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1 **Linkage disequilibrium and population structure in a core collection of *Brassica napus* (L.)**

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25 # Abstract

26 Estimation of genetic diversity in rapeseed/canola is important for sustainable breeding program
27 to provide an option for the development of new breeding lines. The objective of this study was
28 to elucidate the patterns of genetic diversity within and among different structural groups, and
29 measure the extent of linkage disequilibrium (LD) of 383 globally distributed rapeseed/canola
30 germplasm using 8,502 single nucleotide polymorphism (SNP) markers. The germplasm
31 accessions were divided into five subpopulations (P1 to P5) with obvious geographic and growth
32 habit-related patterns. All subpopulations showed moderate genetic diversity (average $H = 0.22$
33 and $I = 0.34$). The pairwise F_{st} comparison revealed a great degree of divergence ($F_{st} > 0.24$)
34 between most of the combinations. The rutabaga type showed highest divergence with spring and
35 winter types. Higher divergence was also found between winter and spring types. Overall, mean
36 linkage disequilibrium was 0.03 and it decayed to its half maximum within < 45 kb distance for
37 whole genome. The LD decay was slower in C genome (< 93 kb), relative to the A genome (< 21
38 kb) which was confirmed by availability of larger haplotype blocks in C genome than A genome.
39 To maximize genetic gain, hybridization between rutabaga and other types are potentially the
40 best option. Hybridization between spring and winter, semi-winter type is also helpful to
41 maximize the diversity in subsequent populations. Low genetic differentiation between both
42 spring type subpopulations (P4 and P3) will accelerate favorable allele accumulation for specific
43 traits in elite lines. The Neighbor-Joining tree and kinship matrix will assist to identify distantly
44 related genotypes from subpopulations to utilize in hybridization. The low levels of LD and
45 population structure make the core collection an important resource for association mapping
46 efforts to identify genes useful in crop improvement as well as for selection of parents for hybrid
47 breeding.

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48 # Introduction

49 Rapeseed/canola (*Brassica napus L*., AACC, 2n = 4x = 38), is a recent allopolyploid of
50 polyphyletic origin that evolved from hybridization events between two parental ancestors of *B.*
51 *oleracea* (Mediterranean cabbage, CC, 2n = 2x = 18) and *B. rapa* (Asian cabbage or rapeseed,
52 AA, 2n = 2x = 20) (1). Rapeseed/canola is the second largest oilseed crops produced in the world
53 after soybean (2). Canola oil is mostly used in frying and baking, margarine, salad dressings, and
54 many other products. Because of its fatty acid profile and the lowest amount of saturated fat
55 among all other oils, it is commonly consumed all over the world and is considered a very
56 healthy oil (3). Canola oil is also rich with alpha-linolenic acid (ALA), which is associated to a
57 lower risk of cardiovascular disease (3). Additionally, canola is utilized as a livestock meal and
58 is the second largest protein meal in the world after soybean (4). In the United States of America,
59 the canola production increased 13.5 folds from five years average of 1991-1995 (0.11 m tons) to
60 five years average of 2015-2019 (1.49 m tons) (5). Due to the growing importance of canola
61 there is a constant need to improve its yield which can be negatively affected by biotic and
62 abiotic stresses. Canola expresses three growth habits, winter, spring and semi-winter. The spring
63 canola is planted in the early spring and harvested in the late spring of the same growing season
64 (6). The winter type canola is seeded in the fall, vernalized over the winter to induce flower and
65 harvested in the summer (6). The semi-winter type is needed for a shorter period of vernalization
66 to induce flower (7). In order to adapt to different growing regions, plants developed systems
67 that sense temperature, light quality, day length, as well as stress signals (8–11). Plants from
68 colder zones require vernalization to flowering (12). Also day length and light quality affect the
69 ability to flower after winter (13). The transition from the vegetative to flowering stage is also
70 controlled by plant hormones and the circadian clock (14, 15). Multiple studies suggest the

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71 Arabidopsis flowering-time gene network might be similar to the one in Brassica (16, 17).
72 Detailed understanding of the nature of genes involved in the growth habits of canola will
73 facilitate development of cultivars adapted to different latitudes (18).

74 Due to limited use of diversified germplasm in breeding program, development of
75 superior cultivars through traditional breeding might become unsuccessful and lead to stagnation
76 in plant improvement (19). The recent origin of *B. napus* as a species and its very recent
77 domestication (400 years ago), as well as selection on few phenotypes (e.g. low erucic and
78 glucosinolate acids, seed yield) contributed to the low diversity which threatens sustainable
79 production of the crop (20). The narrow genetic diversity might also limit the prospects for
80 hybrid breeding where complementing genepools are needed for the optimal exploitation of
81 heterosis (21). Therefore, it is crucial to study, preserve, and even introduce genetic diversity into
82 rapeseed since the diversity is the best source of biotic and abiotic stress resistance, and various
83 agronomical and morphological traits. Canola improvement can benefit from the availability and
84 detailed characterization of genetically diverse germplasm. The knowledge of population
85 structure, genetic relatedness, and patterns of linkage disequilibrium (LD) are also prime
86 requirements for genome-wide association study (GWAS) and genome selection directed
87 breeding strategies (22, 23).

88 Multiple genetic diversity and population structure studies, based on LD, have already
89 provided information in regards to genetic diversity in various *B. napus* collections around the
90 world (24 –27). Unfortunately, there is a limited number of studies investigating the genetic
91 variation of canola germplasms in the U.S. core collection justifying a great need for such a
92 research focus. The LD analysis provides an important insight into the history of the species. It
93 also provides valuable direction to breeders in need to diversify their crop gene pools (28). Here

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94 we report a study revealing population structure and LD pattern of the U.S. core collection with
95 good representation of the genetic diversity present in global rapeseed/canola germplasm
96 accessions.

97 Materials and methods

98 Plant samples and phenotyping

99 A collection of 383 canola germplasm accessions originated in 24 countries comprising
100 spring, winter, semi-winter, and rutabaga types, were collected from North Central Regional
101 Plant Introduction Station (NCRPIS), Ames, Iowa, USA and North Dakota State University
102 (NDSU) (S1 Table). The collection consists of 156 spring, 152 winter, 58 semi-winter, and 17
103 rutabaga types. Growth habit was determined by growing the accessions in a greenhouse for at
104 least two seasons and in a field for two years at two locations. Field flowering time was recorded
105 as number of days from seeding date to flowering date where first flowers opened on half of the
106 plants belonging to a single accession. Spring type accessions flowered within 40-60 days after
107 planting, the semi-winter types flowered from 70 to 110 days. On the other hand, winter types
108 did not flower under field conditions. Vernalization treatment was conducted on greenhouse
109 grown winter type accessions to induce flowering and also to confirm the winter habit type
110 according to Rahman and McClean (2013) (6).

111 Genotyping and sequencing

112 Young leaves were collected from 30 days old plants and flash-frozen in liquid nitrogen.
113 Tubes were stored at -80°C until lyophilized. The lyophilized leaf tissue was ground in tubes

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114 with stainless beads using a plate shaker. DNA was extracted using Qiagen DNeasy Kit (Qiagen,
115 CA, USA) from lyophilized tissue following the manufacturer's protocol. DNA concentration
116 was measured using a NanoDrop 2000/2000c Spectrophotometer (Thermofisher Scientific). The
117 ApekI enzyme was used for GBS library preparation (29). Sequencing of the library was done at
118 the University of Texas Southwestern Medical Center, Dallas, Texas, USA using Illumina HiSeq
119 2500 sequencer.

120

121 SNP calling

122 TASSEL 5 GBSv2 pipeline (30) was used for SNP calling using a 120-base kmer length
123 and minimum kmer count of ten. The reads were aligned to the canola reference genome (31)
124 (available at:
125 ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/686/985/GCF_000686985.2_Bra_napus_v2.0/) using
126 Bowtie 2 (version 2.3.0) alignment tool (32). After passing all the required steps of TASSEL 5
127 GBSv2 pipeline, 497336 unfiltered SNPs were identified. Then VCFtools (33) was used to select
128 bi-allelic SNPs considering the criteria: minor allele frequency (MAF) ≥ 0.05, missing values
129 (max-missing) $\leq$50%, depth (minDP) ≥ 5 and physical distance (thin) ≤ 1000 bp. The SNPs that
130 were located outside chromosomes (i.e. position unknown), were removed. As canola is a self-
131 pollinating crop, the SNPs that were heterozygous in more than 25% of total genotypes, were
132 also removed using TASSEL (34). All these filtering steps resulted in a total of 8,502 SNP
133 markers.

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135 ## Data analysis

136 The core collection was divided into genetic groups using STRUCTURE v2.3.4 (35)
137 software. The admixture model, a burnin period of 10000 and 50000 Monte Carlo Markov Chain
138 (MCMC) iterations with 10 replications per K (K1-K10), were used as parameters for structure
139 analysis. The optimal number of groups was determined based on DeltaK approach (36) which
140 was performed by Structure Harvester (37). The individual Q matrix for the optimal K value was
141 generated utilizing membership coefficient matrices of ten replicates from STRUCTURE
142 analysis using CLUMPP (38). The results of structure analysis was visualized using the Structure
143 Plot v2 software (39). Principal component analysis (PCA) was conducted by covariance
144 standardized approach in TASSEL (34). An unrooted neighbor-joining (NJ) phylogenetic tree
145 was constructed using MEGAX program with 1000 bootstraps (40). Resulting tree was displayed
146 using FigTree V1.4.4 (41).

147 Analysis of molecular variance (AMOVA) was done to partition the genetic variance
148 among the groups identified by STRUCTURE in Arlequin3.5. The average pairwise between-
149 population F_{st} values were also calculated using Arlequin3.5 (42). GenAlex v6.5 (43) was used
150 to estimate percentage of polymorphic loci, number of effective alleles, Shannon's information
151 index, expected heterozygosity and unbiased expected heterozygosity of each marker and
152 subpopulation. The SNP distribution plot was developed using R package CMplot (available at:
153 https://github.com/YinLiLin/R-CMplot). The polymorphism information content (PIC) of
154 markers was calculated using software Cervus (44). Tajima's D value of each group was
155 calculated using MEGAX software (40). The kinship (IBS) matrix was calculated using software
156 Numericware i (45), kinship heatmap and histogram were developed using R package

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157 ComplexHeatmap (46). The level of relatedness (IBS coefficients) was correlated with
158 Shannon's information index (I) and diversity (H) in R v3.5.2 (47). Linkage disequilibrium (LD)
159 pattern of whole collection and different subpopulations were analyzed using PopLDdecay (48).
160 The mean linked LD was calculated by dividing total r^2 value with total number of
161 corresponding loci pair. In this case, $r^2 < 0.2$ was considered only. Same procedure was followed
162 to calculate mean unlinked LD where $r^2 \geq 0.2$ was considered. Haplotype block analysis was
163 done using PLINK (49) with a window size of 5 Mb. Confidence interval (CI) method (50) was
164 used to identify haplotype blocks with high LD. Haplotype blocks (>19 Kb), observed in one
165 subpopulation but not in the other, were considered to be subpopulation-specific block.
166 Haplotype blocks (>19 Kb) shared by more than one subpopulation, were considered to be
167 common to corresponding subpopulations.

168 Results

169 SNP profile

170 The selected 8,502 SNPs were distributed across 19 chromosomes with an average
171 marker density of 1 per 99.5 kb. Chromosome A3 and A4 contained highest (685 SNPs, 8.06%)
172 and lowest (236 SNPs, 2.78%) number of SNPs, respectively. The SNP density was highest on
173 chromosome A7 (71.1 kb) and was lowest on chromosome C9 (134.5 kb) (Table 1, Fig 1). The
174 occurrence of transition SNPs (4,956 SNPs) was more than that of transversions (3,546 SNPs)
175 with a ratio of 1.40. The ratio of transitions to transversions SNPs was higher in A genome (1.41)
176 than that of in C genome (1.38). In both genome, G/C transversions were lowest (4.33% and
177 4.29%), but A/G and C/T transitions occurred in almost similar frequencies (Table 2). The
178 inbreeding coefficient within individuals (F_{st}), inbreeding coefficient within subpopulations (F_{st}),

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179 observed heterozygosity (*Ho*) and fixation index (*F*) of all the markers ranged from -0.45 to 1.00,
180 0 to 0.73, 0 to 0.57 and 0.40 to 1.00, respectively. The Shannon's information index (*I*) of all
181 markers ranged from 0.10 to 0.69 with a mean value of 0.37. The expected heterozygosity (*He*)
182 ranged from 0.05 to 0.50 with a mean value of 0.27. The polymorphic information content (*PIC*)
183 ranged from 0.05 to 0.37 with a mean value of 0.22 (S2 Table). Subpopulation-wise marker
184 diversity parameters are presented in supplementary S3 Table.

185 **Table 1. Chromosomewise distribution of SNP markers.**
186

Chromosome	No. of SNPs	% SNPs	Start position	End position	Length (Mb)	Density (Kb)
A1	440	5.18	149163	35806075	35.7	81.0
A2	392	4.61	13430	34692905	34.7	88.5
A3	685	8.06	2769	49103583	49.1	71.7
A4	236	2.78	32805	23517671	23.5	99.5
A5	413	4.86	18668	31435105	31.4	76.1
A6	448	5.27	120409	36005103	35.9	80.1
A7	384	4.52	85869	27388322	27.3	71.1
A8	281	3.31	231427	27734410	27.5	97.9
A9	541	6.36	81404	45841268	45.8	84.6
A10	305	3.59	133853	22085737	22.0	72.0
C1	445	5.23	86671	50660872	50.6	113.7
C2	589	6.93	92431	68260222	68.2	115.7
C3	651	7.66	3839	80365889	80.36	123.4
C4	634	7.46	138930	70507417	70.4	111.0
C5	366	4.30	26760	44124497	44.1	120.5
C6	414	4.87	275190	45479327	45.2	109.2
C7	518	6.09	271113	62304827	62.0	119.8
C8	383	4.50	57934	46317429	46.3	120.8
C9	377	4.43	920885	51627086	50.7	134.5
Mean	447.47					99.5

187

188 **Fig 1. Chromosome-wise SNP density map.** Frequency of SNPs varies according to color
189 gradient.

190

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191 **Table 2. Transition and transversion SNPs across the genome.**

192

Genome	SNP type	Model	No. of sites	Frequencies (%)	Total (percentage)
A	Transitions	A/G	1195	14.06	2416 (28.3%)
		C/T	1221	14.36	
	Transversions	A/T	457	5.38	1709 (20.1%)
		A/C	424	4.99	
		G/T	460	5.41	
		G/C	368	4.33	
C	Transitions	A/G	1273	14.97	2540 (29.9%)
		C/T	1267	14.90	
	Transversions	A/T	496	5.83	1837(21.6%)
		A/C	482	5.67	
		G/T	494	5.81	
		G/C	365	4.29	

193

194 # Population structure

195 The whole collection was divided into six subpopulations based on structure analysis
196 using the Delta K approach (Fig 2A). Genotypes of different types and origins were well
197 clustered. The winter, semi-winter, rutabaga type genotypes were grouped under subpopulation-1
198 (P1), subpopulation-2 (P2), and subpopulation-5 (P5), respectively. The spring type genotypes
199 were grouped under two subpopulations: subpopulation-3 (P3) and subppulation-4 (P4) whereas
200 subpopulation-6 (P6) composed of only nine genotypes of different types (Fig 2B). Though each
201 subpopulation consisted of genotypes of different origin, P1, P2 and P4 were dominated by
202 European, Asian and American (NDSU breeding lines) genotypes, respectively, whereas P3, P5
203 and P6 were composed of genotypes of mixed origin. We performed principal component
204 analysis (PCA) to show the genetic similarity among subpopulations and genotypes. The first
205 two axes explained 21% of the total observed variation (S4 Table). The PCA revealed that
206 subpopulation P1, P2, P3, P4 and P5 were well clustered and separated from each other, but
207 genotypes of P6 were scattered within other subpopulations (Fig 3). In addition to that, we also

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208 constructed phylogenetic tree based on neighbor joining (NJ) criteria (Fig 4). The output of
209 neighbor-joining (NJ) tree analysis was in line with that of PCA. Based on the PCA and NJ
210 output, we merged the P6 genotypes into P1, P2, P3 and P4 and considered five subpopulations
211 for further analysis and discussions. Based on individual Q matrix, the proportion of pure (non-
212 hybrid) and admixed (containing markers assigned to more than one subpopulation) genotypes in
213 each subpopulation was calculated. The proportion of pure accessions in subpopulations ranged
214 from 41% to 80% at a 0.7 cutoff value and 10% to 35% at 0.9 cutoff value (Table 3).
215 **Fig 2. Bayesian clustering of whole collection using 8,502 SNP markers in STRUCTURE v.**
216 **2.3.4.** (A) Graphical representation of optimal number of clusters (K) determined by Evanno's
217 method, where highest Delta K indicate the number of subpopulations. (B) Estimated population
218 structure (P1 to P6) of 383 canola genotypes on K=6 according to Delta K.

219 **Fig 3. Principal component analysis of SNP diversity based on genetic distance.** Colors
220 represent different subpopulations identified at K = 6 in figure 2. Genotypes belong to P6 (black
221 dots) are scattered within different subpopulations.

222 **Fig 4. Phylogenetic tree based on neighbor-joining (NJ) using information from 8,502 SNP**
223 **markers.** Each branch is color-coded according to membership into the K= 6 subpopulations
224 identified by structure in figure 2. Subpopulations bear same color as in figure 3.

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229 **Table 3. Number of pure and admixed individuals per subpopulation.**

Subpopulations	Total no. of genotypes	0.7 cutoff		0.9 cutoff	
		No. of genotypes	% of from total	No. of genotypes	% of from total
P1	152	89	58.6	42	27.6
P2	52	24	46.2	9	17.3
P3	98	40	40.8	14	14.3
P4	61	30	49.2	6	9.8
P5	20	16	80.0	7	35.0
Total	383	199	52.0	78	20.4

230

231 # Population diversity

232 In all subpopulations, the percentage of polymorphic loci was greater than 75%. It was
233 highest in P1 (99%) and lowest in P5 (75%). The diversity (*H*) of the five subpopulations ranged
234 from 0.19 (P4 and P5) to 0.25 (P2) with an average of 0.22. The Shannon's information index (*I*)
235 ranged from 0.31 (P4 and P5) to 0.40 (P2) with an average of 0.34. The Tajima's D value ranged
236 from -0.70 (P4) to 0.53 (P1) with an average of 0.13 (Table 4).

237
238 **Table 4. Subpopulation-wise diversity parameters.**
239

Subpopulations	Polymorphic loci (%)	*Na* [a]	*Ne* [b]	*I* [c]	*H* [d]	*Uh* [e]	Tajima's D*
P1	99.12	1.99	1.32	0.35	0.21	0.21	0.53
P2	94.32	1.94	1.40	0.40	0.25	0.25	0.30
P3	96.98	1.97	1.35	0.36	0.22	0.23	0.30
P4	80.67	1.81	1.30	0.31	0.19	0.19	-0.70
P5	75.25	1.75	1.31	0.31	0.19	0.20	0.23
Mean	89.27	1.89	1.34	0.34	0.22	0.22	0.13

240 [a] No. of different alleles, [b] No. of effective alleles, [c] Shannon's information index
241 [d] Diversity, [e] Unbiased diversity. SE (standard error) was zero in all cases. Indices calculated
242 using 8191 SNPs with GenAlex v. 6.5. * was calculated with 1000 permutations.

243

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244 ## Population genetic differentiation

245 The analysis of molecular variance (AMOVA) revealed that variance among
246 subpopulations covered 24% of total variation whereas the remaining 76% of total variation
247 accounted for variance among individuals within subpopulations (Table 5) with a F_{st} and *Nm*
248 value of 0.24 and 1.28, respectively.

249 **Table 5. Summary of AMOVA.**
250
251
252
253
254
255
256
257
258

Sources of variation	d.f.	Sum of squares	Variance components	% of variation	F_{st}	Nm
Among subpopulations	4	130814.6	228.5***	23.5	0.24	1.28
Within subpopulations	761	565699.6	743.4	76.5		
Total	765	696514.1	971.9			

259 *** indicates $p < 0.001$ for 1023 permutations

260 All pairwise F_{st} comparisons between subpopulations were significant ($p < 0.01$). All
261 combinations showed a great degree of divergence ($F_{st} > 0.20$) (Wright 1943), except
262 combinations P3 and P4 (0.11), P1 and P2 (0.19). The pairwise $F_{st} > 0.30$ was observed between
263 P1 and P5, P3 and P5, P4 and P5 (Table 6).

264 **Table 6. Genetic differentiation among subpopulations.**

	Subpopulation pairwise *Fst*				
	P1	P2	P3	P4	P5
P1	0				
P2	0.19**	0			
P3	0.25**	0.24**	0		
P4	0.21**	0.24**	0.11**	0	
P5	0.34**	0.24**	0.34**	0.39**	0

265 Diagonal values are pairwise Fst comparisons, performing 1000 permutations using Arlequin v.
266 3.5. **indicates $p < 0.01$

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267 We performed kinship (IBS) analysis to facilitate the individual genotype selection for
268 desirable cross combinations (Fig 5, S5 Table). In whole collection, the IBS coefficients ranged
269 from 1.21 to 1.94. The average coancestry between any two canola genotypes was 1.47. The P2
270 contained almost 50% of total genotypic pairs having IBS coefficients less than 1.50 whereas
271 this portion was comparatively lower in other subpopulations (Table 7, S1 Fig).

272
273 **Fig 5. Heatmap of kinship matrix of entire collection.**

274
275 **Table 7. Summary of subpopulation-wise kinship (IBS) matrix.**

Subpopulations	Whole collection	P1	P2	P3	P4	P5
IBS coefficients range	1.21- 1.94	1.40- 1.94	1.27- 1.93	1.29- 1.93	1.46-1.94	1.35-1.92
Mean of IBS coefficients	1.47	1.58	1.49	1.55	1.62	1.60
Pairs having ≤ 1.50 IBS coefficients (%)	63.9	9.6	50.7	21.7	1.1	18.0
Pairs having > 1.50 IBS coefficients (%)	36.1	90.4	49.3	78.3	98.9	82.0

276
277 We also performed correlation analysis between mean pairwise relatedness (IBS
278 coefficients) among individuals within subpopulation and Shannon's information index (*I*),
279 diversity (*H*). The *I* and *H* were significantly and negatively correlated with relatedness (r= -
280 0.97, -0.98, and p<0.01), respectively.

281 ## Linkage disequilibrium pattern

282 The linkage disequilibrium (LD) pattern was investigated across the entire collection,
283 each subpopulation, genome, and chromosome-wise. LD = r^2 values decreased with the increase
284 of distances. In all cases, mean LD was high (r^2 > 0.22) at short distance bin (0-2 kb) and
285 declined with increasing bin distance (S6 Table). In the entire collection considering both A and
286 C genome, the mean linked LD, mean unlinked LD and loci pair under linked LD was 0.44, 0.02
287 and 1.81%, respectively. The mean linked LD was similar in P3 and P4 (r^2 = 0.45), lower in P2

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288 (r^2= 0.41) and higher in P1 (r^2= 0.48). The loci pair in linked LD was higher in P5 (8.76%) and
289 lowest in P1 (1.52%). The mean linked LD, mean LD and loci pair under linked LD was always
290 higher in all cases in case of C genome than that of A genome (Table 8). We also calculated the
291 LD decay rate. In the whole collection, LD decayed to its half maximum within < 45 kb distance
292 for whole genome, < 21 kb for A genome, and < 93 kb for C genome. In all subpopulations, the
293 distance for LD decay to its half maximum was always higher for C genome than A genome.
294 Each chromosome showed differential rate of LD decay (S2 and S3 Fig). LD persisted the
295 longest in chromosome C1 (348 kb) and C2 (244 kb). The decay distance was shortest in
296 chromosome A5 (13 kb) and A1 (16 kb) (S6 Table). LD decayed to its half –maximum within <
297 29 kb for P1, <45 kb for P2, P3, <101 kb for P4, and <120 kb for P5. In all subpopulations LD
298 persisted also longest in all chromosomes of C genome than that of A genome (Fig 6, S7 Table).
299
300 **Fig 6. Linkage disequilibrium (LD) differences and decay pattern among subpopulations.**
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323 **Table 8. Linkage disequilibrium in the studied collection.**
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Subpopulation	Mean linked LD	Mean unlinked LD	Mean LD	Loci pairs in linked LD (%)	Loci pairs in unlinked LD (%)
			AC_Genome		
Whole collection	0.44	0.02	0.03	1.81	98.2
P1	0.48	0.01	0.02	1.52	98.5
P2	0.41	0.02	0.03	2.65	97.4
P3	0.45	0.02	0.03	1.94	98.1
P4	0.45	0.02	0.04	3.98	96.0
P5	0.43	0.03	0.07	8.76	91.2
			A_Genome		
Whole collection	0.33	0.02	0.02	1.34	98.7
P1	0.38	0.01	0.02	1.12	98.9
P2	0.32	0.02	0.03	2.02	98.0
P3	0.36	0.02	0.02	1.41	98.6
P4	0.40	0.02	0.03	3.45	96.6
P5	0.38	0.04	0.06	7.06	92.9
			C_Genome		
Whole collection	0.50	0.02	0.03	2.21	97.8
P1	0.52	0.01	0.02	1.83	98.2
P2	0.46	0.02	0.04	3.27	96.7
P3	0.50	0.02	0.03	2.35	97.7
P4	0.48	0.02	0.04	4.41	95.6
P5	0.46	0.03	0.08	10.57	89.4

325

326 We also performed haplotype block analysis to investigate LD variation patterns across
327 whole genome. A total 200 blocks covering 18 Mb out of the 976 Mb anchored *B. napus*
328 reference genome (31), were identified. A and C genome contained 67 and 133 haplotype blocks,
329 respectively. The total length of A and C genome specific haplotype blocks were 1.8 Mb and 16
330 Mb, respectively. The total length of haplotype blocks varied greatly from chromosome to
331 chromosome. Total haplotype block length varies from 24 Kb on A1 to 901 Kb on A9 in A
332 genome and in C genome it varies between 40 Kb on C9 to 3,610 Kb on C2 (Table 9). The
333 subpopulation-specific and common haplotype blocks were shown in supplementary S8 Table.
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335 **Table 9. Subpopulation-wise number and length of haplotype blocks (HBs) along**

336 **chromosomes.**

	Entire panel		P1		P2		P3		P4		P5	
Chr.	No[a]	Size[b]	No[a]	Size[b]	No[a]	Size[b]	No[a]	Size[b]	No[a]	Size [b]	No[a]	Size[b]
A1	5	24	6	733	2	5	6	557	5	2080	0	0
A2	8	80	7	96	1	11	8	654	3	15	0	0
A3	6	46	5	29	5	44	8	51	10	927	1	8
A4	5	46	1	27	2	17	0	0	1	6	0	0
A5	7	57	2	13	3	503	5	138	5	427	1	1
A6	9	308	6	564	4	29	5	52	5	51	0	0
A7	8	70	7	72	2	15	7	412	9	1092	1	13
A8	4	304	1	2	3	21	2	234	6	2654	0	0
A9	10	901	7	62	14	1493	6	64	9	1393	1	21
A10	5	29	6	50	2	21	1	6	5	45	1	1
C1	23	3099	14	3314	14	4638	19	2947	20	4295	1	14
C2	26	3611	19	3141	9	4503	22	3756	16	5237	7	3594
C3	14	969	13	930	13	1480	16	1603	9	1070	2	192
C4	16	3440	15	5351	15	5330	14	4502	13	6063	4	3989
C5	9	423	8	410	10	516	7	1148	7	2679	1	2
C6	18	1204	9	972	7	1191	13	1206	15	3357	3	954
C7	17	2394	17	2583	7	70	16	2479	10	1414	1	13
C8	4	893	5	865	10	1289	6	941	7	1143	1	73
C9	6	41	7	49	4	438	7	223	3	11	1	14
AC Genome	200	17938	155	19264	127	21615	168	20975	158	33956	26	8888
A Genome	67	1865	48	1648	38	2160	48	2168	58	8688	5	43
C Genome	133	16073	107	17616	89	19455	120	18807	100	25267	21	8845

337 [a] The number of haplotype blocks on each chromosome.

338 [b] The total length of haplotype blocks for each chromosome in Kb.

339 # Discussion

340 Genotyping-by-sequencing (29) is one approach to obtain high frequency SNPs. The

341 strategy has been used for population genetic studies, association mapping, and proven to be a

342 powerful tool to dissect multiple genes/QTL in many plant species (51–53). We obtained

343 497,336 unfiltered SNPs markers of which 8,502 high quality SNP marker were used for genetic

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344 diversity analysis of 383 genotypes. Delourme et al. (2013) (24) conducted genetic diversity
345 analysis in *B. napus* using 7,367 SNP markers of 374 genotypes. However, different marker
346 technologies such as Single Sequence Repeat (SSR), Sequence Related Amplified Polymorphism
347 (SRAP) markers have been used by other researchers for genetic diversity analysis in *B. napus*.
348 Chen et al. (2020) (54) used 30 SSR markers, Wu et al. (2014) (55) utilized 45 SSR markers,
349 Ahmad et al. (2014) (56) used 20 SRAP markers for genetic diversity and population structure
350 analysis of *B. napus*. Earlier, our group conducted a genetic diversity study of flax using 373
351 germplasm accessions with 6,200 SNP markers.

352 The SNP markers were distributed throughout 19 chromosomes of *B. napus* and the
353 marker density was 1 per 99.5 Kbp. This is comparable density to earlier study conducted by
354 Delourme et al. (2013) (24). Therefore, this marker density provides a sufficient resolution to
355 accurately estimate genome-wide diversity as well as the extent of LD within the genome. The
356 high marker density will also help association mapping studies precisely identify a causal
357 locus/loci or very closely linked loci that can be further used either in MAS or to pinpoint the
358 causative locus (57).

359 The core collection utilized in this study represents mostly adapted lines from various
360 breeding programs rather than wild accessions commonly used in diversity studies. Therefore,
361 sources of variation, markers of interest identified in the collection can be directly used in
362 breeding programs.

363 We have identified higher frequency of transition SNPs over transversion SNPs that is an
364 agreement with Bus et al. (2012) (58), Clarke et al. (2013) (59), and Huang et al. (2013) (60) in
365 *B. napus*. Higher number of transition SNPs over transversion is also reported in other crop
366 species such as *Hevea brasiliensis* (61), *Camellia sinensis* (62), *Camelina sativa* (63), and *Linum*

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367 *usitatissimum* (64). The high number of transition SNPs indicating that this mutation is more
368 tolerable to natural selection (65).
369 We have calculated the polymorphic information content (PIC) and expected
370 heterozygosity (*He*) for each marker. The PIC indicates the usefulness of any marker for linkage
371 analysis, and *He* determines the diversity of haploid markers (66). In our research, the PIC value
372 is ranged from 0.05 to 0.35 indicating that the markers are moderate or low informative. The
373 similar lower PIC value (0.1 to 0.35) was reported by Delourme et al. (2013) (24) in *B. napus*.
374 The lower PIC value was also reported in winter wheat (67), maize (68), flax (64, 69) and rice
375 (70). The lower PIC value is a result of bi-allelic nature of SNP markers and probable low
376 mutation rate (71). In our study, the *He* value of each marker was always greater than
377 corresponding PIC value indicating an average lower allele frequency in our population (66).

378 Population structure and diversity

379 Multiple population-based analyses, including population structure, PCA and NJ tree
380 analysis split the core collection into five distinct clusters. Previously, three clusters have been
381 reported based on winter, spring and semi-winter growth habit types of *B. napus* (20, 24–26,72–
382 74). Our analysis showed a clear trend of the five clusters belonging to winter type (P1), semi-
383 winter type (P2), spring type of mixed origin (P3), spring type developed at NDSU (P4), and
384 rutabaga type (P5) growth habits. Our finding showed strong agreement with other studies to
385 separate winter, semi-winter and spring types. Moreover, we could separate the rutabaga types
386 and divided the spring type into two groups: American origin and the rest of other countries
387 origin. This additional cluster added new information to select germplasm from different
388 subpopulation for increasing genetic diversity in canola breeding program.

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389 We have identified a moderate diversity (average $H = 0.22$) within the subpopulations. *B.*
390 *napus* is capable of self-pollination, and little cross pollination may be occurred by insect. Being
391 a mostly self-pollinated crop a low to moderate subpopulation diversity in *B. napus* is expected.
392 Low to moderate diversity was also found in previous studies (75–77). Along with the
393 reproduction system, one needs to look at evolution and domestication history for explaining low
394 to moderate levels of diversity in *B. napus*. This allopolyploid species originated at
395 Mediterranean coast as a result of a natural cross between *B. rapa* and *B. oleracea* which
396 occurred approximately 0.12 -1.37 million years ago (78, 79). The domestication of *B. napus*
397 occurred very recently, around 400 years ago with the first rapeseed being most likely a semi-
398 winter type due to the mild climate in the region (72, 80). Later on, European growers developed
399 the winter and spring type Brassicas through selection for cold hardiness or early flowering to
400 expand its cultivation in further North in the last century (81). Therefore, the low to moderate
401 diversity in winter and spring *B. napus* can be mostly explained by a recent history of the
402 species, followed by infrequent exchange of genetic material with other Brassicas (24), as well as
403 by the traditional breeding practices selecting for only few phenotypes. In our study, the more
404 diversity in semi-winter type (P2, $H= 0.25$) than winter (P1, $H= 0.21$) and spring (P4, $H= 0.19$)
405 type is supported by its domestication history. We have seen a homogeneity of the diversity
406 indices of different subpopulations indicating that the species is stable enough to avoid the
407 natural loss of genetic variability by genetic drift (82). The *Nm* value was greater than one, which
408 indicates that there was enough gene flow among semi-winter, winter and spring types. These
409 findings also support the evolution of winter and spring types from semi-winter type. In this
410 research, Tajima's D value was calculated to identify different subpopulations with availability
411 or scarcity of rare alleles (83). P4 (spring type originated at NDSU, USA) showed a negative

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412 Tajima's D value indicating presence of more rare and unique alleles and recent expansion of
413 this subpopulation compared to the other subpopulations (84). Recently, the NDSU canola
414 breeding program developed the P4 advanced breeding lines through crossing different genetic
415 resources including winter, spring and semi winter types and subsequent selection. Therefore, the
416 P4 displayed an abundance of rare alleles. The subpopulation P1, P2, P3, and P5 showed positive
417 Tajima's D value indicating an excess of intermediate frequency alleles, which may be caused by
418 balancing selection, population bottleneck, or population subdivision. Previously, negative
419 Tajima's D values were found in spring and winter type B. napus accessions (74). The negative
420 correlation between diversity indices (*H* and *I*) and relatedness (average IBS coefficients)
421 indicates that inbreeding and genetic drift play a significant role in reducing genetic variability in
422 the studied population which results in increased differentiation among sub-populations. Similar
423 phenomenon was also found in flax (64) and *Arapaima gigas* species (85).

424 Parent selection for crossing from diverged population will allow us to create a new
425 population with increased genetic diversity and transgressive segregation, and eventually will
426 increase genetic gain. Pairwise F_{st} statistic, a parameter describing population structure
427 differentiation and the degree of divergence among populations (86), was estimated among five
428 subpopulations. In the present study all pairwise F_{st} values comprising both low and high values,
429 were statistically significant. Similar type results were also found in other studies (74, 87–89).
430 Lower pairwise F_{st} (0.11) was identified between spring type originated in USA (P4) and spring
431 type originated in other countries (P3). This is reasonably justified as both subpopulations
432 comprise of spring type genotypes and germplasm exchanged occurred between USA and other
433 countries. It also indicating that we will not get higher genetic diversity in population if we use
434 only spring types in the crossing program. But this combination is good for accumulating

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435 specific elite trait if the targeted trait is found in members of one and missing from the members
436 of another group. We found spring type (P3 and P4) genotypes are greatly divergent ($F_{st} > 0.20$)
437 from winter and semi-winter type (P1 and P2) genotypes. Utilization of genotypes from these
438 group in crossing program will broaden the genetic base of developed population results in high
439 heterosis. This potentiality has already been proved as hybrids between the Chinese semi-winter
440 and European (including Canada) spring type exhibited high heterosis for seed yield (90). The P5
441 (rutabaga type) showed the higher F_{st} with other subpopulations such as the highest F_{st} was
442 observed between P5 and P4 (NDSU spring type) followed by P3 (other spring type), P1 (winter
443 type) and P2 (semi-winter type). This outcome clearly shows that rutabaga is genetically distinct
444 from spring and winter type canola which is confirmed by previous studies (72, 73, 91). This
445 distinctness of rutabaga can be exploited through heterosis breeding. Several previous studies
446 have already showed rutabaga as a potential gene pool for the improvement of spring canola (92,
447 93). NDSU canola breeding program also utilized winter and rutabaga types in the breeding
448 program for increasing genetic diversity and for improvement of spring canola.

449 Crossing among genotypes within subpopulations is also useful as variation among
450 individuals within subpopulations is greater than that among subpopulations, which was revealed
451 by AMOVA. This finding is also in agreement with the previous findings (54, 77, 94, 95). In this
452 case subpopulations P2, P3 and P1 showing high diversity ($H > 0.20$) could be utilized for
453 cultivar development. Structure, NJ tree and PC analysis revealed that all subpopulations
454 contained both pure (non-hybrid) as well as admixed (share SNPs from different subpopulation)
455 genotypes. For broadening genetic base of population, pure genotypes should be crossed. But for
456 improving or introgression of specific traits, admixed genotypes could also be crossed which will
457 reduce the population size required for phenotypic screening. However, population diversity

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458 indicates the level of similarity or dissimilarity based on alleles that not necessarily come from
459 the same parent or ancestor, this tends to inflate the real differentiation between any two pair of
460 individuals (96). Since a breeder would like to combine positive alleles that historically never
461 have been combined, IBS values are good to decide what individuals will be crossed. Low IBS is
462 the best. But, IBS values in self-pollinated crops tends to be higher than that in cross-pollinated
463 crops as heterozygosity reduces the probability of two alleles at a locus of being identical by
464 state (97). In our study, most of the genotypes had weak relatedness as approximately 64% of
465 pairwise coancestry ranged from 1.21 to 1.50. Crossing among genotypes from subpopulation P2
466 will demonstrate more diversity, than that of other subpopulations, as most genotypic
467 combinations of P2 shows low IBS coefficients than others. This finding is in line with the
468 evolutionary history of B. napus where semi-winter type is the base population containing more
469 divergence. Gradually this diversity is narrowed down in P3 (spring type, mixed origin) and P1
470 (winter type), because genotypic pairs belong to P3 and P1 having high IBS values evolved from
471 semi-winter type (81). Subpopulation P4 exhibited highest number of pairs having IBS > 1.5,
472 which is obvious as these genotypes are advanced breeding lines developed from crossing of
473 same set of parents in different combinations. Genotypic pairs of P5 (rutabaga type) also showed
474 high coancestry which is may be due to the duplicates. We could discard the duplicates during
475 the crossing program.

476 Linkage Disequilibrium

477 Linkage disequilibrium can be defined as the correlation among polymorphisms in a
478 given population (98). The strength of association mapping relies on the degree of LD between
479 the genotyped marker and the functional variant. Linkage disequilibrium analysis provides

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480 insight into the history of both natural and artificial selection (breeding) and can give valuable
481 guidance to breeders seeking to diversify crop gene pools (20). SNPs in strong LD are organized
482 into haplotype blocks which can extend even up to few Mb based on the species and the
483 population used. Genetic variation across the genome is defined by these haplotype blocks.
484 Haplotypes which are subpopulation-specific are defined by various demographic parameters
485 like population structure, domestication, selection in combination with mutation and
486 recombination events. Conserved haplotype structure can then be used for the identification and
487 characterization of functionally important genomic regions during evolution and/or selection
488 (99). Also, the extent of LD needs to be quantified across the genome at high resolution (down to
489 approximately 1 Kbp) (100). The information is important for choosing crossing schemes,
490 association studies and germplasm preservation strategies (101–104).

491 We used markers from across the genome to quantify the LD for the core collection. Low
492 level of LD was evident for each individual subpopulation in A, C and whole gnome. The low
493 level of LD can be due to multiple factors. First of all, canola is a partially outcrossing species
494 with an average of 21-30 % of cross pollination (105–107). The outcrossing occurring in canola
495 leads to more recombination and to a breakdown of haplotype blocks. Secondly, the ancestral
496 history of canola is limited in comparison with other crops, such as rice, common bean, wheat
497 and corn, restricting the selection of desirable haplotypes during the evolution. In other words,
498 there was no adaptation or domestication pressure on the species, which would lead towards
499 positive selection. Third, the only selection pressure imposed on the species for a relatively short
500 time was breeding. However, the breeding practices were biased towards selection of only few
501 phenotypes. Additionally, the short period of time under selection pressure might have not been
502 sufficient to select favorable haplotypes in the genome. Fourth, since canola cultivars with

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503 different growth habits are compatible there has been always gene flow present between them
504 contributing to the low level of LD. The N_m >1 was observed in this study, which supports this
505 gene flow. Finally, the restriction enzyme used to develop the libraries for sequencing of the core
506 collection helped in identification of SNPs largely residing in genic regions, which are prone to
507 high recombination, contributing to the low level of LD.

508 In this study, we have identified that the LD decay in B. napus varied across
509 chromosomes of both A and C genomes. In addition, LD in C genome decayed much slower than
510 A genome. C genome also contained larger haplotype blocks than A genome. This LD patterns
511 are consistent with previous findings (20, 27, 108–110). The slower LD decay and presence of
512 long haplotype blocks in C genome indicates that high level of gene conservation could have
513 resulted from limited natural recombination or could be exchanged of large chromosomal
514 segment during evolution. In the whole genome, presence of subpopulation specific haplotype
515 blocks suggests that these regions had been experienced selection pressure for specific
516 geographic regions adaptation. In all subpopulations, presence of shorter haplotype blocks in A
517 genome than C genome reveals that B. rapa progenitor of B. napus containing A genome, which
518 has been used as oilseed crop and probably being used in hybridization process. Sharing
519 haplotype blocks by different subpopulations specially in C genome also confirms its conserved
520 nature. The low level of LD or haplotype blocks has implications for association mapping and a
521 proper experimentation design is necessary for utilizing a reduced set of markers by tagging
522 major haplotypes (111). Though low LD requires more markers to pinpoint the location of
523 various QTL, but once a marker is found to be significantly associated with a phenotype, there
524 might be a higher probability of identifying the casual gene.

525

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526 # Conclusions

527 This study provided a new insight to select the best parents in crossing plan to maximize
528 genetic gain in the population. The population structure analysis showed a clear geographic and
529 growth habit related clustering. Low LD values indicate that our collection is a valuable resource
530 for prospect association mapping endeavors. The genetic diversity of the core collection of *B.*
531 *napus* was low. Breeding efforts will need to address this issue in order to generate future hardy
532 and high yielding varieties with resistance to many abiotic and biotic stresses. The rutabaga type
533 showed the highest genetic divergence with spring and winter types accessions. Therefore, the
534 breeding strategies to increase the genetic diversity may include generating population from
535 rutabaga and spring crosses, or using rutabaga and winter crosses.
536

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867

868

869 # Supporting information

870 **S1 Table. List of the genotypes analyzed in this study.**

871 **S2 Table. Marker diversity parameters.**

872 **S3 Table. Subpopulation-wise marker diversity parameters.**

873 **S4 Table. (a) Percentage of variation explained by the first 3 axes, (b) Eigen values by axis**
874 **and sample eigen vectors.**

875 **S5 Table. Kinship matrix.**

876

877 **S6 Table. Mean LD values according to distance.**

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878 **S7 Table. Subpopulation-wise and chromosome-wise LD decay rate (Kb) within each**
879 **subpopulation.**

880 **S8 Table. Subpopulation specific and common haplotype blocks.**

881 **S1 Fig. Histogram of IBS coefficients.**

882 **S2 Fig. Chromosome-wise LD decay rate (Kb) in A genome considering whole collection.**

883 **S3 Fig. Chromosome-wise LD decay rate (Kb) in C genome considering whole collection.**

884

885

The number of SNPs within 1Mb window size
0Mb
9Mb
18Mb
27Mb
36Mb
45Mb
54Mb
63Mb
72Mb
80Mb
A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
C1
C2
C3
C4
C5
C6
C7
C8
C9
0
1
4
7
10
13
16
19
22
25
28
31
>31

DeltaK = mean(|L''(K)|) / sd(L(K))

A

Delta K

1000
800
600
400
200
0

3 4 5 6 7 8 9

K

B

1
0.8
0.6
0.4
0.2
0

P1 P2 P3 P4 P5 P6

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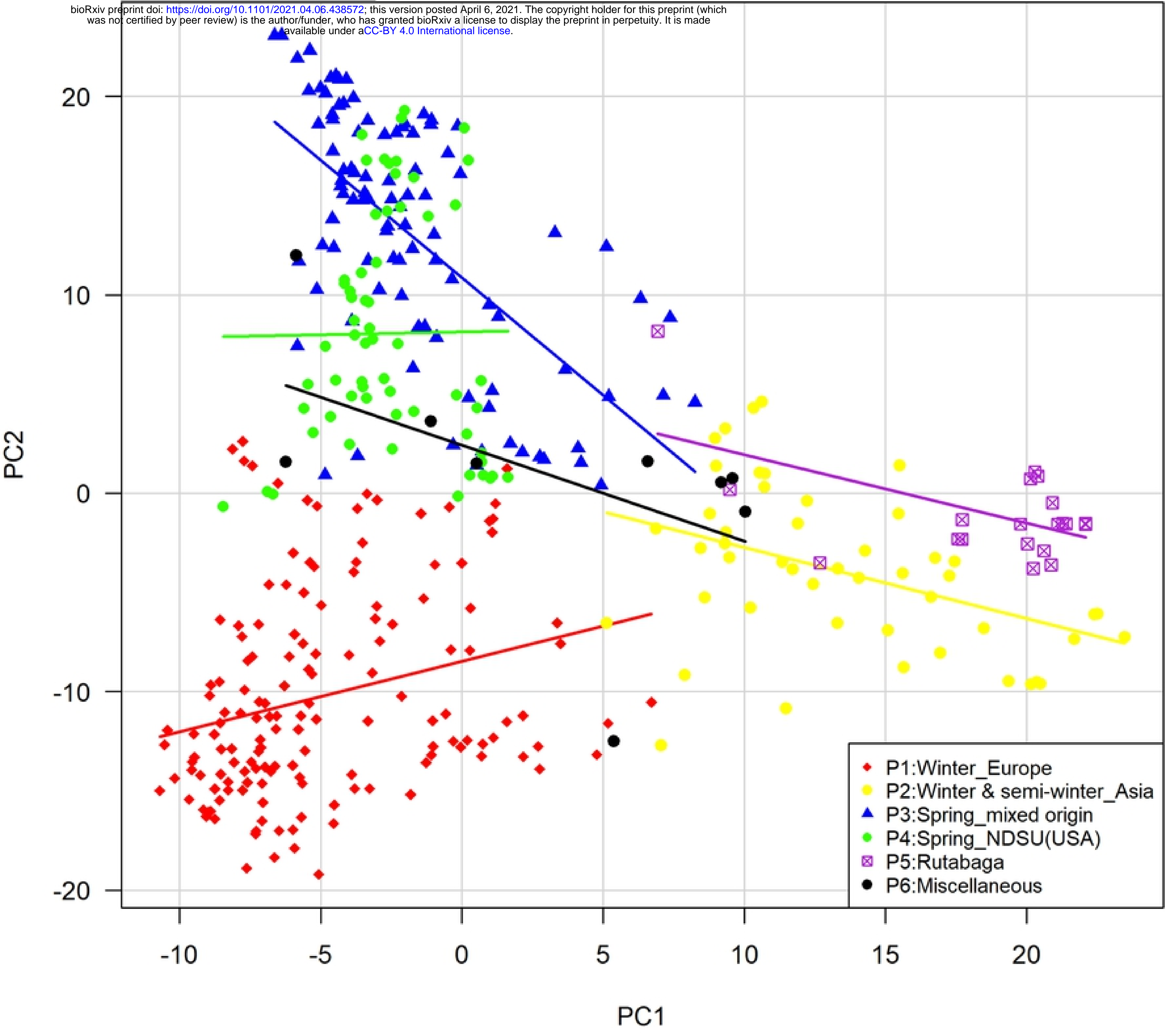
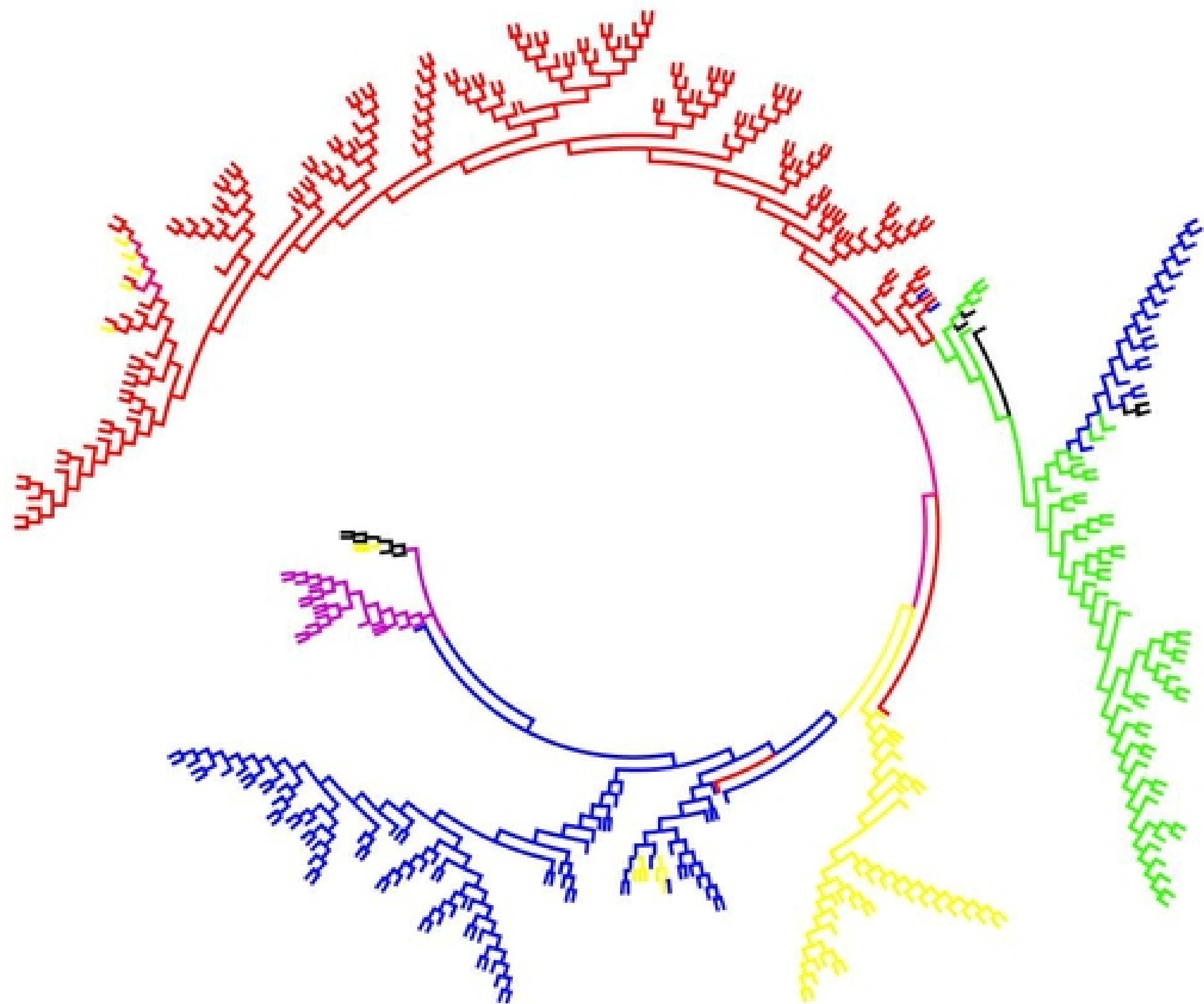
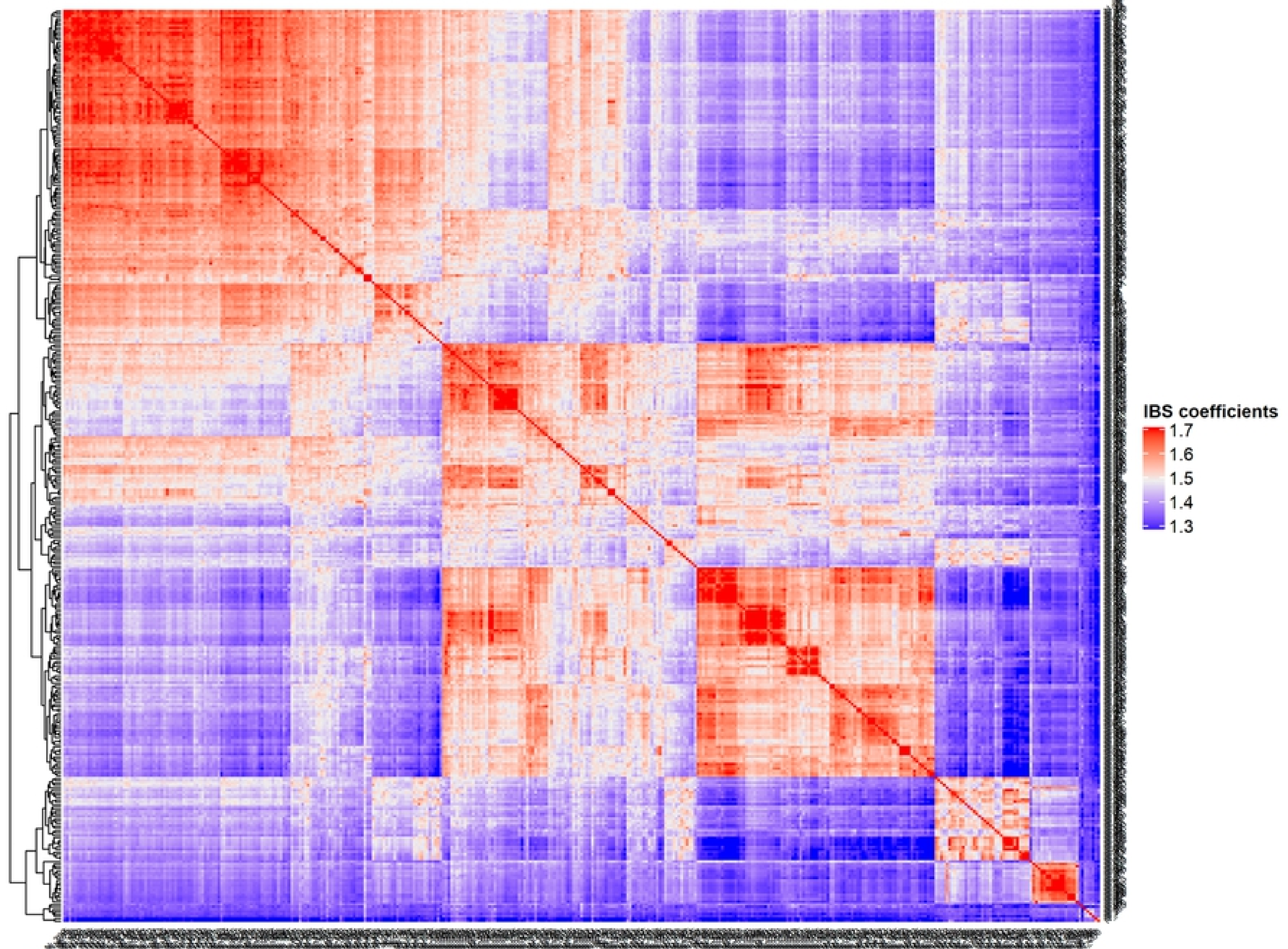
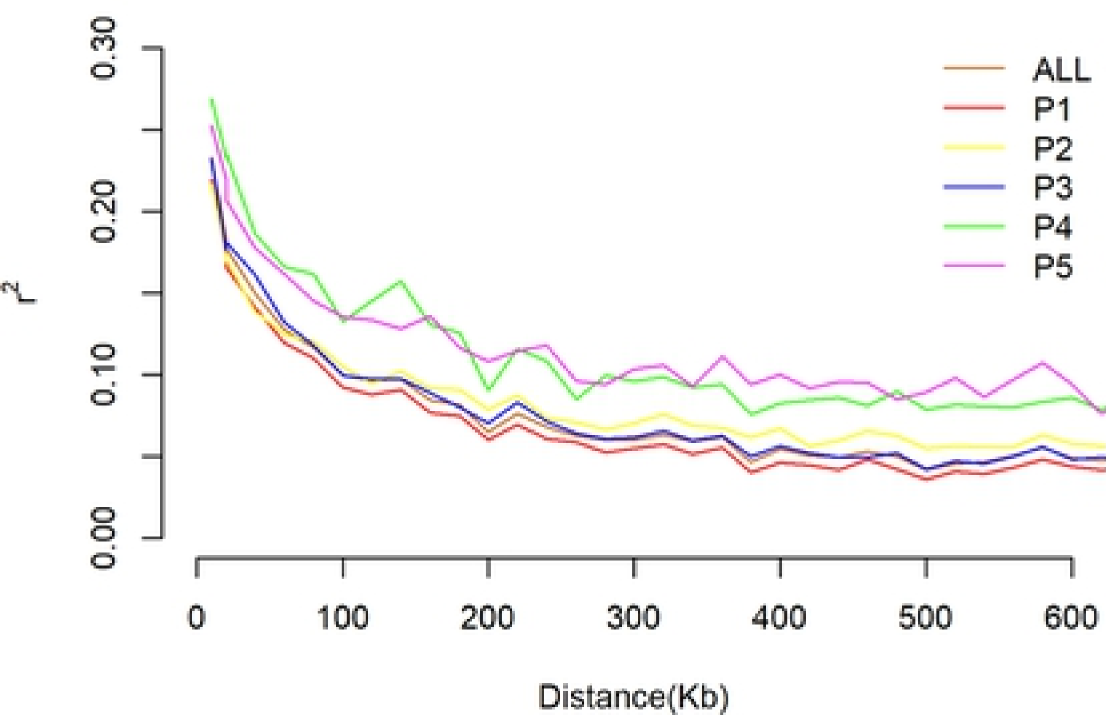
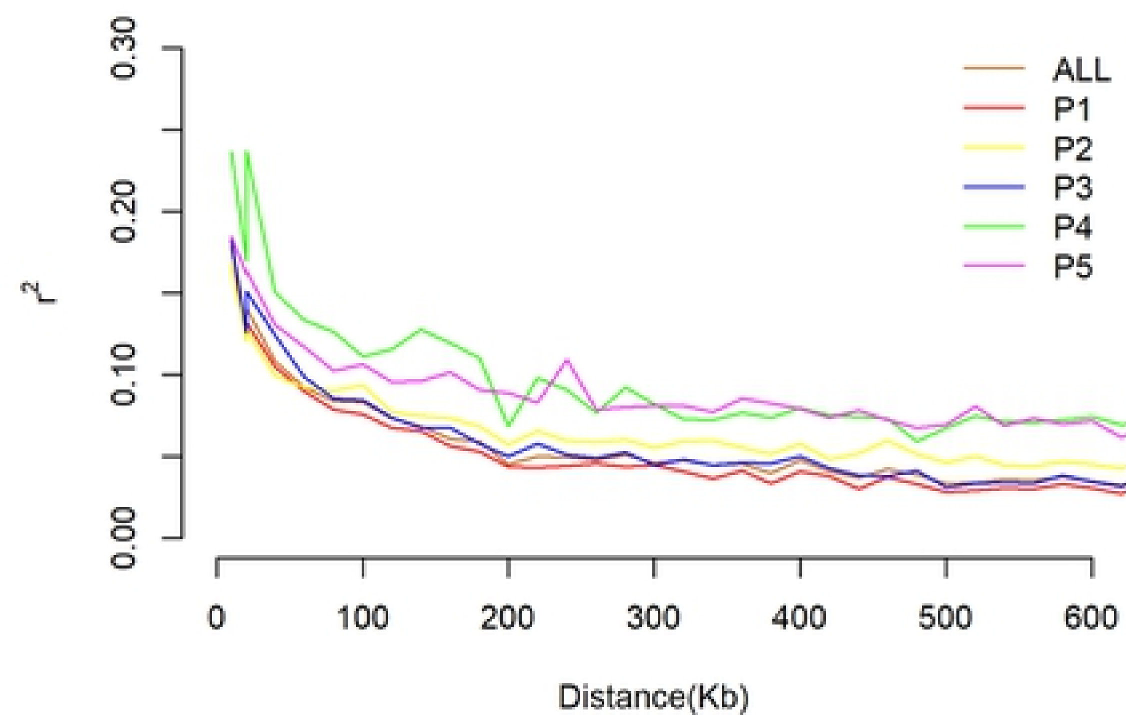
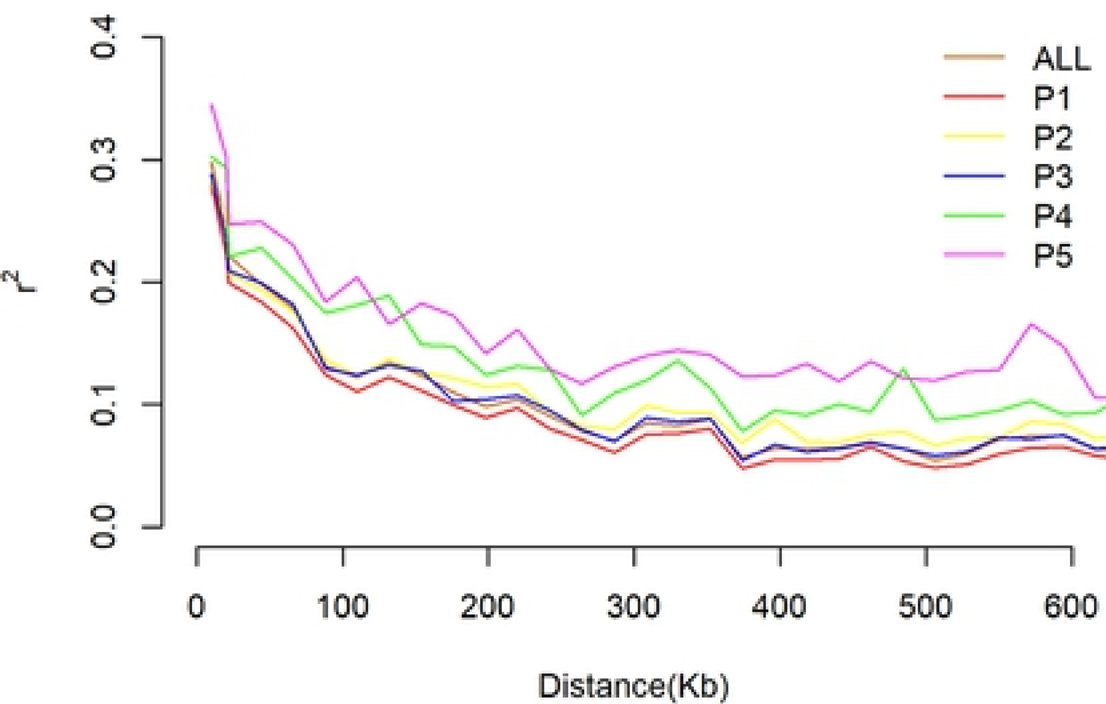



4.0


IBS coefficients
1.7
1.6
1.5
1.4
1.3


LD decay_AC genome
0.30
0.20
0.10
0.00
r^2
ALL
P1
P2
P3
P4
P5
0
100
200
300
400
500
600
Distance(Kb)


LD decay_A genome
0.30
0.20
0.10
0.00
r^2
ALL
P1
P2
P3
P4
P5
0
100
200
300
400
500
600
Distance(Kb)


LD decay_C genome
0.4
0.3
0.2
0.1
0.0
r^2
ALL
P1
P2
P3
P4
P5
0
100
200
300
400
500
600
Distance(Kb)