UNIVERSITI PUTRA MALAYSIA

EFFECTS OF ROOT RESTRICTION AND MYCORRHIZA INOCULATION ON GROWTH, PHYSIOLOGICAL AND YIELD RESPONSES OF CHILLI GROWN IN SOILLESS CULTURE

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By

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July 2018

Chairman : Professor Mohd Razi Ismail, PhD
Institute : Institute of Tropical Agriculture and Food Security

Chilli (*Capsicum annuum* L.) that is used in food preparation for its flavour, aroma, and colour is a fruit vegetable with a high economic value that is cultivated in soilless culture in Malaysia. In soilless culture, the use of high substrate volume of about 6 to 8 liters per plant is closely related to increases in water usage, fertilizer and manpower. Meanwhile, the rising price of substrate and fertilizers coupled with pollution threat due to the disposal of used substrate in the large scale commercial production area results in higher production cost. Therefore, an approach to optimize the volume of substrate and concentration of fertilizer in small container could potentially reduce the production cost in soilless culture meanwhile by understanding physiological mechanism of the growth reduction, root system manipulation by using beneficial microbes such as mycorrhiza could potentially be adopted to improve yield under restricted root condition. Thus, the growth and yield responses of chilli plants grown in small containers were tested using six sizes of polybags. The result from this study showed reductions of stem diameter and total plant biomass of chilli grown in horizontal polybag of 17 cm in length with 32% yield reduction which is associated with root restriction compared to 31.7% yield increment in horizontal polybag of 27 cm in length. The next experiment was conducted to determine the optimum fertilizer concentration in different container sizes chosen from the previous study. Here the effects of 1.5 and 2.5 dS/m fertilizer concentration subjected to different container sizes of 2805 cm^3, 6831 cm^3 and 10557 cm^3 on growth, physiological response and yield of chilli were determined. The result showed reduction of vegetative growth with 24% and 17% reduction of fruit fresh weight in 1.5 dS/m fertilizer concentration and

in 2805 cm^3 container, respectively. Similarly, photosynthetic rate and stomatal conductance were reduced in 1.5 dS/m fertilizer concentration in 2805 cm^3 container. Based on the aforementioned trials, 6831 cm^3 container and 2.5 dS/m fertilizer concentration were found to be the optimum container size and fertilizer concentration for chilli production in soilless culture. The mechanism of growth reduction of plants subjected to root restriction remained unclear, therefore the following study was conducted to determine the physiological mechanism of growth reduction of root restricted chilli plants grown in small PVC column. The result from this study showed sucrose accumulation in the stem with changes in gas exchange parameters while chlorophyll fluorescence was not affected by root restriction. About 50% volume of soilless substrate can be saved with 23% of yield reduction for each plant subjected to root restriction. Based on the potential benefit of soilless substrate saving in small container, the following trial was conducted to minimize the yield gap of root restricted plants through manipulation of the root system to improve the root development by inoculation of beneficial microbes such as mycorrhiza. The result showed that inoculation of mycorrhiza in root restricted plants increased sucrose accumulation in the leaves, stem and fruit at flowering stage yet there were no net increase in yield compared to root restricted plants without mycorrhiza. Manipulation of the root system by mycorrhiza inoculation did not improve the growth and yield of chilli plants grown under restricted root condition.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

KESAN PEMBATASAN AKAR DAN INOKULASI MIKORIZA TERHADAP TINDAK BALAS TUMBESARAN, FISIOLOGI DAN HASIL CILI YANG DITANAM DALAM KULTUR TANPA TANAH

Oleh

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Cili (*Capsicum annuum* L.) yang digunakan untuk rasa, aroma dan warna di dalam penyediaan makanan merupakan sayur-sayuran buah bernilai tinggi yang ditanam menggunakan kultur tanpa tanah di Malaysia. Dalam kultur tanpa tanah, keperluan jumlah substrat yang tinggi kira-kira 6 hingga 8 liter bagi setiap tanaman adalah berkait rapat dengan penggunaan air, baja dan tenaga manusia yang tinggi. Di samping itu, kenaikan harga substrat dan baja ditambah dengan ancaman pencemaran kerana pelupusan substrat terpakai di kawasan pengeluaran komersial berskala besar membawa kepada kos pengeluaran yang lebih tinggi. Oleh itu, pendekatan untuk mengoptimumkan jumlah substrat dan kepekatan baja di dalam bekas kecil berpotensi untuk mengurangkan kos pengeluaran dalam kultur tanpa tanah, manakala dengan memahami mekanisme pengurangan tumbesaran, pengubahsuaian sistem akar dengan menggunakan mikrob bermanfaat seperti mikoriza berpotensi untuk digunakan bagi meningkatkan hasil di dalam keadaan sekatan akar. Oleh itu, tindak balas pertumbuhan dan hasil tanaman cili yang ditanam di dalam bekas kecil telah diuji dengan menggunakan enam saiz polibeg. Keputusan kajian ini menunjukkan pengurangan diameter batang dan jumlah biomas tumbuhan cili yang ditanam di dalam polibeg horizontal dengan panjang 17 cm dengan pengurangan hasil sebanyak 32% yang dikaitkan dengan sekatan akar berbanding kenaikan hasil sebanyak 31.7% di dalam polibeg horizontal dengan panjang 27 cm. Kajian berikutnya telah dijalankan untuk menentukan kepekatan optimum baja di dalam saiz bekas berbeza yang dipilih daripada kajian terdahulu. Di sini, kesan kepekatan baja 1.5 dan 2.5 dS/m di dalam saiz bekas berbeza iaitu 2805 cm^3, 6831 cm^3 dan 10557 cm^3 telah ditentukan terhadap tumbesaran, tindak balas fisiologi dan hasil cili. Keputusannya menunjukkan penurunan pertumbuhan vegetatif dengan 24% dan 17% pengurangan berat buah segar dalam kepekatan baja 1.5 dS/m dan

di dalam bekas 2805 cm^3, masing-masing. Begitu juga, kadar fotosintesis dan konduksi stomata berkurangan dalam kepekatan baja 1.5 dS/m di dalam bekas 2805 cm^3. Berdasarkan ujikaji tersebut, bekas 6831 cm^3 dan kepekatan baja 2.5 dS/m didapati sebagai ukuran bekas dan kepekatan baja yang optimum bagi pengeluaran cili dalam kultur tanpa tanah. Mekanisme pengurangan tumbesaran tanaman yang ditanam dengan sekatan akar adalah tidak jelas, oleh itu, kajian seterusnya telah dijalankan untuk menentukan mekanisme fisiologi pengurangan tumbesaran tanaman cili dengan sekatan akar yang ditanam di dalam kolum PVC kecil. Keputusan kajian ini menunjukkan pengumpulan sukrosa di dalam batang dengan perubahan parameter pertukaran gas sementara fluoresen klorofil tidak terjejas oleh sekatan akar. Sebanyak 50% jumlah substrat tanpa tanah boleh dijimatkan dengan 23% pengurangan hasil bagi setiap tanaman yang ditanam dengan sekatan akar. Berdasarkan manfaat potensi penjimatan substrat tanpa tanah di dalam bekas kecil, kajian berikutnya dilakukan untuk meminimumkan jurang hasil tanaman dengan sekatan akar melalui manipulasi sistem akar untuk memperbaiki perkembangan akar dengan inokulasi mikrob yang bermanfaat seperti mikoriza. Keputusannya menunjukkan bahawa inokulasi mikoriza ke dalam tanaman dengan sekatan akar meningkatkan pengumpulan sukrosa di dalam daun, batang dan buah pada peringkat berbunga tetapi tidak ada kenaikan bersih hasil berbanding tanaman dengan sekatan akar tanpa inokulasi mikoriza. Pengubahsuaian sistem akar melalui inokulasi mikoriza tidak dapat meningkatkan tumbesaran dan hasil tanaman cili yang ditanam di dalam keadaan sekatan akar.

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I certify that a Thesis Examination Committee has met on 6 July 2018 to conduct the final examination of Nurul Idayu binti Zakaria on her thesis entitled "Effects of Root Restriction and Mycorrhiza Inoculation on Growth, Physiological and Yield Responses of Chilli Grown in Soilless Culture" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABBREVIATIONS

%	Percentage
<	Less than
=	Equal to
>	Greater than
≤	Less than and equal to
*	Significantly different at P≤0.05
**	Significantly different at P≤0.01
°C	Degree celcius
µg	Microgram
µmol	micromol
A	Net CO_2 assimilation rate
AAS	Atomic Absorption spectroscopy
ABA	Abscisic acid
AI	Acid invertase
ANOVA	Analysis of variance
ATP	Adenine TriPhosphate
C/N ratio	Carbon/nitrogen ratio
Ca^{2+}	Calcium
CCD	Coconut coir dust
cm	Centimetre
cm^2	Centimeter square
CO_2	Carbon dioxide
DAT	Day after transplant
dS/m	desiSiemen per meter
EC	Electrical conductivity
EFB	Empty fruit bunch
F_m	Maximal fluorescence
F_N	Nitrogen uptake rate per root unit volume
F_o	Minimal fluorescence
F_v/F_m	Quantum yield of PSII
F_W	Transpiration rate per root unit volume
g	gram
H_2O_2	Hydrogen peroxide
K	Potassium
kg	kilogram
L	Litre
LSD	Least Significant Different
m^2	Meter square
m^3	Metre cubic
Mg	Magnesium
mg	miligram
ml	mililitre
mmol	milimole
mS/cm	MiliSiemen per centimetre
N	Nitrogen

n	Number of observation
NFT	Nutrient Film Tecnique
ns/NS	Not significant
P	Phosphorus
pH	Measurement of acidity/alkalinity
PVC	Polyvinyl-chloride
RCBD	Randomized Complete Block Design
SE	Standard error
SuSy	Sucrose synthase
UV	Ultraviolet
V:v	Volume per volume

CHAPTER 1

INTRODUCTION

In Malaysia, vegetables are crucial food crops as they contribute towards development of agricultural sector with a planted area of about 63,569 hectares and total annual production of about 1,195,647 tonnes in 2016 (Department of Agriculture, 2016). Chilli (*Capsicum annuum* L.) is among the most important high value vegetables in Malaysia. It is of economical importance due to its pungency and high level of provitamin A, vitamin C and E, and carotenoids (Perucka and Materska, 2007; Shaha *et al*., 2013). It is either marketed as fresh chilli or processed products such as chilli sauces and chilli pastes. From 2012 to 2016, the chilli production area increased from 2.5 to 3.1 thousand hectares while annual production increased from 40 to 43.7 thousand tonnes (Department of Agriculture, 2016). It is necessary to improve domestic production of chilli to attain the level of self sufficiency. Currently, the self sufficiency level of chilli is only at 51.4% which contributes significantly to the high food import bill (Department of Statistics, 2015).

The adoption of modern cultivation technique can improve plant growth, yield production and income. Substrate culture or fertigation system under protected structure has been used sporadically for commercial vegetables production in Malaysia. The major paradigm shift from the use of soil into container with soilless substrate is to control problems in agriculture, such as soil borne pest and diseases, lack of fertile soil, pollution of freshwater reservoir by discharge of nutritional ion and pesticide residue into the soil, water scarcity and high soil salinity (Raviv and Lieth, 2008). The widespread adoption of soilless culture under protected structures or rain shelters is primarily to replace uncertainty in crop production due to climatic changes such as excessive rainfall, to provide a favorable microclimatic condition for the growth of shoot and root whilst enabling precision crop management to provide a year round of vegetable (Ismail, 2000). Compared to conventional soil based cultivation, this growing system is more cost effective due to efficient and accurate control of water and nutrients and enhanced yield production and quality (Grafiadellis *et al*., 2000; Nicola *et al*., 2005; Nejad and Ismaili, 2014).

Presently, polyethylene bags or polybag filled with coconut coir dust as growing substrate is a crucial input and commonly use in soilless culture production to provide plant support and retain water and nutrient. Container filled with substrate is important variable cost items in soilless culture (Barreto and Jagtap, 2006; Rodriguez *et al*., 2006; Cantliffe *et al*., 2007; Barrett *et al*., 2016). Beside substrate, fertilizers are also crucial inputs and are highest variable cost item in a soilless culture production (Engindeniz and Gul, 2009). Plants in substrate culture are related with root restriction since it has small root system compared to soil cultivation (Ismail and Davies, 1998; Raviv *et al*., 2009).

Growers tend to use large container with higher requirement of substrate to promote root growth and avoid root restriction. However, this creates problem of poor substrate management due to oversupply of substrate which is closely related with higher requirement of water, fertilizer as well as manpower and pollution threat due to the disposal of used substrate in the large scale commercial production area that result in increased cost of production. Besides the problem of poor management of substrate, there are other problems for the growers, which is limited supply of substrate coupled with rising prices of substrate and fertilizer that affect the production cost. Hence, optimization of substrate volume and fertilizer concentration via production of plant in small container size is becoming important issue for the farmers to potentially reduce the high cost of production.

To address these problems, it is obviously important to find a proper management approach that promotes efficient use of substrate to reduce the production cost. Root restriction is important in soilless culture for production of container grown plant with limited substrate such as potted culture. It is a feasible solution to save substrate usage while minimizing the cost of production and controlling environment of root system (Shi *et al*., 2008b). Varying container sizes resulted in variable degree of root restriction (Alsadon, 2000). Previous studies have shown that root restriction did markedly reduced plant growth and total plant yield (Ismail and Dalia, 1995; Xu *et al*., 2001; Saito *et al*., 2008; Massetani *et al*., 2017). Although there was a slight decrease in total yield, but fruit quality and yield efficiency tends to be higher than unrestricted plant (Mandre *et al*., 1995; Atkinson *et al*., 1997; Mataa and Tominaga, 1998; Xie *et al*., 2009).

In a soilless culture, plants are supplied with water including fertilizer by drip irrigation system in container filled with soilless substrate. Small root system of the root restricted plant may be beneficial in increasing efficiency of fertilizer use by targeting applications to plant roots (Mandre *et al*., 1995). Container grown plant requires careful management of fertilizers as the limited root substrate and concentration of essential plant nutrients in small substrate are frequently insufficient to sustain plant growth (Fallovo *et al*., 2009). Fertilizer application in fertigation system is not specific to the optimal electrical conductivity (EC) but a certain range is used. Root restriction usually caused reduction of morphology and physiological process in plant (Ronchi *et al*., 2006; Shi *et al*., 2008a) but does not affect photosynthesis rate (Krizek *et al*., 1985; Kharkina *et al*., 1999). Low EC level of nutrient solution significantly reduced yield of lettuce (Fallovo *et al*., 2009) and rockmelon (Zulkarami *et al*., 2012) however it did not affect the yield of cucumber plant (Al-Harbi, 1994). The extreme stress due to the combination of low nutrient concentration and small container significantly reduced total fruit yield of sweet pepper but increased the translocation of assimilates into the fruits (Xu *et al*., 2001).

These studies demonstrate the potential benefits of root restriction for chilli production in soilless culture. The physiological mechanism of growth reduction remains unclear where there are contradictory finding of nutrient and water resources involvement in various plants under root restriction. Hameed *et al*. (1987) and Tschaplinski and Blake (1985) suggested that water stress led to the reduction of leaf growth and stomatal conductance of tomato and alder seedlings plants, respectively, grown in confined root space of solution culture. However, Krizek *et al*. (1985), Rieger and Marra (1994) and Ismail and Davies (1998) ruled out the involvement of water stress in growth reduction of root restricted soybean, young peach tree and pepper, respectively. Furthermore, Rieger and Marra (1994) and Shi *et al*. (2008a) suggested that factors other than photosynthesis and carbohydrate level were involved in controlling reduction of growth in the root restricted plant.

Several measures are available to manage chilli plant under root restriction particularly by manipulation of root development to enhance the yield gap. Previously, Shi *et al*. (2007) found that reduction of growth in root restricted plant was alleviated by aeration in the root zone of tomato plant grown under hydroponic system. Besides that, the exogenous application of hormones such as gibberellins and cytokinin can partially overcome reduction of growth in root restricted plant (Richards and Rowe, 1977; Carmi and Heuer, 1981). Other techniques of root manipulation such as by boosting more fertilizer, frequent irrigation and inoculation of beneficial microbes can be applied to root restriction. The role of beneficial microbes has been widely investigated in stress plant since the use of biological methods to alleviate the stress effect is more useful, economically and environmentally to the plant (Miransari *et al*., 2008; Al-Khaliel, 2010; Asrar and Elhindi, 2011).

Chilli plants require appropriate amount of substrate hence suitable level of root restriction for chilli plants need to be determine. The information on the appropriate EC of nutrient solution on chilli plants subjected to root restriction will enable growers not only to manage substrate and fertilizer wisely but also enable them to save substrate and fertilizer cost, however the information was limited. Complete understandings of the mechanism of growth reduction in root restriction are still not fully understood therefore to manage plant survival and enhancing yield under root restriction, physiological mechanism and source and sink relationship effect on growth and yield of root restricted chilli need to be determined. Information on improvement of plants yield under root restricted condition are still limited therefore manipulation of the root system by using mycorrhiza which is easy to apply and applicable to the farmer could potentially be adopted to improve yield under root restriction.

The main objective of this study is to determine the effect of root restriction and fertilizer concentration in root restricted chilli plants as well as to understand physiological mechanism of the growth reduction and yield improvement of root restricted chilli plants through inoculation of mycorrhiza.

The main hypotheses to be tested in the present study were:

1) Growing plant in small container size cause root restriction that will reduce plant growth and yield while growing chilli in optimum container size will improve plant growth and yield.
2) Appropriate application of nutrient solution EC level will improve plant growth, physiological response and yield of chilli subjected to root restriction.
3) Selected beneficial microbes will increase plant growth, physiological response and yield of chilli subjected to root restriction.

Looking the economic important of chilli in soilless culture and potential of adopting root restriction, this research was carried out to explore the following objectives:

1) To determine the effects of root restriction on growth and yield of chilli.
2) To determine the effect of low EC of nutrient solution on growth, physiological response and yield of chilli subjected to root restriction.
3) To understand the physiological mechanism and source and sink relationship on the reduction of growth and yield of chilli subjected to root restriction.
4) To determine the effect of manipulation of the root system by beneficial microbes on growth and yield of chilli subjected to root restriction.

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LIST OF PUBLICATIONS

Nurul Idayu, Z., Mohd Razi, I., Yahya, A., Puteri Edaroyati, M.W., Zulkarami, B. (2019). Physiological responses, root morphology and yield of chilli plant (*Capsicum annuum* L.) in response to container size and nutrient solution concentration. *Asian Journal of Crop Science*. Accepted on 26th April 2019.

Nurul Idayu, Z., Mohd Razi, I., Yahya, A., Puteri Edaroyati, M.W., Zulkarami, B. (2020). Effect of root restriction on the growth, photosynthesis rate, and source and sink relationship of chilli (*Capsicum annuum* L.) grown in soilless culture. *BioMed Research International*. Vol. 2020. Article ID 2706937, 14 pages. https://doi.org/10.1155/2020/2706937

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