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RESEARCH ARTICLE

Applicability of Start Codon Targeted (SCoT) markers for the assessment of genetic diversity in bread wheat germplasm

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Abstract Research endeavors focused on examining genetic diversity play a pivotal role in advancing agricultural practices, as they furnish valuable insights for breeding programs and the preservation of germplasm. Wheat holds significant global importance as a cereal crop, serving as a primary source of sustenance for almost one-third of the global human population. In the current study, a total of 80 bread wheat genotypes were characterized using the start codon targeted (SCoT) marker system. A set of 14 polymorphic primers yielded a cumulative count of 399 bands with an average of 28.05 bands per primer. The range of diversity markers, such as polymorphism information content (0.26–0.59), effective numbers of alleles (1.27–1.59), Shannon's information index (0.30–0.51), and gene diversity (0.18–0.34), provided evidence of elevated genetic differences within the examined germplasm. According to the findings of the molecular variance analysis, greater genetic variations are found within populations (92%). The model-based structural approach partitioned the 80 bread wheat genotypes into two distinct populations (Population A and B). The diversity indices based on the structures of the populations revealed that population A had greater diversity compared to population B. Both the principal coordinate analysis and

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the neighbor-joining analysis also differentiated the 80 different bread wheat genotypes based on their respective collection points. The present crop diversity offers great opportunities for enhancing crop quality and increasing tolerance to environmental challenges, both of which will lead to a reduction in the problem of food insecurity.

Keywords *Triticum aestivum* · Germplasm characterization · Molecular markers · Food security

Introduction

Despite the appreciable progress made in the last decades aimed at reducing hunger, more than 800 million individuals globally experience persistent undernourishment (WFP 2020) According to a report shared by FAO (2015), approximately two billion people are facing essential micronutrient deficiencies called "hidden hunger". Based on the presented data, ensuring food security emerges as a critical concern that requires immediate attention. This issue poses a significant threat to the fulfillment of sustainable development goals and gives rise to a range of economic and non-economic challenges (Battersby et al. 2017). Food security occurs if all people, at all times, have economic and physical access to sufficient, safe, and nutritious food that fulfills their dietary requirements for health and active life (FAO 2008). Climate change, increasing population, urbanization, loss of biodiversity, and industrialization are key factors playing a significant role in global hunger. Climate change poses the main threat to food security by affecting agricultural production through unprecedented biotic and abiotic stresses. According to Zhao et al. (2017), approximately a 3.1%–7.4% decline in production has been recorded for various important crops for each degree-Celsius increase in global mean temperature due to climate change. Besides climate changes, the rapidly growing population has become a key concern in present world, and the demand for this rapidly increasing population is a bitter and challenging question for the present breeders.

To feed 9.7 billion people by 2050, the Food and Agriculture Organization of the United Nations (FAO) has stated that there is a need to increase agricultural production by 60% compared to the current level. The scientific community, especially plant breeders, bears a significant responsibility to develop climate-resilient yet high-yielding and highly nutritious crop cultivars. This is crucial in addressing climate change, global hunger, andhidden hunger issues simultaneously. To achieve these objectives, it is imperative to effectively utilize and evaluate plant genetic diversity through germplasm characterization (Baloch et al. 2022; Baloch and Nadeem 2022; Mafakheri et al. 2020; Tabasum et al. 2020; Nadeem 2021). Genetic diversity assessment can be conducted through morphological, biochemical, and molecular markers; however, molecular markers are preferred due to their precision, reliability, reproducibility, and resilience to environmental factors (Nadeem et al. 2018). Different types of molecular markers (SSR, simple sequence repeat; AFLP, amplified fragment length polymorphism; DArT, diversity arrays technology; ISSR, inter simple sequence repeats; and RAPD, randomly amplified polymorphic DNA) have been developed and successfully applied in the marker-assisted breeding program of various crops. These markers are utilized for genetic population analysis, mapping quantitative trait loci (QTL), association analysis of various yield characteristics, and the detection of candidate genes.

Collard and Mackill (2009) developed a new marker system called start codon targeted (SCoT) polymorphism. This marker system is based on the short-conserved region flanking the ATG start codon in plant genes. It is a polymerase chain reaction (PCR) based marker system through which an 18-mer single primer targets the short consensus conserved region flanking the ATG translation initiation codon in plant genes (Collard and Mackill 2009). PCR products resulting from PCR are resolved using standard agarose gel electrophoresis (Guo et al. 2012). This marker system has been found to be cost-effective, resulting in high polymorphism. It is similar to ISSR and RAPD, is easy to use and its primers are universal in plants (Guo et al. 2012; Xiong et al. 2011). This marker has been successfully used in genetic diversity and population structure investigations of various plant species (Zarei et al. 2021; Yilmaz and Ciftci 2021; Tabasi et al. 2020; Al-Qurainy et al. 2015; Guo et al. 2012; Xiong et al. 2011).

Bread wheat (*Triticum aestivum* L.) is one of the most important crops, providing 20% of dietary energy and protein consumption worldwide (Braun et al. 2010). The present hexaploid (bread wheat)

resulted from a hybrid cross between *Triticum. dicoccoides* and *Aegilops tauschii* (Velu et al. 2019). In the year 2020, a total land area of 219,006,893 hectares was used for the cultivation of wheat on a global scale, which ultimately yielding a production of 760,925,831 tons (Salama et al. 2022). To feed 9.7 billion people by 2050, it is estimated that wheat production should increase by 1.7% per year (Leegood et al. 2010). This can be achieved through the development of climate-resilient and high-yielding wheat cultivars. According to the published report (2019) from the IGC (International Grains Council), the global need for wheat is estimated to increase by one billion tons over the next four years. It appears that conventional breeding programs may be sufficient to meet this growing demand (Leśniowska-Nowak et al. 2021). However, breeders express significant concern that ongoing breeding cycles are resulting in a limited genetic diversity within cultivated genotypes. This hindrance in the gene pool poses a noteworthy challenge for future breeding programs, as the remaining variability might not be sufficient (Hegde et al. 2002). Molecular breeding with SCoT markers is a reliable approach for assessing the genetic variability among wheat cultivars (Hamidi et al. 2014) and selecting superior cultivars for further breeding programs (Yalinkiliç et al. 2024). Recently, various investigations worldwide have reported the application of SCoT markers test the diversity in wheat germplasm (Mohamed et al. 2017; El-Moneim et al. 2020; Shaban et al. 2022; Abouseada et al. 2023). Abouseada et al. (2023) investigated the genetic diversity in wheat cultivars using ISSR and SCoT primers with chloroplast DNA barcoding and grain scanning electron microscope (SEM). The investigation highlighted the potential of molecular markers and SEM in characterizing wheat diversity for breeding purposes. The results provided valuable insights into wheat genetic resources, aiding future breeding strategies for improved wheat cultivars. Furthermore, Shaban et al. (2022) assessed the genetic diversity in Egyptian wheat genotypes under drought and watering conditions using ISSR, SCoT and SDS-PAGE techniques. Significant variations were observed during data analysis, with various genotypes showing higher tolerance to drought stress. The researchers concluded that the findings can contribute to wheat breeding programs, enhance resilience to drought and ensure food security in changing climates. In a separate study Alshehri (2019) identified the genetic variation of Saudi wheat genotypes through SCoT and ISSR markers. Their findings contributed to a better understanding of wheat diversity and aided in developing resilient and high-yielding wheat cultivars for sustainable agriculture in Saudi Arabia to meet the needs of the rapidly increasing populations.

Genetic deterioration in cultivated wheat provides a solid foundation for assessing genetic diversity among its relatives. This study also assess the potential for improving the efficacy of plant materials and, consequently, their utility in breeding programs, ultimately contributing to increased food production. In this investigation, we utilized a collection of 80 bread wheat germplasm provided by the United States Department of Agriculture (USDA). As a result, the primary objective of this research was to identify genetic diversity utilizing SCoT markers to facilitate sustainable production for the rapidly expanding global population.

Materials and methods

Plant material and DNA isolation

During the present study, a total of 80 bread wheat genotypes were used for molecular characterization. The plant material used during the study is given in Table 1. These genotypes were provided by the USDA and they originate from several countries and regions: South America (Argentina, Brazil, and Chile), North America (Canada), Southeast Europe (Croatia), Central Europe (Czech Republic), Eastern Europe (Hungary), Australia, East Asia (China), Southern Asia (India), Southern Europe (North Macedonia), Southeastern Europe (Serbia), Western Asia (Turkey), Eastern Europe (Romania, Ukraine), Eastern Europe and North Asia (Russia), Southwest Asia (Saudi Arabia, Yemen), and North America (United States of America, USA). To isolate the genomic DNA, all bread wheat accessions were seeded in the greenhouse, and the newly sprouted and soft leaves of those plants were collected. The DNA extraction was done using the Cetyl trimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1990), with a specific protocol recommended by Diversity Arrays Technology (Nadeem et al. 2021). The quantification of isolated DNA samples was conducted utilizing

Table 1 Details of wheat germplasm collections used in the study

Sr.No	Genotype Code	Genotype name	Sr.No	Genotype Code	Genotype name
1	PI 585037	Argentina1	41	CItr 8899	India1
2	CItr 8438	Argentina2	42	PI 648390	India2
3	CItr 8441	Argentina3	43	PI 648391	India3
4	CItr 5125	Australia1	44	PI 648392	India4
5	CItr 17,385	Australia2	45	PI 648393	India5
6	PI 5078	Australia3	46	PI 648394	India6
7	PI 591911	Australia4	47	CItr 17,379	North Macedonia1
8	PI 591910	Australia5	48	CItr 17,380	North Macedonia2
9	PI 591909	Australia6	49	CItr 17,361	North Macedonia3
10	PI 591908	Australia7	50	CItr 17,368	North Macedonia4
11	–	Australia8	51	CItr 17,373	North Macedonia5
12	PI 584932	Brazil1	52	CItr 17,374	North Macedonia6
13	PI 584927	Brazil2	53	CItr 1513	Russia1
14	PI 584926	Brazil3	54	CItr 7620	Russia2
15	PI 584925	Brazil4	55	CItr 7611	Russia3
16	CItr 6623	Canada1	56	–	Russia4
17	CItr 4930	Canada2	57	CItr 1658	Romania1
18	PI 584986	Croatia1	58	CItr 1663	Romania2
19	PI 584985	Croatia2	59	PI 585019	Saudi Arabia1
20	PI 584984	Croatia3	60	PI 585022	Saudi Arabia2
21	CItr 16,984	Chile1	61	PI 585024	Saudi Arabia3
22	CItr 16,985	Chile2	62	PI 585026	Saudi Arabia4
23	CItr 17,139	Chile3	63	CItr 17,280	Saudi Arabia5
24	CItr 16,959	Chile4	64	CItr 1676	Serbia1
25	CItr 16,964	Chile5	65	CItr 1731	Serbia2
26	CItr 16,970	Chile6	66	–	Serbia3
27	CItr 180	China1	67	PI 585032	Turkey1
28	CItr 8332	China2	68	PI 585030	Turkey2
29	CItr 8589	China3	69	PI 585033	Turkey3
30	CItr 8594	China4	70	PI 585035	Turkey6
31	CItr 8593	China5	71	PI 585027	Turkey7
32	PI 584795	Czech Republic1	72	CItr 8624	Ukraine1
33	PI 584796	Czech Republic2	73	CItr 8627	Ukraine2
34	PI 584797	Czech Republic3	74	PI 5641	Ukraine3
35	PI 584798	Czech Republic4	75	CItr 6247	USA1
36	PI 584789	Czech Republic5	76	CItr 17,316	USA2
37	PI 584790	Czech Republic6	77	CItr 17,249	USA3
38	–	Czech Republic7	78	PI 585213	Yemen1
39	CItr 2029	Hungary1	79	PI 585211	Yemen2
40	CItr 2034	Hungary2	80	PI 585209	Yemen3

–Not available

a 0.8% agarose gel and the NanoDrop instrument (DS11 FX, DeNovix Inc., Wilmington, DE, USA). A final concentration of 5 ng/μL was prepared in order to facilitate subsequent polymerase chain reaction (PCR) analysis.

PCR amplification using SCoT primers

During the preliminary phase of the investigation, a random selection of six DNA samples was done and used in order to identify polymorphic markers

Table 2 Sequence and Annealing temperature of start codon targeted (SCoT) markers used for wheat genetic diversity analysis

Primer name	Sequence	Annealing temperature (^{0}C)
SCoT2	CAACAATGGCTACCACCC	50
SCoT3	CAACAATGGCTACCACCG	50
SCoT12	ACGACATGGCGACCAACG	50
SCoT13	ACGACATGGCGACCATCG	50
SCoT17	ACCATGGCTACCACCGAG	50
SCoT18	ACCATGGCTACCACCGCC	50
SCoT20	ACCATGGCTACCACCGCG	50
SCoT23	CACCATGGCTACCACCAG	50
SCoT24	CACCATGGCTACCACCAT	50
SCoT25	ACCATGGCTACCACCGGG	50
SCoT27	ACCATGGCTACCACCGTG	50
SCoT28	CAACAATGGCTACCACCA	50
SCoT29	CCATGGCTACCACCGGCC	50
SCoT30	CCATGGCTACCACCGGCG	50

suitable for wheat. These selected materials were then subjected to evaluation using a set of 30 SCoT primers in order to obtain polymorphism bands and the 14 highly polymorphic primers were selected (Table 2). The PCR was performed in a 10 µl reaction mixture containing 25–30 ng DNA, 1 ul of 10×PCR buffer containing + KCl and -$MgCl_2$, 1.5 of o.5 µM of each SCoT primer, 1 ul of 0.2 mM dNTPs, 0.15 ul of Taq DNA polymerase (DreamTaq, Thermoscientific), 1 ul of 25 mM of DNTPs, 1 ul of $MgCl_2$, 2.5 ul of DNA (5 ng/ul) and adjust the final volume 10 ul by adding 2.85 ul of distal water. The PCR profile included initial denaturation at 95 °C for 5 min, furthermore the PCR was programmed as follows: amplification for 35 cycles with denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min, after amplification, a final extension step of 72 °C for 8 min was performed. The PCR products were separated by electrophoresis through 2% agarose gel in 1×TBE buffer for 2 h and 30 min at 110 V. GeneRuler DNA Ladder (100–3000 bp) Mix (Thermo Scientific, SM0333) was used to identify band size.

Statistical analysis

The PCR products were scored using a binary approach, where a value of 1 indicated the presence of a band, while a value of 0 indicated its absence. The study examined several diversity measures, including gene diversity (He), Shannon's information index (I), and effective alleles number (Ne), using Popgene ver. 1.32 software (Yeh et al. 2000). The Nei's genetic distance among 80 bread wheat genotypes was also calculated using Popgene ver. 1.32 software. The polymorphism information contents (PIC) was calculated using the formula derived by Roldán-Ruiz et al. (2000) as PIC = 2fi (1—fi). The variable "fi" denotes the frequency of loci that are now present in a molecular marker, whereas the quantity (1 − fi) reflects the frequency of loci that are absent. Principal coordinate analysis (PCoA) and analysis of molecular variance (AMOVA) were performed using the GenAlExV6.5 program. In order to investigate the genetic relatedness between 80 bread wheat genotypes, a neighbor-joining analysis was conducted utilizing the R statistical software. The software known as STRUCTURE was employed in the investigation of the population structure of bread wheat germplasm, as described by Pritchard et al. (2000). The determination of the optimal number of clusters (K subpopulations) was performed by conducting the analysis three times, using the methodology described in the study conducted by Evanno et al. (2005). The range of clusters considered was from 1 to 10. For each run, the burn-in and Markov Chain Monte Carlo (MCMC) were both set to 50,000, and the number of iterations was set to 10. Subsequently, the evaluated results were subjected to processing using STRUCTURE HARVESTER v.0.9.94 (Earl 2012) in order to examine the optimal K value. The R package "pophelper" was utilized to visually represent the ΔK value that was deemed most desirable (Francis 2017).

Results

In this investigation, a total of 14 Start Codon Targeted (SCoT) primers were utilized for the purpose of assessing the genetic diversity and molecular characterization of wheat germplasm collected from various regions of the world. The 14 SCoT primers produced a total of 399 bands with an average of 28.05 bands

Table 3 Genetic diversity characteristics of the wheat germplasm using start codon targeted (SCoT) marker system

Primers	Total Bands	PB	P%	PIC	n_e*	*He**	*I**
Scot2	29	28	96.55	0.53	1.52	0.31	0.47
Scot3	28	24	85.71	0.38	1.43	0.26	0.40
Scot12	31	29	93.55	0.48	1.47	0.29	0.44
Scot13	27	17	62.96	0.36	1.36	0.22	0.34
Scot17	26	24	92.31	0.40	1.39	0.25	0.39
Scot18	22	20	90.91	0.48	1.47	0.27	0.41
Scot20	35	27	77.14	0.37	1.37	0.23	0.36
Scot23	27	25	92.59	0.59	1.59	0.34	0.51
Scot24	28	26	92.86	0.27	1.27	0.19	0.32
Scot25	26	17	65.38	0.36	1.41	0.24	0.37
Scot27	29	22	75.86	0.28	1.29	0.18	0.30
Scot28	29	21	72.41	0.38	1.30	0.20	0.32
Scot29	34	28	82.35	0.29	1.34	0.21	0.34
Scot30	28	21	75.00	0.26	1.30	0.20	0.31
Total	399	329					
Average	28.5	23.5	82.54	0.39	1.39	0.24	0.38

*Polymorphic bands (PB); Polymorphism percentage (*P %*); Polymorphism Information Content (*PIC*); Effective numbers of alleles (n_e); Shannon's information index (*I*); Gene diversity (*He*)

per primer (Table 3). The maximum number of bands was produced by the primers SCoT20 (35 bands), SCoT29 (34 bands), and SCoT12 (31 bands). Furthermore, primers SCoT2, SCoT27, and SCoT28 yielded 29 bands, while SCoT3, SCoT24, and SCoT30 produced 28 bands.Out of 14 SCoT primers, SCoT-18 yileded the minimum number of bands (22). Among the 399 total bands that, a majority of 329 bands, representing an average of 82.54%, exhibited a significant level of polymorphism. It is noteworthy that each primer utilized in the study had a polymorphic band frequency of 23.5%. In addition, none of the single SCoT primers showed 100% polymorphism, and the maximum number of polymorphic bands, 28 of 29, were produced by SCoT2 with an average of 96.55%, followed by SCoT12, which produced 29 of 31 bands with an average of 93.55%, and SCoT24, which produced 26 of 28 bands with an average of 92.86%. The minimum number of polymorphisms was recorded in SCoT13, which produces 17 of 27 bands with an average of 62.96%. Furthermore, the PIC value ranged between 0.59 for the SCoT23 and 0.26 for the SCoT30, with an average PIC value of 0.39 for all tested primers. The maximum number of effective alleles (n_e) of 1.59 was observed with SCoT23, followed by 1.52 with SCoT2, and the minimum n_e *of* 1.27 was recorded with SCoT24. The average n_e of 1.39 was recorded for all utilized SCoT primers. Moreover, the maximum and minimum Shannon's information index (*I*) of 0.30 and 0.51 were recorded for SCoT27 and SCoT23, respectively, and the average *I* for all SCoT primers was 0.38. The maximum gene diversity (*He*) of 0.34 with ScoT23 and minimum of 0.18 with SCoT27 were recorded, and a mean gene diversity value of 0.24 was observed for all SCoT tested primers. During the evaluation of the final consequences, a mean genetic distance of 0.242 was observed, with a minimum genetic distance of 0.08 observed between Saudiarabia-1 and Saudiarabia-5, and a high genetic distance of 0.432 observed between Chile-3 and India-4. Results of the analysis of molecular variance (AMOVA) concluded that a higher number of genetic variations (92%) are present within the assessing population as compared among the populations (8%) (Table 4).

The model-based structure algorithm was performed to check the genetic variation of all tested populations and results revealed that the 80 wheat accessions were divided into two populations

Table 4 Analysis of molecular variance among and within populations in wheat genotypes using the start codon targeted (SCoT) markers

Source	df	SS	MS	Est. Var	% Variations
Among Pops	18	108.281	6.016	0.375	8%
Within Pops	61	271.610	4.453	4.453	92%
Total	79	379.892		4.827	100%

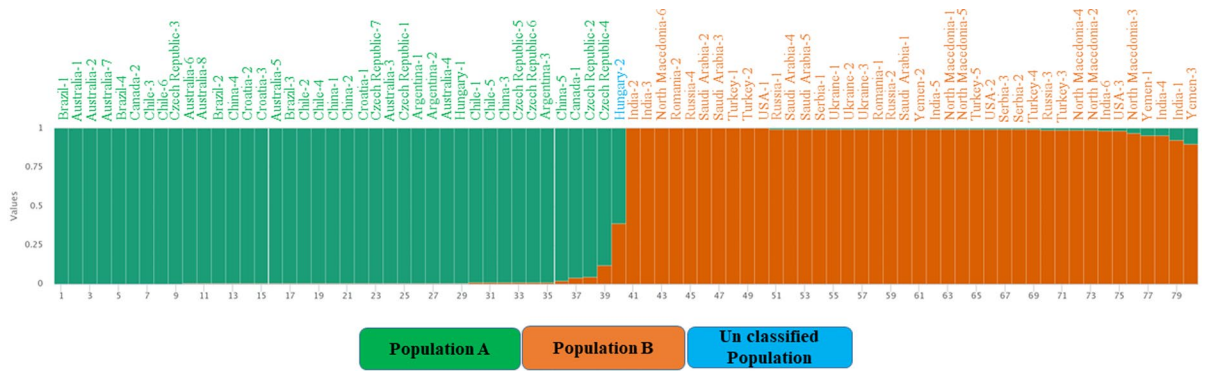

Fig. 1 Population structure of tested wheat germplasm using start codon targeted (SCoT) marker system

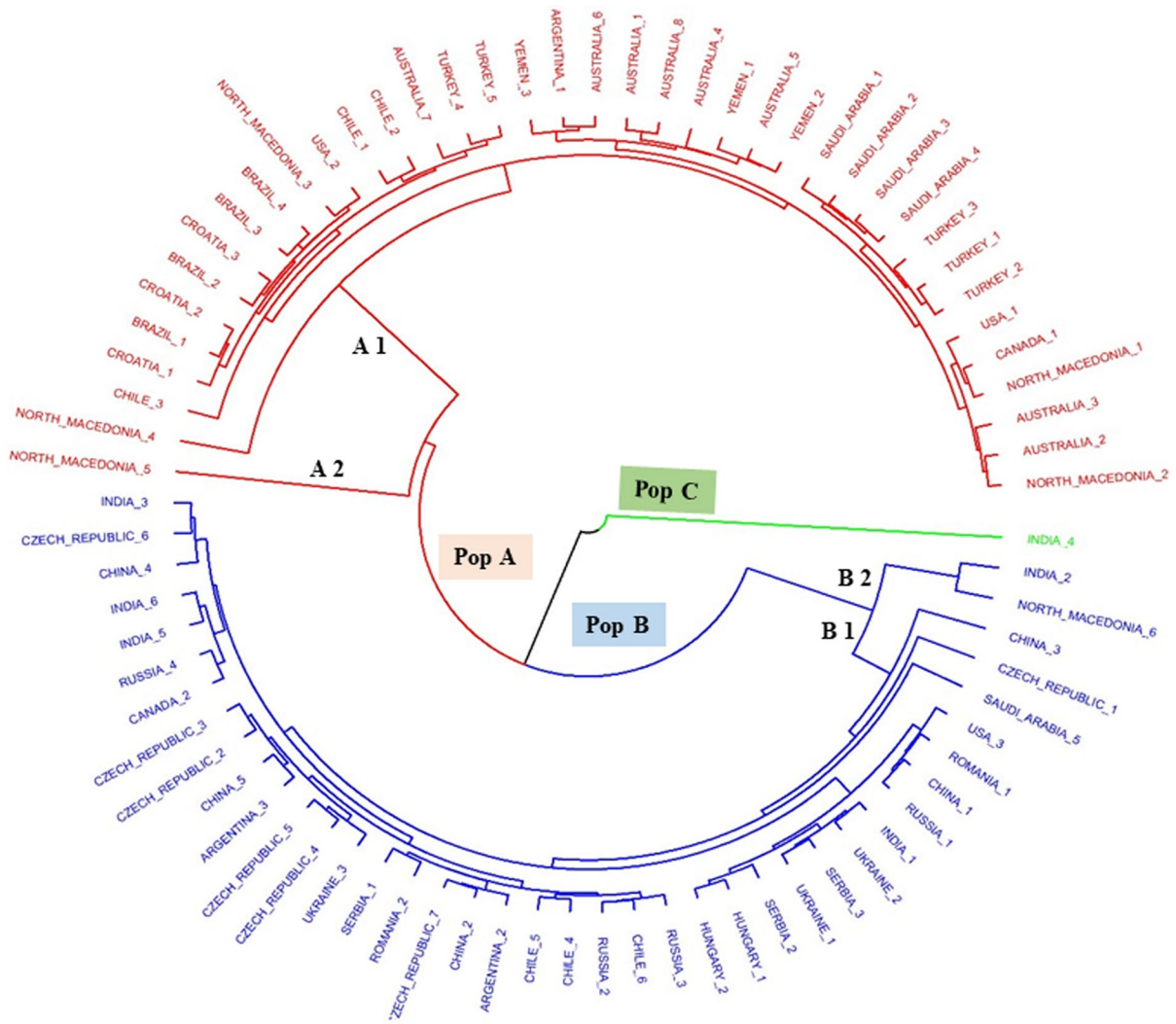

Fig. 2 The neighbor-joining analysis-based clustering of tested wheat germplasm using start codon targeted (SCoT) marker system

(population A and population B) on the basis of their collection regions, while one accession was unclassified (Fig. 1). Furthermore, population A contained a total of 40 wheat accessions; on the other hand, 39 wheat accessions were present in population B with one unclassified population (India 4). Moreover, the mean fixation index was found 0.252 and 0.25 in population A and B respectively. The Neighbor-joining analysis separated the whole germplasm of wheat into three groups (A, B & C) on the basis of their collection points (Fig. 2). The principal coordinate analysis (PCoA) strengthen the clustering of model-based structure algorithm by separating the 80 collected wheat accessions into two populations and results is clearly showed that the population A has more genetic diversity then population B (Fig. 3).

Discussion

Molecular markers are widely observed as the most reliable tools for various genetic research applications, encompassing the characterization of genetic polymorphism at the DNA level, estimation of genetic distance, determination of genetic parentage, gene mapping, analysis of genetic variation, and marker-assisted selection (Nadeem et al. 2018). Several distinct molecular marker technologies have been developed, each based on a slightly different set of guiding principles and methods. Investigation of diversity genetic of bread wheat genotypes is an effective strategy to identify unique populations with specific applications for use in short term and long term purposes of plant breeding (Mhlaba et al. 2018). Therefore, a number of molecular markers such as simple sequence repeats (SSR) (Ahmed et al. 2020), random amplified polymorphic DNA (RAPD) (Nazarzadeh et al. 2020), inter-simple sequence repeats (ISSRs) (Nosair 2021), start codon targeted (SCoT) (Nouri et al. 2021), amplified fragment length polymorphisms (AFLPs) (Roncallo et al. 2019), iPBS-retrotransposons (Nadeem 2021) and sequence related amplified polymorphism (SRAP) (Khaled et al. 2021) marker are well-known and have been explored for their potential use in wheat to assess genetic diversity and their characterizations. Mohammadi et al. (2022) conducted an investigation aimed at exploring the correlation between SCoT markers and agro-physiological traits in durum wheat breeding germplasm. A total of 220

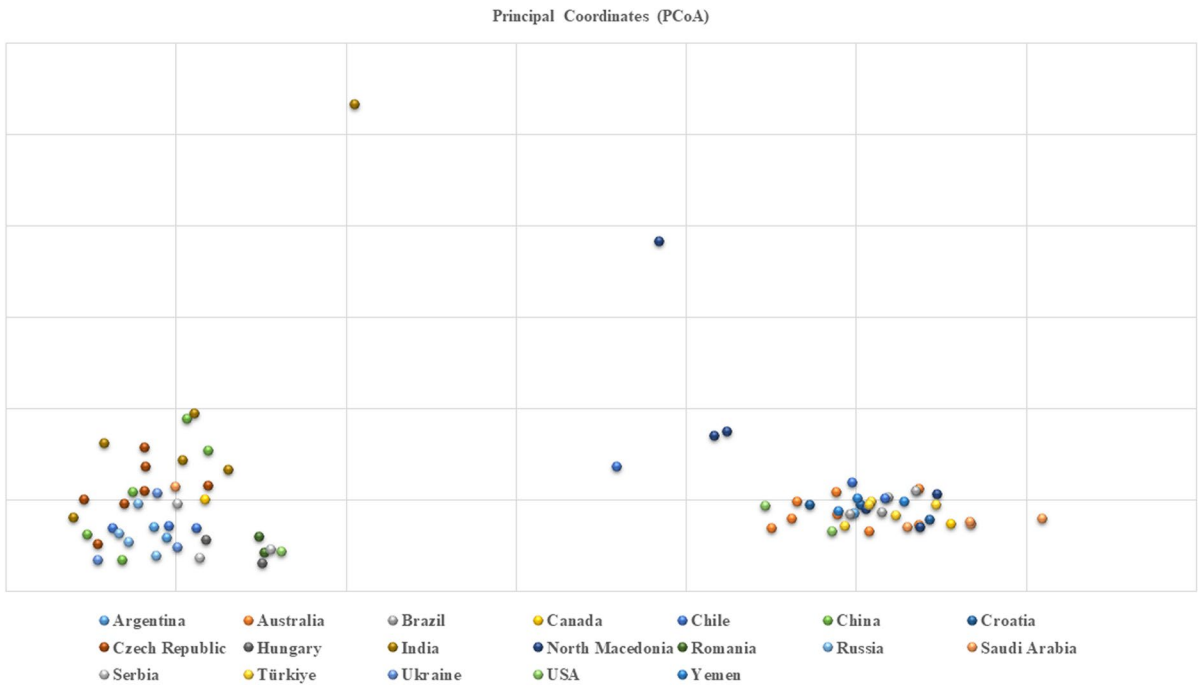

Fig. 3 Principal coordinate analysis (PCoA) of tested wheat germplasm using start codon targeted (SCoT) marker System

durum wheat genotypes, acquired from ICARDA to CIMMYT, underwent evaluation for agronomic, phenological, and physiological traits over two consecutive cropping seasons (2017–18 and 2018–19). Genotyping was performed using five SCoT markers, and a regression analysis method was employed to identify informative markers associated with each trait. Results revealed significant variation in agro-physiological traits among the studied germplasms. ICARDA germplasms, favoring traits like grain yield, 1000-kernel weight, normalized difference vegetation index, plant height, and peduncle length, exhibited distinctions from CIMMYT germplasms, emphasizing the potential for drought tolerance in ICARDA-derived lines. Thirteen SCoT loci demonstrated associations with at least three traits, enhancing parental selection efficiency. Six repeatable SCoT loci emerged as candidate markers for broader germplasm assessments and genome scanning. This study, utilizing phenotypic data and SCoT markers, uncovers substantial diversity in durum wheat breeding germplasm, providing valuable insights for breeding programs, including germplasm grouping, high-yielding genotype development, and cross-breeding initiatives.

During the present study, a total of 14 SCoT primers were utilized to evaluate the genetic diversity and molecular characterization of wheat germplasms. The applied 14 SCoT primers produced a total of 399 bands with an average of 28.05 bands per primer. Total and polymorphic bands reported in this study were higher than in previous research (Pour-Aboughadareh et al. 2017; Mohamed et al. 2017; Ibrahim et al. 2017; Gholamian et al. 2019; Abouseada et al. 2023). Abdel-Lateif and Hewedy (2018) utilized a total of six SCoT primers to assess genetic diversity among eight Egyptian wheat cultivars. They reported that each primer produced an average of 3.6 bands. In another study by Kheibari et al. (2020), SCoT markers were used for the assessment of rainfed durum wheat and the average number of bands was (12.07), which was lower than our study. Alshehri (2019) investigated genetic diversity among different wheat cultivars using SCoT markers, and the average number of bands found in their study was 5.6, while an average of 7.8 bands was found in the study of Shaban et al. (2022), who also used SCoT markers. These results were lower than in our present study. Etminan et al. (2016) found an average of 9 bands using SCoT markers during the assessment of genetic diversity in wheat germplasm. The polymorphism (%) ranged from 62.96 to 96.55% with an average value of 82.54%, which were higher than that reported by Nazarzadeh et al. (2020) using RAPD and ISSR markers, Kumar et al. (2020) using ISSR marker Alshehri et al. (2020), using SCoT and ISSR primers, Mohamed et al. (2017) using SCoT primers, Çifçi and Yağdi (2012) using RAPD markers and Abouseada et al. (2023) using SCoT and ISSR primers. Etminan et al. (2016) found 100% polymorphism using the SCoT markers during the assessment of genetic diversity in wheat germplasm. Nosair (2021) reported 98.57% polymorphism in seven Egyptian wheat (*Triticum aestivum* L) cultivars using Scot markers.

The PIC is a frequently used metric for determining the utility of molecular markers (Houmanat et al. 2016). In the present study, the maximum PIC value recorded was 0.59 for the SCoT23, while the minimum value was 0.26 for the SCoT30. The average PIC value for all tested primers was 0.39. The PIC value of the present investigation (0.39) was in line with Etminan et al. (2016), who also used SCoT markers for the assessment of genetic variability among durum wheat. The mean PIC value reported here were found to be higher than earlier research by Mohamed et al. (2017) and Abouseada et al. (2023). They used SCoT markers for the assessment of wheat germplasm. Ibrahim et al. (2017) also used SCoT markers and revealed a PIC value of 0.24 during the assessment of genetic diversity in wheat cultivars. Gholamian et al. (2019) and Alemu et al. (2020) reported lower PIC values than our research. The higher PIC value showed the effectiveness and utility of SCoT primers for assessing the genetic diversity in wheat germplasm (Yıldız et al. 2022). The highest effective alleles (n_e), 1.59, was recorded with SCoT23, followed by SCoT2, while the average n_e of 1.39 was recorded for all tested SCoT primers. A greater number of effective alleles is favorable as it indicates a higher level of genetic variability. The effective number of alleles (n_e) reported in this study was greater than in previous wheat research (Tajibayev et al. 2023; Nadeem et al. 2021; Marzang et al. 2020) Shannon's information index is an important criterion for recognizing the variation, as it differentiates the genetic diversity in a population by combining abundance and evenness (Ali et al. 2019).

The average Shannon's information index (*I)* for SCoT primers observed at 0.38 was higher than the previous wheat research of different marker systems (Nadeem et al. 2021; Marzang et al. 2020) The result of I was in line with Tajibayev et al. (2023), who used iPBS-retrotransposons markers for the assessment of genetic variation in spring durum wheat. The maximum gene diversity (*h*) of 0.34 was observed with ScoT23, and the minimum of 0.18 was recorded with SCoT27. The mean *h* value of 0.24 was observed for all testedSCoTprimers in wheat diversity, which was greater than Nadeem et al. (2021), who reported 0.21 and 0.20 for landraces and cultivars, respectively. Tajibayev et al. (2023) reported gene diversity of 0.25, which was approximately equal to the gene diversity value of our present study. The average genetic distance was observed to be 0.242 in the present investigation, with the minimum genetic distance of 0.08 (Saudiarabia-1 and Saudiarabia-5) and the maximum genetic distance of 0.432 (Chile-3 and India-4). Results from the Analysis of Molecular Variance (AMOVA) concluded that a higher proportion of genetic variations (92%) exists within the assessing population compared to among the populations (8%) (Table 4). These AMOVA results align with findings by Etminan et al. (2016), Mourad et al. (2020) and Darvıshzadeh and Bernousı (2012). The results of AMOVA were greater than in other wheat research (Nadeem et al. 2021; Tajibayev et al. 2023).

The model-based structure algorithm grouped 80 bread wheat genotypes into two populations (Population A and B) and one unclassified population (Hungary-2) based on a membership coefficient = > 75% (Fig. 1). Population A was found to be almost equal to population B, accounting for 50% (40 genotypes). Population B comprised a total of 39 wheat genotypes, representing 48.75% of all grouped genotypes. Only Population A consisted of accession numbers collected from South America (Argentina, Brazil, and Chile), North America (Canada), Southeast Europe (Croatia), Central Europe (Czech Republic), Eastern Europe (Hungary), Australia, and East Asia (China). Population B consisted of the accession collected from Southern Asia (India), Southern Europe (North Macedonia), Southeastern Europe (Serbia), Southeastern Europe and Western Asia (Turkey), Eastern Europe (Romania, Ukraine), Eastern Europe and North Asia (Russia), Southwest Asia (Saudi Arabia, Yemen), and North America (United States of America, USA). The results of the structure analysis indicate that accessions from the same regions or their neighboring regions demonstrated similarity with each other. For example, in population A, accessions collected from South America showed genetic similarity with North American accessions, while similarity was observed between accessions from Southeast Europe and Central Europe with those from Eastern Europe. A similar level of genetic similarity was also observed in population B and it was confirmed through fixation index resulted from structure analysis. The fixation index (F_{ST}) measures the degree of gene divergence between populations based on allele frequencies. A low level of genetic differentiation is indicated by an F_{ST} of less than 0.05, whereas a moderate level of genetic differentiation is indicated by an F_{ST} of 0.05 to 0.15. Furthermore, a considerable genetic differentiation is indicated by an F_{ST} of 0.15 to 0.25, and a very large genetic differentiation is indicated by an F_{ST} greater than 0.25 (Meirmans and Hedrick, 2011). In this study, we observed F_{ST} value of 0.252 and 025 for population A and B respectively and these values confirmed the presence of moderate to large level of genetic differentiation between both populations. The neighbor-joining dendrogram analysis divided the examined wheat germplasm into three groups (Groups A, B, and C), primarily distinguished by the locations of their respective collection points (Fig. 2). Groups A and B are well-defined such as group A contained of 39 accessions while group B was consisted on 40 accessions of wheat but group C consists only of one accession (India 4). There are various reasons why this single accession is placed in a separate group. It could be due to impurities in its DNA, or significant novelties that differentiate it from all others. This separation was also observed in our model-based structure algorithm, where it was placed in an unclassified population. With few exceptions, the Neighbor-joining clustering method exhibited a clustering pattern similar to the model-based structure, dividing the entire germplasm into two to three groups. Notably, accessions from Chilli, Australia, and Brazil were consistently grouped together, mirroring the patterns observed in population structures. Although certain accessions were placed in-group B in the Neighbor-joining tree, their presence in-group A was evident in the population structure. Despite this discrepancy, these accessions clustered together in the Neighbor-joining method. Notably, accessions

from the same countries consistently grouped together, either among themselves or with accessions from other countries. The clustering of accessions from different regions implies shared genetic similarities (Yildiz et al. 2022). The structure clustering was preferred over other clustering algorithms as it has been demonstrated to be more reliable and significantly more illuminating (Newell et al. 2013; Bouchet et al. 2012). In addition, different diversity parameters were computed for the populations that underwent the structure evaluation, revealing the presence of greater genetic variations. This was further confirmed by the AMOVA study for the structure-evaluated population that there is more genetic variation within populations than among them. The aggregation of model-based structure was validated by the PCoA analysis, which also contributed to the division of the wheat germplasm into two distinct populations (Fig. 3). Hence, it can be argued that the evaluated wheat germplasm possesses a substantial amount of genetic variations within the population (92%), potentially beneficial for future crop breeding efforts.

Conclusion

In conclusion, research employing SCoT markers has revealed significant genetic variability within tested wheat germplasm, presenting great potential for safeguarding forthcoming food sustainability. The investigation of a wide range of genetic resources presents a valuable collection of prospective characteristics, allowing breeders to cultivate enhanced wheat cultivars that possess increased adaptability, disease resistance, and higher yields. Genetic diversity plays a crucial role in mitigating global concerns, including climate change and population expansion, which pose significant threats to food production and sustainability. Through the utilization of the valuable data obtained from SCoT markers, scholars and decision-makers can make substantial advancements in strengthening food security, ultimately ensuring a more resilient and productive agricultural future.

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Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors have read and shown their willingness to publish this manuscript.

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