علم او فن-علمي څېړنيزه مجله

Integrative Analysis of Genetic Diversity, Yield Traits, and Seed Quality in *Brassica napus* L. Cultivars through Phylogenomics

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Abstract

Oilseed rape (Brassica napus L.) is a major oil-producing crop worldwide, valued for its adaptability, yield potential, and seed quality. This study investigated the genetic diversity, phylogenetic relationships, and trait associations of 85 rapeseed cultivars from different geographical regions. Genetic variation was assessed using 5,058 SNP markers and ITS region sequences, and phylogenetic relationships were analyzed using MEGA-X and PowerMarker, resulting in twelve distinct genetic clusters that largely reflected geographical origin and breeding history. Phenotypic evaluations included key agronomic traits (e.g., plant height, silique number, 1000-seed weight) and seed quality traits (e.g., oil, protein, and glucosinolate content). Statistical analysis was performed using analysis of variance (ANOVA) to determine significant differences among clusters ($P < 0.01$). Results showed substantial variation in both agronomic and seed quality traits: Cluster 8 had the highest oil content, while Cluster 1 had the highest protein content. An inverse relationship between oil and protein content was observed, indicating a trade-off between these traits. These findings demonstrate that integrating SNP- and ITS-based molecular markers with phenotypic evaluation effectively identifies genetic relationships and trait variation, providing a robust framework for marker-assisted selection, germplasm improvement, and future breeding programs aimed at enhancing yield, seed quality, and environmental adaptation in Brassica napus.

Keywords: *Brassica napus* L., Genetic diversity, Phylogenetic analysis, SNP markers, Seed quality traits, Agronomic performance.

تحلیل تلفیقی تنوع جنتیکی، خصوصیات عملکرد و کیفیت تخم در واریتی‌های Brassica napus L. با استفاده از فایلوجنومیک

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خلاصه

کلزا (Brassica napus L.) یک محصول عمده‌ی تولیدکننده‌ی روغن در سراسر جهان است که به دلیل سازگاری، پتانسیل عملکرد و کیفیت بذر آن ارزشمند است. این مطالعه تنوع ژنتیکی، روابط فیلوژنتیکی و ارتباط صفات ۸۵ رقم کلزا از مناطق جغرافیایی مختلف را بررسی کرد. تغییرات ژنتیکی با استفاده از ۵۰۵۸ نشانگر SNP و توالی‌های ناحیه ITS ارزیابی شد و روابط فیلوژنتیکی با استفاده از نرم‌افزارهای MEGA-X و PowerMarker تحلیل شد که منجر به تشکیل دوازده خوشه‌ی ژنتیکی متمایز شد که تا حد زیادی منعکس‌کننده‌ی منشأ جغرافیایی و تاریخچه‌ی اصلاحی بودند. ارزیابی فنوتیپی شامل صفات زراعی مهم (مانند ارتفاع گیاه، تعداد غلاف، وزن هزار دانه) و صفات کیفیت بذر (مانند روغن، پروتئین و محتوای گلوکوزینولات) بود. تحلیل آماری با استفاده از آنالیز واریانس (ANOVA) برای تعیین تفاوت‌های معنادار بین خوشه‌ها انجام شد ($P < 0.01$). نتایج نشان داد که تنوع قابل توجهی در هر دو گروه صفات زراعی و کیفیت بذر وجود دارد: خوشه ۸ بیشترین محتوای روغن و خوشه ۱ بیشترین محتوای پروتئین را داشت. یک رابطه‌ی معکوس بین

محتوای روغن و پروتئین مشاهده شد که نشان‌دهنده‌ی وجود یک تعادل بین این صفات است. این یافته‌ها نشان می‌دهند که ترکیب نشانگرهای مولکولی مبتنی بر SNP و ITS با ارزیابی فنوتیپی به طور مؤثری روابط ژنتیکی و تنوع صفات را شناسایی می‌کند و چارچوبی قوی برای انتخاب با کمک نشانگرها، بهبود ژرم‌پلاسما و برنامه‌های اصلاحی آینده به منظور افزایش عملکرد، کیفیت بذر و سازگاری محیطی در Brassica napus فراهم می‌آورد.

کلمات کلیدی: ، تنوع جنتیکی، تحلیل فیلوجنتیکی، نشانگرهای SNP، صفات کیفیت تخم، عملکرد زراعی، Brassica napus L.

1. Introduction

Oilseed rape (*Brassica napus* L.) ranks among the top oil crops worldwide oilseed crops in most of the world's continents, such as Asia, America, Europe, and Australia (Delourme et al., 2013; Zhou et al., 2017; Chen et al., 2014; Rostazada et al., 2023). Among all oil production crops, oilseed rape has the most important role in many countries (Abdelmigid and El-Sayed A., 2016; Fayyaz et al., 2014). In the world ranking, after the soybean, *Brassica napus* L. is the second biggest oil production plant around the world. Canola is also able to be sown and grown in various regions. After the 2011 oil seed rape production, yearly production is over 60 million tons. Canola oil is the most important nutritional oil, principally eatable for food; rapeseed oil can be used as a biofuel; *B. napus* L. oil is an engineering grease and as an infrastructure for polymer synthesis (Allender, 2010). *Brassica napus* L. (AACC, 2n = 38) remains an amphidiploid plant that exists as the outcome of hybridization concerning Brassica *rapa* L. (AA, 2n = 20) plus *Brassic oleracea* L. (CC, 2n = 18) within the previous 10,000 years (Li et al., 2016; Zhou et al., 2017; Chen et al., 2014; Rostazada et al., 2023).

Brassica napus L. Phylogenic analysis relying on internal transcribed spacers and classification was considered to determine the genetic relationships between rapeseed cultivars. The internal transcribed spacer district was sequenced in 85 *Brassica napus* L. cultivars with pedigree histories and various environmental regions. Substantial genetic diversity was detected at intercultivar ranks, supporting the effectiveness of internal transcribed spacer arrangements aimed at founding genetic relations between cultivars. Analysis of internal transcribed spacer arrangements by neighbor joining (NJ) and concentrated parsimony (MP) procedures created dendrograms essentially of related genotypes grouped into two leading groups individually since their environmental basis, portentous of a contracted genetic origin between the considered ecotypes. Rapeseed breeding programs success is largely dependent on the existence of sufficient genetic diversity. *Barassica napus* L. germplasm, phylogeny exploration has been generally used to assess the degree of similarity among plants, but the essential material on ancestry is not continuously true. The spread of DNA markers has allowed the genome to be analyzed for true environmental factors (Bleeker *et al.,* 2008; Rostazada et al., 2023; Bus et al., 2014).

Moreover, it is valuable to study SNP markers of agronomic characteristics in agricultural produc Barassicats through strategies such as genetics, genetic relationships, population structure, and SNP heterozygosity value. SNP markers are nobly enabled and precious for the scrutiny or study of agronomic characteristics in the crop through contrivances like genetic linkage mapping or association mapping. SNP markers are used for a wide range of determinations in *Brassica*, containing rapid credentials of cultivars (Zhou et al., 2017). The polymorphism of internal transcribed spacer arrangements, perceived through variation in size and nucleotide content, completed the genetic diversity between rapeseed cultivars. Information on the associated sequences showed that together, single nucleotide variances and diminutive distances of sequence diversity remained due to supplements or removals existing in the internal transcribed spacer or second internal transcribed spacer regions representing intercultivar differences. Disagreement) was more advanced than the infrageneric dissimilarity of ITS arrangements to the anther angiosperm plant (Baraket *et al.* 2009; Jorgensen *et al.* 2003; Abdelmigid H. M. and El-Sayed A., 2016). The *B.napus* L. yield is connected in the three component parts such as silique number on a single plant, seeds number in each silique, 1000 seeds weight, seeds per each silique, seeds number per single plant, silique the number of traits per single plant has the highest effect on yield (Yang et al., 2012). Parental effects on the F1 generations with different traits It is clear that in the family relationship between the F1 generation and their genitor, the contents of the seeds and oil yield have a distinct correlation coefficient with traits, though the seed yield and yield components of three positive F1 crosses are significantly related to the corresponding maternal and paternal parents. (Shen et al., 2005; Wu et al., 2019).

The topmost measure projects of genome sequencing are administered to know about the evolutionary and instrument of phenotypic diversity construction in rapeseed of the highest quality. *Brassica napus* L. simplifies genome-wide sequence measurement between a wide assortment of diverse materials (Zou et al., 2019; Sun et al., 2017; Allender, 2010). Additional resequencing development of 588 *Brassica napus* L. agreements revealed that winter rapeseed may be the innovative procedure of *Brassica napus* L. and recognized genetic loci connected with stress resistance, seed quality, oil content, and ecotype development by participating in genome-wide association studies, assortment signals, and transcriptome examination (Lu et al., 2019). The previous research showed that the inheritance of cytoplasm was determined to significantly subsidize the construction of the most agronomically significant traits of plants, like yield, resistance in the cold season, grain weight, and milling quality traits. Indica rice-filled grain ratio (Tao et al., 2011; Tao et al., 2004). Soybean seed protein content (Singh, 1972; Liang et al., 2007) Conversely, the genetic diversity, genetic origin population, and population structure of agronomic traits for worldwide *Brassica napus* L. genomes have not been totally analyzed, except for plan height in maize (Khehra, 1976) and oil gratification in rapeseed (Hua et al., 2012).

We hypothesize that combining SNP-based phylogenetic analysis with phenotypic trait assessment across a broad panel of Brassica napus cultivars will provide a comprehensive view of genetic structure and help identify valuable germplasm clusters. Therefore, the objective of this study is to evaluate the genetic diversity, phylogenetic relationships, and associations with key agronomic and seed quality traits across 85 B. napus cultivars from diverse geographic origins using both SNP markers and ITS region sequences. The ultimate goal is to provide a genomic and phenotypic framework to guide rapeseed breeding strategies for enhanced productivity and seed quality.

2. Materials and Methods

2.1. Plant materials

A total of 85 rapeseed cultivars were sownin southwest university agriculture and biotechnology farm, cultivars were selected different regions (table 1, table 2 and table 3). Sowed in two lines, where each line contained ten plants. The distance between each line was 30cm and space between each plant was 15cm five plants from each cultivar groups were sampled for determination of agronomic traits and seed quality.

2.2. Phenotypic traits measurements

2.2.1. Agronomic Traits

For studying different genetic parameters and inter relationships, ten characters were taken into consideration. A pictorial view of observation and data collection is presented in plate 5. The data were recorded on fifteen selected plants from each replication of 31 genotypes on ten following traits. plant height (cm), first branch length (cm), number of branch plant-1, silique length) cm), silique width (cm), single plant yield (gr), silique weight (gr), seed number silique-1 and silique number plant-1.

2.2.2. Seed Quality Data Selection

The seed quality traits, oil, protein, the sum of oil and protein and glucosinolate content as well as saturated fatty acid content in the oil were measured on the 500 seed from each of the five plants categories. The content of oil and protein and glucosinolate measured using the near-infrared reflection of titanium (NIR) using the Foss 6500 system (Daun et al., 1994; Clercq, 2005). Total oil and protein content were calculated by adding Nair results. Fatty Acid Saturated Gas Chromatography Methyl Esters Fatty Acids were determined (Clercq, 2005).

2.3. Phenotypic data statistical analysis

Statistical analysis of the phenotypic data, description average and ANOVA correlation between seed quality and agronomic traits were used (IBM SPSS; VERSION 20) manual option, for seed quality and agronomical traits chart were used Microsoft Excel 2016.

2.4. DNA extraction and phylogenetic analysis

DNA was extracted by using CTAB (Doyle & Doyle, 1990) method from 85 rapeseed cultivar by using 5058 SNP genetic marker. A phylogenetic relationship was analyzed by the POWER MARKER software and MEGA-X v10.0.1 with the manual option. The number of subgroups (K) was set from 1-10 based on models characterized by admixture and correlated allele frequencies.

Table-1: Show name and ID number of rapeseed cultivar from variouse geographical region.

NO.	NAME	AREA	ID	NO.	NAME	AREA	ID
1	ZHEPING NO.4	ANHUI	18Z357	15	QIN YOU 33	SHANXI	18Z370
2	RED OIL NO. 3	JIANGSU	18Z358	16	TIANYOU NO.10	SHANXI	18Z371
3	MEDIUM OIL	HUBEI	18Z359	17	DHERE 33	ZHEJIANG	18Z372
4	DEFANG OIL. 2	SICHUAN	18Z360	18	DEYOU NO.8	SICHUAN	18Z373
5	NINGZA 1818	JIANGSU	18Z361	19	MEDIUM OIL	HUBEI	18Z374
6	FENGYOU 737	SHANXI	18Z362	20	GERMAN OIL1-12	SICHUAN	18Z375
7	DEHUI OIL 50	ANHUI	18Z363	21	SHI LIFENG	JIANGSU	18Z376
8	MISCELLANEOUS OIL 188	HUNAN	18Z364	22	GUANGYUAN 58	HUBEI	18Z377
9	ZHUOYOU 058	GUIZHOU	18Z365	23	HUAXIANG OIL . 11	HUNAN	18Z378
10	HUIHAO OIL 12	ANHUI	18Z366	24	DETIAN 118	SHANXI	18Z379
12	HUYOU 21	ANHUI	18Z367	25	ZHEJIANG OIL	ZHEJIANG	18Z380
13	ZHEJIANG OIL 18	ZHEJIANG	18Z368	26	HUYOU 039	SHANGHAI	18Z381
14	YANGYOU NO. 9	JIANGSU	18Z369	27	ZHEJIANG OIL 50	ZHEJIANG	18Z382

Table-2: Show name and ID number of rapeseed cultivare from variouse geografical region.

No.	Name	Area	ID	No.	Name	Area	ID
1	Zhejiang Oil 51	Jiangsu	18z383	24	Zheping, F219	Anhui	18z412
2	Qin You66	Shanxi	18z384	25	Junlong Oil. 5	Sichuan	18z413
3	Shaanxi oil 0913	Shanxi	18z385	26	Rong oil 15	Sichuan	18z414
4	German oil	Guizhou	18z386	27	Earth 55	Hubei	18z415
5	lazy man jack oil	USA	18z387	28	Rong oil 18	Sichuan	18z416
6	CNPC 828	Henan	18z388	29	Huawanyou.4	Sichuan	18z417
7	Fengyou 737	Henan	18z389	30	Yiyou25	Sichuan	18z418
8	Qinyou No.2	Shanxi	18z390	31	Junlong Oil. 10	Sichuan	18z419
9	American Oil King	USA	18z391	32	Chengyou. 11	Sichuan	18z420
10	Qin You 19	Shanxi	18z392	33	Cotton oil 309	Sichuan	18z421
11	Hardcore Streaming King	Hubei	18z393	34	Subfamily Oil 58	Sichuan	18z422
12	Bright Oil.9	Hunan	18z394	35	Dr. Oil	Sichuan	18z423
13	Con oil 61	Hubei	18z395	36	Mianyou. 12	Sichuan	18z424
14	oil king	Sichuan	18z396	37	Chengyou. 11	Sichuan	18z425
15	Xindu Oil 800	Sichuan	18z397	38	DezhongOil. 1	Sichuan	18z426
16	American Oil	Hubei	18z398	39	Seed oil 998	Sichuan	18z427

17	Fenfei No. 1	Jiangsu	18z399	40	Guohao Oil. 8	Sichuan	18z428
18	Qinrong 2	Shanxi	18z400	41	Miscellaneous Oil No. 9	Sichuan	18z429
19	Dexin oil 53	Sichuan	18z401	42	Rong You No. 13 Long Pod King	Sichuan	18z430
20	Shaanxi oil 0913	Shanxi	18z402	43	Zhejiang University 619	Zhejiang	18z431
21	Tongyouza No. 2	Anhui	18z403	44	Medium oil miscellaneous 19	Hubei	18z432
22	Huayouza No.9	Hubei	18z404	45	Mianxin oil 78	Sichuan	18z433
23	Xiang miscellaneous oil 631	Hunan	18z405	46	Qin You No. 7	Shanxi	18z434

Table-3: Show name and ID number of rapeseeds cultivar from various geographical region.

No.	Name	Area	ID	No.	Name	Area	ID
1	**Yuken 52**	Sichuan	18z406	7	Qin Yan 211	Hubei	18z435
2	Qin You No. 8	Shanxi	18z407	8	Oil Research No. 10	Guizhou	18z436
3	Chuanyou 21	Sichuan	18z408	9	Fengyou 5103	Hubei	18z437
4	Dwarf butter vegetable	Sichuan	18z409	10	Qin You. 11	Jiangsu	18z438
5	Chuanyou 39	Sichuan	18z410				
6	Guohua Oil 1208	Anhui	18z411				

3. Result and Discussion

3.1. Agronomical traits

The agronomic traits results were obtained in 2018 Chongqing (Table 4). The plant height was between 168.8cm and 220.2cm. Plant height is a foundational component of rapeseed architecture, strongly influencing yield potential through its effects on light interception and biomass accumulation. Previous studies have demonstrated substantial genetic variability for plant height and related traits, highlighting the importance of these characteristics in breeding programs (Cui et al., 2025; Xia et al., 2024). The number of branches per plant was between 5.8 and 11. The number of branches per plant and main inflorescence length are major contributors to the formation of the plant canopy and reproductive structures. These traits are often correlated with yield components, including the number of siliques and seeds per plant (Shang et al., 2021). The main inflorescence length was between 46.8cm and 109.2cm, in our results, significant differences among genotypes for branch number and inflorescence length likely reflect underlying genetic control and potential adaptability to the local environment. Branching traits can influence yield both directly and indirectly, as effective branching increases sites for silique formation, which is aligned with findings in other rapeseed populations showing positive correlations between branching and yield components (Shang et al., 2021).

The silique length was between 4.1cm and 8.9cm, the silique width was between 0.3cm and 0.8cm, the silique number per plant was 125-539. The observed variability in silique measurements suggests differing genetic potentials for seed set and fill across genotypes. Previous reports have demonstrated that such silique characteristics are integral components of yield and can be significant pathways for selection in breeding (Shang et al., 2021). Similarly, the broad range in seed number per silique in this study is indicative of considerable diversity in reproductive efficiency among lines. The seed number per silique was 11.9-36.6, the single plant yield was 6.1gr-37.6gr. These traits had significant difference ($P<0.01$) among materials (Table 5 and Table 6). ield per plant, ranging from 6.1 to 37.6 grams, reflects the cumulative effects of these agronomic traits. The significant genotype differences highlight the opportunity for selecting superior lines with higher productivity. This is consistent with studies reporting that agronomic traits such as plant height, branch number, and silique characteristics are key determinants of yield and vary significantly under diverse environmental and genetic backgrounds (Shang et al., 2021; Cui et al., 2025).

Table-4. Summary of the descriptive statistics for agronomic traits and single plant yield.

Trait	**Mean**	**SE**	**Min**	**Max**
PH (cm)	193.4429	8.6393	168.8000	220.2000
NB/P	8.4458	1.1609	5.8000	11.2000
MIL (cm)	77.2586	12.2169	46.8000	109.2000
SL (cm)	5.7828	0.7977	4.1166	8.9120
SW (cm)	0.4926	0.0618	0.3300	0.7560
SN/P	355.6313	86.3595	125.0000	538.6667
SN/S	20.7372	4.3241	11.8833	36.6333
SPY	20.0583	5.9747	6.0600	37.5600

Note: PH, plant height; NB/P, the number of branches per plant; MIL, main inflorescence length; SL, silique length; SW, silique width; SN/P, silique number per plant; SN/S, the number of seed per silique; SPY, single plant yield.

Table-5. Variance of PH, NB/P, BL, SL and SW of 85 *B.napus* varieties.

Source of variance	**DH**	**F**				
		PH	NB/P	BL	SL	SW
Material	82	5.446^{**}	3.585^{**}	4.453^{**}	8.206^{**}	7.718^{**}
Error	332					

$P^{**} < 0.01$; Abbreviations are the same as those given in Table 5.

Table-6. The variance of SN/P and SN/S of 85 B.napus varieties.

Source of variance	DH	F	
		SN/P	SN/S
Material	82	11.172^{**}	8.947^{**}
Error	166		

$P^{**} < 0.01$; Abbreviations are the same as those given in Table 5.

3.2. Seed quality traits

The seed quality traits of Brassica napus genotypes showed considerable variation (Table 7). Oil content ranged from 36.4% to 50.1%, while protein content varied between 17.6% and 26.5%, indicating a negative relationship commonly reported in rapeseed, where higher oil content often corresponds to lower protein levels (Liu et al., 2022). The wide range in yellow seed content (41.4–141.3%) suggests significant variation in seed coat characteristics, which are closely associated with oil and protein composition and overall seed quality (Zhang et al., 2021). Erucic acid content varied markedly from 3.3% to 52.9%, reflecting the presence of both low- and high-erucic acid genotypes, which is critical for breeding edib oil cultivars (Cui et al., 2023). Thioside content ranged from 24.1 to 93.4 µmol/g·cake, demonstrating variability in anti-nutritional compounds, which is important for improving meal quality. Overall, these results indicate substantial genetic diversity in seed quality traits, providing opportunities for selection and breeding of high-quality rapeseed cultivars.

Table-7. Character description and statistics of seed quality traits in 85 *B. napus* varieties.

Trait	Mean	SE	Min	Max
Oil content (%)	42.787	0.343	36.393	50.078
Protein content (%)	21.656	0.235	17.559	26.536
Yellow seed	77.928	1.802	41.359	141.341
Erucic acid (%)	16.252	1.360	3.255	52.872
Thioside (μmol/g·cake)	48.370	2.136	24.092	93.428

The correlation analysis among key seed quality traits in *Brassica napus* cultivars is presented in Table X. Oil content exhibited a strong and highly significant negative correlation with protein content ($r = -0.764$, $p < 0.01$), indicating that an increase in oil content is generally associated with a decrease in protein levels. Oil content showed a weak negative correlation with thioside content ($r = -0.278$, $p < 0.05$) and no significant relationship with seed color or erucic acid content.

Protein content was positively correlated with erucic acid ($r = 0.385$, $p < 0.01$) and thioside content ($r = 0.297$, $p < 0.01$), suggesting that selecting for higher protein may be accompanied by increases in these traits. Yellow-seeded cultivars exhibited significantly lower erucic acid ($r = -0.375$, $p < 0.01$) and thioside contents ($r = -0.441$, $p < 0.01$), highlighting their potential for producing improved oil quality. Notably, erucic acid and thioside content showed a very strong positive correlation ($r = 0.858$, $p < 0.01$), indicating that reduction in one trait is likely to reduce the other. This result is similar whit (Rostazada et al., 2023; Table 8).

Table-8. The correlation analysis of seed quality traits in *B. napus* varieties.

Trait	Oil content	Protein content	Yellow Seed	Erucic acid
Protein content	-0.764**			
Yellow Seed	0.067	0.077		
Erucic acid	-0.194	0.385**	-0.375**	
Thioside	-0.278*	0.297**	-0.441**	0.858**

3.3 Phylogenetic and its impact on agronomical traits and seed quality

This inquarment employed phylogenetic and pedigree analyses using single nucleotide polymorphism (SNP) markers and internal transcribed spacer (ITS) regions to assess the genetic structure of 85 diverse *Brassica napus* L. (rapeseed) cultivars originating from various regions of China. One of the principal objectives was to determine the genetic relationships and regional clustering patterns, which are crucial for understanding cultivar development and directing future breeding programs. According to Aranzana et al. (2003) and Parolin et al. (2002), a foundational aspect of molecular marker-based cultivar identification is the selection of highly polymorphic loci that can accurately differentiate between genotypes. In this study, such markers provided a high-resolution view of the genetic makeup of rapeseed cultivars and revealed significant genetic variation. The hierarchical clustering of the 85 cultivars based on SNP data resulted in twelve distinct clusters.

These clusters were defined based on kinship coefficients, which indicated varying degrees of genetic relatedness among cultivars. Each cluster reflected both the geographical origin and the breeding background of the cultivars. For instance, Cluster 1 grouped three cultivars 18z409 (Dwarf Butter Vegetable) from Sichuan, 18z376 (Shi Lifeng), and 18z399 (Fenfei No. 1), both from Jiangsu. Their grouping suggests a shared genetic lineage possibly arising from similar breeding practices or germplasm exchange between the regions. Cluster 2 included two cultivars: 18z366 (Huihao Oil12) from Anhui and 18z356 (Zhuoyou 058) from Guizhou. Despite being from different provinces, these cultivars shared sufficient genetic similarities to be clustered together, hinting at either a common ancestor or parallel selection strategies for certain agronomic traits. Cluster 3 was notably larger, grouping 14 cultivars from provinces including Shanxi, Shaanxi, Sichuan, and Jiangsu, such as 18z384 (Qin You 66), 18z400 (Qinrong 2), and 18z428. These cultivars showed a strong regional association, suggesting ecotypic adaptation and historical selection pressures that favored particular genetic backgrounds. Cluster 4 incorporated cultivars like 18z418 (Yiyou25), 18z373 (Deyou No.8), and 18z435 (Qin Yan 211) from provinces such as Sichuan, Hubei, Anhui, and Shanxi. Cluster 5 included 16 cultivars spanning Shanghai, Hunan, Zhejiang, and Jiangsu, highlighting the extensive genetic exchange between eastern and central China. Notably, Cluster 6 contained cultivars from Sichuan and Shanxi, such as 18z430 (Rong You No.13 Long Pod King) and 18z379 (Detian 118), again reflecting both geographical proximity and breeding linkages. Clusters 7 through 12 followed similar patterns, where cultivars were grouped based on shared genetic characteristics and geographical origins.

For example, Cluster 7 included 18z397 (Xindu Oil 800), 18z420 (Chengyou 11), and others from Sichuan and Hubei, while Cluster 8 grouped six cultivars like 18z360 (Defang Oil No. 2) and 18z375 (Medium Oil 19) from Sichuan. Interestingly, Cluster 9 grouped three cultivars 18z399 (Fenfei No. 1), 18z388 (CNPC 828) from Henan, and 18z390 (Qinyou No. 2) from Shanxi suggesting potential germplasm sharing across regions. The remaining clusters, including Clusters 10, 11, and 12, consisted of both Chinese and American varieties, indicating the inclusion of foreign germplasm into local breeding programs (Fig. 1).

This pattern of phylogenetic clustering supports previous findings that emphasize the significance of regional breeding history and environmental factors in shaping genetic diversity (Rostazada et al., 2020; Wu et al., 2019; Zhou et al., 2017). The observed grouping is also consistent with the genome-wide shattering gene diversity documented by Afridi et al. (2022), where considerable genetic divergence in *B. napus* and *B. juncea* was attributed to conserved gene motifs such as SHP1, FUL, and IND. These molecular elements likely played a role in defining the clusters in our study. Furthermore, Handa (2007) demonstrated that Japanese rapeseed cultivars exhibited similar clustering patterns due to interspecific hybridization between *B. napus* and *B. rapa*, reinforcing the idea that cross species gene flow and selective breeding contribute to genetic structure. The results of agronomic trait evaluations including oil content, protein content, silique number, seeds per silique, and 1000-seed weight further supported the clustering findings. Notably, Cluster 8 exhibited the highest mean oil content, whereas Cluster 1 had the lowest. Conversely, Cluster 1 had the highest protein content, while Clusters 4, 5, 8, and 10 showed significantly lower protein levels (Fig. 2)

This inverse relationship between oil and protein content suggests a biochemical trade-off, likely rooted in metabolic partitioning within the seed. Such trade-offs have been observed in other crops like soybean and indica rice, where genetic and cytoplasmic factors regulate seed composition (Tao et al., 2011; Singh, 1972). Yield-related traits varied significantly among clusters. For example, cultivars in Cluster 3 and 5 displayed a relatively high number of siliques and seeds per silique, indicating their potential utility in yield improvement programs. These observations align with findings by Yang et al. (2012), who identified these traits as major contributors to yield in rapeseed. Furthermore, the variation in seed size (1000-seed weight) among the clusters points to differing breeding goals, with some programs favoring oil yield while others emphasize seed size or adaptability.

The efficacy of SNP markers in detecting heterozygosity and assessing genetic relationships was clearly demonstrated in this research. SNPs provided a powerful tool for high-resolution mapping, genome-wide association studies (GWAS), and trait dissection, as also discussed by Bus et al. (2014). Their application in this study allowed for precise delineation of cultivar groups and identification of potential parental lines for future crosses. In addition, genotype-by-environment interactions were evident from the observed differences in phenotypic expression among cultivars with similar genetic backgrounds. Some cultivars consistently demonstrated strong trait performance across clusters, suggesting possible roles of epigenetic regulation and gene-environment interactions in trait manifestation. Afridi et al. (2022) noted similar findings in their study of mature siliques in *B. juncea*, where higher gene expression levels of shattering-related genes were linked to environmental influence, implying the role of regulatory divergence. In summary, this integrated analysis of genetic diversity and agronomic traits among 85 *B. napus* cultivars has provided significant insights into the current structure of rapeseed germplasm in China. The identified clusters represent genetically distinct groups shaped by region, breeding strategy, and environmental adaptation. The results underscore the importance of maintaining broad genetic diversity and utilizing SNP markers in modern rapeseed breeding. Specifically, high-oil cultivars in Cluster 8 and high-protein cultivars in Cluster 1 offer valuable genetic material for targeted improvement. These findings will aid in the development of improved varieties with balanced seed quality, high yield potential, and enhanced adaptability.

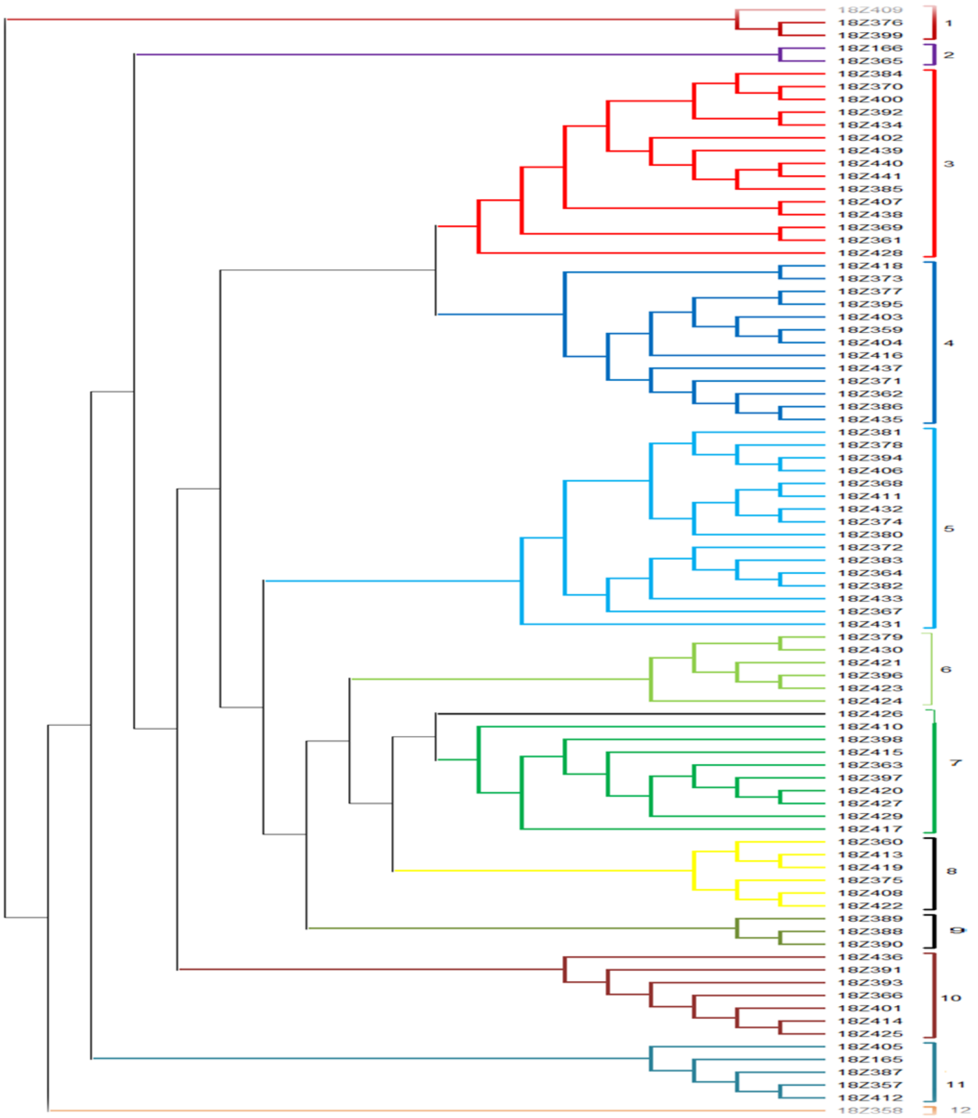

Fig-1: Phylogenetic rare breed grouping in the 85-rapeseed cultivars. Neighbor-joining dendrogram founded on 5058 SNPs obtained from genotyping by sequencing on 85 *Brassica napus* L. genotypes imagined in Generous (V.8.05). Separate collections have remained totaled and color-coded for ease of observing. Each genotype 12 cluster maintainer or restorer in the *cultivar* pollination. (Rostazada et al., 2023).

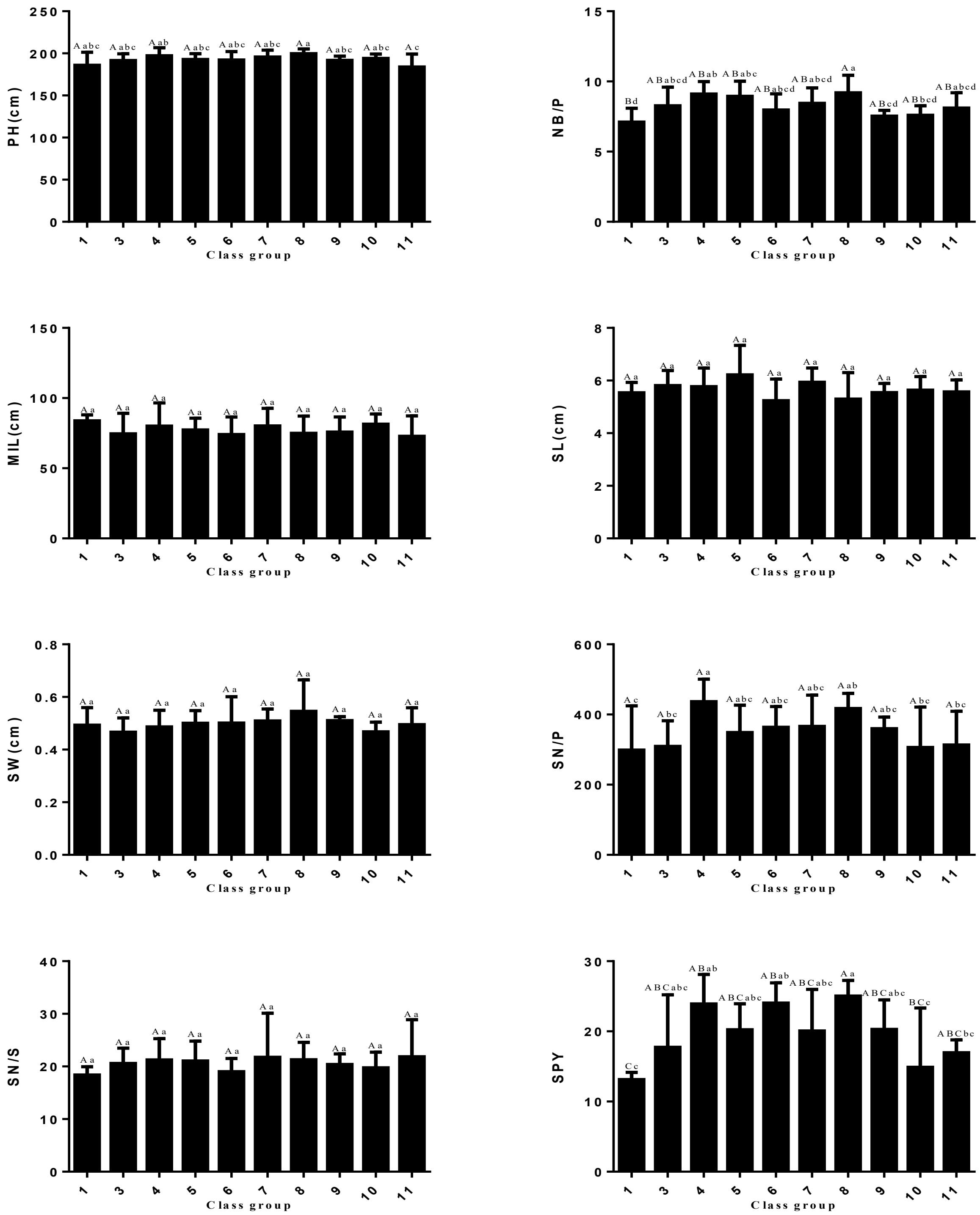

Fig-2: The mean of each agronomic traits in groups. Under the same trait, between different classes were marked with different capital letters indicating that the difference reached a significant level of 1%, and with different.

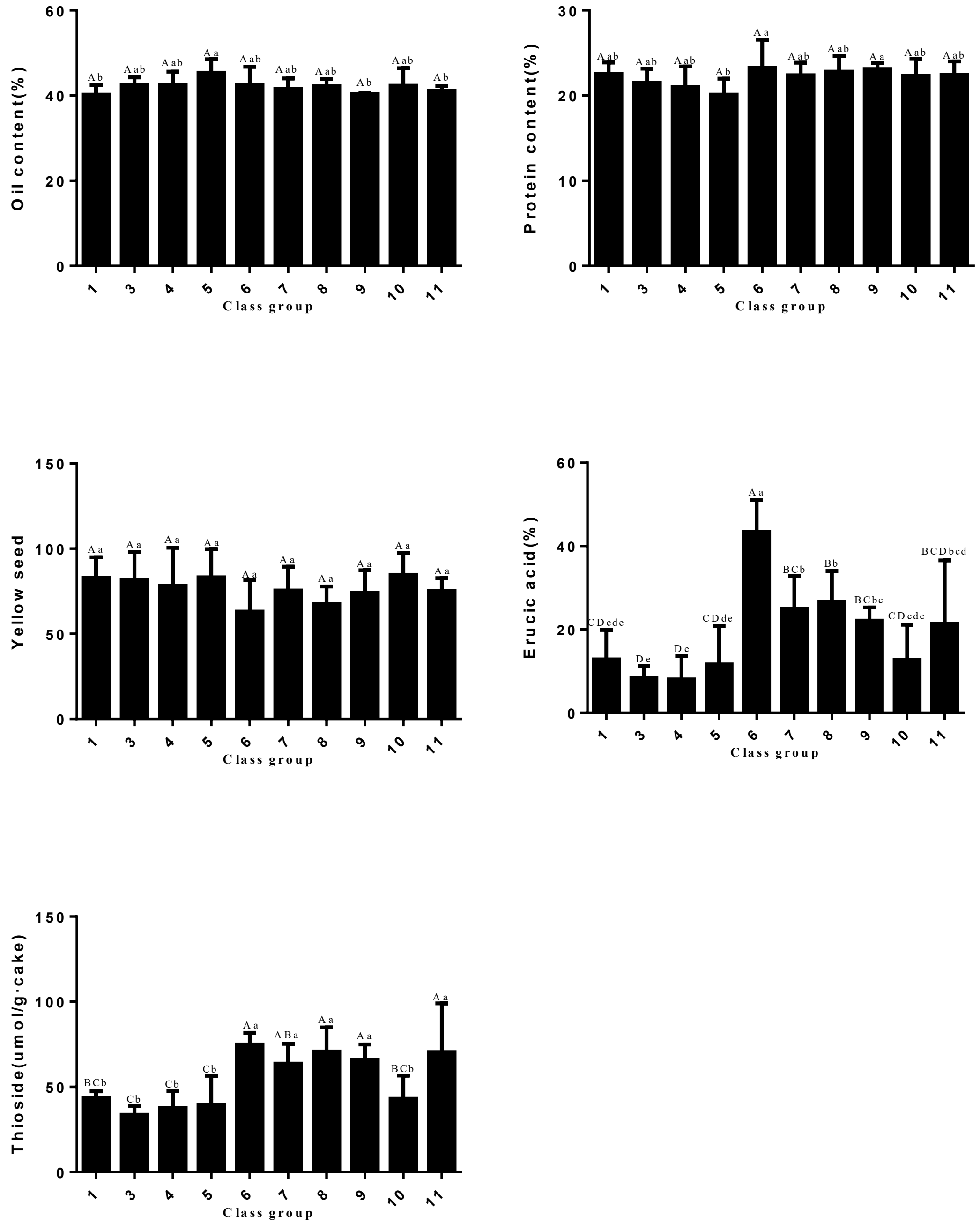

Fig-3: The mean of each seed quality traits in groups. Under the same trait, between different classes were marked with different capital letters indicating that the difference reached the significant level of 1%, and with different lowercase letters indicating.

4. Conclusion

This study provides a comprehensive integrative assessment of genetic diversity, phylogenetic relationships, agronomic performance, and seed quality traits among 85 *Brassica napus* L. cultivars using high-resolution SNP markers and ITS region sequences. The combined molecular and phenotypic analyses revealed a high degree of genetic polymorphism and resolved the germplasm into twelve distinct genetic clusters, reflecting substantial intra-specific diversity shaped by geographical origin, breeding history, and historical germplasm exchange. The clear genetic structuring observed across clusters confirms the effectiveness of SNP- and ITS-based phylogenomic approaches for dissecting population structure and cultivar relationships in rapeseed. Significant variation was detected among cultivars and clusters for key agronomic traits, including plant height, silique number per plant, and seed number per silique, single plant yield, and 1000-seed weight, underscoring the availability of exploitable genetic variability for yield improvement. Likewise, seed quality traits such as oil content, protein content, yellow seed trait, erucic acid.

The consistently observed inverse relationship between oil and protein content across genetic clusters highlights. Cluster-based comparisons further identified valuable germplasm groups, with Cluster 8 showing superior oil content and Clusters 3 and 5 displaying favorable yield-related traits, while Cluster 1 was characterized by high protein content. These results emphasize the potential of cluster-informed selection to guide parental choice, trait introgression, and the design of crossing schemes for targeted breeding objectives. The strong association between molecular clustering and phenotypic performance demonstrates the practical utility of integrating phylogenomic data with field-based evaluations.

Overall, this research establishes a robust genomic and phenotypic framework for rapeseed improvement and highlights the value of high-density SNP genotyping, including 60K SNP chip–based approaches, in modern breeding programs. The findings support the strategic use of molecular markers for marker-assisted selection, population improvement, and future genome-wide association studies (GWAS). By identifying genetically diverse and agronomically superior germplasm, this study contributes to the development of high-yielding, high-quality, and environmentally adaptable *Brassica napus* cultivars, thereby supporting sustainable oilseed production. Despite these strengths, the study was limited by the evaluation of phenotypic traits under a single or limited set of environmental conditions, which may restrict the inference of genotype × environment interactions and the stability of trait expression across environments.

5. Acknowledgment

The authors sincerely appreciate all forms of support and cooperation received during the course of this research

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