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RESEARCH REPORT

Impact of controlled nitrogen application in water solution on seedling growth, tissue and soil nutrient concentrations in vegetative propagation of strawberry

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Abstract
This study was conducted to establish the appropriate nitrogen (N) fertilizer concentration for raising strawberry mother plants of four varieties ('Altaking,' 'Kuemsil,' 'Maehyang,' and 'Vitaberry'). Seedlings were transplanted to a peat moss + perlite medium (7:3, v/v). Then, nutrient solutions containing five different N concentrations (0, 5, 10, 15, and 20 mM) were applied. All the treatments contained equal concentrations of essential nutrients except N. The growth of the mother plants and the occurrence of daughter plants were investigated 90 days after transplanting. Data on root media pH, EC, and concentrations of macro and micro elements, and the tissue nutrient contents of oven dried mother plants were collected and analyzed. The differences observed in the root medium pH among the treatments (5.65–6.10) were not statistically significant. The greatest growth of mother plants in terms of dry weight data and the occurrence of daughter plants in 'Altaking,' 'Kuemsil,' and 'Maehyang' strawberries were observed with 10 mM N, whereas for 'Vitaberry,' the best results were observed with 20 mM N treatments. The tissue N content of 'Altaking,' 'Kuemsil,' 'Maehyang' and 'Vitaberry' when their growth was highest was 2.72, 2.71, 2.69, and 2.74%, respectively, based on the dry weight of the aboveground tissue. Furthermore, the root media nutrient concentrations were 20.1, 20.8, 20.1, and 20.24 mg kg^{-1} NO_3; 32.8, 34.5, 32.7, and 33.1 mg kg^{-1} PO_4; 18.67, 18.68, 18.7, and 18.75 mg kg^{-1} SO_4; and 0.3, 0.4, 0.4, and 0.5 mg kg^{-1} K, respectively. However, the differences observed in the dry weights of the mother plants among treatments (5, 10, 15, and 20 mM N) of the strawberry cultivars were not statistically significant. The above results indicate that 'Vitaberry' strawberries required a higher N concentration in the fertilizer solution than 'Altaking,' 'Kuemsil,' and 'Maehyang' for raising mother plants and inducing daughter plants via vegetative propagation.

Keywords Daughter plant occurrence · Mother plant growth · N concentration · Soilless culture · Tissue nutrient contents

1 Introduction

Strawberry (*Fragaria ananassa*) is one of the main fruit crops grown in South Korea for fresh consumption, processing, and exportation (FAO 2020). It is a high-value fruit rich in vitamins, sugars, minerals, and bioactive compounds, for example, ascorbic acid, folic acid, phenolic compounds, antioxidants, and carotenoids (Chaves et al. 2017). Strawberries are widely cultivated in South Korea, where 97% of their cultivation takes place in greenhouses. Korean farmers have become the leading strawberry growers globally in the past decade (MAFRA 2018). The seedlings are transplanted in September, and the fruit is harvested from December to the following June (Hong and Eum 2020).

The growth and yield of strawberries greatly depends on the different agricultural practices performed from the very beginning of managing seedlings to harvesting of the fruit. Among all the practices, nutrient availability is considered the top factor responsible for increasing growth and development (Negi et al. 2021). Strawberry plant growth is very responsive to nitrogen fertilizer (Jackson et al. 2007).

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The plants need plenty of fertilizer for their growth and development, along with frequent irrigation, because they have an extremely perfunctory root system (Mohamed et al. 2021). The key factor in strawberry cultivation is nutrient management because the plants are very susceptible to disorders caused by nutrients (Ariza et al. 2021). Moreover, the growth and yield of garden strawberries are greatly dependent on the soil moisture content and fertility during the growing period (Zahid et al. 2021). Consequently, to get uniform vegetative growth and good yield with high quality fruit, providing enough nutrients at the seedling stage is essential for proper nourishment (Sharma et al. 2002). Studies have provided evidence that the application of appropriate amounts of nitrogen to strawberry seedlings plays an important role in enhancing vegetative growth (Hargreaves et. al. 2009) and increasing biochemical substances such as proteins, enzymes, nucleic acids, amino acids and chlorophyll in plants (Dae Ho Jung et al. 2021). Achieving greater growth of the plants, as well as higher yield with better quality fruit, requires intensive application of fertilizers, which are expensive and which are also responsible for increasing environmental pollution (Chandini et al. 2019).

However, at present, farmers are also showing eagerness to use fertilizers in amounts that are adequate but not excessive. This is because of the environmental degradation caused by residual effects from many of the different chemical substances used for farming. Moreover, excessive application of various chemicals, along with inorganic fertilizers, can be hazardous to the environment as well as to public health (Hsieh et al. 1995). In contrast, the optimum application of fertilizer boosts proper vegetative growth, which is a prerequisite of an abundant yield with high-quality fruit (Vance et al. 2017; Xu Zhu et al. 2020).

Because of inadequate information regarding nutrient uptake and tissue mineral content, farmers are facing problems growing high-quality fruit in large quantities. This study was conducted to analyze the influence of different nitrogen levels on the growth and development of strawberries. However, the main objective of the study was to investigate the amount of threshold tissue nitrogen that causes reduced growth and induces deficiency symptoms, which could clarify the relationship between plant growth and N concentrations in the tissues or in the petiole sap of aboveground dry matter.

2 Materials and methods

2.1 Experimental site and plant growth conditions

The experiment was conducted using strawberry seedlings inside a greenhouse covered with glass at Chungnam National University (CNU), South Korea (36° 20' N, 127° 26' E) to investigate the influence of different nitrogen levels on the growth and development of strawberry seedlings. Three true-leaf generated strawberry seedlings were transplanted to rectangular plastic pots (length 64.3 cm × width 23.5 cm × height 17 cm) in a a zigzag row (5 seedlings/pot). The pots were filled with peat moss and perlite at a ratio of 7:3 (v/v), which had been irrigated properly before transplanting. During the experiment, the seedlings were irrigated for one week immediately after transplanting. Nutrient solutions (2 L per pot) were applied three times (at 9 am, 1 pm, and 4 pm) every day. Inflorescences and runners were removed and pesticides were applied as and when necessary. During the experiment, the average day and night temperature inside the greenhouse was 24 °C and 14 °C, respectively.

2.2 Preparation of treatment solutions

Five different nitrogen levels (0, 5, 10, 15, and 20 mM) were prepared by modifying Hoagland solution (Hoagland and Arnon 1950). The concentration of other vital macro and micro nutrients were the same for all five nitrogen treatments, which were adjusted according to the work of Choi et al. (2009). The pH was adjusted to 5.8–6.0 using hydrochloric acid (HCl) and the EC was adjusted to 0.6 dS/m by regulating the amount of water. Every treatment was replicated three times, with five plants in each replication.

2.3 Plant sampling and data collection

Ninety days after transplanting, the plants were uprooted and the total aboveground part was harvested. Plant growth was investigated using a variety of parameters; 11 plants were randomly selected from each treatment. The average value of the 11 selected creepers for each treatment was considered the single value of that particular nitrogen concentration. Data were collected on plant height (cm), leaf number, length of leaf (cm), width of leaf (cm), length of petiole (cm), crown diameter (cm), and the fresh and dry weight (g) of the mother plants. Also collected was the number length (cm), fresh weight (g), and dry weight (g) of runners, and the number and fresh weight (g) of the daughter plants.

Root media was sampled and air dried for about 7 d at room temperature until properly dried. A saturated paste of the dried root media was made using distilled water (1:10 w/w). Then, 1–2 drops of soil wetting agent was added and well mixed. To reach equilibrium, the solution was kept at room temperature for 2 h. After that, it was filtered in a test tube using three layers of gauze with the help of gentle pressing. The pH and EC were measured using a pH/EC meter (Multi Meter CP-500L, Istek Co., Seoul, Korea). The concentrations of the ions present in the root media solution were measured using U-chromatography (Basic IC Plus, Switzerland). Each measurement was repeated three times.

To analyze the nutrient contents in tissue, leaves were collected from every treatment, washed using detergent in distilled water, and oven dried at 70 °C for 48 h. Thereafter, the dried leaves were ground using a pestle and mortar, and sieved using a 0.5-mm mesh. Then, 0.5 g of each sample was placed in a small ceramic pot and dry-ashed at 500 °C for 6 h. After cooling, it was burnt using 2 mL of 95% H_2SO_4, filtered through Advantec No. 2 filter paper (Toyo Roshi Kaisha, Ltd., Japan), and finally dissolved in 0.5 N HCl to make 50 mL of solution. The analyses of P, K, Mg, Ca, Fe, Mn, Cu, and Zn were conducted using an Atomic Absorption Spectrophotometer (AA-7000, Shimadzu Co., Japan).

The total nitrogen content was determined by placing 0.1 g of ground leaves in glass tubes, followed by addition of 5 mL sulfuric acid and a salicylic acid mixture (30:1 v/w). Tubes were covered and kept at room temperature for about 1 h. Then, the tubes were placed in digestion blocks (pre-heated at 270 °C) where they were digested for 5 min. After cooling, 2 g of Kjeldahl digestion mixture (K_2SO_4:$CuSeO_3.2H_2O$: pumice in 970:19:11 w/w/w) was added and samples were digested again for about 30 min at 400 °C. Samples were taken out after ensuring complete digestion and cooled at room temperature. To these, 20 mL of distilled water was added and mixed properly. The method used for analyzing total nitrogen was similar to that of Eastin (1978) and the instrument was a Kjeldahl Digestion and Distillation Unit (manufactured in Korea).

2.4 Statistical analysis

The numerical values of the vegetative growth and development, and the nutrient content, were tested using the Duncan multiple range test ($p \leq 0.05$) of CoStat Version 6.311 (CoHort Software, Monterey, California, USA). Furthermore, linear and quadratic regression analyses were carried out to see the trends in seedling growth with respect to differences in the nitrogen concentration.

3 Results and discussion

Based on the findings of Agehara (2021), application of early season N results in a high number of leaves with a large area, along with a great fresh and dry weight of plants, which are responsible for high fruit yield. According to Yoon et al. (2018), application of the proper level of nitrogen could increase strawberry yields by increasing the crown diameter, leaf area, and the fresh and dry weight (i.e., the vegetative growth), thus increasing flowering. It is notable that, while excessive application of nitrogen fertilizer may enhance vegetative growth, it can also cause reduced emergence of daughter plants (Lundblad 2019). According to Santos and Chandler (2009), strawberry cultivars showed differential vegetative growth responses to different rates of nitrogen fertilizers. This result was very similar to that of Sas et al. (2003), who mentioned that strawberry root growth is also influenced by nitrogen fertilizers.

In the current study, different nitrogen levels significantly influenced the average growth of all the strawberry cultivars (Table 1). Among the five nitrogen treatments, the plants treated with no nitrogen had lower growth (i.e., the plant height, fresh weight, dry weight, number of runners, and number and fresh weight of daughter plants) than the other treatments. Different nitrogen concentrations resulted in diverse plant heights among the strawberry cultivars, but there were no significant differences among the four nitrogen treatments (5, 10, 15, and 20 mM) (Fig. 1A). The highest average plant heights were attained with a nitrogen treatment of 10 mM in each cultivar as follows: 'Altaking' 53.6 cm, 'Kuemsil' 56.1 cm, 'Maehyang' 57.9 cm, and 'Vitaberry' 54.7 cm. However, no significant differences were observed among the treatments. The leaf widths were greatest in the seedlings treated with 5 mM nitrogen. Differences in leaf width among treatments were partially significant in 'Altaking and 'Vitaberry' and insignificant in 'Kuemsil' and 'Maehyang.' The greatest growth of mother plants in terms of dry weight for 'Altaking,' 'Kuemsil,' and 'Maehyang' strawberries was observed in the 10 mM N treatment, whereas in 'Vitaberry,' this was observed in the 20 mM N treatment. However, the fresh weight was greatest in seedlings fertilized with 5 mM nitrogen for the 'Altaking' and 'Kuemsil' strawberries, in those fertilized with 10 mM for 'Maehyang,' and in those fertilized with 20 mM for 'Vitaberry' (Fig. 1 B). The crown diameter was not affected by nitrogen treatment. The numerical data indicate that the growth of a strawberry mother plant can be enhanced by increasing the nitrogen concentration in the fertilizer solution to a certain level, after which it starts to decrease. The treatment with 5 mM nitrogen in the fertilizer solution was best with respect to the number and length of runners in all four strawberry varieties. However, the growth in terms of the fresh and dry weights of runners, which are important for the production of daughter plants, was greatest with the 10 mM nitrogen treatment. The number and amount of daughter plants in 'Altaking,' 'Kuemsil,' and 'Maehyang' peaked with 10 mM nitrogen and thereafter began to decline. However, in the case of 'Vitaberry' strawberry seedlings, the highest number of daughter plants occurred with 20 mM of nitrogen in the fertilizer solution (Table 2). According to Sharma et al. (2006), vigorous growth of strawberry seedlings is associated with excessive use of nitrogenous fertilizers, which is in agreement with the results of the present study for 'Vitaberry' strawberry seedlings. The results for 'Altaking,' 'Kuemsil,' and 'Maehyang' in the present study were similar to the results of Choi et al.

Table 1 Influence of different nitrogen concentrations on growth and development of 'Altaking', 'Kuemsil', 'Maehyang' and 'Vitaberry' mother plants at 90 days after transplanting

Nitrogen (mM)	Plant height (cm)	leaf length (cm)	Leaf width (cm)	Crown dia. (cm)	Fresh weight (g)	Dry weight (g)	Plant height (cm)	leaf length (cm)	Leaf width (cm)	Crown dia. (cm)	Fresh weight (g)	Dry weight (g)
	Altaking						Kuemsil					
0	48.7 b[z]	11.6 c	7.8 b	1.23 b	62.5 c	10.0 b	52.4 a	13.1 a	8.7 b	1.20 b	75.4 c	14.2 b
5	48.7 b	12.9 b	9.2 a	1.33 a	89.3 a	14.4 a	55.2 a	13.1 a	9.5 a	1.33 a	98.9 a	17.4 a
10	53.6 a	13.8 a	9.0 a	1.30 a	87.0 ab	14.8 a	56.1 a	13.7 a	9.3 a	1.33 a	97.5 a	17.8 a
15	53.6 a	13.3 ab	9.0 a	1.30 a	86.5 ab	14.8 a	54.3 a	12.9 a	9.1 ab	1.27 ab	93.9 ab	16.3 ab
20	49.9 b	13.1 ab	8.7 a	1.27 a	77.4 b	13.1 a	52.7 a	13.0 a	9.1 ab	1.27 ab	88.5 b	15.7 ab
F-sig	*	**	**	NS	***	*	NS	NS	NS	*	***	NS
Linear	**	*	**	*	***	***	NS	NS	NS	*	**	**
Quadratic	*	***	***	*	***	***	NS	NS	NS	NS	***	*
	Maehyang						Vitaberry					
0	50.4 c	13.2 b	8.3 b	1.1 a	75.8 c	13.6 b	51.2 b	12.9 b	9.3 c	1.2 a	74.3 c	12.8 b
5	51.8 c	13.7 ab	9.3 a	1.2 a	82.4 a	13.9 b	51.4 b	15.1 a	11.1 a	1.4 a	119.3 b	20.0 a
10	57.9 a	14.6 a	9.0 a	1.2 a	89.5 a	15.5 a	54.7 a	15.8 a	11.0 a	1.4 a	129.9 a	21.7 a
15	54.9 b	14.2 a	8.6 ab	1.2 a	81.5 bc	13.5 b	54.5 a	14.8 a	10.3 b	1.3 a	119.4 b	20.7 a
20	53.5 b	14.4 a	8.5 ab	1.2 a	81.6 bc	13.6 b	53.9 a	15.1 a	10.1 b	1.3 a	134.5 a	24.0 a
F-sig	*	*	NS	NS	**	*	*	***	**	NS	***	*
Linear	**	**	NS	*	*	NS	**	*	*	NS	**	**
Quadratic	*	**	NS	NS	*	NS	*	**	***	NS	***	**

NS, *, **, *** Non significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively

[z]Different letters within columns indicate significant difference based on Duncan's multiple range test, $p \leq 0.05$ (n = 3)

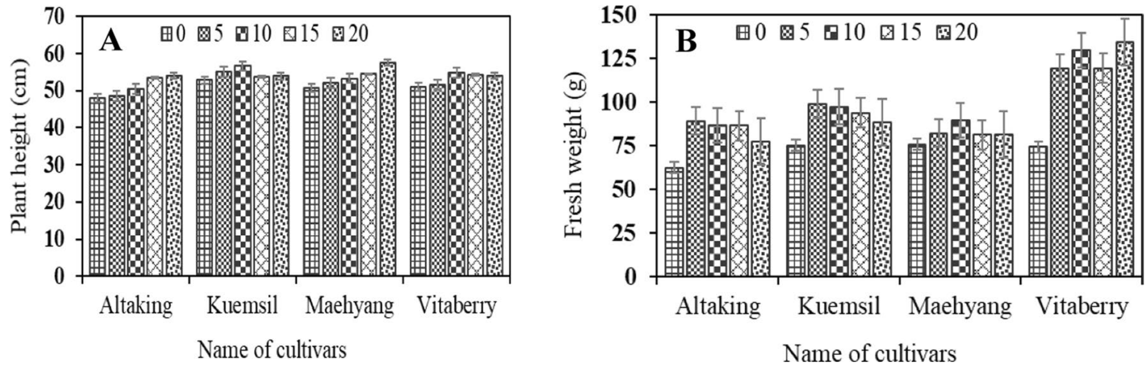

Fig. 1 Plant height (**A**) and fresh weight (**B**) of strawberry mother plant in respect to different nitrogen concentrations at 90 days after transplanting

Table 2 Influence of different nitrogen concentrations on growth and development of strawberry seedlings (runners and daughter plants) at 90 days after transplanting

Nitrogen (mM)	Runner				Daughter plant		Runner				Daughter plant	
	No	Length (cm)	FW (g)	DW (g)	No	FW (g)	No	Length (cm)	FW (g)	DW (g)	No	FW (g)
	Altaking						Kuemsil					
0	5.2 b[z]	644.1 bc	31.3 c	3.7 c	9.2 b	48.3 b	4.9 a	552.4 c	32.4 c	4.5 c	10.4 b	49.2 c
5	6.8 a	818.1 a	40.8 b	5.2 b	11.7 b	62.3 b	5.7 a	734.4 a	42.5 a	6.1 a	9.8 b	58.7 b
10	6.5 a	776.7 a	55.1 a	7.6 a	14.8 a	89.2 a	5.3 a	651.1 b	46.8 a	6.4 a	12.3 a	75.2 a
15	5.3 b	741.4 ab	40.0 b	5.1 b	11.2 b	59.6 b	5.1 a	644.3 b	38.1 bc	5.1 bc	11.2 ab	67.9 ab
20	4.9 b	595.2 c	32.1 bc	4.3 bc	9.7 b	46.8 b	5.0 a	617.9 b	36.0 bc	4.7 c	11.0 ab	61.3 b
F-sig	**	**	***	***	*	**	NS	**	**	*	*	***
Linear	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	*
Quadratic	*	***	**	**	**	*	NS	*	**	*	NS	***
	Maehyang						Vitaberry					
0	4.9 a	656.2 a	38.0 b	5.1 b	12.8 a	60.7 ab	5.7 b	808.2 b	49.2 b	6.3 b	15.2 b	63.8 b
5	5.8 a	726.0 a	41.9 ab	5.3 ab	12.6 a	68.8 ab	6.9 a	848.6 ab	62.4 b	7.9 b	15.9 b	82.5 b
10	5.7 a	704.5 a	49.0 a	6.5 a	13.5 a	79.9 a	6.9 a	865.1 ab	63.4 b	8.0 b	16.0 b	85.9 b
15	5.3 a	693.2 a	40.9 ab	5.2 ab	12.0 a	61.1 ab	6.8 a	834.6 ab	56.9 b	7.2 b	15.2 b	73.3 b
20	5.1 a	606.9 a	36.1 b	4.5 b	10.7 a	56.2 b	7.6 a	915.6 a	74.2 a	9.2 a	23.0 a	124.2 a
F-sig	NS	NS	NS	NS	NS	NS	**	NS	*	*	NS	**
Linear	NS	NS	NS	NS	NS	NS	**	NS	**	**	*	**
Quadratic	NS	NS	NS	NS	NS	NS	**	NS	*	*	*	**

NS, *, **, *** Non significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively

Z Different letters within columns indicate significant difference based on Duncan's multiple range test, $p \leq 0.05$ (n = 3)

FW, DW Fresh weight and Dry weight

(2000, 2009), who stated that strawberry seedlings grow best if they are treated with the suggested nitrogen concentration.

The results regarding the values of pH and EC in the root substrate solution 90 days after transplanting are shown in Fig. 2. The amount and formulation of the nitrogen fertilizer applied has a high impact on nutrient availability and uptake, pH regulation, and acidification of the rhizosphere. Excessive uptake of nitrate is associated with an increase in rhizosphere pH, while ammonium induces acidification. Thus, pH values can increase with increasing amounts of nitrogen fertilizer as plant roots release OH^- ions during anion absorption, such as nitrate (Choi et al. 2009). In the present study, there were no significant differences in root media pH among the nitrogen treatments; however, the pH did rise with increasing nitrogen concentration and was around 5.62–5.87 (Fig. 2A). This result is supported

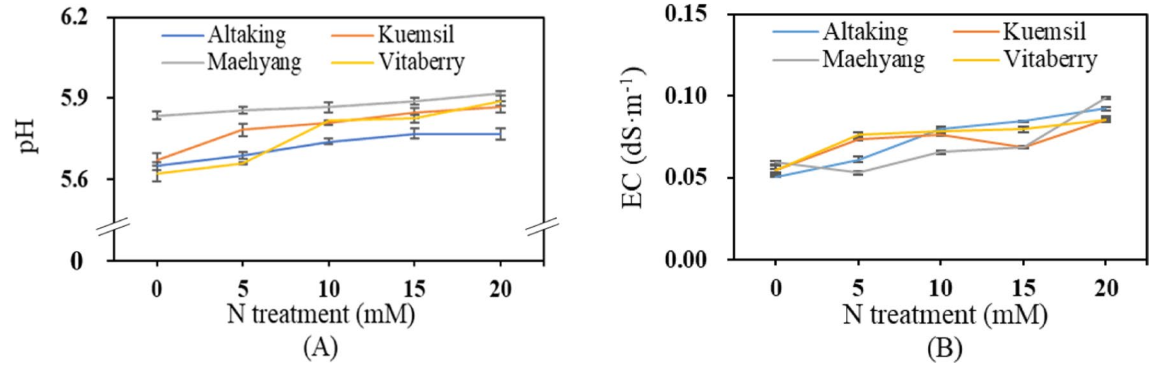

Fig. 2 pH (**A**) and EC (**B**) of root media

by Sas et al. (2003), who stated that pH is correlated with the amount and form of nitrogenous fertilizer applied, and increases with increasing nitrogen concentration in fertilizer solutions. This has a great influence on the availability of other essential nutrients.

On the other hand, the electrical conductivity (EC) varied with nitrogen level and increased with increasing nitrogen concentration (Fig. 2B). This indicates that EC increases with increasing nitrogen concentration in fertilizer solutions, and that the amount of nitrogen has a great influence over the increase in soil-solution EC. Similar results were also found by Choi et al. (2009), that is, an increase in the amount of nitrogen in the fertilizer solution applied is directly responsible for increasing the EC and greatly influences the EC, compared to the effects of other macro elements.

According to Tagliavini (2005), plant growth is affected by the supply of nitrogen fertilizer. Higher amounts of nitrogen in root media enhance N uptake in plants, and relatively more nitrogen is accumulated in older leaves. Very low nitrogen in the soil solution can cause insufficient root development, which can cause poor vegetative growth as well as poor production of daughter plants.

Figure 3 shows the concentrations of macro nutrients present in root media solution at 90 days after transplanting.

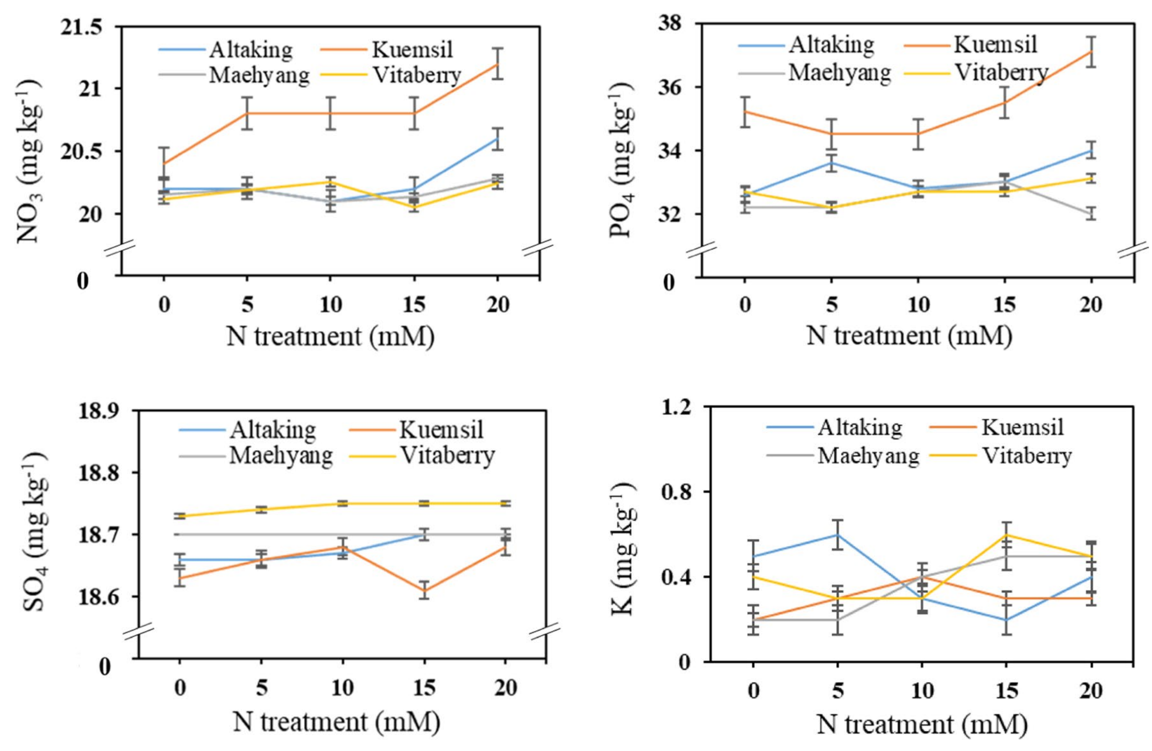

Fig. 3 Concentrations of macro nutrients present in root media at 90 days after transplanting of 0, 5, 10, 15, and 20 mM nitrogen treatments

The greatest growth of mother plants (in terms of dry weight and the occurrence of daughter plants) was observed in the 10 mM N treatment for 'Altaking,' 'Kuemsil,' and 'Maehyang' strawberries. In 'Vitaberry,' the best growth was with the 20 mM N treatment. The nutrient concentration in the soil solution of the above-mentioned nitrogen treatments for 'Altaking,' 'Kuemsil,' 'Maehyang' and 'Vitaberry,' was 20.1, 20.8, 20.1, and 20.24 mg kg^{-1} NO_3; 32.8, 34.5, 32.7, and 33.1 mg kg^{-1} PO_4; 18.67, 18.68, 18.7, and 18.75 mg kg^{-1} SO_4; and 0.3, 0.4, 0.4, and 0.5 mg kg^{-1} K, respectively. Increases in the nitrogen level of the fertilizer solution enhanced the growth of mother plants and the occurrence of daughter plants to a certain degree, but these benefits decreased when the nitrogen level was too high. This result is supported by the work of Libia and Fernando (2014), who also stated that increases in the nitrogen concentration in fertilizer solution significantly increase the total number of runners and daughter plants. Excessive application resulted in a low harvest index and low fruit quality. However, this was not studied in the present experiment and further investigation is needed to observe these effects.

Nitrogen showed a positive interaction with the availability and absorption of phosphorous (P). The concentration of P in the root media increased with increasing nitrogen concentration in the fertilizer and was highest in the 20 mM nitrogen treatment of 'Altaking,' 'Kuemsil,' and 'Vitaberry.' The P concentration was slightly lower in the root media of 'Maehyang' but very similar to that in root media of the other cultivars. The different nitrogen application treatments did not result in any significant differences in the concentrations of potassium (K) and sulfate (SO_4) in the root media, which were almost the same (0.2–0.6 mg kg^{-1} K and 18.63–18.75 mg kg^{-1} SO_4) among all nitrogen treatments and cultivars. Fageria (2006) reported that nitrogen can increase P uptake by enhancing root growth, resulting in increased absorption and translocation of P. He also stated that elevation of the nitrogen concentration in the soil solution can increase or decrease K availability and concentration in the plant tissue (relative to the K concentration). This indicates that nitrogen does not influence the availability of K. He further noted that, nitrogen and sulfur assimilation by seedlings are closely associated, and that nitrogen can regulate sulfur assimilation. However, at adequate concentrations in the nutrient solution, nitrogen does not negatively affect the availability of sulfur.

The tissue nutrient content of all the cultivars was highly influenced by the different nitrogen application concentrations (Table 3). The 0 mM N treatment resulted in the lowest tissue concentrations of macro nutrient, along with the lowest Fe content. The tissue macro nutrient concentrations were higher in the 5 mM N treatment. However, beyond 5 mM, tissue macro nutrient concentrations decreased with increasing nitrogen application, except for the total tissue nitrogen content (T-N), which increased with increasing nitrogen application and was highest in the 20 mM N treatment in all the cultivars. This indicates that an increase in the applied nitrogen level is responsible for increases in the total nitrogen content in the plant. In the case of micro nutrients, the 10 mM N treatment resulted in the highest amount of Mn and Zn in 'Altaking,' 'Kuemsil,' and 'Maehyang.' On the other hand, Mn and Zn were highest in 'Vitaberry' when the nitrogen application concentration was 20 mM. Cu was at its maximum in the 5 mM N treatment and Fe was highest in the 15 mM N treatment. K, P, Ca, Fe, Mn, Cu, and Zn concentrations followed linear and quadratic trends. Nitrogen application concentrations higher than 5 mM decreased the tissue macro nutrient concentrations. The reduction in P concentration with increased nitrogen application could have been due to competition between absorption of NO_3^- and $H_2PO_4^-$ by roots (Choi et al. 2009). However, the soil solution pH was likely responsible for decreasing the amounts of K, Ca, and Mg in the plant tissue. The concentrations of the mentioned macro nutrients declined in the soil solution when the soil become acidic, which could also result in a lower pH in the plant sap. Acidic soils in a root zone create a barrier that prevents the root apex from taking up alkali ions, and excessive nitrogen fertilization can cause rhizosphere acidification (Sas et al. 2003).

The greatest growth of mother plants in terms of dry weight for 'Altaking,' 'Kuemsil,' and 'Maehyang' strawberries was observed in the 10 mM N treatment, whereas for 'Vitaberry,' the best growth was in the 20 mM N treatment (Table 1). The tissue N content after each treatment was 2.72, 2.71, 2.69, and 2.74%, respectively, based on the dry weight of the aboveground tissue. The highest concentrations of macro and micro elements were almost the same in all cultivars (Table 3) and the findings were very close to the optimum level found by Bottoms et al. (2013). Based on these findings, the optimum range of N, P, K, Ca, and Mg in leaf blades was 30, 5–7, 2, 10–25, and 3–4 g kg^{-1}, respectively. The optimum ranges for micro nutrients were 3–15 mg kg^{-1} Cu, 10–30 mg kg^{-1} Zn, 50–300 mg kg^{-1} Mn, and 50–200 mg kg^{-1} Fe. According to Agehara (2021), providing an appropriate rate of nitrogen fertilizer is very important for ensuring satisfactory growth and development, which are influenced by the amount of plant sap in strawberry seedlings.

The concentration of nitrogen applied was equal for all cultivars (0, 5, 10, 15 and 20 mM), and the amount of nitrogen remaining in the root media after seedling growth was higher in the 20 mM N treatment than in the other treatments (Fig. 4). It is noted that, the higher the concentration of nitrogen applied, the higher the ratio of nitrogen remaining in the root media. In the present study, the highest growth of mother plants (dry weight) and occurrence of daughter plants were observed in the 10 mM N treatment

Table 3 Influence of different nitrogen concentrations on tissue nutrient content of strawberry seedlings at 90 days after transplanting

Name of the cultivar	Nitrogen	T-N (%)	K	Ca	Mg	P	Cu (mg kg^{-1})	Fe	Mn	Zn
Altaking	0	1.70 e[z]	1.2 b	0.51 b	0.30 a	0.421 b	4.12 b	48.27 e	168.75 b	13.28 ab
	5	2.30 d	1.9 a	0.93 a	0.32 a	0.643 a	6.88 a	95.13 b	140.91 c	14.34 a
	10	2.72 c	1.8 a	0.85 ab	0.32 a	0.586 a	6.15 a	80.09 c	213.14 a	15.37 a
	15	3.23 b	1.3 b	0.81 ab	0.31 a	0.531 a	4.96 b	180.52 a	102.61 e	10.2 b
	20	3.32 a	1.3 b	0.78 ab	0.31 a	0.511 a	3.36 b	57.14 d	122.35 d	9.86 b
	Linear	***	*	*	NS	*	*	***	***	*
	Quadratic	***	*	*	NS	*	*	***	***	*
Kuemsil	0	2.35 d	1.1 b	0.74 b	0.31 a	0.62 b	4.13 d	57.14 d	263.64 b	14.56 ab
	5	2.71 c	1.7 a	1.38 a	0.31 a	0.92 a	12.86 a	75.00 b	215.96 d	18.51 ab
	10	2.71 c	1.2 b	0.82 b	0.31 a	0.86 a	10.23 b	142.75 a	277.54 a	21.56 a
	15	2.82 b	1.1 b	0.77 b	0.31 a	0.77 ab	8.01 c	143.72 a	253.44 c	15.44 b
	20	3.08 a	1.1 b	0.83 b	0.31 a	0.73 ab	4.68 d	62.34 c	197.23 e	15.85 b
	Linear	***	*	*	NS	*	**	***	***	*
	Quadratic	***	*	*	NS	*	**	***	***	*
Maehyang	0	2.39 d	1.1 b	0.75 b	0.31 a	0.37 c	4.26 c	40.37 e	217.51 b	10.67 a
	5	2.41 d	1.6 a	0.91 a	0.31 a	0.85 a	10.71 a	79.22 c	147.40 c	13.43 a
	10	2.69 c	1.6 a	0.91 a	0.31 a	0.65 b	8.57 b	88.21 b	249.25 a	14.47 a
	15	2.73 b	1.5 a	0.83 b	0.31 a	0.51 bc	5.58 c	133.12 a	127.54 d	13.41 a
	20	2.79 a	1.2 b	0.61 c	0.31 a	0.43 bc	5.03 c	76.52 d	73.50 e	12.73 a
	Linear	***	*	**	NS	**	**	***	***	NS
	Quadratic	***	*	**	NS	**	**	***	***	NS
Vitaberry	0	2.12 e	1.1 c	0.63 c	0.31 a	0.71 c	4.67 b	71.10 d	260.17 b	13.48 b
	5	2.42 d	1.9 a	1.17 a	0.31 a	1.22 a	10.37 a	110.93 b	277.18 a	22.63 a
	10	2.52 c	1.7 a	1.09 a	0.31 a	0.90 b	5.38 b	113.53 b	189.85 c	26.48 a
	15	2.60 b	1.6 ab	0.80 b	0.31 a	0.81 bc	5.44 b	126.84 a	134.18 e	10.19 b
	20	2.74 a	1.3 b	0.68 c	0.31 a	0.78 bc	4.89 b	99.78 c	173.20 d	10.24 b
	Linear	***	**	**	NS	**	*	***	***	*
	Quadratic	***	**	**	NS	**	*	***	***	*

NS, *, **, *** Non significant or significant at $p \le 0.05$, 0.01, and 0.001, respectively

[Z]Different letters within columns indicate significant difference based on Duncan's multiple range test, $p \le 0.05$ (n = 3)

in 'Altaking,' 'Kuemsil' and 'Maehyang' strawberries, and in the 20 mM N treatment for 'Vitaberry' strawberries. This indicates that the nitrogen use efficiency in 'Altaking,' 'Kuemsil' and 'Maehyang' strawberries in the 15 and 20 mM N treatments was low and that the application of fertilizer with higher nitrogen concentrations results in higher nitrogen loss. This result is also supported by Liu et al (2016), who stated that nitrogen use efficiency reduces with increasing nitrogen fertilizer application. However, in the case of 'Vitaberry,' tissue nitrogen content was higher in all nitrogen treatments and the concentration of nitrogen remaining in the root media after seedling growth was lower compared to that of other cultivars. This indicates that 'Vitaberry' needs more nitrogen for its vegetative growth than the other cultivars.

4 Conclusions

These results show that the concentration of nitrogen fertilizer applied to seedlings may be important for ensuring the best growth of mother plants and occurrence of daughter plants. Furthermore, controlling the nitrogen fertilization level based on the absorption characteristics of each variety could increase the number of seedlings and be advantageous for breeding. It can be concluded that proper fertilizer management could be the most important tool for improving the efficiency of fertilizer usage and increasing the growth of strawberry plants. Finally, increasing the rate of early-season N application can enhance vegetative and reproductive growth in terms of the dry weight

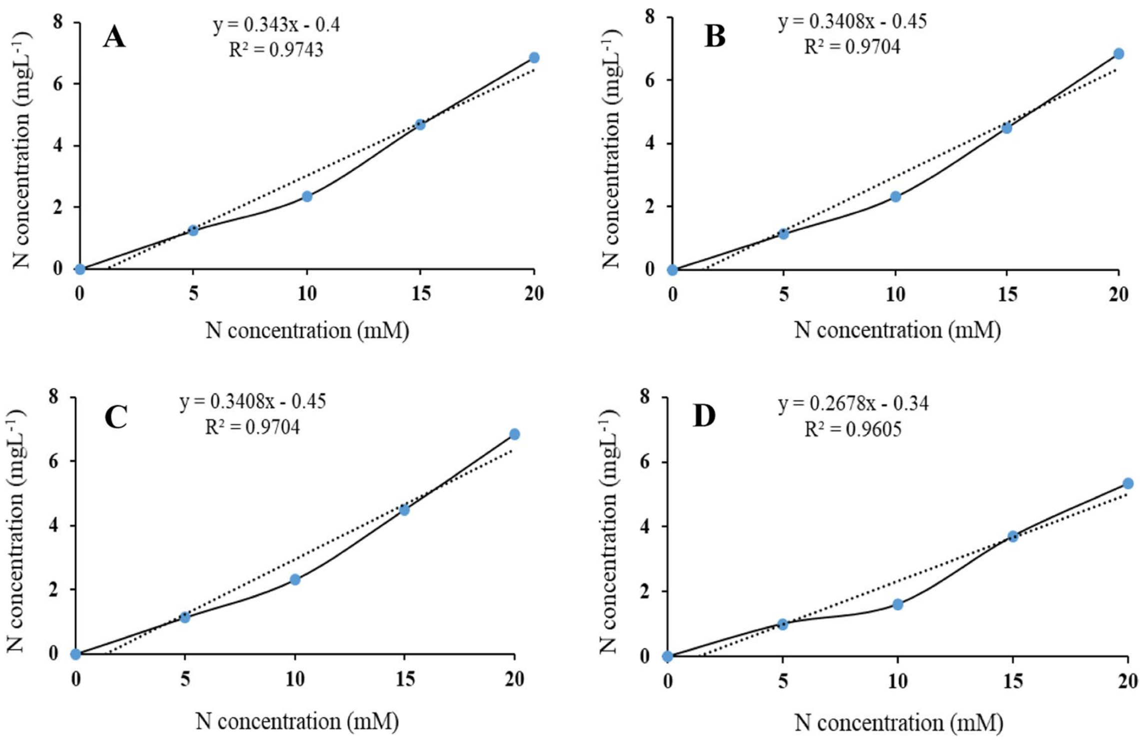

Fig. 4 Correlation between nitrogen concentration applied (mM) and nitrogen remained in root media after seedling growth; 'Altaking' (**A**), 'Kuemsil' (**B**), 'Maehyang' (**C**) and 'Vitaberry' (**D**)

of mother plants and the occurrence of daughter plants. The required N rate varied among the selected strawberry varieties, and appeared to be 10 mM N for 'Altaking,' 'Kuemsil,' and 'Maehyang,' and 20 mM N for 'Vitaberry.' The results indicate that 'Vitaberry' strawberries require a higher concentration of nitrogen in the fertilizer solution than 'Altaking,' 'Kuemsil,' and 'Maehyang' for optimal growth of mother plants and occurrence of daughter plants during vegetative propagation.

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Author contributions JMC designed the research. SF performed the experiments and wrote the manuscript. ISP analyzed the data. All authors read and approved the final manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest.

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