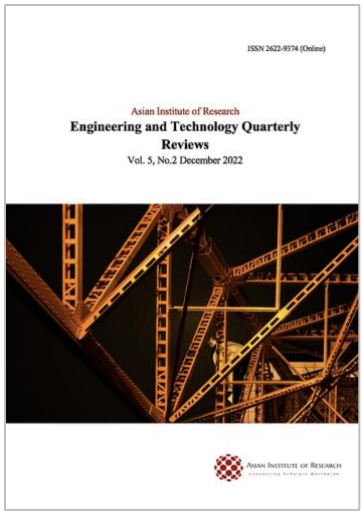

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Assessment of Genetic Configuration Rapeseed (*Brassica napus L.*) Cultivars

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Abstract
Empathetic the inherited configuration of rapeseed cultivars is a precondition to consider phylogenetic pedigree, population structure, haplotype block in the target population. In this investigation, from different regions we have 85 rapeseeds (Brassica napus L.) cultivars. DNA was extracted by using CTAB method. Phylogenetic pedigree, haplotype block and SNP hotspots, Population structure is analyzed by using the 5058 Single Nucleotide Polymorphisms SNP markers to this research 85 rapeseed cultivars were considered. Phylogenetic was analyzed by using Power Marker software and MEGA software, HaploView was using to analysis the haplotype block, population structures were analyzed by using population structure software 2.3.4 manual user as K increasing sidewise from 1 into 10, recording ΔK valency shown at K=2 hence, 85 rapeseed cultivar were divided into two subcategories, P1subgroups including 13 cultivars and P2 subcategory including 72 cultivars, SNP hotspots determined by using map chart software. In this research, the haplotype block analysis presented that the SNP markers number in A sub genome is more than the C sub genome, the block numbers on chromosomes A06 and C03 are greater than the other chromosomes and the lowest block number is on chromosome C09. The hotspots of SNP were defined by SNP position, mutation frequency, and the number of SNPs per 100Kbp on the chromosome. The phylogenetic pedigree was constructed, neighbor-joining was built based on genetic similarity and kinship. In this study, 85 rapeseeds are branched into 12 major groups.

Keywords: *Brassica napus* L. Cultivar, Haplotype Block, SNP Marker, Pedigree. Genetic Structure

1. Introduction

Rapeseed (*Brassica napus* L.), (AA CC, 2n = 38) is an amphidiploid type which is the product of hybridization among *B. oleracea* L. (CC, 2n = 18) and *B. rapa* L. (AA, 2n=20) 10,000 years ago. Rapeseed is the secondary important oilseed producing crop in the world after the soybean, and also its planted in several places in different regions, with a yearly production of over 60 million tons per year after 2011, rapeseed oil is an edible oil, primarily eatable for food (meal) but also can be used non-meal as a Biofuel, rapeseed oil is an industrial grease also as an infrastructure for polymer synthesis (Li et al., 2016; Zhou et al.,2017; Chen et al.,2014) Crop breeding programs are defined by the sighting of three basic parts, such as organization, reproductive and vegetative

organs (Sarlikioti et al., 2011; Li et al., 2016). In this era of technology, plant breeding increments yield collection facility and management, also create a desirable dispersion of carbon and nutrients between seeds and elements (Li et al., 2016). The most crucial purpose of the rapeseed (*Brassica napus* L.) breeding programs are improving the commercial success related traits. Although, on many occasions, such traits can solely be evaluated at an advanced stage of development (Korber et al., 2016). Oilseed rape, is the most embossed oilseed season's growth in the universe's mainland, such as Asia, America, Europe, and Australia (Delourme et al., 2013; Zhou et al., 2017; Chen et al., 2014; Li et al., 2016) . In this accumulation is used to scheme and to be comforted employ of 60k SNP chip also by recently 60k SNP chip (Bus et al., 2014; Chen, 2014). Moreover, valuable to study SNPs markers in agricultural products through strategies such as Genetics, genetic relationship, population structure, SNP marker is nobly enabled precious for the scrutiny or study agronomic characteristics in the crop through contrivance like as not genetic linkage mapping or association mapping.

2. Materials and Methods

2.1 Plant material

The experimentation was organized at Southwest University in the faculty farm of Agronomy and Biotechnology. 85 *Brassica napus* L. cultivars were sown in two lines, where each line contained ten plants. The distance between each line was 30cm and the space between each plant was 15cm.

2.2 Population Structure Analysis

Population structure in the 85 plants and 5058 SNP marker population structure were analysis by using STRUCTURE software V2.3.4, (Pritchard, et al.; 2000) by the manual option, the SNP were coded as follows: A=1, T=2, C=4, G=3, also massing data were coded -9 lengths of the burning period were 100,000 and number 100,000 membership of the genotype was run k=1 to 10 for each kit was replicated 10 times.

2.3 Phylogenetic relationship analysis

A phylogenetic relationship was analyzed by the MEGA-X v10.0.1 with the manual option. The subgroups number (K) was run in to1-10 established supported characterized models by correlated and commixture allele frequencies. Distinctly, three runs were conducted for each K (Zhaoa, et al., 2011).

2.4 Analysis of Haplotype Block Structure

Haplotype block structure in the 85 rapeseed cultivars by the 5058 SNP marker appraise using HAPLOVIEW V4.1 by using four gamete rule options (Barrett et al., 2004). The evaluated and analyzed offered by Gabriel et al., (2002), Haploview software desiderate pairwise associations assuming that the space amongst markers was more than 500 kb resulting in the parameter to evaluation very marker pairs, expressly for a durable LD among markers above 500 kb, this scenery was attuned to zero the analysis mentioned to the explanation by Gabriel et al (2002).

3. Result

3.1 SNP Analysis and Haplotype Block Structure.

Overall of 5058 SNPs were abide by to use to consider the genetic structure. The distribution of SNP in each chromosome is shown in Table1. The chromosome C03 had the most SNPs, and the chromosome C09 had the least SNPs. the SNP density was largest in A10 and least on chromosome C09. In a total of 640 preserved haplotype blocks were identified after assessing 5058 quality of SNPs spread throughout the genome in 85 rapeseed cultivar additives.

Table 1: The distribution of SNP in each chromosom

Chromo-some	No. of SNP	Length of SNP region(bp)	SNP density(bp)	Chromo-some	No. of SNP	Length of SNP region(bp)	SNP density(bp)
A01	217	22920661	105625	C01	291	38016178	130640
A02	205	24734717	120657	C02	278	45644021	164187
A03	415	29652828	71453	C03	432	60554468	140172
A04	225	18704222	83130	C04	358	48892043	136570
A05	281	22923221	81577	C05	182	42637644	234273
A06	305	24307551	79697	C06	218	36358688	166783
A07	317	23709808	74794	C07	248	43849181	176811
A08	212	18493863	87235	C08	262	37660401	143742
A09	230	33526827	145769	C09	134	48333600	360699
A10	248	17234879	69495				
Mean	266	23620858	91943	Mean	267	44660692	183764

The haplotype block position, SNP number, and length, within each block, are also delivered in there were the most haplotype blocks on chromosome A06 and chromosome C03 and the less on chromosome C09. The mean of A-sub genome haplotype blocks was 35 and the mean of C-sub genome haplotype blocks was 32 (Figure 1).

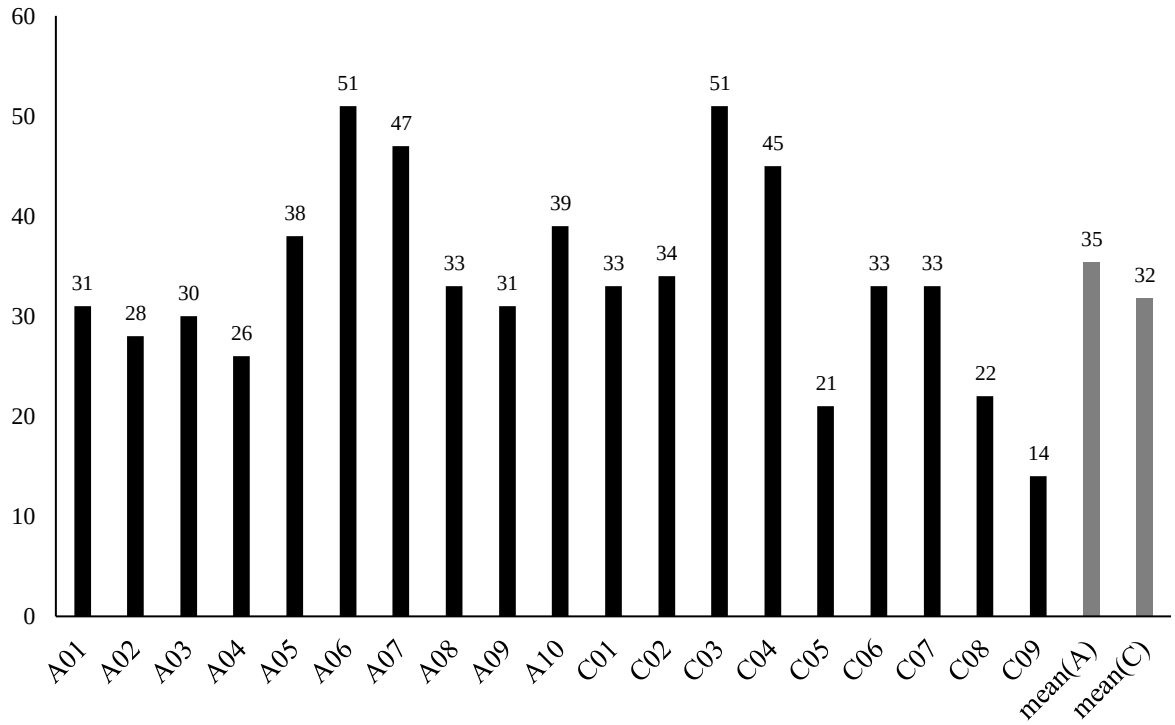

Figure 1: The distribution of haplotype block in each chromosome. Mean (A), the mean haplotype block of A subgenome; mean(C), the mean haplotype block of C subgenome

3.2 Population Structure Analysis.

The 85 rapeseed cultivar population structure was explored in this study, a model based program structure using the STRUCTURE software. It was used to regulate the genetic association among different rapeseed cultivar The LnP (K) values were gradually increased in the case of SNP with K-value start from 1 into 10 (Figure 3b); the number of the population were shown K loci were independent and at Hardy-Weinberg equilibrium. Never the less, the summit of 1K was achieved at K = 2 (Figure 3a). These data in Figure 5B disguised that 85 cultivar lines might be clustered within two categories; first category (population 1) include 13 cultivars and the second category (population 2) include 72 cultivars (Figure 2). By applying a membership threshold of 0.70, the first

group including 13 cultivars and the second group including 72 cultivars were assigned as Group 1 and Group 2, respectively. The remaining 85 accessions were assigned into Mixed Group.

Figure 2: [1]Model-based clustering of k=2 in rapeseed cultivars

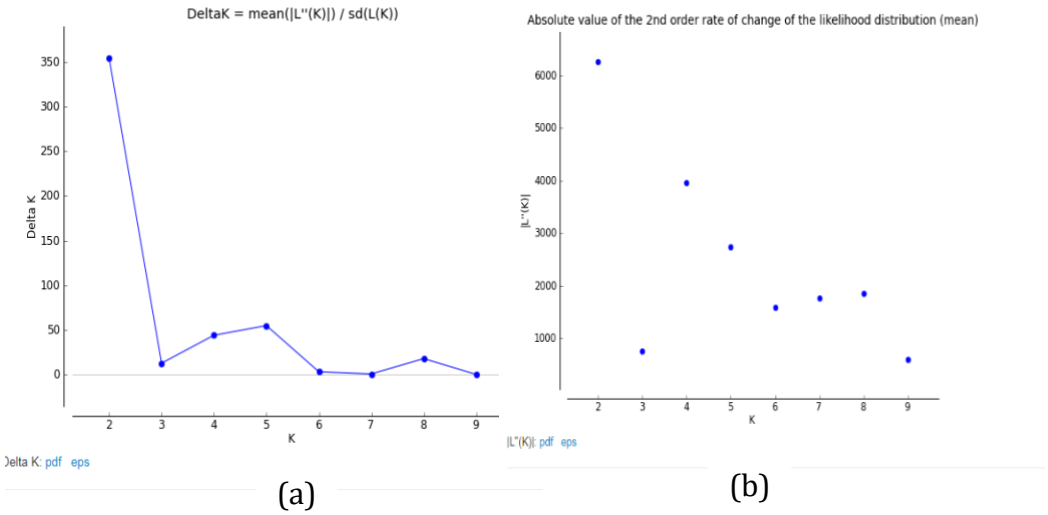

Figure 3: Assessment the 85 rapeseed cultivare population structure using LnP (D) derived Δk for 1 to 10 SNPs.

3.3 Cultivar Identification Pedigree

The SNP markers in the rapeseed cultivar have been generally used for cultivar phylogenetic pedigree identification (Aranzana, et al., 2003; Pritchard, et al., 2002). Considerate the similarity relationships among the 85 rapeseed cultivars, the different geographical area and breeding station. In this study, present that: 18z409 from Sichuan, 18z376 from Shanxi, and 18z399 from Jiangsu, convened in Cluster I. 18Z366 from Anhui, with 18Z365 from Guizhou grouped in second cluster. In the third cluster 18z384, 18z370, 18z400, 18z392, 18z434,

[1] Note−figure 2. Distributed model-based clustering of population structure among 85 *B. napus* L. cultivar out coming from STRUCTURE applied to with 5058 SNP makers, the clusters were obtained at of k=2 where k is an indicator for clusters that can exist in the overall sample, the cluster divided in two genotype group.

18z402, 18z439, 18z440, 18z441, 18z385 from Shanxi, 18z438, 18z369, 18z361 from Jiangsu, 18z428 from Sichuan grouped together. In the cluster four 18z418, 18z373, 18z416 from Sichuan, 18z377, 18z395, 18z359, 18z404, 18z437, 18z435 from Hubei, 18z403 from Anhui, 18z371 and 18362 from Shanxi, 18z386 from Huizhou grouped with each other.

The cluster five 18z381 from Shanghai, 18z3878 and 18z394, 18z364 from Hunan, 18z406 and 18z433 from Sichuan, 18z368, 18z380, 18z372and 18z431 from Zhejiang, 18z411 from Anhui, 18z432, 18z374 from Hubei, 18z383 from Jiangsu and 18z367 from Anhui that variety was grouped in the cluster five. In the cluster six 18z430, 18z421, 18z396, 18z423, 18z424 from Sichuan and 18z379 from grouped together in the cluster six. In the cluster, seventh varieties 18z397, 18z429, 18z417, 18z427, 18z420, 18z425 and 18z410 from Sichuan and two more 18z415, 18z398 from Hubei were grouped in cluster seven. In the cluster eighth included with the 18z360, 18z413, 18z419, 18z375, 18z408, 18z422 from Sichuan were grouped in cluster eighth. Cluster ninth grouped with (18z399 from Jiangsu, 18z388 from Henan and 18z390 from Shanxi). In the cluster tenth 18z401, 18414, 18z425 from Sichuan, 18z393 from Hubei, 18z391 from US and 18Z436 from Guizhou. In the cluster eleventh 18z405 from Hunan, 18z387 from US 18z357 from Anhui and18z412 Anhui. And the cluster twelfth 18z358 from Jiangsu was clustered in the cluster twelfth (Figure5).

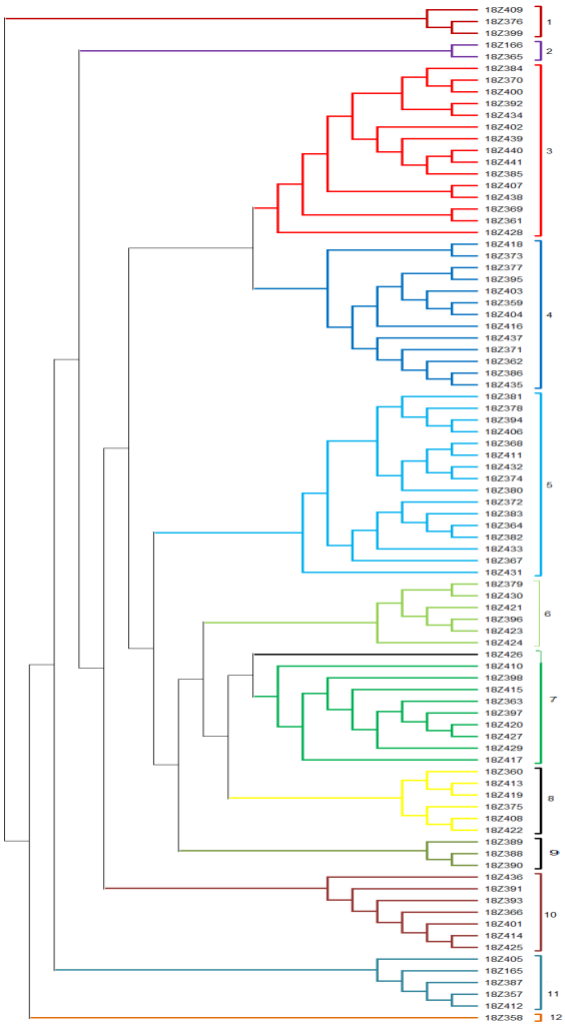

Figure 5: Phylogenetic pedigree clustering in the 85 rapeseed cultivar.

Neighbor-joining dendrogram pedigree calculated by 5058 SNPs markers, obtained from on 85 *Brassica napus L*. cultivars genotypes, have been numbered and color-coded for ease of viewing. divided into12 cluster.

4. Discussion

This research assesses the genetic structure from 85 rapeseed cultivars with the 5058 SNP markers. population structure, haplotype block analysis, pedigree, and assessment SNP population hotspot. SNP markers in different rapeseed cultivar were used for cultivar identification and Haplotype Block Structure, population structure, and phylogenetic analyses. Comparable by Aranzana et al., 2003; Testolin et al., 2000).

In this study, population structure in the 85 rapeseed cultivars population, is increased in the case of SNP with k-value from 1-10 and k value achieved k=2, 85 cultivars branched (clustered) into two groups; the first group includes13 cultivars and the second group contents 72 cultivars. Comparison by (Shi, et al., 2017; Yu, et al., 2006). "Clustering inference performed with possible clusters (K) from 1 to 8 showed that the most significant change of likelihood occurred when K increased from 2 to 3, and the highest ΔK value was observed at K = 2. Both parameters suggested that the 248 genotypes could be assigned into two groups. Using a probability of membership threshold of 70%, 40 and 103 lines were assigned into the two groups" (Wu, et al., 2014). The 248 inbred lines were categorized to two individual's category using the structure, which was consistent with the results of Xiao et al, (2012).

Totally, a haplotype block is a group of SNPs ($r^2 > 0.8$) that be disposed to foldaway over the generations as a block (Zondervan and Cardon, 2004). In this research, we found in haplotype blocks analysis, A sub genome average of haplotype blocks number 35 and haplotype blocks size 3909 kb. In C sub-genome, an average of block number was 32. It's comparable by (Diers and Osborn., 1994; Hasan et al., 2006; Qian et al., 2014**)**. the previous study, presented 25466 haplotype blocks, indicating (12.53% of the *B.napus* L. genome source), most of which extended in size from 0 to 1 Kb. therefore, recognized 3097 preserved haplotype blocks, indicating (15.17% of the rapeseed genome source) by using 24994 SNPs.

There can be numerous description for these result. Firstly, there are many regions in the genome rich in repetitive sequences, where DNA polymerase errors resulting in strand slippage and inequitable exchange can easily occur (Clayton et al., 2016). In this study**,** we found the cultivar accessions that Sichuan, Shanxi, and Jiangsu were of origin grouped in the first cluster which is including three cultivars. Whereas two cultivars were organized in cluster two, from Anhui, Guizhou. In cluster three, nine cultivars from Shanxi, two cultivars from Jiangsu and one Sichuan grouped together. In cluster four, three cultivars from Sichuan, six cultivars from Hubei, one cultivar from Anhui, two cultivars from Shanxi, one cultivar from Huizhou grouped with each other. In cluster five, one from Shanghai three Hunan two from Sichuan, four from Zhejiang, one from Anhui, two from Hubei, one from Jiangsu and one from Anhui those cultivars were grouped in cluster five. And also in other clusters cultivars were groped whit the origin Shanxi, Guizhou, Hubei, Anhui, Jiangsu, Henan, Sichuan, Guizhou. Secondly, the lower the choosy density, the greater the amassing of mutations, and mutated allelic sites in genic regions are usually easily brushed away under the moderately greater selective pressure in these regions. Lastly, some variable regions result from adaptive pressures, whereby mutations in genes related to adaptive capacity are more likely to be retained, as variability may increase survival probabilities with exposure to environmental stress (Hayward et al., 2015; Weigel and Nordborg, 2015)

5. Conclusion

Genomic data provide research novel insight into rapeseed. In the current study, population structure in the 85 rapeseed cultivars population, is increased in the case of SNP with k- value from 1-10 and k value achieved k=2, 85 cultivars divided (clustered) into two groups; the first group includes 13 cultivars and the second group contents 72 cultivars.

In this research, the haplotype block analysis in the rapeseed genome with 5058 SNPs markers, 640 preserved haplotype blokes was identified, in the A subgenome the average of haplotype block number was 35.4 and haplotype block size in the A subgenome was 3909 kb. Also, in the C subgenome, the average of haplotype blocks number is 31.77, an average of haplotype block blocks size is 5673 kb.

The 85 rapeseed cultivar grouped into twelves clusters built on relationship number. The grouping patterns offered a good depiction of relationships between cultivars that originated from different geographical areas and

breeding lines advanced at the research location. 18z409 from Sichuan, 18z376 from Shanxi, and 18z399 from Jiangsu, grouped together in Cluster I. The second group involved of '18Z366 from Anhui, with 18Z365 from Guizhou clustered. In the third group 18z384, 18z370, 18z400, 18z392, 18z434, 18z402, 18z439, 18z440, 18z441, 18z385 from Shanxi, 18z438, 18z369, 18z361 from Jiangsu, 18z428 from Sichuan grouped organized. In the group four 18z418, 18z373, 18z416 from Sichuan, 18z377, 18z395, 18z359, 18z404, 18z437, 18z435 from Hubei, 18z403 from Anhui, 18z371 and 18362 from Shanxi, 18z386 from Huizhou clustered with each other.

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