EFFECT OF DIFFERENT NUTRIENT CONCENTRATION AND GERMINATION SPONGE ARRANGEMENT ON GROWTH AND YIELD OF BUTTERHEAD (*Lactuca sativa var. Butterhead*) IN INDOOR MULTILAYER PLANT FACTORY CONDITION

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ABSTRACT

MARDI developed plant factory to increase vegetable yield per unit area and as a new approach to producing high-value vegetable crops. Seedlings production is vital to produce high quality and potential yield. Transplanting shock occurred when the seedlings were separated from germination sponge. Hence, a study had been conducted to explore the best approach for seedlings production in MARDI plant factory. This study was done in two stages, multilayer plant factory nursery and growth area. The treatments were laid out in two factorial randomised complete block design and replicated four times with lettuce (Lactuca sativa var. Butterhead) as the tested plant. The first factor was nutrient concentration, 0 miliSiemens (T1), 0.5 miliSiemens (T2) and 1.0 miliSiemens (T3) using MARDI leafy vegetable formulation. The second factor was sponge arrangement, single (S1) and regular arrangement (S2). Variables measured were on a three-stage basis; germination, growth and tissue analysis and data were collected at transplanting and harvesting stage. At transplanting, T3 show significant difference with T2 and T1 on day of seed germinate, cotyledon leaf and the actual leaf appears. No significant differences were found on second factors treatment at transplanting and harvesting stage. At harvesting stages, T3 was significantly different in all variables tested. No significant interaction was found on nutrient and sponge arrangement factors. The result shows that 1.0 miliSiemens of the nutrient solution was suitable for the production of healthy and vigorous seedlings for high yield vegetables in multilayer plant factory.

Key words: Butterhead, germination sponge, growth, nutrient concentration, plant factory

INTRODUCTION

Food security is one of the issues for supplying enough nutritional food to 16 billion populations throughout the world. The primary source of natural nutrient and vitamins mostly are from vegetables that we produce on the farm from outside the town. The nutrient is essential for human to stay healthy and fit. As land for agriculture is depreciating because of the introduction of the residential and industrial area, also, the increasing amount of carbon dioxide due to transportation from farm to the consumer will increase the pollution. A new approach for cultivating and producing vegetables are essential to introduce. Hence, the establishment of Plant Factory for producing leafy vegetables was done worldwide (Kozai, 2013).

Plant factory was invented to produce vegetables in close are using multilayer hydroponic with control environment system and artificial lighting using Light Emitting Diode (LED) (Kozai, 2011). MARDI has developed a plant factory since 2018 known as MARDI Plant Factory (MPF). The MPF was established on the Eleventh Malaysia Plan (RMK11) budget. The main purposed of MPF is to produce high-value vegetables in limited space that lead to increase yield per unit area.

Nursery production is one of the sub-areas inside the MPF. Nursery in MPF was designed to produce seedlings from the seed, using multilayer hydroponic system, designated with specific red: blue artificial lighting formulated by MARDI. MPF nursery production is essential to produce healthy and robust vegetable seedlings. Nursery production will affect the end products and yield of the vegetables produce in MPF. Currently, up to 70,000 seedlings can be produced on one-time production in MPF. Development of a standard operating procedure for nursery production in MPF is crucial to achieving cost-effective production. The high-value seedlings selected such as various type of lettuce and kale were sown in the nursery.

As the MPF is new to the researcher, uneven seeds germination and seedling growth happen on the first vegetables production test inside the MPF system. Nutrient is essential on plant growth with favourable microclimatic conditions (Kozai,2012) and plant stress on handling the seedlings from nursery to growth area are two main issues that occur in MPF. Roots are essential in seedlings development (Larson and Nelson, 1984). In the hydroponic system, the roots absorb water and nutrient in the form of ion. Leaf development is depending on environmental factors such as temperature, moisture, mineral elements, and light (Larson and Larson, 1984). Elongating of the leaf is an indicator for the plants is mature enough to be transplanted to the growth area. The conventional hydroponic system used planting pot with light expanded clay aggregate (LECA) as the growing media while multilayer hydroponic system used sponge as the growing medium. The sponge is a good media for MPF production because it easy to handle, absorb water and nutrient efficiently and cheaper compare to LECA. The hydroponic sponge was design to have a piece of sponge, with separation of 112 cubes. However, the sponge arrangement was closed together, so the seedlings root will bind on the side by side, resulting in root damage when we split it and transplant to the growth area. Hence, a study was conducted to search for optimum nutrient solution concentration for seedlings and growth sponge arrangement in nursery production.

MATERIALS AND METHODS

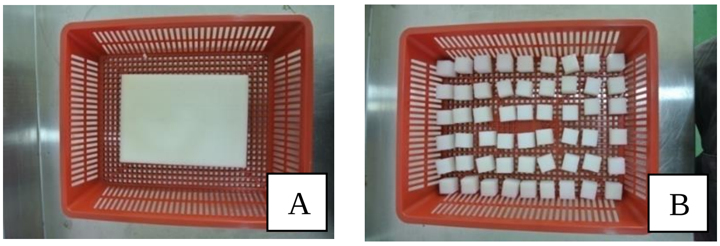
The study was done in July 2019 inside MPF conditions, at two main areas of planting: 1) Nursery, 2) Growth Production Area. Both areas used multilayer hydroponic nutrient film and deep-water culture system with red: blue artificial lighting. *Lactuca sativa* var. Butterhead was used as a test crop.

The nursery is designed with 90 cm (width) x 120 cm (length) x 180 cm (height) with four shelves. Each shelf has six units of four feet LED artificial lighting. The study was done in randomised complete block design with two factors of study, nutrient concentrations, and sponge arrangement.

Treatments are:

Factor 1: Nutrient Concentrations (miliSiemens)
T1: 0.0 (control)
T2: 0.5
T3: 1.0
Factor 2: Sponge arrangements (picture 1)
S1: Split
S2: Normal (control)

Picture 1: normal sponge arrangement (A) and split sponge arrangement (B)



The MARDI leafy vegetables stock A and B hydroponics nutrient solution formulation were used, and the nutrient concentration was determined using Hanna EC meter by adding water according to the stock solution and were adjusted to achieve the treatment nutrient concentration. The pH of the nutrient solution was stabilised at 5.5-6.5 using nitric acid and was determined using Hanna pH meter. The water temperature was at the range of 24-25^{o}C with 5-6 ppm of dissolved oxygen. The nursery area carbon dioxide (CO_2) concentration range was at 400-660ppm. The surrounding environment was set to have 20^{o}C (minimum) and 25^{o}C (maximum) temperature. The relative humidity was at 50-75%. The light supplied at the range of 2-4 $molm^{-2}day^{-1}$ (Kozai, 2005). The sponges were arranged in a 42cm (length) x 32cm (width) x 16cm (height) size basket. The baskets were organised in randomised complete block design with three replications.

The successfully germinated seedlings were transplanted to the production area. The arrangement at the production area was as same as the nursery area. The seedlings were transplanted into the growth tray. The light supplied at the range of 8-12 $molm^{-2}day^{-1}$. At this stage, the nutrient concentration was at the range of 1.2-1.8 miliSiemens/cm. The plant's samples were harvested after 25 days after transplanting (DAT).

Measurement
Nursery: the parameters measured were the day of the seed germinated, percentage of germination, day of the fully grown cotyledon leaf (two leaf blades) and the formation day of the true leaf (four-leaf blades)
Production area: the measurements were done at 25 days after transplanting. The parameters measured were the number of leaves, chlorophyll content using SPAD Konica Minolta SP522, fresh weight of shoot, fresh weight of root, dry weight of shoot, dry weight of root, leaf area. The specific leaf areas were measured using the formula. The formula is as below;

SLA : L_A/L_W , where,
L_A : leaf area (cm^2)
L_W: leaf dry weight (g)
SLA: specific leaf area (cm^2/g)

The shoot and root ratio were calculated using the formula as below:
A= a/b where,
a : dry weight of shoot at 25 DAT
b : dry weight of root at 25 DAT.
A : shoot: root ratio

Data analysis
The data were analysed by analysis of variance using Statistical Analysis Software (SAS) version 9.4. All significant values were analysed using the Least Significance Differences (LSD) at a significant level of $P\leq0.05$.

RESULTS AND DISCUSSION

Germination and day to germinate
Nutrient concentration affects the germination, seed emergence and growth of seedlings on the hydroponic system. Besides, early nutrient supply on seedlings will influence the performance of the plants once transplanted to the production area. In this study, seedlings germinated were calculated after three days of sowing. Both factors treatment signifies the germination rate was on the range of 92.25 to 92.71 per cent (Table 1).

Table 1: Percentage of germination

Treatments	Germination (%)
Nutrient Concentration (T)	
T1(C)	92.48
T2	92.71
T3	92.25
Sponge Arrangement (S)	
S1	92.36
S2(C)	92.59
P≤0.05 LSD	
T*S	ns

The epigeal germination of the seedlings was determined from the emergence either one of radicle or plumule on the sponge starting from the day of sowing. As from table 2, T3 was found to be the least day the seeds to germinate (5.13 days) significantly different with T2 (7.00 days) and T3 (10.00 days).

Cotyledons leaf emerge after the plumule. It was the first part of the plants to emerge from the seeds on the germination process. The cotyledons leaf function in photosynthetic process for a short time before the true leaf emerges. The data show that T3 was the earliest day to cotyledon leaf emergence (7.13 days) significantly different from T2 (9.14 days) and T1 (14.35 days) (Table 2). The presence of true leaf is an indicator of the seedlings maturity to be transplanted into the growth area (picture 2). At this stage, the seedlings were well established to receive higher nutrient concentration and other environmental requirements to grow for productions properly. T3 took 8.38 days for the true leaf to emerge, significantly different from T2 (10.38 days) and T1 (14.35 days) (Table 2).

Table 2: Day to germinate, cotyledon and true leaf emergence

Treatments	Day to Germinate	Day to Cotyledon Leaf Emergence	Day to True Leaf Emergence
Nutrient Concentration (T)			
T1(C)	10.00^{a}	14.35^{a}	16.75^{a}
T2	7.00^{b}	9.14^{b}	10.38^{b}
T3	5.13^{c}	7.13^{c}	8.38^{c}
Sponge Arrangement (S)			
S1	9.33	11.33	12.83
S2(C)	9.41	11.42	12.83
$P \leq 0.05$ LSD	ns	ns	ns
T*S			

The use of electric energy on lighting and planting systems were crucial in nursery and production area. The increased productivity by shortening the day of seedling production will affect the whole process of planting, thus will reduce the energy consumption and cost.

Picture 2: Germination performance on the treatment

Growth effect after transplanting to the production area

The transplanting process is a critical part of the seedlings to grow well in the production area. The indicator for matured seedlings was the emergence of the true leaf. In this stage, the plants need adequate lighting, nutrient, carbon dioxide and suitable environment. In regards to the temperature (24-28^{o}C), relative humidity (60-70%) and carbon dioxide (600-700ppm), the environment on the production area were the same as the nursery production. All seedling samples from the nursery transplanted to production area were 0% of mortality.

Effect on growth and biomass

The samples were harvested and analysed 40 days after sowing (picture 3). The leaf is an important part of plants. Leaf provides food for the whole plants through photosynthesis process. The different nutrient concentration at nursery influenced the leaf number and leaf area variables. The number of leaves in T3 (40.79) was significantly different from T2 (35.63) and T1 (22.63) (table 3). Significant finding also found on T3 (1793.82cm^2) with T2 (1465.14 cm^2) and T1 (531.54 cm^2) on leaf area. The chlorophyll content reading was at the range of 33.08 – 34.82 µmol/m^2 (table 3)

Table 3: Leaf number, leaf area and chlorophyll content

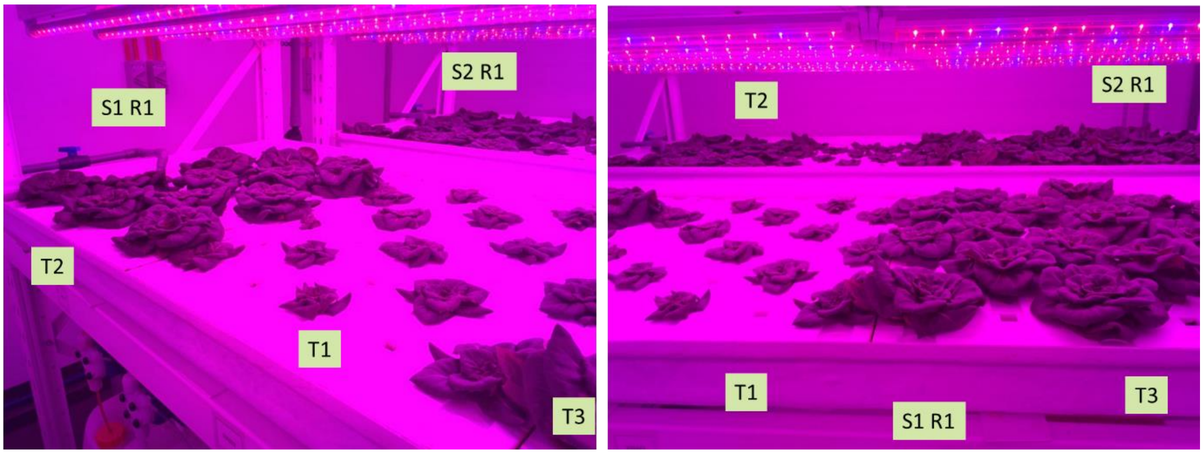
Treatments	Leaf Number	Leaf area (cm^2)	Chlorophyll Content (µmol/m^2)
Nutrient Concentration (T)			
T1(C)	22.63^{c}	531.54^{c}	33.86
T2	35.63^{b}	1465.14^{b}	33.88
T3	40.79^{a}	1793.82^{a}	34.12
Sponge Arrangement (S)			
S1	31.97	1147.36	33.08
S2(C)	34.06	1379.64	34.82
P<0.05 LSD	ns	ns	ns
T*S			

Also, the fresh weight of T3 (82.71g) was significantly different from T2 (70.12g) and T1 (17.88g) (table 4). The plants' biomass was measured from the dry weight of the shoots and roots of the samples. The data show that T3 (2.21g) and T2 (2.85g) were significantly different from T1 (0.95g) on shoots dry weight. Same results were found on roots dry weight, T3 (0.40g) and T2 (0.39g) were significantly different from T1 (0.29g).

Table 4: Shoot fresh weight, shoot dry weight and root dry weight

Treatments	Shoot Fresh Weight (g)	Shoot dry weight (g)	Root dry weight (g)
Nutrient Concentration (T)			
T1(C)	17.88^{c}	0.95^{b}	0.29^{b}
T2	70.42^{b}	2.85^{a}	0.39^{a}
T3	82.71^{a}	2.21^{a}	0.40^{a}
Sponge Arrangement (S)			
S1	51.33^{b}	2.51	0.35
S2(C)	62.67^{a}	1.50	0.39
P<0.05 LSD	ns	ns	ns
T*S			

Picture 3: Effect after transplanting (45 day after sowing)



Effect on specific leaf area

Specific leaf area (SLA) is one of the growth analyses (Evans, 1972) that can be used to quantify the differences between the treatments. SLA is a ratio indicating the leaf area for a specific plant's ratio with the plants dry mass. The measurement was done by using leaf area and dry weight of the samples. The data analysed show that T3 (893.78cm^2/g) and T2 (838.00cm^2/g) were not significantly different from each other but were significantly different from T1 (567.69 cm^2/g) (table 5).

Table 5: Specific leaf area

Treatments	**Specific leaf area (cm^2/g) (L/B)**
Nutrient Concentration (T)	
T1(C)	567.69^b
T2	838.00^a
T3	893.78^a
Sponge Arrangement (S)	
S1	616.75^b
S2(C)	916.23^a
P<0.05 LSD	
T*S	ns

CONCLUSION

The nursery is a crucial area for producing healthy and uniform size seedlings. It is required to supply nutrient from the beginning of sowing the seeds and maintaining the nutrient concentration until transplanting to the production area. No scorching effect was found on all sample's treatment. The best approach to produce healthy and robust seedlings are at the range of 0.5 and 1.0 miliSiemens/cm nutrient solution concentration with regular sponge arrangement. The result will be one of the standard operating procedures to achieve profitable production in indoor multilayer plant factory.

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REFERENCES

Evans, C. 1972. The quantitative analysis of plant growth. Berkeley and Los Angeles; University of California Press.

Kozai, T., Kubota, C., Chun, C., Afreen, F., and Ohyama, K.2000. Necessity and concept of close transplant production system, p.3-19, In: C. Kubota and C. Chun (eds.). Transplant Production in the 21st Century. Kluwer Academic Publishers, The Netherlands.

Kozai, T., 2005. Closed systems for high-quality transplants using minimum resources (In: Plant Tissue Culture Engineering, SBN: 1-4020-3594-2, eds. Gupta, S. and Y. Ibaraki, 480pp.), Springer, Berlin, 275-312.

Kozai, T., 2011. Improving light energy utilization efficiency for a sustainable plant factory with artificial light. Green Lighting Shanghai Forum.

Kozai, T., 2013. Plant factory in Japan: current Situation and perspectives. Chronica Horticulturae. Volume 53:2.

Larson, K,L and Nelson, C,J. 1984. Seedlings Growth. Physiological Basis of Crop Growth and Development. Chapter 4.page 93-129.