

Article

‘Fengyuan No. 1’: A High-Protein, Stress-Tolerant Forage Mulberry Variety from Northern China

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Abstract

The shortage of high-quality forage and the need for alternative protein sources are critical challenges for livestock production in northern China, where cold winters and saline–alkaline soils limit crop options. Mulberry (*Morus alba* L.) is a promising high-protein forage, yet few varieties have been bred for these conditions. Here, we report the breeding of ‘Fengyuan No. 1’, developed through systematic evaluation of 31 mulberry accessions for five forage traits, followed by a 4 × 3 factorial crossing design using four female parents (‘8036’, ‘Dahongpao’, ‘Jinqiang 63’, ‘Hongguo 2’) and three male parents (‘Nongsang 14’, ‘Yu 2’, ‘Yiyuan Huanglu’) selected based on comprehensive evaluation and complementary agronomic traits. Among the nine hybrid families with sufficient seed, family X01 (‘8036’ × ‘Nongsang 14’) ranked first in a three-year (2011–2013) comprehensive evaluation of growth traits using membership function analysis and was subsequently named ‘Fengyuan No. 1’. Multi-location (Shandong, Anhui, Shaanxi) field trials (2018–2021) with ‘Fengchi’ as the control showed that ‘Fengyuan No. 1’ achieved an average annual leaf yield of 12,596.48 kg DM·ha^{−1} (+14.20%) and total branch–leaf biomass of 25,341.95 kg DM·ha^{−1} (+13.36%). Leaf crude protein reached 30.87% on a dry matter basis (+25.64% vs. control). Silage prepared from this variety exhibited excellent fermentation quality (pH 4.10) and a relative feed value of 272.46, exceeding alfalfa silage standards. The variety maintained 100% survival at 0.4% NaCl and a 13.74% biomass advantage over the control on saline–alkaline field soils, with winter-desiccated shoot tips reduced by 53%. These results establish ‘Fengyuan No. 1’ as an elite forage mulberry for the Yellow River Basin and similar ecoregions.

Keywords: *Morus alba*; forage crop; hybrid breeding; crude protein; salt tolerance; cold tolerance; Yellow River Basin



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1. Introduction

The shortage of high-quality roughage is a critical constraint on the development of the livestock industry in northern China [1]. The increasing demand for animal products necessitates the acquisition of economically viable and sustainable feed alternatives to alleviate the contradiction between humans and livestock competing for food [2]. In this context, mulberry (*Morus alba* L.) has emerged as a promising candidate due to its high biomass yield, rapid regrowth, and adaptability to diverse environmental conditions [3,4]. Mulberry leaves are known as “arboreal feed” due to their high crude protein content,

abundance of essential amino acids, and presence of various bioactive substances, making them a potential high-quality protein source for livestock [5,6]. Furthermore, dietary supplementation with mulberry leaves has been shown to improve livestock productivity, feed utilization efficiency, and the quality of animal products, including meat and eggs, in poultry, pigs, cattle, sheep, rabbits, and fish [7–16].

China has a long history of mulberry cultivation for sericulture, with the total cultivation area exceeding 700,000 hectares [17]. However, the traditional focus on silkworm rearing has limited the development of mulberry varieties specifically optimized for forage purposes. Hybrid mulberry, produced by crossing genetically distinct parental lines, exhibits strong heterosis with advantages including simple propagation, low cost, rapid multiplication, and abundant seedling production, making it suitable for high-density, high-yield forage plantations [18]. Hybrid mulberry is characterized by vigorous growth, numerous shoots, early bud burst, and rapid maturation. Because of its strong adaptability, high survival rate, high yield, suitability for high-density planting, mechanized harvesting, and tolerance to repeated cutting, hybrid mulberry has become a preferred type for forage production [19]. Moreover, mulberry leaves are rich in protein, essential amino acids, and bioactive compounds with good palatability, which can effectively improve the quality and flavor of livestock products and enhance disease resistance [7–16].

The northern regions of China, particularly the Yellow River Basin, face unique challenges including cold winters, uneven precipitation, and widespread saline–alkaline soils [20]. Unlike silkworms, which consume only mulberry leaves, herbivorous livestock such as cattle and sheep prefer tender branches and leaves. Therefore, dedicated forage mulberry varieties should possess distinct characteristics compared with traditional sericulture varieties, including upright branch architecture, slender and elongated branches, medium-sized thick leaves with upward-extending leaf posture, high shoot number, and strong tolerance to repeated cutting. To meet the market demand for herbaceous, mechanized harvesting of hybrid mulberry, there is an urgent need to develop forage mulberry varieties with high leaf yield, superior nutritional quality, suitability for high-density planting, drought and wind resistance, and strong cold tolerance for northern China.

Currently, several hybrid mulberry varieties with strong heterosis are cultivated in China, including ‘Guisangyou 12’, ‘Guisangyou 62’, ‘Fengchi’, ‘Kangqing 10’, the ‘Yuesang’ series, and ‘Jisang 3’ [19,21,22]. However, most of these varieties were originally developed for silkworm rearing in subtropical or temperate sericulture regions. When introduced to northern provinces, they often exhibit compromised performance, including poor overwintering survival, reduced spring regrowth vigor, and low biomass productivity under cold and saline stress. Furthermore, existing forage mulberry varieties (or lines) in China were predominantly selected during the 1980s using evaluation criteria designed for sericulture. Differences in certification standards and breeding objectives have resulted in several defects when these older varieties are directly used as livestock feed, such as early lignification of branches, high fiber content, and relatively low crude protein content [23].

To address these challenges, the Shandong Institute of Sericulture has been systematically working on the selection of forage mulberry varieties for northern China since the late 1990s, building on decades of research in mulberry breeding for high yield, superior quality, and strong stress tolerance [24]. Through comprehensive evaluation of germplasm resources and targeted hybridization, we developed a new variety that demonstrates superior agronomic and nutritional properties. In this study, we present the complete breeding history and a comprehensive characterization of ‘Fengyuan No. 1’, a new high-protein, stress-tolerant forage mulberry variety. Our objective was to evaluate its yield potential, nutritional quality, silage fermentation characteristics, and resilience to salt and cold stress,

thereby providing a systematic variety-technology solution for the industrial development of forage mulberry on marginal lands.

2. Materials and Methods

2.1. Germplasm Evaluation and Parental Selection

A total of 31 mulberry accessions, representing diverse genetic backgrounds (including *Morus alba*, *M. multicaulis*, and *M. atropurpurea*) and originally collected from 15 provinces across China, were obtained from the Mulberry Germplasm Repository maintained at the Shandong Sericulture Research Institute, Yantai. All accessions were grown under uniform field conditions at the same experimental station to minimize environmental effects on trait expression.

During the peak vegetative stage, ten plants per accession were randomly selected, and the 4th–5th fully expanded leaves were sampled with three biological replicates. The following five forage-related traits were measured:

(1) Crude protein (CP) content: Determined by the Kjeldahl method ($N \times 6.25$) using an automatic Kjeldahl analyzer (Kjeltec 8400, FOSS, Hillerød, Denmark) on oven-dried (65 °C, 48 h) and ground leaf samples.

(2) Leaf area: Measured on fresh leaves using a portable leaf area meter (LI-3100, LI-COR, USA).

(3) Leaf mass per area (LMA): Calculated as leaf dry weight divided by leaf area ($\text{g}\cdot\text{cm}^{-2}$), reflecting leaf structural investment and resource allocation strategy.

(4) Leaf fresh weight: Recorded immediately after sampling using an analytical balance.

(5) Chlorophyll content (SPAD value): Measured with a portable chlorophyll meter (SPAD-502, Konica Minolta, Japan); three readings were taken per leaf and averaged for each plant.

(6) Data Integration and Comprehensive Scoring

To integrate the five traits into a single comprehensive score for each accession, we applied min–max normalization followed by equal-weight summation:

$$\text{Normalized}_{ij} = \frac{X_{ij} - X_j^{\min}}{X_j^{\max} - X_j^{\min}}$$

$$\text{ComprehensiveScore}_i = \frac{1}{5} \sum_{j=1}^5 \text{Normalized}_{ij}$$

where X_{ij} is the observed value of trait j for accession i , and $X_j^{\min}$ and $X_j^{\max}$ are the minimum and maximum values of trait j among all 31 accessions, respectively. This normalization ensures that each trait contributes equally to the final score regardless of its original measurement unit.

(7) Parental Selection Strategy

Parental selection was based on a comprehensive scoring strategy prioritizing overall performance while ensuring trait complementarity. First, all 31 accessions were ranked by an equal-weight comprehensive score derived from five forage-related traits (CP, leaf area, LMA, leaf fresh weight, and SPAD). Among the top-ranked accessions, we identified high-protein candidates (CP > 16%) and also retained accessions with outstanding complementary traits even if their CP was below 16%, to broaden genetic diversity for specific agronomic characteristics. Based on the comprehensive evaluation, four female parents ('8036', 'Dahongpao', 'Jinqiang 63', and 'Hongguo 2') and three male parents ('Nongsang 14', 'Yu 2', and 'Yiyuan Huanglu') were selected for crossing. Specifically, four female parents were selected: '8036' (CP 19.20%, comprehensive score 0.648, ranked first among high-protein accessions) and 'Dahongpao' (CP 17.90%, score 0.606) as high-protein core

parents; ‘Jinqiang 63’ (CP 14.12%, but the highest overall score of 0.689, with exceptional LMA of $0.1410 \text{ g}\cdot\text{cm}^{-2}$ and leaf fresh weight of 8.53 g) as a complementary parent for leaf structure traits; and ‘Hongguo 2’ (CP 13.50%, a locally adapted variety) as a regional adaptability parent. Among the three male parents, ‘Nongsang 14’ (CP 16.39%) was a nationally certified variety (Guoshen Sange 20000002) with abundant pollen production and synchronized flowering with ‘8036’; ‘Yu 2’ (CP 13.12%) had high chlorophyll content (SPAD 5.26); and ‘Yiyuan Huanglu’ (CP 16.14%) possessed the largest leaf area in the panel ($22,625.91 \text{ mm}^2$), although its pollen quantity at peak anthesis was limited.

The complete evaluation dataset for all 31 accessions is provided in Supplementary Table S1.

2.2. Hybridization and Family Development

Controlled crosses were performed in 2010 using a 4×3 factorial design, generating twelve full-sib families (Table 1). Seeds were harvested in late June and sown in early July 2010. Three families (X05, X08, and X12) produced insufficient seed quantities and were excluded from subsequent field evaluation. The widely planted hybrid variety ‘Fengchi’ (derived from ‘Zhongsang 5801’ $\times$ ‘Yu 82’) served as the control in all comparative trials.

Table 1. Pedigree of the twelve hybrid families and the control variety.

Family Code	Female Parent	Male Parent	Seed Status
X01	8036	Nongsang 14	Sufficient
X02	8036	Yu 2	Sufficient
X03	8036	Yiyuan Huanglu	Sufficient
X04	Dahongpao	Nongsang 14	Sufficient
X05	Dahongpao	Yu 2	Insufficient
X06	Dahongpao	Yiyuan Huanglu	Sufficient
X07	Jinqiang 63	Nongsang 14	Sufficient
X08	Jinqiang 63	Yu 2	Insufficient
X09	Jinqiang 63	Yiyuan Huanglu	Sufficient
X10	Hongguo 2	Nongsang 14	Sufficient
X11	Hongguo 2	Yu 2	Sufficient
X12	Hongguo 2	Yiyuan Huanglu	Insufficient
CK	Zhongsang 5801	Yu 82	Fengchi (control)

2.3. Family Evaluation and Selection

In spring 2011, one-year-old seedlings of the nine families with sufficient seed (X01–X04, X06–X07, X09–X11) and the control ‘Fengchi’ were transplanted to two sites: the Nanyuan Experimental Station in Haiyang and the Institute headquarters in Zhifu, Yantai. A randomized complete block design with six replications (200 plants per plot) was employed. Each experimental unit (plot) contained 200 plants, planted at a density of $66,000 \text{ plants}\cdot\text{ha}^{-1}$ with wide-narrow row spacing (30 cm + 70 cm) and 30 cm within-row spacing, consistent with the regional trial configuration.

From 2011 to 2013, two harvests per year (June and August) were conducted. The following traits were measured: average branch length (ABL, cm), total shoot length per plant (TSL, cm), leaf yield per meter of branch length (LYPM, $\text{g}\cdot\text{m}^{-1}$), and leaf weight per plant (LWP, g). For growth trait measurements (ABL, TSL, LYPM, and LWP), 10 randomly selected plants per plot were individually measured, and the plot mean was calculated from these 10 plants. The plot mean served as the experimental unit for all subsequent statistical analyses (ANOVA, CV calculation, and MFV calculation). Data were pooled across years and sites. The coefficient of variation (CV) was calculated for each trait within each family to assess phenotypic uniformity.

The membership function value (MFV) for each trait was calculated as:

$$MFV_i = \frac{X_i - X_{\min}}{X_{\max} - X_{\min}}$$

where X_i is the observed value for family i , and $X_{\min}$ and $X_{\max}$ are the minimum and maximum values among all families, respectively. The total MFV for each family was the sum of the four trait MFVs. The family with the highest total MFV and the lowest coefficient of variation (CV) was selected and named ‘Fengyuan No. 1’.

2.4. Multi-Location Regional Trials

Regional trials were established in 2016 at three representative sites: (1) Zhaoyuan, Shandong (37.4° N, 120.4° E; warm temperate semi-humid monsoon climate; annual mean temperature 12.5 °C; annual precipitation ~700 mm; frost-free period ~200 days; loam soil, pH 7.2, organic matter 12.5 g·kg⁻¹, total nitrogen 0.85 g·kg⁻¹); (2) Funan, Anhui (32.6° N, 115.6° E; transitional warm temperate–subtropical climate; annual mean temperature 15.2 °C; annual precipitation ~950 mm; frost-free period ~225 days; yellow-brown soil, pH 6.55, organic matter 11.8 g·kg⁻¹, total nitrogen 0.78 g·kg⁻¹); (3) Shiquan, Shaanxi (33.1° N, 108.3° E; subtropical humid monsoon climate; annual mean temperature 14.5 °C; annual precipitation 877–1136 mm; frost-free period 247–290 days; loam soil, pH 7.0, organic matter 13.2 g·kg⁻¹, total nitrogen 0.92 g·kg⁻¹).

‘Fengyuan No. 1’ and ‘Fengchi’ (control) were arranged in a randomized complete block design with five replications, using a planting density of 66,000 plants·ha⁻¹ (wide-narrow row spacing: 30 cm narrow row + 70 cm wide row, with 30 cm within-row spacing). Uniform fertilization and irrigation were applied to both varieties. Leaf yield and total branch–leaf biomass (converted to dry matter basis using variety-specific dry matter content determined at each harvest) were recorded over four consecutive years (2018–2021) with three harvests per year (June, August, and October).

2.5. Agronomic and Morphological Characterization

During the peak vegetative stage in 2018, 30 randomly selected plants per variety were measured for plant height, branch length, internode length, leaf length, leaf width, leaf area (LI-3100, LI-COR, Lincoln, NE, USA), single-leaf weight, number of leaves per plant, and number of branches per plant. Phenological stages (bud burst, leaf unfolding, and maturity) were monitored daily in spring from 2015 to 2018 at the Haiyang site.

2.6. Forage Quality Analysis

The 4th and 5th fully expanded leaves from the apex were collected at each harvest from all trial sites during 2018–2021. Samples were oven-dried at 65 °C to constant weight and ground to pass a 0.5 mm sieve. Crude protein was determined using the Kjeldahl method ($N \times 6.25$) with an automatic Kjeldahl analyzer (Kjeltec 8400, FOSS). Crude fiber was determined by the Weende method, involving sequential digestion with dilute acid and alkali, followed by filtration, drying, and ashing according to the standard procedure (AOAC 978.10). Total flavonoids were measured spectrophotometrically using rutin as a standard [9]. Ash was determined by incineration at 550 °C for 4 h, and reducing sugar was quantified by the 3,5-dinitrosalicylic acid (DNS) method.

2.7. Silage Preparation and Quality Evaluation

Three-year-old plants of ‘Fengyuan No. 1’ and ‘Fengchi’ grown at the Haiyang station were harvested at a plant height of approximately 100 cm with a 5 cm stubble. Fresh branches and leaves were chopped to <1.0 cm and mixed with a commercial silage inoculant (BaoLaiLai Bioengineering Co., Tai’an, China; containing *Lactobacillus plantarum*

and *Lactobacillus buchneri*, $\geq 1 \times 10^5$ CFU·g⁻¹ fresh material) activated in distilled water (100 mg·kg⁻¹, 35 °C, 1 h) and 5% corn flour. Approximately 5 kg of the mixture was packed into food-grade polyethylene bags (40 cm × 50 cm), vacuum-sealed, and stored at 24–33 °C in the dark for 45 days. Three replicates (four bags each) were performed.

After 45 days, the upper 20% of each bag was discarded before sampling. Sensory evaluation was performed according to GB/T 25886-2010 [25], assessing color (20 points), odor (25 points), and texture (10 points). pH was measured with a pH meter (FE28, Mettler Toledo, Zurich, Switzerland). Organic acids (lactic, acetic, and butyric) were quantified by HPLC (Agilent 1260) [10]. Ammonia nitrogen (AN) and total nitrogen (TN) were determined, and the AN/TN ratio was calculated. The comprehensive laboratory score (100 points total) was assigned as follows: lactic acid 12.5 points, acetic acid 12.5 points, butyric acid 25 points, and AN/TN 50 points. Relative feed value (RFV) was calculated as:

$$RFV = \frac{DMI \times DDM}{1.29}, \quad DMI = \frac{120}{NDF(\%DM)}, \quad DDM = 88.9 - 0.77$$

where NDF and ADF were determined using an Ankom Fiber Analyzer. All chemical analyses were performed in triplicate.

2.8. Stress Tolerance Evaluation

Salt tolerance. A pot experiment was conducted in a greenhouse (16 h light/8 h dark, 25 ± 2 °C day/ 18 ± 2 °C night, 60–70% relative humidity, light intensity 400 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Uniform one-year-old seedlings were transplanted into pots (30 cm diameter) containing a 3:1 (v/v) sand:vermiculite mixture. After 30 days of acclimation, plants were irrigated with five NaCl concentrations: 0, 0.2%, 0.3%, 0.4%, and 0.5% (w/w). A completely randomized design with three replicates (each replicate consisting of 10 plants; a total of 30 plants per treatment) was used. After 45 days of salt stress, plant height, leaf number per plant, shoot weight, leaf weight, leaf area, and survival rate were recorded. Salt tolerance indices were calculated as (treatment value/control value) × 100.

Field salinity trial. In addition, a field salinity tolerance trial was conducted at the Dongying experimental station (37.5° N, 118.7° E; warm temperate continental monsoon climate; annual mean temperature 12.8 °C; annual precipitation 560 mm) over four consecutive years (2019–2022). The soil was coastal saline–alkaline with an average electrical conductivity (ECe) of 4.8 dS·m⁻¹ ($\approx 0.3\%$ NaCl), pH 8.2, organic matter 8.6 g·kg⁻¹, and total nitrogen 0.52 g·kg⁻¹. The experimental design was a randomized complete block with three replications, each plot measuring 5 m × 4 m (20 m²), planting density 66,000 plants·ha⁻¹ (30 + 70 cm row spacing, 30 cm within row). Standard agronomic practices were applied uniformly to both varieties. At peak vegetative stage, 10 randomly selected plants per plot were measured for leaf area, leaf number, shoot number, and CP content. Total branch–leaf biomass was determined from a 1 m × 1 m quadrat per plot at the final harvest each year. Data were analyzed using two-way ANOVA with variety and year as fixed factors.

Cold tolerance. Over five consecutive winters (2017/2018, 2018/2019, 2019/2020, 2020/2021, and 2021/2022) at the Haiyang experimental station (36.8° N, 121.2° E; warm temperate semi-humid monsoon climate; annual mean temperature 11.8 °C; annual precipitation ~680 mm; frost-free period ~195 days; average extreme minimum temperature in January −9.5 °C; loam soil, pH 7.1), 30 randomly tagged plants per variety were investigated for the length of winter-desiccated shoot tips (cm) and the proportion of desiccated shoots (%).

2.9. Statistical Analysis

Data were analyzed using SPSS 21.0 (IBM Corp., Armonk, NY, USA). Normality and homogeneity of variances were confirmed before analysis. One-way ANOVA was performed, and means were compared using Fisher's least significant difference (LSD) test at $p < 0.05$. Coefficient of variation was calculated as $(\text{standard deviation}/\text{mean}) \times 100\%$. Regional trial data were pooled across sites and years after confirming variance homogeneity. Graphs were generated with GraphPad Prism 9.0.

3. Results

3.1. Germplasm Evaluation and Parental Selection

Evaluation of the 31 mulberry accessions for five forage-related traits revealed substantial genetic variation (Table S1). Leaf crude protein (CP) content ranged from 9.71% to 20.39%, with a mean of 14.50%. Only 11 accessions (35.5%) exceeded the 16% CP threshold. Leaf area varied from 3963.08 to 22,625.91 mm², leaf mass per area (LMA) from 0.0180 to 0.1410 g·cm⁻², leaf fresh weight from 2.57 to 8.53 g, and chlorophyll SPAD values from 3.99 to 5.81.

Using equal-weight min–max normalization, the top three accessions ranked by comprehensive score were 'Jinqiang 63' (0.689), '8036' (0.648), and 'Dahongpao' (0.606). Although 'Jinqiang 63' achieved the highest overall score, its CP content (14.12%) fell below the 16% threshold. Among the 11 high-protein accessions, '8036' ranked first, followed by 'Dahongpao' (0.606) and '860' (0.576). Based on these results, and considering both overall performance and trait complementarity, four female parents ('8036', 'Dahongpao', 'Jinqiang 63', 'Hongguo 2') and three male parents ('Nongsang 14', 'Yu 2', 'Yiyuan Huanglu') were selected for factorial crossing (see Section 2.1). The complete evaluation dataset is provided in Table S1.

3.2. Family Evaluation and Selection of 'Fengyuan No. 1'

3.2.1. Comparison of Growth Traits Among Families

The three-year (2011–2013) average growth performance of the nine hybrid families and the control 'Fengchi' across two sites is presented in Table 2. Family X01 ('8036' × 'Nongsang 14') consistently outperformed all other families across summer, autumn, and the full growing season. For the total annual period, X01 achieved an average branch length (ABL) of 123.70 cm, total shoot length per plant (TSL) of 1221.80 cm, leaf yield per meter of branch length (LYPM) of 61.57 g·m⁻¹, and leaf weight per plant (LWP) of 737.68 g. These values were significantly higher ($p < 0.05$) than those of the other families for ABL, LYPM, and LWP.

In summer, X01 recorded significantly higher ABL (117.91 cm) and LWP (361.03 g) than all other families. In autumn, X01 showed significantly superior performance in ABL (129.48 cm), LYPM (73.90 g·m⁻¹), and LWP (462.26 g). Notably, X01's annual LWP exceeded that of 'Fengchi' by 19.29%, indicating substantial yield heterosis.

3.2.2. Within-Family Uniformity and Comprehensive Ranking

The coefficient of variation (CV) for each growth trait was calculated across years to assess within-family uniformity (Table 3). Family X01 exhibited the smallest CV among all ten families for the majority of traits over the three-year period: ABL in 2011 (0.94%) and 2013 (1.52%), TSL in 2013 (1.03%), LYPM in 2011 (1.11%), 2012 (0.93%), and 2013 (2.65%), and LWP in 2012 (2.55%) and 2013 (3.69%). Overall, X01 consistently showed the smallest within-family variation, indicating a high degree of phenotypic uniformity essential for a commercially viable variety.

Table 2. Growth performance of nine hybrid families and the control ‘Fengchi’ (pooled data from two sites, 2011–2013).

Season	Trait	CK	X01	X02	X03	X04	X06	X07	X09	X10	X11
Summer	ABL (cm)	106.71 ± 11.14 b	117.91 ± 14.70 a	100.57 ± 13.06 b	104.20 ± 12.06 b	99.87 ± 7.79 b	105.32 ± 12.08 b	87.52 ± 4.46 c	101.70 ± 15.72 b	101.82 ± 5.31 b	104.06 ± 4.32 b
	TSL (cm)	556.00 ± 61.25 ab	591.19 ± 56.06 a	519.12 ± 80.37 b	539.75 ± 59.67 b	534.09 ± 95.10 b	522.31 ± 45.66 b	515.95 ± 43.46 b	518.81 ± 32.27 b	511.09 ± 40.53 b	545.16 ± 74.72 ab
	LYPM (g·m ⁻¹)	53.66 ± 5.12 ab	61.11 ± 4.27 a	47.00 ± 5.41 b	46.55 ± 2.12 b	49.02 ± 7.37 b	53.06 ± 3.58 ab	48.98 ± 9.04 b	54.69 ± 8.57 ab	52.12 ± 3.22 b	51.54 ± 2.42 b
	LWP (g)	297.00 ± 11.76 b	361.03 ± 32.14 a	246.48 ± 55.69 c	250.32 ± 28.24 c	259.57 ± 43.96 bc	278.05 ± 39.02 bc	254.07 ± 58.24 c	283.80 ± 37.06 bc	266.37 ± 23.00 bc	282.19 ± 44.40 bc
Autumn	ABL (cm)	117.07 ± 9.89 b	129.48 ± 11.46 a	112.98 ± 10.65 b	111.78 ± 10.00 b	106.98 ± 4.59 b	112.59 ± 9.22 b	104.38 ± 1.04 b	109.25 ± 19.89 b	110.31 ± 5.03 b	115.53 ± 7.90 b
	TSL (cm)	614.23 ± 76.12 a	630.61 ± 70.85 a	566.65 ± 88.28 a	571.87 ± 47.24 a	574.70 ± 114.50 a	566.75 ± 68.33 a	593.49 ± 94.85 a	579.79 ± 39.68 a	586.40 ± 60.30 a	592.34 ± 67.61 a
	LYPM (g·m ⁻¹)	65.03 ± 5.68 b	73.90 ± 7.82 a	60.65 ± 3.08 b	57.28 ± 3.39 b	57.12 ± 3.20 b	61.29 ± 3.73 b	59.45 ± 3.43 b	64.20 ± 4.07 b	58.41 ± 2.33 b	62.57 ± 1.94 b
	LWP (g)	397.09 ± 32.30 b	462.26 ± 4.91 a	341.28 ± 42.00 c	328.62 ± 44.26 c	326.43 ± 49.18 c	348.92 ± 55.26 bc	354.71 ± 74.97 bc	371.57 ± 29.15 bc	342.76 ± 30.69 c	370.30 ± 16.77 bc
Annual	ABL (cm)	111.89 ± 10.40 b	123.70 ± 12.99 a	106.77 ± 10.41 bc	107.99 ± 9.90 b	103.42 ± 6.17 bc	108.95 ± 10.66 b	95.95 ± 6.30 c	105.48 ± 17.71 bc	106.06 ± 4.23 bc	109.79 ± 6.14 b
	TSL (cm)	1170.22 ± 139.71 ab	1221.80 ± 121.06 a	1085.77 ± 163.93 b	1111.61 ± 129.69 b	1108.79 ± 209.35 b	1089.60 ± 141.43 b	1109.44 ± 157.92 b	1098.60 ± 137.09 b	1097.49 ± 117.67 b	1137.50 ± 155.90 ab
	LYPM (g·m ⁻¹)	54.42 ± 1.33 b	61.57 ± 1.12 a	53.03 ± 6.91 bc	52.64 ± 2.02 c	55.52 ± 3.41 bc	61.20 ± 3.59 bc	60.35 ± 5.86 bc	60.54 ± 1.25 b	55.92 ± 2.04 bc	56.69 ± 2.99 bc
	LWP (g)	618.40 ± 11.73 b	737.68 ± 53.04 a	543.43 ± 83.60 de	521.56 ± 60.08 e	532.13 ± 75.26 de	562.71 ± 74.12 cd	556.02 ± 63.80 d	591.51 ± 57.41 bc	545.39 ± 88.55 de	592.13 ± 62.52 bc

Values are means ± SD of three years (2011, 2012, 2013). Different lowercase letters within a row indicate significant differences among families ($p < 0.05$, LSD test). ABL, average branch length; TSL, total shoot length per plant; LYPM, leaf yield per meter of branch length; LWP, leaf weight per plant.

Table 3. Coefficient of variation (%) and membership function analysis for growth traits of ten families.

Family	2011				2012				2013				MFV				Average MFV	Rank
	ABL	TSL	LYPM	LWP	ABL	TSL	LYPM	LWP	ABL	TSL	LYPM	LWP	ABL	TSL	LYPM	LWP		
CK	6.05	3.74	3.04	5.47	3.12	6.79	2.05	7.52	5.20	2.18	8.62	6.98	0.575	0.621	0.199	0.448	0.461	2
X01	0.94	2.54	1.11	4.02	2.48	2.32	0.93	2.55	1.52	1.03	2.65	3.69	1.000	1.000	1.000	1.000	1.000	1
X02	3.39	3.78	3.03	8.67	3.21	3.87	8.49	6.94	1.74	2.78	7.14	8.45	0.390	0.000	0.043	0.101	0.134	10
X03	2.47	1.63	7.35	11.44	3.22	3.34	7.89	5.46	1.69	1.26	4.95	6.22	0.434	0.190	0.000	0.000	0.156	9
X04	6.74	7.18	4.75	10.29	8.33	8.31	6.26	10.97	1.36	1.04	12.00	11.43	0.269	0.169	0.322	0.040	0.202	8
X06	8.40	3.40	4.85	5.39	2.07	4.49	6.37	10.64	3.78	6.07	4.22	9.00	0.469	0.024	0.959	0.190	0.411	5
X07	3.98	2.82	4.74	6.95	2.43	1.37	4.28	4.56	7.06	4.86	7.73	11.78	0.000	0.174	0.863	0.159	0.299	6
X09	9.00	2.57	4.38	12.19	1.10	3.95	4.97	4.15	3.97	1.77	10.61	10.21	0.343	0.094	0.885	0.324	0.412	4
X10	4.54	7.69	10.43	13.31	6.69	4.27	7.12	7.37	2.02	3.45	3.64	7.21	0.365	0.086	0.368	0.110	0.232	7
X11	4.91	4.82	2.77	5.95	5.07	2.61	6.82	9.15	2.83	1.37	7.41	6.20	0.499	0.380	0.453	0.327	0.415	3

CV: coefficient of variation (%); MFV: membership function value. ABL, average branch length; TSL, total shoot length per plant; LYPM, leaf yield per meter of branch length; LWP, leaf weight per plant.

Membership function values (MFV) for the four annual growth traits were calculated and averaged to provide an integrated ranking of the ten families (Table 3). Family X01 achieved the highest average MFV (1.000), ranking first with a substantial margin. The control ‘Fengchi’ ranked second (0.461), followed by X11 (0.415), X09 (0.412), X06 (0.411), X07 (0.299), X10 (0.232), X04 (0.202), X03 (0.156), and X02 (0.134).

3.2.3. Selection and Morphological Characteristics of ‘Fengyuan No. 1’

The three-year comparative evaluation clearly demonstrated that family X01, derived from the cross ‘8036’ × ‘Nongsang 14’, was the superior genotype. It ranked first in the comprehensive membership function analysis, exhibited the smallest within-family variation, and significantly outperformed both the control and all other families in leaf yield and growth vigor. Based on these results, family X01 was formally named ‘Fengyuan No. 1’ and advanced to multi-location regional trials.

‘Fengyuan No. 1’ has an upright tree architecture with a compact canopy, thick and straight branches, sparse lateral branching, and grey-brown bark. Leaves are dark green, oval to heart-shaped, with a long-caudate apex, coarse wing-like serrations, and a deeply cordate base. The variety is medium-maturing, with bud burst occurring April 11–16 and leaf unfolding April 17–28 at Haiyang, approximately 3–5 days later than ‘Fengchi’. It produces female flowers and purple-black berries. Compared with ‘Fengchi’, ‘Fengyuan No. 1’ showed significant improvements in shoot weight (+11.53%), total shoot length (+17.84%), leaf weight per plant (+15.37%), and single leaf weight (+16.92%) (Table S2).

3.3. Biomass Yield Performance in Regional Trials

Across the four-year (2018–2021) regional trials at three locations, ‘Fengyuan No. 1’ consistently produced significantly ($p < 0.05$) higher leaf and total branch–leaf biomass than ‘Fengchi’ on a dry matter basis (Table 4). The average annual leaf yield across all sites was 12,596.48 kg DM·ha^{−1}, 14.20% higher than the control (11,029.84 kg DM·ha^{−1}). Total branch–leaf biomass reached 25,341.95 kg DM·ha^{−1}, a 13.36% increase over ‘Fengchi’ (22,354.23 kg DM·ha^{−1}). The yield advantage was observed at all three locations, with increases of 13.95% in Shandong, 13.32% in Anhui, and 15.30% in Shaanxi, demonstrating broad adaptability across diverse ecological zones. The superior yield was consistent across all three annual harvests (June, August, and October). Detailed annual data for each site are provided in Table S3.

Table 4. Leaf and total branch–leaf yield of ‘Fengyuan No. 1’ and ‘Fengchi’ across three locations (2018–2021 averages).

Location	Variety	Leaf Yield (kg DM·ha ^{−1} ·yr ^{−1})	Index (%)	Total Branch–Leaf Yield (kg DM·ha ^{−1} ·yr ^{−1})	Index (%)
Shandong	Fengyuan No. 1	12,082.30 ± 297.05 a	113.95	24,286.64 ± 639.53 a	112.19
	Fengchi	10,603.44 ± 261.61 b	100.00	21,648.70 ± 569.75 b	100.00
Anhui	Fengyuan No. 1	12,592.67 ± 309.29 a	113.32	25,442.34 ± 670.15 a	114.98
	Fengchi	11,112.64 ± 274.04 b	100.00	22,127.15 ± 582.56 b	100.00
Shaanxi	Fengyuan No. 1	13,080.87 ± 321.89 a	115.30	26,293.86 ± 692.64 a	112.91
	Fengchi	11,345.04 ± 279.84 b	100.00	23,287.19 ± 613.17 b	100.00
Mean	Fengyuan No. 1	12,596.48 ± 261.91 A	114.20	25,341.95 ± 527.95 A	113.36
	Fengchi	11,020.37 ± 230.23 B	100.00	22,354.23 ± 465.99 B	100.00

Values are means ± SD of four years (2018–2021). Different lowercase letters within a location indicate significant differences between varieties ($p < 0.05$). Uppercase letters indicate significant differences between overall means ($p < 0.05$).

3.4. Forage Nutritional Quality and Silage Fermentation Characteristics

3.4.1. Leaf Nutritional Composition

On a dry matter basis, the average CP content of ‘Fengyuan No. 1’ leaves across the three sites was 30.87%, 25.64% higher than that of ‘Fengchi’ (24.57%) (Table 5). Total flavonoid content averaged 7.16%, significantly exceeding the 6.34% recorded for ‘Fengchi’ (+12.94%). Detailed proximate analysis at the Zhaoyuan site showed that ‘Fengyuan No. 1’ had lower crude fiber (12.83% vs. 15.27%, −15.94%), higher crude fat (6.17% vs. 5.23%), and comparable ash (10.60% vs. 10.50%) and reducing sugar (6.93% vs. 7.57%) relative to the control.

Table 5. Leaf nutritional quality and silage fermentation characteristics of ‘Fengyuan No. 1’ and ‘Fengchi’.

Parameter	Fengyuan No. 1	Fengchi
Leaf nutritional composition		
Crude protein (% DM)	30.87 ± 1.62 a	24.57 ± 1.55 b
Crude fiber (% DM)	12.83 ± 0.75 b	15.27 ± 0.82 a
Crude fat (% DM)	6.17 ± 0.32 a	5.23 ± 0.28 b
Ash (% DM)	10.60 ± 0.55 a	10.50 ± 0.52 a
Reducing sugar (% DM)	6.93 ± 0.41 a	7.57 ± 0.45 a
Total flavonoids (%)	7.16 ± 0.38 a	6.34 ± 0.35 b
Silage fermentation characteristics		
pH	4.10 ± 0.08 b	4.76 ± 0.10 a
Lactic acid (% DM)	4.66 ± 0.18 a	3.85 ± 0.15 b
Acetic acid (% DM)	3.56 ± 0.15 b	4.09 ± 0.16 a
Butyric acid (% DM)	0.01 ± 0.001 b	0.015 ± 0.001 a
AN/TN (%)	6.92 ± 0.35 b	10.59 ± 0.40 a
Laboratory score (100)	82	66.5
Grade	Excellent	Good
Silage nutritional value		
NDF (% DM)	25.10 ± 0.62 b	29.25 ± 0.70 a
ADF (% DM)	19.75 ± 0.48 b	24.40 ± 0.56 a
RFV	272.46 ± 5.32 a	222.28 ± 4.80 b

Values are means ± SD of three replicates for silage parameters. Leaf nutritional data are means across three locations (Shandong, Anhui, Shaanxi) over four years (2018–2021). Different lowercase letters within a row indicate significant differences between varieties ($p < 0.05$). AN/TN: ammonia nitrogen/total nitrogen; NDF: neutral detergent fiber; ADF: acid detergent fiber; RFV: relative feed value.

3.4.2. Silage Fermentation Quality and Relative Feed Value

After 45 days of ensiling, ‘Fengyuan No. 1’ silage exhibited excellent fermentation characteristics (Table 5). The pH was 4.10, significantly lower than the 4.76 recorded for ‘Fengchi’. Lactic acid content was substantially higher in ‘Fengyuan No. 1’ silage, while butyric acid was barely detectable. The ammonia nitrogen to total nitrogen (AN/TN) ratio was 6.92%, considerably lower than the 10.59% of ‘Fengchi’, indicating superior protein preservation during fermentation. Based on the Flieg scoring system, ‘Fengyuan No. 1’ silage was graded “excellent” (laboratory score 82/100), whereas ‘Fengchi’ silage was graded “good” (66.5/100). Sensory evaluation results were consistent with the laboratory assessment (Table S4).

Critically, the relative feed value (RFV) of ‘Fengyuan No. 1’ silage was 272.46, significantly exceeding the 222.28 of ‘Fengchi’ (Table 5). This superior RFV was attributable to lower NDF (25.10% vs. 29.25%) and ADF (19.75% vs. 24.40%) in ‘Fengyuan No. 1’ silage. Both values substantially exceed the threshold for “premium” forage (RFV > 151).

3.5. Stress Tolerance

3.5.1. Salt Tolerance

The salt tolerance of ‘Fengyuan No. 1’ was evaluated under both field and controlled conditions (Table 6). In field trials on saline–alkaline soils (average salinity 0.3%) in Dongying over four consecutive years (2019–2022), ‘Fengyuan No. 1’ exhibited 9.94% greater leaf area, 11.02% more leaves per plant, and 6.16% higher leaf CP content compared with ‘Fengchi’. Total branch–leaf biomass reached 19,287.39 kg DM·ha^{−1}·yr^{−1}, 13.74% higher than that of ‘Fengchi’ (16,957.42 kg DM·ha^{−1}·yr^{−1}).

Table 6. Salt tolerance of ‘Fengyuan No. 1’ and ‘Fengchi’ under field and controlled conditions.

Condition	Parameter	Fengyuan No. 1	Fengchi
Field	Leaf area (cm ²)	181.86 ± 18.19 a	165.42 ± 16.54 b
	Leaf number per plant	85.44 ± 12.82 a	76.96 ± 11.54 b
	Shoot number per plant	6.24 ± 0.94 a	6.04 ± 0.91 a
	Crude protein (% DM)	21.38 ± 1.07 a	20.14 ± 1.01 b
	Total branch–leaf yield	19,287.39 ± 2893.10 a	16,957.42 ± 2544.60 b
Pot (0.4% NaCl)	Survival rate	100.00 a	94.31 ± 0.86 b
Pot (0.5% NaCl)	Survival rate	57.86 ± 0.53 a	43.79 ± 0.40 b
Condition	Parameter	Fengyuan No. 1	Fengchi
Field	Leaf area (cm ²)	181.86 ± 18.19 a	165.42 ± 16.54 b
	Leaf number per plant	85.44 ± 12.82 a	76.96 ± 11.54 b

Field data are means ± SE of four years ($n = 4$). Pot experiment data are means ± SE of three replicates (each replicate = 10 plants; total 30 plants per treatment). Different lowercase letters within a row indicate significant differences between varieties ($p < 0.05$).

In the greenhouse pot experiment with five NaCl concentrations, ‘Fengyuan No. 1’ outperformed ‘Fengchi’ in leaf number, leaf weight, and leaf area across all stress levels (Table 6). At 0.2% and 0.3% NaCl, both varieties maintained 100% survival with no significant difference. At 0.4% NaCl, ‘Fengyuan No. 1’ maintained 100% survival, which was significantly higher ($p < 0.05$) than the 94.31% of ‘Fengchi’. At the most severe stress (0.5% NaCl), it was also significantly higher ($p < 0.05$) than that of ‘Fengchi’ (43.79%). These results consistently indicate that ‘Fengyuan No. 1’ possesses a critical survival threshold at approximately 0.5% NaCl and exhibits robust physiological tolerance to salinity stress.

3.5.2. Cold Tolerance

Winter hardiness was evaluated at the Haiyang site over five consecutive winters (2017–2022) (Table 7). The average length of desiccated shoot tips for ‘Fengyuan No. 1’ was 11.91 cm, which was only 46.91% of the 25.39 cm recorded for ‘Fengchi’. The proportion of shoots affected by winter desiccation was 70.31% for ‘Fengyuan No. 1’, compared with 98.67% for ‘Fengchi’. These data indicate that ‘Fengyuan No. 1’ retains substantially more viable shoot tissue after harsh winter conditions, a trait that is critical for rapid spring regrowth and sustained biomass production in temperate regions.

Table 7. Cold tolerance of ‘Fengyuan No. 1’ and ‘Fengchi’ (2017–2022).

Variety	Desiccated Shoot Tip Length (cm)	Index (%)	Desiccated Shoot Rate (%)	Index (%)
Fengyuan No. 1	11.91 ± 1.20 b	46.91	70.31 ± 3.50 b	71.26
Fengchi	25.39 ± 2.10 a	100.00	98.67 ± 4.20 a	100.00

Values are means ± SD of 30 plants per variety per year over five winters. Different lowercase letters within a column indicate significant differences ($p < 0.05$).

4. Discussion

4.1. Breeding Strategy and Genetic Gain

The systematic screening of 31 germplasm accessions, followed by targeted hybridization, proved highly effective. The complementary cross between the high-protein female parent ‘8036’ (CP 19.20%) and the nationally certified male parent ‘Nongsang 14’ (CP 16.39%) generated strong heterosis, with the X01 family substantially outperforming all others and the benchmark ‘Fengchi’. The low within-family CV (<4% for most traits) ensures uniform field performance, a critical attribute for commercial-scale production. Notably, existing forage mulberry varieties in China were largely selected under sericulture-oriented standards, resulting in high fiber content and early lignification when used for livestock [23]. By incorporating forage-specific criteria—yield, protein content, and silage quality—into the selection process, ‘Fengyuan No. 1’ better meets the requirements of modern animal husbandry.

4.2. Yield Performance and Regional Adaptability

Multi-location trials (2018–2021) demonstrated a consistent yield advantage across three ecologically distinct sites. The average annual total branch–leaf biomass of 25.34 t DM·ha^{−1} exceeded that of ‘Fengchi’ by 13.36%, with similar gains observed at each individual location. This stability across environments indicates broad adaptability, an essential trait for the variable climatic conditions of northern China. The variety’s upright architecture and sparse lateral branching facilitate high-density planting (66,000 plants·ha^{−1}) and mechanized harvesting, aligning with the trend toward herbaceous mulberry cultivation [18].

4.3. Forage Quality and Silage Value

The leaf CP content of 30.87% (DM basis) positions ‘Fengyuan No. 1’ among the highest-protein woody forages available, comparable to premium alfalfa. Combined with low crude fiber (12.83%) and high total flavonoids (7.16%), this profile supports both digestibility and potential health benefits for livestock [7]. When ensiled, the variety produced silage with a low pH (4.10), minimal butyric acid, and a low ammonia-N/TN ratio (6.92%), indicating excellent protein preservation during fermentation. The RFV of 272.46 far exceeds the “premium” forage threshold (>151), attributable to low NDF (25.10%) and ADF (19.75%). These results confirm that ‘Fengyuan No. 1’ silage can serve as a high-quality roughage source for ruminants.

4.4. Stress Tolerance and Marginal Land Potential

Robust salt and cold tolerance are the defining agronomic advantages of ‘Fengyuan No. 1’ for northern China. On saline–alkaline soils (0.3% salinity) in Dongying, the variety maintained vigorous growth with a 13.74% biomass advantage and higher leaf CP than the control over four years. Controlled pot experiments confirmed a survival threshold near 0.5% NaCl, with consistently better performance across all stress levels. This suggests effective physiological mechanisms, possibly involving Na⁺ exclusion or osmotic adjustment, as reported in other tolerant genotypes [26,27]. Winter hardiness was equally notable: desiccated shoot tips were reduced by 53%, ensuring stronger spring regrowth and long-term stand persistence. These combined traits make ‘Fengyuan No. 1’ particularly suitable for the Yellow River Delta and similar marginal environments, where it can support livestock production without competing for arable land.

5. Conclusions

Through systematic evaluation of 31 mulberry accessions and a 4×3 factorial crossing design, we developed ‘Fengyuan No. 1’—a new forage mulberry variety derived from the elite cross ‘8036’ $\times$ ‘Nongsang 14’. This family ranked first among nine hybrid families in a three-year (2011–2013) comprehensive growth evaluation and exhibited the lowest within-family variation, demonstrating excellent uniformity. In subsequent multi-location (Shandong, Anhui, Shaanxi) trials, ‘Fengyuan No. 1’ delivered an average annual leaf yield of 12,596.48 kg DM·ha^{−1} (+14.20% vs. the national benchmark ‘Fengchi’) and total branch–leaf biomass of 25,341.95 kg DM·ha^{−1} (+13.36%). Leaf crude protein reached 30.87% on a dry matter basis (+25.64% vs. control), and silage prepared from this variety achieved a relative feed value of 272.46 with excellent fermentation quality (pH 4.10, low ammonia-N). The variety demonstrated robust salt tolerance (100% survival at 0.4% NaCl; 13.74% biomass advantage on saline–alkaline field soil) and significantly reduced winter desiccation injury (53% less shoot dieback than ‘Fengchi’). These attributes establish ‘Fengyuan No. 1’ as a high-value, resilient forage crop for the Yellow River Basin and similar ecoregions, providing a sustainable solution for utilizing marginal lands and reducing dependence on imported protein feed.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy16181758/s1>, Table S1: Comprehensive evaluation of 31 mulberry accessions for forage-related traits; Table S2: Morphological traits of ‘Fengyuan No. 1’ and ‘Fengchi’ (2015–2018 averages); Table S3: Annual leaf and total branch–leaf yield of ‘Fengyuan No. 1’ and ‘Fengchi’ by year and location (2018–2021); Table S4: Sensory evaluation of silage and silkworm rearing performance of ‘Fengyuan No. 1’ and ‘Fengchi’.

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Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ABL	Average Branch Length
ADF	Acid Detergent Fiber
AN	Ammonia Nitrogen
CP	Crude Protein
CV	Coefficient of Variation
DDM	Digestible Dry Matter
DM	Dry Matter
DMI	Dry Matter Intake

LMA	Leaf Mass per Area
LWP	Leaf Weight per Plant
LYPM	Leaf Yield per Meter of branch length
MFV	Membership Function Value
NDF	Neutral Detergent Fiber
RFV	Relative Feed Value
SD	Standard Deviation
SE	Standard Error
SPAD	Soil Plant Analysis Development (chlorophyll meter value)
TN	Total Nitrogen
TSL	Total Shoot Length per Plant

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