

Article

Screening of Germplasm and Construction of Evaluation System for Autotoxicity Tolerance during Seed Germination in Cucumber

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Abstract: Due to the widespread use of intensive cropping patterns, the problem of continuous cropping obstacle, which is dominated by autotoxicity, has been becoming more and more prominent. Although many methods have been proposed to overcome the continuous cropping obstacle of cucumber, no study has reported the screening and evaluation of cucumber germplasm resistant to autotoxicity. In this study, 28 physiological indices related to the cucumber bud stage under cinnamic acid (CA) treatment were determined. In total, 45 cucumber cultivars were classified into three groups using principal component analysis and cluster analysis, and a model for evaluating cucumber resistance to autotoxicity was developed. The evaluation model was validated using autotoxicity-tolerant and non-autotoxicity-tolerant cultivars. The results showed that the growth of non-autotoxicity-tolerant cultivars was significantly inhibited compared to autotoxicity-tolerant cultivars. This indicated that the evaluation model of cucumber autotoxicity tolerance is reliable. The results of this study provide a valuable reference for the application of cucumber autotoxicity-tolerant germplasm resources and the development of autotoxicity-tolerant genes.

Keywords: *Cucumis sativus* L.; autotoxic stress; germplasm resources; comprehensive evaluation



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1. Introduction

A decrease in crop yield and quality occurs when the same crop is grown continuously on a piece of land, a phenomenon known as continuous cropping obstacle [1]. Continuous cropping obstacle has become increasingly serious with the widespread use of intensive land production methods, especially in facility-based cropping [2]. Cucumber (*Cucumis sativus* L.) is a particularly common continuous monoculture greenhouse vegetable [3]. Although the continuous monoculture model improves land use efficiency, this model poses a major challenge to the sustainability of cucumber facility agriculture [4]. The main causes of barriers to continuous cultivation are soil nutrient imbalances, soil acidification, autotoxicity, and microbial ecological imbalances [5,6]. Autotoxicity, among other things, is an important cause of impediments to continuous cultivation. Autotoxicity occurs as a result of the release of specific chemicals such as cinnamic acid (CA), p-hydroxybenzoic acid (PHBA), and benzoic acid (BA) into the surrounding area [7,8]. These chemicals are actually produced through the volatilization and decomposition of plant leachate, root exudates, and plant residues in the above-ground portion of the plant [9]. Among them, CA is the most toxic to cucumber, adversely affecting plant growth and development through a series of physiological and biochemical changes [10–12]. The most common ways to overcome continuous cropping obstacles in current cultivation methods are crop rotation and grafting. But crop rotation is more time-consuming. For example, melon usually requires a 3–4-year crop rotation to have significant relief from this continuous cropping barrier [13]. The same

grafting has been shown to adversely affect crop quality [14,15]. Therefore, exploring a new approach to effectively mitigate the continuous cropping obstacle of cucumber will be important for sustainable production of cucumber in facilities.

Principal component analysis and cluster analysis have been increasingly used in the screening of crop germplasm resources [16]. These methods are used for dimensionality reduction when there are more evaluation indices, i.e., removing redundancy and leaving the main information that can be maintained, thus realizing the purpose of data compression, denoising, and feature extraction [17]. The evaluation model can be constructed according to the extracted scores of each principal component, and the comprehensive scores can be calculated and ranked [18]. For example, Men et al. [19] used principal component analysis to evaluate the salt tolerance of different cucumber cultivars at the seedling stage and screened the underground dry weight, strong seedling index, and root–crown ratio as representative evaluation indices to construct the evaluation system. Fu et al. [20] classified 12 cucumber cultivars into four categories of strong heat tolerance, moderate heat tolerance, heat tolerance, and heat sensitivity using principal component analysis and subordinate function analysis. Several indicators, such as internode length, relative conductivity, and the content of chlorophyll a, were screened and used to construct an evaluation system. Miao et al. [21] carried out a comprehensive evaluation of the cold hardiness of cucumber at the germination and seedling stages using principal component analysis, affiliation function analysis, and cluster analysis. Chen et al. [22] combined principal component analysis and subordinate function analysis to comprehensively identify and evaluate the salinity tolerance of cereal (*Setaria italica* L. Beauv) during the germination period, screened the evaluation indices, and established a system for evaluating the salinity tolerance of cereal during the germination period. Integrated evaluation methods are well established in these studies on the screening of different resistant cultivars of crops under adversity stress.

Plants have a set of defense mechanisms when subjected to autotoxicity stress [23,24]. Previous studies have emphasized that cucumber autotoxicity tolerance is influenced by genetic factors and environmental conditions [25]. Consequently, efficient production depends on the selection of resistant cultivars, as well as on suitable environmental and management conditions. Nonetheless, comprehensive evaluations of autotoxicity tolerance across diverse cucumber cultivars remain limited, and no studies have been reported on the screening and evaluation of cucumber germplasm for autotoxicity resistance. In this study, we comprehensively evaluated the autotoxic resistance of different cucumber cultivars and constructed an evaluation system under CA-induced autotoxic stress.

Understanding the genetic and environmental factors influencing autotoxicity tolerance can inform variety selection, crop management practices, and breeding programs aimed at developing cucumber cultivars with enhanced resilience to autotoxicity. Consequently, by elucidating the autotoxicity resistance profiles of different cucumber cultivars, this study provides valuable insights for farmers, breeders, and researchers. Ultimately, these findings contribute to the advancement of sustainable cucumber production practices and the promotion of food security.

2. Materials and Methods

2.1. Materials

Cucumber (*Cucumis sativus* L.) cultivars were purchased from the National Resource Germplasm Bank, Tianjin Kerun Cucumber Research Institute, and Vegetable Research Institute of Beijing Academy of Agriculture and Forestry Sciences (Table 1, Supplementary Table S1). Cinnamic acid (CA, 99.5%) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (in Shanghai, China).

Table 1. Information on cucumber cultivars.

No.	Cultivars	Breeding Institutes (Production Unit)	No.	Cultivars	Breeding Institutes (Production Unit)
1	Jinchun5	TJKRCRI	24	LiangyouF17-3	TJYLT Co., Ltd.
2	Jinyou1	TJKRCRI	25	Chaoguan1	SDSGSASI Co., Ltd.
3	Jinyun7	TJKRCRI	26	Bona203	TJDRC
4	Jinyou35	TJKRCRI	27	Jufeng9	SDSGCGASI Co., Ltd.
5	Jinyou3	TJKRCRI	28	Jufengxinxing	SDSGCGASI Co., Ltd.
6	Jinyun4	TJKRCRI	29	TianyuanbaimawangziF1	JXSRLMA Co., Ltd.
7	Jinlü10	TJCRI	30	XianyoudaL307	TJXYDSI Co., Ltd.
8	Jinza2	TJKRCRI	31	Shanglizaoshubai Cucumber	HNZZNZSI Co., Ltd.
9	Jinyan4	TJKRCRI	32	Guifeicui Cucumber	HVRIHBP
10	Xinjinchun4	TJCRI	33	Jieza4	JLVFRI
11	Zhongnong6	CAAS	34	Shengyou68	YNAAS
12	Zhongnong106	CAAS	35	Shengyou8-9	YNAAS
13	Zhongnonglüxiu	CAAS	36	Shengyou58	YNAAS
14	Zhonnong116	CAAS	37	Lüyan3F1	WHGDFACT Co., Ltd.
15	Zhongshou35F1	SDSGXXRH Co., Ltd.	38	LüguanA6	SDAAS
16	Funong1	SDSGHWSI Co., Ltd.	39	LüguanA7	SDAAS
17	Funong4	SDSGHWSI Co., Ltd.	40	Deruite8	TJDRC
18	Fuyang2F1	SDSGHLSI Co., Ltd.	41	Jingyanhanbao5	BJVRI
19	Jingyanlülilinglon	BJVRI	42	Zhongnongcuiyu2	SDAAS
20	Shenglv10-9F1	BJVRI	43	Junyou66	LNVRI
21	Jinyou48	TJKRCRI	44	Jifengcuilüwang	JLVFRI
22	Jinyou10	TJKRCRI	45	DeerLD-3	TJDETSI Co., Ltd.
23	Jinyou401	TJKRCRI			

TJKRCRI: Tianjin Kerun Cucumber Research Institute; CAAS: Chinese Academy of Agricultural Sciences; TJCRI: Tianjin Cucumber Research Institute; SDGSXXRH Co., Ltd.: Shandong Shouguang Xinxinran Horticulture Co., Ltd. (in Shouguang, China); SDSGHWSI Co., Ltd.: Shandong Shouguang Hongwei Seed Industry Co., Ltd. (in Shouguang, China); SDSGHLSI Co., Ltd.: Shandong Shouguang Hongliang Seed Industry Co., Ltd. (in Shouguang, China); BJVRI: Beijing Vegetable Research Institute; TJYLT Co., Ltd.: Tianjin Yilian Technology Co., Ltd. (in Tianjin, China); SDSGSASI Co., Ltd.: Shandong Shouguang Spring and Autumn Seed Industry Co., Ltd. (in Shouguang, China); TJDRC: Tianjin Derui Company; SDSGCGSI Co., Ltd. (in Tianjin, China); Shandong Shouguang Chunguang Seed Industry Co., Ltd. (in Shouguang, China); JXSRLMA Co., Ltd.: Jiangxi Shangrao Limin Agriculture Co., Ltd. (in Shangrao, China); TJXYDSI Co., Ltd.: Tianjin Xianyouda Seed Industry Co., Ltd. (in Tianjin, China); HNZZNZSI Co., Ltd.: Hunan Zhuzhou Nongzhizi Seed Industry Co., Ltd. (in Zhuzhou, China); JLVFRI: Jilin Vegetable and Flower Research Institute; HVRIHBP: Horticultural Vegetable Research Industry of Hebei Province; YNAAS: Yunnan Academy of Agricultural Sciences; WHGDFACT Co., Ltd.: Wuhan Garden Dafeng Agricultural Science and Technology Co., Ltd. (in Wuhan, China); SDAAS: Shandong Academy of Agricultural Sciences; LNVRI: Liaoning Vegetable Research Institute; TJDETSI Co., Ltd.: Tianjin Deerte Seed Industry Co., Ltd. (in Tianjin, China).

2.2. Treatment

In a pre-test, to determine the CA concentration that causes plants not to die but to be stressed in cucumber seeds [24], the seeds of each variety were selected and soaked in warm water, and then soaked in 20 mL of different concentrations of CA solution (the concentration of CA solution was 0, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mmol·L⁻¹, respectively) for 6 h. The seeds were placed in a petri dish with two layers of filter paper (the filter paper was soaked in different concentrations of CA solution) and finally placed at a temperature of 28 °C and a humidity of 65%. The artificial climate (model BIC-400, made by Shanghai Boxun Industrial Co., LTD, the company is in Shanghai, China) with 200 µmol m⁻²s⁻¹ light intensity was used to germinate the seeds for 5 days (the filter paper was kept soaking in CA solution during the germination process). Each treatment consisted of 20 seeds and was repeated three times. The number of germinations was recorded per day. After 5 days, root fresh weight, bud fresh weight, root length, and bud length were measured at the root neck of the cucumber seedlings. It was found that when the concentration of CA solution was increased to 0.8 mmol·L⁻¹, the indices of cucumber seedlings were inhibited to varying degrees. Therefore, 0.8 mmol·L⁻¹ CA solution was used as the autotoxicity stress concentration in this study (data not submitted).

In the main experiment, the seeds of 45 cucumber cultivars (Table 1) were soaked in CA solution ($0.8 \text{ mmol} \cdot \text{L}^{-1}$) and deionized water (CK) for 6 h, respectively. The follow-up process was consistent with the pre-test method. The seeds were placed in an artificial climate for germination, and the number of germinations per day during the germination process was recorded. Each treatment was repeated three times, with 20 seeds per repetition. After 5 days, root length, shoot length, root fresh weight, and shoot fresh weight were measured, and then the amount required to determine antioxidant enzyme, amylase activity, and MDA content was weighed and frozen in liquid nitrogen and placed in a -80°C refrigerator for subsequent analysis.

In order to further verify the reliability of the initial evaluation result, resistant and non-resistant cultivars, as determined from the initial study, were selected for an additional test using the root application of CA. When the cotyledons were fully expanded, the roots were treated with $1.2 \text{ mmol} \cdot \text{L}^{-1}$ CA. Here, we found significant differences in plant phenotypes after 14 days of CA application, so root length, leaf area, stem diameter, and plant height were measured after 14 days. All samples had three biological replicates.

2.3. Determination Method

2.3.1. Determination of Growth Index

Seeds were defined as germinated when the bud length exceeded 1/2 of the seed length. The germination number was counted after treatment for 2–5 days, and the germination potential and germination rate were calculated according to Xiao et al. [26]. After 5 days, the root length, bud length (5 plants were randomly measured in each culture dish, and the average value was obtained), root fresh weight, and bud fresh weight were measured (each treatment of each repeat was a culture dish, first weighed according to the dish, and finally the average fresh weight of each plant was calculated).

2.3.2. Determination of Physiological Indicators

Malondialdehyde (MDA) content was determined by the thiobarbituric acid (TBA) colorimetric method [27], superoxide dismutase activity (SOD) and peroxidase activity (POD) were determined by the method of [28], catalase activity (CAT) was determined by the method of [29], and amylase activity was determined by the 3,5-dinitrosalicylic acid method [30].

2.4. Statistical Analysis

The calculation formula of the relevant indicators is as follows:

Germination potential (%) = the number of germinated seeds on the second day / the total number of seeds $\times 100\%$;

The germination rate (%) = the number of germinated seeds on the 5th day / the total number of seeds $\times 100\%$;

The relative value of index (%) = $\text{CA} / \text{CK} \times 100\%$;

The index toxicity rate refers to the method of Chen et al. [22], where the index toxicity rate (%) (increase rate or inhibition rate) = $|\text{CK} - \text{CA}| / \text{CK} \times 100\%$ (in the formula, the index toxicity rate, increase rate, and inhibition rate all have one meaning, and the numerator represents the absolute value of the difference between CK and CA).

The membership function is an important hierarchical evaluation method with the advantage of high comprehensiveness. It has been increasingly used in research on crop germplasm resources [31,32].

Membership function value calculation formula:

$$\mu(X_j) = (X_j - X_{\min}) / (X_{\max} - X_{\min}) \quad j = 1, 2, 3, \dots, n \quad (1)$$

In the formula, $\mu(X_j)$ represents the membership function value of the j th comprehensive index, X_j represents the j th comprehensive index value, $X_{\max}$ represents the maximum value of the j th comprehensive index, and $X_{\min}$ represents the minimum value of the j th comprehensive index.

Index weight calculation formula:

$$W_j = P_j / \sum_{j=1}^m P_j \quad j = 1, 2, 3, \dots, n \quad (2)$$

In the formula, W_j represents the weight of the j th comprehensive index in all comprehensive indices, and P_j represents the contribution rate of the j th comprehensive index.

According to the membership function value and the weight of each comprehensive index, the comprehensive evaluation value (D) of the self-toxicity tolerance of different cucumber cultivars was calculated:

$$D = \sum_{j=1}^m [\mu(X_j) \times W_j] \quad j = 1, 2, 3, \dots, n \quad (3)$$

The data were analyzed using Excel 2019 software. SPSS 23.0 software was used for significant difference analysis ($p < 0.05$), correlation analysis, principal component analysis, cluster analysis, and stepwise regression analysis [12,20,22].

3. Results

3.1. Correlation Analysis of Various Indicators of Different Cucumber Cultivars under CA Stress

Correlation analyses were performed after the determination of 28 indicators, and a matrix of correlation coefficients was obtained (Supplementary Table S2). Among them, 90 groups of indices reached a significant or extremely significant positive correlation, and 94 groups reached a significant or extremely significant negative correlation. The relative values of germination rate, germination potential, root length, bud length, root fresh weight, bud fresh weight, root–shoot ratio, α -amylase activity, β -amylase activity, and total amylase activity were opposite to their corresponding toxic rate or inhibition rate. The relative values of MDA content, POD activity, SOD activity, and CAT activity showed the same trend as the increase rate. Among them, the increase rate of MDA content was significantly or extremely significantly positively correlated with the toxicity rate of germination, root length, bud length, root fresh weight, and bud fresh weight, and extremely significantly negatively correlated with the increase rate of POD, SOD, and CAT activity. The increase rate of SOD activity was significantly negatively correlated with the toxicity rate of germination and bud length, and significantly positively correlated with the toxicity rate of root fresh weight. These results indicate that cucumber seeds treated with $0.8 \text{ mmol} \cdot \text{L}^{-1}$ CA solution showed reduced POD, SOD, and CAT activities, elevated MDA accumulation, and elevated toxicity rates of germination, root length, stem length, root fresh weight, and stem fresh weight during germination compared to clear water treatment. From the above analyses, it can be seen that there is a complex relationship between the 28 indicators of the 45 cultivars. Therefore, systematic analyses and comprehensive evaluations are needed after the dimensionality reduction process.

3.2. Principal Component Analysis of Various Indices of Different Cucumber Cultivars under CA Stress

In order to comprehensively evaluate the autotoxicity resistance of different cucumber cultivars, principal component analysis was performed on the 28 indicators in this study. The eigen value indicates the magnitude of the amount of variation in the original data in each direction and can be used to determine the number of principal components. The condition that the eigen value was greater than 1 was used to determine the principal component in the data statistics [33]. The results retained 9 principal components with characteristic roots greater than 1, and the cumulative contribution rate reached 91.13%, which met the minimum standard of principal component analysis (cumulative contri-

bution rate $\geq 80\%$) (Table 2). The scores of 9 principal components were obtained using principal component analysis, which were Z1–Z9 (Table 3).

Table 2. Eigen values, contributions of principal components, and cumulative contributions.

Principal Component	I	II	III	IV	V	VI	VII	VIII	IX
Eigen value	6.30	4.72	3.80	2.85	2.40	1.60	1.45	1.34	1.05
Contribution (%)	22.51	16.85	13.56	10.19	8.56	5.73	5.20	4.79	3.74
Cumulative contribution (%)	22.51	39.36	52.93	63.12	71.68	77.40	82.60	87.39	91.13

Table 3. The principal component score values of different cultivars.

No.	Z ₁	Z ₂	Z ₃	Z ₄	Z ₅	Z ₆	Z ₇	Z ₈	Z ₉
1	25.061	7.426	38.382	54.591	2.661	104.669	24.861	59.744	220.965
2	18.143	5.195	23.292	36.267	22.812	82.506	38.620	60.271	244.565
3	20.106	6.159	8.840	18.731	31.420	106.923	−3.287	74.545	177.678
4	6.140	9.353	−0.797	33.579	46.410	93.772	52.939	124.199	261.966
5	4.630	25.641	0.580	23.259	27.773	84.420	65.268	89.463	282.263
6	8.779	18.235	12.647	10.032	16.385	112.009	47.912	96.187	264.947
7	20.353	−2.832	22.810	9.598	39.254	91.388	22.139	33.793	202.477
8	11.583	21.213	6.507	24.618	24.428	87.665	45.350	59.247	258.950
9	53.654	42.631	77.092	73.365	−36.330	151.127	−35.911	114.178	250.063
10	20.279	−13.937	31.185	4.973	36.790	141.979	−1.269	31.758	283.087
11	35.297	32.068	52.411	73.230	15.017	140.915	−39.853	112.753	267.594
12	27.595	−1.319	0.465	12.970	37.583	116.158	15.648	65.776	273.294
13	16.350	4.353	19.455	13.547	9.686	121.629	16.175	82.469	212.177
14	31.198	9.385	34.784	43.267	21.171	83.463	12.347	28.200	217.949
15	9.998	23.463	17.341	19.547	21.810	111.049	59.241	93.036	296.614
16	17.226	13.198	18.285	5.416	17.541	107.228	−11.657	96.365	213.958
17	−6.636	4.911	12.516	−2.630	40.447	82.834	98.765	81.877	252.072
18	21.500	19.239	21.337	34.850	19.004	102.001	4.061	83.530	241.118
19	35.123	0.099	29.745	42.404	28.056	110.560	14.825	24.686	282.031
20	17.657	−2.352	3.108	10.905	32.074	83.218	40.808	61.667	232.877
21	28.140	−10.478	25.626	58.790	8.009	122.645	−17.828	62.115	213.980
22	13.203	2.802	23.011	36.420	18.585	55.887	16.366	30.800	183.931
23	19.271	7.181	31.258	16.303	21.051	98.985	59.237	37.830	205.656
24	17.113	−23.217	5.562	52.892	37.118	136.005	48.680	55.290	235.530
25	28.405	25.618	46.464	58.471	24.524	141.735	−52.733	121.390	281.987
26	16.662	12.272	13.585	28.484	27.284	75.532	43.487	38.557	251.527
27	4.291	−11.472	13.513	4.562	30.170	80.349	16.292	74.389	248.032
28	44.147	7.686	24.110	40.108	20.646	83.781	43.217	73.211	273.856
29	23.427	0.012	8.215	41.296	19.222	78.439	25.454	38.368	262.882
30	5.929	−2.210	17.082	21.773	28.844	97.232	34.270	56.345	220.762
31	30.624	1.333	5.588	21.941	31.143	75.127	5.993	25.278	209.296
32	20.212	−24.542	6.138	16.189	46.063	79.032	48.915	36.956	217.611
33	31.326	10.309	13.282	55.562	−22.350	73.810	59.322	76.822	261.528
34	13.265	−4.653	12.038	30.158	17.611	124.918	55.403	89.765	262.421
35	24.083	−1.522	18.527	−0.543	22.511	55.907	40.083	61.543	166.249
36	23.600	−7.236	26.331	0.284	55.521	97.287	−7.840	−4.808	265.569
37	19.429	24.763	−0.445	31.265	25.620	60.632	6.247	56.591	217.513
38	24.582	−6.892	11.870	6.825	42.949	63.238	25.636	39.514	244.030
39	11.183	−14.402	19.213	21.071	47.459	59.794	−20.791	32.884	209.853
40	30.325	11.668	7.494	9.379	51.595	83.785	32.295	34.515	345.673
41	31.269	−7.391	16.263	9.869	30.455	104.614	30.013	40.989	282.867
42	25.056	−4.848	5.727	4.287	50.636	92.913	62.515	36.378	346.776
43	18.087	−4.805	25.270	35.150	60.891	70.402	−23.890	13.793	331.124
44	43.017	14.977	86.984	110.428	17.877	159.654	−76.240	110.890	319.256
45	25.224	4.937	9.632	21.412	37.917	94.130	12.136	56.727	234.813

3.3. Comprehensive Evaluation of Autotoxicity Tolerance of Different Cucumber Cultivars

The membership function value $\mu(X)$ of each comprehensive trait index was calculated using Formula (1), and the weight of 9 principal components was calculated using Formula (2) according to the contribution rate of principal components. According to the weight of 9 principal components and the membership function value $\mu(X)$ of comprehensive traits, the comprehensive score of each variety was calculated and ranked using Formula (3) (Table 4). A cluster analysis of the comprehensive score D value was carried out. When the Euclidean distance was 12.5, the 45 cucumber cultivars were divided into three different groups. These comprised: (i) two cultivars, ‘Jinyan4’ and ‘Jifengcuilvwang’, with a comprehensive score D of 0.770–0.777; (ii) three cultivars with a general tolerance to autotoxicity, ‘Zhongnong6’, ‘Chaoguan1’ and ‘Jufengxinxing’, with a comprehensive score D of 0.548–0.677; and (iii) 40 self-intolerant cultivars such as ‘Jinchun5’, ‘Jinyou1’ and ‘Jinyun7’, with a comprehensive score D of 0.272–0.499 (Figure 1).

Table 4. Membership function value and comprehensive evaluation value of different cucumber cultivars.

No.	$\mu(X_1)$	$\mu(X_2)$	$\mu(X_3)$	$\mu(X_4)$	$\mu(X_5)$	$\mu(X_6)$	$\mu(X_7)$	$\mu(X_8)$	$\mu(X_9)$	Assessment Values (D)	Rank
1	0.526	0.476	0.446	0.506	0.401	0.470	0.578	0.500	0.303	0.480	8
2	0.411	0.443	0.274	0.344	0.608	0.257	0.656	0.504	0.434	0.418	15
3	0.444	0.457	0.110	0.189	0.697	0.492	0.417	0.615	0.063	0.387	31
4	0.212	0.505	0.000	0.320	0.851	0.365	0.738	1.000	0.530	0.401	26
5	0.187	0.747	0.016	0.229	0.659	0.275	0.809	0.731	0.643	0.402	23
6	0.256	0.637	0.153	0.112	0.542	0.541	0.709	0.783	0.547	0.405	20
7	0.448	0.323	0.269	0.108	0.777	0.342	0.562	0.299	0.201	0.373	35
8	0.302	0.681	0.083	0.241	0.625	0.306	0.695	0.497	0.514	0.405	21
9	1.000	1.000	0.887	0.672	0.000	0.918	0.230	0.922	0.464	0.777	1
10	0.446	0.158	0.364	0.067	0.752	0.830	0.428	0.283	0.647	0.390	28
11	0.696	0.843	0.606	0.671	0.528	0.819	0.208	0.911	0.561	0.677	3
12	0.568	0.346	0.014	0.138	0.760	0.581	0.525	0.547	0.593	0.413	18
13	0.381	0.430	0.231	0.143	0.473	0.634	0.528	0.677	0.254	0.384	33
14	0.628	0.505	0.405	0.406	0.591	0.266	0.506	0.256	0.286	0.480	7
15	0.276	0.715	0.207	0.196	0.598	0.532	0.774	0.758	0.722	0.456	12
16	0.396	0.562	0.217	0.071	0.554	0.495	0.369	0.784	0.264	0.398	27
17	0.000	0.438	0.152	0.000	0.790	0.260	1.000	0.672	0.475	0.306	42
18	0.467	0.652	0.252	0.332	0.569	0.444	0.459	0.685	0.415	0.471	10
19	0.693	0.367	0.348	0.398	0.662	0.527	0.520	0.229	0.641	0.499	6
20	0.403	0.330	0.044	0.120	0.704	0.263	0.669	0.515	0.369	0.344	39
21	0.577	0.209	0.301	0.543	0.456	0.643	0.334	0.519	0.264	0.427	13
22	0.329	0.407	0.271	0.345	0.565	0.000	0.529	0.276	0.098	0.337	40
23	0.430	0.472	0.365	0.167	0.590	0.415	0.774	0.331	0.218	0.419	14
24	0.394	0.020	0.072	0.491	0.755	0.772	0.714	0.466	0.384	0.367	36
25	0.581	0.747	0.538	0.540	0.626	0.827	0.134	0.978	0.641	0.618	4
26	0.386	0.548	0.164	0.275	0.654	0.189	0.684	0.336	0.472	0.401	25
27	0.181	0.195	0.163	0.064	0.684	0.236	0.529	0.614	0.453	0.272	45
28	0.842	0.480	0.284	0.378	0.586	0.269	0.683	0.605	0.596	0.548	5
29	0.499	0.366	0.103	0.389	0.571	0.217	0.581	0.335	0.535	0.390	29
30	0.208	0.332	0.204	0.216	0.670	0.398	0.631	0.474	0.302	0.329	41
31	0.618	0.385	0.073	0.217	0.694	0.185	0.470	0.233	0.238	0.385	32
32	0.445	0.000	0.079	0.166	0.847	0.223	0.715	0.324	0.285	0.303	43
33	0.630	0.519	0.160	0.515	0.144	0.173	0.775	0.633	0.528	0.456	11
34	0.330	0.296	0.146	0.290	0.555	0.665	0.752	0.733	0.533	0.388	30
35	0.510	0.343	0.220	0.018	0.605	0.000	0.665	0.514	0.000	0.346	38

Table 4. Cont.

No.	$\mu(X_1)$	$\mu(X_2)$	$\mu(X_3)$	$\mu(X_4)$	$\mu(X_5)$	$\mu(X_6)$	$\mu(X_7)$	$\mu(X_8)$	$\mu(X_9)$	Assessment Values (D)	Rank
36	0.502	0.258	0.309	0.026	0.945	0.399	0.391	0.000	0.550	0.379	34
37	0.432	0.734	0.004	0.300	0.637	0.046	0.471	0.476	0.284	0.403	22
38	0.518	0.263	0.144	0.084	0.815	0.071	0.582	0.344	0.431	0.357	37
39	0.296	0.151	0.228	0.210	0.862	0.038	0.317	0.292	0.242	0.285	44
40	0.613	0.539	0.094	0.106	0.904	0.269	0.620	0.305	0.994	0.471	9
41	0.629	0.255	0.194	0.111	0.687	0.470	0.607	0.355	0.646	0.418	16
42	0.526	0.293	0.074	0.061	0.895	0.357	0.793	0.319	1.000	0.411	19
43	0.410	0.294	0.297	0.334	1.000	0.140	0.299	0.144	0.913	0.402	24
44	0.824	0.588	1.000	1.000	0.558	1.000	0.000	0.897	0.848	0.770	2
45	0.528	0.439	0.119	0.213	0.764	0.369	0.505	0.477	0.380	0.418	17
Weight	0.247	0.185	0.149	0.112	0.094	0.063	0.057	0.053	0.041		

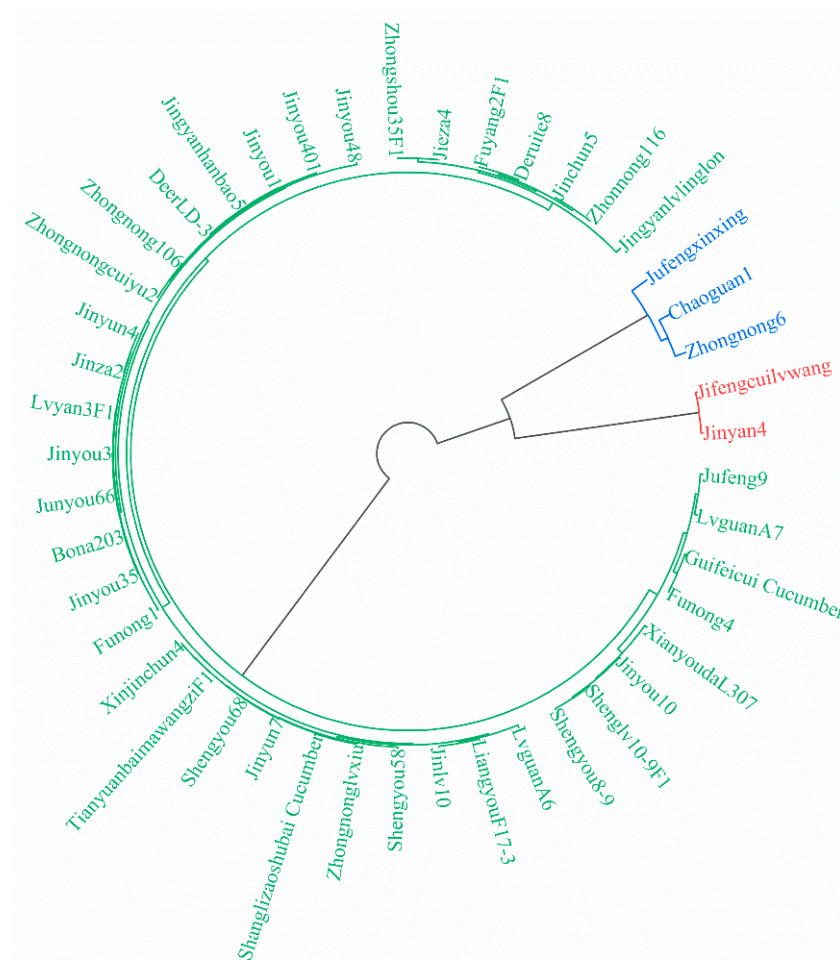


Figure 1. Cluster analysis of the autotoxicity tolerance of different cucumber cultivars. Red indicates 2 cultivars with high autotoxicity tolerance; blue indicates 3 cultivars with average autotoxicity tolerance, and green indicates 40 cultivars with low autotoxicity tolerance.

3.4. Construction of an Autotoxicity Tolerance Evaluation System of Cucumber Cultivars

Stepwise regression analysis was performed with the comprehensive score D as the dependent variable and 28 indicators as the independent variables. Regression analysis equation: $D' = 0.0932 + 0.001X_1 + 0.00019X_4 - 0.00064X_8 + 0.00005X_9 - 0.00028X_{11} + 0.00031X_{13} - 0.0002X_{14} + 0.00005X_{15} + 0.0001X_{16} - 0.00013X_{17} + 0.00017X_{18} - 0.0004X_{20} - 0.00006X_{21} + 0.00012X_{23} + 0.00012X_{24} + 0.00041X_{25} + 0.00042X_{26} + 0.0004X_{27} + 0.00034X_{28}$ ($R^2 = 0.9998$, $p < 0.01$).

Regression analysis excluded nine indicators, including relative germination potential, relative root length, relative root fresh weight, relative shoot fresh weight, germination toxicity rate, shoot length toxicity rate, root–shoot ratio toxicity rate, and β -amylase activity and total amylase activity inhibition rate among the 28 indicators, indicating that these nine indicators have a low contribution rate in the evaluation of cucumber resistance to autotoxicity in this study. The relative germination rate (X1), relative bud length (X4), germination potential toxicity rate (X8), root length toxicity rate (X9), relative root–shoot ratio (X11), root fresh weight toxicity rate (X13), bud fresh weight toxicity rate (X14), MDA content increase rate (X15), relative MDA content (X16), α -amylase activity inhibition rate (X17), α -amylase activity inhibition rate (X18), β -amylase activity inhibition rate (X20), total amylase activity inhibition rate (X21), POD activity increase rate (X23), relative POD activity (X24), SOD activity increase rate (X25), relative SOD activity (X26), CAT activity increase rate (X27), relative CAT activity (X28), and other 19 indicators reached a significant level ($p < 0.01$). It can be used as a representative evaluation index for the evaluation system. The linear analysis of the comprehensive score D and the regression value D' of the 45 cucumber cultivars showed that $R^2 = 0.9998$ (Figure 2). At the same time, the regression accuracy of the comprehensive score D and the regression value D' was analyzed. The estimation accuracy of the regression value and the original value was above 99.0% (Supplementary Table S3), which proved that the retained indices can be used as indices for evaluating cucumber autotoxicity tolerance.

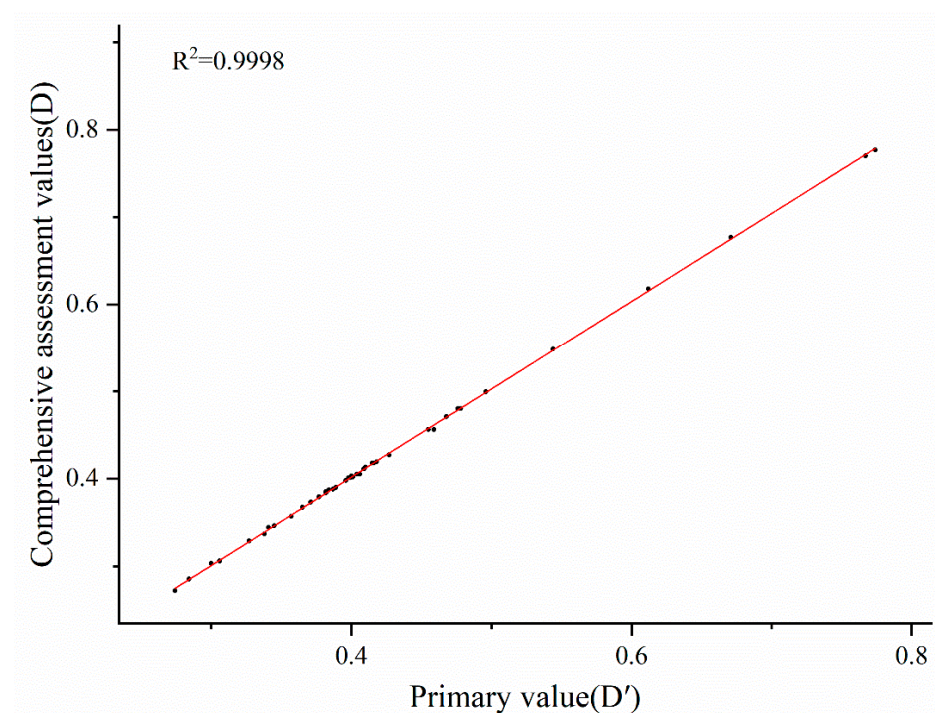


Figure 2. Linear analysis of the comprehensive score values (D) and regression values (D') of 45 cucumber varieties.

We validated the results of the comprehensive evaluation, which showed that (i) CA treatment did not significantly inhibit the growth of 'Jinyan4', the growth of 'Jufengxinxing' was significantly inhibited, but the growth of 'Jufeng9' was more significantly inhibited (Figure 3); (ii) CA treatment did not significantly inhibit plant height, stem diameter, leaf area, and root length of 'Jinyan4'; the growth of 'Jufengxinxing' was inhibited, but the inhibition of 'Jufeng9' was more significant (Table 5). This result coincided with the comprehensive evaluation results, indicating that the constructed cucumber autotoxicity tolerance evaluation system is usable.



Figure 3. Seedling phenotypes of three cultivars differing in autotoxicity tolerance under cinnamic acid (CA) stress. CK: Cucumber seedlings treated with 0 mmol·L^{−1} CA. CA: Cucumber seedlings treated with 1.2 mmol·L^{−1} CA. Note: Cucumber seedlings were treated when the cotyledons were fully expanded, and the treatments were photographed for up to 14 days.

Table 5. Morphological changes in the seedlings of three cucumber cultivars treated with 1.2 mmol·L^{−1} cinnamic acid.

Cultivars and Treatments		Plant Height (cm)	Stem Diameter (mm)	Leaf Area (cm ²)	Root Length (cm)
Jinyan4	CK	13.87 ± 0.36 a	5.30 ± 0.24 ab	22.49 ± 0.59 a	8.90 ± 0.36 a
	CA	13.17 ± 0.36 a	4.77 ± 0.14 bc	20.95 ± 0.77 a	7.93 ± 0.46 ab
Jufengxinxing	CK	13.97 ± 0.42 a	5.43 ± 0.36 a	22.17 ± 0.68 a	8.70 ± 0.78 a
	CA	10.90 ± 0.32 b	4.23 ± 0.05 cd	17.00 ± 0.93 b	6.43 ± 0.14 b
Jufeng9	CK	13.63 ± 0.58 a	5.10 ± 0.18 ab	22.63 ± 0.99 a	8.63 ± 1.05 a
	CA	8.10 ± 0.45 c	4.03 ± 0.14 d	10.07 ± 0.46 c	4.13 ± 0.74 c

Different lowercase letters in the same column indicate a significant difference between the treatments at $p < 0.05$. Data are the mean ± standard error of the mean of at least three different replicates of each treatment.

4. Discussion

Autotoxicity is one of the constraints to the sustainable development of facility cucumber [2]. From the cultivar point of view, the discovery of cultivars with strong autotoxicity tolerance is important for overcoming the continuous cropping obstacle. Significant changes in the external morphology and physiological metabolism of cucumber were observed under autotoxic stress [7,34,35]. In this study, we conducted germination tests after treating the seeds of 45 cucumber cultivars with 0.8 mmol·L^{−1} cinnamic acid and found that the degree of phenotypic and physiological metabolic changes was inconsistent. There were some index changes that were relatively significant, but there were also some index changes that were not significant. In a high-volume cultivar screening exercise, these indices of insignificant variation were meaningless in the screening process. Previous work on varietal screening of cucumber cultivars for salt tolerance [19], heat tolerance [36], and cold tolerance [21] has also pointed out that the occurrence of changes in the response indices to stress varied among the cultivars. Therefore, we used a comprehensive analytical approach based on multiple indicators in order to rapidly and accurately characterize autotoxicity

tolerance in cucumber cultivars. This work was of great importance for the subsequent screening of larger quantities of cultivars.

In autotoxic stress, plants respond to stress through physiological metabolism, and these physiological metabolic processes are interrelated. For example, enhanced SOD, POD, and CAT activity in cucumber seedlings under autotoxic stress resulted in lower levels of MDA and less cell membrane damage [34]. Here, in our work of screening 45 cucumber cultivars, we can understand the correlation between these indices through correlation analysis by referring to the previous research results to analyze the rationality of our data. This will help to ensure that reliable data support is provided for the subsequent screening work. In the results of correlation analysis of this study, we found that SOD, POD, CAT activity and MDA content were elevated during cucumber seed germination under cinnamic acid treatment, which was slightly different from the findings of [34] and similar to those of [10,11]. It was possible that this result was due to the large difference between our cinnamic acid treatment concentration and theirs. In addition, α -amylase activity, total amylase activity, and β -amylase activity of cucumber seeds under autotoxic stress were inhibited to varying degrees during the germination process, which was in general agreement with previous studies [12]. The results of the correlation analysis indicate that our data were reasonable, which was a guarantee for our subsequent work.

Principal component analysis has been increasingly used in the screening of crop germplasm resources [37–41]. This method was suitable for large sample sizes and index sizes in screening exercises. In this study, principal component analysis regrouped the 28 indices into 9 principal components, and the cumulative contribution of the 9 principal components reached 91.13% (when the cumulative contribution was $\geq 80\%$, it was usually considered that these principal components better represented the characteristics of the original data). This result indicates that the reintegrated 9 principal components represent the characteristics of the 28 indices. The score value of each cultivar in each principal component was calculated based on the principal components. This method was limited to the evaluation of the contribution rate of each factor, or it cannot synthesize all principal components for comprehensive evaluation [42]. The affiliation function analysis method can synthesize the effects of multiple factors and make the assessment results more comprehensive and objective [43]. For example, Fu et al. [20] and Miao et al. [21] screened heat-tolerant and cold-tolerant cucumber cultivar germplasm by combining principal component analysis and affiliation function analysis. In this study, the composite evaluation value of each final cultivar was calculated based on the weights and affiliation function values of the 9 principal components. Although the composite evaluation value was calculated, it was not sufficient to visualize the degree of autotoxicity tolerance of these cultivars. Cluster analysis, on the other hand, provides a more intuitive view of the degree of autotoxicity tolerance of these cultivars. For example, Chen et al. [22] used cluster analysis to visualize the salt tolerance of different cereal seeds during germination. In this study, 45 cucumber cultivars were visually categorized into three different autotoxicity tolerance groups by cluster analysis. It is not possible to discern which specific indices contribute to the composite evaluation value in the whole set of evaluation systems described above. The advantage of stepwise regression analysis was that it was able to find the best indicator among a large number of indicators, thus avoiding the overfitting problem [44]. In this study, 19 indices were found to contribute to the composite evaluation value to a large extent after stepwise regression analysis. An evaluation equation for cucumber autotoxicity tolerance was fitted based on the 19 indices. The coefficient of determination of the equation $R^2 = 0.9998$ indicates that the fitted evaluation equation is reasonable. The previous work on the evaluation and screening of germplasm resources ended with the composite evaluation results, but there was a lack of validation of these results [45]. In this study, we conducted a result validation to ensure the reliability of the results. We selected resistant and non-resistant cultivars identified in the preliminary study for additional trials of root-applied CA. The results showed that CA treatment did not significantly inhibit the growth of 'Jinyan4', and the growth of 'Jufengxinxing' was significantly inhibited, but the

inhibition of ‘Jufeng9’ was more significant. This validation indicates that the cucumber autotoxicity tolerance evaluation system we constructed was reliable. The prediction of cucumber autotoxicity tolerance using this evaluation system can greatly simplify the identification work and provide a basis for the selection and breeding of autotoxicity-tolerant cucumber cultivars.

5. Conclusions

In this study, two cucumber cultivars with strong autotoxicity tolerance were screened out using a comprehensive evaluation method, namely “Jinyan4” and “Jifenglvwang”; the autotoxicity tolerance evaluation equation of the cucumber cultivars was fitted using a stepwise regression analysis: $D' = 0.0932 + 0.001X_1 + 0.00019X_4 - 0.00064X_8 + 0.00005X_9 - 0.00028X_{11} + 0.00031X_{13} - 0.0002X_{14} + 0.00005X_{15} + 0.0001X_{16} - 0.00013X_{17} + 0.00017X_{18} - 0.0004X_{20} - 0.00006X_{21} + 0.00012X_{23} + 0.00012X_{24} + 0.00041X_{25} + 0.00042X_{26} + 0.0004X_{27} + 0.00034X_{28}$. The final autotoxicity tolerance validation test of the cucumber cultivars verified that the evaluation equations were feasible. Autotoxicity-tolerant cucumber cultivars and equations for evaluating autotoxicity tolerance in cucumber cultivars provide valuable insights to farmers, breeders, and researchers in overcoming the continuous cropping obstacle.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14051081/s1>, Table S1: Cucumber cultivar characteristics; Table S2: Correlation coefficient of each index; Table S3: Accuracy analysis of regression equation.

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References

1. Yu, J.Q. Autotoxic potential of cucurbit crops: Phenomenon, chemicals, mechanisms and means to overcome. *J. Crop Prod.* **2001**, *4*, 335–348. [CrossRef]
2. Singh, H.P.; Batish, D.R.; Kohli, R.K. Autotoxicity: Concept, organisms, and ecological significance. *Crit. Rev. Plant Sci.* **1999**, *18*, 757–772. [CrossRef]
3. Liu, X.; Li, Y.J.; Ren, X.J.; Chen, B.H.; Zhang, Y.; Shen, C.W.; Wang, F.; Wu, D.F. Long-term greenhouse cucumber production alters soil bacterial community structure. *J. Soil Sci. Plant Nutr.* **2020**, *20*, 306–321. [CrossRef]
4. Xiao, X.; Cheng, Z.; Lv, J.; Xie, J.; Ma, N.; Yu, J. A green garlic (*Allium sativum* L.) based intercropping system reduces the strain of continuous monocropping in cucumber (*Cucumis sativus* L.) by adjusting the micro-ecological environment of soil. *PeerJ.* **2019**, *7*, e7267. [CrossRef] [PubMed]
5. Bai, X.; Gao, J.; Wang, S.; Cai, H.; Chen, Z.; Zhou, J. Excessive nutrient balance surpluses in newly built solar greenhouses over five years leads to high nutrient accumulations in soil. *Agric. Ecosyst. Environ.* **2020**, *288*, 106717. [CrossRef] [PubMed]
6. Zhang, Z.H.; Fan, J.R.; Wu, J.H.; Zhang, L.Z.; Wang, J.R.; Zhang, B.B.; Wang-Pruski, G. Alleviating effect of silicon on melon seed germination under autotoxicity stress. *Ecotoxicol. Environ. Saf.* **2020**, *188*, 109901. [CrossRef] [PubMed]
7. Yu, J.Q.; Matsui, Y. Phytotoxic substances in root exudates of cucumber (*Cucumis sativus* L.). *J. Chem. Ecol.* **1994**, *20*, 21–31. [CrossRef]
8. Wu, F.Z.; Liu, D.; Shi, L.F. Effect of duration of protected cultivation on yield of cucumber. *J. Northeast Agric. Univ.* **1999**, *30*, 245–248. [CrossRef]
9. Kato-Noguchi, H.; Nakamura, K.; Okuda, N. Involvement of an autotoxic compound in asparagus decline. *J. Plant Physiol.* **2018**, *224–225*, 49–55. [CrossRef]
10. Yang, P.; Muhammad, A.N.; Li, F.X.; Bai, L.S.; Li, J. Brassinosteroids regulate antioxidant system and protect chloroplast ultrastructure of autotoxicity stressed cucumber (*Cucumis sativus* L.) Seedlings. *Agronomy* **2019**, *9*, 265. [CrossRef]

11. Bu, R.F.; Xie, J.M.; Yu, J.H.; Liao, W.B.; Xiao, X.M.; Lv, J.; Wang, C.L.; Ye, J.; CalderonUrrea, A. Autotoxicity in cucumber (*Cucumis sativus* L.) seedlings is alleviated by silicon through an increase in the activity of antioxidant enzymes and by mitigating lipid peroxidation. *J. Plant Biol.* **2016**, *59*, 247–259. [\[CrossRef\]](#)
12. Liu, F.H.; Lv, J.; Yu, J.H.; Jin, N.; Jin, L.; Hu, L.L.; Wu, Y.; Liu, X.Q.; Wang, S.Y. Effects of silicon on physiological characteristics of cucumber seed germination under self-toxic stress. *J. Northwest A F Univ. (Nat. Sci. Ed.)* **2020**, *48*, 90–96. [\[CrossRef\]](#)
13. Hu, W.; Zhang, Y.; Huang, B.; Teng, Y. Soil environmental quality in greenhouse vegetable production systems in eastern China: Current status and management strategies. *Chemosphere* **2017**, *170*, 183–195. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Kyriacou, M.C.; Roupael, Y.; Colla, G.; Zrenner, R.; Schwarz, D. Vegetable grafting: The implications of a growing agronomic imperative for vegetable fruit quality and nutritive value. *Front. Plant Sci.* **2017**, *8*, 741. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Miao, L.; Di, Q.; Sun, T.; Li, Y.; Duan, Y.; Wang, J.; He, C.; Wang, C.; Yu, X. Integrated metabolome and transcriptome analysis provide insights into the effects of grafting on fruit flavor of cucumber with different rootstocks. *Int. J. Mol. Sci.* **2019**, *20*, 3592. [\[CrossRef\]](#)
16. Wang, S.; Kang, X.; Dai, J.; Dai, W.; Zhang, J.; Ji, J. Evaluation of areca quality based on principal component and hierarchical cluster analyses in Hainan, China. *HortScience* **2023**, *58*, 699–703. [\[CrossRef\]](#)
17. Gao, B.Y.; Lu, Y.J.; Sheng, Y.; Chen, P.; Yu, L.L. Differentiating organic and conventional sage by chromatographic and mass spectrometry flow injection fingerprints combined with principal component analysis. *J. Agric. Food Chem.* **2013**, *61*, 2957–2963. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Li, J.; Yu, J.H.; Wu, Y.; Tang, Z.Q.; Liu, Z.C.; Lv, J. Quality evaluation of different small fruit watermelon varieties. *China Cucurbits Veg.* **2020**, *33*, 61–67. [\[CrossRef\]](#)
19. Men, L.Z.; Wang, Y.; Gao, Y.E.; Chen, Q.Y.; Tian, Y.Q.; Gao, L.H. Salt tolerance indexes screening of different cucumber cultivars at seedling stage and construction of its evaluation system. *China Veg.* **2013**, *22*, 20–26. [\[CrossRef\]](#)
20. Fu, L.J.; Li, C.X.; Su, S.Y.; Li, Y.H.; Zhou, Y. Screening of cucumber germplasms in seedling stage and the construction of evaluation system for heat tolerance. *Plant Physiol. J.* **2020**, *56*, 1593–1604. [\[CrossRef\]](#)
21. Miao, Y.M.; Ning, Y.; Gao, Y.J.; Shen, J.; Pang, X.; Gui, L.; Cheng, C.Y.; Chen, J.F. Evaluation of cucumber's chilling tolerance at germination and seedling stages. *Chin. J. Appl. Ecol.* **2013**, *24*, 1914–1922.
22. Chen, E.Y.; Wang, R.F.; Qin, L.; Yang, Y.B.; Li, F.F.; Zhang, H.W.; Wang, H.L.; Liu, B.; Kong, Q.H.; Guan, Y.A. Comprehensive identification and evaluation of foxtail millet for saline-alkaline tolerance during germination. *Acta Agron. Sin.* **2020**, *46*, 1591–1604. [\[CrossRef\]](#)
23. Bu, R.F.; Zhang, H.R.; Zhang, S.; Wang, L.S.; Peng, C.Y.; Zhao, X.H.; Zhang, X.N.; Xie, J.M. Silicon alleviates autotoxicity by regulating membrane lipid peroxidation and improving photosynthetic efficiency in cucumber seedlings (*Cucumis sativus* L.). *Sci. Hortic.* **2024**, *325*, 112692. [\[CrossRef\]](#)
24. Meng, X.; Luo, S.L.; Dawuda, M.M.; Gao, X.Q.; Wang, S.Y.; Xie, J.M.; Tang, Z.Q.; Liu, Z.C.; Wu, Y.; Jin, L.; et al. Exogenous silicon enhances the systemic defense of cucumber leaves and roots against CA-induced autotoxicity stress by regulating the ascorbate-glutathione cycle and photosystem II. *Ecotoxicol. Environ. Saf.* **2021**, *227*, 112879. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Xiao, X.; Lv, J.; Xie, J.; Feng, Z.; Ma, N.; Li, J.; Yu, J.; Calderón-Urrea, A. Transcriptome analysis reveals the different response to toxic stress in rootstock grafted and non-grafted cucumber seedlings. *Int. J. Mol. Sci.* **2020**, *21*, 774. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Xiao, X.M.; Ma, N.; Li, J.; Wang, R.; Hu, L.L.; Wu, Y.; Yu, J.H. Effect of autotoxic stress on seed germination and antioxidant property of cucumber scion and rootstock. *China Veg.* **2022**, *9*, 22–29. [\[CrossRef\]](#)
27. Dhindsa, R.S.; Plumb-Dhindsa, P.; Thopre, T.A. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* **1981**, *32*, 93–101. [\[CrossRef\]](#)
28. Song, A.; Li, Z.J.; Zhang, J.; Xue, G.F.; Fan, F.L.; Liang, Y.C. Silicon-enhanced resistance to cadmium toxicity in *Brassica chinensis* L. is attributed to Si-suppressed cadmium uptake and transport and Si-enhanced antioxidant defense capacity. *J. Hazard. Mater.* **2009**, *172*, 74–83. [\[CrossRef\]](#)
29. Gunes, A.; Inal, A.; Bagci, E.G.; Coban, S.; Pilbeam, D.J. Silicon mediates changes to some physiological and enzymatic parameters symptomatic for oxidative stress in spinach (*Spinacia oleracea* L.) grown under B toxicity. *Sci. Hortic.* **2007**, *113*, 113–119. [\[CrossRef\]](#)
30. Bialecka, B.; Kepczyński, J. Germination, α -, β -amylase and total dehydrogenase activities of amaranthus caudatus seeds under water stress in the presence of ethephon or gibberellin A3. *Acta Biol. Cracoviensis Ser. Bot.* **2010**, *52*, 7–12. [\[CrossRef\]](#)
31. Li, F.X.; Zhou, Y.F.; Wang, Y.T.; Sun, L.; Bai, W.; Yan, T.; Xu, W.J.; Huang, R.D. Screening and identification of sorghum cultivars for alkali tolerance during germination. *Sci. Agric. Sin.* **2013**, *46*, 1762–1771. [\[CrossRef\]](#)
32. Han, F.; Zhuge, Y.P.; Lou, Y.H.; Wang, H.; Zhang, N.D.; He, W.; Chao, Y. Evaluation of salt tolerance and screening for salt tolerant accessions of 63 foxtail millet germplasm. *J. Plant Genet. Resour.* **2018**, *19*, 685–693. [\[CrossRef\]](#)
33. Jatav, V.; Singh, D.; Singh, N.; Panchbhairya, A. Principal component analysis in bitter gourd (*Momordica Charantia* L.). *Bangladesh J. Bot.* **2022**, *51*, 1–7. [\[CrossRef\]](#)
34. Wu, F.Z.; Huang, C.H.; Zhao, F.Y. Effects of phenolic acids on growth and activities of membrane protective enzymes of cucumber seedlings. *Sci. Agric. Sin.* **2002**, *35*, 821–825. [\[CrossRef\]](#)
35. Ma, N.; Chen, B.; Yang, H.; Liu, W.; Huang, X.C.; Xiao, X.M.; Xie, J.M. Different response of growth, development and photosynthetic fluorescence characteristics of non-grafted and rootstock grafted cucumber seedling to autotoxic stress. *China Cucurbits Veg.* **2020**, *33*, 17–23. [\[CrossRef\]](#)

36. Tian, X.; Liu, X.Q.; Liu, X.R.; Li, Q.S.; Abd_Allah, E.F.; Wu, Q.S. Mycorrhizal cucumber with *Diversispora versiformis* has active heat stress tolerance by up-regulating expression of both *CsHsp70s* and *CsPIPs* genes. *Sci. Hortic.* **2023**, *319*, 111870. [\[CrossRef\]](#)
37. Yu, Y.; Li, Y.J.; Wang, F.; Wang, N.; Huo, Y.L.; Feng, T. Principal component and cluster analysis of agronomic characters on cucumber germplasm resources in northern China. *China Cucurbits Veg.* **2020**, *33*, 29–34. [\[CrossRef\]](#)
38. Wang, L.Y.; Wang, X.M.; Jing, R.Y.; Guo, Y.X. Comprehensive quality evaluation and analysis of nutrition components of various flaxseed. *Food Mach.* **2021**, *37*, 26–32. [\[CrossRef\]](#)
39. Cao, Q.W.; Du, L.D.; Yang, Z.H.; Li, L.B.; Duan, X.; Yang, W.Q.; Chen, W.; Meng, Z.J. Screening and evaluation of cucumber salt-tolerant germplasm. *J. Nucl. Agric. Sci.* **2022**, *36*, 865–875. [\[CrossRef\]](#)
40. Xu, X.; Liu, M.J.; Wang, J.H.; Sun, Q.; Xiang, C.Y. Differences in vigor of sweet corn seeds under different adverse conditions. *Jiangsu Agric. Sci.* **2019**, *47*, 76–79. [\[CrossRef\]](#)
41. Zhao, W.X.; Liu, X.C.; Li, X.H.; Xu, X.L.; Chang, G.Z.; Liang, S.; Kang, L.Y.; Gao, N.N. Physiological responses of muskmelon seedlings to different adversity stresses and synthetical evaluation of stress resistance. *Southwest China J. Agric. Sci.* **2017**, *30*, 322–326. [\[CrossRef\]](#)
42. Guo, C.; Zhu, L.; Sun, H.; Han, Q.; Wang, S.; Zhu, J.; Zhang, Y.; Zhang, K.; Bai, Z.; Li, A.; et al. Evaluation of drought-tolerant varieties based on root system architecture in cotton (*Gossypium hirsutum* L.). *BMC Plant Biol.* **2024**, *24*, 127. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Tian, H.; Liu, H.; Zhang, D.; Hu, M.; Zhang, F.; Ding, S.; Yang, K. Screening of salt tolerance of maize (*Zea mays* L.) lines using membership function value and GGE biplot analysis. *PeerJ.* **2024**, *12*, e16838. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Bahmani, K.; Izadi-Darbandi, A.; Noori SA, S.; Jafari, A.A.; Moradi, N. Determination of interrelationships among phenotypic traits of iranian fennel (*Foeniculum vulgare* Mill.) using correlation, stepwise regression and path analyses. *J. Essent. Oil Bear. Plants* **2012**, *15*, 424–444. [\[CrossRef\]](#)
45. Zhang, N.; Zhang, H.; Ren, J.; Bai, B.; Guo, P.; Lv, Z.; Kang, S.; Zhao, X.; Yu, H.; Zhao, T. Characterization and comprehensive evaluation of phenotypic and yield traits in salt-stress-tolerant peanut germplasm for conservation and breeding. *Horticulturae* **2024**, *10*, 147. [\[CrossRef\]](#)

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