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Germination and Growth Characteristics of *Perilla frutescens* and Nutrients by the Mixed Seeding Rate of *Medicago sativa* in Indoor Urban Agriculture

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ABSTRACT

Background and objective: This study aims to provide basic data to investigate an efficient and stable environment for indoor urban agriculture by comparing plant growth depending on seeding rate and media nutrients (nitrogen, phosphorous, and potassium) using leguminous plant *Medicago sativa* favorable for nitrogen fixation and Lamiaceae plant *Perilla frutescens* in indoor urban agriculture.

Methods: To investigate plant growth by mixed seeding rate, this study set up 6 treatments: single seeding of *M. sativa* (AC); 2 : 1 *M. sativa* to *P. frutescens* (A_2P_1); 1 : 1 *M. sativa* to *P. frutescens* (A_1P_1); 1 : 2 *M. sativa* to *P. frutescens* (A_1P_2) and single seeding of *P. frutescens* (PC). Experimental plastic containers were completely randomized designed with 9 replications for each seeding rate. Nutrient content of the media and germination and growth parameters of *P. frutescens* were measured.

Results: The nitrogen and phosphorous contents in the media were the highest in the AC treatment, and potassium did not differ according to the mixed seeding rate. Germination percentage and velocity were highest in order of A_1P_2 〉 PC 〉 A_2P_1 〉 A_1P_1 〉 AC due to interspecies competition. Growth of *P. frutescens* was increased with the increasing seeding rate, whereas biomass was higher in A_2P_1 compared to other treatments.

Conclusion: Our results indicated that the 2:1 seeding rate of *M. sativa* and *P. frutescens* was a more effective way to achieve high plant growth and productivity. These results suggest that mixed seeding can promote the interaction of positive neighbor effects in indoor urban agriculture.

Keywords: indoor agriculture, plant growth, media nutrient, nitrogen fixation

Introduction

Recently, the importance of indoor urban agriculture is increasing due to the growing demand for grocerants with improved living standards and income levels. Accordingly, leafy vegetables are receiving attention as fast-growing and easy-to-grow vegetables as well as safe, functional food (Lee and Park, 2014). In particular, indoor plant factories and vertical farms using LED, which is artificial light, are highlighted for year-round stable production (Lee et al., 2020; Park and Oh, 2021; Yang, 2015). However, there is insufficient research on eco-friendly cultivation methods

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since the development of a system for cultivation under structure is covered as a major topic of interest (Lee and Park, 2014). In fact, eco-friendly organic farming accounts for about 20% of the farmers market in Korea, and it is difficult to expand the base in general open fields due to uncertainty in production (Chio and Kim, 2014).

Meanwhile, a mixed sowing cultivation method to increase productivity through plant diversity has recently emerged (Ren et al., 2021). Mixed seeding (mixed sowing, mix-seeding) is a sowing method that increases productivity through interaction between species by mixing and sowing different species (Hinsinger et al., 2011). This farming method is based on the mechanism of adequately regulating the interrelation, or competition, among plants to secure resources and space (Letten et al., 2017). In particular, Leguminosae is known to improve the vegetation environment of the soil and supply the nitrogen source of plants by fixing nitrogen in the air in coexistence with rhizobia *Rhizobium* sp. (Shao et al., 2021). However, most studies on mixed seeding between Leguminosae are focused on the productivity of field crops (Jo, 2009). *Medicago sativa* in particular is a perennial Leguminosae known for its high protein and mineral content as well as excellent soil improvement effects (Bak and Lee, 2021). Therefore, it is necessary to investigate the nutrient changes in the media and the germination and growth characteristics of the major crops through mixed seeding of Leguminosae as companion plants in indoor urban agriculture. Accordingly, this study provides ways to apply existing organic farming techniques to indoor urban agriculture by selecting Leguminosae *Medicago sativa* and Lamiaceae *Perilla frutescens* and examining the effect on the growth of *Perilla frutescens* and the nutrient content within the media depending on the mixed seeding rate of *Medicago sativa*.

Research Methods

Materials

This study used horticultural substrates (Hanpanseung, Samhwa Greentec, Korea) combining organic materials such as peat moss and coco peat and inorganic materials such as vermiculite and perlite for sowing and cultivation (Hanpanseung, Samhwa Greentec, Korea) and perlite (Ecolite, Homan, Korea) as a drain layer for smooth drainage and air permeability. The seeds used in the experiment were *Medicago sativa* and *Perilla frutescens*, and seeds of 450 ± 10 and 300 ± 10 grains/g, germination percentage of 80% or more (Worldseed, Korea) were used, respectively. *Perilla frutescens* is an annual plant in the Lamiaceae family. It has a thin cell wall and easily discharges the nutrients it contains and is thus quickly absorbed by the human body. It is recognized as a health functional food since it is also related to inhibiting cancer cell proliferation, lowering blood pressure, and dissolving blood clots (Jeong et al., 2014), which is why it was selected as the major crop.

Methods

The experimental treatment was implemented by controlling the environmental conditions in the growth chamber (JSGC- 420C, JS Research Inc., Korea). Light conditions were set as temperature 25 °C, relative humidity 50%, and photosynthetic photon flux density (PPFD 297.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$) in a 16-hour. cycle, and dark conditions as temperature 18 °C, relative humidity 50%, and PPFD 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in an 8-hour. cycle (Kim and Lee, 2005). The experiment was conducted for 30 days. For the experimental treatment, a drain layer and bed soil layer were formed with 15 g of perlite and 60 g of horticultural substrate in a 709 mL transparent plastic container with a diameter of 10 cm and height of 14 cm, and then covered up with soil about 3-5 mm thick after sowing seeds.

With the seeding rates of the two species as treatments, we came up with total 5 types: *Medicago sativa* 30 grains in single seeding (Alfalfa Control, AC), *Medicago sativa* 20 grains + *Perilla frutescens* 10 grains (hereinafter A_2P_1), *Medicago sativa* 15 grains + *Perilla frutescens* 15 grains (hereinafter A_1P_1), *Medicago sativa* 10 grains + *Perilla frutescens* 20 grains (hereinafter A_1P_2), and *Perilla frutescens* 30 grains in single seeding (Perilla Control, PC). The cups in which the seeds were sown were completely randomly placed in the growth phase with 9 replications of 30 grains in each treatment.

Media and growth characteristics and statistical analysis

The nutrients of the media were measured using an NPK meter (ZD-1801N, ZD-1802P, ZD-1803K, WANT, China), and nitrogen (N), phosphorus (P), and potassium (K) in the media of the treatments of each mixed seeding rate were measured at 5-day intervals from the sowing date. Plant germination characteristics were investigated at 2-day intervals after sowing, and growth characteristics were investigated at 5-day intervals after seed germination. The seeds were considered germinated when the seed coat was torn and the budlet appeared on the surface of the soil. The number of survey days after sowing, the number of germinations on the day of survey, and total number of germinations were used to calculate germination percentage (GP), germination energy (GE), mean germination time (MGT), and mean germination velocity (MGV) in means (Park and Shim, 2018). Germination energy was calculated as the ratio of the number of seeds until the day in which most seeds were germinated after sowing to the total number of seeds, based on 4 days after sowing.

As growth characteristics, plant height, leaf length, leaf width, number of leaves, fresh weight, and dry weight were measured. Plant height was measured from the bottom to the apex of the plant in media, leaf length was measured from the leaf base where the leafstalk ends to the top of the leaf blade, and leaf width was measured by the maximum length perpendicular to the leaf length. The number of leaves was counted by visually checking the number of leaves of the plant, and fresh weight was measured by using a microelectronic scale (FX-200i, AND, Korea) by harvesting 3 plants in each treatment 30 days after sowing. Dry weight was measured with a microelectronic balance after drying at 70 °C for 72 hours with a hot air circulation dryer (C-DF, CHANG SHIN SCI Co., Korea). As the selection criteria for each item, average-sized leaves were selected from the treatments.

The measured data were statistically analyzed using IBM SPSS Statistics 27 (SPSS Inc., USA). One-way ANOVA was conducted to compare the means between treatments, and the statistical significance level was set to $p < .05$ by Duncan's post-hoc analysis. Moreover, Sigmaplot 12.3 (Systat Software, Inc., USA) was used to graph the results.

Results and Discussion

Nutrient content of the media

The nutrient content of the media according to the mixed seeding rate of *Medicago sativa* and *Perilla frutescens* is shown in Fig. 1. The nitrogen content was highest at 82.11 mg · kg^{-1} in the treatment with single seeding of *Medicago sativa*, and showed statistically significant differences in other treatments in the order of A_2P_1 (77.25 mg · kg^{-1}), A_1P_1 (73.26 mg · kg^{-1}), A_1P_2 (71.55 mg · kg^{-1}), and PC (65.22 mg · kg^{-1}). In other words, higher seeding rate of *Medicago sativa* led to higher nitrogen content after germination than before. This indicates that nitrogen content was increased by increasing fixation and movement according to the nitrogen use principle of Leguminosae (Shao et al., 2021).

Phosphorus content of the media by treatment was relatively higher than nitrogen content, but there was no tendency according to mixed seeding rate, whereas it was highest in AC, which is the *Medicago sativa* single seeding treatment.

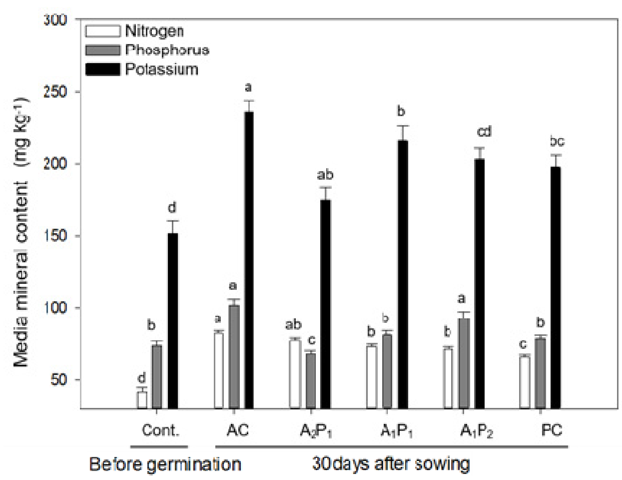

Fig. 1. Comparison on media mineral contents before germination and for 30 days after mixed sowing of *Medicago sativa* and *Perilla frutescens*. Vertical bars give the standard error of the mean. Different letters indicate significant difference among treatments by Duncan's multiple range test at $p < .05$. Cont.: substrate with non-sowing; AC: *M. sativa* control; A_2P_1: 20 M. *sativa* and 10 P. *frutescens*; A_1P_1: 15 *M. sativa* and 15 *P. frutescens*; A_1P_2: 10 *M. sativa* and 20 *P. frutescens*; PC: *P. frutescens* control.

According to a study on the causal interaction in nitrogen fixation (Rotaru and Sinclair, 2009), higher phosphorous content leads to higher nitrogen fixing ability and speed. In addition, arbuscular mycorrhizal fungi (AMF) that coexist with Leguminosae are known to help supply various nutrients, especially phosphorus, to the host plant (Ahn et al., 1992), which may have caused the phenomenon above.

Potassium content was higher than nitrogen or phosphoric acid content and tended to increase overall after sowing than before sowing. Potassium was also highest at 236.63 mg · kg^{-1} in AC with single seeding of *Medicago sativa*. On the other hand, it became lower in treatment with mixed seeding in the order of A_1P_1 (216.19 mg · kg^{-1}), A_1P_2 (202.81 mg · kg^{-1}), and A_2P_1 (175.07 mg · kg^{-1}), not showing a clear tendency depending on the mixing ratio between *Medicago sativa* and *Perilla frutescens*. Considering that phosphoric acid content of *Veronica nakaianum* leaves increases while potassium decreases when there is more nitrogen fertilizer application (Yoo et al., 2021), nitrogen and phosphoric acid increase with nitrogen, while nitrogen and potassium are in a conflicting relationship. Meanwhile, there are contrary results that potassium concentration in chrysanthemums increases with higher nitrogen concentration in nutriculture (Kim et al., 2002). Thus, it is necessary to more closely review literature on interaction of potassium in the media according to mixed seeding with Leguminosae for nitrogen fixation.

Germination and growth characteristics

Seed germination characteristics of *Medicago sativa* and *Perilla frutescens* according to the seeding rate are shown in Fig. 2. All treatments generally showed more than 70% of germination percentage. Although the disclosed germination percentage of the seeds is higher than 80%, it was

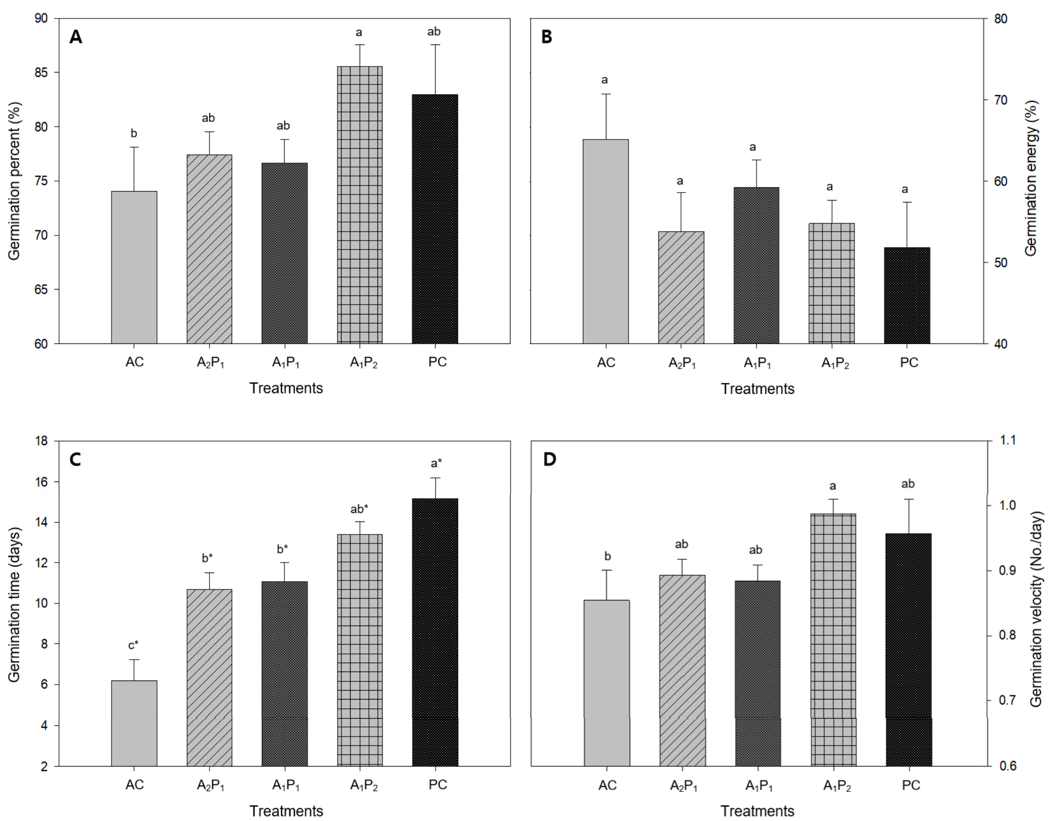

Fig. 2. Comparison on germination percent (A), germination energy (B), germination time (C), and germination velocity (D) in response to mixed sowing of *Medicago sativa* and *Perilla frutescens*. Vertical bars give the standard error of the mean. Different letters indicate significant difference among treatments by Duncan's multiple range test at $p < .05$. AC: *M. sativa* control; A_2P_1: 20 *M. sativa* and 10 *P. frutescens*; A_1P_1: 15 *M. sativa* and 15 *P. frutescens*; A_1P_2: 10 *M. sativa* and 20 *P. frutescens*; PC: *P. frutescens* control.

generally low in this study because seeds were not soaked before sowing (Kim et al., 2020). Meanwhile, the germination percentage was the lowest in the AC treatment and tended to increase in the A_1P_2 treatment with a high seeding rate of *Perilla frutescens*. Germination energy was higher than 50% in all treatments and was the highest at 68.89% in the AC treatment. The mixed seeding treatments tended to be similar without a statistically significant difference.

The mean germination time was slowest at approximately 15 days in the PC treatment but fastest at approximately 6 days in the AC treatment. The mean germination time showed a significant difference among treatments, and *Medicago sativa* turned out to be superior to *Perilla frutescens* although contrary to the results of germination energy. Unlike mean germination time, the mean germination velocity was fastest in the AC treatment and slowest in the treatment with a high ratio of *Perilla frutescens* (Fig. 2). As such, higher seeding rate of *Perilla frutescens* led to higher germination percentage and mean germination velocity, which is a result due to interspecific competitive reaction (Choi, 2007).

Plant height of *Medicago sativa* was the longest at 175.56 mm in the AC treatment, followed by A_2P_1, A_1P_1, and A_1P_2, and the shortest at 92.78 mm in the A_1P_2 treatment. Like plant height, leaf length was relatively high at 10.67 mm in the AC treatment and was lowest in the A_1P_2 treatment with a high ratio of *Perilla frutescens*. This indicates that higher ratio of *Perilla frutescens* affects the growth of *Medicago sativa*. Leaf width was widest in the A_2P_1 treatment (7.78 mm) and narrowest in the A_1P_1 treatment (4.78 mm). The number of leaves was biggest in A_2P_1 (12.67 leaves), followed by AC (10.00), A_1P_1 (9.33), and A_1P_2 (8.33), indicating that higher ratio of *Medicago sativa* led to relatively more leaves. Meanwhile, for fresh weight and dry weight, AC and A_2P_1 were heavier than A_1P_1 and A_1P_2 (Table 1). Thus, it is true that higher seed ratio of *Medicago sativa* indicates better growth overall. Nevertheless, considering that the indicator of the interrelationship between plants is the biomass of the shoot (Ren et al., 2021), setting the ratio of *Medicago sativa* as 65% is adequate for a positive effect between *Medicago sativa* and *Perilla frutescens* in mixed seeding of different species.

There was no significant difference in plant height of *Perilla frutescens* by treatment, but single seeding of *Perilla frutescens* showed highest plant height (161.11 mm), followed by A_2P_1 (156.11 mm), A_1P_1 (150.78 mm), and A_1P_2 (147.33 mm), and A_2P_1 showed a high value for mixed seeding. Leaf length and leaf width were longest in the A_2P_1 treatment at 45.00mm and 26.33mm. The number of leaves, fresh weight, and dry weight showed insignificant differences in numbers among treatments, but the A_2P_1 treatment was heavier than the PC treatment with single seeding (Table 1). Since Leguminosae has a positive effect on the shoot biomass of surrounding plants, it is often cultivated with mixed seeding on agricultural pastures as Leguminosae and gramineous plants (Xi et al., 2017). In particular, arbuscular mycorrhizal fungi (AMF) of Leguminosae is known

Table 1. Growth characteristics of *Medicago sativa* and *Perilla frutescens* grown for 30 days after mixed sowing

Species	Treatments[z]	Plant height (mm)	Leaf length (mm)	Leaf width (mm)	Number of leaves	Fresh weight (g)	Dry weight (g)
Medicago sativa	AC	175.56 a[y]	10.67 a	6.33 ab	10.00 b	0.15 a	0.04 a
	A_2P_1	156.00 a	10.44 a	7.78 a	12.67 a	0.15 a	0.04 a
	A_1P_1	106.11 b	8.33 ab	4.78 b	9.33 b	0.09 b	0.02 b
	A_1P_2	92.78 b	7.56 b	5.89 ab	8.33 b	0.09 b	0.02 b
Perilla frutescens	PC	161.11 a	29.22 b	18.78 b	7.33 b	0.55 bc	0.13 b
	A_2P_1	156.11 a	45.00 a	26.33 a	8.22 a	1.08 a	0.23 a
	A_1P_1	150.78 a	32.11 b	20.67 b	6.22 c	0.64 b	0.13 b
	A_1P_2	147.33 a	29.22 b	16.78 b	6.00 c	0.47 c	0.12 b

[z]AC: *M. sativa* control; A_2P_1: 20 *M. sativa* and 10 *P. frutescens*; A_1P_1: 15 *M. sativa* and 15 *P. frutescens*; A_1P_2: 10 *M. sativa* and 20 *P. frutescens*; PC: *P. frutescens* control.

[y] Different letters indicate significant difference among treatments by Duncan's multiple range test at $p < .05$.

to have various effects since it has a symbiotic relationship with plant roots and helps improve intelligence and recovery due to nitrogen fixation in the air (Shao et al., 2021). Considering that the growth of *Perilla frutescens* is better in mixed seeding than single seeding, especially in the mixing ratio of *Medicago sativa* : *Perilla frutescens* = 2 : 1, mixed seeding of Leguminosae *Medicago sativa* may have played a key role in nutrient absorption necessary for the growth of *Perilla frutescens*. This result implies that high-quality young leafy vegetables can be safely produced through interactions among plants in indoor urban agriculture.

Conclusion

This study suggests an efficient seeding rate for productivity of young leafy vegetables in eco-friendly indoor urban agriculture by comparing the media nutrients and growth characteristics of *Perilla frutescens* according to the mixed seeding rates of Leguminosae *Medicago sativa*. Nitrogen, phosphorus, and potassium contents after germination in the media of *Medicago sativa* and *Perilla frutescens* were higher when there was a higher seeding rate of *Medicago sativa*. On the other hand, higher seeding rate of *Perilla frutescens* led to higher germination percentage and mean germination velocity, and general growth was most satisfactory in the A_2P_1, *Medicago sativa* : *Perilla frutescens* = 2 : 1, except plant height. These results can be applied as an eco-friendly farming method that improves media nutrients and the green manure effect through companion planting of Leguminosae in small-scale indoor crop growing that is recently rising. Meanwhile, mixed seeding of Leguminosae seems to have a positive effect on the growth of companion plants and media, but it is necessary to conduct more detailed media analysis and long-term monitoring for mixed seeding of homogeneous and heterogeneous species.

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