UNIVERSITI PUTRA MALAYSIA

IMPACT OF WATER STRESS AND NITROGEN FERTILIZATION ON GROWTH, BIOCHEMICAL CHANGES AND CARBON ASSIMILATION OF COMMERCIAL STRAWBERRY (Fragaria x ananassa Duch.) PLANTED IN EAST JAVA, INDONESIA

YENNI

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By

YENNI

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

December 2021

DEDICATION

Dedicated to my beloved parents and parents-in-law for their prayers, motivation, support, and advice.

Also specially dedicated to my beloved husband, Joko Purwanto for his endless love, patience, understanding, and motivation.

To my beloved family, who always supports and encourages me.

Also, to my precious teachers, friends, and anyone who supports my study.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

IMPACT OF WATER STRESS AND NITROGEN FERTILIZATION ON GROWTH, BIOCHEMICAL CHANGES AND CARBON ASSIMILATION OF COMMERCIAL STRAWBERRY (*Fragaria x ananassa* Duch.) PLANTED IN EAST JAVA, INDONESIA

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Chairman : Mohd Hafiz Ibrahim, PhD
Faculty : Science

Strawberry has a nutritional content that is beneficial to the human body. In Indonesia, the cultivation of this plant has met with challenges due to climate change. Water stress is one of the climate changes that affect the growth and quality of strawberries. This research was carried out to determine the impact of water stress and nitrogen fertilization on growth, biochemical changes, and carbon assimilation of strawberries. This study was conducted in a glasshouse at the Indonesian Citrus and Subtropical Fruit Research Institute, East Java Indonesia, from June 2018-January 2020. All experiments were arranged in randomized complete block design (RCBD) with three replications. In accomplishing the research, four experiments were carried out. In the first experiment, three cultivars of strawberry (C1=Earlibrite, C2=California, C3=Sweet Charlie and four water stress levels (W1= 100% field capacity (FC), W2=75% FC, W3=50% FC, W4=25% FC) were used to determine the best cultivar under different levels of water stress. The results showed that total chlorophyll and anthocyanin contents were influenced by the interaction effects of cultivars and water stress (WS). WS decreased plant growth, chlorophyll content, leaf gas exchange, leaf relative water content, length, diameter, and weight of fruit but enhanced total soluble solid, anthocyanin, and proline contents. Among the cultivars, California strawberry had highest flowers and fruits number, and anthocyanin content. Hence, this cultivar is recommended to be planted under WS. For the second experiment, two levels of WS (W1=100% FC, W2=75% FC) and four irrigation intervals (II) (F1=daily irrigation; F2 (2 days II); F3 (4 days II); F4 (6 days II) were used to investigate the effect of WS and II on strawberries. The results indicate that W2F4 caused lower growth, yield, chlorophyll content, and leaf gas exchange, but higher water use efficiency (WUE), proline content and fruits total sugar. Increasing WUE and proline indicate that the strawberry plant can adapt (tolerant) to water stress. Therefore,

II can be used as a strategy to vegetate strawberries in the dry season. In the third experiment, four fertilizer sources (P1=without fertilizer, P2=NPK fertilizer, P3=dry goat dung fertilizer, P4=½ NPK+½ dry goat dung fertilizer and WS (W1=daily irrigation 100% FC; W2=2 days II 75% FC; W3=6 II 75% FC) were used to determine the best fertilizer source for the growth, biochemical changes and carbon assimilation of strawberries. It was observed that the interaction of different water stress and fertilizer types had significant effect on all parameters except for the total sugar in strawberry fruits. The application ½ NPK + ½ goat dung fertilizer resulted in higher of plant height, leaves number, biomass, chlorophyll content, sugar content, transpiration rate, photosynthesis rate, and NPK content. Plants that were not fertilized had the lowest growth compared to those fertilized. In the last experiment, five nitrogen fertilizer rates (N1=without N fertilizer, N2=46 kg N/ha, N3 = 92 kg N/ha, N4 = 138 kg N/ha, N5 = 184 kg N/ha) and WS levels (W1=daily irrigation 100% FC; W2=2 days II 75% FC; W3=6 days II 75% FC) were used to determine the best nitrogen fertilizer rate on strawberries. The results showed that strawberry plants subjected to WS and low or no nitrogen fertilizer application had a decline in growth and yield. WS increased WUE and nitrogen use efficiency (NUE) of strawberry plant. Nitrogen increased the growth, chlorophyll, leaf gas exchange, ANR, proline, and yield components of the strawberry under WS. From this research, it can be concluded that the application of nitrogen fertilizer under WS was able to mitigate the negative effect of water stress by maximizing WUE and NUE on the strawberry plants.

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

KESAN KETEGASAN AIR DAN PEMBAJAAN NITROGEN TERHADAP PERTUMBUHAN, PERUBAHAN BIOKIMIA DAN ASIMILASI KARBON TANAMAN STRAWBERI KOMERSIAL (*Fragaria x ananassa* Duch.) YANG DITANAM DI JAWA TIMUR, INDONESIA

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Strawberi mempunyai kandungan nutrisi yang mempengaruhi tubuh manusia. Di Indonesia, penanaman tanaman ini menghadapi cabaran kerana perubahan iklim. Tegasan air adalah salah satu perubahan iklim yang boleh mempengaruhi pertumbuhan dan kualiti strawberi. Kajian ini dijalankan untuk mengetahui kesan tegasan air dan pembajaan nitrogen terhadap pertumbuhan, perubahan biokimia, dan asimilasi karbon tanaman strawberi. Kajian ini dilakukan di sebuah rumah kaca di Institut Penelitian Buah Jeruk dan Subtropika Indonesia, Jawa Timur Indonesia, dari Juni 2018-Januari 2020. Semua eksperimen disusun dalam reka bentuk RCBD dengan tiga replikasi. Bagi menjayakan penyelidikan ini, empat eksperimen telah dilakukan. Dalam eksperimen pertama, tiga kultivar strawberi (C1 = Earlibrite, C2 = California, C3 = Sweet Charlie dan empat tahap tegasan air (W1 = 100% kapasiti had ladang (FC) air, W2 = 75% air FC, W3 = 50 % FC air, W4 = 25% air FC) dilakukan untuk menentukan kultivar terbaik di bawah tahap ketegasan air yang berbeza. Hasilnya menunjukkan bahawa jumlah kandungan klorofil dan antosianin dipengaruhi oleh kesan interaksi kultivar dan tegasan air. Tegasan air menurunkan pertumbuhan tanaman, kandungan klorofil, pertukaran gas daun, kandungan air relatif daun, panjang, diameter, dan berat buah tetapi ia meningkatkan kandungan jumlah pepejal terlarut, antosianin, dan prolin. Strawberi California mempunyai bilangan bunga dan buah tertinggi, dan juga kandungan antosianin. Oleh itu, kultivar ini disyorkan untuk ditanam dalam keadaan tegasan air. Eksperimen kedua, di mana dua tahap tegasan air (W1 = 100% FC, W2 = 75% FC) dan empat jenis selang pengairan (F1 = pengairan harian; F2 (selang 2 hari pengairan); F3 (selang 4 hari pengairan); F4 (selang 6 hari pengairan) digunakan untuk menyiasat kesan tahap tegasan air dan selang pengairan terhadap strawberi. Hasilnya menunjukkan W2F4 mempunyai pertumbuhan, hasil, kandungan klorofil, pertukaran gas daun terendah, tetapi ia meningkatkan WUE, kandungan

prolin dan jumlah gula buah. Peningkatan kandungan WUE dan prolin adalah dua petunjuk yang menunjukkan bahawa tanaman strawberi dapat menyesuaikan diri (toleran) dalam keadaan tegasan air. Penggunaan selang pengairan merupakan strategi untuk menanam strawberi pada musim kemarau. Dalam eksperimen ketiga, empat sumber baja (P1 = tanpa baja, P2 = baja NPK, P3 = baja tahi kambing kering, P4 = ½ NPK + ½ baja tahi kambing kering) dan tahap tegasan air yang berbeza (W1 = pengairan harian 100% FC; W2 = selang 2 hari pengairan 75% FC; W3 = Selang 6 hari pengairan 75% FC) digunakan untuk menentukan sumber baja terbaik untuk pertumbuhan, perubahan biokimia dan asimilasi karbon strawberi. Interaksi tegasan air dan jenis baja yang berbeza telah diperhatikan mempunyai pengaruh yang signifikan kepada semua parameter kecuali pada jumlah gula buah strawberi. Aplikasi ½ NPK + ½ baja tahi kambing meningkatkan tinggi tanaman, jumlah daun, pengeluaran biomas, kandungan klorofil, kandungan gula, kadar transpirasi, kadar fotosintesis, kandungan NPK. Tanaman yang tidak dibaja mempunyai pertumbuhan terendah berbanding dengan tanaman yang dibaja. Dan dalam eksperimen terakhir, lima sumber baja (N1 = tanpa baja N, N2=46 kg N/ha, N3 = 92 kg N/ha, N4 = 138 kg N/ha, N5 = 184 kg N/ha) dan tahap tegasan air berbeza (W1 = pengairan harian 100% FC; W2 = selang 2 hari pengairan 75% FC; W3 = Selang 6 hari pengairan 75% FC)) digunakan untuk menentukan kadar baja nitrogen terbaik untuk strawberi. Tegasan air dan penggunaan nitrogen rendah atau tanpa baja nitrogen merosotkan komponen pertumbuhan dan hasil buah strawberi. Tegasan air meningkatkan WUE and NUE tanaman strawberi. Penggunaan baja nitrogen meningkatkan pertumbuhan tanaman, kandungan klorofil, pertukaran gas daun, ANR, kandungan prolin, dan komponen hasil tanaman strawberi dalam keadaan tegasan air. Dari kajian ini, dapat disimpulkan bahawa penggunaan baja nitrogen dalam keadaan tegasan air dapat mengurangkan kesan negatif defisit air dengan memaksimumkan WUE dan NUE pada tanaman strawberi.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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%	percent
*	significant at 0.05 probability level
**	significant at 0.01 probability level
^{o}C	degree celcius
A	net photosynthetic rates
ABA	abscisic acid
ADP	adenosine diphosphate
ATP	adenosine triphosphate
ANOVA	analysis of variance
ANR	activity of nitrate reductase
AsA	ascorbic acid
B	borron
BNF	biological N fixation
Ca	calcium
CAT	catalase
cm	centimeter
CO_2	carbon dioxide
CH_2O	formaldehyde
Cyd-3-glu	cyanidin-3-glucoside
DMRT	duncan multiple range test
DW	dry weight
FAO	food and agricultural organization
FC	field capacity
Fe	ferrum

FW	fresh weight
g	gram
gs	stomatal conductance
GSH	glutathione
h	hour
ha	hectare
H_2O	water
H_2O_2	hydrogen peroxide
H_2SO_4	sulfuric acid
i.e	that is
ICSFRI	Indonesian citrus and subtropical fruit research institute
II	Irrigation interval
INDPA	indonesian national development planning agency
KCl	potassium chloride
Kg	kilogram
Kg N ha^{-1}	kilogram nitrogen per hectares
L	litre
LR	late ripening
LRCW	leaf relative water content
m	metre
Mg	magnesium
mg	miligram
MG	milk grain
N	Nitrogen
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate hydrogen

$NH4^+$	ammonium
nm	nanometer
NO_3^-	nitrate
NUE	nitrogen use efficiency
OD	optical density
O_2	oxygen
PEG	polyethylene glycol
pH	potential of hydrogen
RCBD	randomized complete block design
ROS	reactive oxygen species
Se	selenium
Si	silicon
SOD	superoxide dismutase
SP36	superphosphate 36
TA	total acidity
TCC	total chlorophyll content
TSS	total soluble solid
TW	turgid weight
WAP	week after planting
WD	water deficit
WS	water stress
WUE	water use efficiency
µg	microgram
$\mu molm^{-2}s^{-1}$	micro mole per meter square per second
$\mu molmol^{-1}$	micro mole carbon dioxide per mole air

CHAPTER 1

INTRODUCTION

1.1 Background

Strawberries (*Fragaria x ananassa* Duch.) have a significant amount of nutrients and valuable antioxidants beneficial to human health (Giampieri et al., 2012). Strawberry is principally a temperate crop, but because it has a short production period (100–120 days), it is considered as a rapid-growing plant in the tropical and subtropical area of the Asian countries. In Indonesia, strawberry plants are distributed in the high areas of Java, Sumatra, Bali, and Sulawesi. Additionally, there are more than 30 varieties of strawberries being developed in Indonesia (Hanif and Ashari, 2013; Kannaujia et al., 2014).

In crop production, plants require water to maintain their growth, as water is a critical requirement in the plant's life cycle (Azam et al., 2016; Joshi and Shrestha, 2017). Water is one of the significant contributors that reduces the productivity and growth of crops when it is unavailable or in deficit levels (Nxele et al., 2017). Plants face water stress if the supply of water to their roots is restricted or when the rate of transpiration increased. Water stress, as well as drought and high soil salinity, are the leading causes of water stress, which can negative impact the agricultural industry and food supply wordwide. For survival purposes, plants attempt to adapt to the water-deficit stress conditions with various physiological and biochemical mechanisms (Lisar et al., 2012; Rahman et al., 2016).

Nitrogen fertilizer inputs are needed to increase crop production. The aim of applying nitrogen fertilizer to plants is to maximize their use by plants. Nitrogen fertilizer is usually applied to plants in various forms, such as ammonium or nitrate. The nitrate form of nitrogen can easily runoff from agricultural land, causing limited nutrient availability for the plant, and the nutrient to leach into water and dissolve into groundwater (Minnesota Department of Agriculture, 2015). Nitrogen is an essential structural material of chlorophyll, nucleic acids, rubisco, proteins, and various hormones. Nitrogen fertilization is a crucial agronomic management to increase plant productivity (Ata-ul-karim et al., 2017) and is the primary nutrient commonly found in inorganic and organic fertilizers (Septianingtyas et al., 2013). Nitrogen fertilizers play an important part in enhancing primary food plants globally (Chardon et al., 2012; Lassaletta et al., 2014).

Nezhadahmadi et al., (2015) and Ghaderi et al., (2015) studied the responses of strawberry plants on different growth parameters under water stress conditions. They found that water stress and stress duration reduced strawberry growth and alleviated the shoots dry weight, total plant dry weight, and fruits weight. On the other hand, Zargar et al., (2017) and Perin et al., (2019) investigated the effects of water stress on stem elongation and plant biomass on strawberry plants. They mentioned that there was a statistically ($p \leq 0.05$) significant reduction in the biomass and stem elongation of strawberry plants under water stress conditions. The research by Rahimi et al., (2013) showed the addition of nitrogen promoted the growth of *Plantago ovata* under different soil water stress conditions and enhanced the shoot-root growth, root to root ratio, and leaf area.

The impact of water stress on biochemical changes at various stages of growth was evaluated in *Vicia faba* and strawberry plants. The results from these studies found that water stress decreased the activity of antioxidant enzymes, but enhanced the protein and proline contents in *V. faba* and strawberry plants. The effects of water stress can also be identified in rice plants. The impact of water stress on rice can cause a reduction in dry weight and relative water content (Parvin et al., 2015; Adak et al., 2018; Kumar et al., 2019; Wijayanto et al., 2021). Additionally, water stress reduced total chlorophyll content, chlorophyll *a* and *b* of *Matricaria chamomilla* and three chickpea (*Cicer arietinum*) cultivars (Mafakheri et al., 2010; Pirzad et al., 2011). In strawberry leaves, high accumulation of peroxidase and antioxidant activities were observed compared to control leaves (70-85% FC) (Sun et al., 2015; Rezaei-Chiyaneh et al., 2018). It was also revealed that the amount of accumulated soluble sugar in *Frankenia laevis* leaves was the highest under 50% water stress and the lowest in the control treatment (without water stress) (Chegah et al., 2013). The influence of water stress was also observed in the total anthocyanin of strawberry cultivars (Adak et al., 2018). The authors found an increase in total anthocyanin content by 34.21% in water stress conditions (16.83mg Cyd-3-glu kg^{-1}) compared to the control (12.54 mg Cyd-3-glu kg^{-1})

At the overall plant level, the impact of drought can be observed in the reduction of photosynthetic carbon assimilation (Zlatev and Lidon, 2012). The investigation on carbon assimilation under water-deficit stress conditions found that the photosynthesis in three cultivars of *C.arietinum* was restricted by water stress due to stomata closure and impaired metabolic processes. Stomatal conductance and transpiration were also reduced in all cultivars of *C. arietinum*. When the plants are subjected to drought stress, one of the plants' first response is the closure of the stomata, which will limit the gas exchange between the atmosphere and the inner part of the leaves. Additionally, other crops such as maize had enhanced WUE under water-deficit stress (Mafakheri et al., 2010; Fghire et al., 2015; Cai et al., 2017). Several studies have shown that the practice of nitrogen fertilization on *Pinus contorta* and white spruce seedlings significantly escalated the rate of net photosynthesis, however, under the condition of nitrogen deficiency, it caused photosynthetic restriction on ambient CO_2 in corn (Yu-zheng & Zhou-ping, 2014; Duan & Chang, 2017).

1.2 Problem statement

In Indonesia, drought is one of the disasters that has a large impact and is the cause of failure in crop production (Surmaini, 2016). East Java Province is also inseparable from drought as a result of the impact of climate change which causes the agricultural sector to be vulnerable to decreased productivity. East Java has a monsoon climate pattern with at least six months of the dry season. The long dry season causes drought in various districts in East Java province. Drought can cause plants to experience water stress (Rahman et al., 2016; DAI, 2018). Water is a crucial limiting factor in many areas that limits strawberry's production and yield. Various studies have explored water stress influence on the physiological-biochemical responses of strawberry plants, such as plant growth, leaf gas exchange, plant height, leaf area, number of leaves, chlorophyll content, fruit yield, net photosynthesis, stomatal conductance, and transpiration (Adak et al., 2018; Dehghanipoodeh et al., 2018).

The deficiency of N will lead to lower yields in plants. N deficiency is also another limiting factor. In addition, N is necessary because if N is not availably or short supply, N will restrict the presence of other elements, for example, it can cause a deficiency of phosphorus (P) so that in this condition, the response of plants to P fertilization is very dependent on the availability of N in the soil (Havlin et al., 2005). According to Chang et al., (2016), the appropriate nitrogen level promotes plant growth and helps plants to withstand stress conditions. Wu et al., (2008) observed that the application of nitrogen at 184 $mg.kg^{-1}$ enhanced the leaf area, net photosynthesis, and chlorophyll content of *Sophora davidii* under water stress.

Nitrogen is the most important nutrient for the growth and development of plants after carbon, hydrogen, and oxygen. However, the use of excessive and inefficient N fertilizers results in an increase in crop production costs which causes economic losses. The nitrogen loss is too large, due to the N application overabundance and low plant population it can be minimized by 15–30% by adopting better agronomic practices, such as optimal nitrogen dosage application. Therefore, further research is needed to obtain appropriate and efficient fertilizer doses to reduce economic losses due to excessive use of fertilizers (Anas et al., 2020). Several researchers have reported the effects of water stress and nitrogen fertilization on plants. Still, there is not enough information about the nitrogen effect associated with water stress on strawberry plants in Indonesia. Additionally, nitrogen fertilizer management affecting water-stressed strawberry plants in Indonesia has not been widely studied. Therefore, further research is needed to determine the effect of nitrogen fertilizer and its relation to water management and how nitrogen metabolism contributes to minimizing the impacts of water stress on strawberry plants in Indonesia. This study was conducted by optimizing the use of water availability combined with the use of nitrogen fertilizer. A deeper and more detailed understanding is needed to determine the impact of water stress on strawberry plants, to help develop strategies to minimize the water stress impact with the use of fertilizers.

This information is expected to be useful for strawberry farmers to increase the yield of the strawberry plant and is of great significance to the sustainable development of agriculture.

1.3 Objectives

The main objective of the study was to determine the impact of water stress and Nitrogen fertilization on strawberry plant's growth, biochemical changes and carbon assimilation in Indonesia.

Therefore, the specific objectives of the study were:

1. To characterize the effect of cultivars and water stress on growth, biochemical changes and carbon assimilation of strawberry.
2. To characterize the effect of water stress and irrigation interval on growth, biochemical changes and carbon assimilation of strawberry.
3. To determine the effect of fertilizer sources on growth, biochemical changes and carbon assimilation on strawberry under water stress.
4. To determine the effect of nitrogen fertilizer rates on growth, biochemical changes and carbon assimilation of strawberry under water stress.

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