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Anatomical and Chemical Properties Analysis of Korean *Broussonetia kazinoki* Bast Fibers and Selection of Superior Individuals

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ABSTRACT

This study aimed to analyze anatomical and chemical properties of *Broussonetia kazinoki* bast fibers collected from 43 regions in Korea to identify superior individuals for industrial applications. Anatomical properties (such as fiber length, width, and cell wall thickness) and chemical compositions (such as holocellulose and lignin contents) were measured. Principal component analysis and K-means clustering were used to classify samples into three clusters (flexibility, structural strength, and durability), with each cluster showing distinct characteristics. Superior individuals were selected based on holocellulose content and fiber length, with samples 6, 34, and 39 identified as optimal candidates. These individuals exhibited high holocellulose contents and diverse fiber lengths, making them suitable for applications in traditional Korean paper (Hanji), artificial leather, and mask filters. These results demonstrate the potential of *B. kazinoki* bast fibers as high-quality and versatile raw materials suitable for various industries.

Keywords: paper mulberry, *Broussonetia kazinoki*, bast fiber, principal component analysis, K-means clustering

1. INTRODUCTION

In recent years, there has been a growing global interest in sustainable development, leading to concentrated efforts to discover renewable materials that can replace petroleum-based resources (Li *et al*., 2021a, 2021b; Lyu *et al*., 2021). This trend has increased interest in natural fiber composites, which are widely used in various industries such as automotive, construction, biomedical, and military sectors (Saleem *et al*., 2020). Natural plant fibers offer various advantages, including low cost, low density, biodegradability, and hypoallergenic properties, making them highly suitable eco-friendly materials (Gao *et al*., 2023). Plant fibers are eco-friendly and renewable materials utilized in various industrial applications, including sound-absorbing materials and fiberboards (Kalasee *et al*., 2023; Mawardi *et al*., 2025; Zyryanov *et al*., 2024). Numerous studies have been conducted to investigate anatomical structures and mechanical properties of plant fibers to accurately understand their characteristics (Andianto *et al*., 2024; Darwis *et al*., 2023). Furthermore, efforts have been continuou-

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sly made to enhance the functionality of plant fibers and expand their industrial applications through treatments such as NaCl and silane (Cha *et al*., 2022; Maras *et al*., 2024; Seo and Kim, 2024; Setyayunita *et al*., 2022a, 2022b). Paper mulberry is one of the plant-based fibrous materials that have been utilized in Korea since ancient times.

Paper mulberry (*Broussonetia kazinoki*), a deciduous broad-leaved shrub belonging to the Moraceae family of the Urticales order (Lee, 2017), has been used historically in Korea for making Hanji, a traditional hand-made paper, using both outer and inner bast fibers (Eichhorn *et al*., 2009). Fibers of *B. kazinoki* are characterized by a high crystallinity that contributes to superior strength of paper produced from them (Khalil *et al*., 2014). Additionally, compared to other wood fibers, *B. kazinoki* fibers have higher cellulose contents but lower lignin contents, resulting in higher polymerization of cellulose that can enhance the durability and preservation of paper (Cho and Choi, 1992; Youn and Cho, 2008).

In Korea, the quality of Hanji is influenced by factors such as the width and length of bast fibers, which vary depending on growing conditions (such as climate and soil) and individual characteristics of plants (Choi *et al*., 2007). These variations in fiber length or cell wall thickness directly affect tensile strength, tear strength, and durability of paper (Zhu *et al*., 2007). Furthermore, chemical properties of bast fibers can significantly impact the yield of pure cellulose during a high-temperature alkaline treatment in Hanji manufacturing process. While alkaline chemical treatments are effective in obtaining high-purity fibers, excessive dissolution of hemicellulose or damage to fibers during this process can affect economic feasibility of paper production (Jung *et al*., 2019; Mun and Lim, 1999).

While *B. kazinoki* fibers have been used traditionally as raw materials for Hanji, they are now being utilized in diverse industrial applications such as artificial leather and mask filters. However, due to variations in bast fiber characteristics among individual plants, ensuring consistent quality during commercialization remains a challenge, even when the same species is used.

Therefore, this study aimed to analyze anatomical and chemical properties of bast fibers from various regions in Korea and to identify superior individuals suitable for industrial applications.

2. MATERIALS and METHODS

2.1. Plants materials

In this study, one-year-old stems of *Broussonetia kazinoki* were collected annually from 2015 to 2019 for experiments. Samples used in this study were transplanted and managed at the Bioenergy Crop Center of the National Institute of Crop Science (Muan-gun, Jeollanam-do, South Korea) after they were collected from 43 regions across Korea (Table 1). Transplanted samples showed successful root establishment and active growth. When collecting 1-year-old stems, individuals with good growth were selected. To ensure uniformity and to accurately assess fibrous properties, stems were harvested between November and February when the growth ended.

2.2. Anatomical analysis of bast fibers

Five selected *B. kazinoki* stems were divided into upper, middle, and lower segments and bast fibers measuring 3 cm and 1.5 cm in length were prepared from each segment for analysis. For delignification, fiber samples were soaked in Schultze's solution (100 mL of 35% HNO_3 with 6 g of $KClO_3$) at room temperature for 7 days. Afterward, they were washed with distilled water and used for measurement.

For staining, delignified bast fibers were immersed in a double-stain solution prepared by mixing 0.5% Astra blue solution (0.5 g Astra blue and 2 mL glacial acetic acid dissolved in 98 mL distilled water) and 1%

Table 1. List of samples used in anatomical and chemical analyses of bast fibers

Pop no.	Collection site	Pop no.	Collection site
1	Korea: Jeollanam-do, Sinan-gun	23	Korea: Gyeongsangbuk-do, Cheongdo-gun
2	Korea: Jeollanam-do, Gochang-gun	24	Korea: Gyeongsangnam-do, Changnyeong-gun
3	Korea: Jeollanam-do, Muan-gun	25	Korea: Jeollanam-do, Goheung-gun
4	Korea: Gyeongsangnam-do, Haman-gun	26	Korea: Unknown
5	Korea: Gyeongsangbuk-do, Cheongdo-gun	27	Korea: Unknown
6	Korea: Gyeongsangnam-do, Changnyeong-gun	28	Korea: Unknown
7	Korea: Gyeongsangnam-do, Changnyeong-gun	29	Korea: Gyeongsangbuk-do, Chungsong-gun
8	Korea: Gyeongsangnam-do, Changnyeong-gun	30	Korea: Jeollanam-do, Jangseong-gun
9	Korea: Gyeongsangnam-do, Changnyeong-gun	31	Korea: Jeollanam-do, Naju-si
10	Korea: Gyeongsangnam-do, Miryang-si	32	Korea: Jeollabuk-do, Imsil-gun
11	Korea: Gyeongsangnam-do, Haman-gun	33	Korea: Gyeongsangnam-do, Hamyang-gun
12	Korea: Gyeongsangbuk-do, Andong-si	34	Korea: Gyeongsangnam-do, Hapcheon-gun
13	Korea: Gyeongsangbuk-do, Chungsong-gun	35	Korea: Gyeongsangnam-do, Miryang-si
14	Korea: Gyeongsangbuk-do, Yeongdeok-gun	36	Korea: Gyeongsangnam-do, Hamyang-gun
15	Korea: Gyeongsangbuk-do, Yeongdeok-gun	37	Korea: Gyeongsangnam-do, Sancheong-gun
16	Korea: Unknown	38	Korea: Gyeongsangnam-do, Hadong-gun
17	Korea: Unknown	39	Korea: Jeollanam-do, Gokseong-gun
18	Korea: Chungcheongnam-do, Gongju-si	40	Korea: Gyeonggi-do, Ganghwa-island
19	Korea: Chungcheongnam-do, Gongju-si	41	Korea: Gyeongsangnam-do, Jinju-si
20	Korea: Daegu Metropoltan	42	Korea: : Jeollanam-do, Muan-gun
21	Korea: Gyeongsangbuk-do, Cheongdo-gun	43	Korea: Gyeongsangnam-do, Changnyeong-gun
22	Korea: Gyeongsangbuk-do, Yecheon-gun		

Safranin solution (1 g Safranin dissolved in 99 mL distilled water) at a 1:1 ratio for 4 minutes. Stained fibers were then observed using an optical microscope (CX23, Olympus, Tokyo, Japan). An eXcope XCAM UHD SE (Sony, Tokyo, Japan) was used to analyze their anatomical characteristics. Fiber length was measured at 40× magnification, while fiber width and lumen width were measured at 400× magnification. Measurements were taken 20 times for each segment (upper, middle, and lower) from five stems to ensure accuracy and reliability. These measurement values were used to calculate various fiber indices, including the Runkel ratio (RR), aspect ratio (AR), flexibility coefficient (FC), and rigidity coefficient (RC; Sadiku *et al*., 2016).

2.3. Climate data collection

Climate data for Muan (Station 699) from 2014 to 2018 were obtained from the Korea Meteorological Administration's data portal. Parameters collected included average temperature, average maximum temperature, average minimum temperature, and total precipitation.

2.4. Chemical analysis of bast fibers

The bark was peeled from collected *B. kazinoki* stems and the outer bark was carefully removed using a knife to isolate bast fibers. Samples were then cleaned, dried, and ground with a microfine grinder followed by sieving using a 40–80 mesh sieve. Chemical composition analysis was performed according to the standards of the Technical Association of the Pulp and Paper Industry (TAPPI). Samples were defatted using organic solvents (TAPPI T 204 cm-97) and used to determine 1% NaOH solubility (TAPPI T 212 om-02), Holocellulose content (TAPPI T 9 wd-75), Klason lignin content (TAPPI T 222 om-02), and ash content (TAPPI T 211 om-02).

2.5. Statistical analysis

Descriptive statistics, including minimum, maximum, mean, and SD, were calculated using R Studio. Multivariate analyses, including principal component analysis (PCA) and k-means clustering, were conducted to classify samples. The elbow method was used to determine the optimal number of clusters. Various R packages, including stats, factoextra, FactoMineR, and cluster, were used for statistical analysis.

3. RESULTS and DISCUSSION

3.1. Correlation between climate factors and the anatomical properties of bast fibers

Anatomical properties of plant fibers are influenced by various factors such as geographic origin, fiber position, climate, and harvest time, all of which can significantly affect the quality of bioproducts made from these fibers (Yan *et al*., 2014). Climate variability, particularly changes in temperature, can promote the growth of certain fiber crops. If the temperature exceeds optimal ranges, it might reduce fiber length and strength. Similarly, changes in precipitation can impact water availability, which in turn can affect fiber growth and quality (Mall *et al.*, 2017).

Bast fibers of *B. papyrifera*, a species in the same genus, have bene reported to have an average fiber length of 10 mm (range, 6 to 20 mm) and an average fiber width of 30 μm (range, 25 to 35 μm; Ilvessalo-Pfäffli, 1995). Over five years of observation, the fiber length of *B. kazinoki* bast fibers peaked at 10.85 mm in 2017. It was the shortest at 8.34 mm in 2015 (Table 2). Fiber width was the largest in 2018 (23.25 μm). It was the smallest in 2015 (18.38 μm). Lumen width was the widest in 2018 (12.44 μm). It was the narrowest in 2015 (9.86 μm). These findings revealed variations of 2.51 mm in fiber length, 4.87 μm in fiber width, and 2.58 μm in lumen width. These variations might be attributed to climate conditions.

The correlation analysis between fiber length and climatic factors showed weak positive correlations of fiber length with average minimum temperature (correlation coefficient: 0.2332) and total precipitation (correlation coefficient: 0.2921; Fig. 1). Previous studies have indicated that wood fiber length is greatly influenced by temperature and that fiber growth can be promoted up to a certain threshold as temperature rises (Jozsa and Middleton, 1994; Kilpeläinen *et al*., 2003). Bast fibers of Kenaf (*Hibiscus cannabinus* L.) are also utilized in various industries through cultivation. The optimal growth temperature for bast fibers of Kenaf (*H. cannabinus* L.) is known to be 22℃–30℃ (Faruk and Sain, 2014; Lewin, 2006). The positive correlation between fiber length and minimum temperatures suggests that excessively high maximum temperatures beyond the optimal range may adversely affect growth. Increased total precipitation promotes growth by enhancing nutrient and water supply, positively influencing cell growth

Table 2. Anatomical properties of *Broussonetia kazinoki* bast fibers and climate factors observed over five years (2014−2018)

Year	Fiber length (mm)	Fiber width (μm)	Lumen width (μm)	Cell wall thickness (μm)	Average temperature (℃)	Average high temperature (℃)	Average low temperature (℃)	Precipitation sum (mm)
2014	9.69 ± 2.11	21.32 ± 4.11	10.18 ± 2.29	5.5745	11.93	23.68	2.22	77.14
2015	10.51 ± 4.53	23.24 ± 9.63	9.86 ± 5.23	5.3848	13.12	24.09	4.17	114.75
2016	10.90 ± 4.03	23.96 ± 8.48	11.28 ± 4.65	5.2852	13.10	23.32	3.71	88.58
2017	11.11 ± 2.44	20.05 ± 3.43	10.64 ± 2.02	4.5789	13.35	24.43	4.09	114.08
2018	9.59 ± 1.28	23.25 ± 2.14	12.44 ± 1.97	5.4015	13.43	24.40	3.46	75.83
Average	10.42	22.47	11.87	5.24	12.99	23.98	3.53	94.08

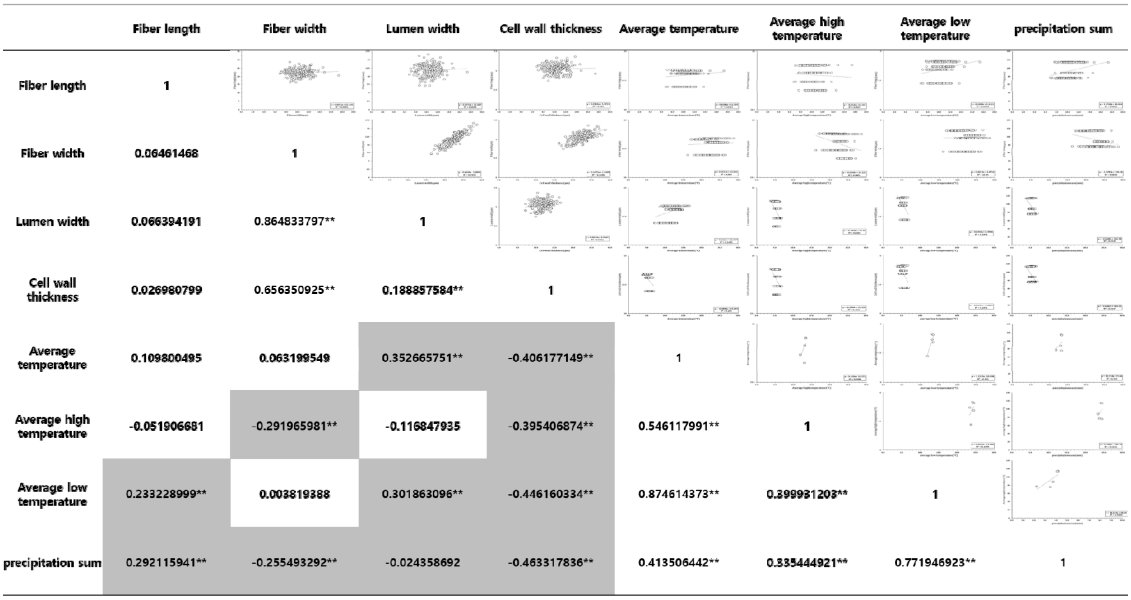

Fig. 1. Correlations between anatomical properties of *Broussonetia kazinoki* bast fibers and climate factors.

(Fritts *et al*., 1991; Honjo *et al*., 2005; Ryan, 2010). In this study, it appeared that an increase in precipitation positively affected cell length.

Fiber width showed a strong positive correlation with lumen width (correlation coefficient: 0.8648). This indicates that wider fibers tend to have larger lumens and thicker cell walls. Fiber width exhibited negative correlations with maximum temperature (correlation coefficient: −0.2919) and total precipitation (correlation coefficient: −0.2554), suggesting that excessive temperatures and precipitation might hinder fiber width development. Although increased precipitation generally promotes fiber growth, excessive or concentrated rainfall may hinder growth (Bakhshi *et al*., 2011).

Cell wall thickness was the only trait that showed significant correlations with all climatic factors. It decreased with increasing temperature and precipitation. This decrease in cell wall thickness might be attributed to increased transpiration caused by high temperatures and excessive water supply. While cell growth may increase, cell wall thickness tends to become thinner under such conditions (Mäkinen *et al*., 2002; Xu *et al*., 2015).

3.2. Analysis of K-means cluster through anatomical and chemical properties of bast fibers by region

Anatomical and chemical properties of bast fibers from 43 regional samples were analyzed and grouped using K-means clustering. A total of twelve variables were examined, including eight related to anatomical characteristics and four related to chemical compositions (Table 3). To reduce dimensionality, PCA was performed before clustering (Table 4). The first three principal components (PC1, PC2, and PC3) had eigenvalues greater than 1, explaining 72.59% of the total variance. PC1 accounted for 34.57% of the variance. It was associated with high positive loadings for RR and RC. It was also associated with high negative loadings for FC and lumen width. PC2 explained 23.77% of the variance. It was positively associated with fiber length, fiber width, and cell wall thickness. PC3 accounted for 14.23% of the variance. It was negatively associated with NaOH solubility.

Based on PCA results, K-means clustering categorized samples into three clusters, with the optimal number of clusters determined using the elbow method (Fig. 2). The three clusters displayed distinct distributions of principal components. Cluster 1 characterized by high FC and lumen width values represented flexible but less strong fibers. Cluster 2 exhibited notable fiber length, width, and cell wall thickness, indicating structurally strong and thick fibers. Cluster 3 showed high RR and RC values, suggesting durable and strong fibers. However, geographic distributions of clusters did not correspond to specific regions. This indicates that traits of bast fibers are influenced more by human-mediated distribution than by geographic factors.

3.3. Selection of superior individuals based on anatomical and chemical properties

Hanji made with *B. kazinoki* as a raw material exhibits differences in anatomical properties, absorption, and ink dispersion depending on compositions and characteristics of the material (Jung, 2007). To standardize the quality of Hanji, which is influenced by properties of its raw materials, 43 samples of *B. kazinoki* bast fibers were analyzed to select superior individuals. During the process of removing non-cellulose components to produce bleached bark (Baekpi) during Hanji manufacturing, raw materials with low intermediate alkali content, low alcohol-benzene extractives, and high cellulose content were found to be suitable for producing high-quality Hanji (Go and Jeong, 2018).

Therefore, in this study, superior individuals were selected based on holocellulose content and fiber length. Holocellulose is a key component required for Hanji manufacturing. Thus, individuals having holocellulose contents within the top 10% were identified first. From these top 10% individuals, one individual each was selected based on fiber length: the shortest, the longest, and an individual with an average fiber length (Fig. 3). This approach ensured that individuals with excellent holocellulose content and diverse fiber length characteristics were secured, providing genetic resources suitable for various industrial applications such as paper production. According to our selection criteria, samples 6, 34, and 39 were identified as superior individuals.

Table 3. Comprehensive analysis of anatomical and chemical properties in 43 *Broussonetia kazinoki* bast fiber samples

ID	Fiber length (mm)	Fiber width (μm)	Lumen width (μm)	Cell wall thickness (μm)	RR	AR	FC	RC	NaOH (%)	Holocellulose (%)	Lignin (%)	Ash (%)
1	11.56 ± 4.2	22.99 ± 5.2	11.19 ± 3.9	5.90	1.05	0.50	0.49	0.51	40.74	83.02	0.69	2.92
2	11.61 ± 4.4	22.96 ± 6.4	12.02 ± 4.3	5.47	0.91	0.51	0.52	0.48	33.00	82.66	0.25	1.80
3	10.39 ± 5.2	20.88 ± 6.5	10.99 ± 4.3	4.95	0.90	0.50	0.53	0.47	38.78	78.05	0.29	2.48
4	9.61 ± 3.5	22.66 ± 5.0	11.99 ± 3.4	5.34	0.89	0.42	0.53	0.47	36.55	86.19	0.23	1.94
5	10.94 ± 3.6	21.48 ± 5.1	10.88 ± 3.8	5.30	0.97	0.51	0.51	0.49	32.43	84.78	0.80	2.57
6	10.26 ± 3.2	22.10 ± 4.2	11.63 ± 3.0	5.23	0.90	0.46	0.53	0.47	39.33	85.10	0.11	3.29
7	10.69 ± 4.4	21.68 ± 5.1	10.67 ± 4.3	5.50	1.03	0.49	0.49	0.51	31.36	80.22	0.34	1.60
8	8.83 ± 2.9	22.25 ± 5.1	11.65 ± 3.3	5.30	0.91	0.40	0.52	0.48	31.65	76.36	0.59	2.11
9	10.27 ± 3.6	22.19 ± 5.5	11.35 ± 3.7	5.42	0.96	0.46	0.51	0.49	38.19	70.61	0.13	2.91
10	11.70 ± 4.0	23.51 ± 5.4	12.21 ± 3.3	5.65	0.93	0.50	0.52	0.48	46.41	71.59	0.09	3.04
11	11.30 ± 4.0	21.37 ± 4.4	10.94 ± 3.5	5.22	0.95	0.53	0.51	0.49	38.56	81.81	0.62	3.04
12	9.51 ± 3.2	21.15 ± 4.9	11.27 ± 4.5	4.94	0.88	0.45	0.53	0.47	41.77	81.73	0.24	2.43
13	10.04 ± 3.6	23.55 ± 5.1	12.47 ± 3.6	5.54	0.89	0.43	0.53	0.47	44.38	84.14	0.12	2.61
14	8.19 ± 2.6	21.84 ± 5.2	11.96 ± 3.6	4.94	0.83	0.38	0.55	0.45	45.65	74.74	0.18	2.35
15	10.47 ± 4.1	21.58 ± 4.6	11.52 ± 4.1	5.03	0.87	0.49	0.53	0.47	36.39	82.05	0.40	2.17
16	9.29 ± 2.8	21.16 ± 4.9	11.33 ± 4.2	4.91	0.87	0.44	0.54	0.46	35.28	81.98	0.43	2.00
17	10.26 ± 3.4	23.84 ± 4.7	12.92 ± 2.9	5.46	0.85	0.43	0.54	0.46	31.69	84.97	0.43	3.36
18	9.59 ± 3.0	21.70 ± 5.2	11.38 ± 4.1	5.16	0.91	0.44	0.52	0.48	33.73	79.50	0.68	2.30
19	9.44 ± 3.1	21.37 ± 4.8	11.00 ± 3.7	5.18	0.94	0.44	0.51	0.49	44.26	83.56	0.33	3.20
20	9.65 ± 4.2	23.30 ± 5.5	12.51 ± 4.4	5.39	0.86	0.41	0.54	0.46	39.97	80.26	0.71	3.80
21	12.79 ± 6.6	23.96 ± 5.1	12.71 ± 3.8	5.63	0.89	0.53	0.53	0.47	46.91	83.84	0.02	3.67
22	9.93 ± 3.9	22.90 ± 6.4	12.75 ± 4.8	5.07	0.80	0.43	0.56	0.44	33.88	76.55	0.22	2.52
23	10.57 ± 3.8	22.01 ± 4.4	11.79 ± 2.8	5.11	0.87	0.48	0.54	0.46	34.17	83.69	0.48	2.00

Table 3. Continued

ID	Fiber length (mm)	Fiber width (μm)	Lumen width (μm)	Cell wall thickness (μm)	RR	AR	FC	RC	NaOH (%)	Holocellulose (%)	Lignin (%)	Ash (%)
24	10.20 ± 2.8	20.98 ± 5.5	10.70 ± 4.0	5.14	0.96	0.49	0.51	0.49	34.13	83.42	0.26	2.23
25	10.66 ± 4.0	22.40 ± 4.7	11.98 ± 3.8	5.21	0.87	0.48	0.53	0.47	33.53	82.84	0.27	2.41
26	11.21 ± 3.9	21.26 ± 4.7	11.42 ± 3.0	4.92	0.86	0.53	0.54	0.46	44.88	80.90	0.48	2.68
27	11.05 ± 4.0	21.47 ± 4.4	11.42 ± 3.2	5.02	0.88	0.51	0.53	0.47	38.08	79.45	0.88	2.69
28	9.80 ± 3.3	21.63 ± 4.5	11.70 ± 3.2	4.97	0.85	0.45	0.54	0.46	43.78	82.34	0.39	3.00
29	10.15 ± 3.5	22.18 ± 4.6	11.85 ± 3.4	5.16	0.87	0.46	0.53	0.47	39.74	83.13	0.15	2.55
30	11.01 ± 4.6	24.00 ± 5.6	13.26 ± 3.9	5.37	0.81	0.46	0.55	0.45	43.38	78.87	0.52	2.88
31	11.36 ± 4.8	24.32 ± 6.7	13.14 ± 5.0	5.59	0.85	0.47	0.54	0.46	43.62	78.55	0.29	2.81
32	11.01 ± 4.1	24.06 ± 4.6	12.63 ± 3.0	5.71	0.90	0.46	0.52	0.47	38.52	80.61	0.78	2.02
33	12.48 ± 4.8	23.63 ± 6.7	12.54 ± 5.2	5.54	0.88	0.53	0.53	0.47	38.66	83.91	0.56	2.26
34	12.81 ± 5.1	24.88 ± 5.6	13.20 ± 3.8	5.84	0.88	0.51	0.53	0.47	40.35	84.99	1.03	2.38
35	11.13 ± 5.0	22.69 ± 5.4	11.87 ± 3.3	5.41	0.91	0.49	0.52	0.48	39.99	83.05	0.75	2.41
36	10.57 ± 4.3	22.38 ± 5.3	11.93 ± 4.1	5.22	0.88	0.47	0.53	0.47	38.34	81.89	0.18	2.39
37	10.66 ± 3.5	21.90 ± 4.6	11.64 ± 3.3	5.13	0.88	0.49	0.53	0.47	41.47	77.18	1.06	2.59
38	10.36 ± 3.8	22.08 ± 4.7	11.60 ± 3.1	5.24	0.90	0.47	0.53	0.47	42.82	78.80	0.37	3.98
39	8.45 ± 2.7	22.60 ± 4.8	12.21 ± 3.3	5.19	0.85	0.37	0.54	0.46	31.59	86.33	0.52	2.35
40	9.12 ± 2.7	23.12 ± 5.2	11.93 ± 3.5	5.59	0.94	0.39	0.52	0.48	32.32	83.37	0.98	1.98
41	10.70 ± 3.5	21.42 ± 4.6	10.36 ± 3.5	5.53	1.07	0.50	0.48	0.52	32.34	83.00	0.33	1.88
42	8.53 ± 2.9	19.93 ± 5.2	10.10 ± 4.0	4.91	0.97	0.43	0.51	0.49	34.47	83.24	0.99	2.35
43	8.33 ± 2.5	22.91 ± 4.4	11.93 ± 3.0	5.49	0.92	0.36	0.52	0.48	34.72	82.13	0.54	2.73

RR: Runkel ratio, AR: aspect ratio, FC: flexibility coefficient, RC: rigidity coefficient.

Table 4. Principal component analysis (PCA) results for anatomical and chemical properties of *Broussonetia kazinoki* bast fibers

Variable	PC1	PC2	PC3
Fiber length (mm)	−0.016858278	0.513223015	−0.182467238
Fiber width (μm)	−0.271223934	0.407395635	0.325309874
Lumen width (μm)	−0.417922033	0.238293348	0.249769811
Cell wall thickness (μm)	0.037952502	0.501273756	0.313361045
RR	0.461351362	0.156109926	−0.00094696
AR	0.122443303	0.361258576	−0.370125671
FC	−0.4618796	−0.155757413	0.00134752
RC	0.462019965	0.155573008	−0.00395912
NaOH (%)	−0.216194508	0.187697723	−0.480910808
Holocellulose (%)	0.077052157	0.040951917	0.327178767
Lignin (%)	0.109422179	0.026174227	0.314914426
Ash (%)	−0.175417187	0.147098099	−0.354676534
Eigenvalue	4.248367093	2.920730104	1.749436627
Proportion of variance (%)	34.57973215	23.77338457	14.23960045
Cumulative variance (%)	34.57973215	58.35311672	72.59271717

RR: Runkel ratio, AR: aspect ratio, FC: flexibility coefficient, RC: rigidity coefficient.

4. CONCLUSIONS

This study analyzed anatomical and chemical properties of *B. kazinoki* bast fibers collected from 43 regions in Korea. Based on this analysis, PCA and K-means clustering were performed to identify superior individuals.

Correlation analysis between climate factors and anatomical properties of *B. kazinoki* bast fibers revealed that average temperature and precipitation had a low positive correlation with fiber length. Cell wall thickness, on the other hand, was directly influenced by temperature and precipitation. This suggests that the growth of *B. kazinoki* is optimized within specific ranges of temperature and humidity and that anatomical traits might be only marginally affected by climate changes.

PCA-based K-means clustering analysis identified three clusters. Cluster 1 showed high values for the FC and lumen width, indicating high flexibility but low strength. Cluster 2 was characterized by prominent fiber length, width, and cell wall thickness, reflecting strong and thick structural fibers. Cluster 3 exhibited high RR and RC, suggesting superior durability and strength. However, the geographical distribution of samples appeared mixed, showed no concentration in specific regions. This might be due to intentional distribution and cultivation of *B. kazinoki* across various regions to secure raw materials for Hanji production.

This study selected superior individuals based on holocellulose content and fiber length of *B. kazinoki* bast fibers. As a result, samples 6, 34, and 39 were identified as superior individuals, showing high holocellulose

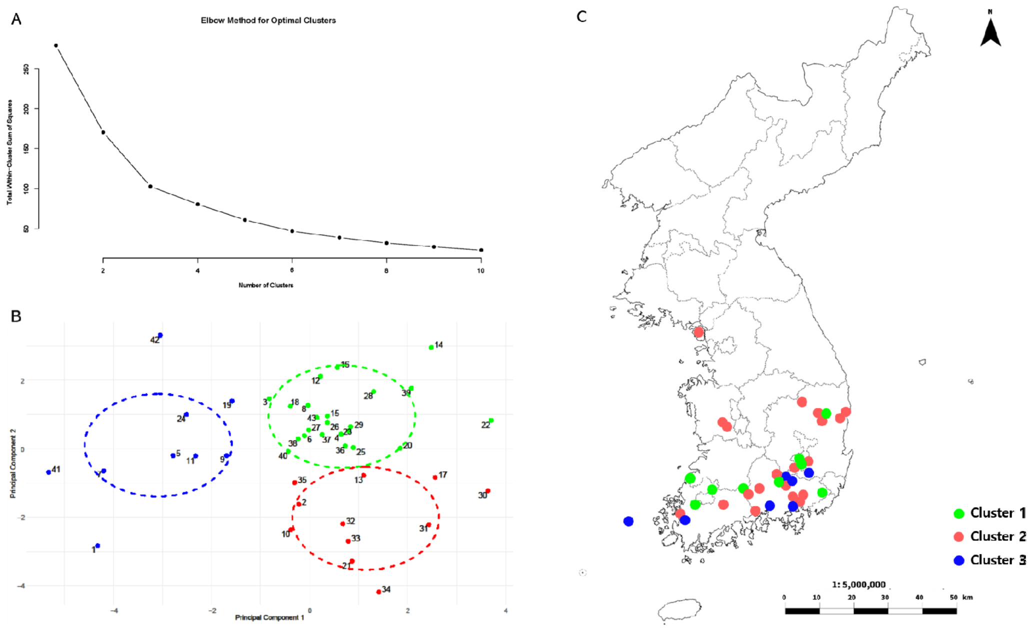

Fig. 2. K-means clustering analysis of anatomical and chemical properties of *Broussonetia kazinoki* bast fibers. (a) Optimal number of clusters determined using the Elbow method; (b) Cluster distribution based on principal component analysis; (c) Geographic distribution.

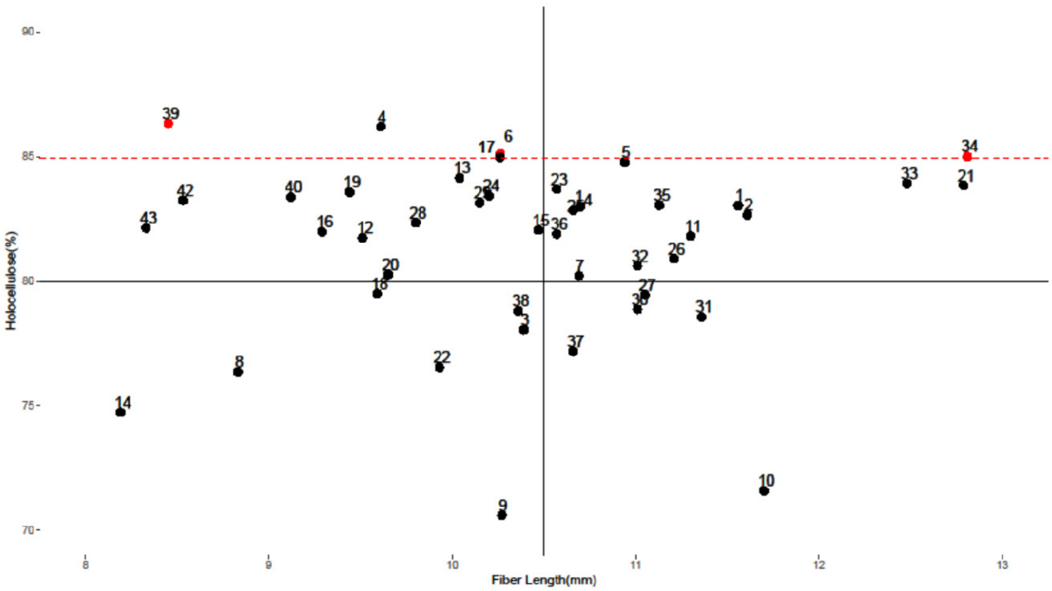

Fig. 3. Selection of superior individuals based on holocellulose contents and fiber lengths of *Broussonetia kazinoki* bast fibers.

contents and diverse fiber length characteristics, making them suitable for industrial applications.

By analyzing anatomical and chemical properties of *B. kazinoki* bast fibers and clustering the samples, this study identified superior individuals suitable for industrial use. Cultivating and propagating these selected individuals could ensure quality uniformity, enhancing the potential of *B. kazinoki* bast fibers in not only the Hanji industry, but also in other sectors such as artificial leather and mask filters. Future studies should track long-term effects of climate changes on growth of *B. kazinoki* and its bast fiber properties. Genetic traits of superior individuals also need to be identified through genetic analysis in the future. These efforts will further optimize the industrial use of *B. kazinoki* bast fibers and promote their development as a sustainable material.

CONFLICT of INTEREST

No potential conflict of interest relevant to this article was reported.

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