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Research Report

Effect of Different Levels of Nitrogen, Phosphorus, and Potassium on Root Activity and Chlorophyll Content in Leaves of *Brassica oleracea* Seedlings Grown in Vegetable Nursery Substrate

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Abstract. Nutrients in the substrate are important for plant growth, particularly during the development of vegetable seedlings. The continued development of intensive methods to produce seedlings has made growing healthy and high-grade seedlings a requirement for nurseries. This study evaluated the effect of nitrogen (N), phosphorus (P), and potassium (K) on the root activity, dry weight, and chlorophyll content in leaves of *Brassica oleracea* seedlings. D-optimal design was used in this study because of its accuracy. A total of 10 treatments (T1–T10) were designed, and different concentrations were chosen in the following ranges: N (0.6-5.0 $g \cdot kg^{-1}$), P (0.2-6.0 $g \cdot kg^{-1}$), and K (0.7-7.0 $g \cdot kg^{-1}$). Our study shows that the application of N, P, and K to the substrate significantly influenced the growth of *B. oleracea* seedlings. T6 significantly increased root activity, root dry weight, chlorophyll content in leaves when compared with the control T1, which had added nutrient concentrations at normal levels. However, the addition of high levels of N (5.0 $g \cdot kg^{-1}$) with high (7.0 $g \cdot kg^{-1}$) or low (0.7 $g \cdot kg^{-1}$) levels of K (T2 and T9) not only significantly decreased the root dry weight but also decreased leaf area. Our results suggest that N, P, and K can be added to the substrate at an appropriate ratio to grow vigorous *B. oleracea* seedlings under intensive seedling production conditions. The present results give insight into the nutrient requirements during early growth of *B. oleracea* seedlings in growth substrate.

Additional key words: *Brassica oleracea*, early plant growth, intensive seedling production, nutrients, vegetable seedling

Introduction

Growing seedlings is an important process in the production of vegetables. Intensive seedling production a leading method used in vegetable plantation because of its advantages in terms of savings in seed, energy, labor, and time, as well as highly efficient usage of soil (Izquierdo, 2000). Currently, intensive seedling production in modern agricultural enterprises is a crucial component in modern agriculture, particularly in vegetable production for which obtaining high-quality seedlings is a crucial step (Lamont, 2005; Narayan and Narayan, 2014). Intensive seedling production has also been used in vegetable facility cultivation in the form of seeds sown in a plastic tray containing 50 or 72 holes. Compared to conventional nutrient soil, growing substrate has several advantages, including saving energy and resources, reducing the cost of breeding, decreasing damage to the developed plant root, shortening the time to adapt to a new soil environment, and maintaining high survival rates (Cui and Wang, 2001; Gruda and Schnitzler, 2004b). The substrate is an important factor in tray seedling production because of its role in strengthening and fixing crop root, supplying the root with water and nutrients, and balancing the availability of water and air (Gruda and Schnitzler, 2004a; Sterrett, 2001). Therefore, the substrate can affect the seedling quality directly.

To date, a substrate composed of peat (turf), vermiculite, and pearlite, mixed in different proportions, has been used to produce seedlings. However, this substrate typically is poor in nutrient content, specifically in the availability of the elements nitrogen (N), phosphorus (P), and potassium (K), which are important during early growth of vegetable seedlings. To satisfy the nutrient demand for seedling growth, spraying nutrient solutions to the substrate has been regularly used (Hao et al., 1998). However, the intensive addition of nutrient solutions has several drawbacks, such as increasing humidity, inducing plant diseases, time consuming, high nutrient loss, and leading to environmental pollution (Kratky et al., 1994; Yanyan and Fupeng, 2011). An alternative to address these problems is the addition of chemical fertilizers in the substrate to supply the required nutrition for seedling growth and development. The fact is that vegetable seedlings only need a certain amount of nutrients during early growth before they are planted into the field (Fageria et al., 2010). Different nutrient amounts and ratios are required during the different stages of vegetable seedling growth and development, specifically. N, P, and K ratios are keys to cultivate strong seedlings in a suitable substrate. Therefore, the concentrations of N, P, and K to be added to the substrate to satisfy the requirements for seedling growth must be determined. In the present study, we used a mixed substrate specifically used to grow vegetable seedlings and composed of peat, vermiculite, and perlite. Levels of N, P, and K in the matrix, as well as their optimal ratios, must be determined to optimize growth conditions.

Cabbage (*Brassica oleracea* var. *capitata* L.) is one of the most important winter vegetables in China (Lee, 1982; Rubatzky and Yamaguchi, 2012), and is valued for its nutritional properties (Rozpądek et al., 2015). This vegetable therefore plays a crucial role in maintaining human health, and constitutes a large proportion of vegetable production worldwide (Lee, 1982; Rakow, 2004). Currently, universal substrates are used to breed *B. oleracea* seedlings in China. Few studies have reported on the specific requirements of N, P, and K levels for *B. oleracea* seedling growth, especially in the substrate composed of peat, vermiculite, and perlite. We therefore set out to study the specific requirements of N, P and K levels and their ratios for early growth of *B. oleracea* seedlings.

Obtaining high quality seedlings is an important prerequisite for intensive seedling production (Hatzig, 2015; Kumar, 2006). The root is a vital part for plant growth, and root activity influences the physiological activity of plant seedlings. Meanwhile, the chlorophyll content reflects the photosynthetic activity in leaves. Thus, criteria based on root health and chlorophyll content report the health of a seedling plant directly and indirectly (Cornelissen et al., 2003). The supply of N, P, and K can affect the plant dry mass, and the chlorophyll content (Gurgul and Herman, 1994). How the soluble N, P, and K contents affect root activity and chlorophyll content during early growth of *B. oleracea* seedlings remains unknown. D-optimal design is a practical method to analyze the effect of different elements on features such as plant biomass and yield because it is specific for analysis of three factors to optimize the best ratios (Bodea and Leucuta, 1997; Eriksson et al., 1998). The main aim of this study was to analyze whether the addition of N, P, and K to the nursery substrate affects the activity of *B. oleracea* seedling roots and chlorophyll content in leaves with the purpose to optimize growth conditions in intensive seedling production.

Materials and Methods

Plant Material and Substrate

Vegetable plants (*Brassica oleracea* Zhong Gan No. 11) were grown in 120 ml substrate in 9 cm diameter plastic pots in a greenhouse from January 03 to October 01, 2015 at the Anhui Academy of Agricultural Sciences, and were watered daily until four leaves had appeared. Vegetable seedling substrate was used with a volume ratio of peat, vermiculite, and perlite at 3:1:1. The mixed substrates were sieved using a 5-mm sieve after being dried and smashed. The bulk density of the resulting substrate was 0.23 $g \cdot (cm^3)^{-1}$, the total porosity was 85.8%, and the pH value was 5.1. According to the volume ratio of 1:10, the electrical conductive (EC) value was 0.19 $mS \cdot cm^{-1}$. The initial characteristics of the substrate were as follows: available nitrogen (NH_4^+), 0.58 $g \cdot kg^{-1}$ (Kuan et al., 2016); available P, 0.15 $g \cdot kg^{-1}$ (Bray and Kurtz, 1945); and K, 0.69 $g \cdot kg^{-1}$ (Kuan et al., 2016). N, P, and K were obtained from urea (46% NH_2), calcium superphosphate fertilizer (14.0% P_2O_5), and potassium sulfate (50.0% K_2O), respectively.

Vegetable Seedling Sampling

A total of 10 seedling plants were collected from each treatment and washed gently under running tap water to remove adhering substrate from the roots. Leaves were collected from each plant from the ten treatments, and rinsed using tap water, and then laid flat on the table. The areas of four expanded leaves were measured using white graph paper (1x1 cm). The leaf area was calculated using the following formula: leaf area (cm^2) = leaf length (cm) × leaf width (cm). The leaf samples were stored at 4°C before analysis. The root was separated from the shoot and oven-dried for 3 days at 70°C. Root fresh weights were recorded after removing all water attached to the root, and after drying, the root dry weights were measured. The experiments were repeated twice during the period from January 03 to October 01, 2015.

Determination of Root Activity

Dehydrogenase activity is considered an indication of root activity (Watanabe et al., 2005), and this activity was determined using the triphenyl tetrazolium chloride (TTC) method (Clemensson-Lindell, 1994; Lindström and Nyström, 1987). Briefly, root samples (0.25 g) were mixed thoroughly with 0.5% TTC solution and transferred to test tubes. After incubation for 1 h at 37°C, the roots were ground in 5 mL of ethyl acetate, and the volume was increased to 10 mL. The color intensity was measured at 485 nm with ethyl acetate as a blank. TTC reduction was calculated using the following formula:

$$Root\ activity = \frac{Amount\ of\ TTC reduction}{Root\ fresh\ weight \times Time},$$

where time is the total reaction time after the root was added to the TTC solution. Ten seedling plants were sampled to determine root activity.

Detection of Chlorophyll in Leaves

The total chlorophyll levels in leaves of *B. oleracea* plants were determined using a KONICA MINOLTA SPAD-502 Plus chlorophyll meter according to the instructions (Konica Minolta, Inc., Japan) because this equipment is fast, easy to use, and can produce reliable estimates of relative leaf chlorophyll content (Akiyama et al., 2001; Richardson et al., 2002). The claimed accuracy of the SPAD-502 Plus is ± 1.0 SPAD units.

Table 1. Random code values in second saturating D-optimal design

Treatments	N	P	K
T1	-1.0000	-1.0000	-1.0000
T2	1.0000	-1.0000	-1.0000
T3	-1.0000	1.0000	-1.0000
T4	-1.0000	-1.0000	1.0000
T5	0.1925	0.1925	-1.0000
T6	0.1925	-1.0000	0.1925
T7	-1.0000	0.1925	0.1925
T8	-0.2912	1.0000	1.0000
T9	1.0000	-0.2912	1.0000
T10	1.0000	1.0000	-0.2912

Table 2. Various levels of N, P, K in the second saturating D-optimal design

Setting Levels	Contents ($g \cdot kg^{-1}$)		
	N	P	K
Maximum level	5.00	6.00	7.00
Minimum level	0.60	0.20	0.70
Zero level	2.80	3.10	3.85
Variation distance	2.20	2.90	3.15

Experimental Design and Statistical Analysis

D-optimal design was employed in this work (De Aguiar et al., 1995; El-Hagrasy et al., 2006). The specific data are listed in Tables 1 and 2. According to the EC value of the nursery matrix, maximum amounts of N, P, and K were added to the substrate based on the combined results of the pre-experiment (data not shown). This addition did not affect the germination rate of seeds. A total of 200 seeds of *B. oleracea* were sown, and more than 90% of the seeds germinated in the treatment with maximum N, P, and K levels when compared with the control. The leaf area and root activity were measured from 10 seedlings in each replication. Chlorophyll was detected from the first to the fourth leaf in each plant. Mean analysis was conducted using One-way ANOVA, followed by Duncan's test at $p < 0.01$ using a statistical analysis software (SPSS version 19.0 Inc., USA).

Results

Growth of *B. oleracea* Seedlings

According to the basic characteristics of the substrate used in this study, the different levels of N, P, and K available in the substrate, and 10 treatments (T1-T10) were established (Table 1). Specific combinations of the 10 treatments are listed in Table 3. T1 (0.6 $g \cdot kg^{-1}$ N, 0.2 $g \cdot kg^{-1}$ P, and 0.7 $g \cdot kg^{-1}$ K) was used as a control treatment because of its lowest content of added elements. Treatment T2-T10 each contained different levels of N, P, and K in the ranges of 0.6-5.0, 0.2-6.0, and 0.7-7.0 $g \cdot kg^{-1}$, respectively. *B. oleracea* seedlings exhibited different growth patterns under the 10 treatments,. As shown in Fig. 1, the seedlings grew well under T5, T6, T7, or T8 treatment. By contrast, T2, T9, and T10 showed poor growth when compared with the control T1.

Root Activity and Dry Weight

T2 (5.0 $g \cdot kg^{-1}$ N, 0.2 $g \cdot kg^{-1}$ P, and 0.7 $g \cdot kg^{-1}$ K) and T9 (5.0

Table 3. Combination of different elements in the second saturating D-optimal design

Elements	Treatments ($g \cdot kg^{-1}$)									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
N	Mi 0.6	Ma 5.0	Mi 0.6	Mi 0.6	Me 3.2	Me 3.2	Mi 0.6	Mc 2.2	Ma 5.0	Ma 5.0
P	Mi 0.2	Mi 0.2	Ma 6.0	Mi 0.2	Me 3.7	Mi 0.2	Me 3.7	Ma 6.0	Mc 2.3	Ma 6.0
K	Mi 0.7	Mi 0.7	Mi 0.7	Ma 7.0	Mi 0.7	Me 4.5	Me 4.5	Ma 7.0	Ma 7.0	Mc 2.9

Four levels were set in the D-optimal design as follows: Mi, Minimum; Mc, Micro-scale; Me, Medium; Ma, Maximum.

Fig. 1. Photo of *Brassica oleracea* seedlings grown in the mixed substrate for 25 days under greenhouse condition. Seedlings of T1–T10 (left to right). A 20-cm length ruler was placed in the middle.

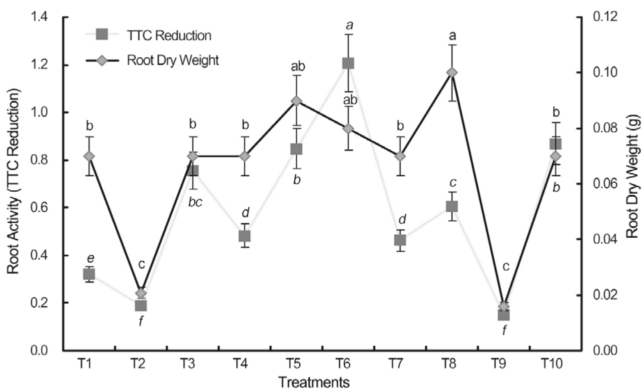

Fig. 2. Triphenyl tetrazolium chloride reduction and root dry weight of *B. oleracea* seedlings in 10 treatments. At least ten seedling plants were sampled to analyze the root activity and dry weight. Values are the means ± standard deviations. Italic characters represent significance testing ($p < 0.01$) of TTC reduction.

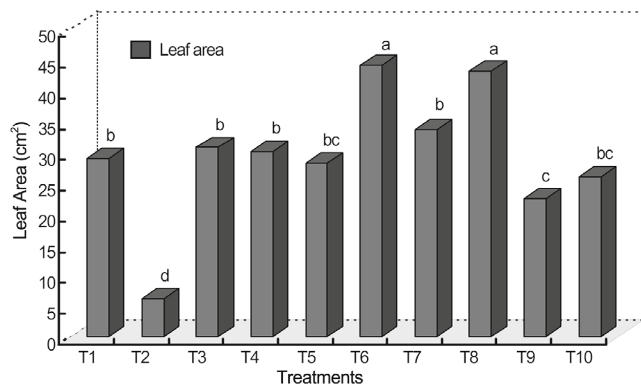

Fig. 3. Leaf area in 10 treatments. Total average was calculated from four leaves in each plant among ten treatments. Significant differences were tested according to Duncan's test at $p < 0.01$ using statistical analysis software (SPSS version 19.0).

$g \cdot kg^{-1}$ N, 2.3 $g \cdot kg^{-1}$ P, and 7.0 $g \cdot kg^{-1}$ K) treatments resulted in reduced root activity, and T9 had the lowest root activity at 0.15. In contrast, T5, T6, T8, and T10 showed good root activity and root dry weight when compared with T1. T2 and T9 showed reduced root dry weights of 20 and 15.8 mg, respectively. Seedlings under T2 and T9 treatment also showed poor root activity (0.19 and 0.15, respectively). Here, we found that root dry weight could affect root activity. T8 seedlings (2.2 $g \cdot kg^{-1}$ N, 6.0 $g \cdot kg^{-1}$ P, and 7.0 $g \cdot kg^{-1}$ K) showed the highest dry weight (100 mg), and T6 treatment (3.2 $g \cdot kg^{-1}$ N, 0.2 $g \cdot kg^{-1}$ P, and 4.5 $g \cdot kg^{-1}$ K) resulted in the highest root activity at 1.21 (Fig. 2). Seedlings with low root dry weight also showed poor root activity (T2 and T9). Although the highest root activity was detected in T6, its root dry weight was only the third largest when compared with that of other treatments.

Chlorophyll and Leaf Area

Treatments, T1, T2, T3, T4, T5, T9, and T10 resulted in small leaf area, and T2 (5.0 $g \cdot kg^{-1}$ N, 0.2 $g \cdot kg^{-1}$ P, and 0.7 $g \cdot kg^{-1}$ K) resulted in the smallest leaf area at 6.2 cm^2 (Fig. 3). T6, T7, and T8 showed larger leaf area, and T6 had the largest leaf area at 44.2 cm^2 (Fig. 3). T2 treatment showed the lowest chlorophyll content at 29.56 SPAD, whereas T6 resulted in the highest chlorophyll content at 45.58 SPAD (Fig. 4). Although T9 and T10 treatments resulted in seedlings with small leaf areas, which were 22.5 and 26.1 cm^2, the leaves did have high chlorophyll content with 46.55 and 48.31 SPAD respectively, when compared with the control T1. Thus, leaf area does not positively correlate with chlorophyll content directly. Additional N (5.0 $g \cdot kg^{-1}$) and P (2.3-6.0 $g \cdot kg^{-1}$) were added in T9 and T10, and the plants showed high chlorophyll values. However, leaf size is significantly affected by the addition of N, P, and K (Fig. 3) in the range of 6.2-44.2 cm^2. Notably, the leaf area in T6 was 1.5-fold higher than that of the control T1 and seven-fold higher than the leaf area in T2.

Analysis by D-optimal Design

Different levels of N, P, and K were established in the following ranges: N (0.6-5.0 $g \cdot kg^{-1}$), P (0.2-6.0 $g \cdot kg^{-1}$), and K (0.7-7.0 $g \cdot kg^{-1}$) (Table 2). T1 was a control treatment without any addition of the three elements. The specific combinations of N, P, and K are listed in Table 3. The following three equations were established according to the data of root activity, leaf area, and amount of chlorophyll value obtained: Root activity equation: $Y_1 = 1.0338 - 0.0009X_1 + 0.0430X_2 -$

$0.1054X_3 - 0.5369X_1^2 + 0.2344X_2^2 - 0.3253X_3^2 + 0.0382X_1X_2 + 0.0264X_1X_3 - 0.2124X_2X_3$, ($p < 0.01$); Leaf area equation: $Y_2 = 42.2946 - 5.6275X_1 + 1.4060X_2 + 4.2271X_3 - 14.2216X_1^2 + 4.3801X_2^2 - 7.9073X_3^2 + 0.7927X_1X_2 + 3.9238X_1X_3 - 0.3017X_2X_3$, ($p < 0.01$); and Chlorophyll equation: $Y_3 = 47.5851 + 3.5287X_1 + 0.7877X_2 + 2.0429X_3 - 5.7693X_1^2 - 1.2483X_2^2 - 2.6192X_3^2 + 5.0697X_1X_2 + 3.4176X_1X_3 - 0.5928X_2X_3$, ($p < 0.01$). These equations were established to describe the correlation of different levels of N, P, and K with seedling growth in nursery substrate.

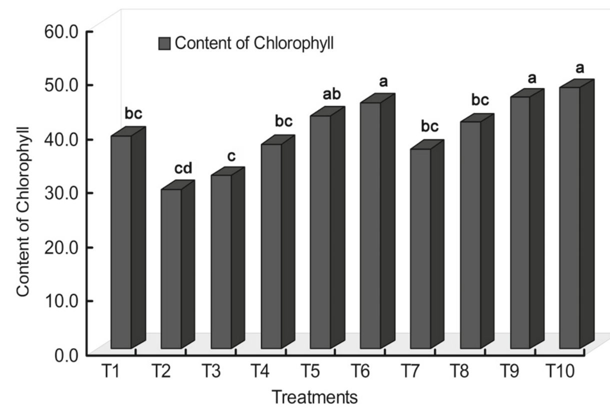

Fig. 4. Chlorophyll value in leaves of *B. oleracea* seedlings in 10 treatments. Significant differences were tested according to Duncan's test at $p < 0.01$ using statistical analysis software (SPSS version 19.0). Units: SPAD.

Discussion

In this study, the stimulating effect of adding elements to the substrate on the growth of *B. oleracea* seedling plants is mainly attributed to the increased root dry weight, root activity and leaf area. Furthermore, we found that element addition increases the photosynthetic capacity of seedling plants through increased chlorophyll values (T6) when compared with the control (T1). The plant mineral status can affect photosynthesis (Longstreth and Nobel, 1980), and the leaf chlorophyll content is high in highly use efficiency of N and K (Minotta and Pinzauti, 1996). In the present work, the growth and photosynthesis content of seedling plants are improved by the addition to the nursery substrate of N, P, and K, which are highly available to seedling plants.

To sustain normal seedling growth and development, plants must develop a robust root system to extract water and mineral nutrients from the substrate. Compared with the control T1, good root growth was detected in three treatments, namely, T5, T6, and T8. These treatments all consisted of N and P additions at the medium and microscale range (Table 3). The influence of supply of P and K at different concentrations on the partitioning of dry matter in bean has been reported previously (Cakmak et al., 1994). Our results are in agreement with previous observations, as we found that different levels of N, P, and K influenced the root dry weight. N is the element that is required in the highest amounts by plants. However, T2 and T9 treatment with the highest level of 5.0 $g \cdot kg^{-1}$ N, showed poor root growth. This observation indicates that early seedling growth in *B. oleracea* seedlings does not require large amounts of N. High levels of N inhibit seedling growth in mixed substrate, although availability of N to roots is required for plant growth (Farage et al., 1998), particularly in the early stage. Nevertheless, the data from our work suggest that *B. oleracea* plants require adequate but not excessive amounts of N to achieve efficient growth and development of seedlings.

It is clear that leaf size is greatly affected by the availability of N, P, and K. T6 and T8 had the largest leaf area when compared with the control treatment T1. This finding suggests that additional supply of a medium concentration of N, low concentration of P, and medium concentration of K is beneficial to increase leaf area. T2 and T9 resulted in small leaf areas, suggesting that high N also inhibits leaf area. However, T5, T6, T8, T9, and T10 had increased chlorophyll content when compared with the control T1 (Fig. 4). The lowest chlorophyll value was detected in T2, suggesting that this treatment is inappropriate to grow seedlings in the nursery substrate.

P is one of the most important elements that significantly affects plant growth and metabolism because it is a component of macromolecular structures (Raghothama, 1999). The lowest amount of P (0.2 $g \cdot kg^{-1}$) was added in T6 and this concentration did not inhibit seedling growth. Most of the P in soils cannot be absorbed by plants due to its insoluble form. Although the P requirement for optimal growth of plants in the soil is in the range of 3-5 $mg \cdot g^{-1}$, root growth is less inhibited under P-deficiency (Lambers et al., 2010). Moreover, despite the fact that the P concentration was lower than the concentration in the previous report, the seedlings still grew well in T6. Therefore, low P concentration does not affect the early growth of *B. oleracea*.

K is the nutrient required in the second largest amount by plants and plays a role in crop survival (Cakmak, 2005). The K requirement for optimal growth is the range of 20-50 $g \cdot kg^{-1}$. However, adding K at these quantities to the substrate is not possible because seedlings can not grow normally under high EC values. In the present study, the maximum K concentration in the substrate was 7.0 $g \cdot kg^{-1}$. When K is deficient, plant growth is retarded (Marschner and Cakmak, 1989), and our findings are consistent with this conclusion. Seedlings under T2 treatment, with low K concentration, showed poor growth.

According to the D-optimal design, three equations were established at $p < 0.01$. These equations express the relation of the three elements to root activity, leaf area, and chlorophyll content, and describe the effect of addition of three elements to the substrate. Different levels of N, P, and K can be added according to these equations. In summary, this research provides evidence that the addition of N, P, and K can increase growth of seedlings grown in nursery substrate, and it stresses their roles in growing seedlings in modern agriculture. This study is the first comprehensive analysis of the effect of N, P, and K elements on cultivation of seedlings for intensive production. Finally, this work provides an understanding of the nutrient requirements of *B. oleracea* seedlings in vegetable nursery substrate.

Conclusion

The application of N, P and K elements to the substrate significantly influenced seedling growth in *B. oleracea*. T6 treatment (3.2 $g \cdot kg^{-1}$ N, 0.2 $g \cdot kg^{-1}$ P, and 4.5 $g \cdot kg^{-1}$ K) significantly increased root activity, root dry weight, and chlorophyll value in leaves when compared with the control T1. However, the addition of high levels of N (5.0 $g \cdot kg^{-1}$) with high (7.0 $g \cdot kg^{-1}$) or low (0.7 $g \cdot kg^{-1}$) levels of K (T2 and T9) not only significantly decreased the root dry weight but also reduced the leaf area. Significant variations between the 10 treatments were detected, suggesting that D-optimal design is a practical method to analyze the effect of N, P, and K on seedling growth. This study is the first to investigate the correlation of different levels of N, P, and K to affect root activity and chlorophyll during early growth of *B. oleracea* seedlings. Our study suggests that seedlings supplied with proper ratios (T6 and T8) of the three elements in the substrate exhibit good growth patterns. This study also demonstrates that N, P, and K addition can help grow strong seedlings in intensive seedling production. Future studies are underway to assess the effect of the three elements in the seedlings when plants are transplanted into field conditions.

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