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ARTICLE

Effects of Nitrogen Application Rate on Yield and Quality of Different Genotypes of Foxtail Millet

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ABSTRACT

In order to elucidate the response mechanism of yield and quality of different genotypes of foxtail millet to nitrogen application. In this study, plant physiology and metabolomics were used to study the effects of different amounts of nitrogen (0, 75, 150, 225 and 300 kg hm^{-2}) on agronomic characters, yield and quality of Jingu 21 and Zhangza 10. The results showed that with the increase of nitrogen application, the plant height of different genotypes of foxtail millet increased gradually, and the content of stem diameter, yield, protein, fat, lysine, phenylalanine, isoleucine, arginine, aspartic, glutamic, glycine, and proline content of different genotypes of foxtail millet showed an increasing and then decreasing trend. The highest yield was recorded in Jingu 21 at 150 kg hm^{-2}, and the highest yield was recorded in Zhangza 10 at 225 kg hm^{-2} of nitrogen application. Yield of Jingu 21 was positively correlated with protein and tryptophan content (r = 0.91). The yield of Zhangza 10 was positively correlated with fibre content (r = 0.89). The protein content of different genotypes of foxtail millet were negatively correlated with the peak viscosity (PV), trough viscosity (TV) and breakdown value (BD). The results of this study clarify that the optimal nitrogen application of Jinguu 21 was 150 kg hm^{-2}, and that of Zhangza 10 was 225 kg hm^{-2}. The regulation effect of nitrogen on foxtail millet was clarified, which laid the theoretical and technical foundation for foxtail millet cultivation with high yield and high quality.

KEYWORDS

Foxtail millet; nitrogen application rate; yield; quality

1 Introduction

Foxtail millet has been cultivated in China for 6000–7000 years and is rich in beneficial vitamins, proteins, fats, and other nutrients, which have a variety of nutritional and medicinal values [1]. China currently preserves 70% of the world's foxtail millet germplasm resources [2]. Foxtail millet is a drought-resistant and barren-tolerant environmentally friendly crop [3], which has been gradually developed as a new C_4 model plant [4]. This is of great significance to dryland ecological agriculture, food production diversity and food security [5].

Nitrogen is a key factor affecting crop growth and development [6]. Zhou et al. showed that nitrogen application could increase the grain yield and the total above-ground nitrogen uptake at the maturity of

winter wheat [7]. Reasonable nitrogen application rate can improve the plant height and stem diameter of quinoa [8], as well as the spike length and spike branch of rice plants [7]. After nitrogen application, the number of rice panicles and grains per panicle increased, and the population quality improved significantly [9]. At the same time, it can promote the accumulation of assimilates in early and late rice [10] and the transport of dry matter from vegetative organs to grains [11], thus increasing the yield. However, when nitrogen application exceeds a certain range, crop yield decreases rather than increases [12,13]. The results showed that thousand-grain weight and grain per spike of wheat increased first and then decreased after nitrogen application [14]. Zhang et al. showed that the increase in grain yield was small when nitrogen application was greater than 90 kg hm^{-2} [15], and others showed that the increase in hybrid foxtail millet yield was not significant when nitrogen application was more than 200 kg hm^{-2} [16].

Reasonable nitrogen fertilizer regulation can increase crop quality [17]. Proper increase of nitrogen supply can promote nitrogen absorption by plants, improve nitrate reductase activity, and thus promote NO^{-3} absorption and transformation, provide more amino acid and protein supply for organ building [18], promote wheat starch accumulation and increase protein content [19]. Liu et al. showed that with the increase of nitrogen application, the protein content in rice grain was significantly increased, and the starch content was significantly decreased [20]. Hu et al. showed that with the increase in nitrogen application, the amino acid content of rice generally showed an increasing trend and with glutamic acid content being the highest and methionine content being the lowest [21]. Yang et al. showed that the total amino acid content of summer peanuts increased first and then stabilized gradually after nitrogen application [22]. High nitrogen levels will reduce starch synthesis rate, increase the proportion of amylopectin short branch and amylose long branch [23], reduce amylopectin content and inhibit FV and SB and affect gelatinization quality [17]. The rise in production costs and environmental pollution have a negative impact on the sustainable development of agriculture [24]. Emissions from the production and application of nitrogen fertilizer can have harmful effects on ecosystems [25].

Currently, the application of nitrogen fertiliser to foxtial millet production is mainly focused on agronomic characters [9], quality [19], nitrogen utilisation [17] and yield, photosynthetic characteristics [15,16] and water utilisation efficiency [16]. The effect of nitrogen application on amino acid content and RVA of foxtail millet has been less studied. In this article, different genotypes of foxtail millet were used as materials, and different levels of nitrogen application were set up. Plant physiology and non-targeted metabolomics were used to study the effects of nitrogen application on agronomic characters, quality, amino acid content and RVA of different genotypes of foxtail millet. The regulation effect of nitrogen on the quality formation and nitrogen metabolism of foxtail millet was clarified, and the optimal nitrogen application rate was selected. To establish a theoretical foundation for the cultivation of foxtail millet that achieves both high yield and superior quality.

2 Materials and Methods

2.1 Experiment Overview

The experiment was conducted in 2020 and 2021 at Dingxiang Experimental Base, Agricultural Gene Resources Research Center of Shanxi Agricultural University, and the air temperature and precipitation during the reproductive period of the foxtail millet are shown in Fig. 1. The experimental materials were provided by Agricultural Gene Resources Research Center of Shanxi Agricultural University (Taiyuan, China). The test varieties were selected from Jingu 21 and Zhangza 10. Jingu 21 is the most planted area of conventional foxtail millet varieties in Shanxi Province and Zhangza 10 is a high-quality hybrid foxtail millet. Based on the research of Zhang et al. [15] and previous experiments, the following treatments were set up: Jingu 21: 0 kg hm^{-2} (JGF1), 75 kg hm^{-2} (JGF2), 150 kg hm^{-2} (JGF3), 225 kg hm^{-2} (JGF4), 300 kg hm^{-2} (JGF5); Zhangza 10: 0 kg hm^{-2} (ZZF1), 75 kg hm^{-2} (ZZF2), 150 kg hm^{-2} (ZZF3), 225 kg hm^{-2} (ZZF4), 300 kg hm^{-2} (ZZF5). The experimental plots were organized into randomized

block designs with each treatment replicated thrice for accuracy and reliability, plot size of 5 m × 6 m, density of 300,000 plants hm^{-2}, row spacing of 50 cm, and nitrogen fertilizer of urea (N: 46.4%). The seeds were sown on 23th May in 2020 and 25th May in 2021, and the experimental crops were cultivated in strict adherence to the local agricultural technical protocols.

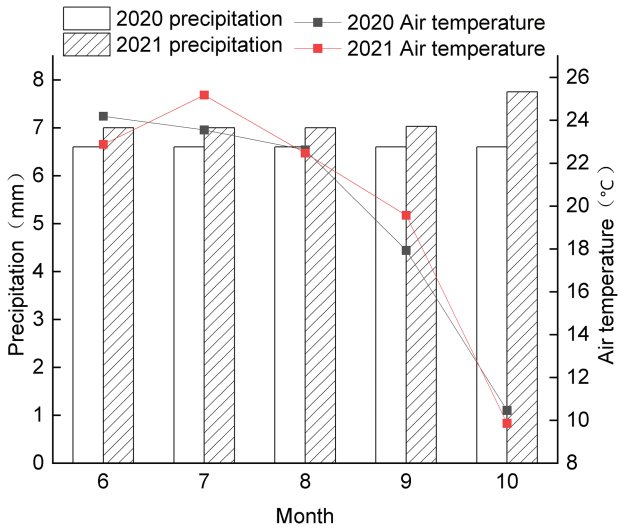

Figure 1: Air temperature and precipitation during foxtail millet growth period from June to October in 2020 and 2021

2.2 Indicators and Methods of Determination

2.2.1 Determination of Agronomic Characters

After maturity, 10 foxtail millet plants were taken from each plot for testing, and agronomic characters such as plant height, stem diameter, pitch number, and spike weight were measured. The measurement method was as follows: the length from the tillering node to the longest leaf tip of the plant was measured by a tape measure to be the plant height (cm); the diameter of the middle stem of the third section of the main stem of the millet was measured by a vernier caliper as the diameter of the millet stem (mm); the number of internodes from the bottom to the top stem of foxtail millet is the number of nodes; the spikes were cut from the stem of the spike and weighed by the analytical balance as the spike weight (g). The investigation standards of agronomic characters refer to foxtail millet Germplasm Resources Description Specification and Data Standard [26].

2.2.2 Yield Determination

Harvested by hand at maturity, removing 2 rows of side rows, harvested, threshed, and the seeds weighed for yield after natural air drying.

2.2.3 Determination of Protein, Fat, Starch, and Fiber Content

The protein components in the sample were separated following the method of Wieser et al. [27]. The absorbance of the sample at 595 nm was determined by ultraviolet spectrophotometer. The standard curves of different concentrations were fitted and the content of each protein component was calculated based on the formula: protein content (mg/g) = C × V/M. Here, C is the result calculated based on the standard curve; M is the actual weighed mass; V is the total volume of the extract.

The fat content was determined using the Soxhlet extraction method, in accordance with the national standard GB 5009.5-2016 [28]. A thoroughly mixed sample weighing between 2 to 5 g was placed into a

filter paper cartridge. This cartridge was then positioned in the extraction cylinder of the Soxhlet extractor. Anhydrous ether or petroleum ether was added until it filled two-thirds of the volume of the receiving flask, introduced from the upper end of the condenser tube of the extractor. The mixture was heated on a water bath, allowing for extraction at a rate of 6 to 8 cycles per hour for a duration of 6 to 10 h. At the conclusion of the extraction process, a drop of the extraction solution was obtained using a frosted glass rod. When the solvent in the receiving flask remained at approximately 1 to 2 mL, it was evaporated on the water bath and subsequently dried at 100°C ± 5°C for 1 h. The sample was then cooled in a desiccator for 30 min before being weighed. This process was repeated until a constant weight was achieved.

$$X = \frac{m1 - m0}{m2} * 100$$

In the formula: X—fat content in the sample (%);

$m1$—quality of bottles and fats received (g);

$m0$—quality of the receiving bottle (g);

$m2$—quality of sample (g).

The starch content was determined by visible spectrophotometry according to Zhuang et al. [29] using the starch content detection kit provided by Solarbio Company. 0.05 g of the sample was pulverized, 1 mL of the reagent was added and transferred to the EP tube. 80°C water bath for 30 min, centrifuge at 3000 g for 5 min, retain precipitate, then add 0.5 mL double distilled water water bath for gelatinization for 15 min, add reagent 2 after cooling, water bath for 15 min, shake 3–5 times, centrifuge at 8000 g for 15 min after cooling, take 0.2 mL of supernatant and 1 mL of working solution to EP tube, water bath at 95°C for 10 min, measure the absorbance value at 620 nm after cooling, and then draw a curve to calculate the starch content.

The fiber content was measured according to the national GB/T 5009.10-2003 [30]. Colorimetry was performed on a spectrophotometer at a wavelength of 620 urn, the extinction value was measured with the sample, and the corresponding cellulose content was found on the curve, and then all in accordance with the following formula calculates the cellulose content in the sample.

$$X = \frac{A * 10^{-6} * C * 100}{B}$$

In the formula: A—the cellulose content value found on the curve (μg);

B—sample weight (g);

10^{-6}—coefficient for converting μg into g;

C—sample dilution factor;

X—cellulose content in the sample (%).

2.2.4 Determination of Amino Acid Content

After collection, the samples were sent to Sanshu Biotechnology Co, Ltd., Shanghai, China for analysis. Various free amino acids were extracted and measured using the method of Kovacs et al. [31]. The instrument systems used for data acquisition mainly include ultra-high performance liquid chromatographs (Vanquish, UPLC, Thermo, Carlsbad, CA, USA) and high-resolution mass spectrometers (QExactive, Thermo, USA). The parameters of the liquid chromatography were set as follows: The column was Waters BEH C18 (50 * 2.1 mm, 1.7 μm). In the mobile phase, phase A is ultra-pure water containing 0.1% formic acid, and phase B is acetonitrile containing 0.1% formic acid. The flow rate was set to 0.5 mL/min. The column temperature was maintained at 55°C. The sample size was 1 μL.

The quantitative analysis was carried out by external standard method, the fitting curve was constructed by standard substances of different concentrations, and the data provided by the instrument was processed. Amino acid content (ug/g) = (C × V × F × Mw)/M. Molar content (nmol/mg) = (C × V × F)/M. Mw is the molecular weight of the amino acid. C is the concentration of the sample read by the instrument, in nmol/mL. V is the final constant volume of the sample, the unit is mL. F is the dilution ratio of the sample. M is the weighing mass of the sample in mg.

2.2.5 Determination of RVA

The RVA data were measured according to Qi et al. [32]. RVA data properties of starch were evaluated using (RVA-4) (Perten Instruments Australia, Macquarie Park, NSW, Austrilia). The starch sample was mixed with 25 mL distilled water to bring the total weight to 28 g. The starch was balanced at 50°C for 1 min, then heated at 12 °C/minute to 95°C and kept at 95°C for 2.5 min. It is then cooled to 50°C at a rate of 12 °C/min and held at 50°C for 2 min. Record peak viscosity (PV), trough viscosity (TV), breakdown value (BD), final viscosity (FV), setback (SB), peak time (PT), and pasting temperature (PTM).

3 Data Processing

SPSS 27.0 and Excel 2022 software were used for statistical analysis of experimental data, including one-way ANOVA, principal component analysis, correlation analysis and cluster analysis. At the level of $p < 0.05$, the significant difference between the treatment groups was tested by the least significant difference method. In addition, the Origin 2024 software is used for drawing. All indicator data are presented as three repeated mean ± standard errors.

4 Results and Analysis

4.1 Effects of Nitrogen Application Rate on Agronomic Characters of Different Foxtail Millet Genotypes

Different nitrogen application rates can have a significant effect on the agronomic characters of foxtail millet (Table 1). With the increase of nitrogen application rate, the plant height of different genotypes of foxtail millet showed a gradual upward trend in the two years and the JGF5 and ZZF5 treatments reached the maximum. In 2020, the stem diameter of Jingu 21 showed a gradual upward trend and the JGF5 treatment was the largest. The stem diameter of Zhangza 10 showed a trend of increasing first and then decreasing in both years and the ZZF4 treatment was the largest. There was no significant difference in the number of foxtail millet nodes between different genotypes in two years. In 2020, the spike weight of Jingu 21 showed a gradual upward trend and the JGF5 was the largest. In 2021, the spike weight of Jingu 21 showed a trend of increasing first and then decreasing and the JGF4 was the largest. The spike weight of Zhangza 10 showed a trend of increasing first and then decreasing in both years and the ZZF4 was the largest.

Table 1: Effects of nitrogen application on agronomic characters of different genotypes of foxtail millet

Year	Treatment	Plant height (cm)	Stem diameter (mm)	Pitch number	Spike weight (g)
2020	JGF1	168.00 ± 3.00^{d}	21.74 ± 0.55^{b}	12.33 ± 0.58^{a}	26.74 ± 0.37^{c}
	JGF2	176.67 ± 2.08^{c}	22.83 ± 0.69^{b}	12.00 ± 0^{a}	27.89 ± 0.46^{b}
	JGF3	179.33 ± 3.06^{c}	24.93 ± 1.23^{a}	12.67 ± 1.53^{a}	28.20 ± 0.23^{b}
	JGF4	190.33 ± 4.04^{b}	24.98 ± 0.76^{a}	12.67 ± 1.15^{a}	28.30 ± 0.15^{b}
	JGF5	197.00 ± 2.65^{a}	25.18 ± 0.64^{a}	12.67 ± 0.58^{a}	28.78 ± 0.18^{a}

(Continued)

Table 1 (continued)

Year	Treatment	Plant height (cm)	Stem diameter (mm)	Pitch number	Spike weight (g)
	ZZF1	161.67 ± 2.51^{e}	26.21 ± 0.30^{c}	12.33 ± 0.58^{a}	27.85 ± 0.18^{b}
	ZZF2	165.67 ± 1.53^{d}	27.34 ± 0.19^{b}	12.67 ± 0.58^{a}	28.52 ± 0.33^{b}
	ZZF3	169.33 ± 1.15^{c}	27.68 ± 0.41^{ab}	13.00 ± 0^{a}	30.13 ± 0.97^{a}
	ZZF4	172.67 ± 0.58^{b}	27.89 ± 0.40^{ab}	12.67 ± 0.58^{a}	31.08 ± 1.33^{a}
	ZZF5	176.67 ± 2.08^{a}	27.41 ± 0.27^{a}	13.00 ± 0^{a}	28.55 ± 0.91^{b}
2021	JGF1	173.00 ± 1.73^{d}	22.45 ± 0.39^{c}	12.33 ± 0.58^{a}	26.66 ± 0.18^{c}
	JGF2	178.67 ± 1.53^{d}	23.95 ± 0.07^{b}	12.67 ± 0.58^{a}	27.13 ± 0.50^{c}
	JGF3	186.00 ± 3.61^{c}	26.03 ± 0.54^{a}	12.67 ± 0.58^{a}	28.96 ± 0.32^{a}
	JGF4	193.33 ± 2.08^{b}	26.29 ± 0.30^{a}	13.00 ± 0^{a}	28.67 ± 0.32^{a}
	JGF5	198.33 ± 2.08^{a}	26.26 ± 0.14^{a}	13.00 ± 0^{a}	27.97 ± 0.57^{b}
	ZZF1	173.67 ± 3.06^{c}	26.71 ± 0.22^{d}	12.33 ± 0.58^{a}	28.24 ± 0.27^{c}
	ZZF2	167.67 ± 0.58^{bc}	27.27 ± 0.17^{c}	12.67 ± 0.58^{a}	29.13 ± 0.30^{b}
	ZZF3	169.67 ± 4.93^{b}	28.54 ± 0.27^{a}	12.33 ± 0.58^{a}	31.56 ± 0.46^{a}
	ZZF4	177.33 ± 1.53^{a}	28.77 ± 0.21^{a}	12.67 ± 0.58^{a}	31.84 ± 0.50^{a}
	ZZF5	178.67 ± 2.89^{a}	28.18 ± 0.10^{b}	13.33 ± 0.58^{a}	29.56 ± 0.63^{b}
Average		176.63	26.00	12.63	28.41
Coefficient of variation (%)		6.39	7.70	4.60	4.40

Note: Within the same column, distinct letters signify notable disparities in treatments for the same variety at the $p < 0.05$ level.

4.2 Effect of Nitrogen Application Rate on Yield of Different Genotypes of Foxtail Millet

Different nitrogen application rates can have a significant effect on the yield of foxtail millet (Fig. 2). The yield of different genotypes of foxtail millet increased first and then decreased with the increase of nitrogen application rate. In 2020 and 2021, the highest yield of JGF3 treatment was 6005.75 and 6109.8 kg hm^{-2}, respectively, which increased by 13.07% and 11.57% compared with the control. The highest yield of ZZF4 treatment was 7083.5 and 6892.95 kg hm^{-2}, respectively, which increased by 11.46% and 6.82% compared with the control.

4.3 Effects of Nitrogen Application Rate on Quality of Different Genotypes of Foxtail Millet

Different nitrogen application rates can have a significant effect on the quality of foxtail millet (Table 2). With the increase of nitrogen application rate, the protein and fat content of different genotypes of foxtail millet increased first and then decreased. The protein content of JGF3 treatment was the highest in both years and the protein content of ZZF4 treatment was the highest in both years. The fat content of JGF4 and ZZF4 treatments was the highest in both years. The starch content of JGF5 treatment was the highest in two years and the starch content of ZZF3 treatment was the highest in two years. In 2020, the fiber content of JGF4 treatment was the highest. In 2021, the fiber content of Jingu 21 was the highest without nitrogen application and the fiber content of ZZF4 treatment was the highest in both years.

4.4 Effect of Nitrogen Application Rate on Amino Acid Content of Different Genotypes of Foxtail Millet

Different nitrogen application rates can have a significant effect on the amino acid content of foxtail millet (Tables 3 and 4). With the increase of nitrogen application rate, the lysine, phenylalanine,

isoleucine, arginine, aspartic, glutamic, glycine and proline content of different genotypes of millet showed a trend of increasing first and then decreasing in two years. Compared with the control, the contents of essential amino acids (threonine, lysine, valine, tryptophan, isoleucine, phenylalanine, methionine) treated by JGF4 and ZZF4 increased by 3.53% and 3.56% (Jingu 21) and 1.57% and 1.19% (Zhangza 10), respectively. The content of non-essential amino acids (aspartic, glycine, arginine, proline, glutamic, serine, histidine, cysteine) treated by JGF4 increased by 4.02% and 4.05% in two years, respectively. In 2020, the content of non-essential amino acids treated by ZZF4 increased by 1.33% and the content of non-essential amino acids in Zhangza 10 was the highest without nitrogen application in 2021. The contents of 15 amino acids in the grains of different genotypes were different. The highest was glutamic and proline content, followed by aspartic acid and serine content, and the lowest was lysine content.

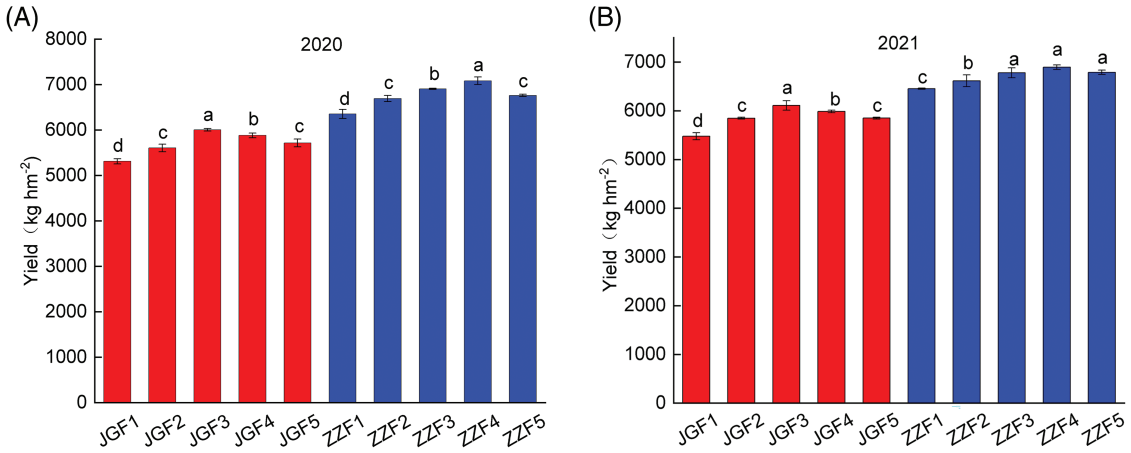

Figure 2: Effects of different nitrogen application rates on foxtail millet yield of different genotypes of foxtail millet. (A) Yeld of different genotypes of foxtail millet in 2020 (B) Yield of different genotypes of foxtail millet in 2020

Table 2: Effects of different nitrogen application rates on grain quality of different genotypes of foxtail millet (%)

Year	Treatment	Protein content	Fat content	Starch content	Fiber content
2020	JGF1	11.23 ± 0.12^{c}	4.01 ± 0.04^{b}	71.00 ± 0.12^{a}	1.22 ± 0.04^{bc}
	JGF2	11.76 ± 0.14^{b}	4.49 ± 0.03^{a}	70.46 ± 0.15^{b}	1.28 ± 0.06^{ab}
	JGF3	11.99 ± 0.04^{a}	4.42 ± 0.06^{a}	70.16 ± 0.09^{c}	1.20 ± 0.05^{bc}
	JGF4	11.98 ± 0.03^{a}	4.44 ± 0.06^{a}	70.07 ± 0.09^{c}	1.33 ± 0.05^{a}
	JGF5	11.63 ± 0.09^{b}	3.98 ± 0.04^{b}	70.65 ± 0.10^{b}	1.17 ± 0.02^{c}
	ZZF1	11.63 ± 0.05^{b}	4.33 ± 0.05^{c}	70.79 ± 0.06^{b}	1.74 ± 0.06^{c}
	ZZF2	11.05 ± 0.04^{d}	3.78 ± 0.01^{e}	70.85 ± 0.04^{b}	1.70 ± 0.02^{c}
	ZZF3	11.21 ± 0.06^{c}	4.21 ± 0.09^{d}	71.12 ± 0.08^{a}	1.96 ± 0.01^{b}
	ZZF4	11.83 ± 0.02^{a}	4.71 ± 0.03^{a}	70.10 ± 0.03^{d}	2.13 ± 0.05^{a}
	ZZF5	11.59 ± 0.06^{b}	4.45 ± 0.02^{b}	70.27 ± 0.06^{c}	1.93 ± 0.03^{b}

(Continued)

Table 2 (continued)

Year	Treatment	Protein content	Fat content	Starch content	Fiber content
2021	JGF1	11.10 ± 0.14^{c}	4.01 ± 0.03^{c}	71.03 ± 0.04^{a}	1.47 ± 0.31^{a}
	JGF2	11.81 ± 0.35^{a}	4.46 ± 0.51^{a}	70.44 ± 0.01^{c}	1.26 ± 0.04^{ab}
	JGF3	11.91 ± 0.09^{a}	4.38 ± 0.03^{b}	70.24 ± 0.09^{d}	1.16 ± 0.05^{b}
	JGF4	11.88 ± 0.09^{a}	4.47 ± 0.02^{a}	70.14 ± 0.08^{d}	1.38 ± 0.04^{ab}
	JGF5	11.52 ± 0.06^{b}	3.97 ± 0.02^{c}	70.75 ± 0.06^{b}	1.17 ± 0.03^{b}
	ZZF1	11.74 ± 0.06^{a}	4.35 ± 0.03^{c}	70.71 ± 0.06^{b}	1.69 ± 0.05^{c}
	ZZF2	11.17 ± 0.08^{c}	3.80 ± 0.02^{e}	70.74 ± 0.07^{b}	1.76 ± 0.06^{c}
	ZZF3	11.11 ± 0.04^{c}	4.16 ± 0.02^{d}	71.19 ± 0.03^{a}	1.95 ± 0.1^{b}
	ZZF4	11.74 ± 0.07^{a}	4.68 ± 0.01^{a}	70.17 ± 0.05^{d}	2.16 ± 0.02^{a}
	ZZF5	11.44 ± 0.08^{b}	4.43 ± 0.02^{b}	70.44 ± 0.07^{c}	1.95 ± 0.05^{b}
Average		11.57	4.28	70.57	1.58
Coefficient of variation (%)		2.75	6.30	0.52	22.34

Note: Within the same column, distinct letters signify notable disparities in treatments for the same variety at the $p < 0.05$ level.

Table 3: Effect of nitrogen application on amino acid composition of different genotypes of foxtail millet in 2020 (μg/mg)

Amino acid		JGF1	JGF2	JGF3	JGF4	JGF5	ZZF1	ZZF2	ZZF3	ZZF4	ZZF5
EAA (Essential amino acids)	Thr	40.67^{b}	41.67^{a}	42.00^{a}	42.00^{a}	41.67^{a}	40.67^{a}	39.00^{c}	40.00^{b}	41.00^{a}	41.00^{a}
	Val	51.67^{b}	51.67^{b}	52.00^{b}	53.00^{a}	52.33^{ab}	51.00^{a}	39.00^{a}	40.00^{a}	41.00^{a}	41.00^{a}
	Trp	15.00^{a}	14.33^{b}	15.00^{a}	15.00^{a}	15.00^{a}	14.67^{a}	15.00^{a}	14.00^{b}	14.00^{b}	14.00^{b}
	Lys	15.67^{c}	16.00^{c}	16.00^{c}	17.33^{a}	16.67^{ab}	17.67^{d}	15.00^{c}	19.67^{ab}	20.67^{a}	18.67^{bc}
	Phe	59.33^{c}	60.67^{b}	61.67^{a}	62.00^{a}	61.67^{a}	57.57^{b}	54.67^{d}	57.00^{c}	59.00^{a}	58.00^{a}
	Ile	37.67^{b}	38.67^{ab}	38.67^{ab}	39.00^{a}	37.67^{b}	38.67^{ab}	37.00^{d}	38.00^{c}	39.00^{a}	38.33^{bc}
	Met	34.00^{d}	35.33^{c}	36.00^{b}	36.00^{b}	37.33^{a}	31.00^{a}	30.67^{ab}	30.00^{b}	31.33^{a}	31.00^{a}
NEAA (Non-essential amino acids)	Arg	29.33^{b}	31.00^{a}	30.67^{a}	31.33^{a}	28.67^{b}	33.33^{b}	29.33^{c}	34.00^{b}	36.00^{a}	34.00^{b}
	His	22.00^{a}	22.00^{a}	22.00^{a}	22.00^{a}	22.00^{a}	22.00^{a}	21.00^{a}	22.00^{a}	22.00^{a}	22.00^{a}
	Asp	78.33^{c}	79.33^{bc}	80.00^{ab}	81.33^{a}	79.00^{bc}	80.00^{b}	77.33^{c}	79.67^{b}	82.00^{a}	80.33^{b}
	Cys	23.00^{b}	22.33^{c}	23.00^{b}	23.00^{b}	25.00^{a}	19.67^{a}	20.00^{a}	19.00^{b}	18.00^{c}	19.00^{b}
	Glu	209.00^{c}	214.67^{b}	219.67^{a}	219.33^{a}	212.67^{b}	213.33^{ab}	203.67^{c}	206.00^{c}	214.00^{a}	211.00^{b}
	Gly	26.00^{b}	26.67^{ab}	26.67^{ab}	27.33^{a}	26.00^{b}	28.00^{b}	26.00^{c}	28.00^{b}	29.67^{a}	28.33^{b}
	Pro	81.33^{c}	82.33^{bc}	83.33^{ab}	84.33^{a}	82.33^{bc}	79.00^{bc}	76.33^{d}	77.67^{cd}	80.67^{a}	30.33^{ab}
	Ser	53.33^{c}	54.67^{b}	56.00^{a}	55.67^{a}	56.00^{a}	51.67^{a}	51.00^{a}	49.67^{b}	51.00^{a}	51.33^{a}
EAA		254.01	258.34	261.34	264.33	262.34	251.25	230.34	238.67	246.00	242.00
NEAA		522.32	533.00	541.34	544.32	531.67	527.00	504.66	516.01	533.34	476.32

Note: Thr: Threonine; Val: Valine; Trp: Tryptophan; Lys: Lysine; Phe: Phenylalanine; Ile: Isoleucine; Met: Methionine; Arg: Arginine; His: Histidine; Asp: Aspartate; Cys: Cysteine; Glu: Glutamic; Gly: Glycine; Pro: Proline; Ser: Serine. Within the same column, distinct letters signify notable disparities in treatments for the same variety at the $p < 0.05$ level.

Table 4: Effect of nitrogen application on amino acid composition of different genotypes of foxtail millet in 2021 (μg/mg)

Amino acid		JGF1	JGF2	JGF3	JGF4	JGF5	ZZF1	ZZF2	ZZF3	ZZF4	ZZF5
EAA (Essential amino acids)	Thr	40.44^{d}	41.56^{b}	41.89^{a}	41.89^{a}	41.19^{c}	40.89^{a}	39.44^{c}	40.00^{c}	41.00^{a}	40.33^{b}
	Val	51.22^{b}	51.89^{a}	52.00^{a}	52.44^{a}	52.11^{a}	51.44^{a}	49.56^{d}	50.89^{b}	51.11^{ab}	50.33^{c}
	Trp	14.22^{a}	14.67^{a}	15.00^{a}	14.44^{a}	14.92^{a}	14.78^{a}	15.00^{a}	14.11^{b}	14.00^{b}	14.00^{b}
	Lys	17.00^{ab}	17.00^{ab}	15.44^{c}	17.33^{a}	17.08^{ab}	17.67^{c}	15.78^{d}	19.67^{ab}	20.78^{a}	18.89^{b}
	Phe	59.44^{c}	60.78^{b}	61.33^{ab}	61.67^{a}	61.19^{a}	58.44^{a}	55.44^{d}	56.67^{c}	58.67^{a}	57.44^{b}
	Ile	37.22^{b}	38.67^{a}	38.33^{a}	38.67^{a}	37.19^{b}	39.00^{a}	37.33^{c}	37.67^{bc}	38.78^{a}	38.11^{b}
	Met	33.89^{d}	35.33^{c}	36.11^{b}	35.89^{b}	37.11^{a}	31.33^{a}	30.89^{b}	30.00^{c}	31.22^{ab}	31.00^{ab}
NEAA (Non-essential amino acids)	Arg	29.89^{bc}	30.78^{b}	29.78^{c}	31.44^{a}	28.78^{c}	33.56^{b}	30.11^{c}	33.67^{b}	36.00^{a}	34.00^{b}
	His	21.89^{a}	22.00^{a}	22.00^{a}	22.11^{a}	22.00^{a}	22.00^{a}	21.00^{c}	21.78^{b}	22.00^{a}	21.89^{ab}
	Asp	78.44^{b}	79.56^{a}	79.56^{a}	80.56^{a}	78.47^{b}	80.67^{ab}	78.44^{d}	79.22^{c}	81.33^{a}	80.00^{b}
	Cys	22.67^{c}	22.67^{c}	23.56^{b}	22.89^{c}	24.78^{a}	19.67^{ab}	20.00^{a}	19.22^{b}	18.22^{d}	18.67^{c}
	Glu	205.56^{c}	216.33^{a}	218.00^{a}	216.56^{a}	210.56^{b}	215.89^{a}	205.56^{d}	204.11^{d}	211.89^{b}	208.56^{c}
	Gly	26.44^{c}	26.67^{b}	26.22^{bc}	27.22^{a}	26.00^{c}	28.22^{b}	26.44^{c}	28.11^{b}	29.33^{a}	28.22^{b}
	Pro	80.67^{c}	82.56^{b}	83.33^{ab}	83.89^{a}	81.47^{c}	79.56^{a}	77.22^{b}	77.44^{b}	80.11^{a}	79.22^{a}
	Ser	52.67^{c}	55.00^{b}	56.00^{a}	55.00^{b}	55.19^{b}	52.33^{a}	51.44^{b}	49.44^{d}	50.78^{ab}	50.44^{c}
EAA		253.43	259.01	260.1	262.33	260.79	253.55	243.44	249.01	255.56	211.99
NEAA		518.23	535.57	538.45	508.23	527.25	531.90	510.21	512.99	529.66	521.00

Note: Thr: Threonine; Val: Valine; Trp: Tryptophan; Lys: Lysine; Phe: Phenylalanine; Ile: Isoleucine;Met: Methionine; Arg: Arginine; His: Histidine; Asp: Aspartate; Cys: Cysteine; Glu: Glutamic; Gly: Glycine; Pro: Proline; Ser: Serine. Within the same column, distinct letters signify notable disparities in treatments for the same variety at the $p < 0.05$ level.

4.5 Effects of Nitrogen Application Rate on RVA of Different Genotypes of Foxtail Millet

The amount of fertilizer can have a significant effect on the RVA of foxtail millet (Fig. 3). With the increase of nitrogen application rate, the PV, TV, BD and FV of Jingu 21 showed a trend of decreasing first and then increasing and JGF3 treatment was the lowest. The PV, TV, BD and FV of Zhangza 10 decreased gradually with the increase of nitrogen application rate, and ZZF5 treatment was the lowest. There was no significant difference in the SB and PT of different genotypes of foxtail millet. The PTM of Jingu 21 showed a trend of increasing first and then decreasing and the PTM of Zhangza 10 showed a trend of decreasing first and then increasing.

4.6 Correlation Analysis and Principal Component Analysis

The correlation analysis of millet quality index and amino acid index (Fig. A1) showed that protein content was significantly positively correlated with most amino acid content (Fig. A1A), among which the positive correlation with glutamic content was the strongest (r = 0.98), and the negative correlation with starch content was the strongest (r = −0.97). Starch content was significantly negatively correlated with other amino acid contents except cysteine content. Starch content had the strongest positive correlation with PV (r = 0.91) and the strongest negative correlation with glutamate content (r = −0.97). The yield had the strongest positive correlation with the protein content and tryptophan content (r = 0.91), and the strongest negative correlation with starch content (r = −0.89).

Protein content was positively correlated with most amino acids (Fig. A1B). Glutamic content had the strongest positive correlation (r = 0.96) and the strongest negative correlation with starch content (r = −0.75). The starch content had the strongest positive correlation with cysteine content (r = 0.59) and the strongest negative correlation with proline content (r = −0.79). The yield had the strongest positive correlation with fiber content (r = 0.89) and the strongest negative correlation with cysteine content (r = −0.75).

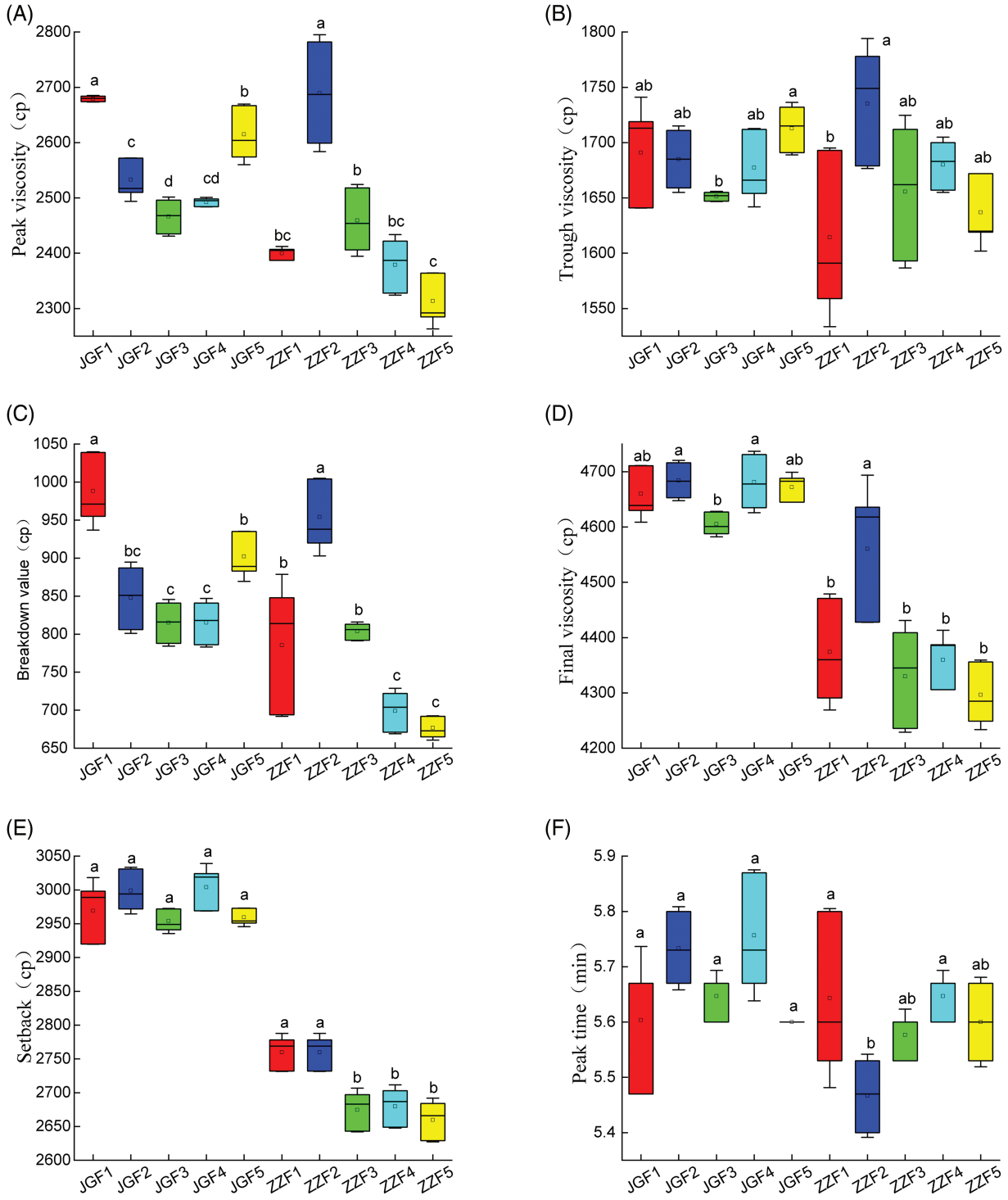

Figure 3: (Continued)

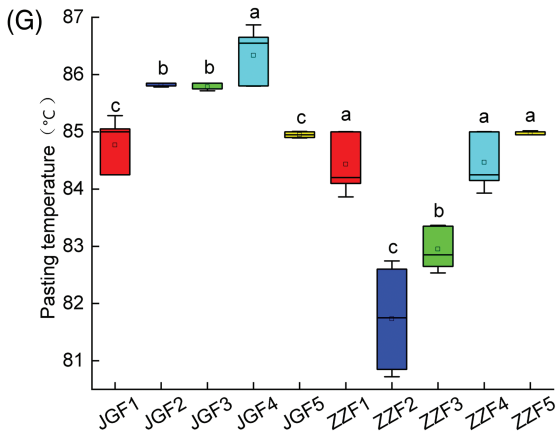

Figure 3: Effect of nitrogen application on RVA of different genotypes of foxtail millet. (A) PV (B) TV (C) BD (D) FV (E) SB (F) PT (G) PTM

Principal component analysis was performed on the quality index and amino acid content of foxtail millet (Fig. 4). The results showed that in 2020, the contribution rates of PC1 and PC2 of Jingu 21 accounted for 57.0% and 20.2%, respectively, and the cumulative contribution rate was 77.2% (Fig. 4A). The contribution rate of Zhangza 10 was 68.2% and 16.9%, respectively, and the cumulative contribution rate was 85.1% (Fig. 4B). The contents of cysteine, tryptophan, methionine, serine, phenylalanine, aspartic acid, threonine and protein in Jingu 21 and Zhangza 10 were significantly positively correlated with PC1 and PC2.

In 2021, the contribution rate of PC1 and PC2 of Jingu 21 accounted for 54.9% and 25.9%, respectively, and the cumulative contribution rate was 80.8% (Fig. 4C). The contribution rate of Zhangza 10 was 59.7% and 26.6%, respectively, and the cumulative contribution rate was 86.3% (Fig. 4D). Aspartic, proline, isoleucine, threonine and glutamic content in Jingu 21 and Zhangza 10 were significantly positively correlated with PC1 and PC2.

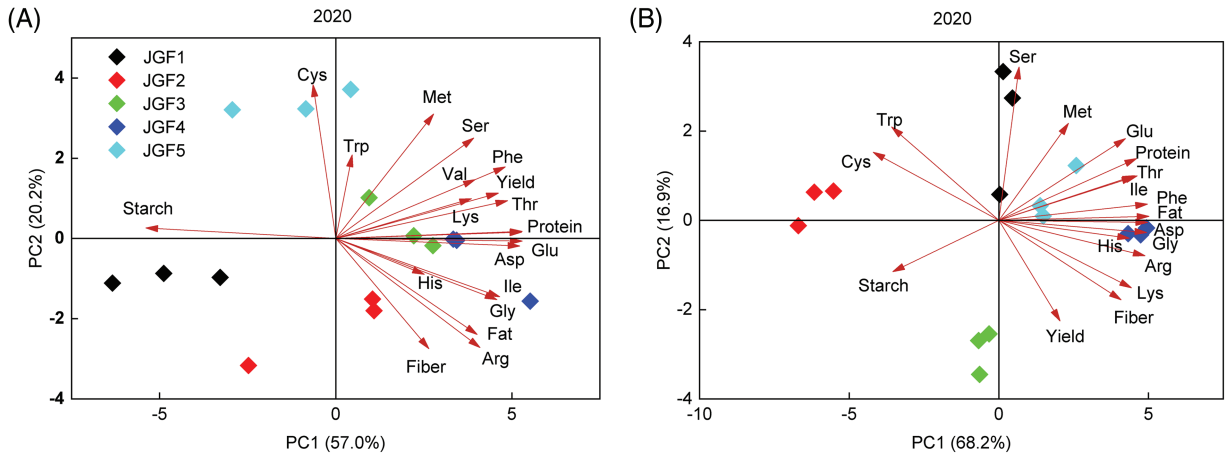

Figure 4: (Continued)

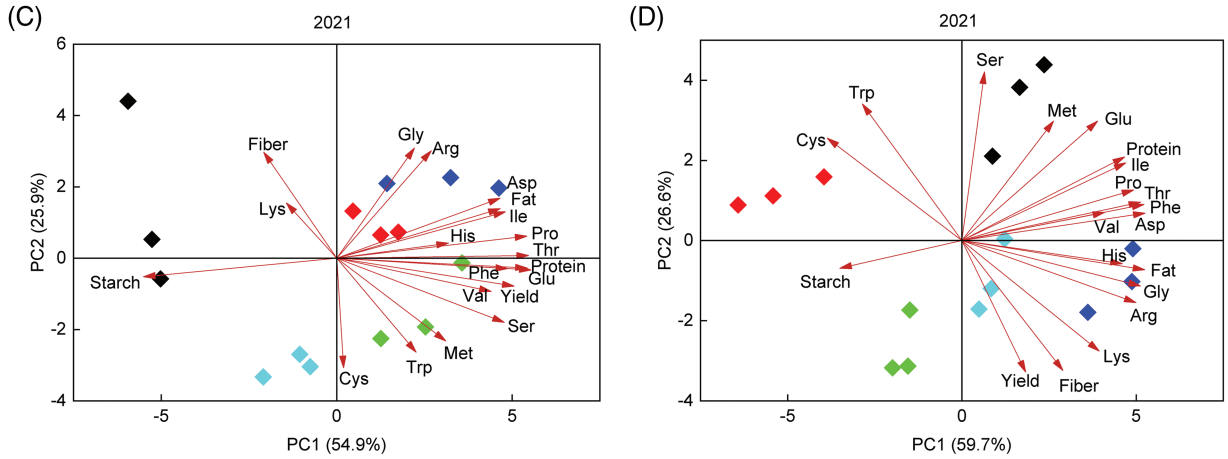

Figure 4: Principal component analysis of grain quality and amino acid content of different genotypes. (A) 2020 Jingu 21 (B) 2020 Zhangza 10 (C) 2021 Jingu 21 (D) 2021 Zhangza 10

4.7 Cluster Analysis

The agronomic characters, quality, amino acid content and RVA indexes of different genotypes of foxtail millet were analyzed by cluster analysis (Fig. 5). The results show that when the distance between classes is 15. The above indicators are divided into five categories. The first category included aspartic content, isoleucine content, fat content, histidine content, PT, valine content, tryptophan content, proline content, phenylalanine content, glutamic content and protein content. The second category included node number, spike weight, lysine content, glycine content, arginine content, stem diameter, yield and fiber content. The third category includes starch content. The fourth category includes PV, BD and TV. The fifth category included cysteine content, methionine content, serine content, FV, SB, plant height and tryptophan content.

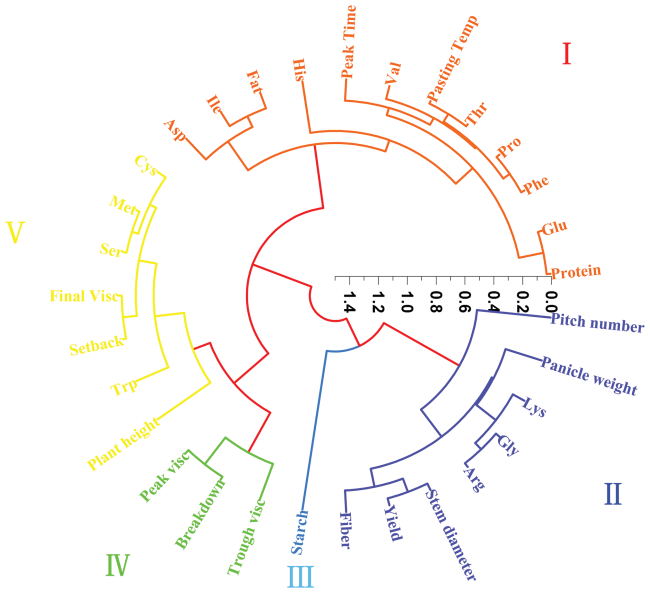

Figure 5: Cluster analysis of agronomic characters, yield, quality, amino acid content and RVA of different genotypes of foxtail millet

5 Discussions

5.1 Effects of Nitrogen Fertilizer on Agronomic Traits and Yield

Nitrogen application effectively maintained the number of spikes harvested per unit area of wheat [33]. It increased the spike weight of wheat by 42.6% [34] and increased the plant height and stem diameter of quinoa [8]. In this study, it was found that the plant height of different genotypes of foxtail millet increased in two years after nitrogen application. The stem diameter of different genotypes of foxtail millet was thickest with nitrogen application of 225 kg hm^{-2} and the spike weight of Zhangza 10 was heaviest with nitrogen application of 225 kg hm^{-2}. The heaviest spike weight of Jingu 21 at 300 kg hm^{-2} of nitrogen application in 2020 and 225 kg hm^{-2} of nitrogen application in 2021 may be due to the lower precipitation in 2020, which resulted in damage to the floral organs affecting pollination, degradation of glumes and reduction in the number of grains in the spike and the percentage of fruit set [35].

Increased application of nitrogen fertilizer increased spring maize kernel yield by 6.80% to 24.66% [34]. This study showed that the highest yield (6907.25 kg hm^{-2}) was recorded in Zhangza 10 when nitrogen was applied at a rate of 225 kg hm^{-2} and Zhangza 10 had the highest yield (6109.8 kg hm^{-2}) when nitrogen was applied at a rate of 150 kg hm^{-2}. Reasonable application of nitrogen fertilizer will have higher root cortical aeration tissues, promote soil excavation and deep soil nitrogen acquisition, thereby increasing the amount of nitrogen absorption and utilization efficiency [36] and increase the re-assimilation ability of leaf NH^{4+} [37]. It also promotes and assimilate accumulation [10] and dry matter transport from vegetative organs to seeds [11], thereby increasing reservoir capacity [38] and coordinating the source/reservoir ratio [39], promoting the drive to drive nitrogen transport from vegetative organs to seeds [40]. In addition, it can effectively regulate the morphological development and biomass distribution of millet during growth [41]. Nitrate nitrogen can induce the expression of cytokinin synthesis genes *IPT3* and *CYP735A* [42]. This study showed that the yield of different genotypes of foxtail millet was reduced at a nitrogen application rate of 300 kg hm^{-2}. Under the condition of high nitrogen, the root activity and effective root absorption area were improved, decreasing photosynthesis rate, unbalanced carbon and nitrogen metabolism and function of root cells may have changed [43,44]. In addition, the activity of nitrate reductase (NR), a key enzyme in nitrogen metabolism, was reduced and nitrogen uptake was reduced [36]. As a result, the nitrogen retention in leaves and stem sheathing at maturity will increase, leading to the growth of stems and leaves without efficient flow to ear [45] and the proportion of dry matter allocated to economic organs will decrease [46]. This resulted in the decrease of seed setting rate and panicle number [47], which was not conducive to the improvement of cash crop yield [48].

5.2 Effect of Nitrogen Fertilizer on Quality

Reasonable application of nitrogen fertilizer had a significant regulatory effect on grain quality [17]. Sun et al. [49] showed that protein content had the largest loading in the first principal component contribution Nitrogen application could enhance protein content in rice [50] as well as soybean [51]. Nitrogen application in low-fertility wheat fields has a greater effect on the enhancement of protein factors, and nitrogen application in medium-high-fertility wheat fields can increase protein factors [49]. Moderately increasing nitrogen levels could increase the contents of soluble protein and free amino acid in peanut organs, and increase the activities of nitrate reductase, glutamine synthetase and other nitrogen assimilases [52]. It can also increase the storage of nitrogen in the vegetative organs of plants before flowering and delay their senescence [53], which is conducive to the uptake and assimilation of nitrogen and promotes the accumulation of nitrogen in the plant [54], and increase the synthesis of proteins in the seeds [52]. The results of this study showed that the protein content of different genotypes of foxtail millet showed a trend of increasing and then decreasing, and the protein content of different genotypes of foxtail millet decreased at a nitrogen application rate of 300 kg hm^{-2}. This may be because too much nitrogen fertilizer is applied during the vegetative growth stage, resulted in excessive nitrogen absorption and excessive

nutrient consumption, which is not conducive to the carbon and nitrogen metabolism of plants in the grouting stage [55]. As a result, more sugars are converted into organic acids by enzymatic conversion, which affects the transfer of photosynthetic products to grains [56], resulting in excessive crop populations [55], intensifying competition for source pools [57] and reducing grain quality [58].

Han et al. showed that the crude fat content of foxtail millet showed an increasing and then decreasing trend with the increase of nitrogen application and the fat content was highest at a nitrogen application rate of 180 kg hm^{-2} [59]. The starch content of rice was the lowest when the nitrogen rate was 400 kg hm^{-2} [60]. We found that the starch content of different genotypes of grains decreased at a nitrogen application rate of 225 kg hm^{-2}, which may be due to the fact that starch is affected by the environment, cultivar, and nitrogen application [61] and also because the relative improvement of nitrogen metabolism during the filling process suppresses carbon metabolism [62]. The relative improvement in nitrogen metabolism during ensiling suppresses carbon metabolism [62], nitrate in leaves regulates nitrate reductase activity, which can cause more sugars to undergo enzymatic conversion to organic acids, affecting starch accumulation [56] and higher levels of nitrate also lead to unfavorable starch synthesis due to the inhibition of nitrate reductase on the subunit of ADP-glucose pyrophosphorylase [63].

Bullman et al. showed that the phenylalanine content increased proportionally with the increase in nitrogen fertilizer supply [64]. We found that the phenylalanine content of different genotypes of foxtail millet increased first and then decreased after nitrogen application. It may be due to the fact that the amino acids in roots can be used as precursors for plant hormone synthesis and can regulate nitrogen transport from source to sink [65]. Too little secretion of roots under nitrogen deficiency conditions may change the direction of assimilate transport, and the secretion of basic amino acids will lead to a decrease in grain amino acid content [66]. Wang et al. showed that the contents of aspartic, serine, glutamic, glycine, isoleucine, tryptophan, histidine and arginine were significantly positively correlated with the amount of nitrogen application [67]. Nokerbekova et al. showed that nitrogen application could increase the content of lysine, phenylalanine and valine in sweet sorghum [68]. In this study, it was found that the content of lysine, phenylalanine and valine in sweet sorghum increased when the nitrogen rate was 225 kg hm^{-2}. This may be attributed to the increased application of nitrogen fertilizer, which improved the assimilation indexes of crop nitrogen metabolism, such as nitrogen accumulation in the ground and seeds and the activity of glutamine synthetase in leaves, promoted the absorption and assimilation of nitrogen by plants [69] and enabled the grains to accumulate sufficient NH^{4+}−N [36]. At the same time, increasing the activity of Nitrate Reductase and Glutamine Synthetase [54] promotes nitrogen metabolism [69]. Carbohydrate-derived carbon skeleton and nitrogen contribute to the biosynthesis of amino acids [70]. As the main subunit of all protein molecules, the content of amino acids increases with the increase of protein content [68]. The single form of NO^{3-} −N can also promote the increase of GS activity in leaves, promote the enhancement of nitrogen metabolism, and enhance nitrogen metabolism and amino acid synthesis and transformation [71].

Matsumoto et al. [72] showed that essential amino acids such as isoleucine and valine in millet had a significant enhancement effect on protein synthesis. This study showed that protein content showed a significant positive correlation with the content of most amino acids (Fig. A1). It may be because nitrogen application increased the activity of nitrate reductase (NR) and glutamine synthetase (GS) [52], promoted the absorption and assimilation of nitrogen and increasing protein content [73]. As the main transport form of nitrogen assimilation in plants [74]. amino acid content is the main subunit of all protein molecules [68], which is involved in the absorption and assimilation of nitrogen and is associated with the synthesis and degradation of proteins in organs [39].

5.3 Effect of Nitrogen Fertilizer on RVA

Lei et al. [75] used principal component analysis and cluster analysis to determine the protein content, BD, PV, FV, and SB as the key indicators for evaluating the quality of steamed grain rice. Rice with better eating quality generally showed high PV and BD, while the opposite was true for poor-quality rice [62]. Hu et al. showed that the BD of rice decreased with the increase in nitrogen application [76], and the PV and FV of buckwheat decreased [77]. Excessive nitrogen application inhibited the FV, SB and TV of millet [17]. In this study, we found that the PV, TV and BD of Jingu 21 showed a tendency to decrease and then increase. It may be because nitrogen application reduced the content of amylose [50] and amylopectin [78]. The lower the amylose content, the softer and more sticky after cooking [79] and the higher the PT [58]. Amylopectin has more branch structure, which makes rice have a soft taste [80]. The more the content of short and medium chains of amylopectin, the better the cooking and eating quality [81].

In this study, we found that the PV, FV and BD of Zhangza 10 showed a tendency of increasing and then decreasing. Proteins affect the amount of water absorbed by the rice, thus affecting the pasting behavior of starch [82]. The protein content of grain increased with the increase of nitrogen application [83]. Higher protein content and higher disulfide bonds limit the starch/water interaction, limiting the adhesion and eventually losing the protein's network effect [82] and thus reduce viscosity [17]. The taste quality of rice is closely related to the amino acid content of rice [84], amino acid content is positively correlated with the PV and negatively correlated with the FV [85]. In this study we found that the amino acid content was negatively correlated with the PV, probably due to differences in stratification, and it remains to be investigated whether it is affected by the inherent characteristics of the variety [85].

6 Conclusion

The plant height of different genotypes of foxtail millet was proportional to the amount of nitrogen application. The contents of protein, fat, isoleucine, arginine, aspartic acid, glutamic acid and glycine in different genotypes of foxtail millet increased first and then decreased under nitrogen application. The PV, TV, BD and FV of Jingu 21 decreased first and then increased, while that of Zhangza 10 decreased gradually. The analysis of correlation results showed that the protein content of different genotypes of millet was significantly positively correlated with most amino acid content. The positive correlation between the yield of Jingu 21 and the content of protein and tryptophan was the strongest ($r = 0.91$). The positive correlation between the yield of Zhangza 10 and fiber content was the strongest ($r = 0.89$). Under the experimental conditions, the optimal nitrogen application rates of Jinguu 21 and Zhangza 10 were 150 and 225 kg hm^{-2}, respectively. It provides a theoretical basis for rational fertilization and high-yield and high-quality cultivation techniques of foxtail millet.

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Availability of Data and Materials: All the data supporting the findings of this study are included in this article.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest to report regarding the present study.

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Appendix A

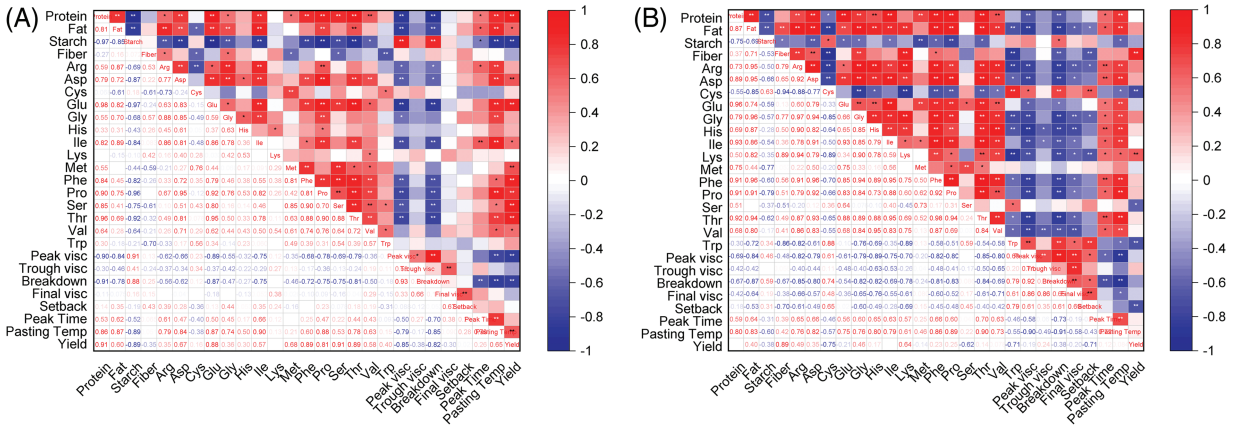

Figure A1: Correlation of quality, amino acid content and RVA in different genotypes of foxtail millet. (A) Jingu 21 (B) Zhangza 10. Note: *p <= 0.05, **p <= 0.01. Red indicates a positive correlation. Blue indicates a negative correlation. The lighter the color, the weaker the correlation