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ARTICLE

The Effects of Planting at Varying Seedling Ages on the Agronomic Traits and Nutritional Components of Stem

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ABSTRACT: This study aimed to elucidate the effects of varying seedling ages at planting on the agronomic traits and nutrient content of stem lettuce. The early-maturing variety “WS120” and the late-maturing variety “WS1” were employed as experimental materials. Four seedling age treatments were established at 20, 25, 30, and 35 d. By measuring the agronomic traits and nutrient content of the stem lettuce, we employed correlation analysis, principal component analysis, cluster analysis, and the membership function method for a comprehensive evaluation. This study aims to elucidate the optimal planting age for stem lettuce in plateau regions. By addressing the issues of inconsistent quality and yield that arise from arbitrary selection of planting age in production, we seek to establish a theoretical foundation for the development of a high-quality and efficient cultivation technology system for this crop. The results of the correlation analysis revealed that a total of 18 pairs of indicators in WS1 exhibited significant correlations, comprising 8 pairs that were positively correlated and 10 pairs that were negatively correlated. In WS120, 25 pairs of indicators reached a significant correlation level, with 13 pairs positively correlated and 12 pairs negatively correlated. Principal component analysis identified 14 agronomic traits and 4 quality indicators, which were consolidated into 3 principal components, achieving cumulative contribution rates of 94.068%. Cluster analysis results indicated that WS1 could be categorised into two groups based on different treatments. At 30 d and 35 d, the samples were grouped together due to superior root-related indicators and nutritional components, whereas at 25 d and 20 d, they formed a separate group owing to enhanced agronomic traits. WS120 was classified into Group I, reflecting relatively favourable agronomic traits at 25 d and 20 d. Group II (30 d) was characterised by all indicators falling within the mid-range. Class III (35 d) was distinctly categorised due to elevated levels of vitamin C, soluble sugars, and soluble proteins. A comprehensive evaluation employing the membership function method indicated that the overall performance of WS1 and WS120 under varying treatments was ranked as follows: 25 d > 30 d > 20 d > 35 d. It can be utilised directly to inform production practices and holds considerable practical importance for advancing the high-quality and efficient cultivation of stem lettuce, thereby contributing to the growth of the plateau vegetable industry.

KEYWORDS: Stem lettuce; seedling age; agronomic traits; nutrient content; comprehensive evaluation; WS1; WS120

1 Introduction

Lactuca sativa L. is a biennial herb belonging to the Lactuca genus, indigenous to the Mediterranean coast. It was introduced to China during the Sui and Tang Dynasties [1]. Lettuce can be categorised into two types [2], leaf lettuce and stem lettuce. The latter is primarily consumed as a vegetable due to its tender, fleshy stems. Stem lettuce exhibits remarkable adaptability to various environmental conditions, allowing for year-round cultivation [3], which has led to its widespread cultivation in China. This vegetable possesses significant nutritional and economic value. Currently, in response to the growing consumer demand for high-quality vegetables, the cultivation area of stem lettuce continues to expand [4].

In recent years, the widespread adoption of facility cultivation techniques and the promotion of intensive seedling models have markedly enhanced the seasonal adaptability of stem lettuce cultivation. Consequently, multi-cropping patterns, including those for spring, autumn, and overwintering, have become increasingly prevalent [5,6]. However, significant variations in the growth rates of stem lettuce seedlings arise under different cultivation seasons and planting patterns. The absence of a unified standard for defining optimal seedling age often leads to issues such as reduced yield and diminished quality, which frequently occur due to improper selection of seedling age in production [7,8]. Previous studies have demonstrated that both excessively young and overly mature seedlings can negatively impact crop growth [9]. Tomato seedlings that are too young exhibit weak stress resistance, rendering them susceptible to diseases and environmental factors following transplanting, which leads to diminished growth vigour and poor resilience in the early stages [10]. Mishra Ay et al. found that when the age of rice seedlings is inappropriate, their incomplete root and stem development hampers their ability to adapt to the environment, consequently inhibiting growth [11]. Excessive seedling age presents notable challenges. As seedling age increases, the bolting rate of onions rises markedly [12]. In late-maturing rice varieties, older seedlings exhibit a more pronounced decline in yield following transplantation [13]. Research conducted by Jan B on sweet corn revealed that older transplanted seedlings demonstrated the poorest performance in growth indicators associated with various phenological periods [14]. The appropriate age for transplanting is crucial. Seedlings at the optimal age exhibit robust root vitality and well-differentiated tissue structures. Following transplantation, these seedlings can swiftly acclimatise to their environment, minimising physiological stress during the seedling stage. This adaptation subsequently enhances nutrient absorption and dry matter accumulation, thereby ensuring the crops maintain a healthy growth state [15].

This study established various transplanting treatments based on seedling age to systematically evaluate the agronomic traits and nutrient content of stem lettuce. The objective is to identify the optimal transplanting age for stem lettuce, thereby providing a scientific basis and technical support for precise seedling cultivation and transplanting. This approach aims to enhance yield stability and economic benefits while promoting the high-quality development of the vegetable industry.

2 Material and Methods

2.1 Test Materials

The varieties tested were WS1 and WS120, supplied by the College of Agriculture and Forestry Sciences at Qinghai University. WS1 is characterised as a late-maturing variety, exhibiting green pointed leaves, white skin, and green flesh. In contrast, WS120 is an early-maturing variety, distinguished by its purple-green pointed leaves and white skin with green flesh.

2.2 Experimental Design

The experiment was conducted at the Horticultural Innovation Base of Qinghai University from April 2025 to June 2025. The experimental materials were uniformly cultivated in a seedling greenhouse and sown in 72-hole black PVC (Polyvinyl Chloride) plastic trays. Daytime temperatures were maintained between 18°C and 20°C, while nighttime temperatures ranged from 8°C to 10°C. The growth period to achieve 4–5 true leaves is approximately 25 d. Subsequently, the plants are transplanted into the greenhouse as flat beds with film mulching at intervals of 20, 25, 30, and 35 d, respectively (Table 1). Following planting, the test area of the plot measured 25 m^2, with dimensions of 5 m in length and 5 m in width. The planting density was set at 35 × 35 cm, with three repetitions arranged in a random block design. The soil organic matter content in the experimental area was measured at 20.28 g/kg, with a pH value of 8.12. The soil also contained 1.17 g/kg of total nitrogen, 2.18 g/kg of total phosphorus, and 22.50 g/kg of total potassium. Additionally, the available nitrogen was recorded at 69 mg/kg, available phosphorus at 65 mg/kg, and available potassium at 229 mg/kg [16].

Table 1: Age treatment of experimental seedlings.

Crop Variety	Processing	Transplanting Time
WS1	20 d	2025.4.10
	25 d	2025.4.15
	30 d	2025.4.20
	35 d	2025.4.25
WS120	20 d	2025.4.10
	25 d	2025.4.15
	30 d	2025.4.20
	35 d	2025.4.25

Note: “d” represents the number of days for seedling cultivation.

2.3 Index Measurement and Methods

Determination of Agronomic Traits: During the organ harvest period of stem lettuce, five plants were randomly selected from each plot for analysis. The assessment of agronomic traits was performed using measurement techniques, employing vernier calipers, tape measures, and balance scales as the primary instruments. Ten indicators were evaluated, specifically plant height, leaf length, leaf width, petiole width, petiole thickness, the number of stems and leaves, length of fleshy stems, thickness of fleshy stems, weight of a single stem, and weight of a single plant. All agronomic traits were investigated in accordance with the “Specification for Description and Data Standards of Lettuce Germplasm Resources” (ISBN: 9787109115033) [16].

Root morphologically related indicators, including root surface area (mm^2), total root length (mm), average root diameter (mm), and number of root tips (count), were assessed at 20, 25, 30, and 35 d following seedling cultivation. The root system was scanned and analysed using a root scanner.

Quality index measurement involved the determination of soluble protein using both the Coomassie brilliant blue method and the G-250 method. Vitamin C levels were assessed with the Boxbio kit from Beijing. The content of soluble sugar was also measured using the Boxbio kit from Beijing. Additionally, vitamin E content was evaluated using the Subcoine kit.

2.4 Data Processing and Analysis

Data processing was executed using Microsoft Excel 2019, while statistical analysis was undertaken with SPSS SS 20.0 software, and graphing was performed using Origin 2021. Fuzzy membership function analysis was applied to each agronomic trait and nutritional index. The formula for the membership function is given by: $\mu(Xi) = (Xi - Xmin)/(Xmax - Xmin)$, where Xi denotes the i-th comprehensive index value, and Xmax and Xmin represent the maximum and minimum mean values of this index, respectively [17].

3 Results and Analysis

3.1 The Influence of Planting at Different Seedling Ages on Agronomic Traits

Significant differences exist in the effects of planting at various seedling ages on the agronomic traits of stem lettuce. WS1 (Fig. 1) exhibited the following results during the organ harvest period: no significant differences in plant height were observed among the treatments. In the case of the 20 d seedling age treatment, leaf length, leaf width, and petiole thickness were 7.88%, 13.74%, and 24.2% greater than those recorded for the 35 d seedling age treatment, respectively. Nonetheless, no significant differences were noted in leaf length, leaf width, petiole width, petiole thickness, or the number of leaves for the treatments at 25 d and 30 d of seedling age. The length of succulent stems peaked at 20 d, measuring 36.77 cm, which was significantly greater than the lengths observed at 25 d, 30 d, and 35 d, with increases of 6.89%, 13.38%, and 10.88%, respectively. The thickness of the fleshy stems was greatest at 25 d, recorded at 65.30 mm, and was significantly higher than that at 20 d, 30 d, and 35 d, with increases of 12.65%, 9.85%, and 16.89%, respectively. Regarding yield-related indicators, the 25 d treatment exhibited the most favourable results: the weight of a single stem reached 897.67 g, which was 16.41% higher than that of the 35 d treatment ($p < 0.05$). The weight of a single plant was 1946.67 g, reflecting increases of 9.81%, 8.73%, and 16.32% compared to the treatments at 20 d, 30 d, and 35 d, with significant differences observed.

During the organ harvest period of stem lettuce products of WS120 (Fig. 2), the maximum plant height was observed at the 20 d treatment, reaching 56.67 cm. No significant differences were noted in leaf length and petiole width across the treatments. However, leaf width and the number of leaves were 14.75% and 32.56% greater, respectively, under the 20 d treatment compared to the 35 d treatment. The petiole thickness was greatest at the 25 d treatment, exhibiting a 19.13% increase over the 35 d treatment. Additionally, the length of fleshy stems was longest under the 20 d treatment at 36.50 cm, which represented a significant increase of 18.71% compared to the 35 d treatment. The thickness of the fleshy stems measured 55.19 mm after 25 d of treatment, which was significantly greater than that observed at 20 d and 35 d, representing increases of 7.72% and 14.15%, respectively. Regarding yield-related indicators, the treatment at 25 d yielded the most favourable results: the weight of a single stem was 542.67 g, reflecting increases of 13.39% and 38.7% compared to the 20 d and 35 d treatments ($p < 0.05$). Additionally, the weight of a single plant reached 961.33 g, which constituted increases of 15.95%, 14.32%, and 39.15% in comparison with the 20 d, 30 d, and 35 d treatments, respectively, with significant differences noted.

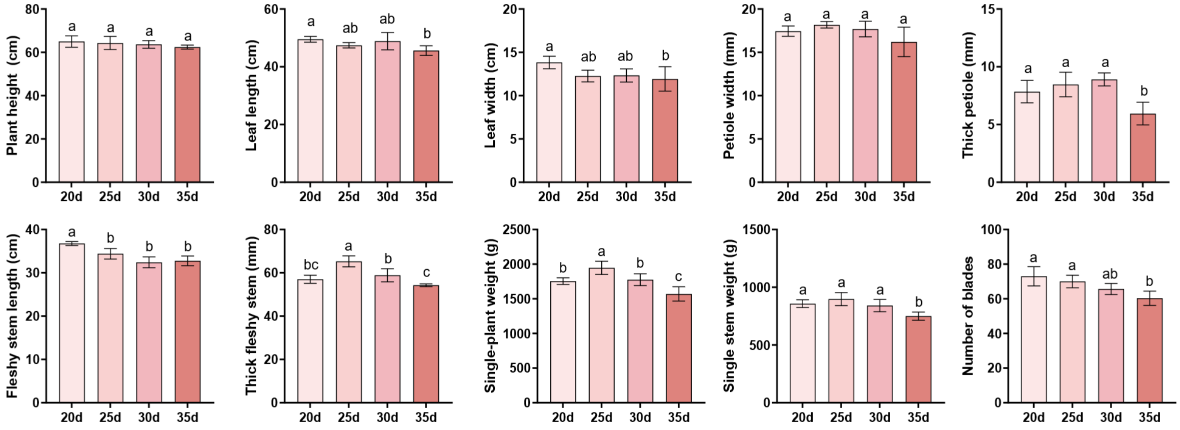

Figure 1: The influence of planting at different seedling ages on agronomic traits of WS1. Note: Different lowercase letters indicate significant differences between different treatments ($p < 0.05$).

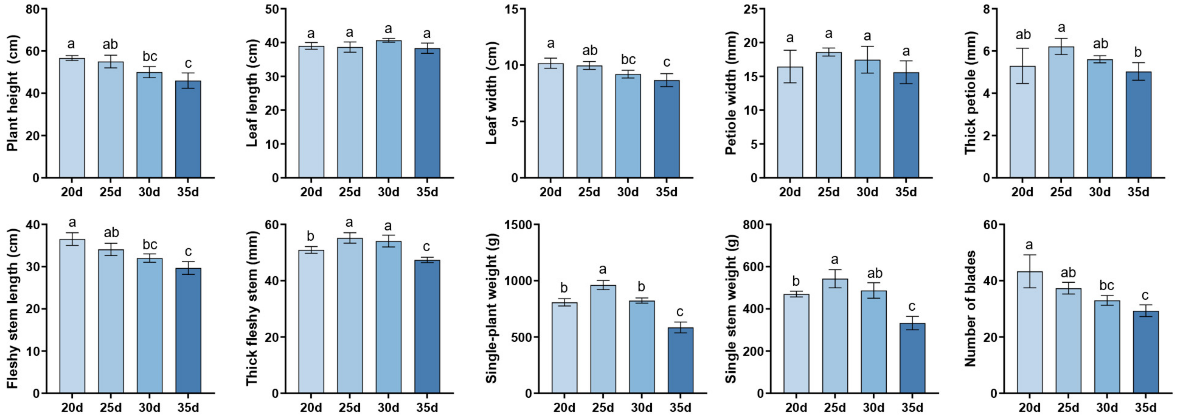

Figure 2: The influence of planting at different seedling ages on agronomic traits of WS120. Note: Different lowercase letters indicate significant differences between different treatments ($p < 0.05$).

3.2 The Influence of Planting at Different Seedling Ages on Root Morphology

Planting at varying seedling ages significantly influences the root morphology of stem lettuce (Figs. 3 and 4). As the number of d of seedling age increases, the root length, average diameter, surface area, and root tip number of WS1 and WS120 all reached their maximum values at the 35 d treatment and their minimum values at the 20 d treatment. Specifically, WS1 exhibited increases of 78.73%, 47.54%, 87.85%, and 61.78% under the 35 d treatment compared to the 20 d treatment. Similarly, WS120 demonstrated increases of 79.22%, 55.79%, 90.46%, and 64.52%, respectively.

3.3 The Influence of Planting at Different Seedling Ages on Nutritional Components

Planting at different seedling ages has a significant impact on the changes in nutrient content of stem lettuce (Fig. 5).

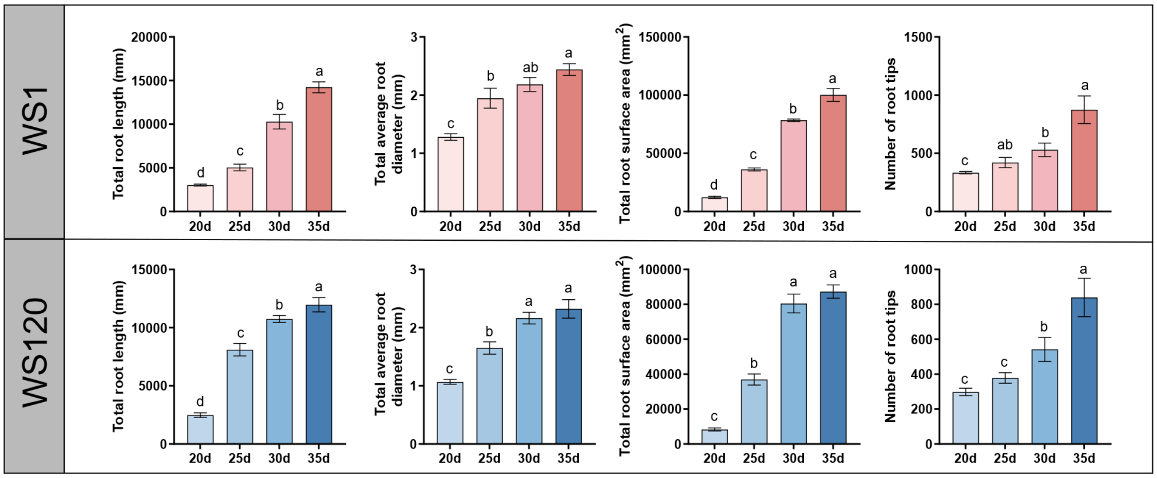

Figure 3: The effects of planting at different seedling ages on the root morphology of WS1 and WS120. Note: Different lowercase letters indicate significant differences between different treatments ($p < 0.05$).

During the organ harvest period of stem lettuce products, the soluble sugar content of WS1 (16.79 mg/g) following a 30 d treatment was significantly greater than that observed after a 20 d treatment, reflecting an increase of 31.69%. Additionally, vitamin E content (47.30 μg/g) at 25 d was significantly higher than at 20 d, with an increase of 13.59%. However, the levels of soluble protein and vitamin E did not exhibit significant differences across various seedling ages post-planting.

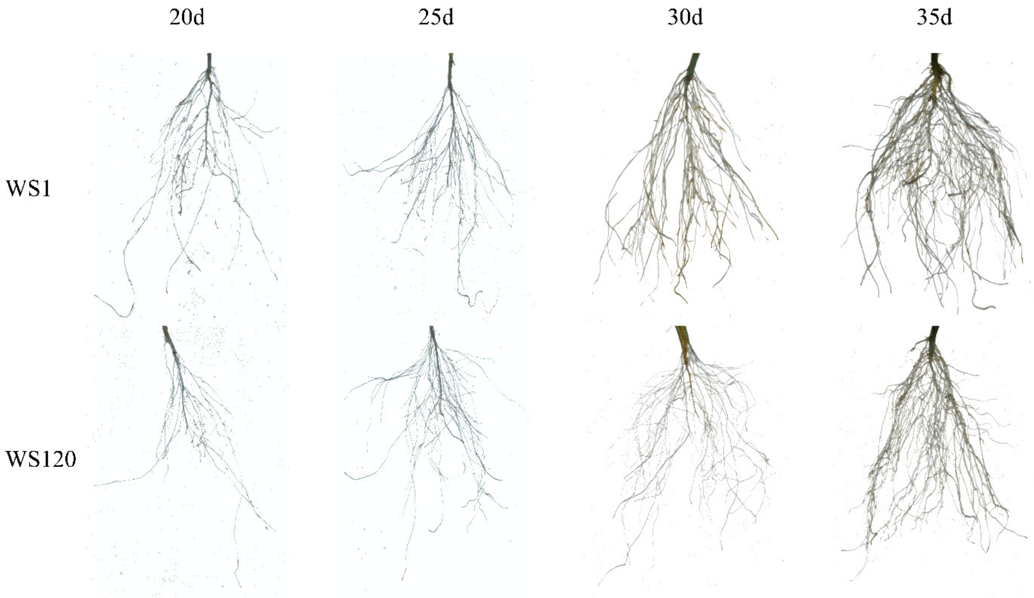

Figure 4: Root morphology diagrams of WS1 and WS120 at different seedling ages after planting.

There was no significant change in the soluble sugar content of WS120. The soluble protein content increased significantly from 20 d to 35 d, reaching 71.37 mg/g, which represents an increase of 11.99%. Vitamin C attained its peak concentration of 0.94 mg/g at 35 d of treatment, which was significantly higher than the levels observed at 20 d, 25 d, and 30 d, with increases of 19.15%, 10.64%, and 13.83%, respectively. Conversely, Vitamin E was significantly higher at 25 d, measuring 53.24 μg/g, than at 35 d, reflecting a decrease of 23.57%.

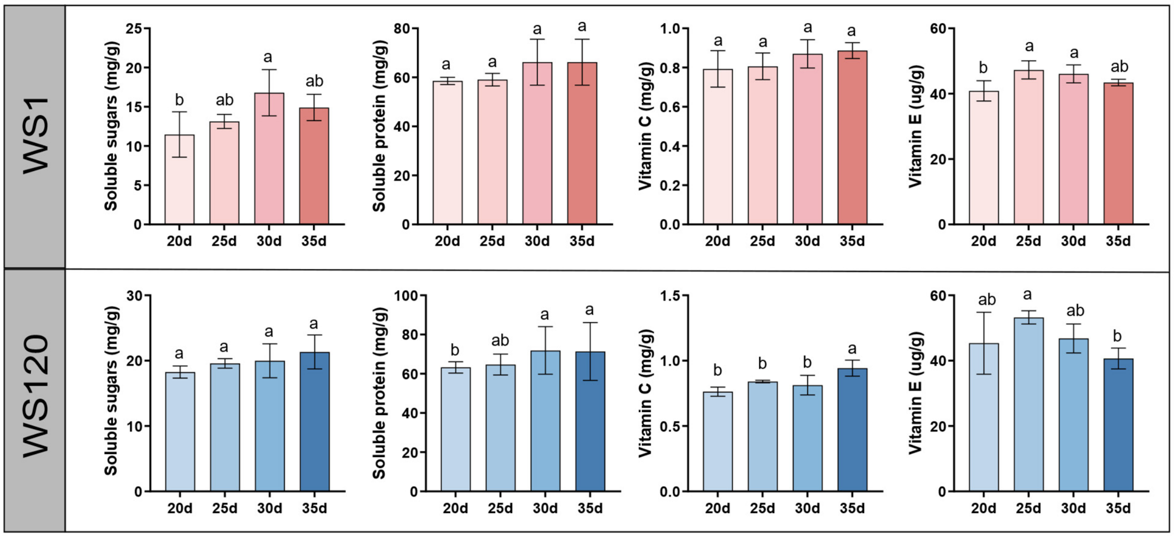

Figure 5: The influence of planting at different seedling ages on the nutritional components of WS1 and WS120. Note: Different lowercase letters indicate significant differences between different treatments ($p < 0.05$).

3.4 Correlation Analysis

Pearson correlation analysis was performed on the pairs of agronomic trait indicators and nutrient content for WS1 and WS120 (Fig. 6). The findings revealed a notable correlation between WS1 and the agronomic trait indicators, with a total of 11 pairs exhibiting significant correlations. Among these, four pairs demonstrated positive correlations: plant height with the number of leaves, petiole width with the weight of a single stem, the weight of a single stem with the weight of a single plant, and root length with root surface area. Traits including leaf count, petiole width, and root length function synergistically to enhance the photosynthetic surface area, facilitate the accumulation of individual stem weight and overall plant biomass, and ultimately drive plant growth and yield development. Conversely, seven pairs displayed negative correlations, specifically plant height with root length, root surface area with root tip number, leaf length with root tip number, succulent stem length with average root diameter, and leaf number with both root length and root surface area. A significant correlation was observed between nutrient contents, with vitamin C exhibiting a positive correlation with soluble protein. This suggests that the two experience synergistic alterations in antioxidant defence and nitrogen metabolism. Plants characterised by robust protein synthesis and heightened metabolic activity typically exhibit enhanced antioxidant capacity, which facilitates the accumulation of vitamin C. A total of six pairs were identified between agronomic trait indicators and nutrient content. Notably, the number of leaves demonstrated a negative correlation with both vitamin C and soluble protein. In contrast, vitamin C and root length were positively correlated with root surface area, as were soluble protein and root length.

The correlation among agronomic trait indicators of WS120 is markedly evident, with a total of 18 pairs exhibiting significant correlations. Of these, nine pairs demonstrate positive correlations, including plant height with single stem weight, single plant weight, leaf width, petiole width, petiole thickness, succulent stem length, leaf number, single stem weight with single plant weight, root length with average root diameter and root surface area, as well as average root diameter with root surface area. Conversely, nine pairs show negative correlations, notably between plant height and root tip number. Additionally, the length of fleshy stems is negatively correlated with the length of the root system, the average diameter of the root system, the surface area of the root system, and the number of root tips. Furthermore, the weight of a single stem is negatively correlated with the number of root tips. The number of leaves also exhibits a negative correlation with the length of the root system, the average diameter of the root system, and the surface area of the root system. No significant correlations were observed among the nutrient contents. A total of seven pairs exist between agronomic trait indicators and nutrient content. Among these, leaf width, petiole width, and petiole thickness exhibit a positive correlation with vitamin E. Additionally, soluble sugar is positively correlated with the average diameter of the root system, while soluble protein correlates positively with root surface area. Conversely, the length of fleshy stems and the number of leaves show a negative correlation with soluble sugar. Excessive vegetative growth in the aboveground parts will consume a large amount of photosynthetic products, which in turn leads to a reduction in the accumulation of soluble sugar.

3.5 Cluster Analysis

A systematic cluster analysis was performed on various treatments of WS1 and WS120, utilising 14 agronomic trait indicators and 4 nutrient content measures (Fig. 7). WS1 was categorised into two distinct groups based on the treatments applied. The first group, comprising data from 30 d and 35 d, exhibited larger values for root-related indicators; however, the agronomic traits were at their lowest levels. This suggests that a more extensive root system at the time of planting correlates with weaker plant performance during the harvest period of the product organ. In contrast, the second group, which includes data from 20 d and 25 d, displayed relatively favourable agronomic traits, while the remaining indicators were positioned in the mid-range.

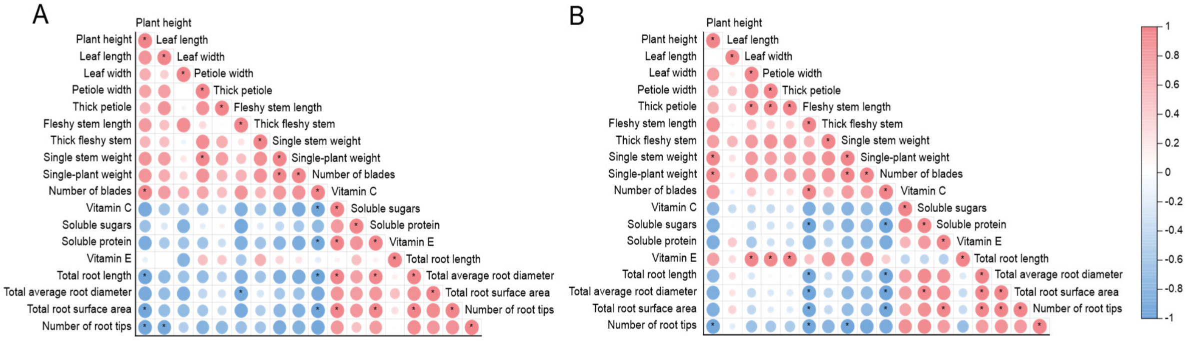

Figure 6: Correlation analysis of agronomic traits and nutritional components of different varieties of stem lettuce. Notes: (**A**): WS1; (**B**): WS120; $^{*}p \leq 0.05$.

WS120 was categorised into three groups, with the first group comprising 20 d and 25 d. Within this group, the agronomic traits associated with plant height, single stem weight, single plant weight, length of fleshy stem, thickness of fleshy stem, leaf width, petiole width, and petiole thickness at 25 d all attained their maximum values, whereas the levels of other indicators remained relatively moderate. This observation suggests that WS120 is beneficial for the enhancement of agronomic traits under the 25 d treatment. Group II corresponds to 30 d; in the third group (35 d), the concentrations of vitamin C, soluble sugar, and soluble protein are at their highest, and the root-related indicators also reach their maximum values. This finding indicates that as the seedling age increases, the levels of nutrients and root-related indicators improve, while other indicators remain comparatively low.

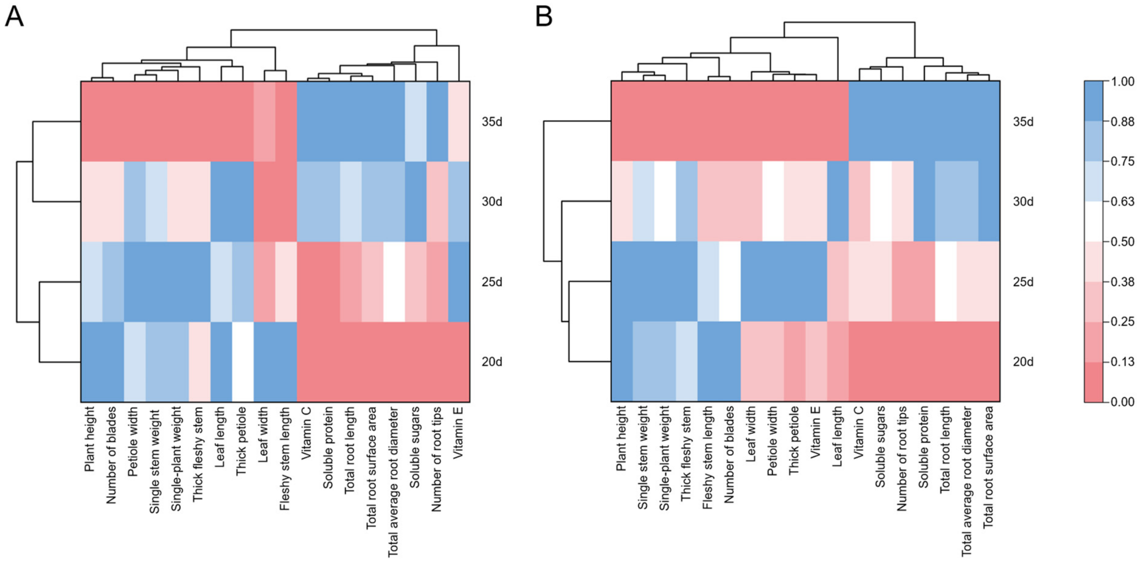

Figure 7: Cluster analysis of agronomic traits and nutritional components of different varieties of stem lettuce. Notes: (**A**): WS1; (**B**): WS120.

3.6 Analysis of Agronomic Traits and Principal Components of Nutritional Components

Following the standardisation of the measured values for 14 agronomic trait indicators and 4 nutrient contents of WS1 and WS120, principal component analysis was performed using SPSS software. This analysis yielded three principal components with eigenvalues exceeding 1 (Table 2). The results indicate that the eigenvalue of the first principal component (F1) was the highest, at 10.281, with a contribution rate of 57.117%. F1 exhibited a positive correlation with aboveground morphological indicators and biomass, while demonstrating a negative correlation with nutrient components and root morphology. The eigenvalue of the second principal component (F2) was 4.625, corresponding to a contribution rate of 25.692%. The third principal component (F3) was primarily associated with petiole width, petiole thickness, soluble sugar, vitamin E, and other indicators, reflecting characteristics related to "leaf development and sugar accumulation." The cumulative contribution rates of the three principal components reached 94.068%, suggesting that these components comprehensively encapsulate all information from the original indicators and provide a foundation for subsequent comprehensive evaluation.

Table 2: Principal Component analysis.

Indicator	**Principal Component**		
	F1	**F2**	**F3**
Plant height	0.089	0.061	−0.028
Leaf length	0.077	0.122	−0.049
Leaf width	0.088	0.073	−0.099
Petiole width	0.055	−0.042	0.379
Thick petiole	0.079	0.079	0.131
Fleshy stem length	0.074	−0.121	−0.11
Thick fleshy stem	0.079	0.054	0.223
Single stem weight	0.086	0.097	0.039
Single-plant weight	0.083	0.11	0.027
Number of blades	0.088	0.086	−0.077
Vitamin C	−0.061	0.139	−0.013
Soluble sugars	−0.087	−0.058	0.134
Soluble protein	−0.089	0.053	0.063
Vitamin E	0.007	−0.077	0.435
Total root length	−0.062	0.152	0.099
Total average root diameter	−0.049	0.175	0.12
Total root surface area	−0.058	0.164	0.079
Number of root tips	−0.06	0.159	−0.091
Characteristic value	10.281	4.625	2.027
Contribution rate (%)	57.117	25.692	11.259
Cumulative contribution rate (%)	57.117	82.809	94.068

3.7 Comprehensive Evaluation

The 14 agronomic trait indicators and 4 nutrient contents assessed in WS1 and WS120 were thoroughly evaluated through the calculation of the membership function (Table 3). The comprehensive scores for the 14 agronomic traits and 4 quality indicators of WS1 ranged from 0.596 to 0.833, with a mean of 0.732. The performance hierarchy was as follows: 25 d > 30 d > 20 d > 35 d. In contrast, the comprehensive scores for WS120 varied from 0.205 to 0.481, with an average of 0.355. The overall performance also followed the same order: 25 d > 30 d > 20 d > 35 d. Notably, both WS1 and WS120 achieved their highest scores at the seedling age of 25 d, recording values of 0.833 and 0.481, respectively, thereby securing the top rank.

Table 3: Comprehensive evaluation of membership functions.

Crop Variety	Treatment	FAC-1	FAC-2	FAC-3	μ1	μ2	μ3	Score	Ranking
WS1	20 d	1.33643	−0.1971	−1.22021	1.000	0.490	0.000	0.741	3
	25 d	1.1671	0.27158	0.67573	0.944	0.643	0.702	0.833	1
	30 d	0.47129	1.02306	0.65349	0.713	0.889	0.694	0.759	2
	35 d	−0.21359	1.36416	−0.59994	0.486	1.000	0.230	0.596	4
WS120	20 d	−0.02107	−1.69625	−0.94074	0.550	0.000	0.104	0.346	3
	25 d	−0.21199	−0.95573	1.47901	0.486	0.242	1.000	0.481	1
	30 d	−0.85002	−0.19727	0.74897	0.275	0.490	0.730	0.388	2
	35 d	−1.67815	0.38755	−0.7963	0.000	0.681	0.157	0.205	4

4 Discussion and Conclusion

4.1 The Influence of Planting at Different Seedling Ages on Agronomic Traits and Nutrient Content

This study demonstrates that both the early-maturing variety “WS120” and the late-maturing variety “WS1” attained the highest comprehensive scores when planted at a seedling age of 25 d. This finding suggests that this specific seedling age is critical for achieving high-quality and high-yield stem lettuce. Such results may be closely linked to the physiological condition of the seedlings and their capacity for acclimatisation following transplantation. As the seedling raising time extends, the growth of the plant roots is restricted by space [18]. Impaired growth and development ensue as a consequence. Research conducted by Modupeola et al. indicates that younger seedlings exhibit more vigorous growth post-planting, resulting in higher yields [19]. Vaishnava et al. corroborated this in their onion experiments, demonstrating that all growth and yield traits significantly declined with increasing seedling age [20]. These findings align closely with the planting outcomes observed at 30 and 35 d of seedling age in the present study. The research conducted by A. Kalisz on broccoli transplanting indicates that early planting enhances growth vigour, as evidenced by increased stem height, stem thickness, and leaf number. This finding aligns closely with the outcomes of prior studies [21]. The influence of seedling age on quality formation: Previous studies have shown that in the cultivation of broccoli, appropriately delaying sowing is conducive to improving quality [22]. In this experiment, the effect of varying seedling ages on nutrient content was not significant. This lack of impact may be attributed to the absence of fertilisation or drought stress during the plants’ growth. Liu Shaohuan et al. [23] indicated that the degree of aging in sweet pepper tray seedlings is positively correlated with seedling age. An excessively prolonged seedling age can exacerbate plant aging and diminish adaptability to external environmental conditions. Conversely, a seedling age that is too short may result in the incomplete development of various plant organs, which is detrimental to subsequent growth. In this study, the treatment at 25 d suggested that seedlings at this age possess a well-developed root system, with balanced growth of both above-ground and below-ground parts, resulting in vigorous vegetative growth. Consequently, this stage facilitates the synchronous enhancement of agronomic traits and nutrient content, leading to relatively favourable later growth. These findings align with previous research.

4.2 Correlation Analysis and Comprehensive Evaluation Based on Planting at Different Seedling Ages

This study employed principal component analysis, cluster analysis and comprehensive evaluation to systematically assess the effects on the growth and quality of stem lettuce planted at different seedling ages. Principal component analysis identified 14 agronomic traits and 4 nutritional component indicators, which were condensed into 3 principal components, achieving a cumulative contribution rate of 94.068%.

This finding suggests that the selected principal components comprehensively encapsulate the information contained in the original data, thereby mitigating the limitations associated with single-indicator evaluations. This aligns with the research conducted by Tian Xueke et al. [16]. who, in their assessment of phenotypic traits and nutritional quality of leaf lettuce germplasm resources, extracted principal components related to yield and nutritional components from 16 indicators via principal component analysis, attaining a cumulative contribution rate of 95.44%. This further substantiates the reliability of this methodology in the comprehensive evaluation of Lactuca crops. The results of the correlation analysis revealed a notable correlation between WS1 and WS120 concerning agronomic trait indicators. Specifically, there were 4 and 9 pairs of significant positive correlations, respectively, alongside 7 and 9 pairs of negative correlations. Differences may exist in the co-growth or mutual inhibition between the aboveground components, such as plant height, number of leaves, and petiole width, and the underground root systems, which include root length, root surface area, and number of root tips. When leaf development is robust, it can provide adequate carbon sources to the root system, thereby supporting nutrient absorption and facilitating coordinated growth. Conversely, if the aboveground vegetative growth becomes excessively vigorous, it may inhibit the elongation, thickening, and expansion of the root system's absorption area, ultimately exerting a detrimental effect on the overall morphogenesis of the plant. Cluster analysis classifies resources according to the similarity of their traits, thereby reflecting the degree of kinship [24,25]. This study demonstrated a strong alignment with the comprehensive evaluation results of the membership function, elucidating the optimal performance of the WS1 and WS120 varieties at seedling ages of 20 d and 25 d. This consistency affirmed the objectivity and stability of the evaluation outcomes. The research methodology aligns with established practices in the screening of tomato germplasm resources. Through cluster analysis, 169 tomato resources were categorised into 10 principal groups, each corresponding to distinct germplasm types and agronomic characteristics. The taste and flavour of tomato fruits were thoroughly assessed using the membership function method, thereby providing a theoretical and material foundation for the genetic enhancement of high-quality tomato resources and the development of new varieties [26]. The effective utilisation of the membership function method in this study has been corroborated by research on other vegetable crops. This method has demonstrated its objectivity and efficacy as a comprehensive evaluation tool in the variety screening of crops, including cucumber [27] and cabbage [17].

4.3 The Potential Value of Lettuce for the Lower Stem When Planted at Different Seedling Ages

This research holds considerable theoretical and practical importance for the efficient cultivation of stem lettuce. Through a comprehensive evaluation, the effects of seedling age on root morphology, agronomic traits, and nutrient content of stem lettuce were systematically elucidated. It was established that the optimal planting age for both the early-maturing variety "WS120" and the late-maturing variety "WS1" is 25 d. This seedling age achieves an optimal balance among "above-ground growth, root development, and quality formation." Consequently, the promotion of this technology aligns with the objectives of "enhancing quality and efficiency" and "efficient resource utilisation" within China's vegetable industry. Such advancements can facilitate the precise transformation of the industry and support the standardised development of plateau characteristic vegetables, thereby possessing significant practical implications.

Stem lettuce flourishes in climatic conditions characterised by ample sunlight, appropriate temperatures, and a suitable duration of light exposure. It is particularly well-suited for cultivation in plateau environments. By capitalising on the advantages offered by these plateaus, the promotion of this technology can enhance both the quality and efficiency of the stem lettuce industry, while simultaneously reducing costs and increasing revenue. To provide practical support for the establishment of a standardised production system, the core concepts can also serve as references for the cultivation of similar vegetables in diverse regions, thereby broadening the applicability of these findings. Future research may build upon this foundation to further investigate the interaction effects between optimal seedling age and cultivation practices, including planting density, fertiliser and water management, and temperature and light regulation. Additionally, it could examine the variations in seedling age control strategies across different ecological regions and seasonal conditions, offering both theoretical support and practical guidance for the development of a more comprehensive standardised production technology system for stem lettuce. Furthermore, an in-depth exploration of the physiological and biochemical mechanisms through which seedling age influences the growth and development of stem lettuce could elucidate its internal regulatory processes.

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References

1. Zdravković J, Aćamović-Đoković G, Mladenović J, Pavlović R, Zdravković M. Antioxidant capacity and contents of phenols, ascorbic acid, β-carotene and lycopene in lettuce. Hem Ind. 2014;68(2):193–8. [CrossRef].
2. Wei J, Dai Z, Zhang Q, Yang L, Zeng Z, Zhou Y, et al. Seed multispectral imaging combined with machine learning algorithms for distinguishing different varieties of lettuce (*Lactuca sativa* L.). Food Chem X. 2025;27:102399. [CrossRef].
3. Huang Y, Li Y, Liu Z, Chen W, Wang Y, Wang X, et al. Combined analysis of the transcriptome and metabolome provides insights into the fleshy stem expansion mechanism in stem lettuce. Front Plant Sci. 2022;13:1101199. [CrossRef].
4. Wang GB. Cultivation Techniques for early spring high-yield lettuce in greenhouses. Agric Technol Serv. 2008;(02):64–76. (In Chinese). [CrossRef].
5. Parkell N, Hochmuth R, Laughlin W. Overview of lettuce production systems and cultivars used in hydroponics and protected culture in Florida. EDIS. 2015;2015(3):6. [CrossRef].
6. Gonzaga NR, Pepito SL, Octavio RP, Gonzaga AB, Rogers GS. Growth and yield performance of lettuce (*Lactuca sativa* L.) under protected and conventional cultivation. Ann Trop Res. 2017;39(Supplement B):137–43. [CrossRef].
7. Liu Q, Zhou X, Li J, Xin C. Effects of seedling age and cultivation density on agronomic characteristics and grain yield of mechanically transplanted rice. Sci Rep. 2017;7(1):1–10. [CrossRef].
8. Kunting W, Mengzhu L, Yuan F, Ping L, Haiyan W, Qun H, et al. Effects of sowing rate and seedling age on seedling quality, yield and processing quality of mechanically transplanted hybrid rice. China Rice. 2024;30(3):91. (In Chinese). [CrossRef].
9. Brar SK, Mahal SS, Brar AS, Vashist KK, Sharma N, Buttar GS. Transplanting time and seedling age affect water productivity, rice yield and quality in north-west India. Agric Water Manag. 2012;115:217–22. [CrossRef].

10. Thomas P, Upreti R. Influence of seedling age on the susceptibility of tomato plants to Ralstonia solanacearum during protray screening and at transplanting. Am J Plant Sci. 2014;5(12):1755–62. [CrossRef].
11. Mishra A, Salokhe VM. Seedling characteristics and the early growth of transplanted rice under different water regimes. Exp Agric. 2008;44(3):365–83. [CrossRef].
12. Khan NH, Khan MA, Attaullah ZB, Shafiullah MZ, Ali A. Preventing bolting in onion (*Allium cepa* L.) bulb crop; effect of transplanting date and seedling age. Pak J Bot. 2024;56(4):1485–90. [CrossRef].
13. Fei TE, Huizhe CH, Yanhua ZE, Xueqing CA, Defeng ZH. Effect of different seedling age on the growth and yield of double cropping of late rice. Agric Sci Technol. 2015;16(7):1385–9. [CrossRef].
14. Jan B, Bhat MA, Bhat TA, Yaqoob M, Nazir A, Bhat MA, et al. Evaluation of seedling age and nutrient sources on phenology, yield and agrometeorological indices for sweet corn (*Zea mays saccharata* L.). Saudi J Biol Sci. 2022;29(2):735–42. [CrossRef].
15. Bhattacharya A. Singh DN. Effect of Age of Seedlings at transplanting on growth and yield of rice varieties (*Oryza Sativa*). Exp Agric. 1975;11(1):65–74. [CrossRef].
16. Tian XK, Zhong QW, Sun XM, Zhang GN, Yang SP, Tan L, et al. Comprehensive evaluation of phenotypic traits and nutritional quality of leaf lettuce germplasm resources. Jiangsu J Agric Sci. 2022;38(05):1330–9. (In Chinese).
17. Song C, Ye X, Liu G, Zhang S, Li G, Zhang H, et al. Comprehensive evaluation of nutritional qualities of Chinese cabbage (*Brassica rapa* ssp. pekinensis) varieties based on multivariate statistical analysis. Horticulturae. 2023;9(12):1264. [CrossRef].
18. Keever GJ, Kessler Jr JR, Fain GB, Mitchell DC. Seedling developmental stage at transplanting affects growth and flowering of medallion flower and globe amaranth. J Environ Hortic. 2015;33(2):53–7. [CrossRef].
19. Modupeola TO, Takim FO, Akintoye HA, Olaoye GO. Effects of transplanting age on growth and yield of tomato varieties commonly grown in south-western nigeria. Niger J Hortic Sci. 2019;24(3):13–25.
20. Vaishnava D. Effect of date of nursery sowing and seedling age on growth, yield and quality of rabi onion in Chhattisgarh plains [doctoral dissertation]. Raipur, India: Indira Gandhi Krishi Vishwavidayalaya (IGKV); 2012.
21. Kalisz A, Sękara A, Grabowska A, Cebula S, Kunicki E. The effect of chilling stress at transplant stage on broccoli development and yield with elements of modeling. J Plant Growth Regul. 2015;34(3):532–44. [CrossRef].
22. Grabowska A, Sekara A, Kalisz A, Kunicki E, Wojciechowska R, Kopta T. Optimisation of transplant age in combination with dark-chilling to enhance the biological quality of broccoli cultivated in summer. Not Bot Horti Agrobot Cluj-Napoca. 2018;46(2):494–500. [CrossRef].
23. Liu S, Zhao R, Gao L, Chen JQ. Effects of different seedling ages on the formation of aged seedlings in sweet pepper trays. Northwest Agric J. 2010;19(07):103–6. (In Chinese). [CrossRef].
24. Wang Y, Wang C, Zhang H, Yue Z, Liu X, Ji W. Genetic analysis of wheat (*Triticum aestivum* L.) and related species with SSR markers. Genet Resour Crop Evol. 2013;60(3):1105–17. [CrossRef].
25. Kumar M, Prakash S, Kumar A, Kumar B, Gangwar L, Maurya U, et al. Harnessing genetic diversity in papaya: Cluster analysis and its implications for breeding. Int J Adv Biochem Res. 2024;8(12):409–12. [CrossRef].
26. Li Y, Xiao J, Ma Y, Tian C, Zhao L, Wang F, et al. Identification and evaluation of phenotypic characters and genetic diversity analysis of 169 tomato germplasm resources. Sci Agric Sin. 2024;57(18):3671–83. (In Chinese). [CrossRef].
27. Li J, Li J, Yang P, Fu H, Yang Y, Liu C. Screening of germplasm and construction of evaluation system for autotoxicity tolerance during seed germination in cucumber. Agronomy. 2024;14(5):1081. [CrossRef].