealing genetic diversity among 131 safflower accessions



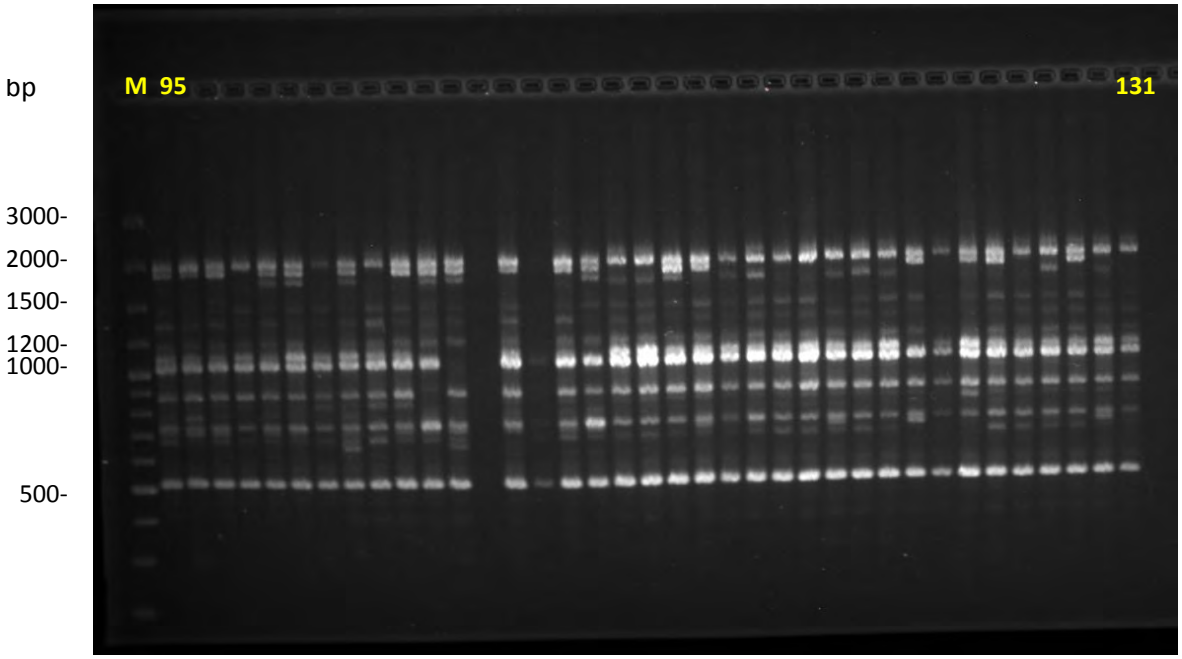
Appendix IV Picture 2c: Gel imaging picture of the primer ISSR810 revealing genetic diversity among 131 safflower accessions



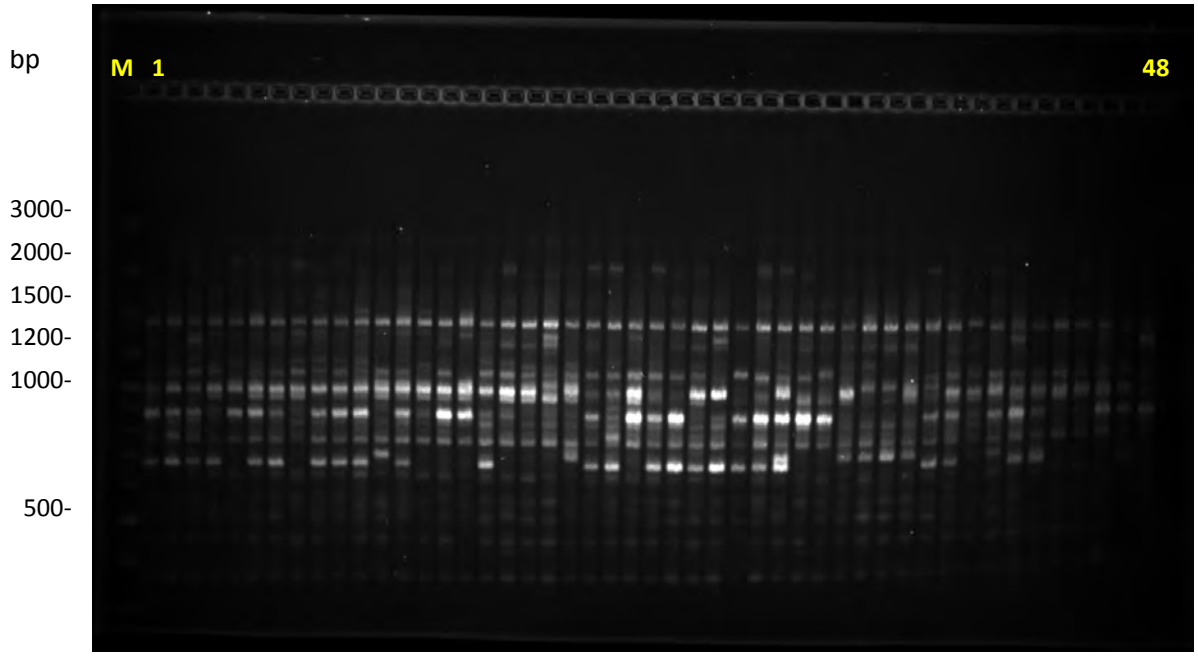
Appendix IV Picture 3a: Gel imaging picture of the primer ISSR811 revealing genetic diversity among 131 safflower accessions



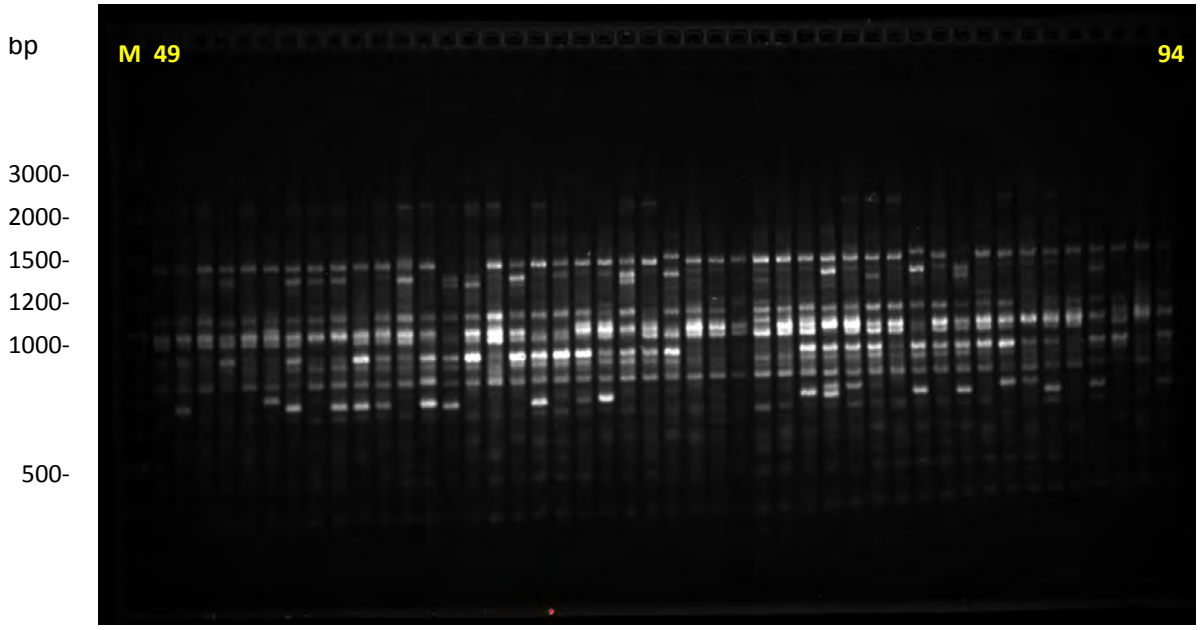
Appendix IV Picture 3b: Gel imaging picture of the primer ISSR811 revealing genetic diversity among 131 safflower accessions



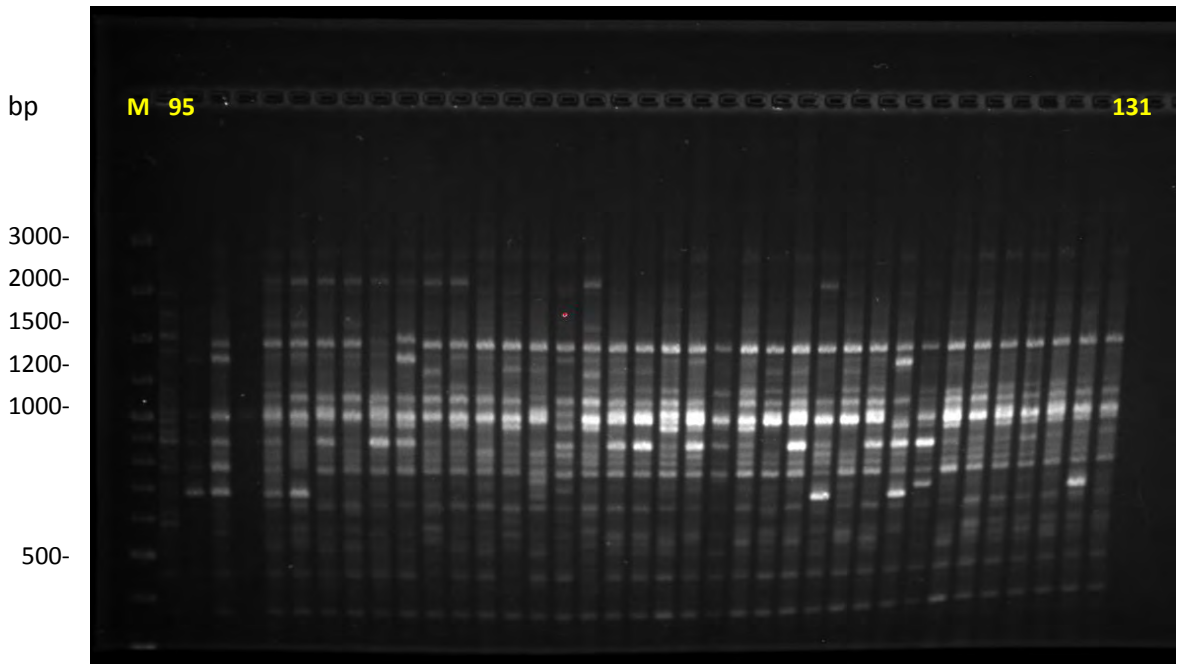
Appendix IV Picture 3c: Gel imaging picture of the primer ISSR811 revealing genetic diversity among 131 safflower accessions



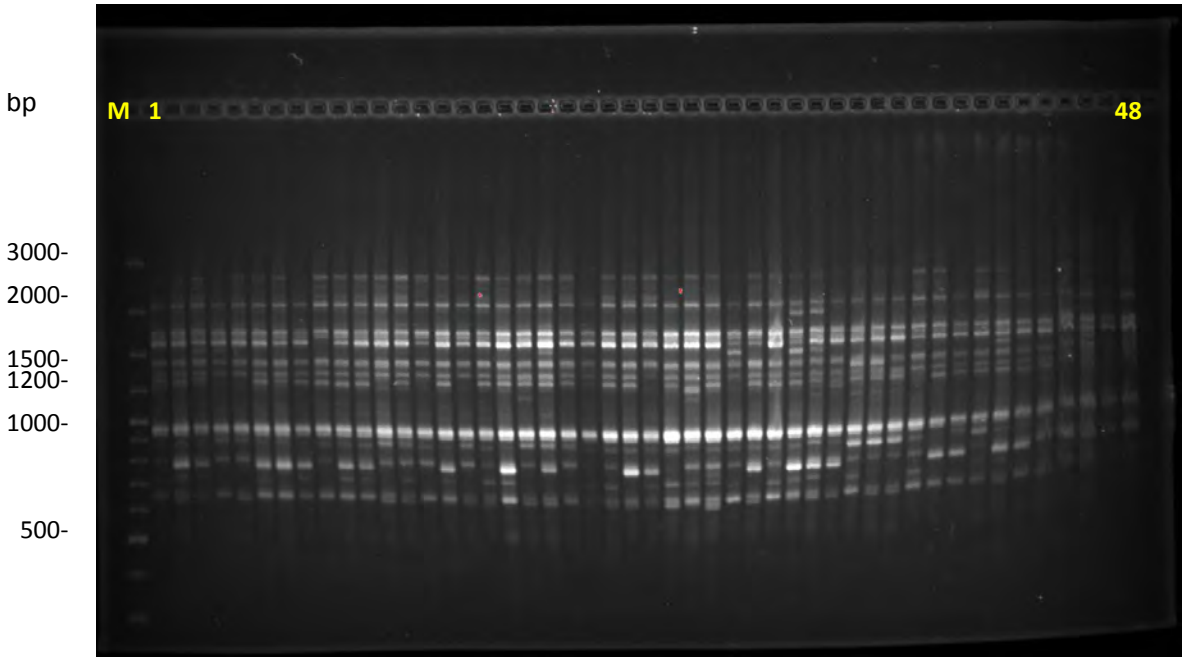
Appendix IV Picture 4a: Gel imaging picture of the primer ISSR812 revealing genetic diversity among 131 safflower accessions



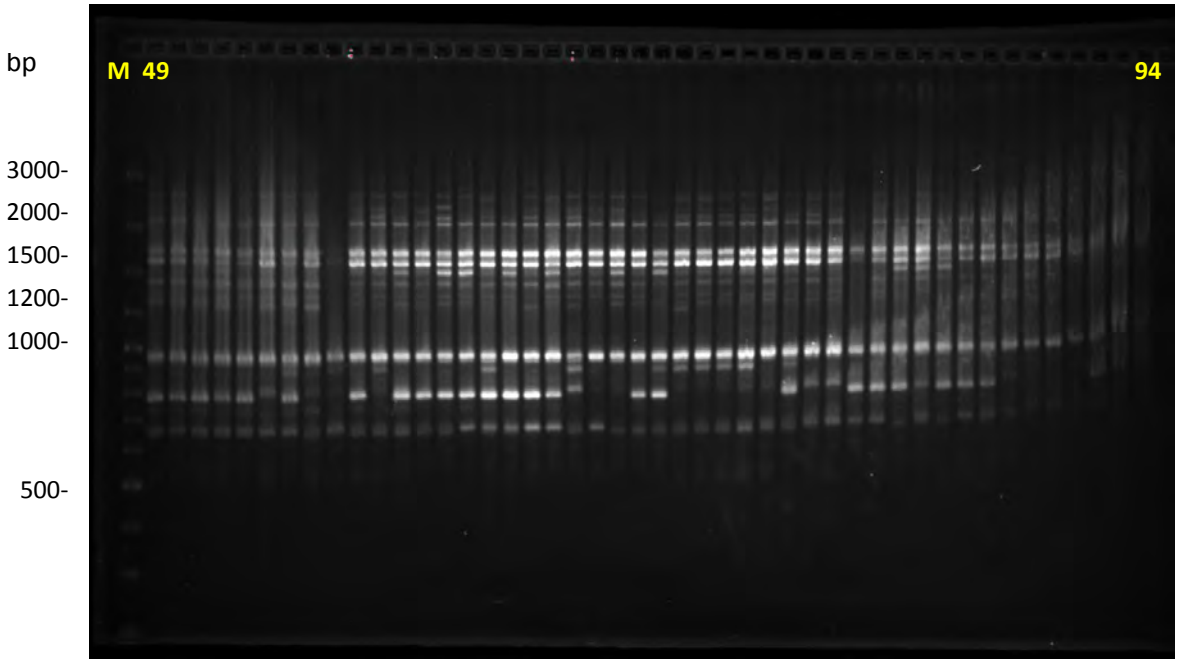
Appendix IV Picture 4b: Gel imaging picture of the primer ISSR812 revealing genetic diversity among 131 safflower accessions



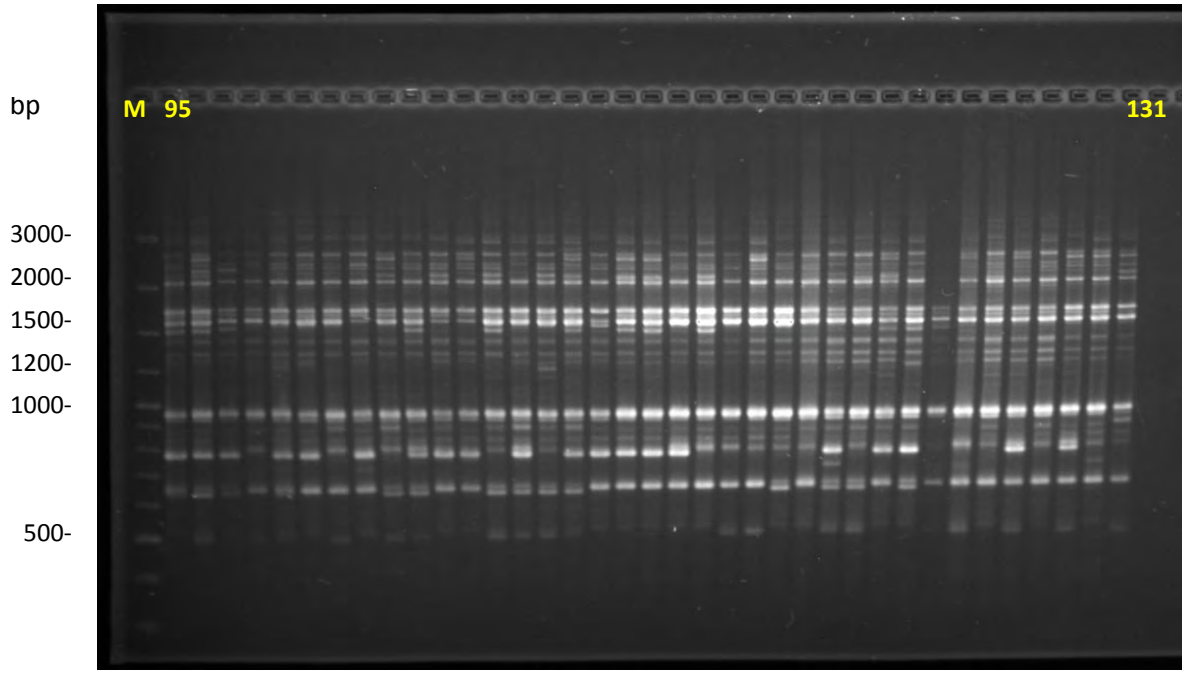
Appendix IV Picture 4c: Gel imaging picture of the primer ISSR812 revealing genetic diversity among 131 safflower accessions



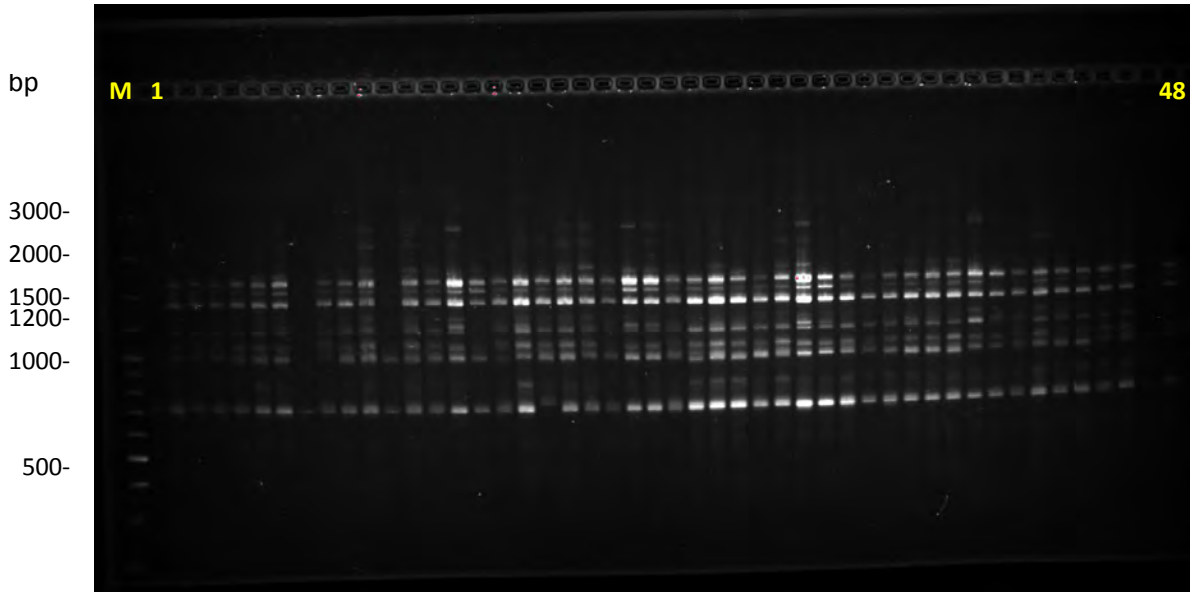
Appendix IV Picture 5a: Gel imaging picture of the primer ISSR817 revealing genetic diversity among 131 safflower accessions



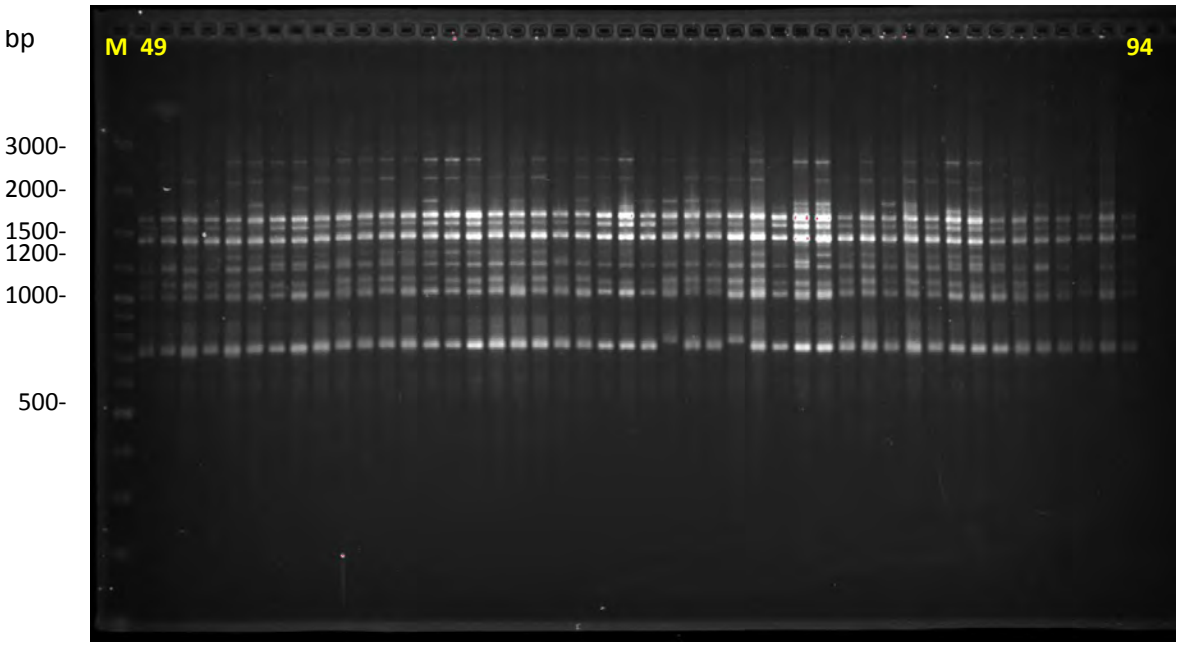
Appendix IV Picture 5b: Gel imaging picture of the primer ISSR817 revealing genetic diversity among 131 safflower accessions



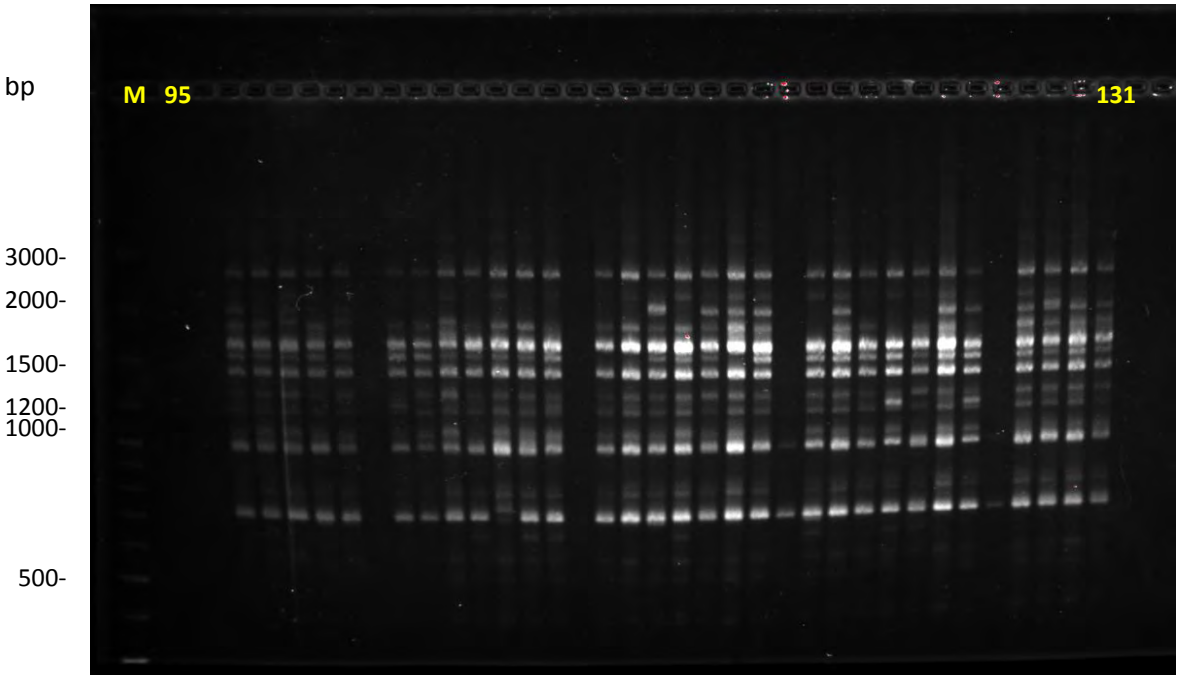
Appendix IV Picture 5c: Gel imaging picture of the primer ISSR817 revealing genetic diversity among 131 safflower accessions



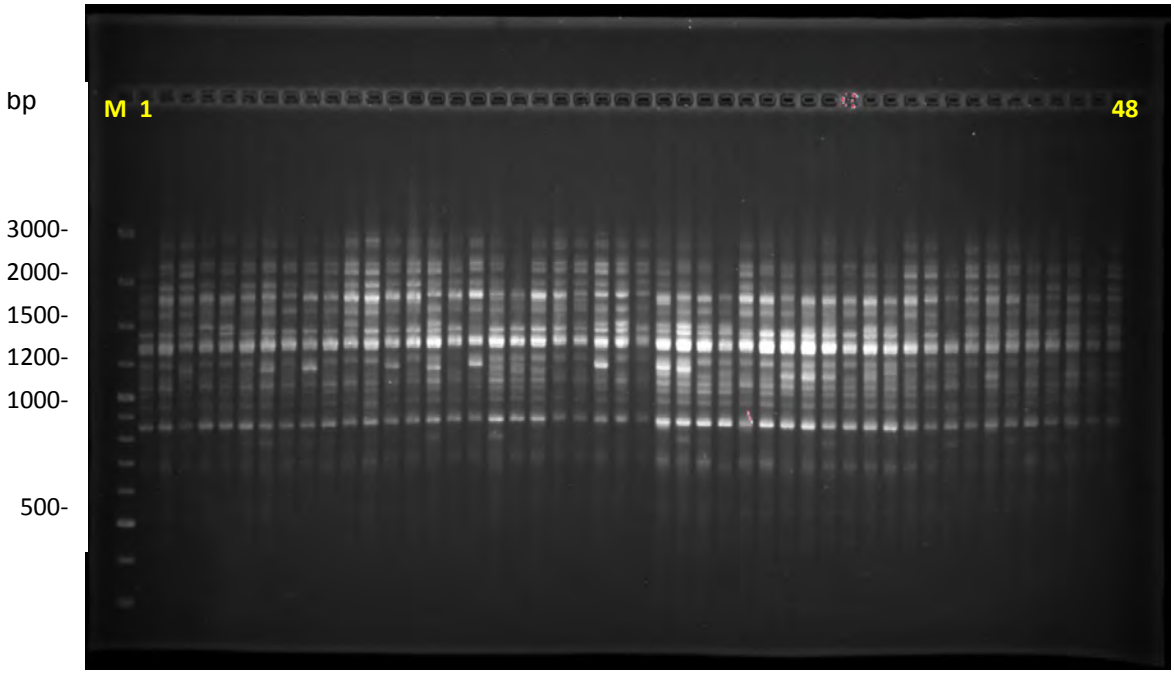
Appendix IV Picture 6a: Gel imaging picture of the primer ISSR818 revealing genetic diversity among 131 safflower accessions



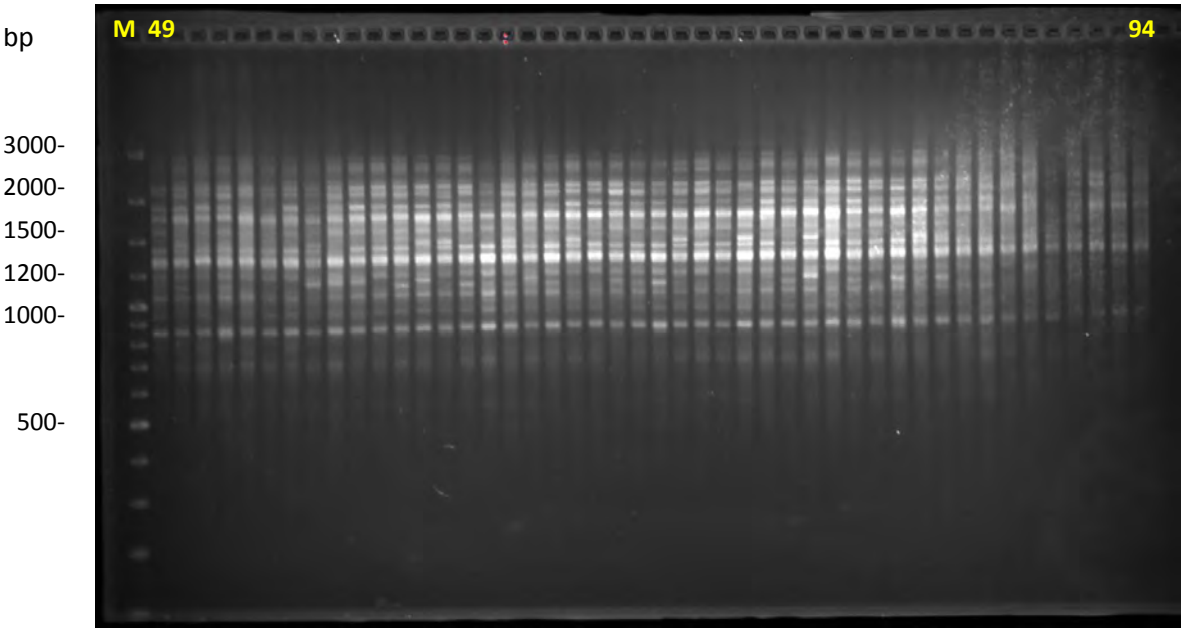
Appendix IV Picture 6b: Gel imaging picture of the primer ISSR818 revealing genetic diversity among 131 safflower accessions



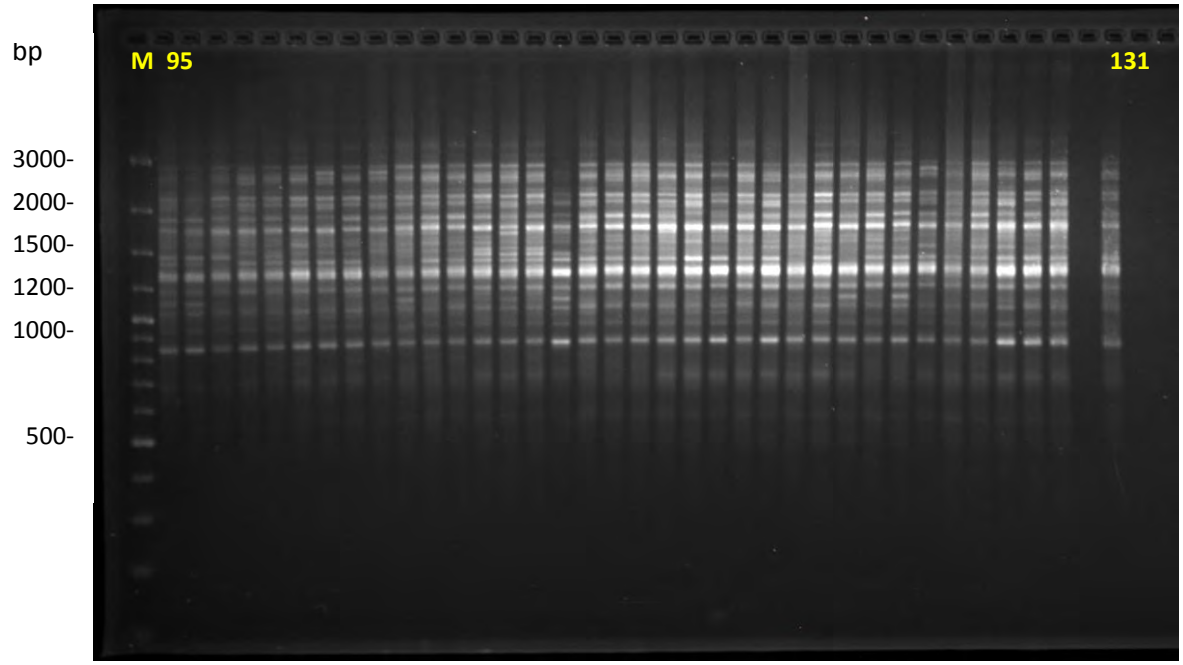
Appendix IV Picture 6c: Gel imaging picture of the primer ISSR818 revealing genetic diversity among 131 safflower accessions



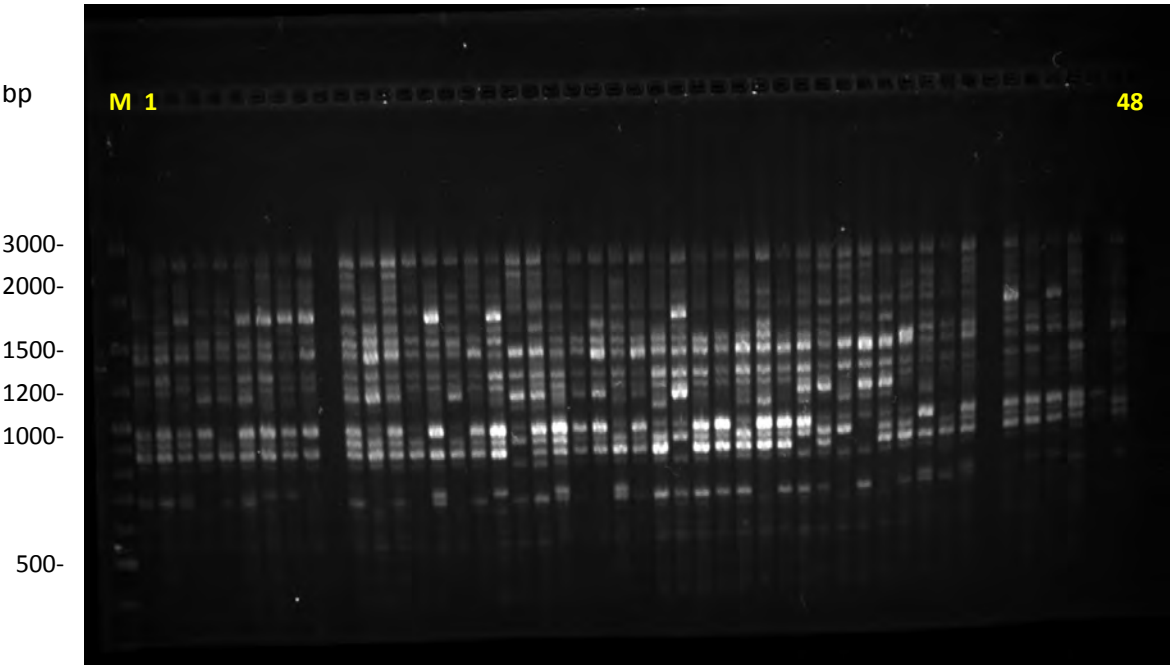
Appendix IV Picture 7a: Gel imaging picture of the primer ISSR819 revealing genetic diversity among 131 safflower accessions



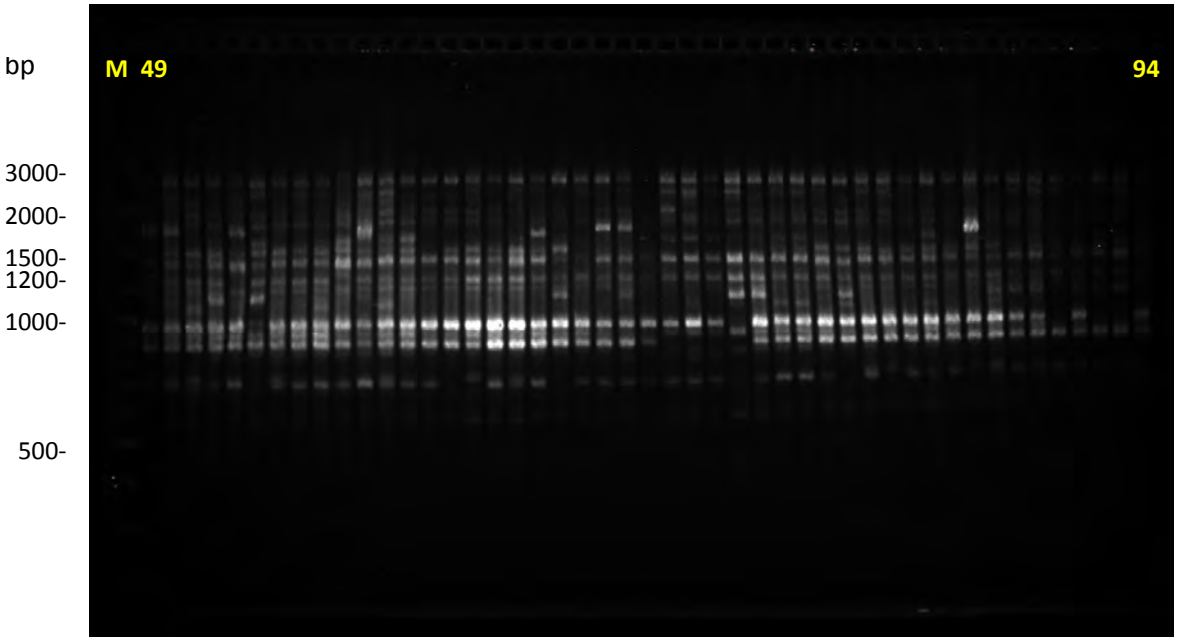
Appendix IV Picture 7b: Gel imaging picture of the primer ISSR819 revealing genetic diversity among 131 safflower accessions



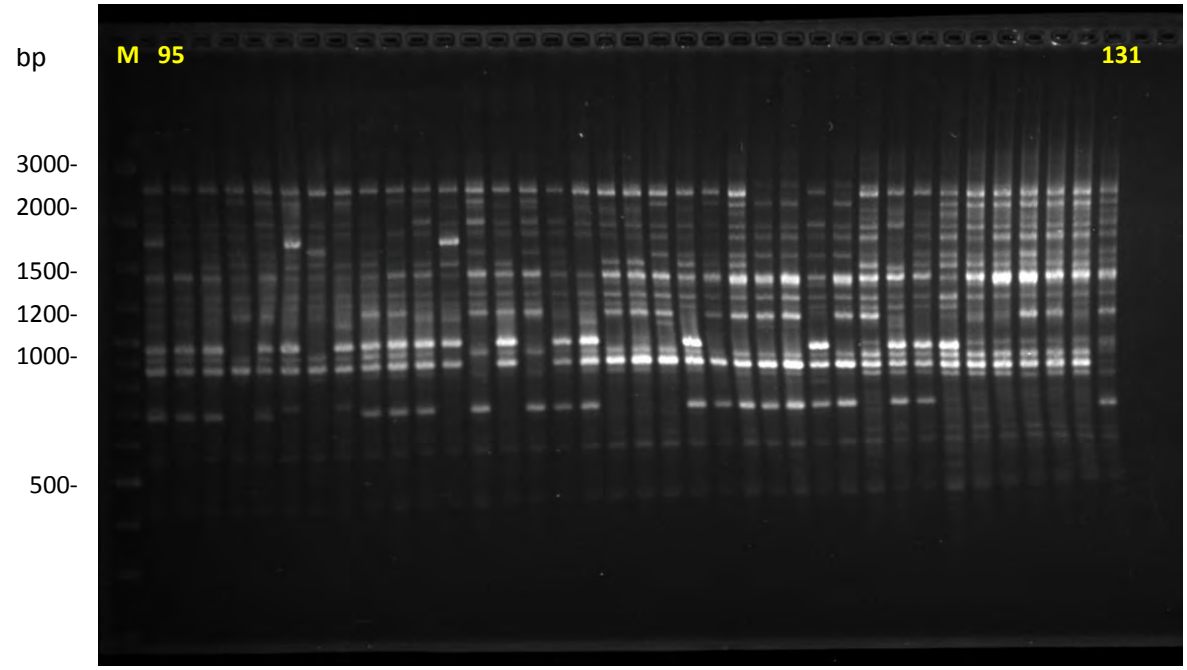
Appendix IV Picture 7c: Gel imaging picture of the primer ISSR819 revealing genetic diversity among 131 safflower accessions



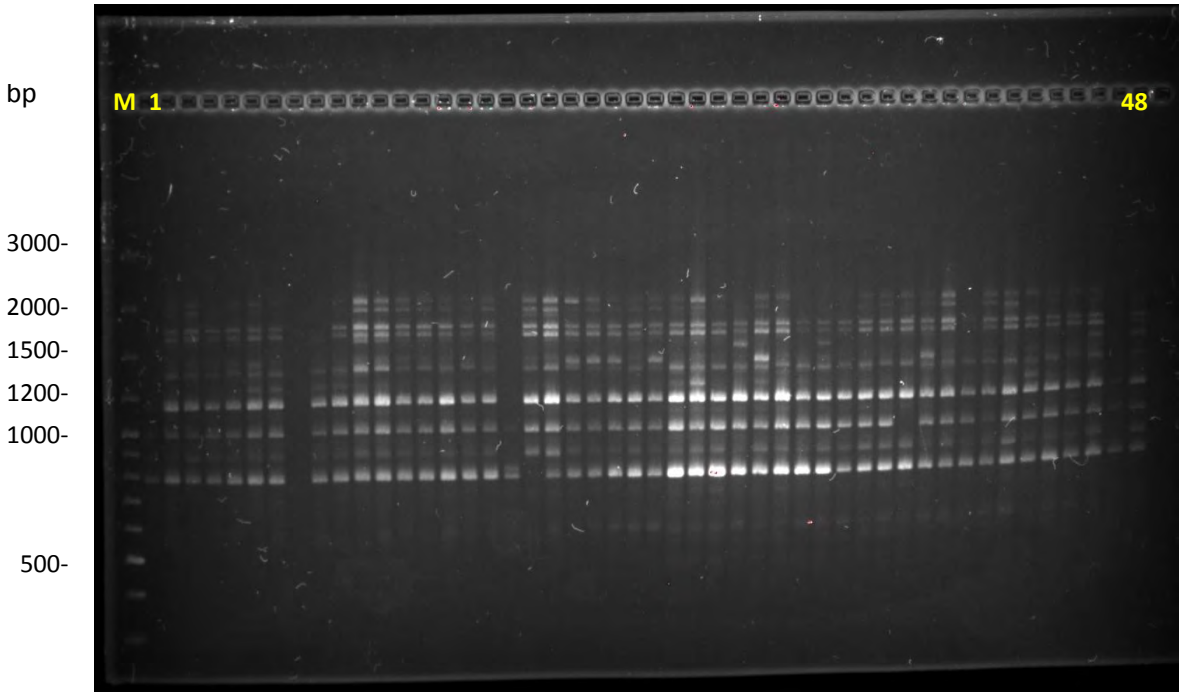
Appendix IV Picture 8a: Gel imaging picture of the primer ISSR827 revealing genetic diversity among 131 safflower accessions



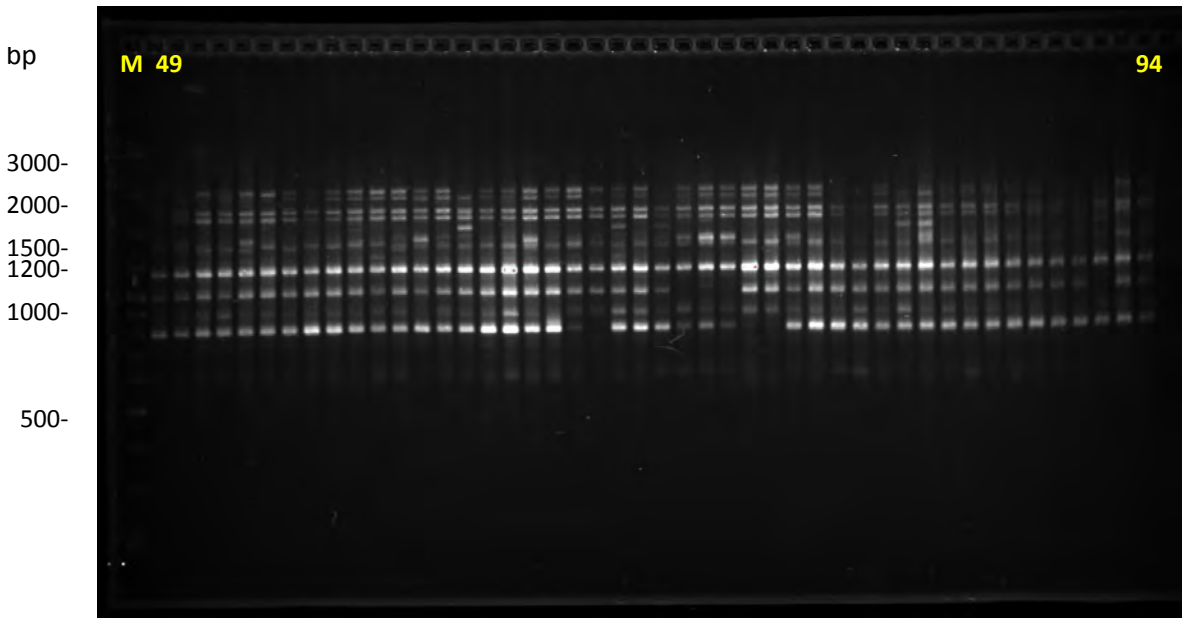
Appendix IV Picture 8b: Gel imaging picture of the primer ISSR827 revealing genetic diversity among 131 safflower accessions



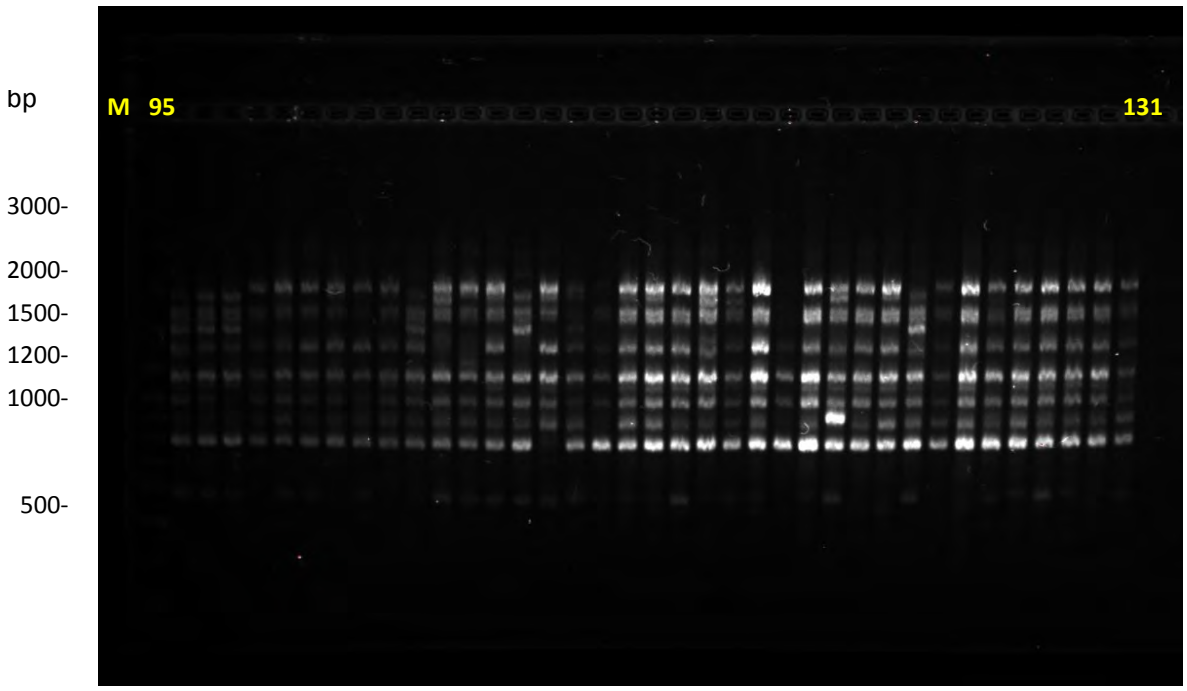
Appendix IV Picture 8c: Gel imaging picture of the primer ISSR827 revealing genetic diversity among 131 safflower accessions



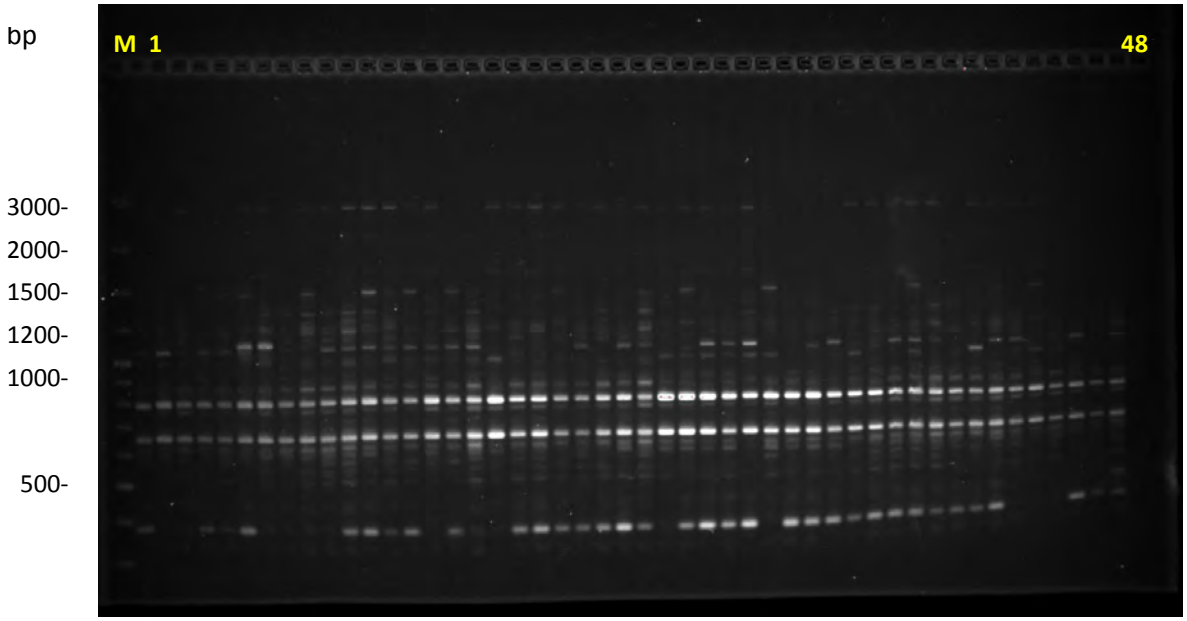
Appendix IV Picture 9a: Gel imaging picture of the primer ISSR830 revealing genetic diversity among 131 safflower accessions



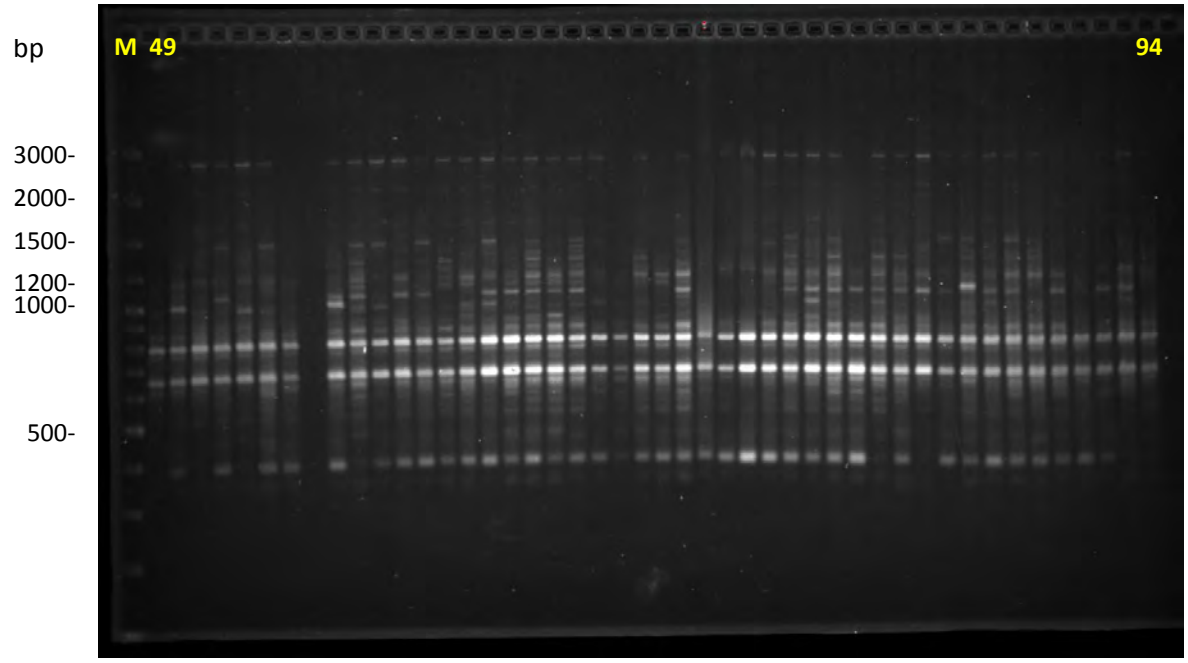
Appendix IV Picture 9b: Gel imaging picture of the primer ISSR830 revealing genetic diversity among 131 safflower accessions



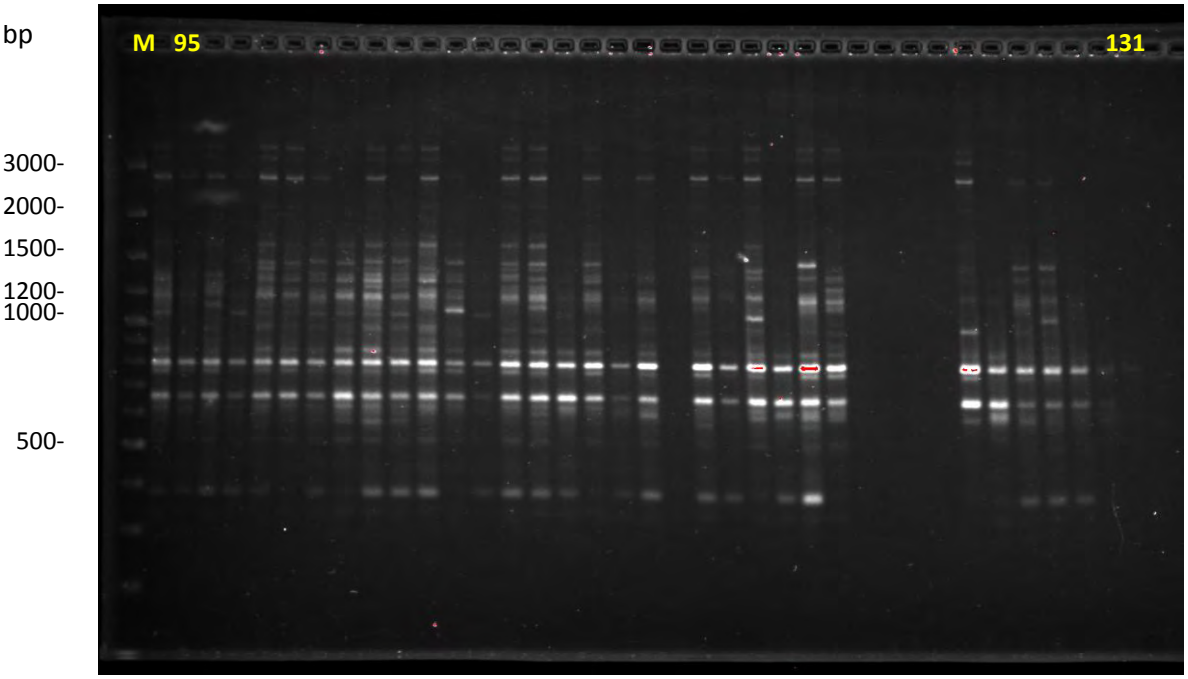
Appendix IV Picture 9c: Gel imaging picture of the primer ISSR830 revealing genetic diversity among 131 safflower accessions



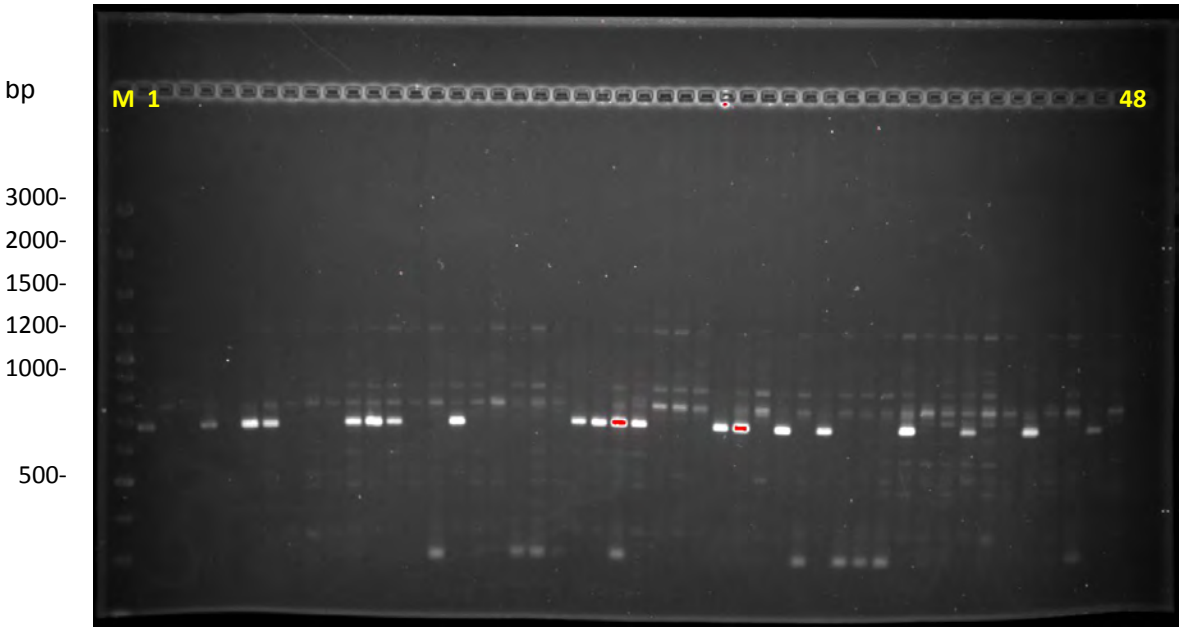
Appendix IV Picture 10a: Gel imaging picture of the primer ISSR834 revealing genetic diversity among 131 safflower accessions



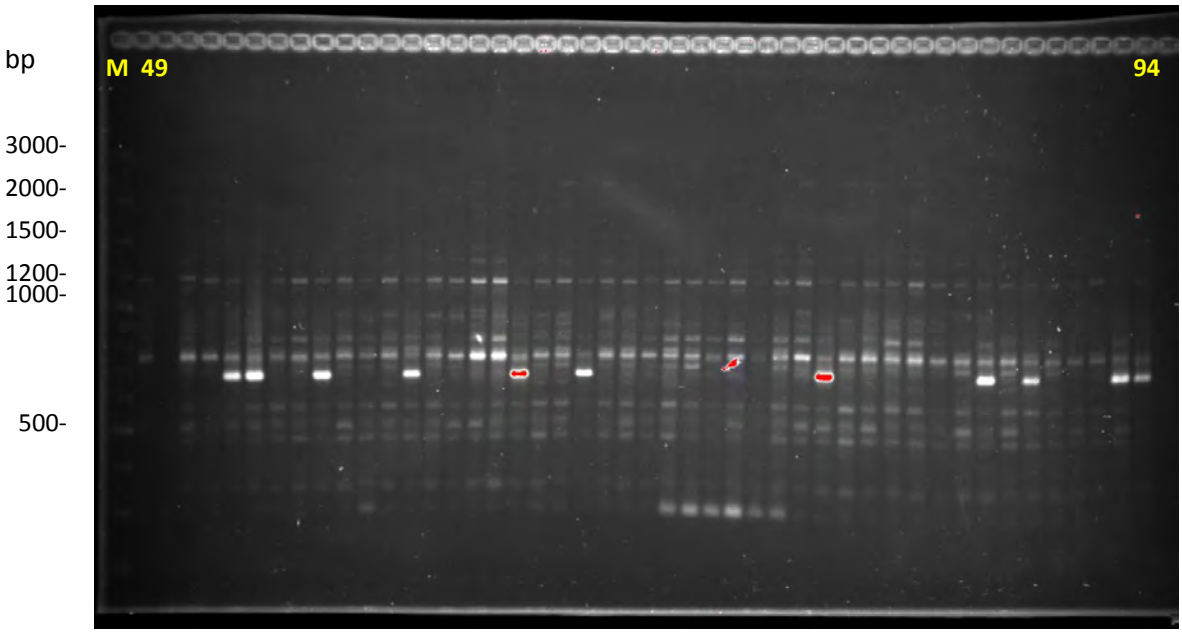
Appendix IV Picture 10b: Gel imaging picture of the primer ISSR834 revealing genetic diversity among 131 safflower accessions



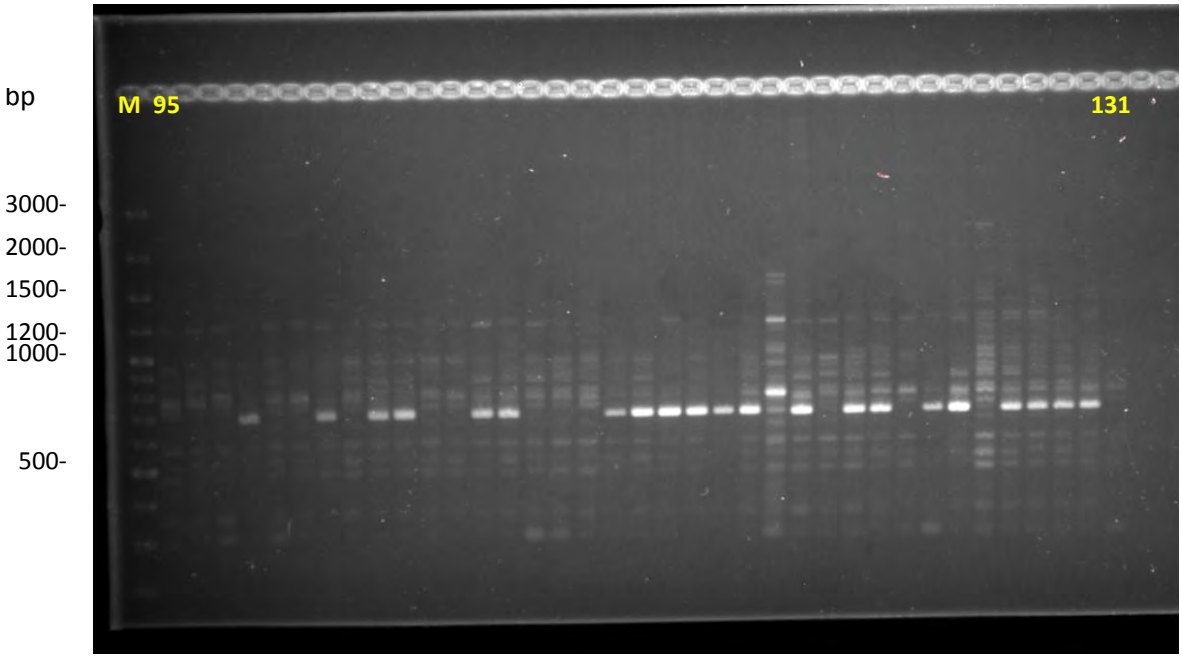
Appendix IV Picture 10c: Gel imaging picture of the primer ISSR834 revealing genetic diversity among 131 safflower accessions



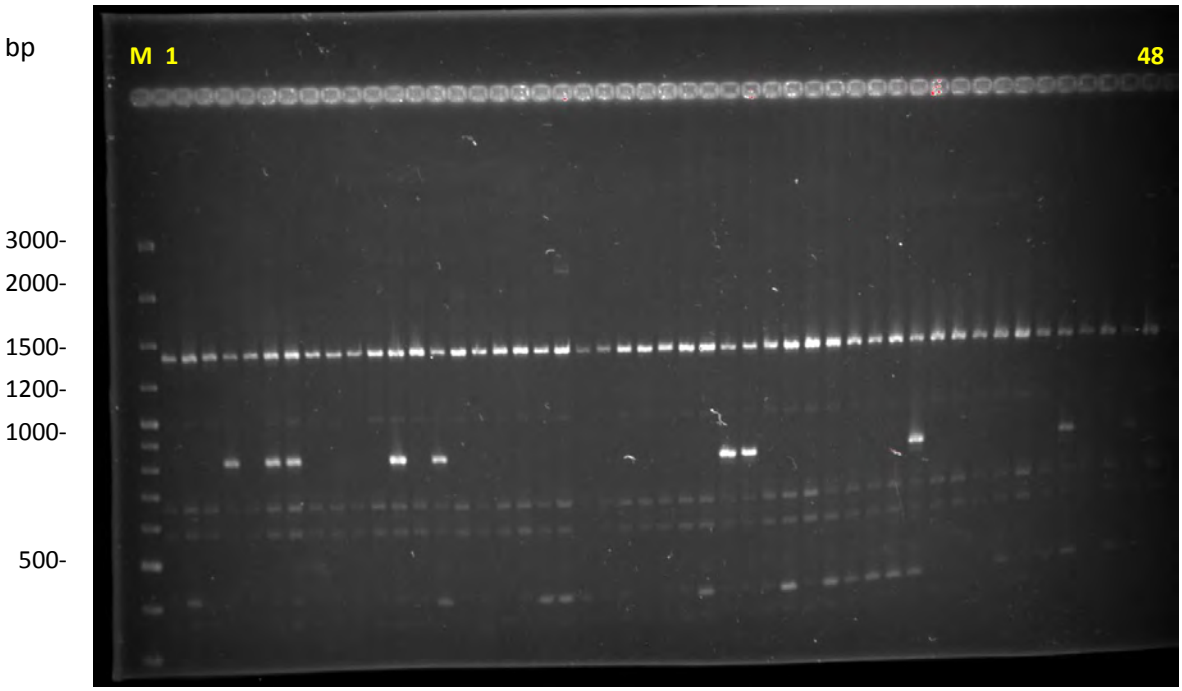
Appendix IV Picture 11a: Gel imaging picture of the primer ISSR840 revealing genetic diversity among 131 safflower accessions



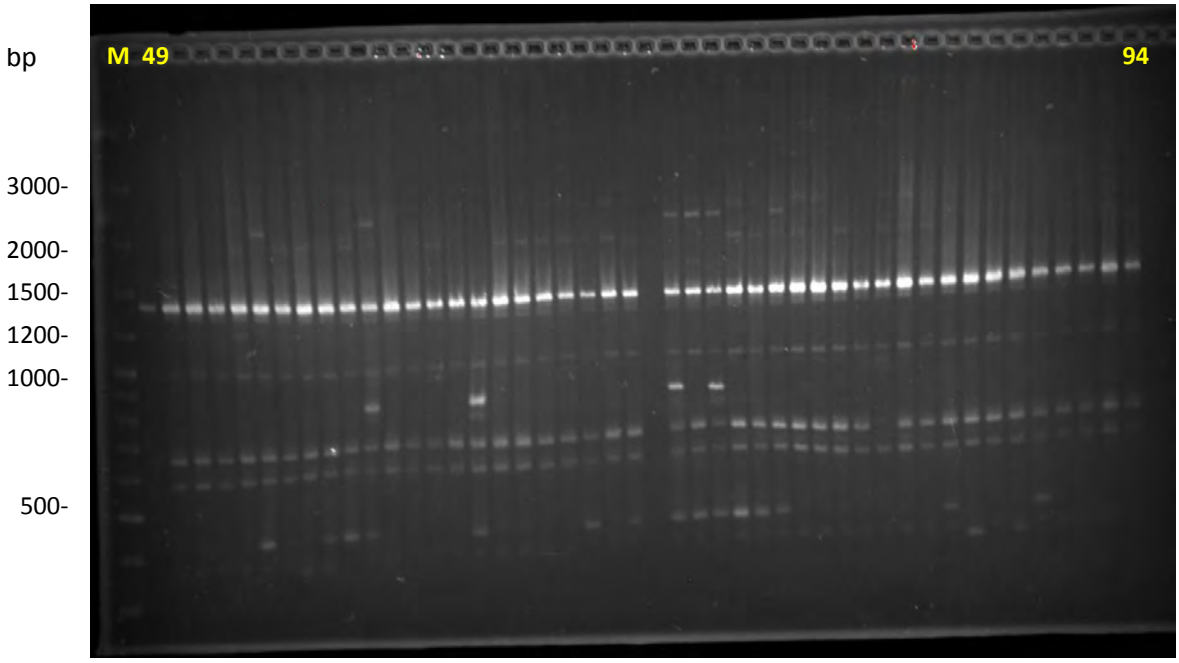
Appendix IV Picture 11b: Gel imaging picture of the primer ISSR840 revealing genetic diversity among 131 safflower accessions



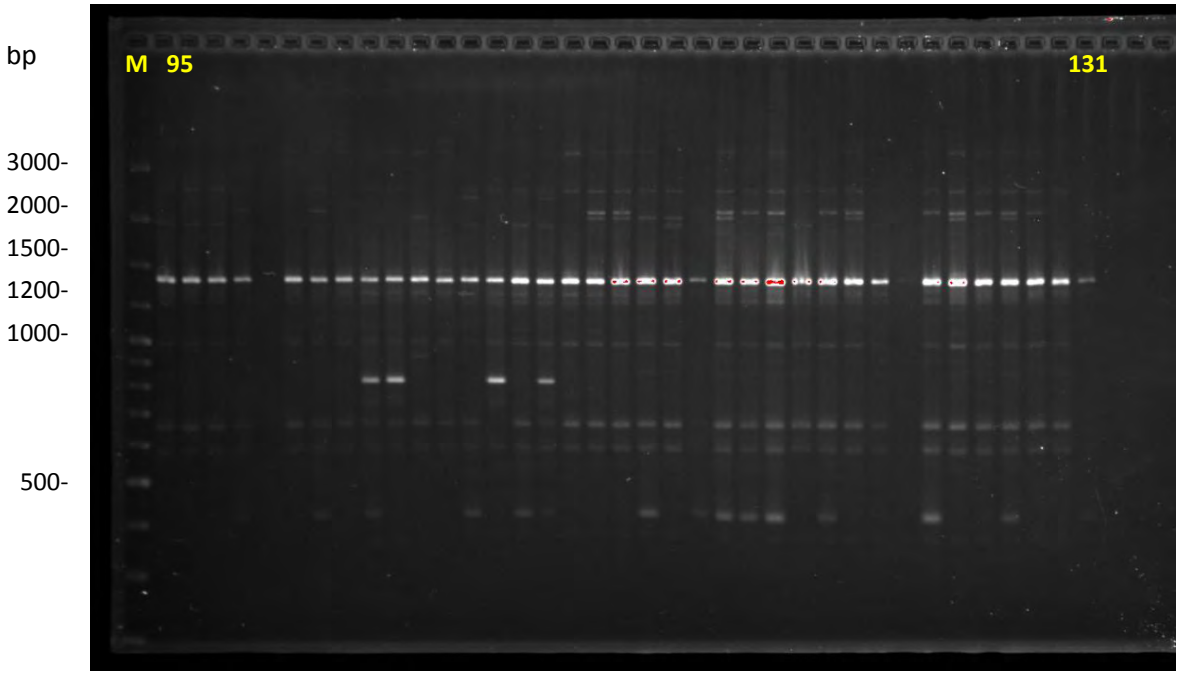
Appendix IV Picture 11c: Gel imaging picture of the primer ISSR840 revealing genetic diversity among 131 safflower accessions



Appendix IV Picture 12a: Gel imaging picture of the primer ISSR868 revealing genetic diversity among 131 safflower accessions



Appendix IV Picture 12b: Gel imaging picture of the primer ISSR868 revealing genetic diversity among 131 safflower accessions



Appendix IV Picture 12c: Gel imaging picture of the primer ISSR868 revealing genetic diversity among 131 safflower accessions

Investigation of Morpho-agronomic Performance, Genetic Diversity and Similarity Centers Exploration in International Safflower Panel

ORIGINALITY REPORT

15%	9%	13%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	krishikosh.egranth.ac.in Internet Source	1%
2	www.frontiersin.org Internet Source	1%
3	Muhammad Azhar Nadeem, Ephrem Habyarimana, Vahdettin Çiftçi, Muhammad Amjad Nawaz et al. "Characterization of genetic diversity in Turkish common bean gene pool using phenotypic and whole-genome DArTseq-generated silicoDArT marker information", PLOS ONE, 2018 Publication	1%
4	Ünal Karık, Muhammad Azhar Nadeem, Ephrem Habyarimana, Sezai Ercişli et al. "Exploring the Genetic Diversity and Population Structure of Turkish Laurel Germplasm by the iPBS-Retrotransposon Marker System", Agronomy, 2019 Publication	1%

PLOS ONE

RESEARCH ARTICLE

Mobile genomic element diversity in world collection of safflower (*Carthamus tinctorius* L.) panel using iPBS-retrotransposon markers

Fawad Ali[1,2], Abdurrahim Yılmaz[1], Muhammad Azhar Nadeem[1], Ephrem Habyarimana[3], Ilhan Subaşı[4], Muhammad Amjad Nawaz[5], Hassan Javed Chaudhary[2], Muhammad Qasim Shahid[6], Sezai Ercişli[7], Muhammad Abu Bakar Zia[8], Gyuhwa Chung[9]*, Faheem Shehzad Baloch[1]*

1 Department of Field Crops, Faculty of Agricultural and Natural Science, Bolu Abant İzzet Baysal University, Bolu, Turkey, 2 Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan, 3 Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria—Centro di ricerca cerealicoltura e colture industriali, Bologna, Italy, 4 Central Research Institute for Field Crops, Ankara, Turkey, 5 Education and Scientific Center of Nanotechnology, Far Eastern Federal University, Vladivostok, Russian Federation, 6 State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, South China Agricultural University, Guangzhou, China, 7 Department of Horticulture, Faculty of Agriculture, Ataturk University, Erzurum, Turkey, 8 Department of Plant breeding and genetics, Muhammad Nawaz Sharif University of Agriculture, Multan, Pakistan, 9 Department of Biotechnology, Chonnam National University, Chonnam, Republic of Korea

* balochfaheem13@gmail.com (FSB); chung@chonnam.ac.kr (GC)

Abstract

Safflower (*Carthamus tinctorius* L.) is a multipurpose crop of dry lands yielding very high quality of edible oil. Present study was aimed to investigate the genetic diversity and population structure of 131 safflower accessions originating from 28 different countries using 13 iPBS retrotransposon markers. A total of 295 iPBS bands were observed among which 275 (93.22%) were found polymorphic. Mean Polymorphism information content (0.4[illegible]) and diversity parameters including mean effective number of alleles (1.3[illegible]), mean Shannon's information index (0.33), overall gene diversity (0.19), Fstatistic (0.2[illegible]), and inbreeding coefficient (1.00) reflected the presence of sufficient amount of genetic diversity in the studied plant materials. Analysis of molecular variance (AMOVA) showed that more than 40% of genetic variation was derived from populations. Model-based structure, principal coordinate analysis (PCoA) and unweighted pair-group method with arithmetic means (UPGMA) algorithms clustered the 131 safflower accessions into four main populations A, B, C, D and an unclassified population, with no meaningful geographical origin. Most diverse accessions originated from Asian countries including Afghanistan, Pakistan, China, Turkey, and India. Four accessions, Turkey9, Afghanistan4, Afghanistan2, and Pakistan24 were found most genetically distant and might be recommended as candidate parents for breeding purposes. The findings of this study are most probably supported by the seven similarity centers hypothesis of safflower. This is a first study to explore the genetic diversity and population structure in safflower accessions using the iPBS-retrotransposon markers. The information provided in this work will therefore be helpful for scientists interested in safflower breeding.

OPEN ACCESS

Citation: Ali F, Yılmaz A, Nadeem MA, Habyarimana E, Subaşı I, Nawaz MA, et al. (2019) Mobile genomic element diversity in world collection of safflower (*Carthamus tinctorius* L.) panel using iPBS-retrotransposon markers. PLoS ONE 14(2): e0211985. https://doi.org/10.1371/journal.pone.0211985

Editor: [illegible]

Received: November 13, 2018

Accepted: January 24, 2019

Published: February 27, 2019

Data Availability Statement: All relevant data are within the manuscript.

Funding: The authors express their gratitude to TUBITAK (The Scientific and Technological Research Council of Turkey) for providing a doctoral fellowship to Fawad Ali under the TUBITAK-2215 fellowship program for international researchers.

Competing interests: The authors have declared that no competing interests exist.

TÜBİTAK

Turkish Journal of Agriculture and Forestry

[illegible]

Research Article

Turk J Agric For [illegible]

[illegible]

Investigation of morphoagronomic performance and selection indices in the international safflower panel for breeding perspectives

[illegible]

Faheem Shehzad BALOCH[1,*]

[illegible]

Received: [illegible] • Accepted/Published Online: [illegible] • Final Version: [illegible]

Abstract: [illegible] ... programs.

Key words: [illegible]

1. Introduction

Agronomic crops are grown on a large scale for consumption purposes because they provide food, feed, grain, oil, and fiber. [illegible] (Sahin et al., [illegible]; [illegible] 2018; [illegible] et al., 2018).

About [illegible] of the global vegetable oil [illegible] is derived from [illegible] main crops: soybeans, oil palm, rapeseed, and sunflowers. [illegible] (Murphy, [illegible]). However, these underutilized oilseed crops represent a good source of genetic diversity and adaptation to diverse agroecological zones (Padulosi et al., [illegible]; [illegible], 2000; Ozdemir et al., 2018).

Safflower (*Carthamus tinctorius* L.) is an underutilized oilseed crop and belongs to the family *Asteraceae* (Knowles, [illegible]; Ali et al., 2019). It is known as one of the oldest crop [illegible] (Knowles and Ashri, [illegible]). Safflower was first domesticated and grown because of its flowers for dyes, food coloring, and various medicinal uses, but it is also grown as an oilseed crop. Safflower is preferred [illegible] semiarid conditions that represent important scenarios of climate change (Weiss, 2000).

Safflower accessions belonging to specific geographical locations present similarities on the basis of their

* Correspondence: balochfaheem13@gmail.com

Euphytica (2019) 215:85
https://doi.org/10.1007/s10681-019-2409-9

ORIGINAL ARTICLE

Molecular characterization of genetic diversity and similarity centers of safflower accessions with ISSR markers

Fawad Ali[1,2] · Muhammad Azhar Nadeem[1] · Ephrem Habyarimana[3] · Abdurrahim Yilmaz[1] · Muhammad Amjad Nawaz[1] · Iftikhar Hussain Khalil[4] · Sezai Ercişli[5] · Gyuhwa Chung[6] · Hassan Javed Chaudhary[7] · Faheem Shehzad Baloch[1,8]

Received: July 2019 / Accepted: 19 November 2019 / Published online: 30 December 2019
© Springer Nature B.V. 2019

Abstract

Crop genetic resources are vital inputs in crop genetic improvement. In this study, genetic diversity, population structure and similarity centers for 131 safflower accessions obtained from 28 countries were investigated using 12 ISSR markers. A sum of 201 ISSR bands were observed among which 168 (83.84%) were found polymorphic. Many polymorphic information content (PIC = 0.449) and diversity parameters including mean effective number of alleles (1.6551), mean Shannon's information index (0.557), mean observed heterozygosity (0.356), and mean overall gene diversity (0.377) showed a great level of genetic diversity in the studied safflower materials. Model-based structure, unweighted pair-group method with arithmetic means, and principal coordinate analyses clustered all accessions into three main populations, A, B, and C and an unclassified population. Accessions originated from Asian countries like Pakistan and Central Asia were found more diverse. Three accessions, Pakistan-11, Israel-1, and Pakistan-26, were found most genetically distant and might be used as potential sources for genetic combinations in safflower breeding activities. Analysis of molecular variance revealed highly significant differentiation among the identified populations and population/country combinations. The results presented in this work most probably supported the hypothesis of seven similarity centers of safflower but need to be validated with further confirmed investigations. The information provided herein is expected to be helpful for the scientific community interested in safflower breeding.

Keywords: Genetic differentiation · Genetic resources · Molecular markers · Population structure · Safflower breeding

1 Introduction

The fast growth of the world's population requires sufficient availability of food in the form of cereals and other nutrients (Long et al. 2015). Daily average of per capita available calories across the world was 2,789 kcal in the year 2000, and it is believed that it will become 3130 kcal by year 2050 (FAO 2009), highlighting a steady increase in food demand paralleling the growth of world's population. A steady increase in the production of safflower has been observed during the last two decades to mainly meet the vegetable oil shortage. However, according to FAO's 2018–2027 projections, the increase in oilseed production will amount 1.2% annually, implying a great drop relative to the last decade. Vegetable

✉ Fawad Ali
fawad.ali@sydney.edu.au

✉ Faheem Shehzad Baloch
faheemsbaloch@gmail.com

[1] Department of Field Crops, Faculty of Agriculture and Natural Science, Bolu Abant Izzet Baysal University, Bolu, Turkey

Department of Plant Breeding, Quaid-i-Azam University, Islamabad, Pakistan

[2] CIHEAM-Bari, Mediterranean Agronomic Institute of Bari, Valenzano, Bari, Italy

[3] Vavilov Research Center for Genetic Resources, Federal Scientific Center of Vegetable Growing, All-Russian Research Institute of Vegetable Breeding, Moscow, Russia

[4] Department of Plant Breeding and Genetics, University of Agriculture, Peshawar, Pakistan

[5] Department of Horticulture, Faculty of Agriculture, Ataturk University, Erzurum, Turkey

[6] Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, Seongbuk-gu, Republic of Korea

[7] Faculty of Agricultural Sciences and Technologies, Sivas Cumhuriyet University, Sivas, Turkey

plants

MDPI

Article

Genetic Diversity, Population Structure and Marker-Trait Association for 100-Seed Weight in International Safflower Panel Using SilicoDArT Marker Information

Fawad Ali [1,2], Muhammad Azhar Nadeem [3], Muzaffer Barut [2,4], Ephrem Habyarimana [5], Hassan Javed Chaudhary [1], Iftikhar Hussain Khalil [6], Ahmad Alsaleh [7], Rüştü Hatipoğlu [4], Tolga Karaköy [3], Cemal Kurt [4], Muhammad Aasim [3], Muhammad Sameeullah [2], Ndiko Ludidi [8], Seung Hwan Yang [9], Gyuhwa Chung [9,*] and Faheem Shehzad Baloch [3,*]

1. Department of Plant Sciences, Quaid-I-Azam University, Islamabad 45320, Pakistan; [illegible] (F.A.); [illegible] (H.J.C.)
2. Department of Field Crops, Faculty of Agriculture and Natural Science, Bolu Abant Izzet Baysal University, 14030 Bolu, Turkey; [illegible] (M.B.); [illegible] (M.S.)
3. Faculty of Agricultural Sciences and Technologies, Sivas University of Science and Technology, [illegible] Sivas, Turkey; [illegible] (M.A.N.); [illegible] (T.K.); [illegible] (M.A.)
4. Department of Field Crops, Faculty of Agriculture, Çukurova University, [illegible] Adana, Turkey; [illegible] (R.H.); [illegible] (C.K.)
5. CREA Research Centre for Cereal and Industrial Crops, 40128 Bologna, Italy; [illegible]
6. Department of Plant Breeding and Genetics, The University of Agriculture, Peshawar 25130, Pakistan; [illegible]
7. Science and Technology Application and Research Center (BILTEM), Yozgat Bozok University, 66200 Yozgat, Turkey; [illegible]
8. Department of Biotechnology and Centre of Excellence in Food Security, University of the Western Cape, Robert Sobukwe Road, Bellville 7530, South Africa; [illegible]
9. Department of Biotechnology, Chonnam National University, Chonnam 59626, Korea; [illegible]

* Correspondence: [illegible] (G.C.); [illegible] (F.S.B.)

Received: 8 April 2020; Accepted: 19 May 2020; Published: 21 May 2020

Abstract: Safflower is an important oilseed crop mainly grown in the arid and semi-arid regions of the world. The aim of this study was to explore phenotypic and genetic diversity, population structure, and marker-trait association for 100-seed weight in 94 safflower accessions originating from 26 countries using silicoDArT markers. Analysis of variance revealed statistically significant genotypic effects ($p \leq 0.01$), while Turkey samples resulted in higher 100-seed weight compared to Pakistan samples. A Constellation plot divided the studied germplasm into two populations on the basis of their 100-seed weight. Various mean genetic diversity parameters including observed number of alleles (1.96), effective number of alleles (1.54), Shannon's information index (0.48), expected heterozygosity (0.32), and unbiased expected heterozygosity (0.32) for the entire population exhibited sufficient genetic diversity using [illegible] silicoDArT markers. Analysis of molecular variance (AMOVA) revealed that most of the variations (9[illegible]%) in world safflower panel are due to differences within country groups. A model based structure grouped the 94 safflower accessions into populations A, B, C and an admixture population upon membership coefficient. Neighbor joining analysis grouped the safflower accessions into two populations (A and B). Principal coordinate analysis (PCoA) also clustered the safflower accessions on the basis of geographical origin. Three accessions; Egypt-5, Egypt-2, and India-2 revealed the highest genetic distance and hence might be recommended as candidate parental lines for safflower breeding programs. The mixed linear model, i.e., the Q + K model, demonstrated that two DArTseq markers (DArT-45483051 and DArT-15672391) had