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GENETIC DIVERSITY OF WHITE CABBAGE (*Brassica oleracea* var. *capitata* subvar. *alba*) INBREED LINES USING SRAP MARKERS

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Abstract: Genetic diversity assessment is crucial for effective breeding programs and the conservation of plant genetic resources. This study aimed to characterize the genetic diversity of 24 cabbage (*Brassica oleracea* var. *capitata* subvar. *alba*) inbred lines using Sequence-Related Amplified Polymorphism (SRAP) markers. A total of 45 SRAP primer combinations were employed, resulting in the amplification of 258 bands, of which 194 (75.2%) were polymorphic. The polymorphism information content (PIC) values ranged from 0.03 to 0.42, with a mean value of 0.20, indicating relatively low genetic diversity among the studied inbred lines. The major allele frequency (MAF) values varied between 0.54 and 0.99, with an average of 0.83, further confirming the limited genetic diversity. The effective allele number (NE), gene diversity (H), and Shannon information index (I) averaged 1.40, 0.23, and 0.35, respectively. Principal component analysis (PCA) revealed that the first seven principal component axes accounted for 90.59% of the total variance among the cabbage lines, demonstrating that the genetic diversity could be largely explained along a few dimensions. STRUCTURE analysis identified three major genetic clusters, with Cluster 3 exhibiting the highest proportion of genetic composition (40.3%) and the highest level of genetic differentiation (mean Fst = 0.4080). The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering analysis, based on the Dice similarity method, produced a dendrogram depicting the genetic relationships among the inbred lines. The Mantel test value r for the UPGMA clustering was 0.78, indicating a good fit between the dendrogram and the original similarity matrix. The study highlights the utility of SRAP markers in assessing genetic diversity and relationships among cabbage inbred lines, providing valuable information for breeding programs and genetic resource management. The identification of genetically distinct clusters and the quantification of genetic variation within and among these clusters can guide future breeding efforts and facilitate the development of improved cabbage varieties with desirable traits.

Keywords: *Brassica oleracea*, Genetic diversity, Inbred lines, SRAP markers, STRUCTURE analysis, UPGMA clustering

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1. Introduction

White cabbage (*Brassica oleracea* var. *capitata*) is an important vegetable species cultivated and consumed worldwide. This plant, which belongs to the Brassicaceae family, is of great importance for health and nutrition due to its high nutritional value, containing various antioxidants, vitamins (especially vitamin C and vitamin K), minerals and fiber (Fahey et al., 2001). In addition, studies have suggested that white cabbage may have protective effects against several types of cancer (Verhoeven et al., 1996). Cabbages are categorized into white cabbage (*B. oleracea* var. *capitata* subvar. *alba*), red cabbage (*B. oleracea* var. *capitata* subvar. *rubra*), and savoy cabbage (*B. oleracea* var. *capitada* subvar. *sabauda*) (Nieuwhof, 1969). White cabbage constitutes the most consumed group of cabbage, utilized either as a table vegetable or in the pickling industry. In Türkiye, the total production of white cabbage is 597910 tons. The provinces with the highest production are listed as Samsun (143241 tons), Niğde (135495 tons), Bursa (34830 tons), Antalya (25116 tons), Mersin (22534 tons), and Afyonkarahisar (22465 tons) (Anonymous, 2021).

Plant breeding involves genetic studies aimed at developing more productive and resilient plant varieties. While traditional breeding methods rely on phenotypic selection and hybridization techniques, the use of molecular markers has increased significantly in modern breeding studies. Molecular markers are used for various purposes, including detection of genetic variation, gene mapping, pedigree analysis, and improving the efficiency of breeding programs (Collard and Mackill, 2008).

Molecular marker techniques play a major role in revealing genetic diversity and relationships by detecting differences at the DNA level. Commonly used molecular marker techniques include RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeats), RAPD (Random Amplified Polymorphic DNA), and SRAP

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(Sequence-Related Amplified Polymorphism). Each marker has unique advantages and limitations. For example, SSR markers exhibit a high degree of polymorphism, while AFLP markers provide high reproducibility and comprehensive genetic information (Powell et al., 1996). SRAP markers are often preferred in plant genetics and breeding studies. SRAP typically targets exon regions to obtain polymorphic bands, thereby providing high accuracy in determining genotypic differences (Li and Quiros, 2001). The simplicity, speed, low cost and high reproducibility of SRAP markers make this technique ideal for genetic diversity analysis. A study by Ferriol et al. (2003) reported that the SRAP marker technique provided more information than AFLP, while another study by Liu et al. (2008) indicated that the SRAP marker system produced relatively more informative bands and was more efficient compared to ISSR and RAPD marker systems. SRAP technology, which was initially detailed by Li and Quiros (2001) in Brassica, is a marker system that preferentially amplifies open reading frames (ORFs). This technique utilizes two types of primers: forward primers, which are 17 base pairs long and have 14 nucleotides rich in C and G with 3 selective bases at the 3' end, and reverse primers, which are 18 base pairs long with 15 nucleotides rich in A and T (Ferriol et al., 2003).

This study aims to determine the molecular diversity of white cabbage breeding lines using SRAP markers. Detection of genetic diversity is essential for plant breeding because plant populations with a broad genetic base are more resilient to environmental changes and diseases. In this context, our study aims to provide important data for the conservation of white cabbage genetic resources, to guide breeding efforts and to develop new lines. Our study will provide a better understanding of the genetic diversity of cabbage and integrate this knowledge into practical breeding applications. As a result, we aim to make a significant contribution to the conservation of white cabbage genetic resources and the support of sustainable agricultural practices.

2. Materials and Methods

A total of 24 white cabbage inbred lines, derived from the cabbage genetic pool at the Black Sea Agricultural Research Institute, were used for this study (Table 1).

2.1. SRAP Analysis of Inbred Lines

The mini-prep method of Haymes (1996) was used for DNA isolation and DNA extraction from young leaves of cabbage seedlings. Cabbage inbred lines were characterized using 45 SRAP primer combinations (Table 2). Polymerase chain reaction (PCR) experiments were carried out using a total reaction mixture volume of 15 µL. This mixture consisted of 7.5 µL PCR master mix (Dream Taq Green Master Mix, ThermoScientific), 1 µL forward (ME) and 1 µL reverse (EM) primers (10 pmol each), 3 µL genomic DNA (15-20 ng), and 2.5 µL deionized water. The PCR protocol was performed as follows: An initial predenaturation step was performed at 94 °C for 2 minutes. This was followed by 5 cycles of denaturation at 94 °C for 1 minute, annealing at 35 °C for 1 minute, and extension at 72 °C for 1 minute. This was followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 50 °C for 1 minute, and extension at 72 °C for 1 minute. Finally, a post extension step at 72 °C for 5 minutes was performed (Yildiz et al., 2011).

2.2. Gel Electrophoresis

The PCR products obtained from the SRAP evaluation were separated by gel electrophoresis. A 3% agarose gel (Fisher BioReagents) containing 1X TAE (Tris-Acetic Acid-EDTA) buffer was prepared, and the samples were loaded onto the gel. Electrophoresis was performed on a SCIE-PLAS system (Hu20) at 100 V and 300 mA for 4 hours. After electrophoretic separation, the gel was stained with ethidium bromide (10 mg/ml) for 20 minutes and then washed with distilled water. The stained gel was then visualized under a UV transilluminator (Syngene-Ingenius). The resulting gel image was analyzed, with distinct bands scored as 1 (present) and 0 (absent). This scoring system was used to generate a binary data matrix for further data analysis.

Table 1. White cabbage inbred lines used in the study

Inbred lines	Consumption purposes	Precocity
40	for stuffing	Mid-season
501	for pickling	Mid-season
518	for pickling	Mid-season
22-1	for pickling	Mid-season
A119	for stuffing	Early-maturing
A126	for stuffing	Early-maturing
A145	for pickling	Early-maturing
A168	for pickling	Early-maturing
A322	for stuffing	Early-maturing
A387	for stuffing	Early-maturing
A62	for pickling	Mid-season
BLMY-4	for stuffing	Early-maturing
BY27-2	for stuffing	Late-maturing
EXT	for pickling	Early-maturing
FG	for stuffing	Early-maturing
P43-1	for stuffing	Mid-season
P59-1	for stuffing	Early-maturing
P91	for pickling	Mid-season
P95	for stuffing	Early-maturing
TAR	for pickling	Early-maturing
W37	for pickling	Mid-season
WEİ	for pickling	Mid-season
YBB-35	for stuffing	Late-maturing
ZL-3	for pickling	Late-maturing

Table 2. Genetic diversity of cabbage inbred lines revealed by SRAP markers

Primer	TBN	PBN	PIC	MAF	NE	H	I
Me01Em02	7	5	0.26	0.78	1.36	0.21	0.32
Me01Em03	4	2	0.08	0.95	1.15	0.11	0.19
Me01Em04	3	2	0.14	0.90	1.35	0.20	0.30
Me01Em05	3	2	0.22	0.86	1.49	0.27	0.40
Me01Em08	5	5	0.28	0.75	1.87	0.46	0.66
Me02Em09	3	3	0.31	0.65	1.80	0.44	0.64
Me02Em10	2	2	0.23	0.81	1.36	0.26	0.43
Me02Em11	4	2	0.20	0.82	1.37	0.21	0.30
Me02Em12	4	3	0.21	0.88	1.08	0.08	0.15
Me02Em13	6	3	0.16	0.85	1.29	0.17	0.26
Me03Em02	4	4	0.19	0.84	1.36	0.21	0.33
Me03Em03	2	2	0.42	0.54	1.82	0.45	0.64
Me03Em04	3	2	0.09	0.94	1.26	0.16	0.26
Me03Em05	4	3	0.19	0.86	1.52	0.29	0.42
Me03EM10	10	10	0.25	0.80	1.41	0.26	0.42
Me03Em13	4	4	0.21	0.82	1.54	0.31	0.46
Me04Em10	5	4	0.19	0.88	1.41	0.26	0.40
Me05Em02	5	5	0.27	0.78	1.74	0.41	0.59
Me05Em03	9	5	0.12	0.90	1.21	0.13	0.21
Me05Em04	12	8	0.14	0.88	1.28	0.18	0.29
Me05Em05	7	6	0.24	0.77	1.49	0.30	0.46
Me05Em08	5	3	0.24	0.77	1.30	0.19	0.29
Me06Em09	5	4	0.06	0.97	1.15	0.12	0.21
Me06Em10	6	3	0.11	0.90	1.36	0.20	0.29
Me06Em11	7	4	0.15	0.89	1.39	0.21	0.31
Me06Em12	8	7	0.25	0.79	1.48	0.28	0.43
Me14Em02	9	7	0.19	0.84	1.44	0.26	0.39
Me14Em03	3	1	0.03	0.99	1.01	0.01	0.03
Me14Em04	6	6	0.24	0.78	1.61	0.36	0.54
Me14Em05	4	2	0.15	0.86	1.26	0.15	0.23
Me14Em08	10	10	0.35	0.67	1.62	0.36	0.53
Me16Em10	9	8	0.26	0.76	1.47	0.27	0.41
Me17Em05	5	5	0.24	0.82	1.46	0.26	0.41
Me18Em09	7	5	0.16	0.88	1.32	0.18	0.28
Me18Em10	5	4	0.28	0.74	1.46	0.26	0.38
Me18Em11	2	1	0.19	0.77	1.32	0.20	0.29
Me19Em02	11	8	0.23	0.81	1.23	0.17	0.28
Me19Em03	11	10	0.32	0.73	1.44	0.27	0.41
Me19Em04	5	4	0.20	0.84	1.39	0.25	0.38
Me19Em05	7	5	0.22	0.80	1.33	0.21	0.32
Me21Em09	5	4	0.20	0.78	1.49	0.28	0.40
Me21Em10	6	4	0.20	0.83	1.38	0.23	0.35
Me21Em11	6	2	0.07	0.94	1.08	0.06	0.10
Me21Em12	6	3	0.14	0.88	1.39	0.22	0.31
Me21Em13	4	2	0.11	0.91	1.32	0.18	0.27
Total	258	194	-	-	-	-	-
Average	5.73	4.31	0.20	0.83	1.40	0.23	0.35

TBN= total band number, PBN= polymorphic band number, MAF= major allele frequency, NE= effective allele number, H= gene diversity (Nei, 1973), I= Shannon information index

2.3. Data Analysis

The polymorphism rate (Pr) was calculated for each primer. PopGene v.1.32 software (Yeh et al., 2000) was used to calculate genetic diversity parameters, including the effective number of alleles (Ne) (Kimura and Crow, 1964), gene diversity (H) (Nei, 1973), and Shannon's information index (I) (Lewontin, 1972), for the inbred cabbage lines under study. Polymorphism information content (PIC) and major allele frequency (Maf) values were obtained using PowerMarker v.3.25 software (Liu and Muse, 2005). Cluster analysis was performed using the unweighted pair-group method with arithmetic mean

(UPGMA) (Sneath, 1979), and a correlation matrix was generated using the Dice module of the NTSYSpc v.2.02 package (Rohlf, 2000). This analysis elucidated the genetic relationships among the cabbage lines. Additionally, multivariate analysis was performed using Principal Component Analysis (PCA) based on the correlation matrix in NTSYSpc, and a PCA scatterplot was generated using Past 3 statistical software. In further, a STRUCTURE analysis was performed on the cabbage lines using the Bayesian clustering algorithm implemented in the STRUCTURE 2.3.4 software tool, as introduced by Pritchard et al. (2000). The analysis was performed with a burn-in period of 10.000 iterations and 100.000 Markov Chain Monte Carlo (MCMC) repetitions after burn-in, with five iterations.

3. Results

3.1. Characterization of Cabbage Lines by SRAP Markers

A total of 258 bands were obtained from 45 combinations of SRAP primers, of which 194 (75.2%) showed a polymorphism among the cabbage breeding lines (Table 2). Based on primers, the highest total number of bands was observed in Me05Em04 primer at 12 and the lowest number of bands was observed in Me02Em10, Me03Em03 and Me18Em11 primers at 2. Polymorphism information content (PIC) values ranged from 0.03 to 0.42. The mean PIC value was calculated as 0.20. The highest PIC value was determined in the Me03Em03 primer with 0.42 and the lowest in the Me14Em03 primer with 0.03. The major allele frequency (MAF) values changed between 0.54-0.99 (average 0.83) indicating relatively low genetic diversity among cabbage lines. The effective allele number (NE) ranged from 1.01 to 1.87 with an average NE value of 1.40. Gene diversity (H) ranged from 0.01 to 0.46 and Shannon information index (I) ranged from 0.03 to 0.66. The mean values of H and I were calculated as 0.23 and 0.35, respectively. These results indicate that the genetic diversity of cabbage breeding lines was successfully characterized using SRAP markers.

The results of the principal component analysis (PCA) showed that the data from the SRAP markers could be largely represented by the first seven principal component axes (Table 3). The eigenvalues associated with these axes were 19.76, 0.47, 0.38, 0.38, 0.32, 0.29, and 0.27, respectively. These values indicate that the first seven principal component axes account for 90.59% of the total variance among the cabbage lines, demonstrating that the genetic diversity within these lines can be largely explained along a few dimensions.

Two-dimensional scatter plot in Figure 1 represents the genetic variation among cabbage inbred lines based on PCA. The plot reveals distinct clusters of inbred lines. The lines such as BLMY-4, FG, and WEI cluster closely together, indicating high genetic similarity. In contrast, lines such as 501, W37, and BY27-2 are positioned far apart from the main cluster, suggesting significant genetic differences.

Table 3. Principal components revealed by SRAP primers

PC axises	Eigen values	Variation (%)	Cumulative variation (%)
1	19.76	82.35	82.35
2	0.47	1.98	84.32
3	0.38	1.59	85.91
4	0.32	1.32	87.23
5	0.29	1.20	88.43
6	0.27	1.13	89.57
7	0.25	1.03	90.59

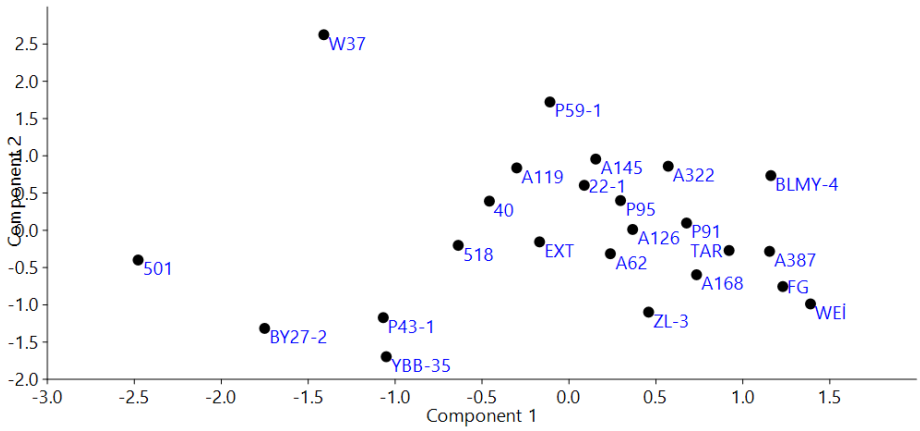

Figure 1. Scatter plot of inbred lines by PCA.

The STRUCTURE analysis of the SRAP marker data revealed the genetic structure of the inbred lines, identifying three major clusters based on the K-means method (Figure 2). The Q matrix represents the individual membership coefficients, indicating the proportion of an individual's genome that belongs to each of the inferred genetic clusters (Figure 3). Further assessment of the genetic diversity among the cabbage inbred lines using STRUCTURE, as summarized in Table 4, revealed distinct patterns among the three identified clusters. Cluster 1 exhibited an average distance (AD) of 0.273 between individuals within the same cluster, indicating a moderate level of genetic variation. The proportion of the genetic composition (PGC) in this cluster was 0.311, suggesting that approximately 31.1% of the genetic material of the inbred lines was represented in this cluster. The mean value of Fst for Cluster 1 was 0.0122 indicating minimal genetic differentiation from other clusters. Cluster 2 demonstrated a slightly lower average distance (AD) of 0.264 compared to Cluster 1, reflecting slightly reduced genetic variation within the cluster. The proportion of the genetic composition (PGC) in Cluster 2 was 0.286, showing that 28.6% of the genetic material was encompassed in this cluster. The mean Fst value for Cluster 2 was 0.0774, indicating a moderate level of genetic differentiation. Cluster 3 showed the lowest average distance (AD) of 0.178, indicating the least genetic variation among individuals within this cluster. However, it had the highest proportion of genetic composition (PGC) at 0.403, signifying that 40.3% of the genetic material of the inbred lines was present in this cluster. Notably, Cluster 3 exhibited a mean Fst value of 0.4080, which is substantially higher than those of the other clusters, indicating a significant level of genetic differentiation.

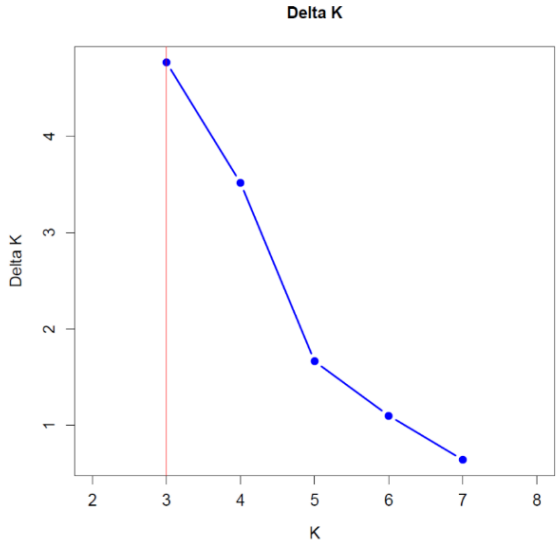

Figure 2. Optimum cluster number obtained by STRUCTURE analysis.

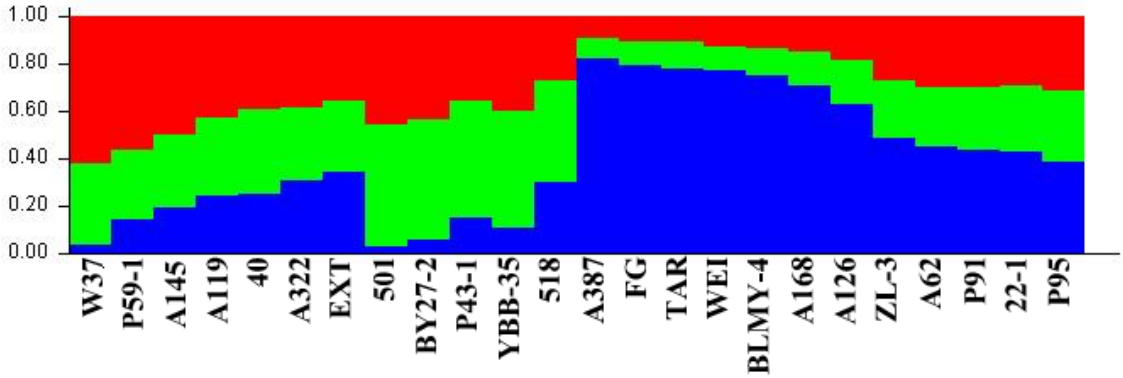

Figure 3. The assignment probabilities of each individual obtained by STRUCTURE.

Table 4. Genetic diversity of cabbage inbred lines revealed by SRAP markers

Clusters	AD	PGC	Mean values of Fst
1	0.273	0.311	0.0122
2	0.264	0.286	0.0774
3	0.178	0.403	0.4080

AD= average distance between individuals in same cluster, PGC= proportion of the genetic composition of inbred lines in each cluster.

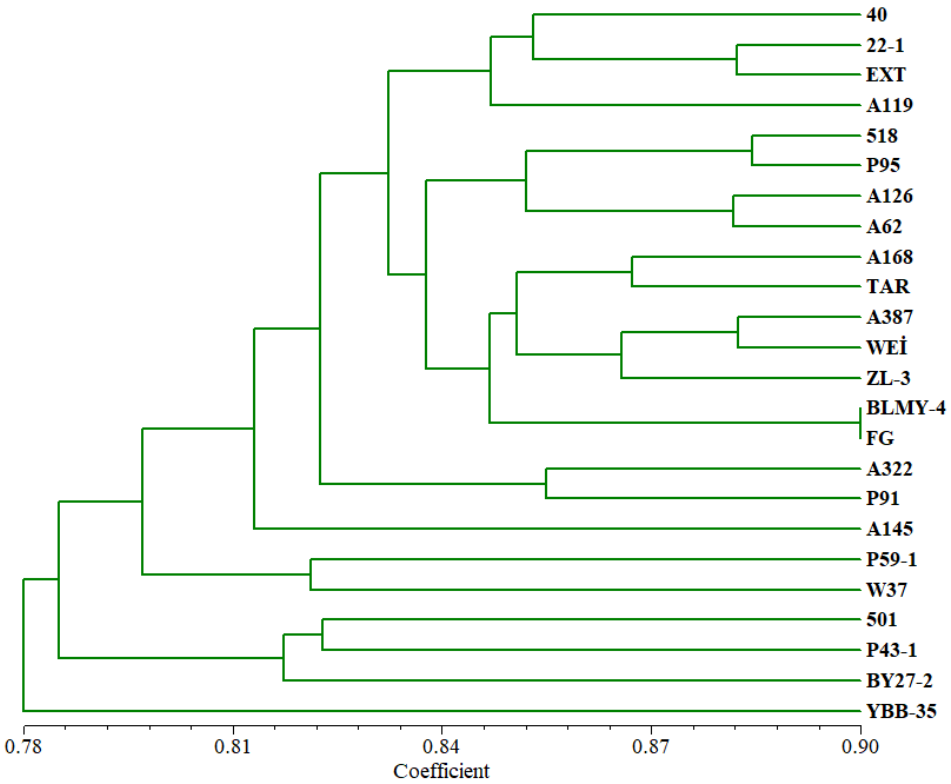

Figure 4. UPGMA cluster constructed from SRAP marker data.

The Mantel test value r for the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering analysis using the Dice similarity method was calculated to be 0.78. The dendrogram shown in Figure 4 represents the genetic relationships among 24 cabbage inbred lines based on SRAP marker data. The inbred lines BLMY-4 and FG are the most genetically similar, as indicated by their tight clustering towards the top right of the dendrogram. Similarly, lines 22-1 and EXT, A126 and A62, and W37 and 501 also show high genetic similarity due to their close proximity in the dendrogram. On the other hand, the inbred lines 501 and P91 are the most genetically distant from each other, as indicated by their positions on opposite ends of the dendrogram branches. BY27-2 and YBB-35 are also relatively genetically distant from the other lines, forming their own separate cluster at a lower similarity coefficient. Several clusters of inbred lines exhibit moderate genetic similarity. For example, the cluster including 40, 22-1, and EXT, as well as the cluster including A126, A62, A168, TAR, and A387, show intermediate levels of genetic relatedness. The cluster including A322, P91, and A145 also indicates moderate genetic similarity among these lines.

4. Discussion

Hybrid vegetable varieties are preferred by producers for their high yield, disease resistance, and uniform product quality. White head cabbage can be cultivated in almost every region of Turkey, with provinces such as Samsun, Amasya, Tokat, Niğde, and Nevşehir standing out in production. While the proportion of local hybrid varieties was significantly high in the hybrid varieties of the main vegetable crops grown in Türkiye, such as tomato, pepper and melon, this proportion remained at a very low level in cabbage. To increase this rate, some agricultural research institutes and universities in Turkey are conducting breeding studies on cabbage. The Black Sea Agricultural Research Institute is prominent in developing hybrid varieties of white head cabbage in Turkey. The findings of our study will greatly contribute to the breeding efforts carried out at this institution. The effectiveness of breeding programs planned for developing hybrid varieties with high adaptability to global climate change depends on selecting the most suitable parents (Schnable and Springer, 2013; Kadam and Lorenz, 2018). Choosing genetically different parents in hybrid variety development contributes to a high heterosis effect (Labroo et al., 2021). Therefore, the genetic characterization of breeding lines is of great importance. In our study, the genetic relatedness among 24 cabbage inbred lines in the genetic resource of the Black Sea Agricultural Research Institute was screened using SRAP markers. Our findings indicated that SRAP markers were effective in differentiating cabbage inbred lines. The genetic similarity among the inbred lines ranged from 78 to 90, indicating relatively low variability. In a genetic diversity study conducted on Brassicas (*Brassica napus*), it was reported that 20 SRAP primer combinations successfully differentiated 60 accessions (Ahmad et al., 2014). Researchers reported that the genetic similarity among accessions ranged from 40 to 100. In a genetic diversity study conducted with SRAP markers on another Brassica species (*Brassica juncea* L.), a total of 286 bands were obtained, with an

average number of bands per primer combination being 9.23 (Li et al., 2018). The similarity among the 44 accessions was determined to range from 0.61 to 0.89. In a diversity study comparing SSR and SRAP markers among different Brassica species, it was reported that 36 SSR primers produced 133 polymorphic bands, while 43 SRAP primer combinations produced 268 polymorphic bands. SRAP primers were reported to be more successful than SSR primers in determining variation among Brassica species (Zhang et al., 2017). Another study investigating the effectiveness of SRAP and SSR markers for the diversity of elite *Brassica napus* breeding lines found that 8 SSR primers explained 96.08% of the variation among the breeding lines, while 12 SRAP primer combinations explained 98.72%, indicating that SRAP primers were nearly as successful as SSR primers (Zang et al., 2019). In the PCA applied to the SRAP marker data for the characterization of cabbage breeding lines, the first component explained 82.35% of the variation among the lines. This finding is consistent with the results obtained from the diversity study of Brassica napus varieties with SRAP markers (Framarzpour et al., 2021). STRUCTURE analysis showed that cabbage breeding lines were genetically divided into three groups. The findings revealed significant genetic variability among the breeding lines we have. Different cabbage species have been successfully differentiated with SRAP (Wu et al., 2009) and SSR (Pipan et al., 2024; Malik et al., 2024) markers in Brassica germplasms. The SRAP markers we used, were also successful in differentiating elite inbred cabbage lines.

5. Conclusion

The study of the genetic diversity of inbred cabbage lines using SRAP markers has provided comprehensive information on their genetic composition. Key genetic diversity metrics, including PIC, MAF, NE, H, and I, were evaluated. The mean PIC value of 0.20 (range: 0.03 to 0.42) indicates moderate genetic diversity, with primers like Me03Em03 showing high discriminatory power (PIC=0.42). An average MAF of 0.83 suggests a predominance of certain alleles, reflecting lower overall genetic diversity. The mean NE, H and I values further confirm the presence of genetic variability among the inbred lines. Principal component analysis showed that genetic diversity could be largely explained along a few dimensions. The cluster analysis using STRUCTURE and UPGMA showed distinct genetic groupings, with three major clusters identified. Cluster 3 showed the highest genetic differentiation, indicating significant divergence within this group. The Mantel test value (r=0.78) from the UPGMA clustering analysis underscores the robustness of the genetic relationships observed among the cabbage lines. The dendrogram further supports these findings, showing clear genetic differences and similarities among the lines. In conclusion, SRAP markers have been found to be a valuable tool for characterizing the genetic diversity of inbred cabbage lines. The moderate level of genetic diversity observed suggests potential for selective breeding and genetic improvement programs.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	E.E.	C.T.
C	70	30
D	90	10
S	100	
DCP	30	70
DAI	50	50
L	20	80
W	80	20
CR	80	20
SR	80	20
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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This study was summarized from the second authors MSc thesis under the supervision of the first author.

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