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RESEARCH ARTICLE

Genetic diversity and population structure analysis of bread wheat (*Triticum aestivum* L.) germplasms as revealed by inter simple sequence repeat (ISSR) markers

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Abstract Wheat is one of the most important staple food crops in Ethiopia and elsewhere in the world. Knowledge of its genetic diversity provides breeders a chance to develop new, improved cultivars with desirable traits. In this research the genetic diversity and population structure of 180 bread wheat germplasms representing eight populations was analyzed using 10 Inter simple sequence repeat (ISSR) markers used. Based on diversity indices all the ISSR markers were polymorphic and highly informative. High within-populations genetic diversity with a mean allele values of 1.47, effective alleles of 1.33, Shannon's Information index of 0.29, Nei's gene diversity of 0.19, and percent polymorphic loci of 80.5% were obtained in this research. Among the populations, those from the International Septoria Observation Nursery (ISEPTON) showed relatively, the highest observed number of alleles (1.47), effective number of alleles (1.33), Shannon's Information index (0.4) and Nei's gene diversity (0.19). Thus, the ISEPTON lines could be a good source of useful alleles to be used in wheat improvement programs. Analysis of molecular variance revealed that 95% of the total genetic variation resides within populations, while the remaining 5% is accounted to among populations. Clustering using unweighted pair group method with arithmetic mean, principal coordinate analysis and population structure analysis did not group the wheat populations into genetically distinct clusters following their breeding objectives. In the present research adequate polymorphism and reproducible fingerprint profiles were explored from of the genetic structure analysis in the wheat germplasms. The information obtained could thus be useful in future bread wheat breeding programs as well it its sustainable use.

Keywords Bread wheat · Genetic diversity · Gene diversity · ISSR · PCoA · Population structure

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Introduction

Wheat is one of the most important cereal crops grown around the world, and it is an essential

component of most food industries that feed billions of people (Dawlah et al. 2015). In 2017, over 219 million ha of agricultural land in the world was planted with wheat, with a total expected production and productivity of 771.7 MT and 3.5 t/ha, respectively. China, India, and Russia are among the world's top wheat-producing countries (FAO 2017). Common wheat, also known as bread wheat, accounts for 95% of wheat production worldwide and is the dominant wheat type in Sub-Saharan Africa (Gemechu and Tadese 2018). Next to South Africa, Ethiopia is the second-largest wheat producer in Africa (FAO 2017). The importance of wheat is not only for making bread, biscuits, and pastry products but also for the production of starch and gluten (Hanson 2022).

Since 1991, the amount of wheat consumed in Sub-Saharan Africa (SSA) has significantly increased due to rising urbanization and income levels. The situation has resulted in a change in people's dietary preferences from eating grains such as maize, sorghum, and millet to wheat and rice, as well as tuber crops like cassava, sweet potato, and yam. The Sub-Sharan Africa region imports more than 15 million tons of wheat grain each year, as the region's local wheat output has ffallen short to keep up with the rising demand (Listman 2019). By 2025, wheat consumption in Africa is projected to reach 76.5 MMT, of which 48.3 MMT, which accounts for 63.3% of wheat demand should be covered through imports (Tadesse et al. 2018). In Ethiopia, wheat is the main staple food for more than 76% of the Ethiopian population, and it also used as animal feed and for income generation (FAO 2018). It ranks fourth after Teff, Maize, and Sorghum in area coverage and third after Maize and Teff in total production (CSA 2010). Ever-increasing demand for wheat and its production challenges due to climate change, increased cost of inputs, and increased intensity of abiotic (such as soil acidity, drought, and heat) and biotic (such as diseases and pests) stresses, make the wheat demand–supply chain very volatile and at times lead to social instabilities (Bayeh 2010). The lower productivity could be attributed to other additional factors, such as limited access to advanced production technologies and low agricultural inputs (Anteneh and Asrat 2020).

The major wheat-growing areas in Ethiopia are located between 6 and 16° N and 35 and 42° E and at altitudes ranging from 1500 to 3000 m above sea level. The most suitable areas for wheat production, however, fall between 1900 and 2700 m. Irrespective of the significant increase in wheat cultivation land and production in Ethiopia, the national average of wheat productivity in Ethiopia stands at 3.0 t ha^{-1}, far lower than the global average of 3.55 t ha^{-1} resulting in a production deficit to meet the rising local demand (Gemechu and Tadese 2018). Ethiopia satisfies 25–35% of its domestic wheat demand through commercial imports and food aid (Agriculture Global Practice 2018).

Improving wheat production and productivity involves exploring the available genetic resources, and their wise management. Knowledge of the genetic diversity of a crop helps breeders to choose desirable parents for breeding programs and gene introgression from distantly related germplasm to maintain sustainable crop productivity. The presence of genetic diversity offers the opportunity for plant breeders to develop new and improved cultivars with desirable characteristics including high yield potential, large seed, biotic stress resistance (disease and insect resistance) as well as tolerance to abiotic stresses like drought, cold and salinity (Allard 1999).

DNA markers are widely used to identify genetic similarities and differences among plant genotypes that are resistant to changing environmental conditions. Molecular marker-based characterization does not require previous pedigree information (Frisch et al. 1999), and it has played a crucial role in the conservation, and use of plant genetic resources in trait improvement programs for various crops (Tirfessa et al 2020). So far, several DNA markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), simple sequence repeat (SSR), and single nucleotide polymorphisms (SNP)s have been widely used to address challenges that occur in changing ecology and agriculture. ISSR are among those extensively used in plant research, particularly for the evaluation of genetic variability in wheat (Najaphy et al. 2011; Najaphy et al. 2012; Ng and Tan 2015; Rafiq et al. 2016). They are useful genetic tools to assess genetic variation both within and among populations, being highly polymorphic, more accessible projects, not requiring prior sequence information, and high reproducibility (Vijayan 2005). However, their application to address bread wheat genetic diversity in Ethiopia is scanty. Therefore, this research aimed to investigate the extent and pattern of

genetic variability and population structure of bread wheat lines in Ethiopia using ISSR markers.

Materials and methods

Plant material

In this research, a total of 180 bread wheat germplasms, 13 commercial cultivars (COV), and 167 recombinant inbreed lines obtained from CIMMYT were used. In the 167 CIMMYT genotypes, 49 were from the International Bread Wheat Screening Nursery (IBWSN), 56 from the International Septoria Observation Nursery (ISEPON), 14 from the High Rain Wheat Yield Trial (HRWYT), 34 from the High-Rain Wheat Screening Nursery (HRWSN), five from an adaptation trial (ADT), six from the national cultivar trial (NVT), and the remaining three genotypes were from a preliminary cultivar trial (PVT). Accordingly, the research materials were grouped into 8 populations (ADT, COV, HRWSN, HRWYT, IBWSN, ISEPTON, NVT, and PVT) based on their origin.

Genomic DNA extraction

For genotyping, high molecular weight genomic DNA (gDNA) was extracted from 2-week-old silica gel dried leaf samples pooled from five plants per line using a modified cetyl trimethyl ammonium bromide (CTAB) procedure (Ogbonnaya et al. 2001; Mekonnen et al. 2017). The quantity and quality of the extracted DNA were checked using Nanodrop spectrophotometry and electrophoresis on a 1% agarose gel, respectively.

ISSR genotyping and data scoring

Thirty ISSR primers ranging from 16 to 25 base pairs (bp) in length and representing di, tri, and tetramer repetitions were tested for polymorphism and reproducibility. Out of the 30 primers examined, 10 reproducible ones with distinct banding patterns were used. These were dissolved in 10 pmol/l concentration of sterilized, double-distilled water. For optimum amplification, several amounts of template DNA, dNTPs, and Taq polymerase were examined. Polymerase chain reaction (PCR) was carried out in a total volume of 20 µL containing 2.5 µL of 10×PCR buffer, 2.5 µL MgCl2 (25 mM), 0.5 µL dNTP mixture (10 mM), 0.5 µL primer (15 pmol/µL), 0.3 µL *Taq* DNA polymerase (1 U/µL), 2 µL of template DNA (80 ng/µL) and 11.7µL double-distilled water. Optimization of the PCR condition (annealing temperature) was performed on a Biometra 2003 T3 thermocycler (USA). The PCR amplification program involved an initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 min, optimized primer annealing conditions (45–52 °C for 1 min), and primer extension at 72 °C for 2 min, followed by a final extension step of 10 min at 72 °C. Amplification products were fractionated by loading 10 µl amplification product of each sample with 2 µl 6X loading dye on 1.67% agarose gel electrophoresis supplemented with ethidium bromide in 1×TBE buffer at 120 V for 2 h. The gel was visualized under UV light and subsequently photographed using a BIO-RAD Gel Doc TM EZ Imaging System. A 100-base-pair DNA ladder was used to estimate the amplification size.

For scoring purpose, an ISSR marker was used as dominant marker, and hence, each locus was considered as a bi-allelic locus with one amplifiable and one null allele. Scoring was performed manually for each primer based on presence (1) and absence (0) or ambiguous (?), and each band was regarded as a locus. The band scoring resulted in a "0" and "1" data matrix that was used for further analysis.

Statistical analysis

Different statistical software packages were used to research genetic diversity and population structure of the bread wheat germplasms. Locus based diversity parameters including number of observed alleles (Na), effective number of alleles (Ne), Shannon's information statistic (I), Nei's genetic diversity (h), total genetic diversity (H_T), genetic differentiation (H_S), genetic differentiation statistics by locus (Gst), estimate of the number of migrants (gene flow) (Nm) and percent polymorphic loci (PPL) were computed using POPGENE version 1.31 software (Yeh et al. 1999). Within population genetic diversity parameters such as number of observed alleles (Na), Effective number of alleles (Ne), Shannon's Information index (I), Nei's genetic diversity (h) and Percent of polymorphic Loci (PPL)

across loci were computed using GenAlEx ver. 6.501 software (Nei et al. 1983; Peakall and Smouse 2006). The polymorphism information content (PIC) was computed using the formula: PIC = 2f (1 − f) (23) where f is the frequency of occurrence of polymorphic bands in different primers. The same software was used to compute the analysis of molecular variance (AMOVA) and the estimation of the variance components. Clustering was carried out based on the unweighted pair group method with arithmetic mean (UPGMA) using molecular evolutionary genetics analysis (MEGA) software (Sudhir et al. 2018). Bayesian model-based clustering with presumed K populations was employed for 180 wheat germplasm using STRUCTURE, version 2.3.4 (Pritchard et al. 2000; Hubisz et al. 2009). The run parameters were 80,000 iterations of burn-in with 80,000 Monte Carlo Markov Chain (MCMC) iterations. Using web-based STRUCTU RE HARVESTER (Earl and VonHoldt 2011), K values from 1 to 10 were used with 15 independent runs for each K. A bar plot for the optimum K was determined using the Clumpak beta version (Kopelman et al. 2015).

Result

ISSR primers and their banding patterns

The 10 primers used revealed polymorphism ranging from 200 to 3200 bp in the amplified DNA fragments. Figure 1 presents a sample of scoreable polymorphic bands that were generated by primer UBC 834. Among the tested ISSR markers, 10 primers that relatively highly polymorphic, and reproducible with clear bands and an unambiguous amplification, generated a total of 166 bands (Table 1). The number of scorable bands for individual primers ranged from 12 to 25, with an average number of bands of 16.6 per primer. The highest number of bands was generated by primer UBC 873 with 25 bands, followed by primer UBC 834 with 21 bands. Whereas, the lowest amplification was recorded for primer 844, which generated 12 bands (Table 1). The fragment size amplified with these primers ranged from 150 to 1600 bps. The 10 primers were composed of di- and tetra-nucleotide sequences, and those with dinucleotide motifs (AG)n and (CT)n produced a high level of polymorphism. Table 1 presents the ISSR primers

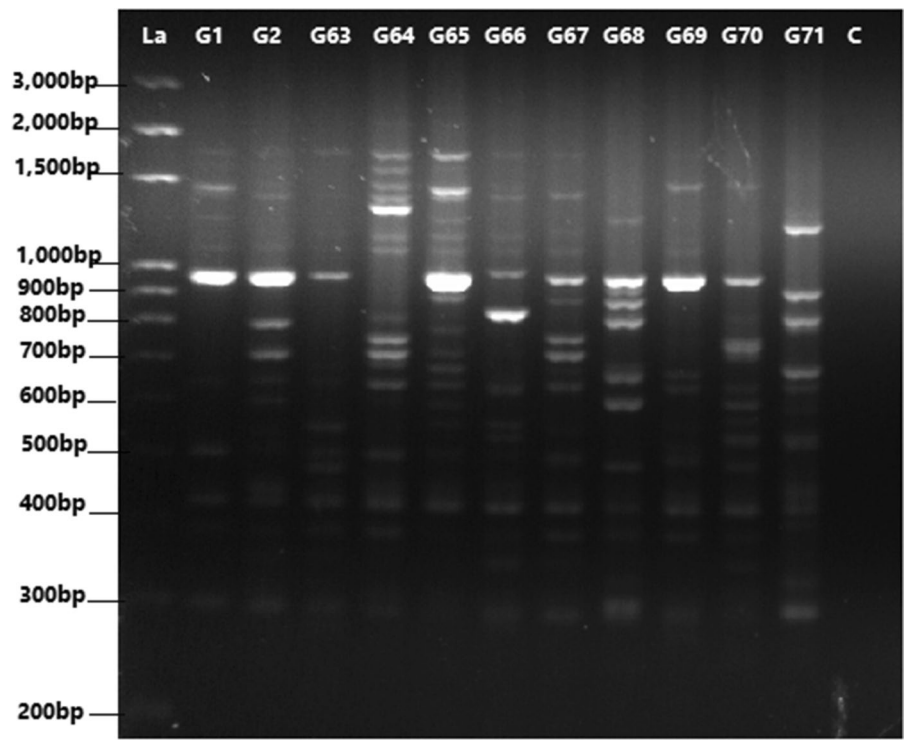

Fig. 1 Banding pattern of inter simple sequence repeat (ISSR) primer UBC 834 in 11 bread wheat genotypes: La: DNA ladder, G1: genotype 1, G2: genotype 2, G63: genotype 63, G64: genotype 64, G65: genotype 65, G66: genotype 66, G67: genotype 67, G68: genotype 68, G69: genotype 69, G70: genotype 70, G71: genotype 71 and C: negative control

Table 1 Reproducible and polymorphic inter simple sequence repeat (ISS)R primers with their primer motif, annealing temperature (°C), amplification patterns, fragment size and scorable bands

Primer	Primer motif	Annealing temperature (°C)	Amplification pattern	Fragment size (bp)	Scorable bands
UBC 822	(AC)8T	49	Reproducible and polymorphic	500–1500	13
UBC 825	(AC)7T	48	Reproducible and polymorphic	200–1400	14
UBC 826	(AC)8C	52	Reproducible and polymorphic	150–2000	13
UBC 834	(AG)8GTT	52	Reproducible and polymorphic	200–2100	21
UBC 836	(AG)8GTA	49	Reproducible and polymorphic	150–1400	20
UBC 844	(CT)8RC	48	Reproducible and polymorphic	400–2600	12
UBC 848	(CA)8NG	48	Reproducible and polymorphic	200–2100	14
UBC 853	(TC)8RT	49	Reproducible and polymorphic	350–2500	13
UBC 857	(AC)8YG	52	Reproducible and polymorphic	300–2000	21
UBC 873	(GACA)4	45	Reproducible and polymorphic	150–1900	25

N: (A, T, G, C); R: (A, G); Y: (C, T)

used, their sequence, repeat motif, amplification patterns and fragment sizes.

ISSR based Genetic diversity in bread wheat germplasms

The analysis revealed that the ISSR markers used were highly polymorphic and informative with a mean number of alleles of 1.90 (range 1.62–2.00), effective number of alleles of 1.43 (range 1.05–1.78), Shannon's Information index of 0.26 (range 0.04–0.42), Nei's gene diversity of 0.40 (range 0.09–0.61), and gene flow of 5.34 (range 2.02–14.64) (Table 2). The highest number of allele (Na = 2.00), effective number of allele (Ne = 1.78), gene diversity (h = 0.61 and Shannon's information index (I = 0.42) were obtained for primer 834, while primer UBC-853 resulted in least values for the corresponding genetic diversity indices (Table 2). The maximum percentage of polymorphic loci (100%) was produced by UBC-834, followed by UBC-857 (90.5%). However, primers UBC-826 and UBC-853 generated the least percentage of polymorphic loci, accounting for 61.5% and 50.00%, respectively. The locus polymorphic information content ranged from 0.34 (UBC-853) to 0.43 (UBC-834) with an overall mean value of 0.21 (Table 2). Markers

Table 2 Informativeness and other genetic diversity summary statistics of inter simple sequence repeat (ISSR) loci

Primer	Na	Ne	I	H	Ht	Hs	Gst	Nm	PPL (%)	PIC
UBC 822	1.85	1.34	0.22	0.34	0.16	0.15	−0.45	7.98	84.6	0.14
UBC 825	2.00	1.27	0.20	0.35	0.17	0.14	0.15	3.24	85.7	0.13
UBC 826	2.00	1.27	0.20	0.35	0.17	0.14	0.15	3.24	61.5	0.13
UBC 834	2.00	1.78	0.42	0.61	0.44	0.36	0.17	6.06	100	0.43
UBC 836	1.95	1.50	0.29	0.44	0.28	0.21	0.21	3.43	90	0.26
UBC 844	1.83	1.50	0.29	0.43	0.22	0.18	0.17	4.83	83.3	0.22
UBC 848	2.00	1.60	0.36	0.54	0.34	0.26	0.21	2.02	71.4	0.29
UBC 853	1.62	1.05	0.04	0.09	0.04	0.04	0.08	14.67	53.8	0.07
UBC 857	1.95	1.50	0.29	0.44	0.28	0.21	0.21	3.43	90.5	0.13
UBC 873	1.84	1.47	0.28	0.43	0.29	0.24	0.23	4.52	84	0.34
Mean	1.90	1.43	0.26	0.40	0.24	0.19	0.11	5.34	80.5	0.21

Na: Observed number of alleles; Ne: Effective number of alleles; I: Shannon's Information index; h: Nei's gene diversity; H_T:Total genetic diversity H_S: genetic differentiation; Gst: Genetic differentiation statistics by locus; Nm: estimate of the number of migrants (gene flow) from Gst where Nm = 0.5(1 − Gst)/Gst, PPL: Percent of polymorphic Loci, PIC: polymorphic information content

with a PIC value between 0.25 and 0.5 were considered moderately informative, and less than 0.25 were considered less informative. Accordingly, 40% of the used ISSR markers showed moderate PIC (Table 2). Moreover, 50% of the primers (5) demonstrated PIC values greater than the average value (0.21).

Genetic variability within and among populations

The average within population genetic diversity estimates for the varying population sizes ranging from 3 to 56 were: estimated allele frequency (p and q), p of 0.54 and q of 0.46, observed alleles of 1.47, effective alleles of 1.33 Shannon's information index of 0.29, Nei's gene diversity value of 0.19, unbiased expected heterozygosity of 0.20 and percentage of polymorphic loci of 65.06% (Table 3). The ISEPTON population showed the highest observed number of alleles, effective number of alleles, Nei's gene diversity, Shannon's information index, and PPL while IBWSN and HRWSN populations ranked second and third, respectively. The highest and the lowest Nei's gene diversity were observed in the populations of ISEPTON and PVT, respectively. Fifty percent of the research populations displayed a genetic diversity greater than mean value (h = 0.19) and the PPL per population was between 15.06% (PVT) and 83.13%% (ISEPTON) with an overall mean of 57.23%. A summary of the different genetic diversity estimates over all loci across populations is presented in Table 3.

Banding patterns across populations

In the banding pattern analysis of 180 germplasms that were grouped into eight populations, HRWSN represented the highest number of bands (165), followed by ISEPTON and IBWSN with 164 and 163 bands, respectively, while ADT comprises the lowest number of bands (120). The highest mean heterogeneity (0.19) was observed in COV, followed by NVT and PVT with mean heterogeneities of 0.18 and 0.14, respectively. Private bands used for distinguishing populations from each other were not identified; all populations had no private band (Fig. 2).

Genetic relationships between the populations

Estimates using genetic distance (below diagonal) and gene flow (above diagonal) showed the highest genetic distance of 0.14, 0.12, and 0.1 between the populations of PVT and COV, PVT and DAT, and HRWSN and PVT, respectively. A low genetic distance (0.01) was observed between the populations of IBWSN and HRWSN (Table 4).

Table 3 Allelic patterns and diversity indices across eight wheat populations estimated from 10 inter simple sequence repeat (ISSR) markers

Popn	N	Band frequency	P	Q	Na	Ne	I	h	uHe	PPL (%)
DAT	5	0.65	0.62	0.38	0.87	1.10	0.08	0.06	0.06	15.06
COV	13	0.64	0.54	0.46	1.34	1.33	0.28	0.19	0.20	51.20
HRWSN	34	0.62	0.51	0.49	1.60	1.39	0.33	0.22	0.23	65.66
HRWYT	14	0.62	0.49	0.51	1.78	1.43	0.38	0.25	0.26	78.92
IBWSN	49	0.62	0.50	0.50	1.77	1.41	0.38	0.25	0.25	78.92
ISEPTON	56	0.63	0.50	0.50	1.82	1.44	0.40	0.26	0.27	83.13
NVT	6	0.67	0.58	0.43	1.41	1.32	0.27	0.184	0.20	50.00
PVT	3	0.66	0.59	0.41	1.18	1.24	0.20	0.138	0.17	34.94
Mean	22	0.64	0.54	0.46	1.47	1.33	0.29	0.19	0.20	57.23

N: sample size, Na: Observed number of alleles, p & q: Estimated Allele Frequency, Ne: Effective number of alleles, h: Nei's gene diversity, I: Shannon's Information index uHe:Expected and Unbiased Expected Heterozygosity and PPL: percentage of polymorphic loci. Na: No. of Different Alleles, Ne: No. of Effective Alleles: 1/(p^2 + q^2), I: Shannon's Information Index: − 1* (p * Ln (p) + q * Ln(q)), He = Expected Heterozygosity: 2 * p * q, uHe: Unbiased Expected Heterozygosity: (2N/(2N − 1)) * He Where for Diploid Binary data and assuming Hardy–Weinberg Equilibrium, q: (1 − Band Freq.) ^0.5 and p: 1 − q

Population abbreviations are: *ADT* adaptation trial, *COV* commercial verities, *HRWSN* high rain weed trial screening nursery, *HRWYT* high rain weed yield trial, *IBWSN* international bread wheat screening nursery, *ISEPTON* international Septoria observation nursery, *NVT* national verification trial, *PVT* primary verification trial

The pairwise coefficient of genetic differentiation between the populations ranged from 0.000 (PVT and NVT) to 0.410 (between PVT and DAT). The highest and statistically significant genetic differentiation, (PhiPT = 0.410, $p = 0.017$) was observed between populations of PVT, and DAT, implying the lowest gene flow between them. The second-highest genetic differentiation with a gene flow rate of 0.365 was observed between the populations of NVT and DAT. The genetic differences between populations of HRWYT and IBWSN, NVT and PVT, HRWYT and NVT, ISEPTON and PVT, HRWSN and PVT,

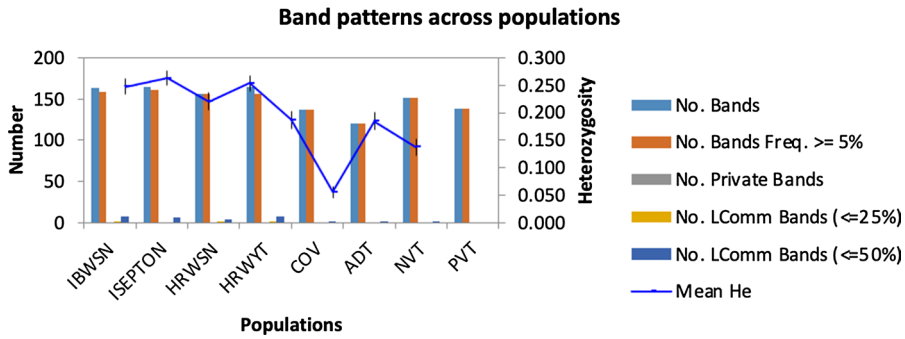

Fig. 2 Band pattern across populations, Population abbreviations are: *ADT* adaptation trial, *COV* commercial verities, *HRWSN* high rain weed trial screening nursery, *HRWYT* high rain weed yield trial, *IBWSN* international bread wheat screening nursery, *ISEPTON* international Septoria observation nursery, *NVT* national verification trial and *PVT* primary verification trial

Table 4 Pairwise Nei's measures of genetic distance

Pop ID	DAT	COV	HRWSN	HRWYT	IBWSN	ISEPTON	NVT	PVT
DAT	****							
COV	0.06	****						
HRWSN	0.05	0.03	****					
HRWYT	0.07	0.05	0.02	****				
IBWSN	0.05	0.03	0.01	0.02	****			
ISEPTON	0.06	0.03	0.01	0.02	0.01	****		
NVT	0.07	0.06	0.04	0.04	0.05	0.05	****	

Population abbreviations are: *ADT* adaptation trial, *COV* commercial verities, *HRWSN* high rain weed trial screening nursery, *HRWYT* high rain weed yield trial, *IBWSN* international bread wheat screening nursery, *ISEPTON* international Septoria observation nursery, *NVT* national verification trial, *PVT* primary verification trial

Table 5 Population genetic differentiation measured by PhiPT (below the diagonal) between the eight bread wheat populations with *p* values above the diagonal

Pop ID	DAT	COV	HRWSN	HRWYT	IBWSN	ISEPTON	NVT	PVT
DAT	****	0.036	0.003	0.001	0.001	0.001	0.006	0.017
COV	0.118	****	0.123	0.001	0.001	0.001	0.001	0.024
HRWSN	0.183	0.029	****	0.001	0.001	0.012	0.015	0.304
HRWYT	0.257	0.166	0.082	****	0.406	0.200	0.352	0.272
IBWSN	0.233	0.140	0.078	0.000	****	0.168	0.200	0.100
ISEPTON	0.174	0.107	0.043	0.006	0.006	****	0.213	0.269
NVT	0.365	0.204	0.104	0.005	0.019	0.021	****	0.388
PVT	0.410	0.173	0.024	0.024	0.061	0.028	0.000	****

ISEPTON and NVT, ISEPTON and NVT, HRWYT and ISEPTON, COV and HRWS, and IBWSN and PVT were not statistically significant ($p > 0.05$) (Table 5).

Genetic relationship within and among populations

An analysis of molecular variance (AMOVA) was performed to observe variation among and within-populations. Analysis of molecular variance using the 166 ISSR bands generated by the ten primers in eight bread wheat populations revealed that a higher percent (95%) of the total genetic variation was attributed to within-population genetic variability, leaving only 5% for among-populations genetic variations. The populations showed statistically significant low genetic differentiation (FST value of = 0.054; $p < 0.001$). The AMOVA results revealed highly significant genetic differences ($p < 0.001$) among the eight bread wheat populations (Table 6).

Cluster analysis using PCoA and UPGMA

PCoA is a technique frequently used in multivariate statistics to display the pattern of genetic structure and similarly to determine the amounts of variance described per component and cumulatively (Mekonnen et al. 2020 and references therein). In the current research, PCoA explained 34.46% of the total genetic variations, and the first three axes (1, 2, and 3) accounted for 19.63%, 14.83%, and 9.29%, respectively. It clustered the entire population into three subgroups with high genetic admixture. PCoA clustered the ADT, COV, and NVT populations together. However, individuals from HRWYT, IBWSN, and ISEPTION populations showed nearly a uniform distribution on the two-dimensional coordinate plane without distinct population clustering. None of the clusters were composed entirely of germplasms from a particular population (Fig. 3).

The dendrogram generated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) grouped the eight populations into three major clusters (C1, C2, and C3), each of which was further grouped into two sub-clusters. One hundred eighteen (66%) of the germplasms were assigned to C3 followed by C2 and C1, which were composed of 38 (21%) individuals. The lowest numbers (24 or 13%) of germplasm were assigned to cluster C2 (Fig. 3). None of the major clusters were composed of individuals from a single population, indicating the presence of high genetic admixture. The dendrogram generated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) also grouped the eight populations into three major clusters (Fig. 4).

Population genetic structure analysis

It is known that testing for population structure is important while conducting association investigations and drawing relationships between markers. Accordingly, wheat germplasm can be efficiently categorized using population structure analyses, and maximum membership probability in STRUCTURE. The NJ, UPGMA clustering, and PCoA failed to group the wheat populations, indicating the presence of high genetic intermixing among the eight bread wheat populations. The Bayesian model-based population structure of the 180 wheat genotypes was applied using Structure v2.3.4 software, and the K value was used to estimate the number of clusters of the isolates. The output of the structure harvester revealed that the delta K (ΔK) values reached a sharp peak at K = 3 (Fig. 5A). The Clumpak result (bar plot) detected a greater degree of genetic admixture between the three subpopulations (Fig. 5B).

Table 6 Analysis of molecular variance (AMOVA) within and among the research populations

Source of variation	df	SS	MS	Est. Var	PV (%)	FST	*p* value
Among Pops	7	317.93	45.42	1.21	5	0.05	$p < 0.001$
Within Pops	172	3640.34	21.17	21.17	95		$p < 0.001$
Total	179	3958.26		22.37	100		

df degree of freedom, *SS* sum of squares, *MS* mean square, *Est. Var.* estimated variation, *PV* percent of variation, *FST* Fixation indices

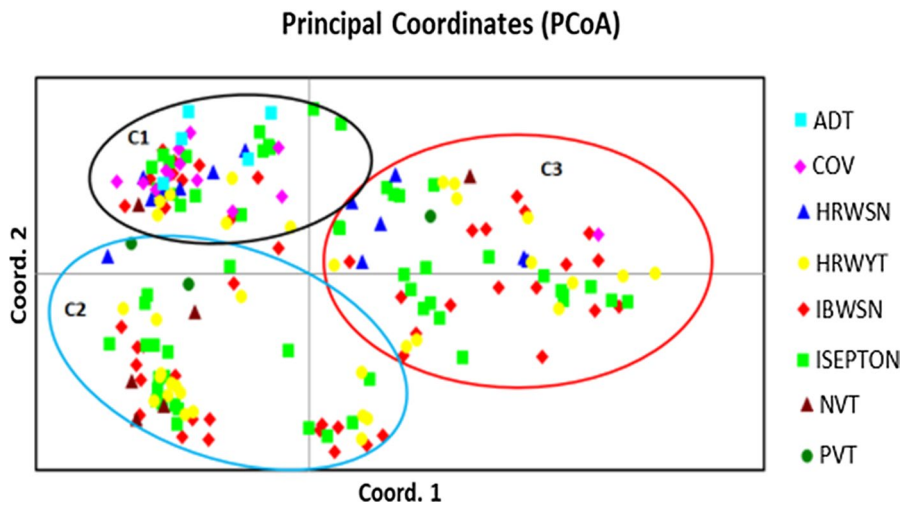

Fig. 3 Principal coordinate analysis (PCoA) of the 180 bread wheat germplasms as revealed by 10 inter simple sequence repeat (ISSR) markers,samples coded with the same symbol and color belongs to the same population. Population abbreviations are: *ADT* adaptation trial, *COV* commercial cultivars, *HRWSN* high rain weed trial screening nursery, *HRWYT* high rain weed yield trial, *IBWSN* international bread wheat screening nursery, *ISEPTON* international Septoria observation nursery, *NVT* national verification trial and *PVT* primary verification trial

Discussion

Genetic diversity in wheat has been increasingly narrowed down due to different reasons, modern breeding practices being the major one (Apoorva et al. 2014). For the improvement of existing germplasm genetic diversity investigations are important (Zeb et al. 2009). In the current research, the genetic diversity and population structure analysis of 180 bread wheat germplasms were conducted so that they could be used in the selection of parents with desirable traits for forthcoming breeding programs and also for sustainable use of this valuable genetic resource.

Polymorphism level of the ISSR markers

In this research, ISSR primers with dinucleotide motifs (AG)n and (CT)n produced a high level of polymorphism in bread wheat, indicating that dinucleotide primers are more suitable for amplification. The result is in agreement with other investigations (Carvalho et al. 2009; Najaphy et al. 2011). In this research, of the total 166 DNA fragments (bands) produced, 134 (80.5%) fragments were polymorphic, and the polymorphism among germplasms ranged from 53.8 to 100% with an average of 80.5%, and the mean number of bands per primer was 16.6. Likewise, polymorphism among germplasms was in line with Ateş-Sönmezoğlu et al. (2022), which was 75%. The total number of bands observed in this research was substantially higher than reported by Abdollah et al. (2011) who testified a total of 86 bands, with the percentage of polymorphic bands ranging between 60 and 100 and an average of 80.2% using 10 ISSR primers. The mean number of bands per locus observed in the present research (16.6) was considerably higher than the level (8.6 bands per primer) reported by Abdollah et al. (2011). The difference was attributed to the high number of genotypes used in the present research, marker polymorphism differences, and also genotype variations.

In this research, the polymorphic information content of the used ISSR loci was found across populations range from 0.07 to 0.43, with an overall mean of 0.21. The moderate PIC observed in most of the ISSR primers could be attributed to the diverse nature of the bread wheat germplasm and/or the highly informativeness of the ISSR markers used. The ISSR markers used are highly informative, and have been found to be useful genetic tools for wheat genetic structure analysis.

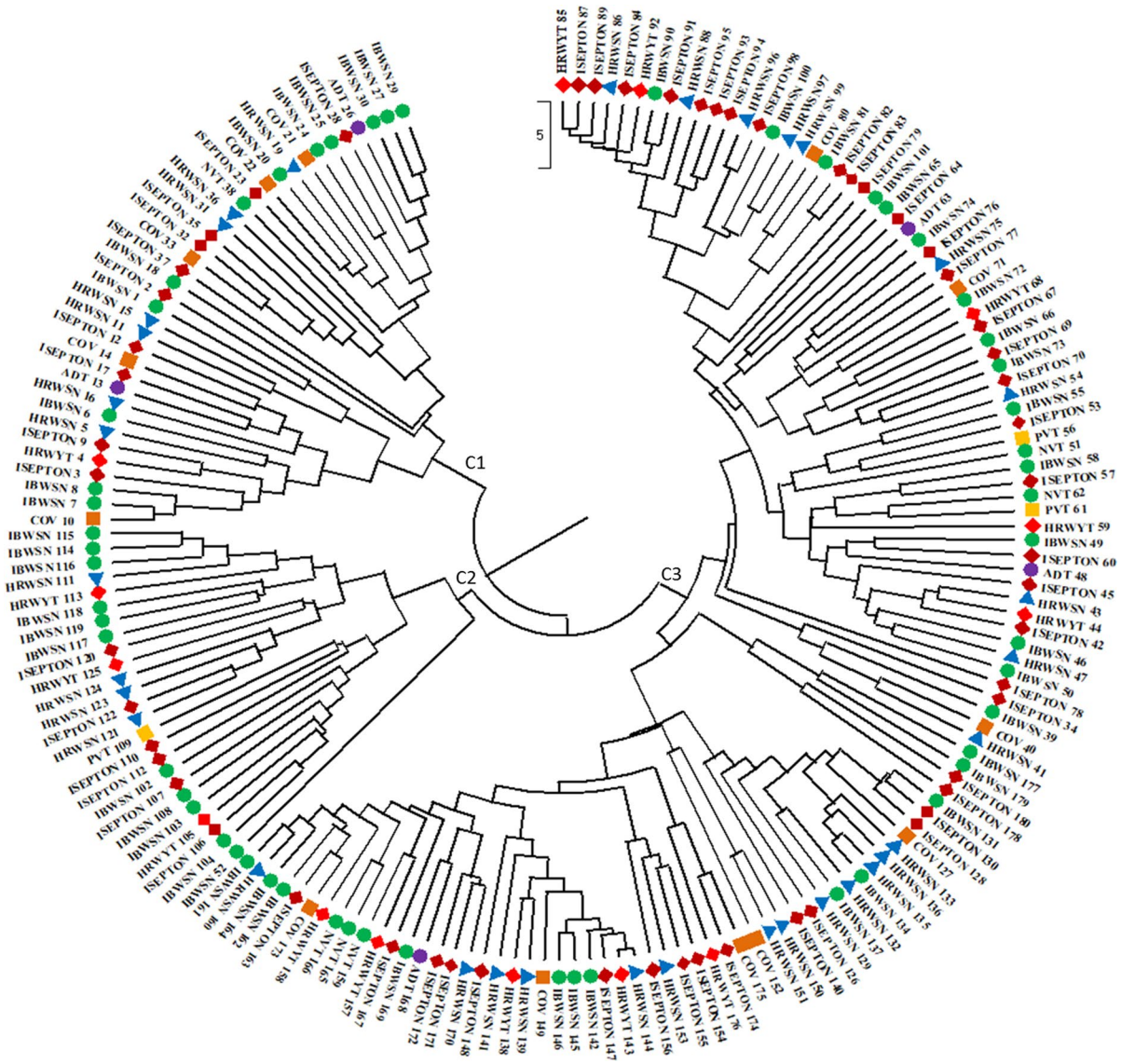

Fig. 4 The neighbour joining-based clustering of 180 bread wheat from the eight populations. Samples coded with the same symbol and color belongs to the same population. Population abbreviations are: *ADT* adaptation trial, *COV* commercial verities, *HRWSN* high rain weed trial screening nursery, *HRWYT* high rain weed yield trial, *IBWSN* international bread wheat screening nursery, *ISEPTON* international Septoria observation nursery, *NVT* national verification trial, *PVT* primary verification trial

Population genetic diversity

Worldwide wheat production is facing challenges due to the impact of abiotic and biotic stresses. Consequently, it is important to look for germplasm with the highest genetic diversity of important traits through research for effective conservation and improvement of existing germplasm. Wheat germplasms are thus used in many wheat breeding programs because they have a unique potential and diversity of essential genes that influence both biotic and abiotic stressors (Manickavelu et al. 2016; Tehseen et al. 2022). It could be useful for exploring genetic constituents and identifying loci, which are then used to improve wheat performance in breeding programs. In the present research genetic diversity in the studied wheat

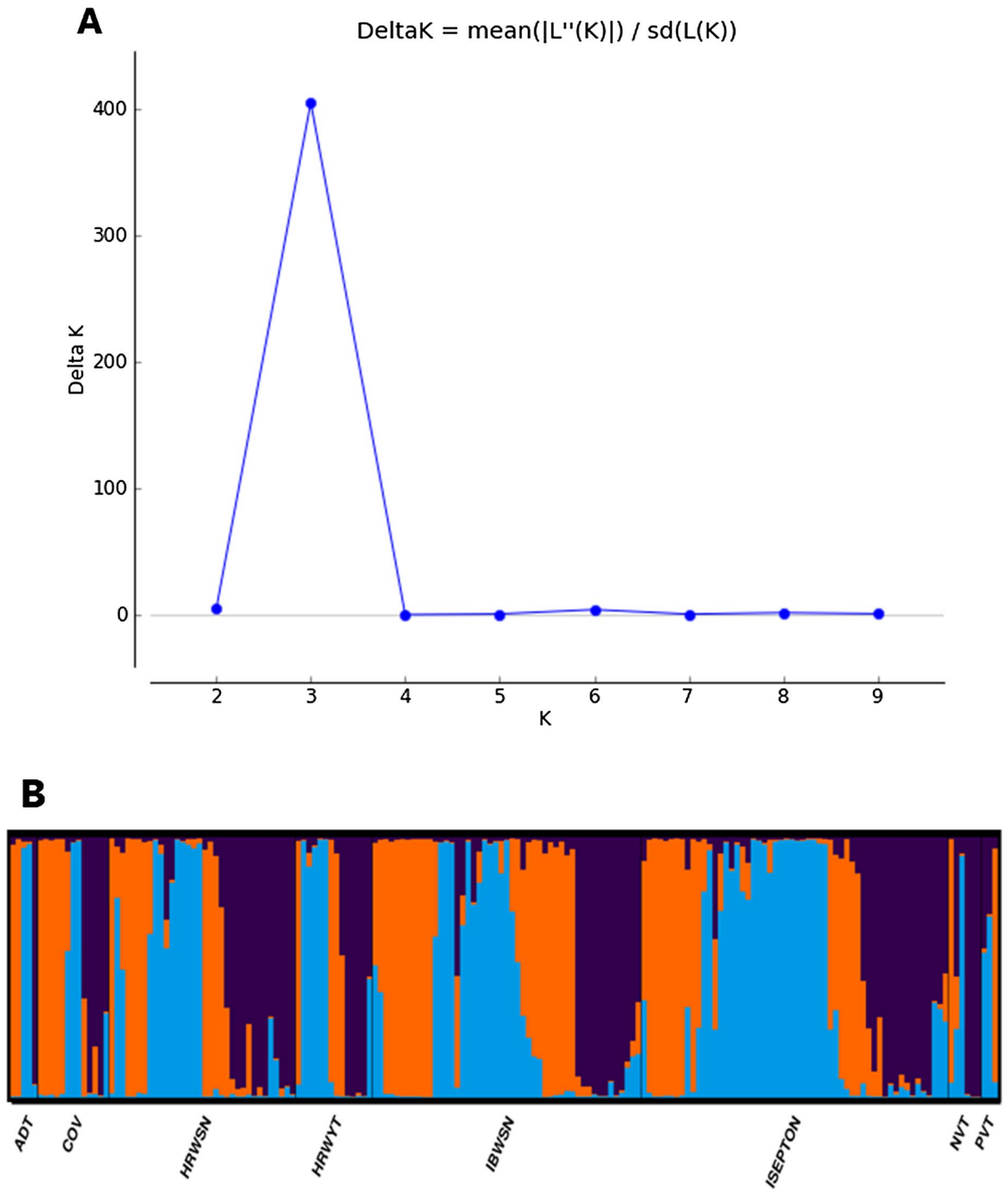

Fig. 5 Population structure analysis of 180 bread wheat germplasms, **A** best estimated delta K value and **B** estimated population structure for K = 3. The blue, orange and black colures represent genetic groups designated by Structure Harvester. Population abbreviations are: *ADT* adaptation trial, *COV* commercial verities, *HRWSN* high rain weed trial screening nursery, *HRWYT* high rain weed yield trial, *IBWSN* international bread wheat screening nursery, *ISEPTON* international Septoria observation nursery, *NVT* national verification trial, *PVT* primary verification trial. (Color figure online)

populations was determined, and gene diversity and Shannon's information index were found to be 0.19 and 0.29, respectively. The levels of gene diversity and Shannon's information index obtained in the current research differed from those reported by (Abouized et al. 2013), most likely due to substantial gene flow, which reduced the amount of variation among groups. The average genetic diversity across populations observed in the present research was in agreement to the level reported by Fatih et al., (2022), which was 0.33. In line with this, (Abou et al. 2013; Abdel and Hewedy 2018) reported that ISSR markers are highly efficient in discriminating wheat genotypes. The observed population genetic diversity in this research was consistent with Haliloglu et al., (2023) results for gene diversity (0.095) and Shannon's information index (0.114).

Among the eight studied wheat populations, the ISEPTON population showed the highest observed number of alleles (Na = 1.82), effective number of alleles (Ne = 1.44), Nei's gene diversity (h = 0.26), Shannon's information index (I = 0.40) and PPL (83.13%). This could likely be due to its highest population size as compared to the other populations. This implies that ISEPTON populations could be a good source of valuable alleles for wheat improvement as compared to other populations.

Population genetic structure

Analysis of molecular variance (AMOVA) revealed a higher (95%) genetic diversity within the population than among populations (5%), likely due to high sexual recombination within the population and high gene flow among populations. Similarly, higher genetic diversity within populations was reported by Sofalian et al., (2008). The high gene flow (Nm = 5.34) could be attributed to the genetic diversity differences within and among populations. Basically, a high level of genetic differentiation among populations is inversely proportional to gene flow (Wang et al. 2012; Dalvand et al. 2018). Genetic diversity is considerably influenced by gene flow, which encompasses several mechanisms of gene exchange among populations (Slatkin 1987). In the present research, pollen movement among populations, seed exchange practices between communities, and common marketing as well as socio-economic conditions may have facilitated the high gene flow (Nm = 5.34) among populations, leading to lower among-population genetic differentiation (Fst: 0.05).

Principal coordinate analysis and UPGMA also confirmed the presence of higher genetic variation at the population level than among populations, where the individuals of the different populations failed to form distinct clusters (Pandian et al. 2019), rather were mixed up along the axis. PCoA resulted in three clusters, where none of the clusters were composed of germplasm entirely from a particular population. This implies the existence of a significant intermixing of genetic backgrounds among the populations. Likewise, STRUCTURE analysis supported PCoA, and signifying the presence of high genetic relationships among the studied wheat populations, likely due to the presence of higher gene flow. The STRUCTURE analysis detected three sub-groups ($\Delta K = 3$), with a greater degree of genetic admixture. Similar high genetic admixture and weak population clustering in wheat were reported by Deepender et al., (2019). In contrast, a more structure, i.e., 5–6 genetically distinct groups in accordance with their geographical area of sampling, was reported for Indian and Turkish wheat genotypes (Khan et al. 2015). This kind of sharp clustering might be due to low genetic intermixing and low gene flow.

Conclusion

In this research, we profiled the genetic diversity and population structure of 180 bread wheat genotypes were examined and analyzed using highly informative ISSR markers to generate valuable information for wheat breeding programs and conservation purposes. The analysis revealed that markers used were entirely highly informative, and hence, very suitable tools to describe genetic diversity and population structure of bread wheat germplasms. The research also revealed how the bread wheat genotypes are structured, and their potential to contribute to the wheat improvement programs through selection breeding, and also designing proper management strategies. Among the eight studied populations, ISEPTON showed relatively higher Shannon's Information Index (I = 0.4) and Ne's gene diversity, implying that it could serve as a good source of desirable genes for wheat improvement program. UPGMA based clustering, PCoA analysis, and STRUCTURE analysis inadequately grouped

the populations, confirming high genetic intermixing and high gene flow among populations. The STRUCTURE analysis confirmed the studied bread wheat populations shared genetic background that originated from three sub-populations. Finally, we recommend further research using high-density marker that includes large sets of bread wheat genotypes to generate a better insight from the whole nation to disclose the genetic structure of wheat in the country, and also for development of best-performing cultivars.

Author contribution Conceptualization: GA, TM, KT, TH, MK; Data curation: GA; Formal analysis: GA; Funding acquisition: KT; Methodology: GA, TM; Resources: TM, KT, TH, MK; Software: GA; Supervision: TM, KT, TH, MK; Validation: TM, TH; Writing: original draft: GA; Writing: review and editing: TM, TH, MK, KT.

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Declarations

Conflict of interest None.

Ethical approval The conducted research does not involve human participants or animals.

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