FOXTAIL MILLET (*SETARIA ITALICA* (L.) P. BEAUVOIS) QUALITY RESPONSE TO FERTILIZER LEVELS, HERBICIDE, AND SELENIUM

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Abstract. Foxtail millet (*Setaria italica* (L.) P. Beauvois) is a high-nutrition food source. It is commonly consumed in Africa, Asia, Central America, and South America. The key to popularize high-quality foxtail millet in accordance with local conditions is quantifying the impacts of cultivation conditions on its quality. Here, we determined the effects of different fertilizer levels nitrogen (N), tetraphosphorus decaoxide (P_2O_5), and potassium oxide (K_2O), tribenuron-methyl (TBM) herbicide, and *selenium* (Se) on the quality of foxtail millet Jingu 54 using a quadratic general rotation combination design. The first principal component, which could explain 57.41% of the total variance of grain quality, was chosen as the comprehensive quality of Jingu 54 via principal component analysis. The effects of fertilizer levels and Se on comprehensive quality of Jingu 54 were significant ($P < 0.05$), except for TBM. The effects of N × K_2O, N ×TBM, and K_2O × TBM interactions reached significance for the comprehensive quality of Jingu 54 ($P < 0.05$). The grain quality of Jingu 54 was predict by regression equation ($P = 0.0048$, $R^2 = 0.8451$). Recommended cultivation conditions are 108.31 kg ha^{-1}, 94.80 kg ha^{-1}, 105.03 kg ha^{-1}, 88.08 g ha^{-1}, and 18.48 g ai ha^{-1} for N, P_2O_5, K_2O, Se, and TBM, respectively. The maximum predicted comprehensive quality of Jingu 54 was 56.85. This study lays a theoretical foundation for achieving high quality Jingu 54 in the field.
Keywords: *Jingu 54, comprehensive quality, principal component analysis, response surface methodology, tribenuron-methyl herbicide*

Introduction

Foxtail millet (*Setaria italica* (L.) P. Beauvois) is known as the first of five cereals grain has high nutritional value (Sachdev et al., 2021). It is second only to wheat and maize in dry farming of north China and characterized by small genome, short growth cycle and tolerance to drought stress (Jones and Liu, 2009; Yang et al., 2012; Veeranagamallaiah et al., 2008). Some studies have investigated the effects of cultivation conditions on crop quality in wheat (Zòrb et al., 2018; Xia et al., 2019), maize (Chilimba, et al., 2012; Guo et al., 2020), and rice (Chen and Chen, 2019; Huang,

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2020), but few have examined the cultivation conditions on quality of foxtail millet. Moreover, existing studies have focused on the relationship between individual quality indicators of foxtail millet and individual cultivation conditions by using statistical methods such as simple correlation analysis, which lacks comprehensiveness and is insufficient at explaining the complex interaction between different factors (Powers et al., 2020).

Principal component analysis (PCA) is a widely used statistical method for reducing variable dimensionality. Actually, as a multivariate correlation method, PCA disintegrates a few of inter-correlated variables into smaller sets of clusters which are composed of variables with lower or no degree of correlation (Anju and Banerjee, 2012). Similarly, as a statistical modeling and analysis technique, response surface methodology (RSM) is very popular in optimizing multiple variables (Mao et al., 2018; Montgomery, 2008). Hence, RSM has widespread application prospects in processing biotechnology, such as protein extraction, biofuel production, fermentation, and enzyme immobilization (Feng and Zhang, 2020; Abdel-Fattah et al., 2002; Liu et al., 2003; Adinarayana and Ellaiah, 2002). Moreover, RSM had been used on seedlings, and canola (*Brassica napus* L.) (Dong et al., 2011; Koocheki et al., 2014) culture, Chinese white poplar (Populus tomentosa Carr.). However, its application in optimization of foxtail millet cultivation conditions is barely explored.

Improper fertilization levels and fertilization methods will reduce the efficiency of fertilizers and also increase environmental pollution, thus hampering the quality of foxtail millet. The effects of nitrogen (N), phosphorus (P), potassium (K), and their interaction on the quality of millet have rarely been reported. In addition, exogenous selenium (Se) can increase the content of lutein in tomato (Pezzarossa et al., 2013). It was reported that foliar application Se increased the concentration of iron and zinc in colored-grain wheat (Xia et al., 2019). Whether Se addition also benefits the quality of foxtail millet and affects yellow pigment content in foxtail millet grains is unknown. Finally, while the effect of herbicides on yield components has been reported (Guo et al., 2019; Suganthi et al., 2013; Robinson et al., 2013), its impact on the quality of foxtail millet is not well-understood.

The foxtail millet variety used in this study is Jingu 54, which was breeded from Jingu 21 (a dominant foxtail millet in China) and Jingu 20 (high yielding and drought tolerance). Jingu 54 show a specific characteristic in high yielding, quality and Se enrichment. However, the sown area of Jingu 54 in Shanxi province is about 0.5 million acres. The object of this study was to (1) obtain a comprehensive quality measurement of foxtail millet Jingu 54 by PCA, and (2) assess the effects of fertilizer levels, tribenuron-methyl (TBM) herbicide, and Se on quality of foxtail millet. We carried out field experiments based on 5-factor-5-level quadratic general rotary combination design. Based on the experimental results, the optimal cultivation conditions were determined, thus providing a guidance for future quality improvement of foxtail millet.

Materials and methods

Experimental site and materials

Field experiments were carried out in the Agricultural Experimental Station of Shanxi Agricultural University in Taigu County, Jinzhong City, Shanxi, China. An average annual rainfall of 462.9 mm and 9.9 °C of the annual average temperature are

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owned by the study site which has a temperate continental climate. The meteorological data of the experimental locations throughout the growth season of foxtail millet (May-September) in 2019 are shown in *Table 1*.

Table 1. *Meteorological data of the experimental sites during growing season of foxtail millet (May-September) in 2019*

Month	Precipitation (mm)	Temperature (℃)		≥ 20 ℃ accumulated temperature (℃)	Sunshine hours (h)
		Min	Max		
5	30.1	2.6	35.1	329.1	277.0
6	41.6	12.7	35.2	708.0	224.2
7	63.8	12.1	37.0	768.8	229.9
8	42.2	10.5	33.8	706.8	214.0
9	73.9	7.4	34.2	218.4	217.0

The test variety was the foxtail millet Jingu 54. Strawberries were cultivated for rotation with foxtail millet Jingu 54 in the experimental site. Soil texture was red sandy loam and with a medium organic matter content (17.9 g kg^{-1}). Soil texture was characteristic of red sandy loam and a medium organic matter content (17.9 g kg^{-1}). Soil pH was 8.2. Soil involves original soil-available K_2O (93 mg kg^{-1}), N (76 mg kg^{-1}), and P_2O_5 (29 mg kg^{-1}) (Soil Survey Staff, 2014).

Experimental design

Quadratic general rotation combination design with a 5-factor-5-level was implemented to optimize fertilizer levels (N, P_2O_5, and K_2O), herbicide, and Se. The five independent factors (x_1 to x_5) were studied at five different levels (coded: −2, −1, 0, + 1, and + 2, respectively) (*Table 2*), at the central point with six repetitions and two replications at the axial and factorial points, respectively (*Table 3*). The recommended applicable dose of the herbicide was form 13.5 g ha^{-1} to 22.5 g ha^{-1}. A total of 32 treatment combinationswith three replications were run in a completely randomized block design and protection rows were set around the experimental site. Each plot was 3 m × 6 m in size. The plant density and the apllied row distance were 330,000 plants per hectare and 23 cm, respectively. The best combination in 2019 was chosen for the verification test in 2020, planting on May 18, with a plot size of 6 m ×6 m = 36 m^2 and 6 plots.

Table 2. *Levels and codes of five experimental factors*

Code	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Se (g ha^{-1})	Tribenuron-methyl (g ai ha^{-1})
–2	0	0	0	0	0
–1	69	36	37.5	60	20
0	138	72	75	120	40
1	207	108	112.5	180	60
2	276	144	150	240	80
△j	69	36	37.5	60	20

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Table 3. *Program and experimental results of quadratic general rotation design for quality traits comprehensive quality of foxtail millet*

No	x_1	x_2	x_3	x_4	x_5	Protein (%)	Fat (%)	Yellow pigment (mg kg^{-1})	Folic acid (ug g^{-1})	Se (ug kg^{-1})	Alkali	Gel	Amylose	PC1
1	1	1	1	1	1	9.9	5.28	11.48	1.76	48.59	2.6	95.43	16.46	50.72
2	1	1	1	-1	-1	10.96	5.6	12.25	1.79	48.59	3.31	99.7	17.76	54.15
3	1	1	-1	1	-1	10.47	4.5	10	1.86	49.15	2.98	85	14	43.88
4	1	1	-1	-1	1	10.15	5.28	11.56	1.83	50.07	2.76	95.43	16.6	50.58
5	1	-1	1	1	-1	9.95	4.96	11.13	1.89	49.15	2.64	91.15	15.88	48.07
6	1	-1	1	-1	1	10.55	5.49	12.2	1.85	48.04	3.04	98.16	17.67	53.29
7	1	-1	-1	1	1	9	4.99	11.01	1.87	50.26	2	91.5	15.69	47.51
8	1	-1	-1	-1	-1	10.72	4.83	10.6	1.8	48.96	3.15	89.44	14.99	46.93
9	-1	1	1	1	-1	9.05	5.45	12.22	1.83	46	2.04	97.65	17.7	52.95
10	-1	1	1	-1	1	9.41	5.28	11.39	1.72	48.78	2.27	95.43	16.32	50.40
11	-1	1	-1	1	1	9.63	5.23	11.67	1.83	46.93	2.42	94.74	16.79	50.87
12	-1	1	-1	-1	-1	10.66	5.46	11.89	1.77	48.22	3.11	97.82	17.15	52.76
13	-1	-1	1	1	1	9.44	4.68	10.31	1.84	49.52	2.29	87.39	14.52	44.97
14	-1	-1	1	-1	-1	9.08	5.5	12.02	1.75	46.93	2.05	98.33	17.37	52.86
15	-1	-1	-1	1	-1	9.52	5.15	11.46	1.86	49.15	2.35	93.72	16.43	49.57
16	-1	-1	-1	-1	1	10.01	5.21	11.44	1.76	46.56	2.67	94.4	16.4	50.65
17	-2	0	0	0	0	9.63	5.41	12.07	1.81	46	2.42	97.14	17.45	52.73
18	2	0	0	0	0	10.5	4.91	10.83	1.87	51	3	90.47	15.38	47.18
19	0	-2	0	0	0	9.6	5.32	11.91	1.78	48.78	2.4	95.94	17.18	51.33
20	0	2	0	0	0	10.8	5.59	11.99	1.7	48.41	3.2	99.53	17.31	53.74
21	0	0	-2	0	0	11.15	5.19	11.29	1.82	49.33	3.44	94.23	16.15	50.20
22	0	0	2	0	0	9.11	5.47	12.14	1.84	47.11	2.07	97.99	17.56	52.79
23	0	0	0	-2	0	10.01	5.4	12.27	1.9	47.11	2.67	96.97	17.78	52.78
24	0	0	0	2	0	10.39	5.05	11.52	1.89	49.89	2.93	92.35	16.54	49.14
25	0	0	0	0	-2	10.09	5.65	12.37	1.84	46	2.73	100.38	17.95	54.86
26	0	0	0	0	2	9.68	5.17	11.38	1.83	49.33	2.45	93.89	16.29	49.59
27	0	0	0	0	0	11.62	5.65	12.4	1.8	48.22	3.75	100.38	18.01	54.99
28	0	0	0	0	0	11.81	5.62	12.6	1.83	49.52	3.87	99.87	18.34	54.77
29	0	0	0	0	0	10.61	5.74	12.59	1.81	48.78	3.07	101.58	18.31	55.30
30	0	0	0	0	0	11.37	5.72	12.73	1.86	48.78	3.58	101.24	18.56	55.59
31	0	0	0	0	0	12	5.49	12.25	1.81	48.59	4	98.16	17.76	53.78
32	0	0	0	0	0	11.18	6	13	1.79	47.48	3.45	105	19	57.99

x_1 – N, x_2 – P_2O_5, x_3 – K_2O, x_4 –Se, x_5 –tribenuron-methyl, PC1-the first principal component

Uniform seeds were sown on May 6, 2019 using a 2BX-3 small seeder (College of Engineering, Shanxi Agricultural University). Seedlings with at least three fully expanded leaves were thinned in accordance with plant spacing. One half of N was applied as a basal fertilizer, and the other half as a top-dressing at the jointing-booting stage. K_2O and P_2O_5 were sereved as supplement natural fertilizers. Fertilizers included urea (N 46%), triple superphosphate (P_2O_5 42%), and sulfate of potash (K_2O 50%). Different dosages of TBM herbicide (10%) and water as control were applied on foxtail millet Jingu 54 seedlings at five-leaf stage. Different dosages of selenite selenium (Na_2SeO_3) were sprayed on the leaf surface during the filling stage. Plots were irrigated and prepared by rotary tillage before sowing. Weed control was undertaken by inter-tillage twice during the experimental period.

The response surface design, which (1) significantly reduces the number of experiments (n = 32) without loss of information when compared to the 5-factor-5-level

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full factorial design (n = 5^5); (2) simultaneously analyzes the effects of linear, quadratic, and interaction terms of target factors; and (3) obtains a prediction model with a curved surface, is the main advantage of the current study. Although the orthogonal design may decrease the number of trials as well, it only examines the isolated experimental sites one at a time. In contrast, the response surface design enables for ongoing analysis of the experimental levels throughout the optimization process. The latter approach may produce equal variances at experimental sites with equal distance to the center point, overcoming the limitation of orthogonal design methods and improving optimization accuracy. Using a response surface design, optimal conditions for comprehensive quality of foxtail millet can be found. The research took into account all interactions between fertilizer levels, Se, and herbicide, yielding more accurate findings than prior studies that just looked at single-factor impacts (Yang et al., 2018).

Measurement

The gel consistency was determined using a method described by Tran et al. (2011) with slight modifications. Finely grounded foxtail millet grain (100 mg) in duplicate were putted into 13 mm × 100 mm tubes which were respectively filled with 200 μL ethyl alcohol (95%), 0.025% thymol blue, 2.5 mL 0.15 N KOH. The tubes were mixed using a Vortex Genie mixer and then placed in a vigorously boiling water bath for 8 min, held at room temperature for 5 min, and cooled in an ice water bath for 20 min. After this, tubes were laid horizontally on a light box on top of graphing paper. Measure the distance that the gel migrated in the tube after 1 h.

The amylose content in foxtail millet starches was determined according to the procedure of the American Association of Cereal Chemists (2000).

The alkali digestion value was measured from twenty intact and fully mature foxtail millet grains of uniform size that were placed in a Petri dish. 10 mL 1.7% potassium hydroxide (KOH) solution was added to each Petri dish until the grains were completely submerged. The grains remained dispersed to facilitate decomposition and covered with a lid. The samples were placed in a 30 °C thermostat incubator (BIC-300, Shanghai Boxun Industry & Commerce Co., Ltd. Medical Equipment Factory) for 6 h and the decomposition of each grain was then observed. The degree of decomposition was recorded according to *Table 4*. The alkali digestion value was calculated as follows: $A = \sum (G \times N)/7$, where A is alkali digestion value, G is grade of each grain, and N is number of grains at the same degree.

Table 4. *Standard for alkali digestion value of foxtail millet grain samples*

Standard	Degree of decomposition	Definition
1	Grain unchanged	White core in grain
2	Grain expanded	White core in grain, with powdery ring
3	Grain expanded, with incomplete or narrow ring	White core in grain, with flocculent or nebulous ring
4	Grain enlarged, with complete and wide ring	Cotton white core in grain, with nebulous ring
5	Grain cracked, with complete and wide ring	Cotton white core in grain, with clear ring
6	Grain partially dispersed and dissolved, blended with ring	Cloud white core in grain, with no ring
7	Grain completely dispersed	Both core and ring disappeared in grain

The crude protein and fat content of grain samples were determined by the methods described in Association of Official Analytical Chemists (AOAC, 2000). Percentage

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crude protein (CP%) was determined by the Kjeldahl method and calculated based on the percentage N (N%) obtained: CP% = N% × 6.25. Fat content (%) was detected by the Soxhlet extraction technique.

Total 500 mg finely grounded foxtail millet grain (was acid-digested with 5 mL 70% superior-grade pure nitric acid and 2 mL 30% hydrogen peroxide to measure Se. With ultrapure water (18.2 MΩ cm^{-1}) the digested samples were diluted to 25 mL. We analyzed the concentration of Se in the digestion solution by atomic fluorescence spectrometry (AFS-933, Beijing Jitian Instrument Co., Ltd.).

Folic acid was determined in exactly 2.5 g foxtail millet grain. Sample was weighed into 25 mL 0.1 mol L^{-1} *potassium dihydrogen phosphate* (KH_2PO_4) and incubated in a 50 °C thermostat water bath for 8 h. The sample was then centrifuged in a high-speed refrigerated centrifuge (Neofuge 15R, Shanghai Lishen Scientific Equipment Co., Ltd.) at 5000 rpm for 10 min. The supernatant was collected, followed by addition of 0.5 g aniline-treated activated C. The mixture was thoroughly vortexed and then heated to boiling in a water bath (DK-S26, Shanghai Jinghong Experimental Equipment Co., Ltd.) for 10 min. The sample was filtered and the supernatant was discarded. The residue was washed five times with 7 mL 3% ammonia: 70% ethanol. The eluate was evaporated and concentrated to 5 mL, followed by addition of 1 mL 2% glacial acetic acid. Thereafter, 0.04% potassium permanganate ($KMnO_4$) was added dropwise until the color of the solution no longer changed. Furthermore, 3% hydrogen peroxide (H_2O_2) was added until the color of $KMnO_4$ faded. The solution was diluted to a volume of 10 mL. The fluorescence intensity was measured using a fluorescence spectrophotometer (BioSpectrometer fluorescence, Germany Eppendorf) at Ex = 370 nm and Em = 443 nm (Shao et al., 2014).

The content of grain yellow pigment was determined using the method described by Ning et al. (2016). The absorbance was measured at 450 nm using a spectrophotometer (UV-2400, Shanghai Sunny Hengping Scientific Instrument Co., Ltd.).

Statistical analysis

All treatments were performed at triplicate and results presented as mean values. We collected the crops (2 m × 2 m) in the middle of each plot for lab analysis to minimize the marginal impact. Using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA), all data analysis were processed. Comprehensive quality of foxtail millet was obtained by principal component analysis (PCA). Quadratic regression model was used for optimizing the cultivation conditions of foxtail millet for fertilizer levels, herbicide and Se. Effects of factorial interactions on grain quality were based on contour plots with Graphpad prism 8.0.

Results

PCA of quality indicators of foxtail millet

To analyze the relationship between the cultivation conditions and grain quality of foxtail millet, PCA was used. Only two eigenvalues were > 1 and explained 82.34% of the total variance in grain quality (*Table 5*). As a result, the first two eigenvalues were picked out for further analysis; other small but non-zero eigenvalues were discarded to set up some potential contributing source factors. From the rotated component matrix (*Table 6*), it was noticeable that all quality parameters were clearly illustrated by the

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first two principal components. The first principal component (PC1) explained 57.41% of the variance including protein, fat, folic acid, gel consistency, amylase, alkali digestion value, yellow pigment, and Se. Obviously, the second principal component (PC2), accounting for 24.92% of the variance, had high loadings for protein, Se, and alkali digestion value. Consequently, PC1 was chosen to represent the comprehensive quality of foxtail millet.

Table 5. *Eigenvalues, contribution, and cumulative contribution of the correlation matrix of indicators for comprehensive quality of foxtail millet*

Principal component	**Eigenvalue**	**Contribution proportion (%)**	**Cumulative (%)**
F1	4.5926	57.4080	57.4080
F2	1.9943	24.9285	82.3365
F3	0.9334	11.6670	94.0035
F4	0.4571	5.7140	99.7175
F5	0.0225	0.2818	99.9993
F6	0.0000	0.0004	99.9997
F7	0.0000	0.0002	99.9999
F8	0.0000	0.0001	100.0000

Table 6. *Eigenvectors of selected principal components for comprehensive quality of foxtail millet*

Quality indexes	**Eigenvector**	
	F1	**F2**
protein	0.2246	0.6032
fat	0.4583	-0.0798
Yellow pigment	0.4522	-0.0800
Folic acid	-0.1509	0.1444
Se	-0.2182	0.4753
Alkali digestion value	0.2243	0.6033
Gel consistency	0.4582	-0.0803
Amylose	0.4522	-0.0790

Effects of fertilizer levels, Se and hericide on grain comprehensive quality of foxtail millet

Except for TBM, the effects of fertilizer levels and Se on comprehensive quality of foxtail millet were significant ($P < 0.05$). The effect on comprehensive quality was the most significant for Se ($P = 0.0014$), followed by N ($P = 0.0135$), K_2O ($P = 0.0180$), and P_2O_5 ($P = 0.0341$) (*Table 7*). In the design range, the effects of the five factors on comprehensive quality showed a parabolic trend, yet with various patterns (*Fig. 1*). With increasing K_2O, comprehensive quality first increased rapidly; when K_2O exceeded 0.5, the quality showed a slow decline. With increasing P_2O_5 and N, comprehensive quality climbed slightly and then gradually decreased. With increasing Se and TBM, comprehensive quality increased slightly and then plummeted.

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Table 7. *Test of significance for the coefficients of regression equation for comprehensive quality of foxtail millet*

Term	Sum of square	DF	Mean square	Partial correlation	F-value	P-value
x_1	18.3750	1	18.3750	-0.6631	8.6328	0.0135*
x_2	12.4416	1	12.4416	0.5891	5.8452	0.0341*
x_3	16.4011	1	16.4011	0.6418	7.7054	0.0180*
x_4	38.4054	1	38.4054	-0.7882	18.0434	0.0014**
x_5	6.7416	1	6.7416	-0.4728	3.1673	0.1027
$x_1 \times x_1$	60.8736	1	60.8736	-0.8498	28.5992	0.0002**
$x_2 \times x_2$	18.5659	1	18.5659	-0.6650	8.7225	0.0131*
$x_3 \times x_3$	32.6839	1	32.6839	-0.7633	15.3553	0.0024**
$x_4 \times x_4$	41.4913	1	41.4913	-0.7995	19.4932	0.0010**
$x_5 \times x_5$	22.3593	1	22.3593	-0.6989	10.5047	0.0079**
$x_1 \times x_2$	1.8225	1	1.8225	-0.2687	0.8562	0.3746
$x_1 \times x_3$	25.0000	1	25.0000	0.7186	11.7453	0.0057**
$x_1 \times x_4$	2.6082	1	2.6082	-0.3166	1.2254	0.2919
$x_1 \times x_5$	25.8064	1	25.8064	0.7241	12.1242	0.0051**
$x_2 \times x_3$	1.9600	1	1.9600	0.2779	0.9208	0.3579
$x_2 \times x_4$	1.0712	1	1.0712	0.2092	0.5033	0.4928
$x_2 \times x_5$	0.0016	1	0.0016	-0.0083	0.0008	0.9786
$x_3 \times x_4$	1.5006	1	1.5006	-0.2454	0.7050	0.4190
$x_3 \times x_5$	14.2884	1	14.2884	-0.6156	6.7129	0.0251*
$x_4 \times x_5$	0.1190	1	0.1190	0.0711	0.0599	0.8174

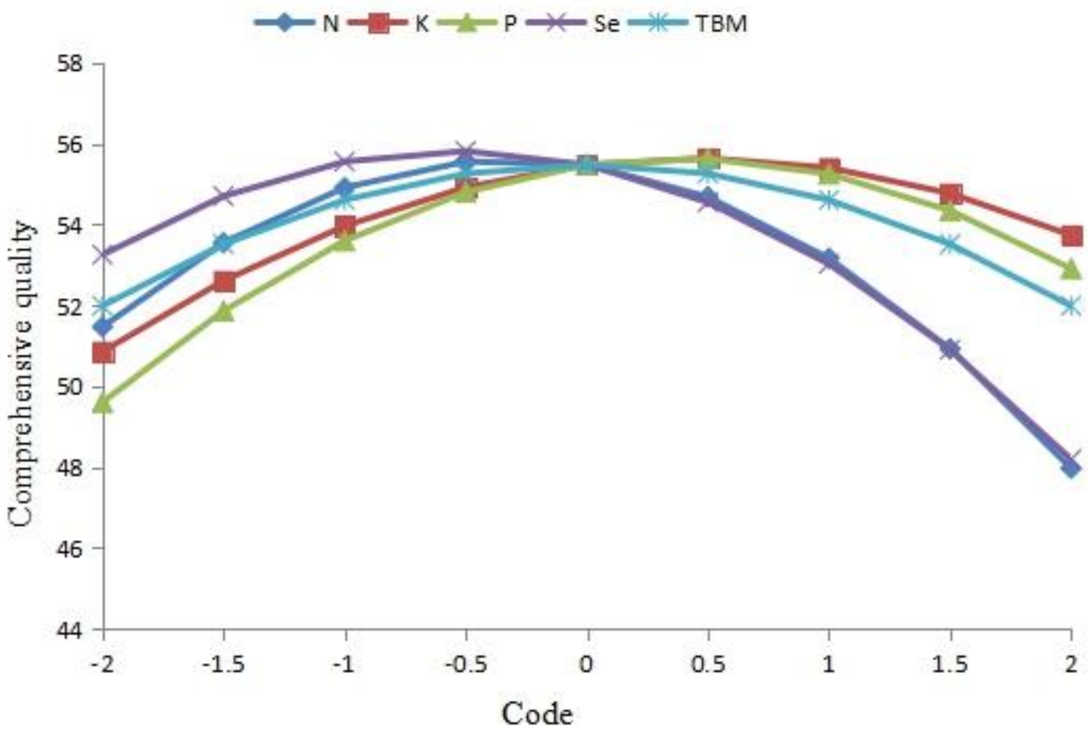

Figure 1. *Effects of single factors on comprehensive quality of Jingu 54*

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The effects of N × K_2O, N ×TBM, K_2O ×TBM interactions reached statistical significance for comprehensive quality of foxtail millet (*Table 7*). The contour plots (*Fig. 2*) were mapped to show the interactive effects of N × K_2O, N ×TBM, K_2O ×TBM.

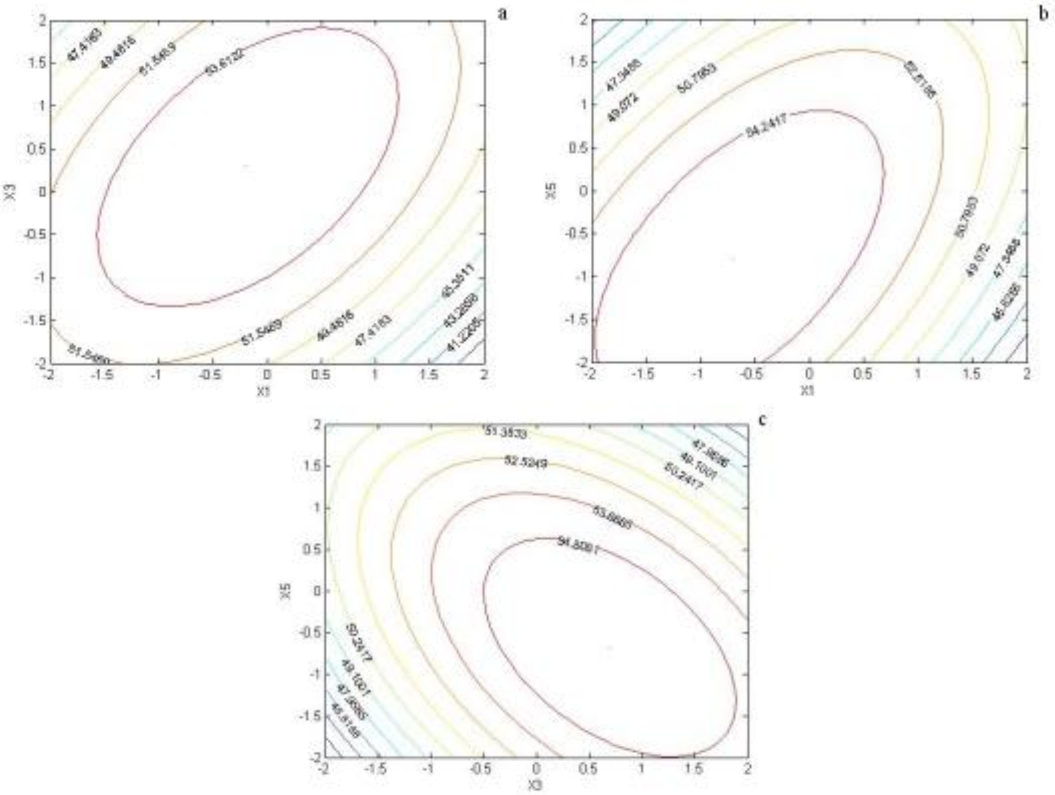

Figure 2. *Significant effects of factor interactions on comprehensive quality of Jingu 54. a: N × K_2O; b: N ×TBM; c: K_2O × TBM*

When P_2O_5, TBM and Se were fixed at the zero level, the comprehensive quality of foxtail millet decreased and then increased with the increase of N and K_2O (*Fig. 2a*). The effect of N was greater at higher K_2O levels than at lower K_2O levels. At lower N levels, enriching K_2O caused little change in the comprehensive quality; at higher N levels, comprehensive quality first increased rapidly and then decreased slowly with the increase of K_2O.

When P_2O_5, K_2O, and Se were fixed at the zero level, increasing N caused a dramatic comprehensive quality increase at high TBM levels, but not at low TBM levels. With continued increase in N application, there was a downward trend in comprehensive quality (*Fig. 2b*). When TBM levels was increased at lower N levels, the comprehensive quality first increased slowly and then decreased rapidly. An opposite trend was observed in the comprehensive quality with increasing TBM levels at higher N levels.

When N, P_2O_5, and Se were fixed at the zero level, comprehensive quality began to increase quickly and then dropped as TBM levels were increased at an appropriate (or lower) level of K_2O. When TBM levels were reduced at higher K_2O levels, comprehensive quality was rapidly increased after a slow reduced (*Fig. 2c*).

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Response of comprehensive quality of foxtail millet to fertilizer, herbicide and Se

To obtain a practicable and effective model, the actual responses should be suitable for existing linear, two factor interactions, cubic, or quadratic model. The quadratic model was selected and validated in the analysis of variance. The results included Fisher variation ratio (*F* value), probability value (*P* value), lack of fit, and adjusted *R*-squared (R_{Adj}^2). The second-order polynomial equation for grain quality follows:

$$y = 55.48 - 0.88x_1 + 0.72x_2 + 0.83x_3 - 1.27x_4 - 0.53x_5 - 1.44x_1^2 - 0.80x_2^2 - 1.06x_3^2 - 1.19x_4^2 - 0.87x_5^2 - 0.34x_1x_2 + 1.25x_1x_3 - 0.40x_1x_4 + 1.27x_1x_5 + 0.35x_2x_3 + 0.26x_2x_4 - 0.01x_2x_5 - 0.31x_3x_4 - 0.95x_3x_5 + 0.09x_4x_5 \quad \text{(Eq.1)}$$

where *y* is the predicted response of grain quality and x_1, x_2, x_3, x_4, and x_5 are coded values of N level, P_2O_5 level, K_2O level, Se level, and TBM level, respectively.

The statistical significance of *Equation 1* was evaluated by an *F*-test. The F-test of the response surface variances showed that the equation (R^2_{adj} was 0.8451) was statistically valid ($P < 0.05$), and lack of fit item test was not significant ($P > 0.05$). The optimal values of the selected factors in their respective coded values were: x_1 = -0.4303, x_2 = 0.6334, x_3 = 0.8007, x_4 = -0.5320, and x_5 = -1.0761. Accordingly, the actual N level, P_2O_5 level, K_2O level, Se level, and TBM level were 108.31 kg ha^{-1}, 94.80 kg ha^{-1}, 105.03 kg ha^{-1}, 88.08 g ha^{-1}, and 18.48 g ai ha^{-1}, respectively. The maximum predicted comprehensive quality of foxtail millet was 56.85. Multivariate quadratic regression indicated that the relationship between the five factors and comprehensive quality of Jingu 54 was significant, which can be used for forecast of production.

Verification of Jingu 54 cultivation conditions

The theoretically optimum combination was not included in the 32 experimental treatment combinations developed in 2019. Six plots with an area of 6 m × 6 m were chosen in May 2020 to further validate the optimum conditions of 108.31 kg ha^{-1}, 94.80 kg ha^{-1}, 105.03 kg ha-1, 88.08 g ha^{-1}, and 18.48 g ha^{-1} for N, P_2O_5, K_2O, selenium, and tribenuron-methyl, respectively. The comprehensive quality of Jingu 54 was 57.46, and the error with the projected value was 1.07 percent, which was extremely near to the model value, confirming the model's practicability.

Discussion

Cultivation conditions have a significant impact on the grain quality of foxtail millet. Existing research on the connection between cultivation circumstances and foxtail millet quality has often relied on simple correlation analysis, which cannot show the intricate interaction of internal regulation (Powers et al., 2020). Multiple statistical analyses were used in this study to gradually show that fertilizer levels, herbicide, and Se influence the grain quality of foxtail millet.

Both the quality indicators of foxtail millet and the growing circumstances were multidimensional factors in the research. As a result, determining optimum cultivation conditions influencing grain quality of foxtail millet proved challenging. PCA was initially utilized in the research to get the main component reflecting the comprehensive quality of foxtail millet. Furthermore, the most important cultivation factors influencing foxtail millet quality were identified, and an optimum connection model was developed

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using regression analysis. The optimum growing conditions were established, and the impacts of one-factor and two-factor interaction on comprehensive quality of foxtail millet were investigated. The above-mentioned progressive system analysis is also a quantitative comparison study of the development trend in a dynamic process. This technique overcomes the drawbacks of utilizing single indicators and methodologies, having limited and dispersed cultivation circumstances, and a lack of thorough research and assessment of the crop-cultivation connection. As a result, the results reached here are more trustworthy and may better represent the real circumstances, which meet the requirements of this research. Furthermore, the findings provide a logical decision-making foundation for future research and analysis of foxtail millet.

Foxtail millet grain quality is a multifaceted characteristic that includes nutritional quality, as well as cooking and eating quality (Suman et al., 2015). Based on the PCA findings, we chose PC1 to represent the comprehensive quality of foxtail millet since it contributed 57.41 percent of the total variance. This component comprised nutritional quality (protein, fat, yellow pigment, folic acid, and Se) as well as cooking and eating quality (gel consistency, amylase, alkali digestion value), and therefore accurately reflected the foxtail millet quality.

Reasonable fertilization settings may enhance crop quality while reducing nutrient loss and avoiding possible environmental issues (Wang et al., 2011). Our findings revealed that single factors N, P, and K had a substantial impact on the comprehensive quality of Jingu 54, and that the quality rose initially and then declined as fertilization increased (Li, 2008). This may be because enhanced soil fertility raised the pace of grain filling, delayed the appearance of the filling phase, extended the filling time, and accelerated the accumulation of nitrogen, phosphorus, and assimilates in grains, all of which are beneficial to grain quality formation (Li, 2008). Nitrogen and potassium may boost photosynthetic capacity, enhance pigments that absorb light energy, improve carbohydrate synthesis and transformation, and boost grain nutritional quality. However, overuse of a particular nitrogen fertilizer resulted in a decrease in quality (Wang et al., 2011). Tang et al. (2019) obtained similar results while researching the potential function of nitrogen fertilizer in rice quality regulation. Guo et al. (2020) demonstrated that judicious nitrogen fertilizer application may enhance the concentration of certain nutritional components in maize kernel and improve its quality. Zӧrb et al. (2018) demonstrated that nitrogen fertilizer aided protein accumulation in wheat. Chen and Chen (2019) discovered that phosphate fertilizer may enhance the amount of starch, crude protein, and amino acids in rice, as well as boost phosphorus absorption in rice and straw. Results indicated that the impact of N, P, and K on foxtail millet quality was at the following sequence N > P > K, which was inconsistent with previous finding on beta-carotene and lutein of foxtail millet (Dong et al., 2018). The explanation for the discrepancy may be because the research was more thorough in its assessment of the plant's overall quality, including lutein.

Appropriate nitrogen, phosphorus, and potassium ratios may significantly enhance crop growth and development, accelerate the accumulation and transit of photosynthetic products after anthesis, and improve grain nutritional quality (Wang et al., 2010). According to the findings, the combination between N and K had a substantial impact on the quality of Jingu 54. When the potassium level is low, an excessively high nitrogen level will result in a decrease in quality, while when the potassium level is high, the higher the nitrogen level is within a specific range, the better the quality. The findings indicated that crop quality could not be improved in the presence of low

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nitrogen, but that quality deteriorated significantly as potassium treatment increased, suggesting that excessive potassium application should be avoided in the presence of low nitrogen. Because N and K complement each other in the process of plant metabolism, maintaining the proper N/K ratio is essential to guarantee excellent quality (Wang et al., 2011). But the effects of the interaction of phosphorus, nitrogen, and potassium did not reach statistical significance for comprehensive quality of Jingu 54. There are many studies on the use of N, P, and K fertilizers in combination, although most of them focus on the impact of fertilization on crop yield or fertilizer efficiency. According to Wang et al. (2010) the interaction of N and P had a substantial impact on rice production, while the interaction of N and K was not significant. Wang et al. (2011) demonstrated that when the K level was low, a high N level resulted in a lower yield, while a higher N level in a specific range resulted in a greater yield, which was comparable to the findings of our research. However, research on the effect of N, P, and K interactions on crop quality is limited, making it difficult to determine a reasonable fertilization ratio for crop quality. This research provides a certain reference for the investigation of the effects of combined application of nitrogen, phosphorus, and potassium on crop quality.

This study revealed a significant role of Se in the comprehensive quality of Jingu 54. In consistent with our finding, previous studies have shown that exogenous Se can increase the content of lutein in tomato (Pezzarossa et al., 2013). Similarly, Se (at 0-810 kg ha^{-1}) increased the content of lutein in carrots by 94.2% (Biacs et al., 1995). However, other trials have shown that exogenous Se has no effect on the content of lutein in cabbage (Lefsrud et al., 2006) or tomato (Pezzarossa et al., 2014). The reason for the inconsistency of these results may be due to the concentrations of exogenous Se and/or the characteristics of the target crop. So far, there are few reports on the effect of Se on foxtail millet, especially regarding to their yellow pigment content. Previous investigation on corn shows that Se application on leaf surface does not influence mineral content in corn kernels (Wang et al., 2013). Studies on various crops such as corn (Chilimba et al., 2012), wheat (Broadley et al., 2010), and rice (Boldrinet et al., 2013) showed that the Se content in the grain has a linear relationship to the concentration of exogenous Se. This research also found that when selenium content rose, the quality of Jingu 54 improved at first, then declined. To avoid the negative health consequences of deficiency or excessive selenium intake appropriate dose should be chosen when applying selenium fertilizer to crops, so that selenium may completely exercise its biological activities of anti-oxidation and immune promotion (Boldrinet et al., 2013). The combination of selenium with nitrogen, phosphorus, potassium, and herbicide did not achieve a significant level of influence on the comprehensive quality. But most of them adopted orthogonal design without analyzing the interaction between factors (Long et al., 2019).

Weed competition has become an important limiting factor for crop growth and development, with chemical herbicides controlling weeds in the field while also reducing crop yields and quality (Iqbal et al., 2019; Javaid and Tanveer, 2013). Because yield and quality are two determinants of millet value (Chauhan and Openga, 2012), it is critical to investigate the effects of herbicides. The findings indicated that the quality of Jingu 54 rose initially and subsequently deteriorated as TBM herbicide concentration increased. Malalgoda et al. (2020) discovered that incorrect administration of fensulfuron-methyl may have a serious impact on agricultural seed protein content. The impact of herbicide stress on agricultural seed mineral content was suggested by Gugala

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et al. (2010, 2012) and Ning et al. (2015). The interaction between TBM and N (and K) had a substantial effect on the comprehensive quality of Jingu 54 in this research. The quality of Jingu 54 improved quickly initially and then declined slowly as TBM concentration increased at high nitrogen levels, indicating that nitrogen may mitigate pesticide damage at specific concentrations. When TBM was raised to a specific level at high potassium levels, the quality of Jingu 54 dropped quickly, which may be attributable to the suppression of potassium on TBM degradation (Huang, 2020). The effect of herbicides on crop quality can only be fully appreciated via a comprehensive investigation of soil and crop nutritional status, which necessitates further research.

Conclusion

It is the first time to report the effects of different fertilizer levels (N, P_2O_5, and K_2O), TBM herbicide, and Se on comprehensive quality of foxtail millet Jingu 54. The optimal application of N, P, K, Se and herbicides could reduce waste, reduce pollution, and protect the ecological environment without affecting the quality of foxtail millet. Additionally, foxtail millet grain was reported to be enriched with Se through exogenous Se under the foliar application of Se. Our study established a multivariate quadratic regression model of comprehensive quality, which showed statistical significance and thus could be used for production prediction. Based on the regression model, optimal cultivation conditions were obtained for Jingu 54 under the following experimental conditions: N level, P_2O_5 level, K_2O level, Se level, and TBM level as 108.31 kg ha^{-1}, 94.80 kg ha^{-1}, 105.03 kg ha^{-1}, 88.08 g ha^{-1}, and 18.48 g ai ha^{-1}, respectively. The maximum predicted comprehensive quality of foxtail millet was 56.85. Our study provides a valuable practice for the optimized cultivation management and targeted planting of high-quality foxtail millet. Because of the impact of soil environment and fertilization, the development of grain quality is a complicated process that has to be researched more thoroughly.

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APPENDIX

Figure A1. *A diagram of the used experimental design (a quadratic general rotation combination design with a 5-factor-5-level was used, with 32 treatments and three replications). 96 units were run in a fully randomized block design, with protection rows placed around the experimental location)*

	3 m × 6 m		Protection row			
	5 ($A_4B_2C_4D_4E_2$)		30 ($A_3B_3C_3D_3E_3$)		14 ($A_2B_2C_4D_2E_2$)	
	7 ($A_4B_2C_2D_4E_4$)		28 ($A_3B_3C_3D_3E_3$)		17 ($A_1B_3C_3D_3E_3$)	
	32 ($A_3B_3C_3D_3E_3$)		24 ($A_3B_3C_3D_5E_3$)		12 ($A_2B_4C_2D_2E_2$)	
	25 ($A_3B_3C_3D_3E_1$)		13 ($A_2B_2C_4D_4E_4$)		26 ($A_3B_3C_3D_3E_5$)	
	10 ($A_2B_4C_4D_2E_4$)		1 ($A_4B_4C_4D_4E_4$)		22 ($A_3B_3C_5D_3E_3$)	
	21 ($A_3B_3C_1D_3E_3$)		31 ($A_3B_3C_3D_3E_3$)		9 ($A_2B_4C_4D_4E_2$)	
	16 ($A_2B_2C_2D_2E_4$)		7 ($A_4B_2C_2D_4E_4$)		18 ($A_5B_3C_3D_3E_3$)	
	8 ($A_4B_2C_2D_2E_2$)		2 ($A_4B_4C_4D_2E_2$)		29 ($A_3B_3C_3D_3E_3$)	
	12 ($A_2B_4C_2D_2E_2$)		27 ($A_3B_3C_3D_3E_3$)		17 ($A_1B_3C_3D_3E_3$)	
	9 ($A_2B_4C_4D_4E_2$)		23 ($A_3B_3C_3D_1E_3$)		2 ($A_4B_4C_4D_2E_2$)	
	1 ($A_4B_4C_4D_4E_4$)		5 ($A_4B_2C_4D_4E_2$)		27 ($A_3B_3C_3D_3E_3$)	
	4 ($A_4B_4C_2D_2E_4$)		15 ($A_2B_2C_2D_4E_2$)		6 ($A_4B_2C_4D_2E_4$)	
	26 ($A_3B_3C_3D_3E_5$)		11 ($A_2B_4C_2D_4E_4$)		30 ($A_3B_3C_3D_3E_3$)	
	18 ($A_5B_3C_3D_3E_3$)		8 ($A_4B_2C_2D_2E_2$)		19 ($A_3B_1C_3D_3E_3$)	
	25 ($A_3B_3C_3D_3E_1$)		16 ($A_2B_2C_2D_2E_4$)		32 ($A_3B_3C_3D_3E_3$)	
Protection row	13 ($A_2B_2C_2D_4E_2$)	Aisle	4 ($A_4B_4C_2D_2E_4$)	Aisle	15 ($A_2B_2C_2D_4E_2$)	Protection row
	3 ($A_4B_4C_2D_4E_2$)		6 ($A_4B_2C_4D_2E_4$)		24 ($A_3B_3C_3D_5E_3$)	
	28 ($A_3B_3C_3D_3E_3$)		19 ($A_3B_1C_3D_3E_3$)		1 ($A_4B_4C_4D_4E_4$)	
	22 ($A_3B_3C_5D_3E_3$)		14 ($A_2B_2C_4D_2E_2$)		32 ($A_3B_3C_3D_3E_3$)	
	30 ($A_3B_3C_3D_3E_3$)		9 ($A_2B_4C_4D_4E_2$)		16 ($A_2B_2C_2D_2E_4$)	
	21 ($A_3B_3C_1D_3E_3$)		25 ($A_3B_3C_3D_3E_1$)		11 ($A_2B_4C_2D_4E_4$)	
	3 ($A_4B_4C_2D_4E_2$)		11 ($A_2B_4C_2D_4E_4$)		7 ($A_4B_2C_2D_4E_4$)	
	27 ($A_3B_3C_3D_3E_3$)		2 ($A_4B_4C_4D_2E_2$)		20 ($A_3B_5C_3D_3E_3$)	
	20 ($A_3B_5C_3D_3E_3$)		28 ($A_3B_3C_3D_3E_3$)		29 ($A_3B_3C_3D_3E_3$)	
	6 ($A_4B_2C_4D_2E_4$)		26 ($A_3B_3C_3D_3E_5$)		8 ($A_4B_2C_2D_2E_2$)	
	23 ($A_3B_3C_3D_1E_3$)		5 ($A_4B_2C_4D_4E_2$)		31 ($A_3B_3C_3D_3E_3$)	
	17 ($A_1B_3C_3D_3E_3$)		22 ($A_3B_3C_5D_3E_3$)		3 ($A_4B_4C_2D_4E_2$)	
	31 ($A_3B_3C_3D_3E_3$)		15 ($A_2B_2C_2D_4E_2$)		21 ($A_3B_3C_1D_3E_3$)	
	4 ($A_4B_4C_2D_2E_4$)		24 ($A_3B_3C_3D_5E_3$)		14 ($A_2B_2C_4D_2E_2$)	
	20 ($A_3B_5C_3D_3E_3$)		10 ($A_2B_4C_4D_2E_4$)		19 ($A_3B_1C_3D_3E_3$)	
	18 ($A_5B_3C_3D_3E_3$)		12 ($A_2B_4C_2D_2E_2$)		23 ($A_3B_3C_3D_1E_3$)	
	13 ($A_2B_2C_4D_4E_4$)		29 ($A_3B_3C_3D_3E_3$)		10 ($A_2B_4C_4D_2E_4$)	
			Protection row			

The numbers in the table are treatment codes, which are the same as the treatment codes in *Table 2* of the main text. A_1, A_2, A_3, A_4, and A_5 correspond to nitrogen fertilizer levels of 0, 69, 138, 207, and 276 kg ha^{-1} correspondingly; B_1, B_2, B_3, B_4, and B_5 equate to 0, 36, 72, 108, and 144kg ha^{-1} of phosphate fertilizer, respectively; C_1, C_2, C_3, C_4, and C_5 equate to 0, 37.5, 75, 112.5, and 150kg ha^{-1} of potash fertilizer, respectively; D_1, D_2, D_3, D_4, and D_5 equate to 0, 60, 120, 180, and 240kg ha–1 of selenium fertilizer, respectively；E_1, E_2, E_3, E_4, and E_5 correspond to 0, 20, 40, 60, and 80g ai ha^{-1} of the various herbicide levels

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Figure A2. *The photos of the experimental culture or equipment*

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