Doctoral (PhD) Dissertation

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Performance Studies of Soybean (G*lycine max* (L.) Merr.) in the Field and Using a Soilless Culture System in a Controlled Environment

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DEDICATION

I thank the Almighty for choosing me for this journey of my PhD, granting me faith and stamina to believe in myself and to complete it successfully. Without His blessings, this would not have been possible. I would like to dedicate this dissertation to my family and friends. I have a special feeling of gratitude for my loving husband, Mohammad Fauzi, who gave me tremendous encouragement and unconditional support. This work is also dedicated to my wonderful four children, Syabil Faiq, Aniq Isyad, Faiha Ufairah, and Fieyaz Ulayya Szofia, who inspire me and make me so proud. I also dedicate this work, special to my beloved mother, Maimun Hassan, for her endless love, prayers, and support. Finally, this dissertation is dedicated to my relatives and friends for helping me and for sharing their words of advice and encouragement to finish this work.

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LIST OF ABBREVIATIONS

AOSA	:	Association of Official Seed Analysts
%	:	Percent
°C	:	degree Celsius
CSIR	:	Council for Scientific and Industrial Research
cm	:	centimeter
dS/m	:	deciSiemens per meter
EDTA	:	Ethylenediaminetetraacetica acid
F	:	F statistic
FAO	:	Food and Agriculture Organization of the United Nations
IBM	:	International Business Machines
K_A	:	Active earth pressure coefficient
kg N/ha	:	kilogram nitrogen per hectare
L	:	liter
M	:	mean
MOFA	:	Ministry of Foreign Affairs
m	:	meter
µM	:	micro mol
ml	:	milliliter
mm	:	millimeter
NIR	:	Near Infra-Red
P	:	Probability level
SD	:	Standard deviation
SPSS	:	Statistical Program for Social Sciences
Std	:	Standard deviation
t/ha	:	Tonne per hectare
V	:	Volt
W	:	Watt

CHAPTER 1

INTRODUCTION AND OBJECTIVES

Soybean (*Glycine max* (L.) Merr) is one of the most valuable crops in the world, not only as an oilseed crop and feed for livestock and aquaculture but also as a good source of protein for the human diet. Soybean is the basis of human diets in many countries because of its great nutritional and functional benefits as well as its low cost. Soybeans are high in protein, carbohydrates, lipids, and bioactive compounds (HERCIA et al. 2011). The chemical content of soybeans is approximately 38% protein, 27% carbohydrate, 19% oil, 12% water, and 4% ashes (PROTERRA FOUNDATION 2020). Soybean also contains a greater amount of Ca, P, and vitamins (ADU-DAPAAH et al. 2004, MoFA and CSIR 2005). The oil produced from soybeans is free of cholesterol and highly digestible. As a result, soybeans are widely used in the production of food products, either directly as tofu, soy sources, soy milk, fermented bean paste, and tempeh, or indirectly as a huge source of livestock feed. In many countries, a byproduct of soybean oil known as soybean cake is used as a high-protein animal feed. Soybean oil cake can contain up to 47% protein, while soybean flour can include up to 40% protein (USTIMENKO-BAKUMOVSKY 1983). Other than edible products for humans and livestock, soybean is also used for non-edible products such as wax, construction materials, cosmetics, plastics, and biofuel (MPEPEREKI et al. 2000, CAMINITI et al. 2007).

Another advantage of soybean is that it improves soil fertility by fixing atmospheric nitrogen (N). The soybean will create a symbiotic relationship with beneficial bacteria in order to convert an inaccessible form of N into plant-available N. After harvest, parts of the fixed N in the roots, as well as those that remain in the haulms, are returned to the soil, thereby enhancing soil fertility. This reduces the amount of N fertilizer growers need to apply to their fields in order to increase yield. This has indirectly reduced dependence on other N sources, such as from fertilizer and soil. This is more beneficial in some locations, particularly among small growers, where soils lack nutrients and inputs are costly.

Soybean is a herbaceous, annual legume crop of the Leguminosae or Fabaceae family. The average plant height of soybeans can reach between 0.9 and 1.5 m and can have up to 20 nodes depending on the variety, conditions, and cultural practices (DRAKE STOWE and VANN 2022). The erect stems are covered in thick brown hair, and the leaves are compound with three leaflets. The flower is tiny, white to purple, and borne individually or in small groups in the axils. When fully mature and dried, the fruits are large, hairy, flattened pods about 10 cm long. The dried pods contain 1 to 4 yellow or black seeds.

Soybean is derived from the Japanese word meaning salt-soy sauce (HYMOWITZ 1970). The soybean is a subtropical plant native to Southeast Asia that was domesticated in China around 8000 years ago (SINGH 2017). Soybean was introduced to Europe around 1713 and to North America in 1765 (COBER et al. 2009). However, the origin countries produce just 45% of total soybean production (JOY et al. 1998). The rest of soybean production become major in Brazil, the United States, Argentina, and India. Soybean has good market demand, and these countries are presently exporting soybeans to many countries due to the high demand. Currently, soybean is cultivated over an area of 136.14 million ha, with a global production of 388.01 million metric tonnes in 2022/23 (USDA 2023b). Global soybean production is expected to reach a record 410.6 million metric tonnes in 2023/24, an increase of 11% from 2022/23 (USDA 2023c). After China, the European Union is the second largest soybean importer, with 13,900 metric tonnes in 2022/23. However, the European Union is the leading producer of soybean oil, with 2,785 metric tonnes in 2022/23 (USDA 2023c). European countries are also the largest importers of soybean-based products in the world. This scenario shows that soybean consumption in Europe is widespread but highly dependent on imported supplies. Therefore, the cultivation of soybeans in European countries can be expanded to reduce the dependence on imported supplies to meet domestic demand.

The whole cultivation process should be given full attention in order to sustain or enhance high-quality soybean production. Soybean cultivation must consider many aspects, including environmental conditions and agronomic practices. Environmental factors that can affect soybean growth, yield and quality include soil type, temperature, humidity, water availability, day length and other factors. Soybean growth, yield and quality are also strongly influenced by planting techniques or systems, variety selection, seedling production, nutrient management, weed control and pest and disease management (MATSUO et al. 2016; GULLUOGLU et al. 2017). Even cultural practices and environmental conditions can interact to influence soybean production.

The cultivation system that is widely used for soybean is conventional system in various environmental conditions. Soybean can be grown on well-drained, fertile loam soils in warm climates such as the tropics, subtropics, and temperate zones (FAO 2023). However, significant changes have occurred in several agricultural areas recently, causing conventional cultivation systems can not be done properly. It is due to various problems such as the reduction of agricultural land due to rapid development, the decrease in the level of soil fertility because of continuous cultivation, the presence of pests and diseases caused by organisms and nematodes, frequent drought conditions, and the unpredictability of climate and weather patterns. These issues have an impact on agricultural productivity and quality. Therefore, there is an alternative cultivation system that is currently used for most plants, which is in a closed environment using a soilless cultivation system.

A soilless cultivation system in a controlled environment ensures that planting can be done anywhere and anytime throughout the year. It can also produce high and consistent yields and quality. This is because the required quality of many crops can be achieved by engaging different substrates and modifying cultivation techniques. The soilless culture technique has been implemented in crop production systems all over the world and is now heavily used in greenhouse vegetable production in Europe, the United States, the Middle East, Japan, and Canada (HOCHMUTH et al. 2002). In Japan, there has recently been an increasing interest in using soilless culture systems for growing soybean in the greenhouse, especially for the production of vegetable soybean (edamame), which involves harvesting immature seeds (HATA and FUTAMURA 2020). However, cultivation utilizing this approach for soybean grain yield (mature seeds) has not been widely studied, and the related information is still limited and difficult to obtain.

Soybean planting in a controlled environment utilizing a soilless culture technique allows for the manipulation of several crucial factors, including temperature, that can not be manipulated in an open field or conventional cultivation system. Temperature is a major component influencing plant development. The rate of plant growth is affected by the temperature around the plant, and each species has a temperature range that is represented by a minimum, maximum, and optimal. Temperature influences plant growth, beginning with germination and seedling emergence. Germination is the initial step of the life cycle of plants and is an important and sensitive stage of plant development (DE VILLIERS et al. 1994). Some plant seeds germinate at a broad range of temperatures, while others germinate only at a narrow range of temperatures (BURIRO et al. 2011 and MULLER et al. 2013). Therefore, the importance of optimum temperatures starts once at the germination and seedling emergence stage, as it will affect the development of more vigorous and viable seedlings for good growth in the following stage and subsequently increase the yield and quality of the plant.

Variety selection, in addition to environmental considerations, is crucial for both conventional planting systems and closed-environment systems. The maturity duration is one of the selection criteria for variety selection. Some varieties have a significantly short maturity period and are best suited for low-rainfall areas or planting late in the season. Short maturity varieties are also appropriate for controlled environment planting in order to save on operating costs. Late mature varieties are less suitable for dry conditions, although they frequently generate higher grain, fix more N, and contribute more soil fertility than early maturing varieties (N2AFRICA 2014). Furthermore, most varieties require nutrients and respond differently to nutrient application. Nutrients, including N, are an essential element for production that has great interactions that are able to have either a positive or negative effect on crop production depending on the amount, dose, crop growth stage, combination, and balance (LI et al. 2009).

Weed management is an agronomic factor that also influences soybean cultivation. It is especially critical when utilizing a conventional planting technique. This is due to competition between soybeans and weeds for input sources such as nutrients (SHAFAGH-KOLVANAGH et al. 2008). When weeds are not well controlled, nutrient application, particularly N, can encourage weed growth rather than crop yield since weeds outcompete for N. Therefore, it is important to investigate the impact of nutrient and weed management in soybean cultivation, particularly in open-field planting. Weeds can be controlled while planted in a controlled environment using soilless culture since the presence of weeds is minimal and is possible to prevent. Thus, nutrient requirements for specific varieties are important to study either in the open field or in a controlled environment. Meanwhile, weed control on specific varieties is also necessary to be studied only in open field planting.

Thus, three experiments were conducted on several important factors that affect the growth, yield, and quality of soybeans for certain varieties in both cultivation systems: open field (one experiment) and controlled environment (two experiments). The general aim of these studies is to investigate the performance of soybean varieties in the field and using a soilless culture system in a controlled environment as influenced by factors such as nutrients, weed canopy, variety, and temperature. The specific objectives are specified as follows:

i. To evaluate the effect of nitrogen nutrition and weed canopy on yield formation and chemical composition of soybeans grown in the field

ii. To investigate the influence of temperature and variety in a controlled growth chamber on the germination rate, seedling length, and viability of soybeans

iii. To study the response of growth, yield components, yield, and chemical compositions to the nutrient concentration of two soybean varieties grown under soilless and controlled environment conditions

CHAPTER 2

LITERATURE REVIEWS

2.1 Economic importance of soybean

Soybean is the most economically important bean in the world, providing vegetable protein to millions of people as well as ingredients for hundreds of chemical products. This is because soybean is one of the richest and cheapest sources of protein and has become a staple in the diets of people and animals around the world. Soybeans are a good source of protein for diabetics because they contain no starch. Furthermore, soybeans are used not only as a vegetable protein source but also as a functional food to prevent degenerative diseases such as coronary heart disease and hypertension (MUCHLISH ADIE and KRISNAWATI 2014). Isoflavones, which are found in soybeans, appear to have antioxidants functions. Modern research has resulted in a remarkable number of uses for soybeans. Its oil can be used to make margarine, shortening, and vegetarian and vegan cheeses. Industrially, the oil is used as an ingredient in fertilizers, paints, adhesives, cloth sizing, linoleum backing, and fire extinguisher fluids (TIKKANEN 2023). As a result of its high demand and wide range of applications, soybean has become a strategic commodity in recent years.

Global oilseed production reveals that soybean oil is the main source of oilseed production when compared to oilseed production from other plant sources (rapeseed, sunflower seed, and others). Oil production from soybeans is predicted to grow by 11% in 2023/24 to almost 411.0 million metric tonnes (MT) (Figure 1) (USDA 2023a). Total consumption of oilseeds, including soybeans, is also increasing and is anticipated to rise by 4% in 2023/24 (Figure 1). The global supply and distribution of soybean meal in terms of production, import, export, and domestic consumption show a total global production of 251.5 thousand MT in March 2022/23. China is the world's largest producer of soybean meal and has the highest domestic consumption (Table 1) (USDA 2023c). However, the European Union is the world's largest importer of soybean meal, with a total of 64.3 thousand MT in 2022/23, and Argentina is the world's largest exporter, with a total of 24.9 thousand MT (Table 1). As a result, it is clear that the demand for soybean-based food is high, and it is expected to rise further in the coming year.

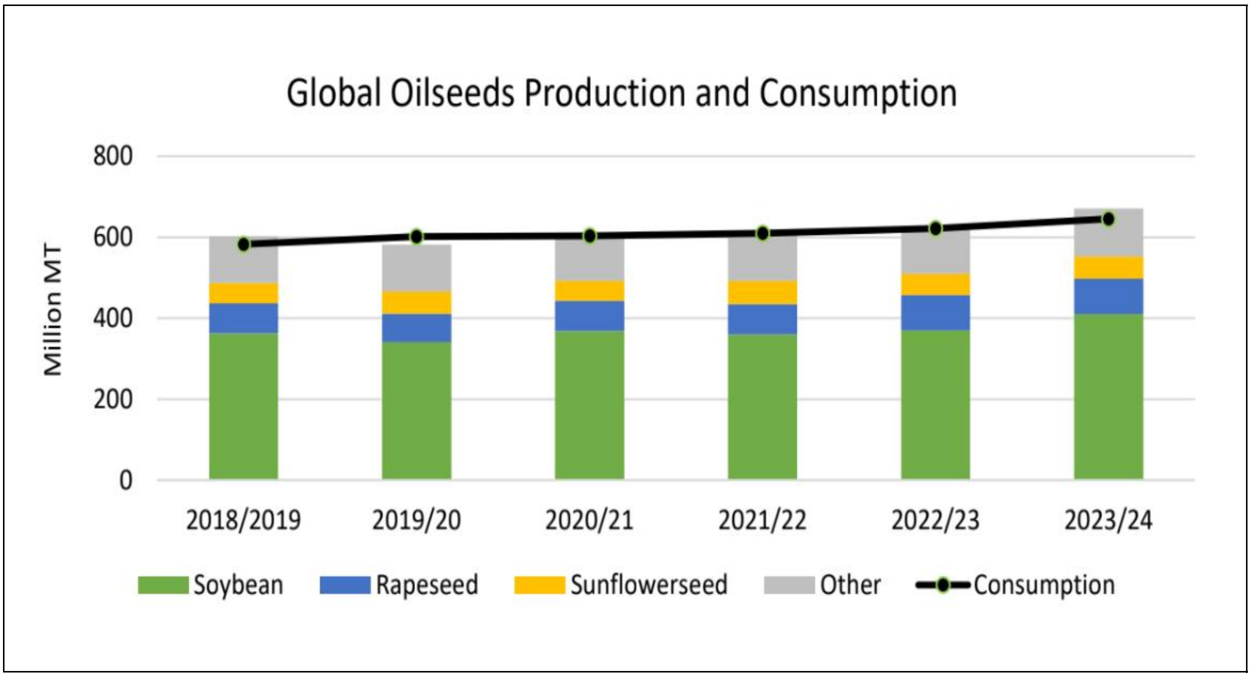


Figure 1. Global oilseeds production and consumption (Source : USDA 2023c)

Because of the increased demand for soybean-based foods, the need for soybean grain supply is also increasing. As a result, the planting area has expanded. The global soybean planting area has expanded from 129 million hectares in 2020/21 to 135.6 million hectares in February 2022/23, with a production of 383.01 million MT (Table 2) (USDA 2023b). Brazil has the largest soybean planting area, with 43.4 million hectares in February 2023, producing about half of the world's production (Table 2).

Thereby, as the human population grows, global crop demand, including soybeans, will be unavoidable in the future. In addition to population growth, agricultural production has not kept pace with estimated demand (TILMAN et al. 2011). Therefore, it is critical to increase soybean production to meet the growing demand for grains and soybean-based products. Although soybean crop yields to 2050 were projected to increase by 1.3% per year (RAY et al. 2013), currently there are several global challenges, particularly regarding the issue of environmental conditions such as climate change and soil fertility problems, which tend to not only limit production but also interfere with the quality of grain yield. Therefore, continuous research on various aspects of soybean production, including soybean cultivation, is critical to overcome current and future issues and constraints.

Table 1. Soybean meal: World supply and distribution (Thousand Metric tons) (Source: USDA 2023a)

	2018/19	2019/20	2020/21	2021/22	Mar 2022/23	Apr 2022/23
Production						
China	67,320	72,468	73,656	69,300	72,864	72,072
United States	44,283	46,358	45,872	47,005	47,663	47,617
Brazil	32,960	36,225	36,047	39,307	40,876	41,263
Argentina	31,500	30,240	31,320	30,287	27,495	24,960
European Union	11,850	12,324	12,482	12,166	11,574	11,575
India	7,680	6,890	8,000	6,800	7,760	7,760
Mexico	4,860	4,740	4,900	5,020	5,136	5,136
Other	33,577	36,151	35,896	36,306	38,101	37,222
Total	234,030	245,396	248,173	246,191	251,469	247,605
Imports						
European Union	17,197	16,329	16,504	16,704	16,400	16,000
Indonesia	4,449	5,043	5,356	5,535	5,750	5,700
Vietnam	5,127	5,336	5,200	5,531	5,300	5,300
Philippines	2,935	2,906	2,839	2,895	2,800	2,800
Thailand	2,889	2,854	2,687	3,077	2,800	2,650
United Kingdom	2,147	2,133	2,214	2,015	2,265	2,100
Mexico	1,887	1,818	1,854	1,827	1,850	1,850
Japan	1,596	1,858	1,839	1,699	1,850	1,700
Korea, South	1,855	1,992	1,727	1,726	1,825	1,700
Colombia	1,433	1,509	1,607	1,831	1,700	1,650
Other	22,606	21,502	23,330	24,083	21,758	21,323
Total	64,121	63,280	65,157	66,923	64,298	62,773
Exports						
Argentina	28,833	27,461	28,325	26,589	24,900	22,400
Brazil	16,095	17,499	16,576	20,207	21,100	21,400
United States	12,141	12,549	12,406	12,269	12,428	12,428
Paraguay	2,332	2,138	1,916	1,270	2,000	2,000
Bolivia	1,638	1,723	2,117	2,075	1,675	1,675
Other	7,019	6,566	8,113	6,336	6,080	5,977
Total	68,058	67,936	69,453	68,746	68,183	65,880
Domestic Consumption						
China	66,405	71,507	72,678	68,872	72,514	71,922
United States	32,901	34,444	34,179	35,352	35,743	35,743
European Union	27,867	28,267	28,392	28,042	27,392	27,042
Brazil	17,645	18,500	19,200	19,550	19,850	19,850
Mexico	6,625	6,650	6,725	6,875	6,950	6,950
India	5,530	5,780	5,850	6,288	6,725	6,725
Vietnam	5,780	6,130	6,330	6,385	6,260	6,485
Indonesia	4,625	4,900	5,200	5,600	5,750	5,700
Thailand	4,300	4,650	4,700	4,900	4,850	4,900
Japan	3,450	3,655	3,621	3,638	3,695	3,663
Russia	3,500	3,500	3,475	3,450	3,650	3,650
Egypt	3,325	3,450	3,600	3,650	3,825	3,600
Iran	3,325	3,400	3,430	3,500	3,575	3,550
Argentina	3,126	3,200	3,275	3,325	3,325	3,325
Philippines	2,925	2,975	2,900	2,950	2,885	2,975
Other	38,720	39,879	40,770	41,415	41,886	41,116
Total	230,049	240,887	244,325	243,792	248,875	247,196
Ending Stocks						
Brazil	3,537	3,773	4,062	3,624	3,344	3,654
Argentina	2,988	2,568	2,289	2,797	2,068	2,033
Vietnam	842	952	616	697	249	484
European Union	1,278	790	537	595	547	428
Thailand	276	468	560	556	93	396
Other	7,399	7,622	7,661	8,032	6,663	6,608
Total	16,320	16,173	15,725	16,301	12,964	13,603

Most countries are on an October/September Marketing Year (MY). The Mexico and Thailand are on a September/August MY. Canada is on an August/July MY. Paraguay, Vietnam and the Philippines are on a January/December MY and Bolivia is on a March/February MY.

Table 2. Soybean area, yield and production (Source: USDA 2023c)

Country / Region	Area (Million hectares)				Yield (Metric tons per hectare)				Production (Million metric tons)				Change in Production			
	2020/21	Prel. 2021/22	2022/23 Proj. Jan	2022/23 Proj. Feb	2020/21	Prel. 2021/22	2022/23 Proj. Jan	2022/23 Proj. Feb	2020/21	Prel. 2021/22	2022/23 Proj. Jan	2022/23 Proj. Feb	From last month MMT	From last month Percent	From last year MMT	From last year Percent
World	129.00	130.89	136.14	135.55	2.86	2.74	2.85	2.83	368.52	358.00	388.01	383.01	-5.00	-1.29	25.02	6.99
United States	33.43	34.93	34.94	34.94	3.43	3.48	3.33	3.33	114.75	121.53	116.38	116.38	0.00	0.00	-5.15	-4.24
Total Foreign	95.57	95.96	101.20	100.62	2.66	2.46	2.68	2.65	253.77	236.47	271.63	266.63	-5.00	-1.84	30.17	12.76
South America																
Brazil	39.50	41.50	43.40	43.40	3.53	3.12	3.53	3.53	139.50	129.50	153.00	153.00	0.00	0.00	23.50	18.15
Argentina	16.47	15.90	16.30	15.90	2.81	2.76	2.79	2.58	46.20	43.90	45.50	41.00	-4.50	-9.89	-2.90	-6.61
Paraguay	3.15	3.30	3.45	3.45	3.14	1.27	2.90	2.90	9.90	4.20	10.00	10.00	0.00	0.00	5.80	138.10
Bolivia	1.43	1.40	1.43	1.43	2.32	2.14	2.17	2.17	3.32	3.00	3.10	3.10	0.00	0.00	0.10	3.33
Uruguay	0.95	1.10	1.10	1.10	1.88	2.80	2.09	2.09	1.79	3.07	2.30	2.30	0.00	0.00	-0.77	-25.08
East Asia																
China	9.88	8.42	10.27	10.27	1.98	1.95	1.98	1.98	19.60	16.40	20.33	20.33	0.00	0.00	3.93	23.99
Korea, South	0.06	0.05	0.06	0.06	1.47	2.06	1.73	1.73	0.08	0.11	0.10	0.10	0.00	0.00	-0.02	-14.41
Korea, North	0.16	0.17	0.17	0.16	1.40	1.15	1.44	1.15	0.23	0.19	0.24	0.18	-0.06	-24.37	-0.01	-5.26
Japan	0.14	0.15	0.15	0.15	1.54	1.63	1.55	1.55	0.22	0.24	0.23	0.23	0.00	0.00	-0.01	-2.52
India	12.92	12.50	12.70	12.70	0.81	0.95	0.94	0.94	10.45	11.90	12.00	12.00	0.00	0.00	0.10	0.84
Canada	2.04	2.08	2.12	2.12	3.12	2.99	3.09	3.09	6.36	6.22	6.54	6.54	0.00	0.00	0.32	5.13
Former Soviet Union - 12																
Russia	2.71	2.99	3.30	3.30	1.59	1.59	1.67	1.67	4.31	4.76	5.50	5.50	0.00	0.00	0.74	15.55
Ukraine	1.46	1.44	1.50	1.35	2.05	2.64	2.40	2.37	3.00	3.80	3.60	3.20	-0.40	-11.11	-0.60	-15.79
European Union	1.00	0.98	1.06	1.06	2.61	2.76	2.34	2.34	2.60	2.71	2.47	2.47	0.00	0.00	-0.24	-8.87
Southeast Asia																
Indonesia	0.39	0.35	0.33	0.33	1.22	1.21	1.21	1.21	0.48	0.43	0.40	0.40	0.00	0.00	-0.03	-5.88
Vietnam	0.04	0.03	0.03	0.03	1.61	1.61	1.60	1.60	0.06	0.05	0.05	0.05	0.00	0.00	-0.01	-9.43
Thailand	0.03	0.03	0.03	0.03	1.63	1.63	1.63	1.63	0.05	0.05	0.05	0.05	0.00	0.00	0.00	0.00
Burma	0.14	0.13	0.13	0.13	1.04	1.04	1.04	1.04	0.14	0.14	0.13	0.13	0.00	0.00	-0.01	-3.70
Serbia	0.22	0.22	0.22	0.22	2.89	2.45	2.50	2.50	0.64	0.54	0.55	0.55	0.00	0.00	0.01	1.85
Mexico	0.16	0.18	0.15	0.12	1.58	1.57	1.50	1.54	0.25	0.29	0.23	0.19	-0.04	-17.78	-0.10	-35.76
Africa																
South Africa	0.83	0.93	1.10	1.10	2.29	2.38	2.05	2.05	1.90	2.20	2.25	2.25	0.00	0.00	0.05	2.27
Nigeria	1.10	1.20	1.20	1.20	1.01	0.93	1.04	1.04	1.11	1.12	1.25	1.25	0.00	0.00	0.13	11.91
Zambia	0.16	0.30	0.38	0.38	1.81	1.35	1.27	1.27	0.30	0.41	0.48	0.48	0.00	0.00	0.06	15.57
Uganda	0.05	0.05	0.05	0.05	0.60	0.60	0.60	0.60	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00
Middle East																
Iran	0.07	0.07	0.08	0.08	2.41	2.29	2.27	2.27	0.17	0.16	0.17	0.17	0.00	0.00	0.01	6.25
Turkey	0.03	0.03	0.04	0.04	3.79	3.91	4.00	4.00	0.11	0.13	0.14	0.14	0.00	0.00	0.02	12.00
Others	0.49	0.46	0.49	0.49	2.06	2.03	2.05	2.05	1.01	0.94	1.01	1.01	0.00	0.10	0.07	7.78

2.2 Conventional and controlled environment agriculture systems

2.2.1 Conventional agriculture system

There are several agricultural systems used in crop production around the world to ensure a continuous supply of raw materials. The current agricultural system is constantly being improved to achieve high yields and quality while at the same time overcoming current agricultural issues. The conventional agricultural production system is the main cropping system that is widely practiced around the world. According to AL-KAISI and LAL (2021), conventional agricultural systems are described as open systems in which the main purpose is the production of food, such as grain and vegetables, which is eventually removed from the field. They also described conventional agriculture as an imbalance between input and output because it removes soil, water, nutrients, and energy in addition to food products. Furthermore, there is a heavy reliance on synthetic fertilizers and other agricultural chemicals, as well as intensive tillage and mono- or limited-rotation cropping systems.

Although agricultural inputs are extensively used in this conventional cultivation system, it is an essential system since it is widely used in the production of safe and nutritious food. Additionally, this system provides the most support for farmers' livelihoods. Nowadays, modern technologies such as mechanical planting and crop management are widely incorporated into conventional agriculture for more sustainable production and to overcome any constraints. Each agricultural practice certainly has its advantages and disadvantages. Aside from improving and developing technology for conventional agricultural systems, suitable alternative systems can also be used. Indoor farming, also known as a controlled environment system, is one of the increasingly popular alternative cultivation strategies in this era.

2.2.2 Controlled environment agriculture system

Controlled environment agriculture, which includes greenhouses, high-tunnels, vertical farms, and plant factories, is a sophisticated and intensive form of hydroponically-based agriculture that is increasingly being recognized as an important strategy for addressing global food challenges (SAAD et al. 2021). Controlled environment agriculture is further classified depending on the growth medium and production technology. Almost entirely in a controlled environment agriculture system applies soilless culture as a growth medium. Cultivation using soilless cultures is becoming more popular in many countries. This is because this cultivation technique reduces pesticide contamination of the soil, requires less water, and produces more uniform crops (TOMASI et al. 2015). Furthermore, a soilless system in a controlled environment can generate high and consistent quality as well as a continuous supply of raw materials. Soilless cultivation in a controlled environment covers only 95,000 hectares throughout the world (GULLINO et al. 2019), but it is gaining popularity in the horticulture sector due to its cultural advantages. Soilless agricultural production in a controlled environment is a very promising technology for increasing the cultivation of numerous cash crops (TZORTZAKIS et al. 2020).

Soybean is one of the potential crops that can be grown under the system since it can utilize nitrogen (N) from biological N_2 fixation and reduce the requirement for mineral nitrogen fertilization. Additionally, soybean is one of four potential species studied for cultivation in the Bio-regenerative Life-Support System (BLSS) (PARADISO et al. 2013). Therefore, both conventional and controlled environments using soilless systems are important, particularly for commercial production. The controlled environment using a soilless culture system is one of the most common production techniques in today's horticulture industry because it can result in higher yields, even in areas with poor planting conditions, and it is also one of the most sustainable and environmentally friendly crop production systems.

2.3 Growth and development of soybean

Understanding the stages of growth can assist growers in carrying out more timely production practices, which leads to greater yields. There are three stages of growth and development of a plant, namely germination, vegetative (V), and reproductive (R). Numbers are used to identify each vegetative stage once the first trifoliate leaves have fully expanded. The growth phases become reproductive when the plant begins to set flowers. For soybean plants, the normal vegetative stages are VE (emergence), VC (cotyledon), V1, V2, and V3 (Figure 2). Meanwhile, the reproductive stages include R1, R2, R3, R4, R5, R6, R7, and R8 (Figure 2). If at least 50% of the plants examined are at a specific stage, the entire field is assumed to be at that stage (McCLURE 2022).

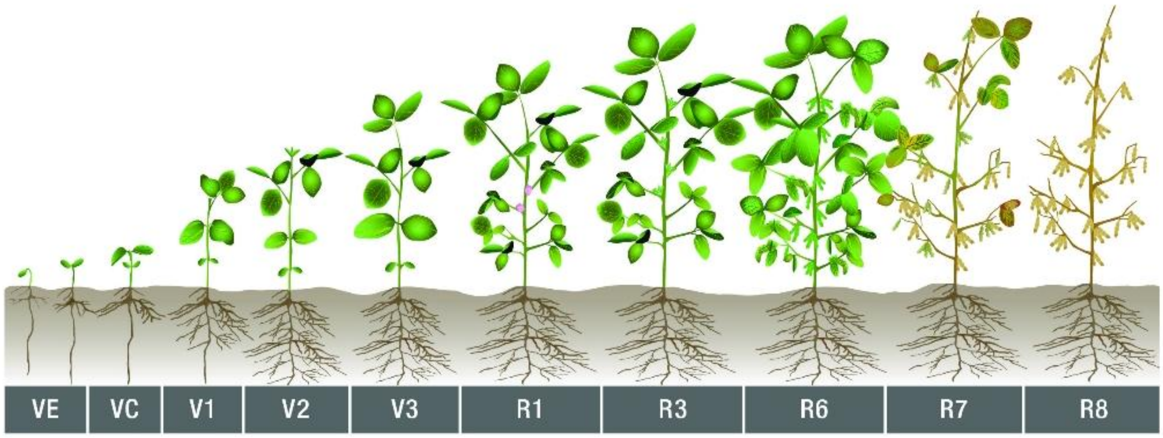


Figure 2. Growth stages in soybeans (Source: UNIVERSITY OF ILLINOIS 1999)

Soybean growth habit, which describes the degree of overlap between vegetative and reproductive development, is either determinate or indeterminate. Determinate varieties complete vegetative growth when the plant reaches the reproductive stage, whereas indeterminate growth varieties have simultaneous vegetative growth and flowering throughout the reproductive stage (NLEYA et al. 2019). Therefore, soybean growth and development need to be understood due to specific considerations that must be taken into account during various stages of soybean growth, and every stage has its own set of specific actions and concerns. Hence, soybean producers must pay attention to each stage of soybean growth and development so that every necessary thing may be undertaken according to the stage of plant growth and any problems can be overcome to achieve good growth, high yield, and good quality.

2.3.1 Germination process

Germination is the first essential stage of planting. According to MUTHIAH et al. (1994), there are three phases of soybean germination. The initial stage is water absorption by the endosperm. The amount of water absorbed is approximately 50% of the seed weight (UNIVERSITY OF WISCONSIN 2015). The second stage is development, which occurs when meristematic activities start, and the third stage is growth, which occurs when the radical begins to elongate and push through the seed coat. The radical is the first structure to emerge from the germination seed, generally within 48 hours of sowing under ideal conditions (PURCELL et al. 2014) (Figure 3). The seedling structure that emerges from the soil or substrate is known as the hypocotyl. As the hypocotyl develops, it produces a crook that pulls the cotyledons from the soil. After emerging through the soil surface, the cotyledons unfold, synthesize chlorophyll, and begin to photosynthesize. The cotyledons are high in protein and oil and serve as the main source of nutrition for the growing seedling for the first 7 to 10 days.

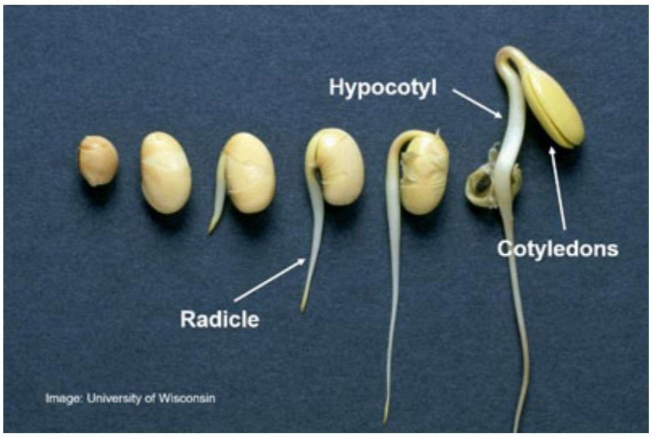


Figure 3. Germination stages of soybean (Source: UNIVERSITY OF WISCONSIN 2015)

2.3.2 Vegetative stages

Soybean vegetative phases begin with the emergence of cotyledons from the soil surface, which is referred to as the VE stage. When the unifoliate leaves unfold, the plant has reached the VC stage. The plant is said to be in the V1 stage when the unifoliate leaves have fully grown. The soybean plant has unifoliate leaves and two trifoliate leaves, and the edges of the young developing trifoliate are not touching; the plant is at V2 (Figure 4) (PURCELL et al. 2014). The nodes above the unifoliate leaves and vegetative growth are identified from V2 to the highest node of the plant (Vn). Table 3 describes the vegetative stages of soybeans and their importance for timing management decisions.

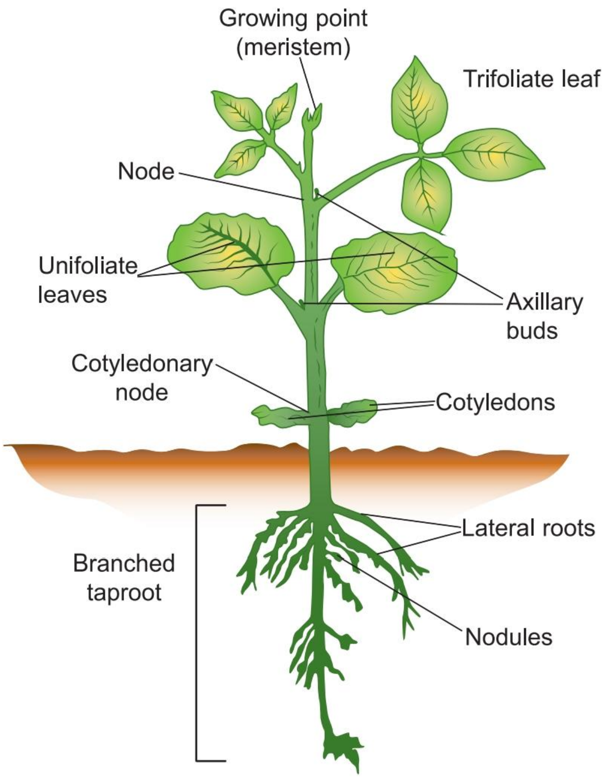


Figure 4. Vegetative structures of a young soybean plant (Source: PURCELL et al. 2014)

Table 3. Description of soybean vegetative stages (Source: PEDERSEN 2009)

STAGE	ABBREVIATED STAGE DESCRIPTION	DESCRIPTION
VE	Emergence	Cotyledons appear above the soil surface.
VC	Cotyledon	Unifoliate leaves unrolled so that the leaf edges do not touch.
V1	1st trifoliate leaf	Fully developed (leaflets unrolled so edges do not touch) trifoliate leaf.
V2	2nd trifoliate leaf	Two fully developed trifoliate leaves. Early N-fixing root nodules becomes functional.
V3	3rd trifoliate leaf	Three fully developed trifoliate leaves. V3 is the cutoff stage for some post herbicides.
V(n)	(n) trifoliate leaf	"n" number of fully developed trifoliate leaves.

2.3.3 Reproductive stages

Soybean becomes reproductive at first bloom, or R1, when 50% or more of the plants have at least one flower on the main stem. When predicting soybean reproductive growth, only plants with intact main stems should be considered. Stages R1-R2 define flowering, R3-R4 define pod development, R5-R6 define seed development, and R7-R8 define plant maturity (Table 4).

Table 4. Description of soybean reproductive stages (Source: PEDERSEN 2009)

STAGE	ABBREVIATED STAGE DESCRIPTION	DESCRIPTION
R1	Beginning bloom	One flower opens at any node on the main stem.
R2	Full bloom	A flower opens at one of the top two nodes on the main stem with a fully developed trifoliate. *Beginning of rapid nutrient accumulation to vegetative parts.*
R3	Beginning pod	Pod 3/16 inch long at one of the four uppermost nodes with fully developed trifoliate. *Fungicide application timed at R3-R4.*
R4	Full pod	Pod 3/4 inch long at one of the four uppermost nodes with fully developed trifoliate.
R5	Beginning seed	Seed 1/8 inch long in a pod at one of the four uppermost nodes with fully developed trifoliate. *Apply insecticide when economic thresholds are reached.*
R6	Full seed	Green seed fills pod cavity in a pod on one of the four uppermost nodes with fully developed trifoliate. *Irrigation terminated at R6.5.*
R7	Early maturity	One normal pod on main stem turns mature brown color.
R8	Full maturity	95 percent pods on main stem reach mature brown color.

2.4 Factors influencing the germination and seedling emergence of soybeans: Variety and temperature

Seed germination and seedling establishment influence the productivity of crops (HOPPER et al. 1979, SHARMA et al. 2014). Many factors affect seed germination, including variety and various environmental conditions. One of the main and important environmental factors affecting germination other than water and oxygen is temperature (ANONYMOUS 2023a). Each variety has different characteristics genetically, either physical or chemical differences. It can also interact with environmental conditions such as temperature, which can affect growth and yield. Different temperatures have a great influence on germination and plant growth (YUFENG 2015).

Several studies have confirmed the effects of temperature, such as duration and intensities of temperature, on soybean seed growth at different developmental stages, including germination and seedling emergence (GIBSON 1992, TACARINDUA et al. 2013, NAGAKAWA et al. 2020). In addition to germination rate, certain soybean varieties are also reported to have different seed characteristics, such as vigour and viability. However, seeds will only grow more vigorously and viable under optimal conditions, which are essential indications for measuring seed quality and seed emergence in the field (GUO et al. 2018, TATIC et al. 2012). However, when exposed to unpredictable environmental conditions such as temperature stress (low or high temperatures), the pattern of seed development and emergence is affected.

The optimum temperature for germination of most soybean varieties was 25 °C, and although soybeans can germinate at soil temperatures as low as 10 °C, but germination is slow (AWAN et al. 2014, JAGDISH 2020). According to SZCZERBA et al. (2021), germination and seedling percentages for all tested soybean varieties at 25 °C were considerably greater than at 10 °C and 15 °C. At 25 °C, the germination percentage ranged between 97.5 and 100%. BEGUM et al. (2022) also reported that the germination percentage of two soybean varieties (PI408105A and PI416937) peaked at 25 °C, whereas the germination percentage of the other two varieties peaked at 20 °C (Figure 5). Numerous studies have found that a temperature of 25 °C is best for the germination of soybeans, peas, beans, and maize (LADRO et al. 2022, ZAITER et al 1994, WANG et al. 2018). However, there are certain soybean varieties whose germination performance is good at low and high temperatures, but up to a certain level.

The germination rate drops at low temperatures and increases with increasing temperatures until it reaches the optimum level, but at high temperatures exceeding the limit, the germination rate declines due to seed damage (FU et al. 2017). However, it depends on the variety of the plant. SZCZERBA et al. (2021) discovered that no soybean varieties germinated after two days at low temperatures (10 °C), but one had better germination, with nearly double the amount of germinated seeds than other varieties. However, when they evaluated the seeds that germinated after two days at 10, 15, and 25 °C to measure the seedling number, they discovered that at lower temperatures, 10 and 15 °C had lower seedling percentages, with 10 °C having the lowest for all varieties (Figure 6). Among the four varieties, one (Petrina) had a lower seedling percentage at all temperatures than the others. HATFIELD and EGLI (1974) discovered that soybean hypocotyl elongation was very slow at 10 °C and reached a maximum at 30 °C. ALM et al. (1993) similarly found that when the temperature climbed from 10 to 25 °C, the seedling elongation rate for maize and soybean increased. According to JIA et al. (2020), they discovered that two types of cabbage seeds had a reduced germination rate, and the seedlings were thin and weak at the low temperatures (15 °C), but the other two germinated well, and the difference was not significant with the germination at the optimal temperature (25 °C). Low temperatures (below 10 °C) during seed germination also significantly reduced the germination ability of other legume plants, such as lupine (PLAZEK 2018a, PLAZEK 2018b).

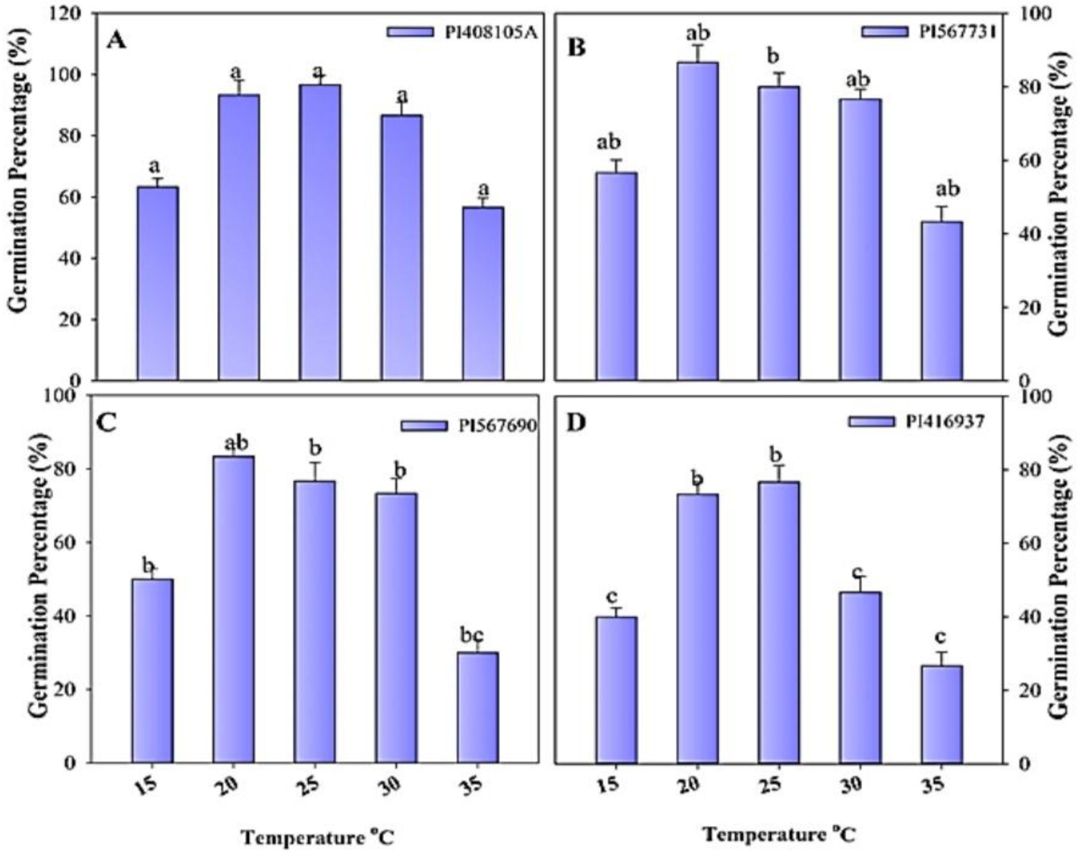


Figure 5. Effect of different temperature regimes on the seed germination percentage of four soybean varieties. Each data value is the mean of three replicates, and different letters indicate significant differences at $p < 0.05$ (Source: BEGUM et al. 2022)

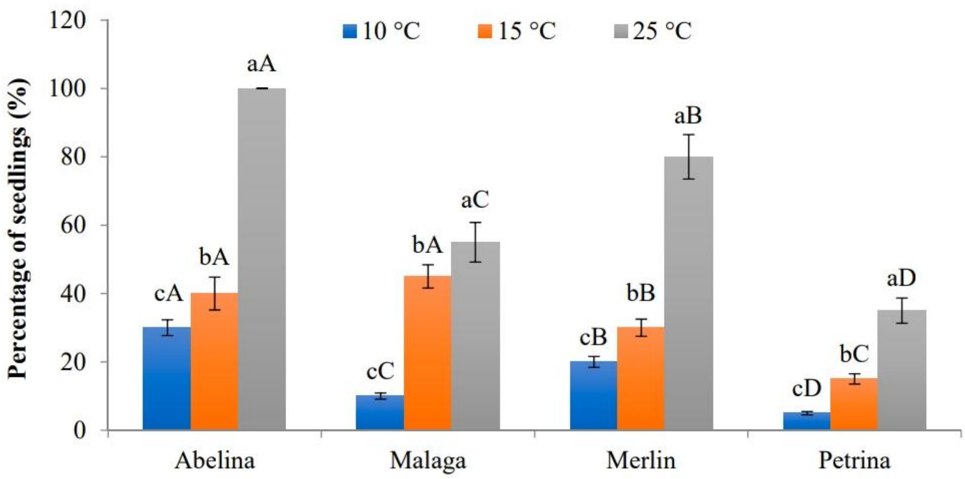


Figure 6. Percentage of seedlings of different soybean varieties at different temperatures. The values for each variety followed by the same lowercase letter do not differ significantly, while the means for the same temperature marked with the same uppercase do not differ significantly according to Duncan's multiple range test ($p < 0.05$) (Source: SZCZERBA et al. 2021)

Higher temperatures effect the depletion of free and bound water inside biomolecules, leading to seed dryness and tighter biochemical packing. The high temperatures associated with soybean growth development were observed in controlled chambers and a greenhouse (NAGAKAWA et al. 2020). Previous research on the influence of high temperatures on seed germination found that most seed germination performances declined and were worse at high temperatures. Temperatures above 30 °C restrict soybean seed viability, resulting in reduced germination. As a result, the quantity of stachyose and phytic acid in soybean seed diminished, making the process of membrane biogenesis and germination more difficult (REN et al. 2009). BEGUM et al. (2022) observed that at high temperatures of 35 °C, the germination rate (Figure 5) and seedling length (Table 5) of four soybean varieties were the lowest, followed by 15 °C. According to RAY et al. (2015), who conducted a study on temperature stress on soybeans, the viability and vigour of soybean seeds were lowered when temperature stress increased from 40 to 60 °C. However, BEENA and JAYARAM (2010) found that there are soybean seeds that germinate up to 70% with a seed vigour index of 12.87% at temperatures up to 50 °C. Nevertheless, when the temperature was raised to 60 and 70 °C, both decreased significantly.

Table 5. Effect of different temperatures on the seedling length (cm) of four soybean varieties. The data presented are the means of each of three replicates, and different letters indicate significant differences at $p < 0.05$ (Source: BEGUM et al. 2022)

Cultivars	Temperature regimes				
	15 (°C)	20 (°C)	25 (°C)	30 (°C)	35 (°C)
PI408105A	4.67 ± 0.18 a	12.00 ± 0.37 a	10.43 ± 0.32 a	6.07 ± 0.29 a	1.60 ± 0.30 a
PI567731	2.30 ± 0.25 b	9.47 ± 0.31 b	8.30 ± 0.15 b	5.27 ± 0.13 b	0.73 ± 0.22 b
PI567690	1.73 ± 0.07 c	8.73 ± 0.17 b	7.40 ± 0.09 c	4.03 ± 0.25 c	0.83 ± 0.14 b
PI416937	1.17 ± 0.77 d	7.33 ± 0.29 c	6.27 ± 0.24 d	3.33 ± 0.19 d	0.60 ± 0.28 b

Hence, it can be concluded that most soybean seeds germinate and emerge successfully at temperatures ranging from 20 to 25 °C. Therefore, most of the previous research suggests that the temperature range is the ideal temperature for the soybean germination stage. At low and high temperatures, most soybean varieties show reduced germination rates and seedling emergence, which is under temperature stress conditions. However, there were soybean seeds that could germinate and emerge at low temperatures, as well as those that could germinate and emerge at high temperatures, but only up to a certain point. At high temperatures, this is most likely due to seedling drying, which may be due to conformational changes that result in molecular deterioration of biochemicals within seeds under high temperatures and varied times. Furthermore, the characteristics of the variety itself influence germination and seedling emergence. Varieties with low lipid content in oilseed plants, such as soybeans, can cause seed germination to be slower than varieties with high lipid content (MIQUEL and BROWSE 1994).

2.5 Effect of agronomic factors on growth, yield, and chemical composition of soybeans planted in open fields: Nitrogen application and weed management

2.5.1 Nitrogen fertilizer

Nitrogen (N) is one of the most important nutrients for plant development. N requirements are crucial, particularly during the vegetative period. At this stage, the plant is actively growing. N fertilizer is commonly used in crop production in one or more of the following forms: nitrate (NO_3), ammonia (NH_3), ammonium (NH_4), or urea (COCNH). Each form has specific properties that determine when, where, and how various fertilizer materials can be utilized (MENGEL 1986). Primary specific N fertilizer sources applied for crop production include ammonium liquor, ammonium nitrate, ammonium sulphate, anhydrous ammonia, aqua ammonia, natural fertilizers, and nitric acid (CHEREMISINOFF and ROSENFELD 2011).

In soybean production, other than obtaining N from available N in the soil or from supplied N fertilizer, the soybean plant also obtains N from the atmosphere because the soybean plant can form a symbiotic relationship with N-fixing bacteria known as *Bradyhizobium japonicum*, which colonizes the roots of the soybean plant and forms nodules. The bacteria use these nodules to convert or fix N_2 gas from the atmosphere to ammonium (NH_4+) (SHOBER and TAYLOR 2014). HARDY et al. (1971) found that N_2-fixation began 14 days after sowing only when soybeans were grown at optimal temperature and moisture conditions. Therefore, the application of N fertilizer to soybean plants needs to consider its ability to fix N and the availability of N in the soil. This is because certain soybean varieties do not require additional N fertilizer and are adequate sources of N_2 fixation and N from the soil. However, due to genetics, irrigation supply, climatic conditions, or other factors, soybeans require additional N application to create a higher yield since N_2 fixation and soil N may not be sufficient to fulfil crop demands at high yields (SHOBER and TAYLOR 2014).

2.5.2 Effect of nitrogen application

Nitrogen (N) fertilizer, when applied at the proper rates, has been shown to improve agricultural production (CHANDIO et al. 2022). It has been claimed that about 50% of the improvement in grain yield is due to genetic development, while the other 50% is attributed to sustainable management practices, including nutrient application (HENCHION et al. 2017). Good N fertilization practices, including recommended techniques and rates, are critical not only for increasing crop yield but also for preserving soil and environmental health (PANHWAR et al. 2019 and SHAH et al. 2019). According to STITT and KRAPP (1999), GOOD et al. (2004), DING et al. (2005), and DIAZ et al. (2006), N may promote root development, increase volume, area, diameter, total and main root length, dry mass, and consequently increase nutrient uptake, nutritional balance, and dry mass production. However, inefficient application of synthetic fertilizers, primarily nitrogenous fertilizers, has a negative impact on crop growth and yield. Slow plant growth and early leaf senescence caused by N deficiency might result in lower crop yield and quality (DONG et al. 2012). Meanwhile, excessive N fertilizer application prolongs the vegetative growth period, delays maturation (HODGES et al. 2002), reduces sugar content, attracts insect pests, and promotes disease outbreaks. It is expected that cumulative N fertilization may cause an enhancement of 23-60% of nitrous oxide (N_2O) emissions by 2030 (FAO and WHO 2009).

Soybean responses to N fertilizer might vary depending on the fertilizer type and rate, technique of application, and developmental stage during application (KASCHUK et al. 2016). Previous research has shown that the effect of N fertilization on soybean growth, yield, and nutritional content varies, with some soybeans responding positively and others responding negatively. Concerning the response of N fertilization to soybean plant growth, previous research has shown that N fertilization has a positive effect on yield components such as plant height, pod number/plant, and seed weight/plant (FALIGOWSKA and SZUKALA 2010). KUBAR et al. (2021) conducted a study in China and reported that, in comparison to the control (0 N), the maximum value for the number of pods/plant was 21.02 at high N (225 kg N/ha) but was not significantly different at 150 and 300 kg N/ha (Table 6). Similarly, the number of grains/pods was significant with others, with the largest number of grains/pods being 225 kg N/ha (Table 6). LORENC-KOZIK and PISULEWSKA (2003) also proved that increased N fertilization greatly increases the number of pods/plants. This is supported by research by CALISKAN et al. (2008) and CHAFI et al. (2012), which revealed that N fertilization increases both the number of pods/plants and the thousand grain weight. The effects of N fertilization on soybean growth, such as plant height, pod number/plant, and grain number/pod, are mostly positive. This indicates that the nutrient N is necessary during the vegetative and early reproductive stages.

Table 6. Effect of different nitrogen rates on the grain yield components of soybean (Source: KUBAR et al. 2021)

N (kg ha^{-1})	Pods plant^{-1}	Number of grains pod^{-1}	100-grain weight (g)	Grain yield (kg ha^{-1})
0	18.70 ± 1.02b	2.36 ± 0.22c	29.06 ± 1.20b	3001.48 ± 49.78c
75	19.86 ± 1.07b	2.73 ± 0.15b	30.43 ± 1.28a	3162.09 ± 52.62b
150	20.32 ± 0.60a	2.66 ± 0.09b	30.06 ± 1.38a	3208.24 ± 61.50a
225	21.02 ± 0.74a	3.03 ± 0.18a	32.02 ± 0.35a	3233.62 ± 60.40a
300	20.56 ± 0.62a	2.62 ± 0.35b	30.21 ± 1.63a	3221.89 ± 60.45a

The data is the average of the three repeats. Different lowercase letters indicate the significant (p£0.05) differences among the N rates and growing stages of soybean, means with similar letters denotes the no significant different among the treatments $p > 0.05$ using the HSD test in SPSS

A previous study on the effect of N fertilization on yield demonstrates that the response of soybean production to N fertilizer planted in Alabama varies on cultivation location, the variety used, and application time (WOOD et al. 1993). Two of the five locations evaluated showed a negative response to grain yield. Five locations that showed positive effects were also inconsistent in grain yield due to location and soybean variety interactions. They also observed that when N was applied during the R5 stage, grain yields dropped for all tested varieties. According to LORENC-KOZIK and PISULEWSKA (2003), N application rate that positively affects soybean production varies at low N, which is between 30 kg N/ha and 60 kg N/ha. The use of high rates of N fertilizer, as studied by HATAMI et al. (2009) in Kashmir, revealed that increasing the N fertilizer rate up to 150 kg/ha enhanced soybean production significantly. They also found that N fertilizer increased not only grain yield but also dry matter accumulation.

According to studies conducted by MOURTZINIS et al. (2018) across the United States, applying up to 120 kg/ha N fertilizer enhanced the yield of grains. They examined several N rates ranging from 0 to 560 kg/ha. According to KUBAR et al. (2021), a high rate of N treatment of 225 kg N/ha resulted in the greatest soybean grain yields of 3233.62 kg/ha, but there was no significant difference in grain yield between 225 and 300 kg N/ha (Table 6). Based on these observations, it is obvious that applying N fertilizer increases soybean output, and the optimal amount varies depending on the area and variety chosen. The rate of N that has positive effects ranges from 30 to 150 kg N/ha. However, certain soybean varieties have a favourable effect at a high rate of up to 225 kg N/ha.

The effect of nutrients on other crops, such as maize, demonstrates that fertilizer dose, genotype, and crop year have impacts on the NPK utilization of applied fertilizer. After a certain level of fertilizer application, NPK utilization decreased (PEPÓ and KARANCSI 2017). Research on hybrid maize found that climatic conditions significantly impact the response of maize hybrids to fertilizer and nutrient utilization (SZELES et al. 2019). They confirm that increasing the N + constant proportion PK treatment combination can boost hybrid tolerance to environmental stress factors in most hybrids evaluated. Another study on three nutrient levels in maize found that in the control plots, all three nutrient levels were significantly different from each other. The yield on the plots treated with 80 kg N/ha + PK was 6.25 t/ha, whereas the yield on the plots treated with 160 kg N/ha was 11.64 t/ha (RÁTONYI et al. 2020). The study on sweet sorghum also revealed that fertilizers had a substantial influence on yields, as the maximum biomass was produced at 100 kg N/ha in both years (2009 and 2010) (KOVACS and GYURICZA 2012). Therefore, nutrient application, including N, must consider the genetic factors of the variety itself, environmental factors, and other cultural practices so that the uptake of nutrients by plants becomes more effective for growth and development to achieve optimum yields and quality.

Meanwhile the influence of N on soybean seed composition, such as protein and lipid content, also varies among varieties and conditions (McClURE et al. 2017, ASEEFA et al. 2018). According to SLIWA et al. (2015) and POPOVIC et al. (2016), site conditions, including the amount and distribution of rainfall, significantly influence the quantity and quality of soybean seed. A research done by ETONE EPIE et al. (2022), the effect of N fertilizer on different soybean varieties and environments in the Southeastern United States differed for protein concentration, oil concentration, and protein and oil yield. Although N treatment effected seed protein content in two sites, the variations across these conditions were not consistent. According to BOBRECKA-JAMRO and PIZŁO (1996), the highest dosages of N significantly increased the total protein content of the seeds while decreasing the crude fat content. They also revealed that the basic chemical composition of grains varied during the period of the investigation. The greatest total protein content was reached in 2012, and the highest crude fat content was recorded in 2014. However, VALINEJAD et al. (2013) and FERREIRA et al. (2016) found that N fertilization did not influence the chemical composition of soybean seeds.

For other crops, such as wheat, an N fertilizer rate of 120 kg/ha had no impact on yield, but it had a positive effect on protein and gluten content (SZENTPÉTERY et al. 2005). This is supported by the findings of ESER et al. (2020), who discovered that increasing the level of N topdressing on winter wheat had a significant effect on grain protein content in all examined varieties, whether in split or undivided dose applications. Therefore, based on the previous research, the effect of N on chemical composition differs, with some studies showing an increase in protein and fat content and others not. However, it has been shown that when protein levels increase, fat content decreases.

2.5.3 Effect of weed management

Weed control plays an important role in soybean cultivation, particularly with conventional approaches. Weed infestation is a major problem for almost all field crops. Similarly, in soybean plants, weed infestation is a major cause of low production (WALLACE et al. 2018). According to weed surveys in the dry year (2000) and wet year (2001), the yield of the cultivated crop (winter wheat) and weed condition are both influenced by soil humidity (PERCZE et al. 2005). KRISTÓ et al. (2022) discovered that the number of shoots, spikelets, grains, and grain weight of linked winter wheat with weeds was significantly lower than pure wheat.

Weeds are regarded as the most serious concern in all major soybean producing countries. Even with advanced technologies, farmers report significant losses because of weed influence. Weeds alone are estimated to produce a 37% loss in soybean yield (OERKE et al. 2004). Weeds can reduce soybean yield by up to 80% in some regions if not managed properly (GAZZIERO 2004). Weeds influence soybeans because they compete for limited light, water, fertilizer and space. According to KNEZEVIC (2014), the combination of pre-emergence herbicide application and mechanical weed control is not a fixed method that must be modified based on the type of crop, planting operation, and seasonal conditions.

According to MARANGONI et al. (2013), weed management (weeded or unweeded) has a significant impact on soybean yield. They evaluated the effect of weed control on different soybean varieties and discovered that certain varieties had a positive effect on yields while others had a negative effect. When no weed management was implemented, yields were reduced by 30%. Weeds, in addition to limiting the yield of crops, can also cause other issues such as reduced grain quality, loss and difficulties while harvesting, and becoming a host of pests and diseases. Weeds also produce toxins that are extremely harmful to crop growth. Weed control in soybean crops can be managed by the use of one or more of the following methods: preventative, mechanical, chemical, biological, and cultural (BENNETT and SHAW 2000).

A study conducted by CARRANZA et al. (1995) found that as the weed population increased, relative intraspecific competition (yield loss per weed unit) dropped. One of the major methods of weed management on fields with smaller planted areas is mechanized cultivation. However, the main drawback of this approach is the difficulty in controlling weeds in crop rows, the poor efficacy when conducted in wet conditions (wet soil), and the inefficiency in managing weeds that reproduce by vegetative parts (SILVA et al. 2007). PIRES (2005) investigated the competitive ability of soybean varieties against weeds and discovered that in the presence of weeds, all varieties showed yield decreases of roughly 480 kg/ha.

The effect of weed management on the chemical content of soybean crops also revealed that when chemical control was used for weed management, the fat content in seed was 12.9-18.3% and the protein content was 29.9-31.5% (ARIUNAA et al. 2016). They discovered no significant difference in protein and fat content between unweeded and chemical control using herbicides. According to PEER et al. (2013), research using weed control methods (weedy, hand weeding, integrated hand weeding and herbicide, and herbicide) showed that integrated hand weeding and herbicide recorded comparable lipid content in soybean grain, and the weedy plot recorded the lowest lipid percentage. Therefore, weed management is an integral part of soybean production. It is very clear that weed control greatly affects soybean growth and yield, but the effect on chemical composition varies depending on the weed management strategy used.

2.6 Factors affecting the growth, yield, and chemical composition of soybeans planted using a soilless culture system in a controlled environment: Variety and nutrient solution

2.6.1 The use of soilless culture

Soilless culture generally refers merely to growing cultures with nutrient solutions without a support medium like soil. Soilless culture is widely used in indoor farming, particularly for producing short-term crops like vegetables. This protected cultivation method can control the growth conditions by managing the environment, nutrient solution requirements and growing medium (BLANK 1999). Soilless cultures are usually divided into substrate cultures (artificial, mineral or organic growing media or a mixture thereof) and water cultures or hydroponics, in which the roots are partially or fully immersed in the nutrient solution, according to the type of plant carrier (SAVVAS et al. 2013a). Changes in horticultural crop quality parameters can be influenced by the growing substrate (GRUDA 2009). The classification of soilless culture systems is shown in Figure 7. The selection of a soilless culture must be suitable for the type of plant and the equipment of the system.

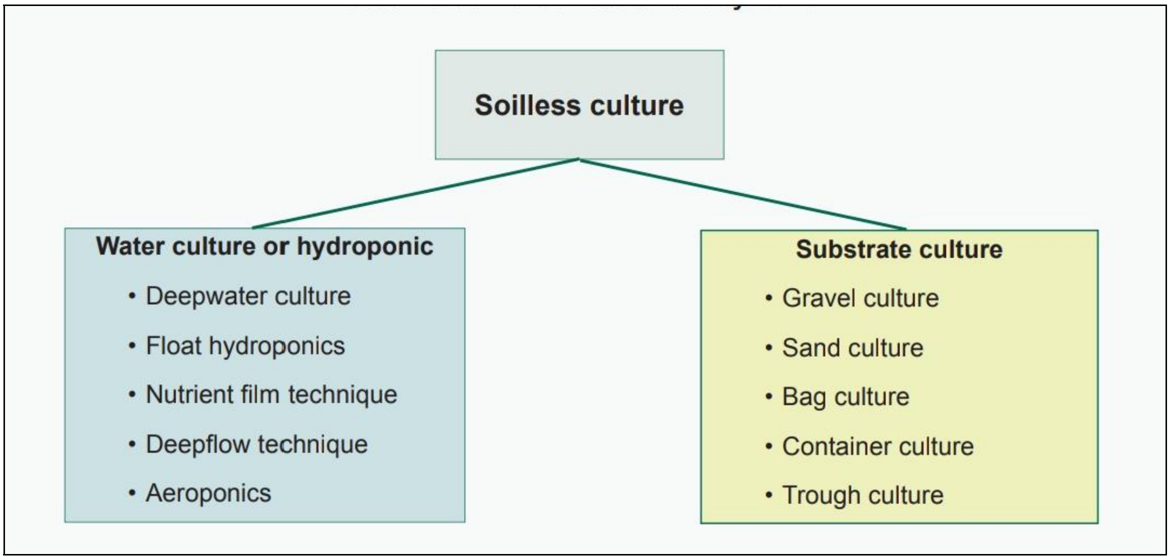


Figure 7. Classification of soilless culture systems (Source: SAVVAS et al. 2013a)

For the use of soilless substrate as a growing medium, the choice of material to be used depends on the plant species to be grown. The qualities of the substrate must fulfil the needs of plant production. To produce sustainable future possibilities, the substrate should also be ecologically friendly and consumer-oriented (GRUDA 2012). A previous study has shown that particle sizes ranging from 1 to 4 mm are appropriate for the soilless substrate. The substrate should have physical and chemical properties such as a uniform texture that drains well while retaining nutrients and water, a low bulk density between 190 and 700 kg/m^3, high porosity between 50% and 85%, particle-size distribution to maintain a good balance between air and water retention (between 0.25 and 0.5 mm), pH between 5.0 and 6.5, and a low content of soluble salts (PARDOSSI et al. 2011).

There are two types of substrates, which are organic and inorganic. Organic substrates include sawdust, peat moss, coco peat, woodchips, fleece, marc, bark, biochar, etc., whereas inorganic substrates are perlite, vermiculite, zeolite, gravel, rockwool, sand, glass wool, pumice, sepiolite, volcanic tuff, hydrogel, and expanded clay (OLYMPIOS 1992, GRUDA et al. 2006, NICHOLS and SAVIDOV 2010, OLLE et al. 2012). A preliminary investigation showed that the grain yield of two legume varieties, snap beans, surpassed 6 t/ha when cultivated in gravel culture (GARCIA and PINCHINAT 1973). MAJDI et al. (2012) conducted an experiment to determine the efficacy of substrate and cultivar selection and discovered that a combination of peat and perlite had the greatest impact on the growth characteristics and yield of green pepper. POPESCU et al. (1995) conducted studies on sweet pepper production using different organic soilless substrates compared to the soil under high plastic tunnels and discovered that plants grown on organic substrates produced twice as much as plants grown in soil. Therefore, there are several soilless substrate options that can be adapted in a controlled environment and can be suited to the type of plant.

Furthermore, inorganic substrate cultures, such as expanded clay aggregate or clay mineral aggregate, are widely used because their spherical form and porosity help provide an appropriate balance of oxygen and water, preventing plant roots from becoming excessively dry or drowning. The clay mineral aggregate substrate releases almost no nutrients and has a pH of 7.0 (SAVVAS et al. 2013b). The other characteristic is that they have a large pore space, which allows better solution flow. They are also rarely clogged or blocked. As a result, water drains very effectively (THAKULLA 2021). They may also be sterilized and cleaned for reuse. Hence, because it can be reused, this substrate has a long lifespan, and the cost of the culture medium can be reduced since it can be used repeatedly.

2.6.2 Effect of variety

In addition to the type of plant, the selection of appropriate plant varieties based on genetic factors also needs to be considered for planting in a controlled environment using soilless culture. Among the criteria for variety selection are maturity period and plant size. A shorter maturation period tends to be appropriate for cultivation in a controlled environment since it reduces the usage of inputs and saves time and cost on crop and planting system maintenance.

Plants with lower heights should be chosen because, in addition to easing plant management, it also saves space if a multi-layer planting system is implemented. However, variety can also interact with other factors, such as environmental factors and cultural practices, in affecting growth, yield, and quality.

According to previous studies, variety affected not only the number of pods/plants but also the 100-grain weight, as stated by AGEGN et al. (2022). The 100-grain weight is important because the grain size of a soybean can be determined (CHOI et al. 2021). According to CHOI et al. (2021), soybean varieties that have a larger grain size can produce more protein but lower lipid content. Also, SABATINO et al. (2019) discovered a significant interaction between tomato variety and molybdenum (Mo) solution concentration with regard to total yield. The variety with the highest total yield was applied with 2.0 µM Mo/L, whereas the variety with the lowest total yield was applied without nutrient concentration. They also found an interaction between the tested variety and the nutrient concentration, which resulted in different behaviour (total yield, above-ground biomass, ascorbic acid, N, and Mo fruit content) of the tested varieties when subjected to different nutrient solution concentration levels. CIRIELLO et al. (2020) conducted research on three varieties of sweet basil grown in three nutrient solutions with crescent electrical conductivity (EC: 1, 2, and 3 dS/m) and discovered that the combination of variety Aroma 2 and the EC of 2 dS/m resulted in the highest production, both in terms of fresh weight and dry biomass.

This means that, in addition to selecting high-yielding varieties, appropriate genetic traits of the varieties are necessary for adaptation to the controlled environment since some variety characteristics may affect growth and production. Another aspect that influences plant performance in this system, such as nutrient management, must be considered to enhance crop yield and quality.

2.6.3 Effect of nutrient solution

Nutrient management is an agronomic practice that impacts crop productivity, including productivity in a controlled environment. According to SINHA et al. (2020), the frequency and amount of nutrients provided under soilless culture systems are determined by substrate type, crop type, container size, irrigation system, and existing environmental conditions. Although optimal nutrition is easy to reach in soilless cultivation, improper nutrient solution management can harm plants and lead to failure. The success or failure of a soilless culture is mostly determined by the strict nutrient management programme (PRAKASH et al. 2020). In a soilless system, nutrients are supplied to the plant in solution form by irrigation water. Soilless cultivation allows for direct control of the nutrient solution, enabling modifications in composition and concentration to achieve predictable results about the dry matter content, nitrate content, or other organoleptic properties of produce (FREZZA et al. 2005).

Extremely low nutrient solution concentrations commonly inhibit plant growth (SAVVAS and ADAMIDIS 1999). On the other side, excessively high nutritional solution concentrations produce osmotic stress, ionic toxicity, and growth limitation. A high concentration of nitrogenous fertilizer also enhances vigorous growth, which reduces light intensity penetration to the entire canopy due to dense foliage and hence reduces ascorbic acid accumulation in shadowed areas. Increased plant growth caused by nitrogenous fertilizer increases the concentration of nitrate in plant tissue while decreasing the concentration of ascorbic acid, which may have a twofold negative effect on the quality of plant foods (LEE and KADER 2000).

The nutrient solution can be formulated with a mixture of several fertilizer sources or using fertilizers that have been formulated and are commercially available on the market. The most important aspects for nutritional solution selection are a potential hydrogen (pH) range of 5.5-6.5 and an electrical conductivity (EC) range of 1.2-2.5 dS/m. WHEELER et al. (2008) used a nutrient solution containing substances such as 7.5 mM N, 3.0 mM potassium (K), 0.5 mM phosphorus (P), 2.5 mM calcium (Ca), 1.0 mM magnesium (Mg), 1.0 mM sulphur (S), 60 µM iron (Fe), 7.4 µM manganese (Mn), 0.96 µM zinc (Zn), 1.04 µM copper (Cu), 7.13 µM boron (B) and 0.01 µM molybdenum (Mo) in their study on the performance of wheat, potato, tomato, lettuce, and soybean (variety: McCall and Hoyt) that were carried out in a biomass production chamber using nutrient film technique. The pH was controlled to 5.8 by the automatic addition of 0.4 mM nitric acid and EC to 1.2 dS/m. According to BASAL et al. (2020), who studied the effect of water stress using polyethylene glycol (PEG) to control the water level on the growth of the soybean variety ES Mentor and Pedro used a nutrient solution containing substances such as 2.0 mM calcium nitrate [$Ca(NO_3)_2$], 0.7 mM potassium sulphate (K_2SO_4), 0.5 mM magnesium sulphate ($MgSO_4$), 0.1 mM potassium dihydrogen phosphate (KH_2PO_4), 0.1 mM potassium chloride (KCl), 10 µM boric acid (H_3BO_3), 0.5 µM manganese sulphate ($MnSO_4$), 0.5 µM zinc sulphate ($ZnSO_4$), 0.2 µM copper sulphate ($CuSO_4$) and 10^{-4} M ferric-ethylenediaminetetraacetic acid (Fe-EDTA). There are various formulated fertilizers available on the market that are commonly used in soilless systems, and one of them is 'Advance Hydroponics of Holland' (Dutch Formula). This hydroponics fertilizer, which comprises three parts mineral fertilizers (Grow, Bloom, and Micro), was invented in Holland in 1993 (ANONYMOUS 2018).

Previous research has shown the influence of different levels of nutrient solutions on certain plants in controlled conditions using soilless culture. The concentration of nutrients in a solution affects the growth and components of crops such as spinach, tomato, cucumber, salvia, bean, artichoke, wasabi, and lettuce (OZTEKIN et al. 2018, SAKAMOTO et al. 1999, WANZHENG et al. 2011, KANG and VAN IERSEL 2004, VALDEZ et al. 2002, ROUPHAEL et al. 2012, HOANG et al. 2019, SHINOHARA and SUZUKI 1981). A high concentration of micro-elements in a solution causes an increase in the synthesis of protein components and proteins, which harms the synthesis of carbon-based substances such as vitamin C (CARIS-VEYRAT et al. 2004). A study by SMITH et al. (2022) discovered an increase in pod number when the fertilizer concentration was increased from low (10%), medium (50%), and high (100%) for common bean plants using a soilless culture mixture of sand and vermiculite at a rate of 50:50. VALDEZ et al. (2002) reported that increasing nutrient solution levels dramatically reduced the vine length of the legume crop (snap bean) (Table 7). They used the nutrient solution Enshi-shoho, which is widely used in Japan. They reported that leaf area and root fresh and dry weight increased with increasing nutrient levels. They also observed a significant reduction in harvested pods in the 1/4 strength treatment, while the number of pods obtained from the 1/2 to 2 strength treatments was comparable (Table 8).

Table 7. Effect of nutrient solution (Enshi-shoho solution) levels on the growth components of snap beans (Source: VALDEZ et al. 2002)

Nutrient level (strength)	Vine length (cm)	Leaf count[z]	Leaf area[y] (dm^2)	Shoot FW ($g \cdot plant^{-1}$)	Shoot DW ($g \cdot plant^{-1}$)	Root DW ($g \cdot plant^{-1}$)	Pod DW ($g \cdot plant^{-1}$)	Total DW ($g \cdot plant^{-1}$)
1/4	80.2	19.0	23.7	87.6	15.2	2.22	18.2	35.6
1/2	79.0	19.8	30.8	90.4	15.7	2.25	25.8	43.8
1	77.1	20.0	32.6	91.2	15.9	2.33	24.8	43.0
1½	74.5	20.5	33.9	101.8	17.7	2.49	23.0	43.2
2	72.4	21.0	34.6	103.7	18.0	2.66	24.2	44.9
$LSD_{0.05}$	3.14	NS	3.51	2.02	0.27	0.33	1.17	1.27

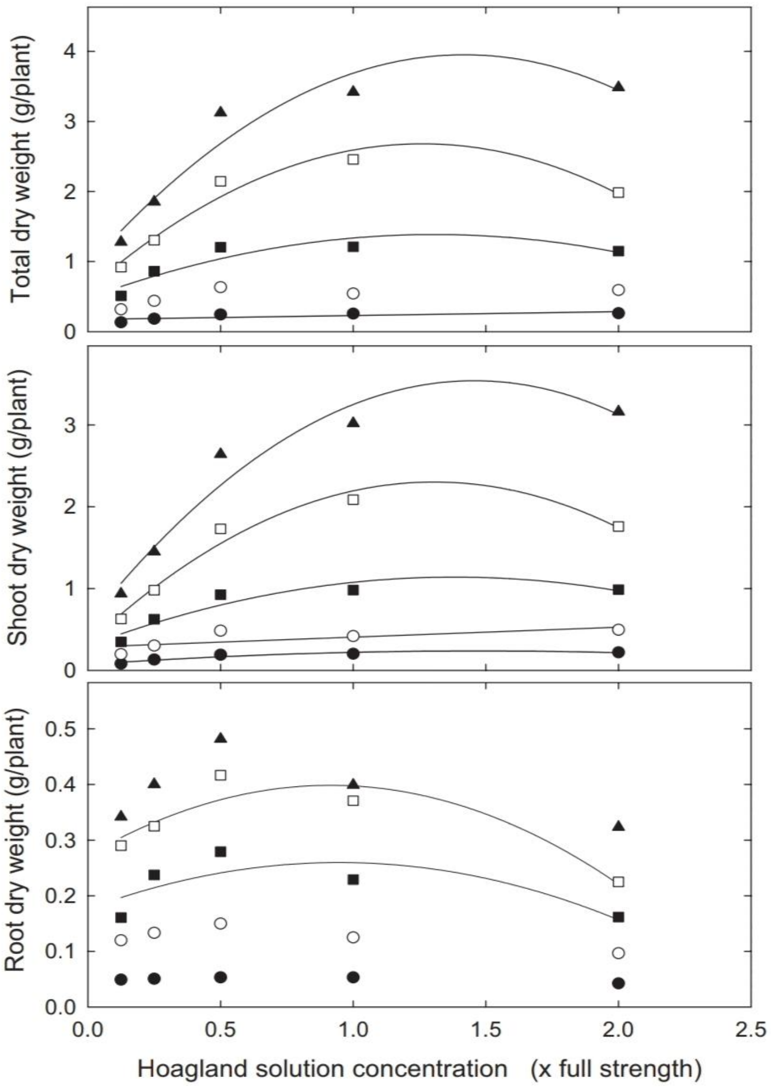
[z] Leaf count means total number of unfolded trifoliate leaves at harvest.
[y] Total leaf area of trifoliate leaves at maturity.
NS, not significant.

Table 8. Effect of nutrient solution (Enshi-shoho solution) levels on the reproductive components (Source: VALDEZ et al. 2002)

Nutrient level (strength)	No. of flowers	No. of pods	Pod set (%)	Pod FW ($g \cdot pod^{-1}$)	Fresh pod yield ($g \cdot plant^{-1}$)
1/4	79	49	62.0	5.9	289.1
1/2	92	74	80.4	5.9	436.6
1	103	71	68.9	6.2	440.2
1½	98	70	71.4	6.3	441.0
2	99	70	70.7	6.1	427.0
$LSD_{0.05}$	11.40	9.71	4.3	NS	79.6

NS, not significant.

KANG and VAN IERSEL (2004) found that nutrient solution concentration had a significant quadratic influence on salvia (*Salvia splendens*) root dry weight at 44 and 51 days after transplanting (Figure 8). They used a fertilizer source from the Hoagland solution, which is also available on the market. They discovered comparable patterns in shoot and total dry weight. From 0.125 to 1.0x nutritional solution concentrations, shoot and total dry weight increased. Nevertheless, there was little or no additional increase in dry weight from 1.0 to 2.0x concentrations (Figure 8). They also discovered that nutrient content had a significant impact on the shoot:root ratio. They observed that when nutrient content increased, the shoot:root ratio increased. An increase in shoot:root ratio with increasing fertilizer concentration is common (MAK and YEH 2001, SATTELMACHER et al. 1990). Water and nutritional deficiencies can significantly reduce the shoot:root ratio (FINDENEGG 1990). A plant with a high shoot:root ratio has a bigger proportion of shoots than roots, and plants with a higher proportion of shoots are better equipped to collect light energy and grow larger (FINDENEGG 1990). The use of a hydroponics nutrient solution concentration with high EC in tomatoes restricted plant growth but increased the level of sugars and lycopene in tomato fruits, and thus fruit quality (WU and KUBOTA 2008). In strawberries, flower bud initiation was promoted by treatment with low nutrient solution concentrations (SAROOSHI and CRESSWELL 1994, LIETEN 2002, GALLACE et al. 2017).



The effects of nutrient solution concentration on root, shoot, and total dry weight of salvia. The plants were subirrigated with 0.125, 0.25, 0.5, 1.0 or 2.0 × full strength of Hoagland solution as needed. Data were collected at 30 (●), 37 (○), 44 ■), 51 (□), and 58 (▲) d after transplanting. Lines indicate linear or quadratic significance within a sampling date; $P \leq 0.05$.

Figure 8. The effect of nutrient solution concentration (Hoagland solution) on root, shoot, and total dry weight of the salvia crop at different days after transplanting (Source: KANG and VAN IERSEL 2004)

Thus, based on previous findings, nutrient solutions for crops using the soilless culture method in a controlled environment can be formulated to meet crop demand. Even the nutrient solutions can be obtained from those available on the market. However, the most important thing is to ensure the concentration of the solution is suitable for crop type and variety, as well as that the pH and EC readings are at an optimal level and within the appropriate range. The effect of nutrient concentration on most plants shows an increase in growth and yield performance with an increase in nutrient concentration up to a certain level and a decrease in growth and yield performance when nutrient stress occurs.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Research 1: Effect of nitrogen application and weed canopy on yield formation and chemical composition of soybeans under open-field planting

3.1.1 Experimental site

The first experiment is a field experiment that was conducted in May 2020 at the experimental plot, Institute of Agronomy, Hungarian University of Agriculture and Life Sciences (MATE), Gödöllő, Hungary (47.46'N, 19.4014 'E, 242 m above sea level) (Figure 9). This experiment was carried out in order to achieve the first objective of this study. The soil type at the experimental site is brown forest soil (Chromic Luvisol), and the textural classification of the soil is sandy loam. The properties of the soil are shown in Table 9. The meteorological data during the planting season (May to October 2020) is shown in Figure 10 (temperature) and Figure 11 (rainfall and rainy days). The average temperature in the sowing month (May) was 15 °C, with 42.8 mm of rain. Temperatures were over 20 °C from June to September, with the highest average temperature was 25 °C in August. The month with the most precipitation was June, with an average of 139.31 mm of rain and around 16 rainy days.

Figure 9. Satellite view of the experimental plot, Gödöllő, Hungary

Table 9. Soil properties of the experimental plot at the Hungarian University of Agriculture and Life Science, Gödöllő, Hungary

Properties	Average
Humus (%)	1.32
pH	7.08
K_A	40
Sand (%)	49
Silt (%)	25
Clay (%)	26
$CaCO_3$	0

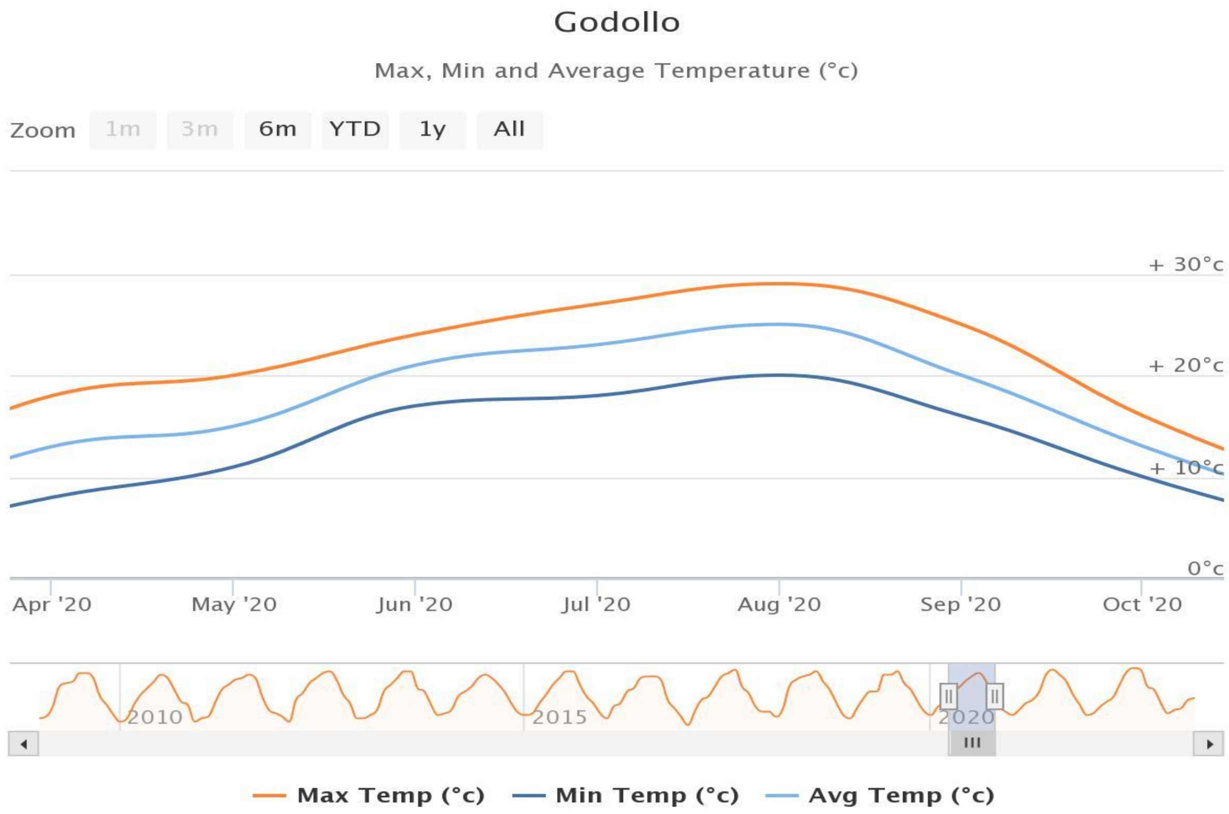


Figure 10. Max, min, and average weather temperature April - October 2020 at Gödöllő, Hungary (Source: worldweatheronline.com)

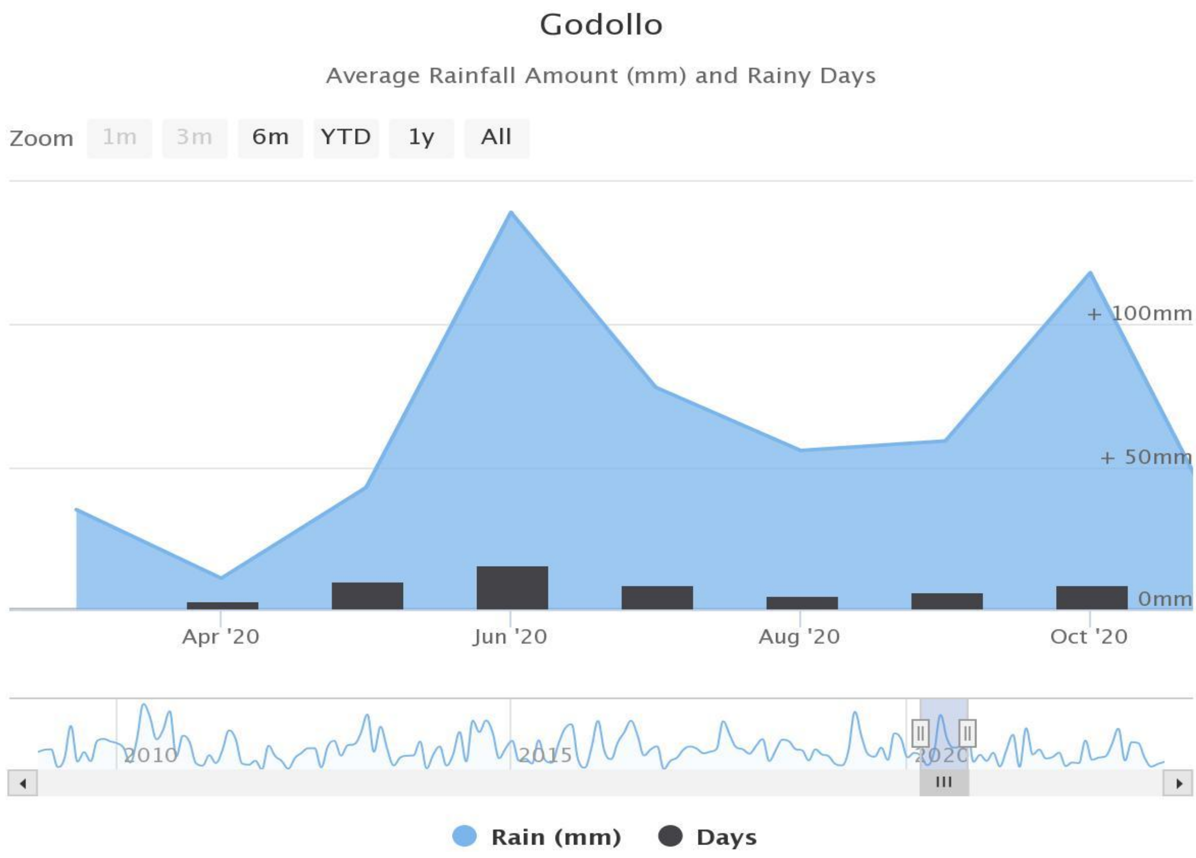


Figure 11. Rainfall and rain days from April 2020 to October 2020 at Gödöllő, Hungary (Source: worldweatheronline.com)

3.1.2 Soybean cultivar, plant population, and experimental design

A soybean variety, ES Gladiator, was used in this study and was planted with a scheduled plant density of 540,000 viable germs per hectare. The specific characteristics of the variety are provided in Table 10. The treatments of this experiment comprise two nitrogen (N) fertilizer rates (0 and 200 kg N/ha) and three weed canopy treatments (weedy, hand weeded, and mechanically weeded). The N fertilizer source in this study was ammonium nitrate (33.5% N) that was applied once at 60 days after sowing. Meanwhile, the weed canopy treatments were done every two weeks. The experimental design was arranged in a split plot design with four replications. In this experimental design, N fertilizer was assigned to the main plot and the weed canopy to the sub-plot.

Table 10. Specific characteristics of the ES Gladiator soybean variety

Characteristic	Detail
Origin	France
Maturity time (day)	133
Height (cm)	99.5
Flowering (day)	35.2
Pod colour	Brown
Seed colour	Yellow
Pod opening (1-9)	8.2
Yield (t/ha)	3.78
Thousand grain weight (g)	188.3
Stability (1-10)	8
Protein content (%)	37.6
Lipid content (%)	21.2

3.1.3 Cultural practices

The experimental plot was cleared, ploughed, and rotor-tilled, and the seedbed was prepared before planting. The basic fertilizer treatments were applied to the experimental field in following the usual practices (BIRKAS et al. 2004), based on crop requirements. A pre-emergent weed control was used to eliminate weeds. Soybean seeds were planted at a depth of 3 cm. Eleven weeks after planting, the plants were supplied with 200 kg N/ha, the control had no nutrient supply. The N dosage was selected to meet the aims of the study to evaluate the effect of a high dose of N fertilizer on the yield and chemical content of soybeans. Weeds were controlled every two weeks according to the weed canopy treatments, which were weedy, hand weeded, and mechanically weeded. The mechanically weeded treatment was done by an inter-row handy hoeing machine. The plants were then harvested manually. Planting and harvest dates were respectively May 25 and October 7.

3.1.4 Measurements

At harvest, all plants in a sampling area of 1.5 m^2 per plot were harvested to calculate grain yield. Pods from harvested plants were oven-dried immediately at a temperature of 50 °C for two days for grain yield determination. The dried pods were then hand-threshed, and the grains were weighed to calculate the grain yield/m^2. The grains were ground for moisture content and chemical composition of protein and lipid determinations using the NIR Product Analyzer (INSTALAB 600). The average moisture content of dried grain was 4.65%. The protein and lipid contents were expressed as percentages, and protein and lipid yields were calculated based on their contents multiplied by dry grain yield. All seed samples were analyzed at the Crop Production Laboratory at the MATE Institute of Agronomy.

3.1.5 Statistical analysis

Statistically, a one-way between treatments ANOVA was conducted to compare the effect of the different nutrition supply and weed canopy on pod number, grain yield, protein content, lipid content, protein yield, and lipid yield. ANOVA was performed at the $p = 0.05$ level of significance to determine whether the treatments were different. Post hoc comparisons using the least significant difference (LSD) test were made at $p < 0.05$. The significance level used in the statistical studies was 5%. For the statistical evaluation of our results, the Explore and ANOVA modules of the IBM SPSS V.23 software were used.

3.2 Experimental Research 2: Influence of temperature and varieties on seed germination of soybeans at different germination times

3.2.1 Experimental location and growth conditions

The second experiment was designed to meet the second objective of this study. The experiment was carried out in the Crop Production Laboratory of the Institute of Agronomy, Szent Istvan Campus, Hungarian University of Agriculture and Life Sciences (MATE), Gödöllő, Hungary (47°35’37” N, 19°21’55” E).

The germination test was conducted according to general laboratory standards. Two soybean varieties with different seed sizes were used in this study. Seeds of both varieties that were in good condition with a germination rate above 90% were selected and treated using a 5% hypo solution to prevent the formation of fungi during the germination test period. The seed treatment was done by soaking the seeds in the solution for 3 minutes and then rinsing them with distilled water. After being cleaned and rinsed, the seeds were placed in 13.5 cm Petri dishes. The Petri dishes were lined with Whatman filter paper (AOSA 1992), which was moistened with 10 ml of distilled water. The Petri dishes were then sealed with parafilm to prevent water evaporation and exposed to different temperatures according to the treatment that represented suboptimal, optimal, and high temperatures in the plant growth chamber for 12 days.

3.2.2 Treatments and experimental design

Three factors that influence the germination of soybean seeds were tested in this study, namely temperature, variety, and germination time. Two soybean varieties (Martina and Johanna) were exposed to three different levels of temperatures, which were 15, 25, and 35 °C. There were four germination times based on the number of days after sowing, which were days 3, 5, 7, and 9. Table 11 displays the physical and chemical properties of the investigated soybean varieties. This experiment was arranged according to a completely randomized block design with four replicates, in which each Petri dish contained six seeds, and the total number of seeds per treatment was 24.

Table 11. Specific characteristics of the Martina and Johanna soybean varieties

Characteristics	Martina Variety	Johanna Variety
Origin	Hungary	Hungary
Maturity time (day)	138	140
Height (cm)	105.9	90.9
Flowering (day)	33.3	34.3
Pod colour	Brown	Brown
Seed colour	Yellow	Yellow
Pod opening (1-9)	8.9	9.0
Yield (t/ha)	3.95	4.06
Thousand grain weight (g)	187.1	193.4
Stability (1-10)	8.0	8.4
Protein content (%)	35.3	36.5
Lipid content (%)	23.2	22.3

3.2.3 Data collection

Observation and data collection were recorded for germination rate (%), total seedling length (cm), and viability (%). All these parameters were recorded on days 3, 5, 7, and 9 after sowing, except for the viability data (%), which was recorded on day 12. Seeds were considered germinated if radicles with a size of 1 mm or greater emerged from the seeds. Meanwhile, in this experiment, viability refers to the capability of the soybean seeds to germinate, survive, and produce healthy seedlings that were recorded when the germinated seeds produced a shoot (plumule), which was after 10 days of sowing.

3.2.4 Statistical Analysis

All the recorded data were subjected to a three-way analysis of variance (ANOVA) using IBM SPSS V.23 software (SPSS Inc., Chicago, IL, USA). The data presented are the mean values of the main effects of temperature, variety, and day, as well as the means of interaction effects. At a probability level of 0.05, the least significant difference (LSD) test was applied to compare treatment mean differences.

3.3 Experimental Research 3: Response of growth, yield, and chemical composition to nutrient concentrations of soybean varieties grown using a soilless culture system in a controlled environment

3.3.1 Experimental setup

This experiment was carried out in a controlled environment growth chamber at the experimental plot, Institute of Agronomy, Hungarian University of Agriculture and Life Sciences (MATE), Szent Istvan Campus, Gödöllő, Hungary, which is located at 47°46′ N, 19°21′ E, and 242 m above sea level (Figure 12). The size of the chamber is 4 m x 1.8 m (Figure 13). The chamber is equipped with planting tools, including pots with a capacity of 10 L and dimensions of 19 cm in diameter by 22 cm in height, 117 cm × 60 cm fertilizer solution tanks, 1000 L/h water pumps (Newa Maxi IP68; 220–240 V, 13 W), a drip irrigation system, and a timer. A total of 24 pots were placed on top of 4 nutrient solution tanks, where 6 pots were placed for each tank. Each tank was installed with a water pump that pumps the fertilizer solution in the tank through a drip irrigation system to each pot controlled by a timer. The arrangement of some equipment in this planting system is shown in Figure 14.

Figure 12. Satellite view of the controlled growth chamber (in the circle) at the experimental field, Institute of Agronomy, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

Figure 13. The controlled growth chamber at the experimental field

Figure 14. Inside the controlled growth chamber that was equipped with a complete planting system

In order to optimize the growing conditions for soybean growth, the chamber was installed with air conditioning to control the temperature, fluorescent lamps to supply enough light, fans for a ventilation system, and an exhaust fan for airflow. The growth chamber's temperature was 22 °C during the day and 16 °C at night. Each nutrient concentration treatment (main plot) had two fluorescent lamps installed, each 58 watts, with a combination of red and blue lights. The lamp was turned on automatically for 16 hours at 950 Lux and turned off automatically for 8 hours at night. The humidity percentage in the chamber was between 40 and 60%. Before planting, pots were filled with substrate culture as a growing medium, which used expanded clay aggregate. The research was carried out from January 2022 to November 2022 to achieve the third objective of the study.

3.3.2 Experimental treatments and design

The variety of Martina and Johanna was used in this experiment and was supplied with different nutrient solution concentrations (0, 50, 100, and 150%). The liquid fertilizer of 'Advance Hydroponics of Holland' (Dutch Formula) was used as a source of nutrients. The pH of the nutrient solution for all concentrations ranged from 6 to 6.5, while the electrical conductivity (EC) ranged from 1.0 to 1.8 ds/m. This fertilizer is suitable for hydroponics cultivation and consists of three different formulas, namely formula 1 (Grow), 2 (Bloom), and 3 (Micro). Table 12 indicates the nutrient content of each of the three formulas.

The use of fertilizer was in combination, and the rate was according to the growth stage of the soybean plant. Formula 1 was applied during the first vegetative stage (V1), the second vegetative stage (V2), the third vegetative stage (V3), the fourth vegetative stage (V4), the fifth vegetative stage (V5), and the flowering stage. The fertilizer formulas 2 and 3 were given at all stages of plant growth, including the end of the flowering stage. Identification of each of these stages is crucial, especially when deciding when to apply nutrients. If a minimum of 50% of the examined plants reach that stage, the whole field can be considered to be at that stage. Each fertilizer formula needs to be diluted with water, and in this study, as much as 25 L of water was used in each fertilizer tank. Therefore, the amount of fertilizer used based on 25 L of water and according to the plant growth stage and treatment is shown in Table 13. The nutrient solutions started to be supplied to the plants for all treatments after 10 days of germination.

This study was designed in a split plot experimental design with three replications. The first factor (nutrient concentration) was arranged as a main plot, while the second factor (variety) was arranged as a sub-plot.

Table 12. Nutrient content (%) in Dutch Formula fertilizers

Nutrient	Nutrient Content (%)		
	Formula 1 (Grow)	Formula 2 (Bloom)	Formula 3 (Micro)
Nitrate (NO_3)	1.8	0.3	4.5
Ammonium (NH_4)	0.6	0.4	0
Phosphorus pentoxide (P_2O_5)	4.4	5.7	0
Potassium oxide (K_2O)	7.4	5.3	3.0
Magnesium oxide (MgO)	0.8	2.1	0
Sulfur trioxide (SO_3)	2.2	5.6	0
Calcium oxide (CaO)	0	0	6.0
Boron (B)	0	0	0.015
Molybdenum (Mo)	0	0	0.01
Copper (Cu)	0	0	0.006
Manganese (Mn)	0	0	0.04
Zinc (Zn)	0	0	0.02
Iron (Fe)	0	0	0.15

Table 13. The total of Dutch Formula fertilizers that were diluted in 25 L of water according to nutrient concentration treatments and different plant growth stages

Plant Stage	Treatment	Formula 1 (Grow)	Formula 2 (Bloom)	Formula 3 (Micro)
Growing stage (V1, V2)	0%	0	0	0
	50%	9.38	4.63	4.63
	100%	18.75	9.25	9.25
	150%	28.13	13.88	13.88
Growing stage (V3,V4,V5)	0%	0	0	0
	50%	18.75	9.38	9.38
	100%	37.5	18.75	18.75
	150%	56.25	28.13	28.13
Flowering stage	0%	0	0	0
	50%	10	20	10
	100%	20	40	20
	150%	30	60	30
End of flowering stage	0%	0	0	0
	50%	0	37.5	12.5
	100%	0	75	25
	150%	0	112.5	37.5

3.3.3 Planting and crop management

Soybean seeds of both varieties that have a growth rate of more than 90% were used in this study. A total of 8 seeds were sown directly into the pot by planting the seeds 3 cm deep into the growing medium. Six seedlings that were healthy and growing uniformly were retained in the pots, while the other two were uprooted and discarded after 10 days of planting.

For the first 10 days of planting, during the germination period, each pot was irrigated without nutrients three times daily for 30 minutes per irrigation. Irrigation with fertilizer solution was then given according to the treatment and growth stage of the plant from the 11th day after planting. The fertilizer solution was automatically given three times daily for 30 minutes per irrigation. The solution was manually replaced weekly by pumping it out of the tank and refilling it with a new fertilizer solution. The aim was to maintain the proper range for the EC and pH readings. Fallen leaves in and around the pot were collected and disposed of to prevent the growth of fungi that would damage the plant.

3.3.4 Harvesting and sampling

Soybean plants are mature when the seeds, pods, and stem turn yellow. However, harvesting should be conducted when the soybean pods are completely dry and turn brown. In this experiment, plants were harvested at the age of 162 days after planting. After the pods were harvested, they were oven-dried for two days at 50 °C to reduce the moisture in the seeds and reach the appropriate moisture level of less than 13%. This purpose was to prevent seed damage, mould, and insect attacks. After the pods were completely dried, the seeds were removed from the pods and stored in a covered and dry area.

After all the pods were harvested, six plants in the pots were uprooted and separated into two parts: above ground (leaves and stem) and below ground (root). Both parts were then oven-dried at 50 °C for two days for dried weight measurement.

3.3.5 Data collections

The data measurements were started a week after nutrients were supplied. All data were collected during vegetative growth, during harvest, and after harvest. The vegetative growth data included plant height, number of leaves, soil plant analysis development (SPAD) reading, and leaf area. For SPAD reading measurements, the green leaf colour intensity of fully expanded second trifoliate leaves was measured using a SPAD-502 chlorophyll meter (Minolta Camera, Japan). Meanwhile, leaf area was obtained using a non-descriptive method by directly measuring the maximum length and width of the leaves on the plant. The vegetative growth data were recorded for all 6 plants in each pot every week until the plants produced flowers, with 5 weeks of data measurement (Week 1, Week 2, Week 3, Week 4, and Week 5).

The collected data during and after harvest include root length, root weight, shoot weight, shoot:root ratio, yield components, yield, and chemical composition. Root length, or deep root, was measured vertically, which was conducted during harvest. Meanwhile, shoot (above the ground) and root (below the ground) weights were measured by weighing the weights of both parts of the plant after drying. When the root and shoot weights were measured, the shoot:root ratio was calculated and recorded. Data on yield components, such as the number of pods/plants and the number of grains/pods, were recorded during harvesting. Meanwhile, yield data such as grain weight/pod, grain yield/pot, and 100-grain weight were recorded three days after harvest, when the pods were dried. The grains from each treatment were then grounded to measure their chemical composition, including protein and lipid content. Protein and lipid content expressed as percentages were measured in the laboratory using a NIR Product Analyzer (INSTALAB 600). The yields of protein and lipids were then calculated by multiplying their contents by grain yield.

3.3.6 Statistical analysis

All the recorded data were statistically analyzed using IBM SPSS V.23 software (SPSS Inc., Chicago, IL, USA). The results presented for vegetative growth measurements are the mean value data for the main effects of nutrient concentration, variety, weeks and the mean value of interaction effects. Meanwhile, the root, shoot weights, shoot:root ratio, yield components, yield and chemical composition data are presented as averages for the main effects of nutrient concentration, variety, and the interaction of the main effects. At the significance level of $p < 0.05$, a three-way analysis of variance (ANOVA) was carried out to compare the effects of nutrient concentration, variety, and weeks on vegetative growth parameters, and a two-way ANOVA was used on other parameters. Post hoc comparisons were then performed for all parameters using the least significant difference (LSD) test at $p < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Experimental Research 1: Effect of nitrogen application and weed canopy on yield formation and chemical composition of soybeans under open-field planting

4.1.1 Pod number

The pod number performance of plots was rather diverse in accordance with the vegetation period and the treatments applied (Figure 15). In general, it can be stated that the highest pod numbers were developed by plants in hand weeded plots. Nitrogen applications did not have a direct effect on pod numbers. The number of pods increased with time in most applications, however, this consequent increment within treatment was not significant, as demonstrated by Figure 15.

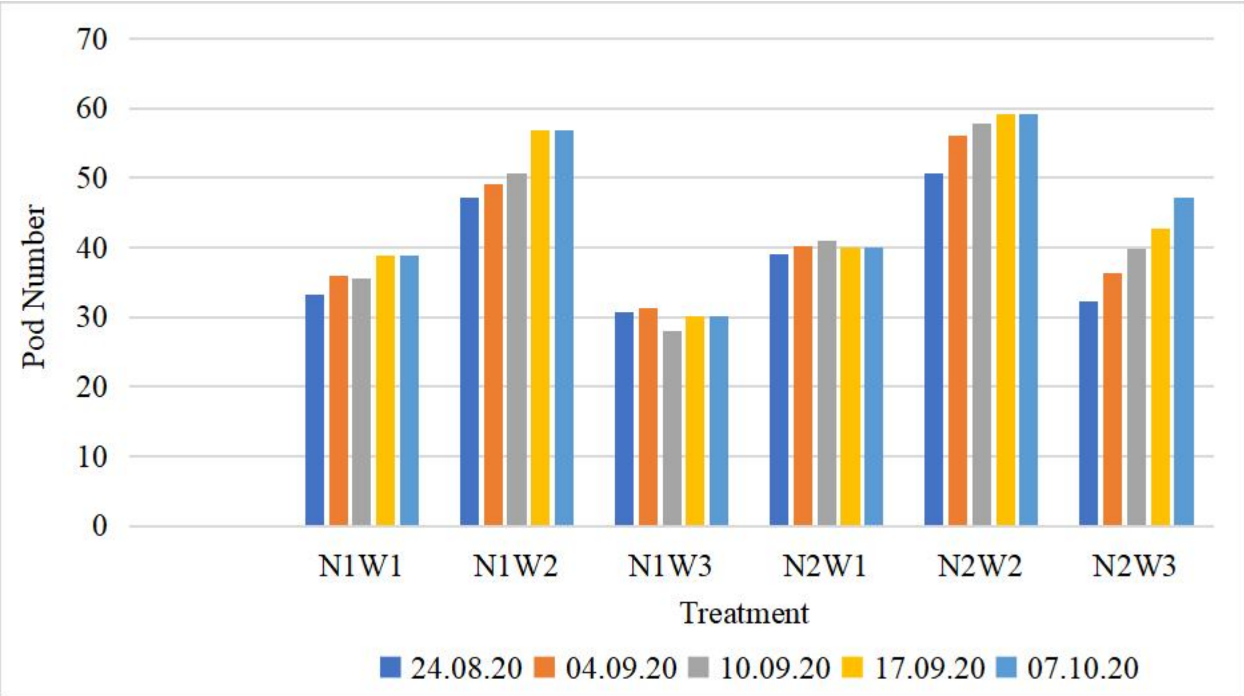


Figure 15. The increment of pod number by treatments. N1: 0 kg N/ha; N2: 200 kg N/ha; W1: Weedy; W2: Hand weeded; W3: Mechanically weeded

4.1.2 Grain yield (g/m^2)

The grain yield was not significantly different between nutrition treatments. However, the yield of soybeans with the treatment of 200 kg N/ha was 14.78% higher than that of the control (0 kg N/ha). Several previous studies have demonstrated that soybean yields give different responses to nitrogen (N) nutrition. Some studies show a positive response, and some show a negative response. A study conducted by WOOD et al. (1993) in Alabama found that the response of soybean yield to N nutrition depends on the location of cultivation, the variety used, and also the time of application. There were two out of the seven locations tested that showed a negative response regarding grain yield. Five locations that had a positive effect were also inconsistent in grain yield as there was interaction with soybean varieties. They also found that grain yield decreased when N was applied at the plant stage of R5 for all varieties tested. Therefore, they concluded that N application was not recommended because of the inconsistency of grain yield response.

Similarly, KASCHUK et al. (2016) found that application of N fertilizer did not cause an increase in yield on the two soybean varieties studied, whether N fertilizer was supplied at sowing time, during reproductive stages, or both. In contrast, TAYLOR et al. (2005) in Alabama found that N application increased seed yield regardless of planting date, variety, or location. N application of 60–70 kg/ha maximized yield and R1 dry matter accumulation. They concluded that N can be a viable input for double-cropped soybeans at an optimal economic rate of 59 kg/ha. Meanwhile, HATAMI et al. (2009) in Kashmir found that soybean yield increased significantly with the increase of N fertilizer up to 150 kg/ha. They also found that not only the grain yield increased, but N fertilizer promoted dry matter accumulation and plant growth. According to the research done by MOURTZINIS et al. (2018) across the United States, grain yield increased when N fertilizer was used up to 120 kg/ha. They tested different rates of N, from 0 to as much as 560 kg/ha.

Different grain yield responses to N application show that the success of N application on soybeans is highly dependent on the variety and also on the cultivation location. The positive response of soybean grain yield to N fertilizer is probably due to a low-nodulation variety or caused by an environmental limitation on soybean growth. Both of these factors have restricted N fixation, resulting in a positive response to N fertilizer.

The grain yields of soybeans were significantly different for the applied treatments of the weed canopy (Figure 16). A post hoc comparison using the LSD test indicated that the mean value for hand weeded was 51.52 g/m^2, which significantly differed from weedy (29.69 g/m^2) and mechanically weeded (28.32 g/m^2). However, results for the weedy canopy did not significantly differ from those of the mechanically weeded. According to research done by MARANGONI et al. (2013), weed management had a significant effect on the grain yield of soybeans. The yield of soybeans in the absence of coexistence with weeds (weeded) was higher compared to the yield of soybean in coexistence (unweeded) with weeds. The yields were reduced by 30% when no weed control was performed. Similar results were found by

NEPOMUCENO (2007), who evaluated weed interference in soybeans in a conventional sowing system and reported a 32% drop in the yield of the crop when it coexisted with weeds throughout its cycle. PIRES (2005) evaluated the competitive potential of soybean cultivars against weeds, and it was observed that all cultivars in the presence of weeds displayed yield reductions of approximately 480 kg/ha. As a result of these studies, it is clear that weeds interfere with the yield of soybeans. In fact, mechanical weeding, where the stump or root of the weed is still left in the ground, also interferes with the soybean yield. One of the possible reasons behind the drop in yield is competition between crops and weeds for sources of nutrients, water, and light.

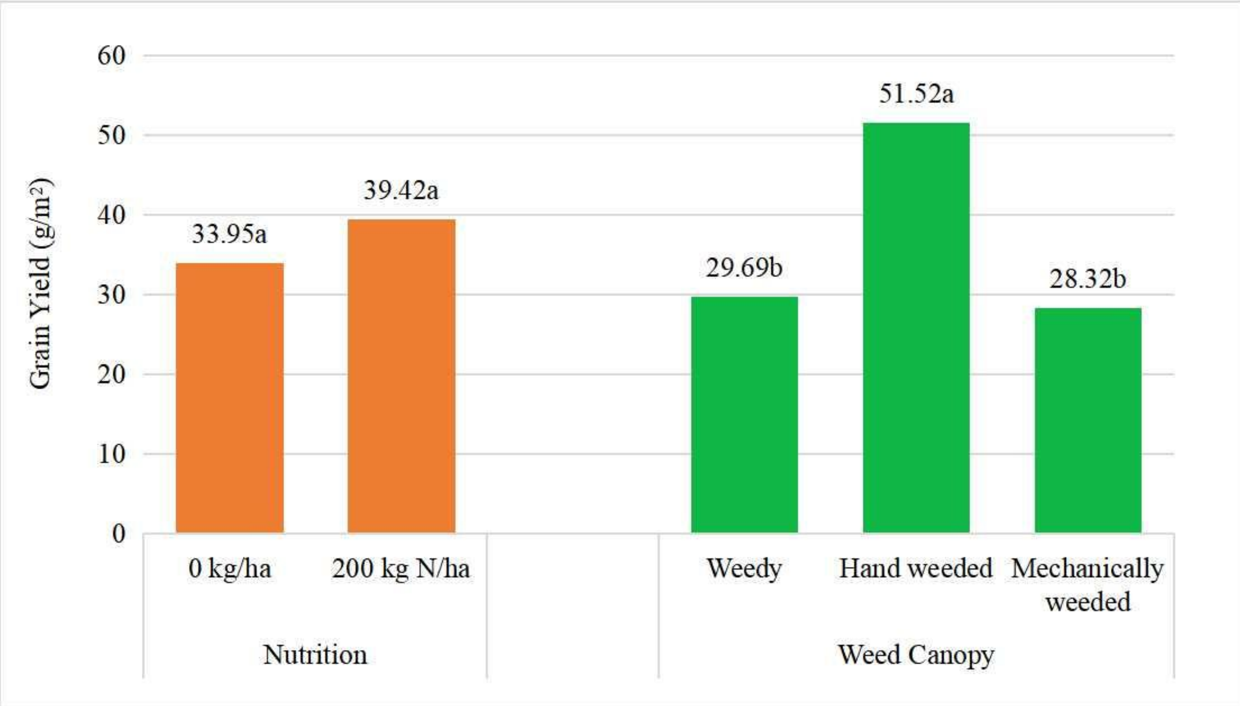


Figure 16. Effect of nutrition and weed canopy on grain yield (g/m^2) of soybeans. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

4.1.3 Protein and lipid content (%)

The results of protein content at different nutrition and weed canopy treatments are shown in Figure 17. Nutrient supply had no significant effect on protein content, the mean score difference between the two treatments was only 0.63%. According to the results, neither weed canopy treatment had any effect on the protein content. The LSD test showed that the mean score for mechanically weeded (M = 47.31, SD = 2.55) was the highest but not significantly different from weedy (M = 45.80, SD = 1.03), which did not significantly differ from hand weeded treatment (M = 45.03, SD = 2.38).

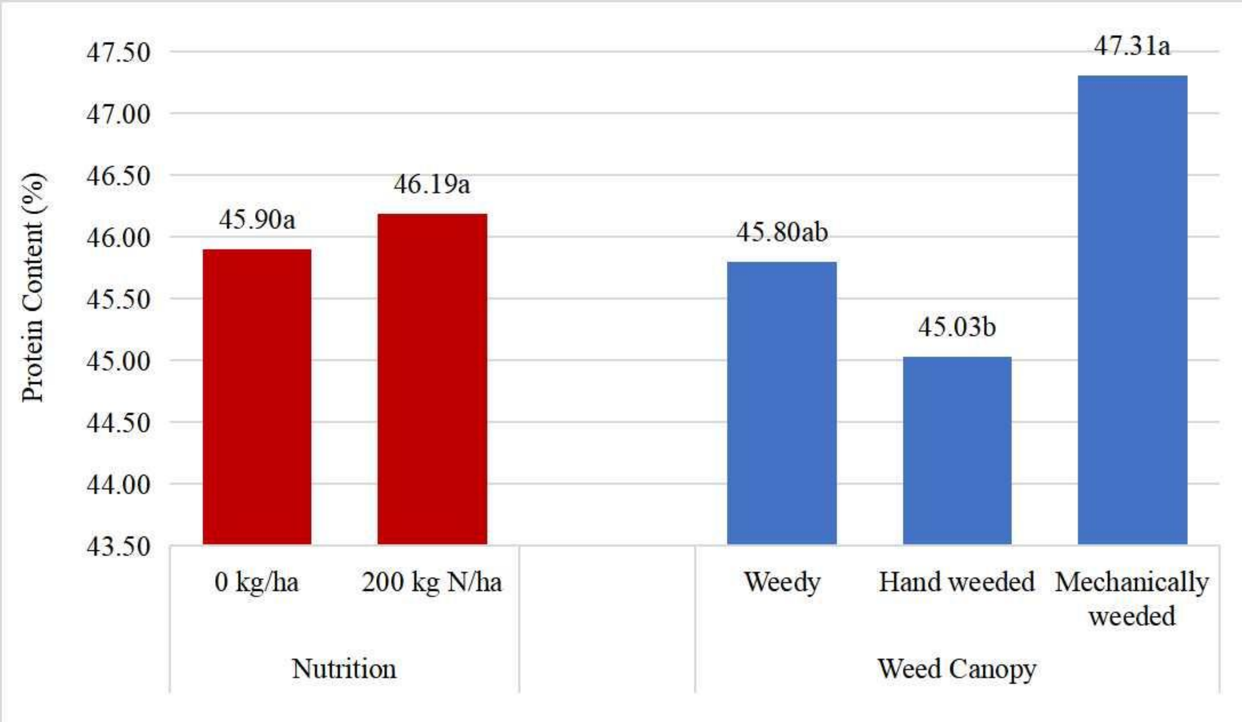


Figure 17. Effect of nutrition and weed canopy on protein content (%) of soybeans. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

The effects of nutrition and weed canopy on lipid content are presented in Figure 18. There were also no significant differences between nutrition supplies or weed canopy treatments regarding lipid contents. The mean values of lipid content for no nutrient supply treatment (0 kg N/ha) and 200 kg N/ha supplementation were 12.81% and 12.72%, respectively. The mean values of lipid content under different treatments of weed canopy were 13.17% (weedy), 13.04% (hand weeded), and 12.08% (mechanically weeded).

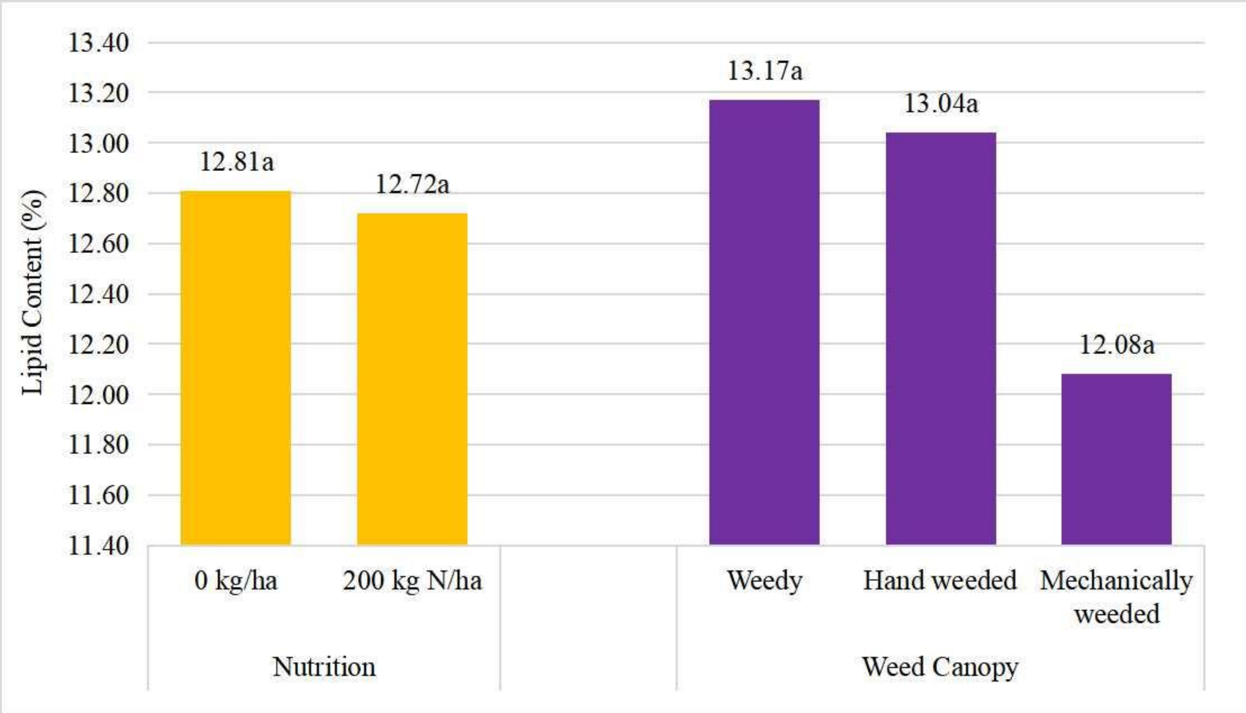


Figure 18. Effect of nutrition and weed canopy on lipid content (%) of soybeans. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

Several previous studies reported similar results for the response of the protein, oil, or lipid content of soybeans to N fertilizer. According to the research done by WOOD et al. (1993), N fertilization had no significant effect on the protein and oil concentrations of six of the seven soybean varieties tested. They indicate that N fertilization would not be an effective means of altering protein and oil concentrations of soybeans in Alabama, a selection of varieties with the desired oil and protein concentrations would be a more reliable method for producing premium soybeans based on seed composition.

TAYLOR et al. (2005) found that N applied to late-planted soybeans in the Deep South, Alabama, had no impact either on seed yield and quality or on protein and oil contents. Meanwhile, KAUR et al. (2017) reported that N application at 179 kg/ha on clay soil reduced seed protein by 1.05% compared to unfertilized soybeans, however, it increased oil content by 0.7%. Soybean seed composition showed inconsistent responses to N fertilization, probably due to climatic conditions such as lower temperatures and higher precipitation during seed filling, which may reduce protein and oil concentrations, whereas high air temperatures and moderate rainfall during the seed filling period can result in higher protein concentrations in soybean seeds (BENNETT and KRISHNAN 2005).

There is limited data on the effect of weeds on the protein and lipid contents of soybeans. However, there is a study done by ARIUNAA et al. (2016) on weed management using chemical control. They found that the fat contents of the seeds were 12.9–18.3% and the protein contents were 29.9–31.5%. They found no significant difference between control (unweeded) and chemical control using herbicides on either protein or fat contents. Therefore, the presence of weeds does not affect the protein and fat contents of soybeans, the inconsistency in chemical composition may be due to other factors such as environmental limitations like drought or water stress conditions. Water stress during the early reproductive stages resulted in a 16% decrease in seed protein (ROTUNDO and WESTGATE 2009). However, oil concentrations of two varieties responded to drought stress in an opposite trend to protein concentration, drought stress increased oil concentration, regardless of N application and rate (BASAL et al. 2020). According to the research done by PEER et al. (2013), weed control methods (weedy, hand weeding, integrated hand weeding and herbicide, and herbicide) showed that integrated hand weeding and herbicide recorded comparable lipid content in soybean seed, and the lowest lipid percentage was seen in the weedy plot.

In this study as well, the protein content is greater and the lipid content is lower than the standard average of protein and lipid content for soybean plants. As discussed in Chapter 2 (Literature Review), this is likely due to different varieties and environmental factors such as differences in soil type, weather, climate, and agronomic practices that are used directly or indirectly to influence the production of different chemical compositions for soybean.

4.1.4 Protein and lipid yield (g/m^2)

There were no significant differences in protein yields under different nutritional conditions. The results in Figure 19 show that the application of 200 kg N/ha gave a 14.69% higher soybean yield than without nutrient supply treatment. The results also revealed that the protein yields under different weed canopy conditions (Figure 19) were significantly higher at the hand weeded treatment (23.35 g/m^2), followed by the weedy treatment (13.73 g/m^2), and the mechanically weeded treatment (13.37 g/m^2). However, there was no significant difference between weedy and mechanically weeded treatments.

The lipid yield of samples showed a similar trend to that of the protein yield (Figure 20). No significant difference was shown between the nutrition treatments, but a significant difference was shown between the weed canopy treatments. The lipid yield of soybeans supplied with 200 kg N/ha was 12.71% higher compared to no nutrient supply condition. Meanwhile, the LSD test results on the weed canopy showed that hand weeded conditions (6.51 g/m^2) provided significantly higher yields than weedy (3.88 g/m^2) and mechanically weeded conditions (3.23 g/m^2). However, the weedy condition did not significantly differ from the mechanically weeded condition.

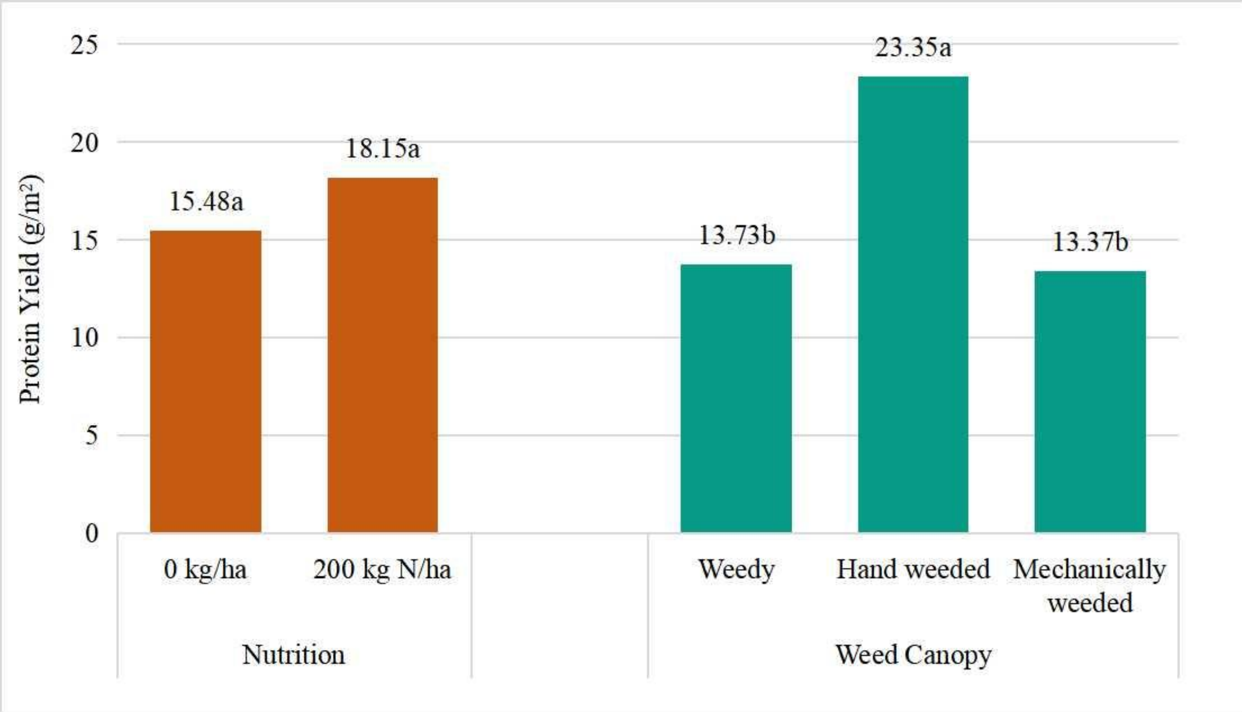


Figure 19. Effect of nutrition and weed canopy on protein yield (g/m^2) of soybeans. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

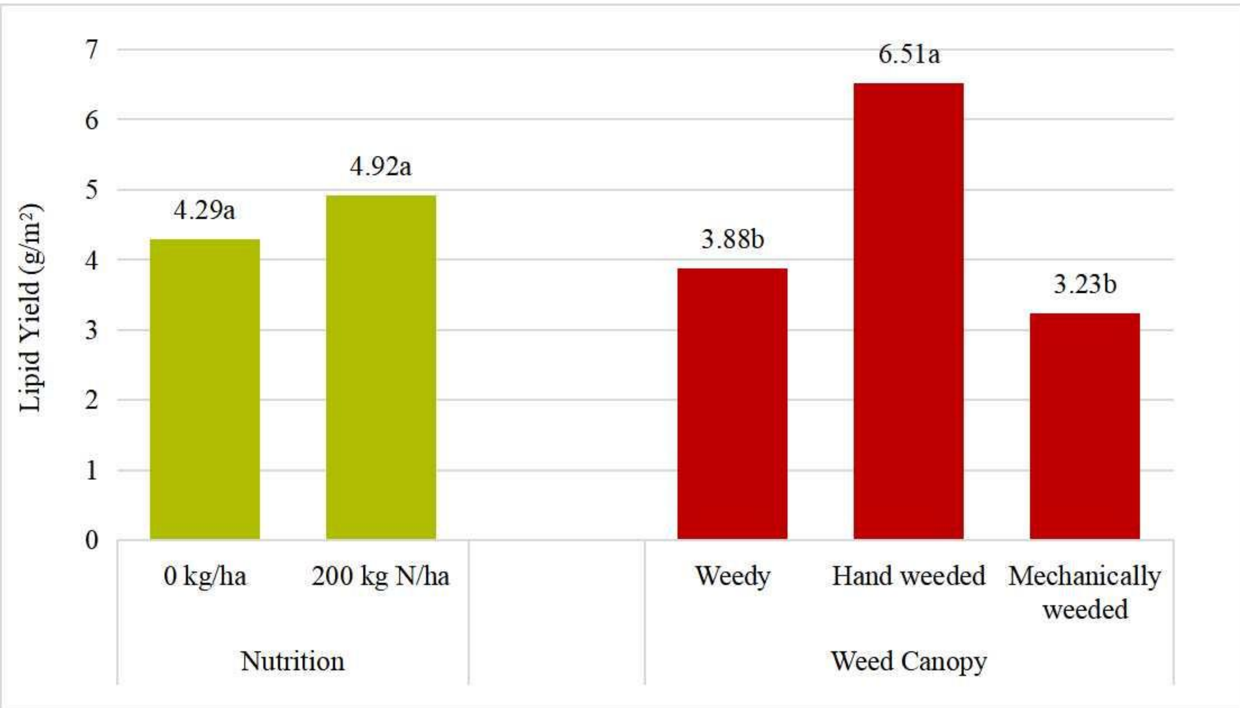


Figure 20. Effect of nutrition and weed canopy on lipid yield (g/m^2) of soybeans. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

Protein and lipid yields were calculated on the basis of concentration multiplied by grain yield. Although the concentrations of protein and lipid did not show significant differences under weed canopy conditions, the high grain yield for hand weeded treatments caused higher protein and lipid yields than for other treatments. Soybean production based on chemical composition yield is important for the production of processed food products and oils.

4.2 Experimental Research 2: Influence of temperature and varieties on seed germination of soybeans at different germination times

4.2.1 Results

4.2.1.1 Germination rate (%)

According to the ANOVA table (Table 14) below, there were significant effects of the day after sowing, temperature, and variety on the germination rate of soybeans at $p < 0.05$. However, the two main factors, which were temperature and variety, showed a significant ($p < 0.05$) interaction effect. Meanwhile, there was no significant interaction between day x temperature, day x variety, and day x temperature x variety on the germination rate.

Table 14. Analysis of variance (ANOVA) for the germination rate of soybeans as affected by day, temperature, and variety

Source	Sum of Squares	df	Mean Square	F	Sig.
Day (D)	2352.54	3	784.18	7.58	0.00
Temperature (T)	15568.00	2	7784.00	75.28	0.00
Variety (V)	6112.04	1	6112.04	59.11	0.00
D x T	343.08	6	57.18	0.55	0.77
D x V	691.38	3	230.46	2.23	0.09
T x V	2465.33	2	1232.67	11.92	0.00
D x T x V	890.25	6	148.38	1.44	0.21
Error	7445.00	72	103.40		
Total	35867.63	95			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

The effect of day on the germination rate is shown in Figure 21. Both varieties showed an increase in germination rate from Day 3 (69%) to Day 9 (82%). The germination rate on Day 3 was the lowest and significantly differed from Days 5, 7, and 9. However, the rates on Days 5, 7, and 9 were not significantly different from each other. The Martina variety had a higher germination rate (85%) compared to the Johanna variety (70%) (Figure 22). The varieties interacted with temperatures and had a significant effect on the germination rate (Figure 23). The Martina reached 100% germination at a temperature of 15 °C and 96% at a temperature of 25 °C. Meanwhile, the Johanna variety showed a much lower germination percentage than Martina, which was 74% at 15 °C and 76% at 25 °C. The germination dramatically decreased when both varieties were exposed to high temperatures (35 °C), and the germination only achieved 61% for Martina and 58% for Johanna.

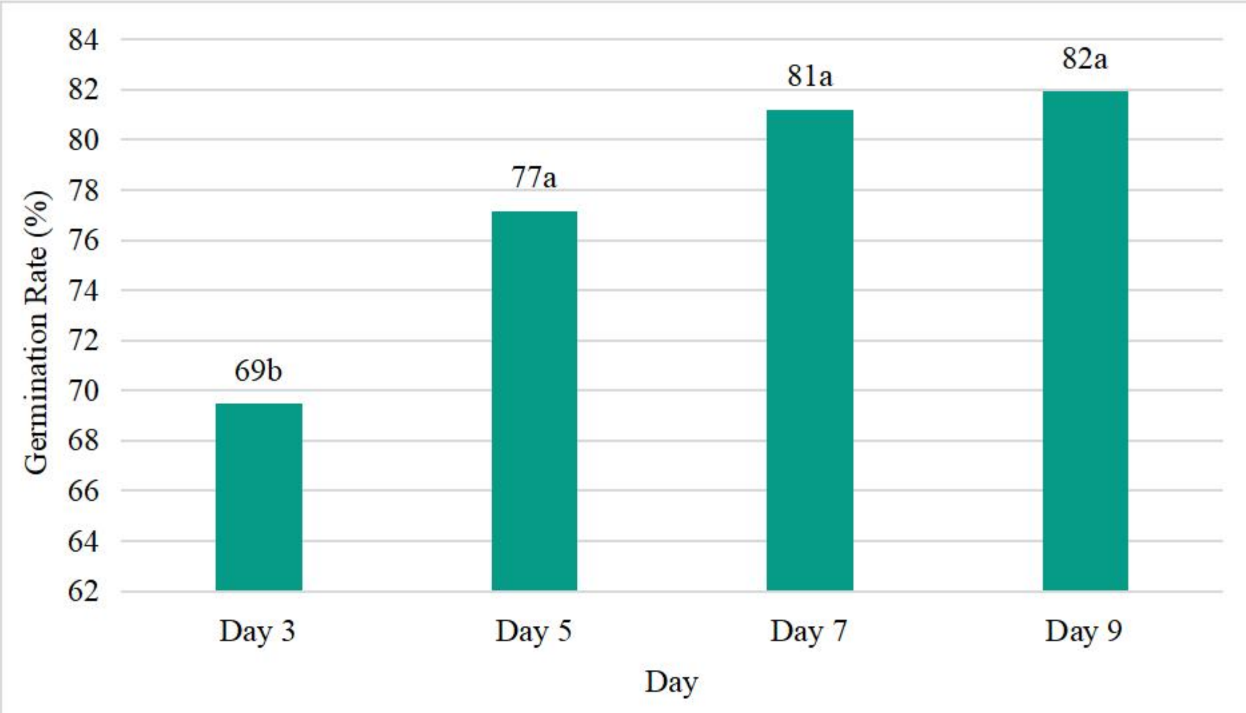


Figure 21. Effect of day on germination rate of soybeans. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

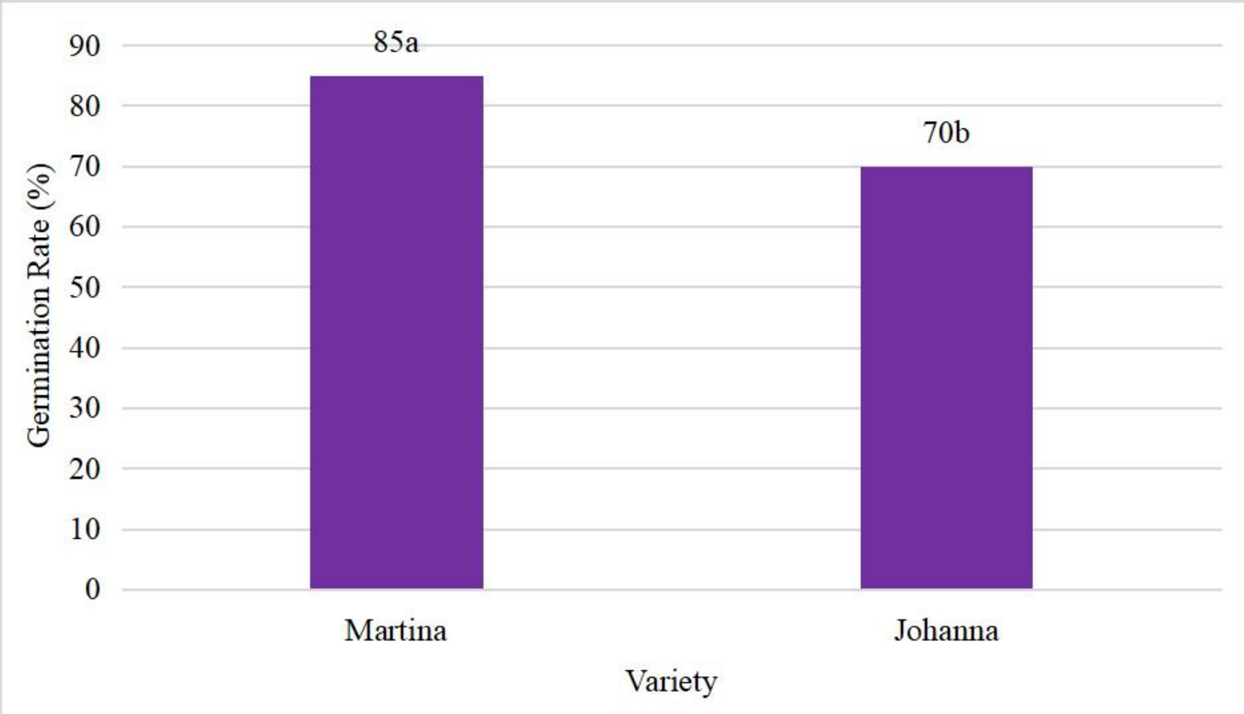


Figure 22. Effect of variety on the germination rate of soybeans. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

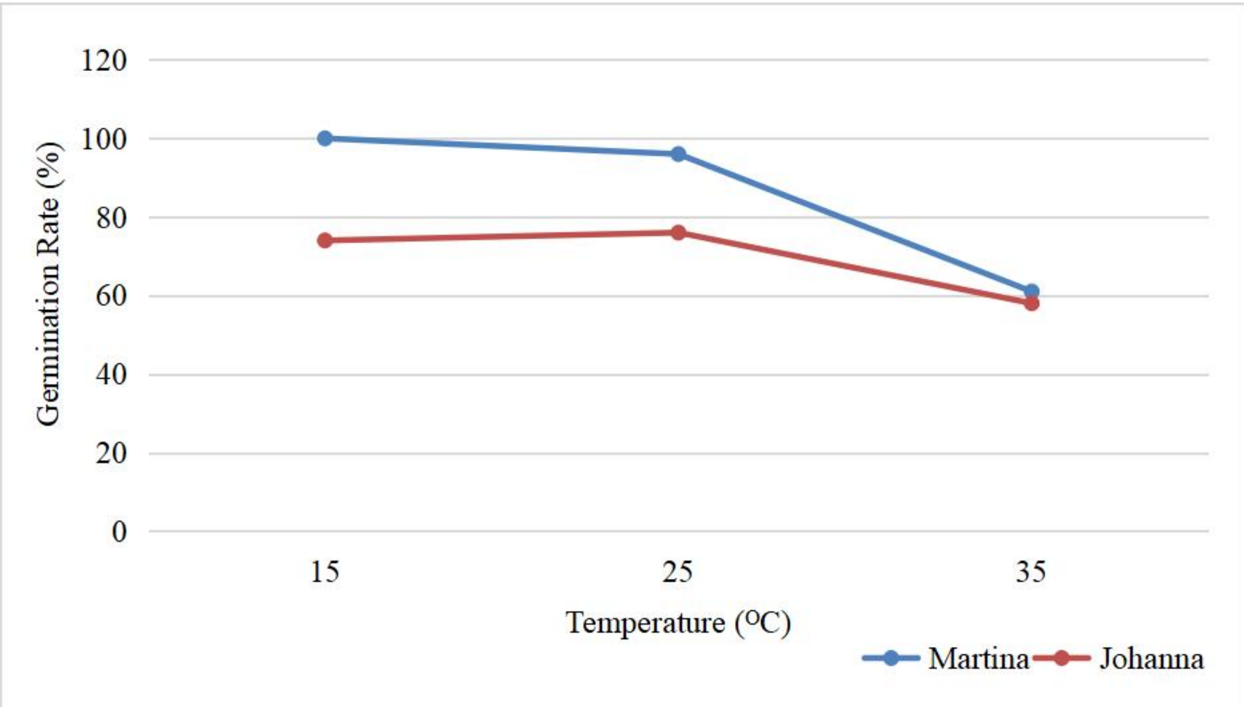


Figure 23. Interaction effect of temperature and variety on the germination rate of soybeans

4.2.1.2 Total seedling length (cm)

The analysis of variance (ANOVA) on the total seedling length showed that all main and interaction effects gave significant results at $p < 0.05$ (Table 15). Therefore, the results were only shown and discussed for the interaction effect between three factors (day, temperature, and variety).

Table 15. Analysis of variance (ANOVA) for the total seedling length of soybeans as affected by day, temperature, and variety

Source	Sum of Squares	df	Mean Square	F	Sig.
Day (D)	223.88	3	74.63	1093.00	0.00
Temperature (T)	239.44	2	119.72	1753.00	0.00
Variety (V)	47.46	1	47.46	694.90	0.00
D x T	72.22	6	12.04	176.24	0.00
D x V	5.41	3	1.80	26.39	0.00
T x V	16.10	2	8.05	117.90	0.00
D x T x V	4.45	6	0.74	10.86	0.00
Error	4.92	72	0.07		
Total	613.87	95			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

The interaction graph between day, temperature, and variety on total seedling length is shown in Figure 24. The total seedling length increased until Day 9 for both varieties when exposed to temperatures of 15 °C and 25 °C. The longest total seedling length was detected at a temperature of 25 °C, which was 10.83 cm (Martina) and 6.55 cm (Johanna). However, the total seedling length increased only until Day 7 for both varieties when exposed to a high temperature of 35 °C. The total seedling length at a temperature of 35 °C was also the shortest for both varieties compared to the length at temperatures of 15 °C and 25 °C. On Day 9, the total seedling length at the temperature of 35 °C was only 2.63 cm for the Martina and 1.58 cm for the Johanna. Overall, the Martina variety showed a longer total seedling length on varying days (3, 5, 7, 9) and at all temperatures compared to the Johanna variety. The difference in total seedling length between both varieties on Day 9 was 1.08 cm, 4.28 cm, and 1.05 cm, respectively, at temperatures of 15, 25, and 35 °C.

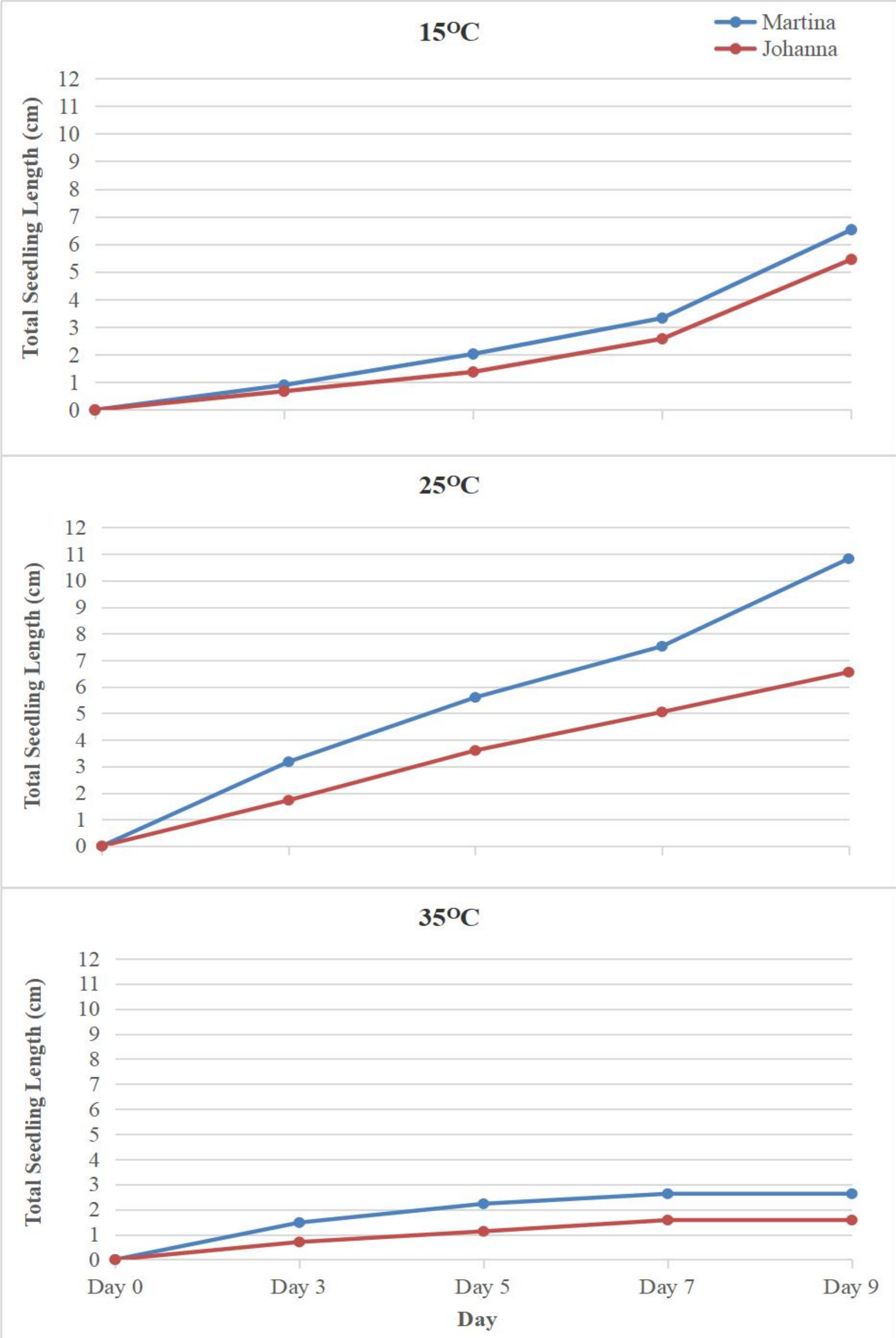


Figure 24. Interaction effect of day, temperature, and variety on the total seedling length of soybean

4.2.1.3 Viability (%)

The viability results revealed that the main effect of temperature and variety had a significant value at $p < 0.05$ (Table 16). Meanwhile, there was no significant interaction between temperature and variety.

Table 16. Analysis of variance (ANOVA) for the viability of soybeans as affected by temperature and variety

Source	Sum of Squares	df	Mean Square	F	Sig.
Temperature (T)	45046.33	2	22523.17	324.20	0.00
Variety (V)	416.67	1	416.67	5.99	0.03
T x V	272.33	2	136.17	1.96	0.17
Error	1250.50	18	69.47		
Total	46985.83	23			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

The effect of temperature on the viability of soybeans is shown in Figure 25. At temperatures of 15 °C and 25 °C, the percentage of viability for both varieties was not significantly different. However, the percentage of viability at those two temperatures was significant with the percentage of viability under a temperature of 35 °C (0%). In addition, variety also had a significant effect on viability. The Martina variety performed better and was more viable than the Johanna variety (Figure 26). The difference in the viability percentage of the Martina and Johanna varieties was 8%.

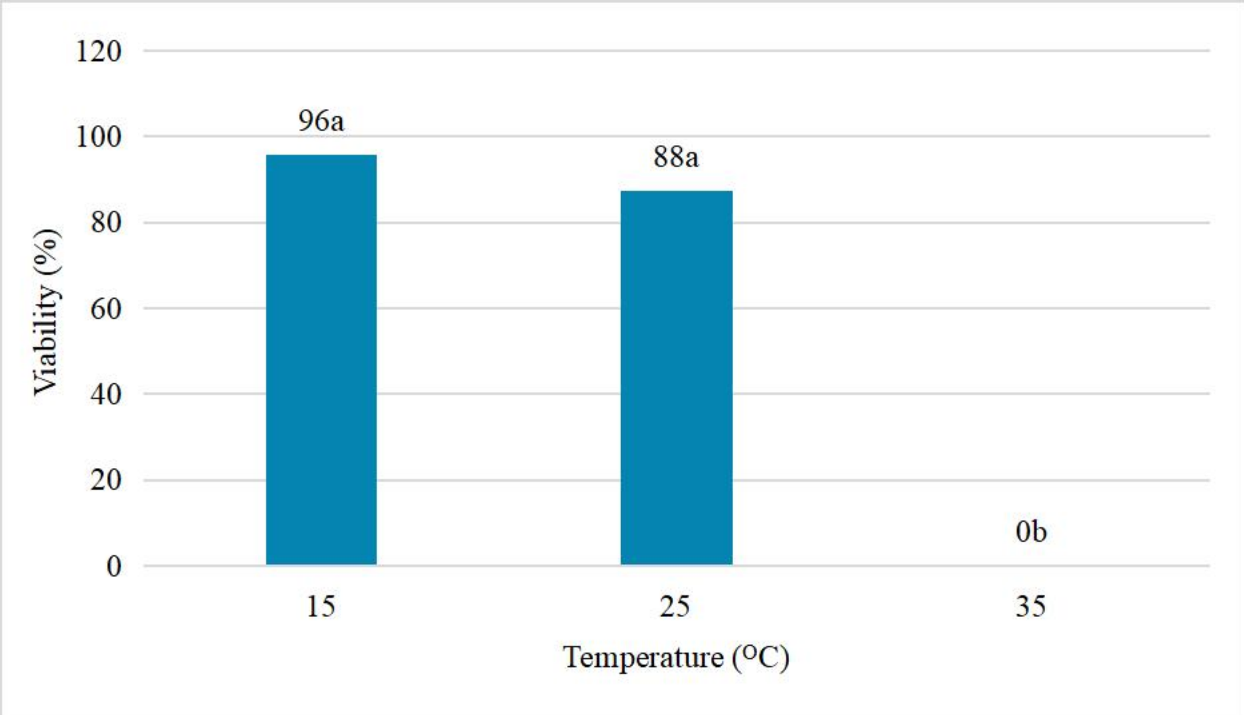


Figure 25. Effect of temperature on the viability of soybeans. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

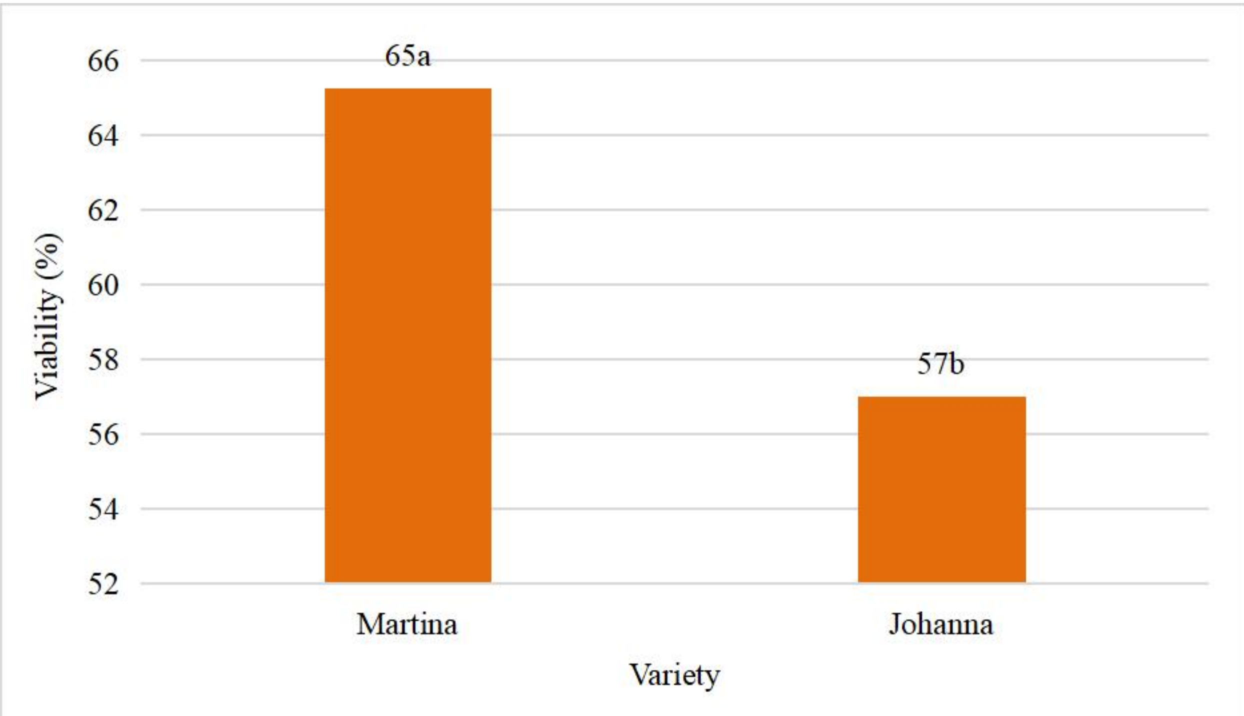


Figure 26. Effect of variety on the viability of soybeans. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

4.2.2 Discussion

4.2.2.1 Germination time affected the germination rate

According to the findings of our study, the percentage of germination rate peaked at 82% on Day 9, but there was no significant difference between the germination rate on Day 5 and Day 7. This means that seeds from both varieties completed germination as early as the fifth day. Other research on soybean seeds discovered that the number of germinated soybeans increased as the number of days increased (RAY et al. 2015). A study involving several maize varieties revealed that all varieties began germination on the third day. All the varieties reach 100% germination on the 12th day, except one variety that germinated on the 9th and one variety on the 7th day (OMAR et al. 2022).

The percentage of germination that increases with time is typical in the initial stages of plant growth. However, the increase will vary depending on the type of plant and its variety. GLORIA and OSBORNE (2014) and WAINWRIGHT et al. (2012) reported that species that germinate earlier than other species can benefit from early access to some resources, space, and reduced competition during the establishment of the early stage. Apart from that, information on germination percentage is important not only to understand the early growth stages of soybeans but also to be used in the determination of seed rate for field or next crop planting. If the seed has an 80% germination rate, 70 to 80 kg of seed per hectare is required (JAGDISH 2020).

4.2.2.2 Temperature and variety interacted on germination rate

The study also discovered that temperature and variety interacted strongly in affecting the germination rate. The Martina variety had a higher germination rate not only at optimal temperatures (25 °C) but also at low temperatures (15 °C). SZCZERBA et al. (2021) similarly found an interaction between temperature and variety in the germination of four varieties of Polish soybean seeds. Their result found that at 15 °C, only one variety germinated more than 50%, and the other three varieties germinated between 19.9% and 26.7%. At 25 °C, the seeds of the two varieties germinated 100%, and the seeds of the other two varieties germinated 98%. Their findings revealed that the responses of soybean seeds were highly different when influenced by low and optimal temperatures, but there was also a variety responding well to low temperatures.

Studies on maize varieties also discovered a significant interaction between temperature and variety on germination percentage (ALI 2018). The study found that at a temperature of 10 °C, all 20 tested varieties were able to germinate by 93-100%. On the other hand, at temperatures of 8.6 °C and 7.2 °C, the difference in germination percentage in most varieties was very significant, with some varieties reaching only 7% germination and others reaching up to 85% germination. LIU et al. (2021) also found a significant impact on the interaction between temperature and variety on the germination percentage of common grass.

For the effect of high temperatures on seed germination, previous studies reported that most seed germination performances dropped and were lower at high temperatures. Likewise, our research observed that the germination rate decreased and was lowest at a temperature of 35 °C. This finding was supported by a study conducted by RAY et al. (2015) on the effects of temperature stress on the germination of soybean seeds. They discovered that the number of seedlings that emerged at high temperatures of 40, 50, and 60 °C was lower than the control. The range of germinated seeds was between 60 and 40 seeds per 100 seeds at those high temperatures.

Most research, including ours, found that temperatures influenced most of the germination of plant varieties. When exposed to a certain temperature, the seed may react in the formation of the chemical composition, subsequently affecting germination. Most varieties have their own particular characteristics and differ from one another in terms of physical or chemical composition. In oil-seed plants, such as soybean varieties that contain low lipids, the germination process is slow. This was reported by MIQUEL and BROWSE (1994), who conducted a study on two *Arabidopsis thaliana* varieties and discovered that cultivars with low lipid content germinated slower at temperatures of 10 °C and 6 °C. This confirmed our findings that the Johanna variety showed a low percentage of germination at all tested temperatures (15, 25, and 35 °C), which is one of the causes probably due to the fact that the lipid content of the Johanna variety was lower than the Martina varieties (data not included).

4.2.2.3 Germination time, temperature, and variety interacted on total seedling length

Total seedling length, which includes root and shoot length, is one of the indicators used to determine seedling vigour (REDOÑA and MACKILL 1996). Seedling vigour is defined as a seed's ability to emerge rapidly from soil or water, mainly referring to early seedling growth (HUANG et al. 2004). In our study, total seedling length increased with increasing time up to Day 9, when seeds were exposed to low (15 °C) and optimal (25 °C) temperatures. Therefore, both of the tested soybean varieties (Martina and Johanna) showed good seedling vigour because they can survive at a critical stage of early plant growth when exposed to low and optimal temperatures. However, seedlings for both varieties were more vigorous at 25 °C than at 15 °C because they had a longer seedling length at all germination periods (Day 3, 5, 7, and 9) under the temperature of 25 °C. This finding stands in line with previous research on soybean germination, which found that the optimal temperature for germination for most varieties of soybeans was 25 °C. Although soybeans can also easily germinate at low temperatures (10 °C), germination is quite slow (JAGDISH 2020, ANONYMOUS 2023b). The Martina variety was more vigorous than the Johanna variety because it emerged faster and had a longer total seedling length at each temperature and germination time.

When seeds were exposed to high temperatures (35 °C), the total seedling length increased until Day 7, and the length was also the shortest at this temperature. According to a study conducted on temperature stress (40, 50, and 60 °C) in soybeans by RAY et al. (2015), there was a significant decrease in vigour when temperature and time (the number of days) were increased. This is most likely due to the drying of seedlings, which may be caused by conformational changes and hence the molecular deterioration of biochemicals within seeds under high temperatures and varying times.

4.2.2.4 Temperature and variety affected the percentage of seed viability

Our findings also revealed that the main factor of temperature affected the seed viability of soybeans. The percentage of viability at low temperatures was not much different from the percentage of viability at optimal temperatures. However, at high temperatures, no seeds were viable, although some seeds germinated, the germinated seeds only survived until Day 7 and then died. Also, our investigation found that the more viable variety was Martina, with a different viability percentage from the Johanna variety of 8%. Viability refers to whether a seed is alive or not, and the percentage of viable seeds is not necessarily the same as the percentage of germinated seeds (BASARA et al. 2002, ANONYMOUS 2023c). This difference is probably because some seeds are immature or dormant.

According to the findings of SZCZERBA et al. (2021) on the percentage of germinated soybean seeds exposed to temperatures of 10, 15, and 25 °C, the percentage of germinated seeds from four soybean varieties was highest at 25 °C, followed by 15 °C and 10 °C. They discovered that seeds from the Abelina variety were the most viable when compared to the other three varieties, with the Petrina variety producing the lowest percentage of seedlings, which was less than 40%. The results of numerous publications confirmed that a temperature of 25 °C, also used in our experiment, is the most beneficial for the germination of thermophilic plant species such as soybeans (HATFIELD and EGLI 1974, LADROR et al. 2022), peas, beans (ZAITER et al. 1994), and maize (WANG et al. 2018). The ideal germination temperature is an important factor that influences further plant development, and seed viability is one of the indications used to evaluate further plant growth.

4.3 Experimental Research 3: Response of growth, yield, and chemical composition to nutrient concentrations of soybean varieties grown using a soilless culture system in a controlled environment

4.3.1 Results

4.3.1.1 Plant height (cm)

The result of the studies showed that there was no significant difference between nutrient concentration treatments in plant height at the vegetative stage of soybeans grown using a soilless culture in a controlled environment (Table 17). The study also showed that there was no significant difference between soybean varieties in plant height. However, the main effect of the week significantly affected the plant height at $p < 0.05$. As seen in Figure 27, the plant height increased every week until the fifth week.

Table 17. Analysis of variance (ANOVA) for plant height of soybean as affected by nutrient concentration, variety, and week number

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	43.6	3	14.53	2.10	0.11
Variety (V)	15.48	1	15.48	2.23	0.14
Week (W)	1609.26	4	402.32	58.04	0.00
N x V	127.52	3	42.51	6.13	0.00
N x W	24.91	12	2.08	0.30	0.99
V x W	14.39	4	3.60	0.52	0.72
N x V x W	32.43	12	2.70	0.39	0.96
Error	554.51	80	6.93		
Total	2422.13	119			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

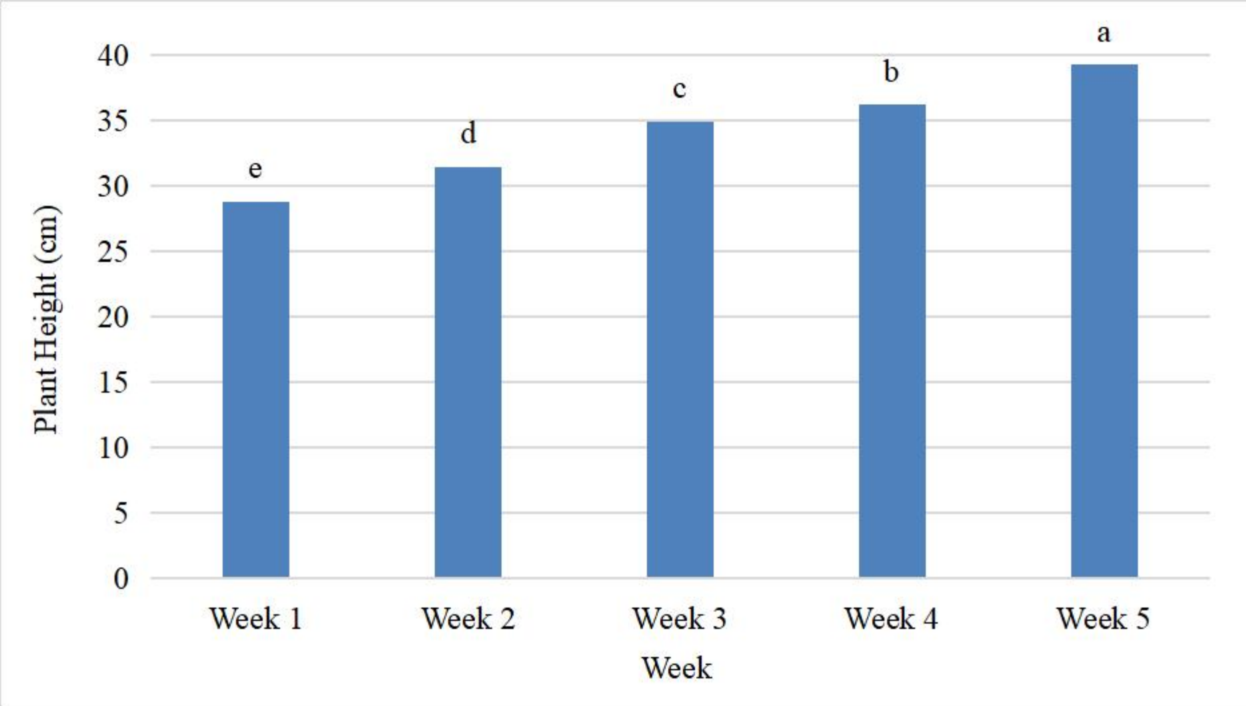


Figure 27. Effect of week number on plant height at early growth of soybeans grown using soilless culture. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

There was also an interaction effect between nutrient concentration and variety on soybean plant height (Table 17). The interaction effect is shown in Figure 28. The plant height of the Martina variety was higher than the Johanna variety when no nutrient (0%) was applied. When nutrients were applied up to 50% of the complete plant requirement, the Martina and Johanna varieties showed almost the same height, with a value of 34.17 cm and 34.21 cm, respectively. However, the plant height of the Martina variety was higher (36 cm) than the Johanna variety (32.35 cm) when nutrients were supplied at a concentration of as much as 100%. The Johanna variety was then higher in plant height (35.88 cm) compared to the Martina variety (33.86 cm) at a nutrient concentration of 150%.

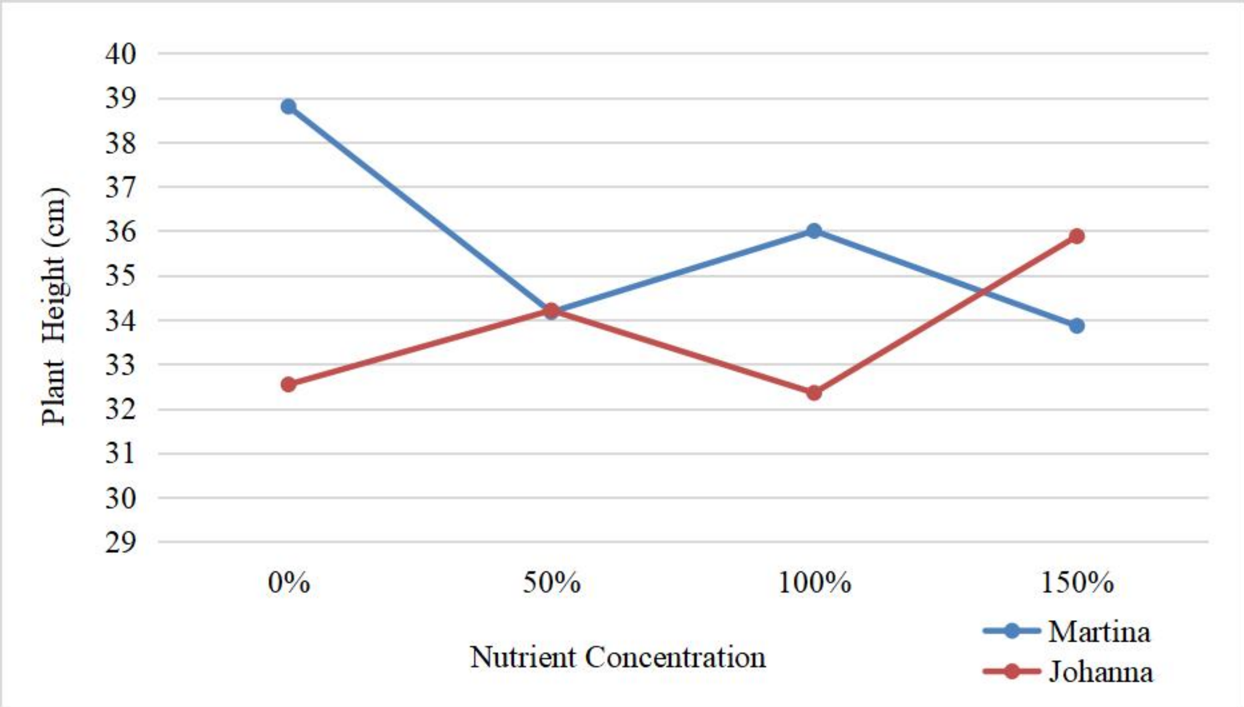


Figure 28. Interaction effect of nutrient concentration and variety on plant height at early growth of soybeans planted under soilless conditions

4.3.1.2 Number of leaves

Meanwhile, the three main effects of nutrient concentration, variety, and week have a significant impact on the average number of leaves (Table 18). However, the number of leaves of the soybean plants with treatment without nutrient (0%) and with 50% nutrient were not significantly different at $p < 0.05$, the number of fully expanded leaves for both treatments was found to be 7 leaves (Figure 29). They were the lowest and very significant at $p < 0.05$ compared with the number of leaves at 100% and 150%. Soybeans treated with 100% and 150% nutrient concentrations produced 9 leaves, two more than plants treated with 0 and 50% nutrients. The effect of variety on the number of soybean leaves at the early growth stage showed that both varieties of Martina and Johanna have a significant difference at $p < 0.05$. The number of leaves for the Johanna variety was greater than the number of leaves for the Martina variety, which respectively had 9 and 8 leaves.

Table 18. Analysis of variance (ANOVA) for the number of leaves of soybean as affected by nutrient concentration, variety, and week number

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	45.76	3	15.25	21.28	0.00
Variety (V)	3.01	1	3.01	4.20	0.04
Week (W)	1085.00	4	271.25	378.49	0.00
N x V	4.56	3	1.52	2.12	0.10
N x W	14.87	12	1.24	1.73	0.08
V x W	13.87	4	3.47	4.84	0.00
N x V x W	6.40	12	0.53	0.74	0.70
Error	57.33	80	0.72		
Total	1230.79	119			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

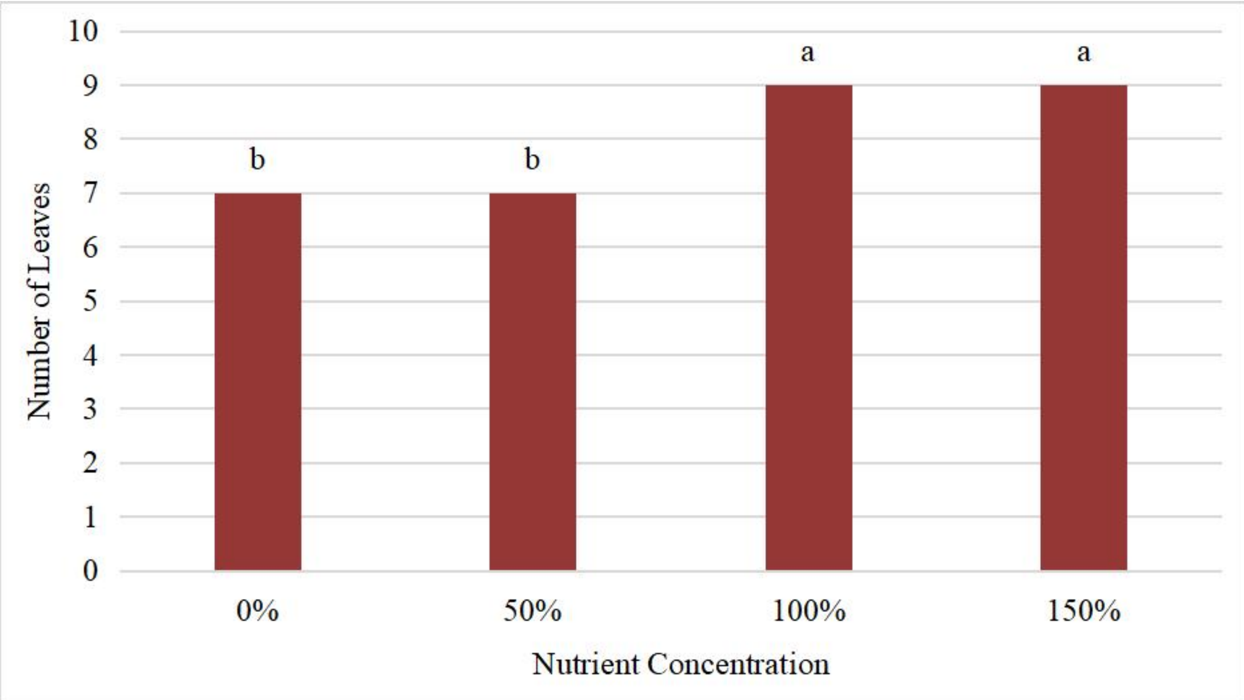


Figure 29. Effect of nutrient concentration on the average number of leaves at early growth of soybeans planted under soilless culture conditions. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

There was also a significant interaction between variety and week on the number of leaves. The number of leaves for both varieties increased with an increasing number of weeks (Figure 30). In Week 1, the number of leaves for the Johanna variety, was higher than the Martina variety, with 5 and 3 leaves respectively. Meanwhile, the number of leaves for both varieties was 6 leaves in the second week and 8 leaves in the third week. However, the Johanna variety increased to 10 leaves and exceeded the number of leaves for the Martina variety (9 leaves) in Week 4. The number of leaves for the Martina variety then continued to increase in Week 5 and reached a higher level than the Johanna variety, which had 13 leaves for Martina and 12 leaves for Johanna.

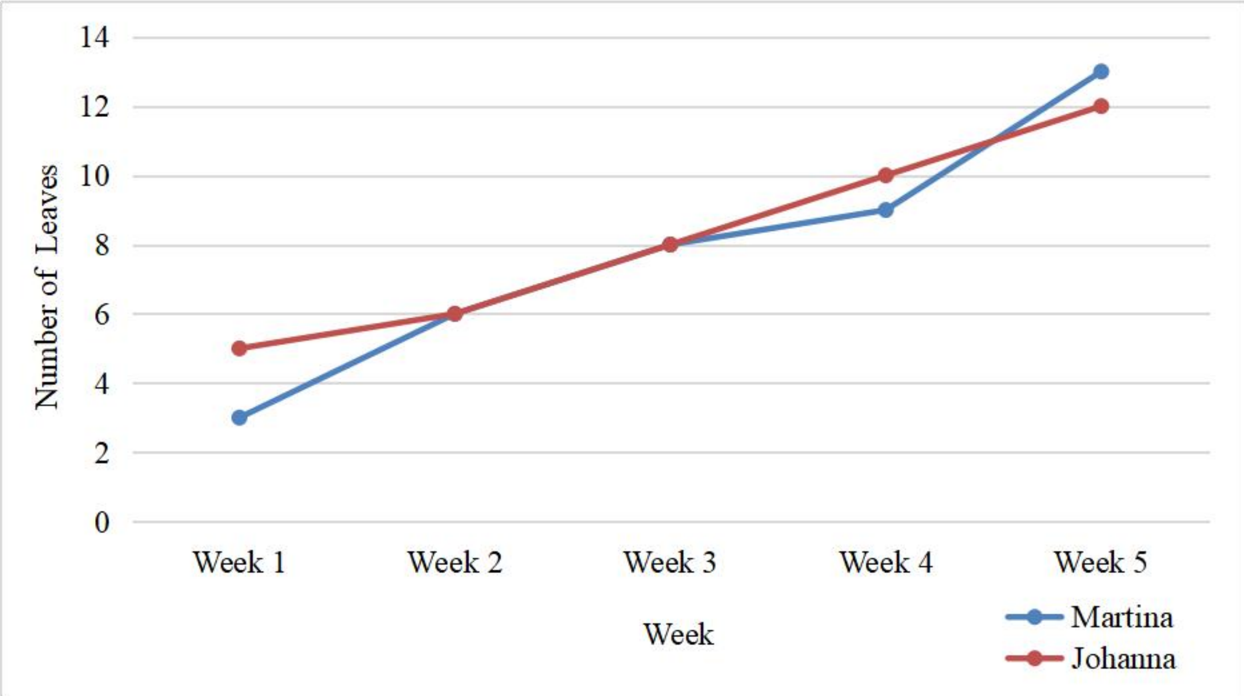


Figure 30. Interaction effect of variety and week on the number of leaves at early growth of soybeans planted under soilless conditions

4.3.1.3 SPAD reading

There were no significant differences in the main effect of nutrient concentration and variety on SPAD reading at the early growth stages of soybeans (Table 19). However, the main effect of the week number was significant ($p < 0.05$) on SPAD reading. A significant interaction was also found between variety and week number on the SPAD reading (Table 19). The SPAD reading for both varieties increased weekly until Week 3 (Figure 31). In Week 1, Week 2, and Week 3, the Johanna variety gave a higher SPAD reading than the Martina variety. In Week 1, the SPAD reading for the Johanna variety was 35.17, and the SPAD reading for the Martina variety was lower by 2.49 than the Johanna variety. The SPAD reading in Week 2 was 36.70 for the Johanna variety and 35.93 for the Martina variety. In Week 3, the SPAD reading was at its maximum value, where Johanna and Martina, respectively, had a SPAD reading of 38.65 and 38.21. The decrease in SPAD reading was found for both varieties in Week 4 and Week 5. The Johanna variety decreased drastically and had a lower SPAD reading compared to the Martina variety in Weeks 4 and 5.

Table 19. Analysis of variance (ANOVA) for SPAD readings of soybeans as affected by nutrient concentration, variety, and week number

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	14.38	3	4.79	2.57	0.60
Variety (V)	3.23	1	3.23	1.73	0.19
Week (W)	284.24	4	71.06	38.08	0.00
N x V	13.13	3	4.38	2.35	0.08
N x W	22.72	12	1.89	1.01	0.44
V x W	53.45	4	13.36	7.16	0.00
N x V x W	15.08	12	1.26	0.67	0.90
Error	149.30	80	1.87		
Total	555.52	119			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

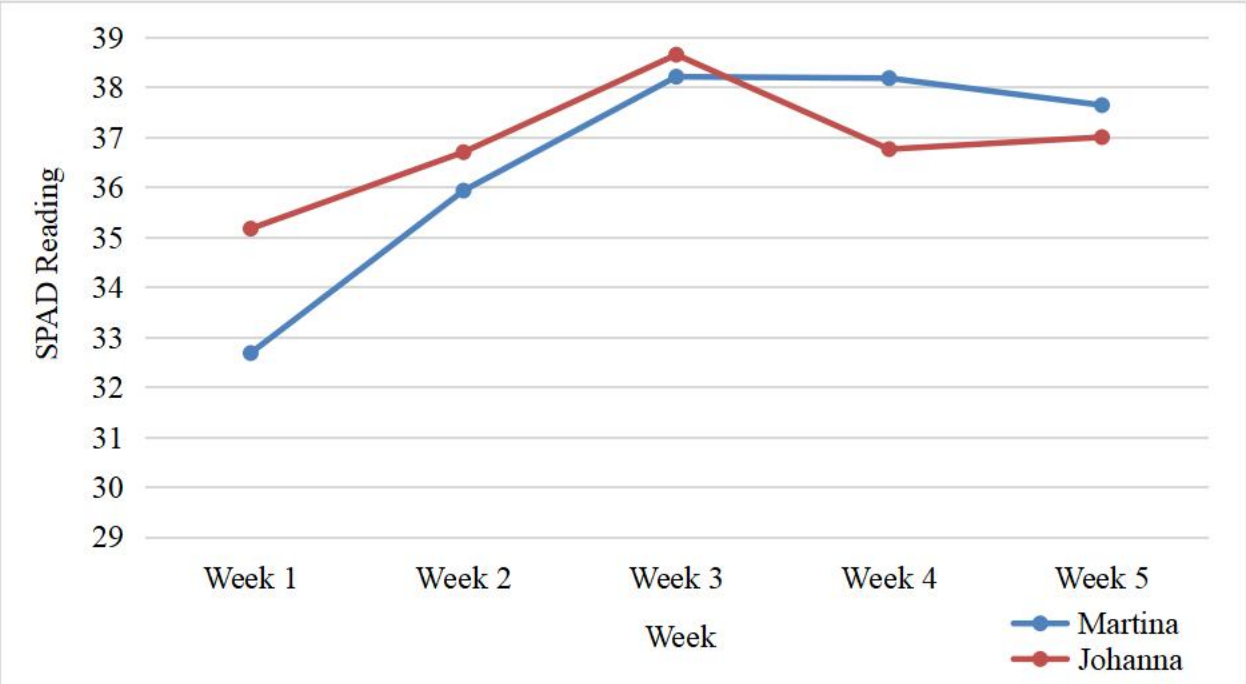


Figure 31. Interaction effect of variety and week on SPAD reading at early growth of soybeans planted under soilless conditions

4.3.1.4 Leaf area (cm^2)

All three factors that were tested, which were nutrient concentration, variety, and week, showed significant effects on leaf area (Table 20). However, there was no significant interaction between the treatments for all tested factors. The effect of nutrient concentration on leaf area showed an increase in leaf size with increasing nutrient concentration (Figure 32). The leaf size was the largest and most significant in the early growth of soybeans, which was supplied with nutrients that were more than the full plant requirement (150%). The leaf area of the soybean with a nutrient concentration of 150% was 37.40 cm^2, which was 1.98 higher than the size of the leaf with the concentration of the full plant requirement (100%) at 35.42 cm^2. Meanwhile, without nutrient treatment (0%) showed the smallest soybean leaf size, which was 30.21 cm^2, and had a significant difference at $p < 0.05$ with other treatments. The leaf area for the 50% treatment was the second lowest, with a leaf size of 32.37 cm^2. The main effect of variety on the leaf area of soybeans showed that the Martina variety had a larger leaf area than the Johanna variety, which was 36.66 cm^2 and 31.05 cm^2, respectively. Meanwhile, the result for the effect of week on leaf area showed a significant difference at $p < 0.05$ between weeks (Figure 33). The leaf area increased significantly from Week 1 until Week 3 and slightly decreased in Week 4. However, the leaf area in Week 4 was not significantly different from the leaf area in Week 3. The leaf area continued to decrease in Week 5 but did not show a significant difference from Week 4 and Week 2.

Table 20. Analysis of variance (ANOVA) for leaf area of soybeans as affected by nutrient concentration, variety, and week number

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	915.34	3	305.11	30.96	0.00
Variety (V)	943.94	1	943.94	95.80	0.00
Week (W)	644.78	4	161.20	16.36	0.00
N x V	62.77	3	20.92	2.12	0.10
N x W	37.56	12	3.13	0.32	0.98
V x W	8.34	4	2.09	0.21	0.93
N x V x W	27.01	12	2.25	0.23	1.00
Error	788.30	80	9.85		
Total	3428.02	119			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

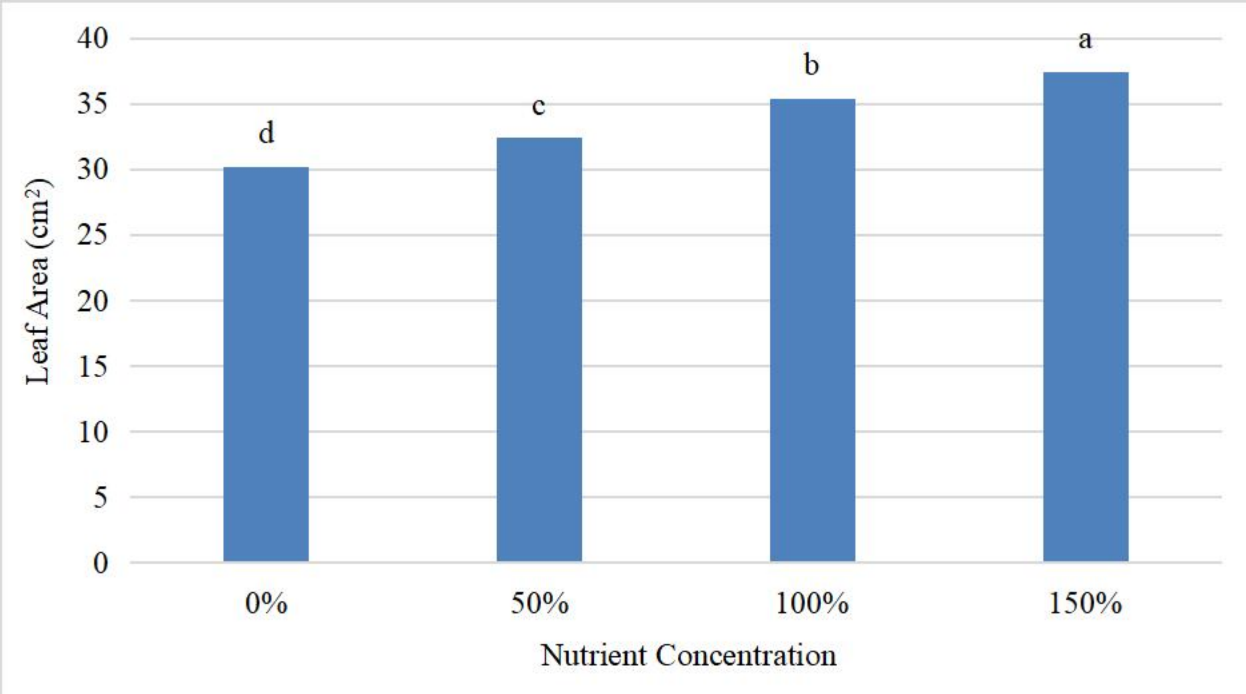


Figure 32. Effect of nutrient concentration on leaf area at early growth of soybeans planted under soilless culture conditions. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

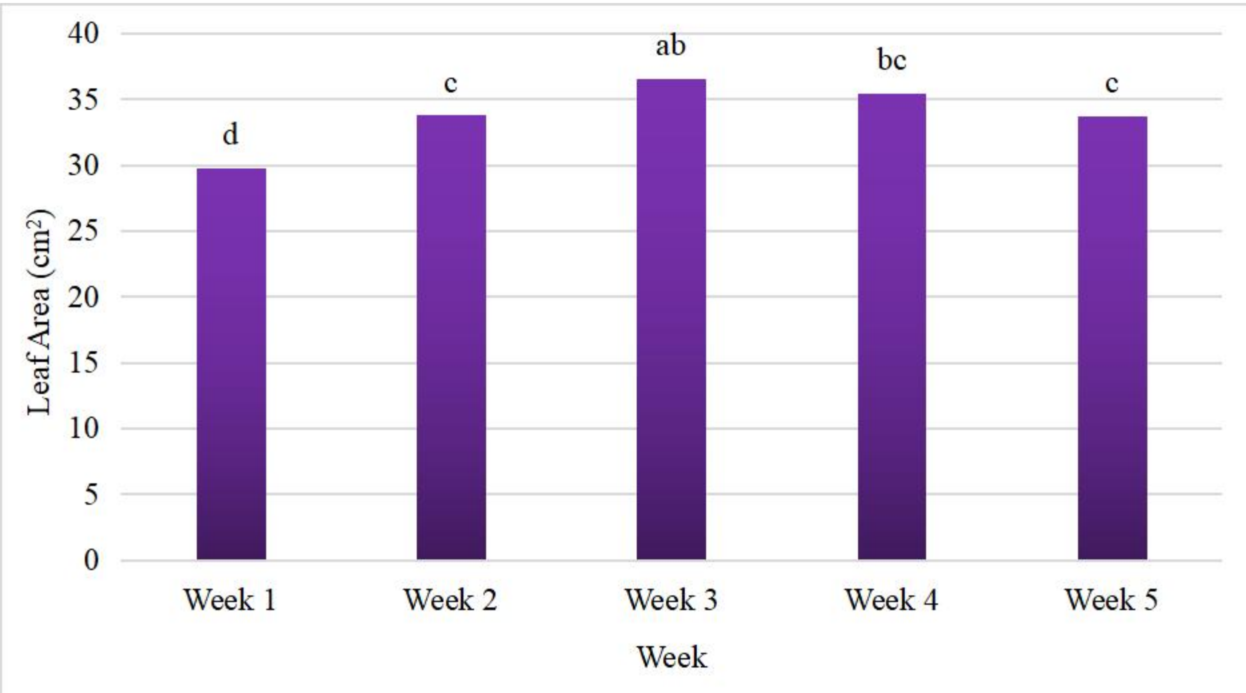


Figure 33. Effect of week on leaf area at early growth of soybeans planted under soilless culture conditions. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

4.3.1.5 Root length (cm)

Based on the analysis of variance (ANOVA) in Table 21, nutrient concentration and variety of treatments have a significant interaction at $p < 0.05$ on root length. The Martina variety had deeper roots than the Johanna variety at 0% nutrient concentration, with a length of 12.56 cm and 10.22 cm, respectively (Figure 34). On the other hand, at a nutrient concentration of 50%, the Johanna variety had deeper roots than Martina. The highest root lengths for both varieties, 16.48 cm for Johanna and 15.61 cm for Martina, were observed in this treatment. Similar results were obtained at 100% and 150% concentrations, where the Johanna variety had deeper roots than the Martina variety. However, the root length of both varieties increased as the nutrient concentration treatments increased from 0 to 50%. When the nutrient was increased to 100%, the root length of both varieties was shorter but not significant than the root length under the treatment of 150%.

Table 21. Analysis of variance (ANOVA) for root length of soybean as affected by nutrient concentration and variety

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	65.24	3	21.75	22.15	0.0001
Variety (V)	0.02	1	0.02	0.02	0.894
N x V	11.87	3	3.96	4.03	0.026
Error	15.71	16	0.98		
Total	92.84	23			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

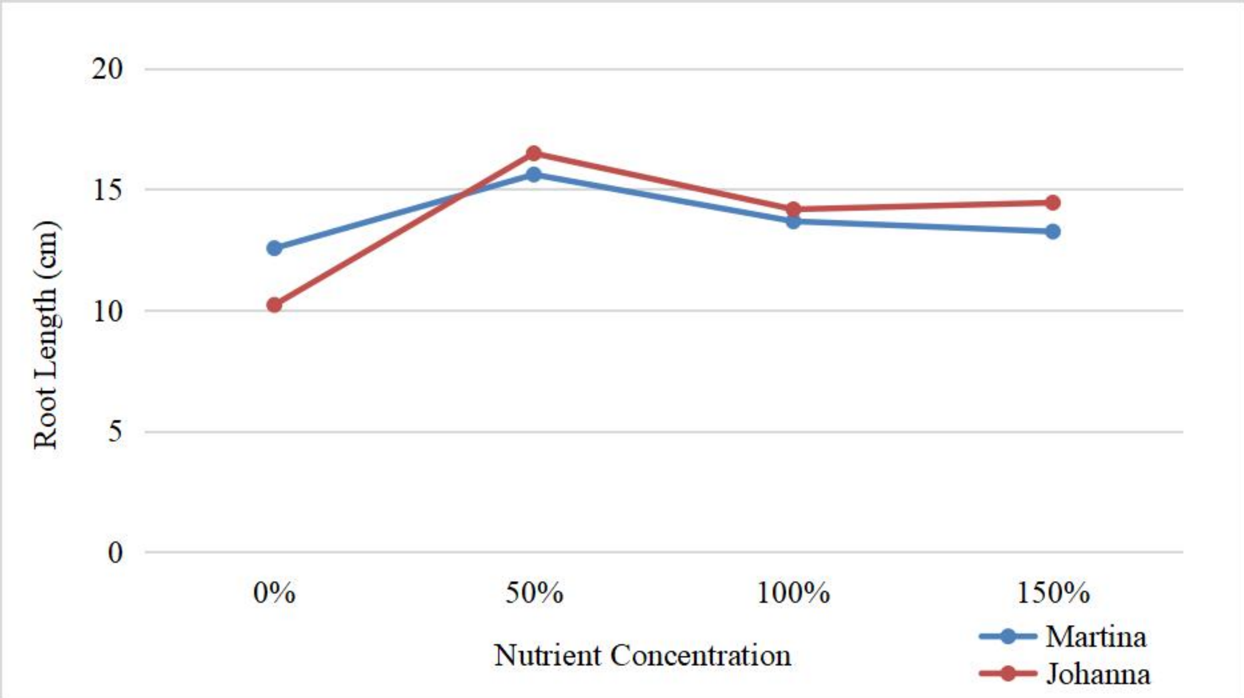


Figure 34. Interaction effect of nutrient concentration and variety on root length of soybeans grown under soilless conditions

4.3.1.6 Shoot weight/plant (g), root weight/plant (g) and shoot:root ratio

There was a significant effect ($p < 0.05$) on shoot weight (Table 22) and root weight (Table 23) when different nutrient concentrations and different varieties were evaluated. Meanwhile, there was no significant interaction effect between the two factors on shoot and root weight. According to Figure 35, shoot weight in all four nutrient concentration treatments showed a significant difference at $p < 0.05$. The shoot weight was the lowest when no nutrient (0%) was supplied to the plant. When the nutrient concentration was increased to 50%, 100%, and 150%, the shoot weight increased, with the highest shoot weight at 150% concentration. The different trend was shown by root weight when different nutrient concentrations were applied. Root weight was the highest under the nutrient concentration of 50% and significant ($p < 0.05$) with other nutrient concentrations (Figure 35). However, root weight under the 0% concentration was the lowest, but not significantly different with the 150% treatment.

Meanwhile, the comparison between the two soybean varieties regarding shoot and root weight showed that the shoot and root of the Martina variety were heavier than the Johanna variety (Figure 36). Both varieties showed that the shoot and root weight were significant at $p < 0.05$.

Table 22. Analysis of variance (ANOVA) for shoot weight of soybeans as affected by nutrient concentration and variety

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	7.74	3	2.58	232.29	0.0001
Variety (V)	1.09	1	1.09	98.40	0.0001
N x V	0.06	3	0.02	1.72	0.202
Error	0.18	16	0.01		
Total	9.06	23			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

Table 23. Analysis of variance (ANOVA) for root weight of soybeans as affected by nutrient concentration and variety

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	0.01	3	0.00	19.25	0.0001
Variety (V)	0.01	1	0.01	58.13	0.0001
N x V	0.00	3	0.00	0.87	0.478
Error	0.00	16	0.00		
Total	0.02	23			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

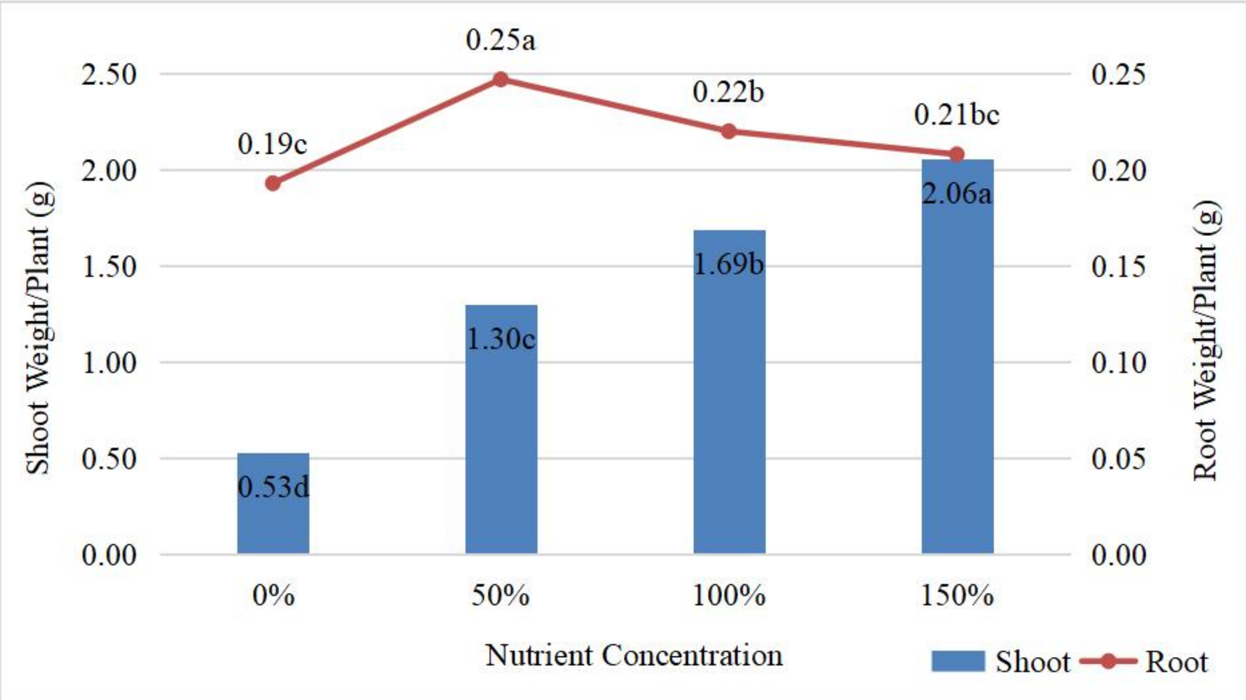


Figure 35. Effect of nutrient concentration on shoot weight and root weight of soybeans grown using soilless culture. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

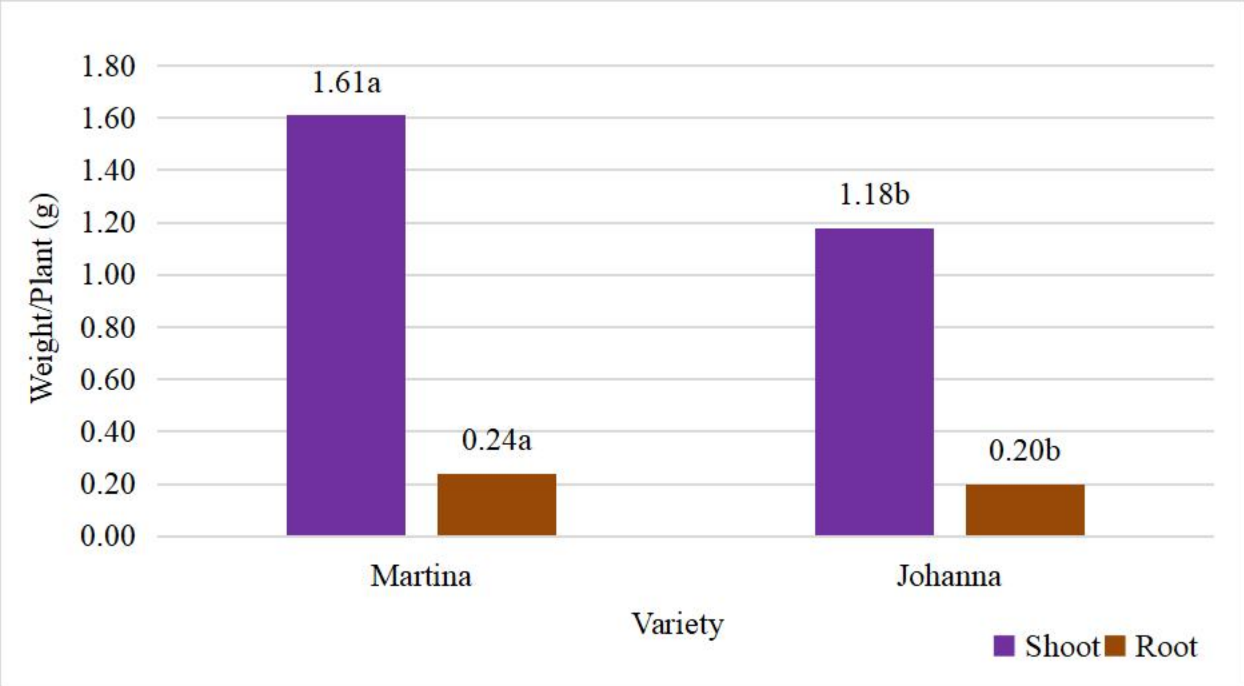


Figure 36. Effect of variety on shoot weight and root weight of soybeans grown using soilless culture. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

Based on the ANOVA table in Table 24, the different nutrient concentrations and variety treatments showed a very significant difference ($p < 0.05$) in the shoot:root ratio. However, there was no significant interaction effect between the two factors. The shoot:root ratio value increased with increasing nutrient concentration from 0 to 150%, while the treatment without nutrients (0%) gave the lowest shoot:root ratio. The plant with the nutrient concentration treatment of 150% had the highest, with a difference in shoot:root ratio with the 0% treatment of as much as 7.16 (Figure 37). Meanwhile, the Martina variety showed a higher and significant ($p < 0.05$) shoot:root ratio compared to the Johanna variety (Figure 37).

Table 24. Analysis of variance (ANOVA) for shoot:root ratio of soybean as affected by nutrient concentration and variety

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	171.59	3	57.20	935.21	0.0001
Variety (V)	4.30	1	4.30	70.33	0.0001
N x V	0.33	3	0.11	1.78	0.192
Error	0.98	16	0.06		
Total	177.19	23			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

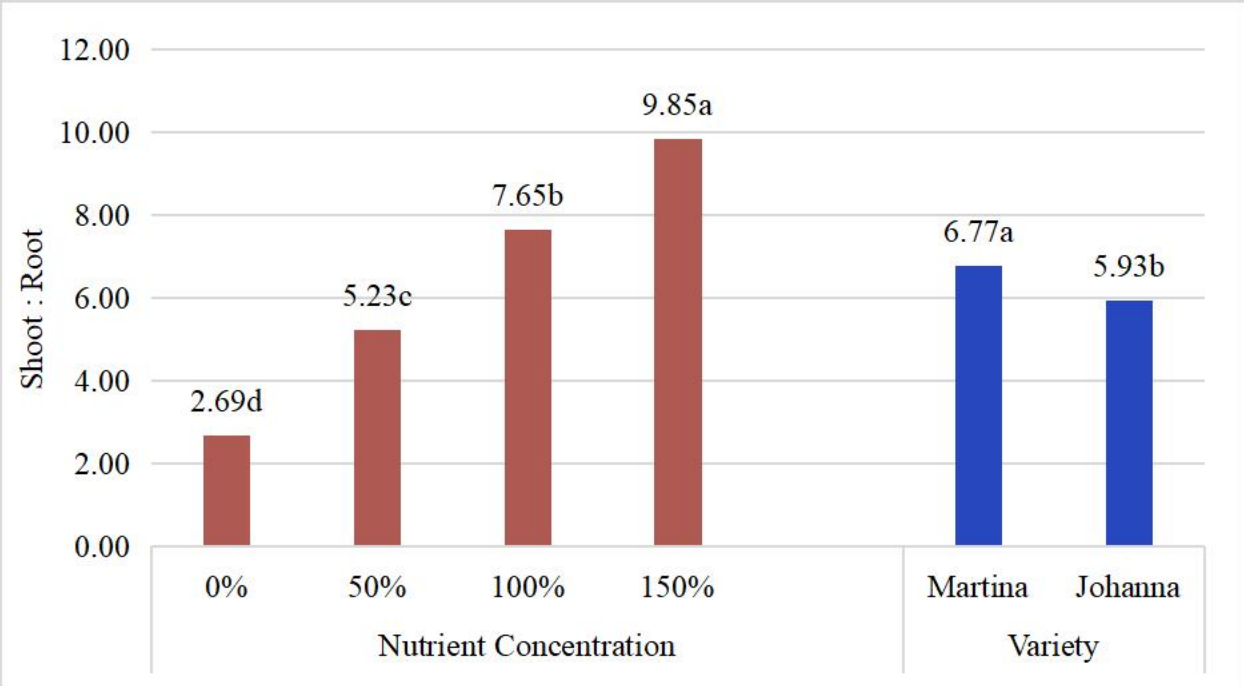


Figure 37. Effect of nutrient concentration and variety on the shoot:root ratio of soybeans grown using soilless culture. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

4.3.1.7 Number of grains/pods, grain weight/pod (g) and number of pods/plants

The results on the yield components, such as the number of grains/pods, grain weight/pod and the number of pods/plants, showed that the nutrient and variety treatments significantly affected all three recorded parameters on the yield components (Table 25). Table 25 also shows an insignificant interaction between the nutrient concentration and variety of all three yield components.

Results on the number of grains/pods showed that the soybean plant with the 0% nutrient treatment had the lowest number of grains/pods, which was only one grain per pod, and it was very significant compared to the other nutrient concentration treatments. The other nutrient concentration treatments, 50%, 100%, and 150%, have two grains per pod (Table 25). As for the Johanna and Martina varieties, the number of grains in both varieties was insignificant and had two grains per pod (Table 25).

The results for grain weight/pod showed that the soybean with a 150% nutrient concentration treatment had the highest grain weight/pod, followed by the 100% treatment (Table 25). However, the two treatments were not significantly different from one another. Meanwhile, soybeans grown without nutrient treatment (0%) had the lowest grain weight/pod followed by treatments of 50%, but both treatments were insignificant, with treatments of 100% and 150%. At the same time, the grain weight/pod under the 100% and 150% treatments were not significantly different. According to variety treatment, no significant difference was observed between Martina and Johanna's grain weight/pod, where the average grain weight/pod of the two varieties was 0.271g (Table 25).

The other parameter of the yield component was the number of pods/plants. The result showed that the number of pods/plants for soybeans with a 150% nutrient concentration was the highest, followed by 100%, 50%, and 0% (Table 25). All the treatments were significant at $p < 0.05$. Based on Table 25, the two tested varieties significantly differed in several pods/plants' indicators. It revealed that the Johanna variety had more pods/plants with one pod difference than the Martina variety.

Table 25. Effect of nutrient concentration and variety on number of grains/pods, grain weight/pod and number of pods/plant of soybeans grown under soilless conditions

Treatment	Number of Grains/Pods	Grain Weight/Pod (g)	Number of Pods/Plants
Nutrient concentration			
0%	1b	0.143c	5d
50%	2a	0.232b	7c
100%	2a	0.342a	11b
150%	2a	0.367a	14a
Grand mean	1.75	0.271	9
Significance	**	*	**
Variety			
Martina	2a	0.269a	9b
Johanna	2a	0.273a	10a
Grand mean	2	0.271	9
Significance	ns	ns	**
N x V	ns	ns	ns

Mean values with different letters are significantly different by LSD, ns = not significant, *significantly different at $p < 0.05$, **significantly different at $p < 0.01$

4.3.1.8 100-grain weight (g)

In contrast to the results for 100-grain weight (g), according to the ANOVA table in Table 26, the nutrient concentration and variety factors significantly affect 100-grain weight. However, the interaction between nutrient concentration and variety still did not show significant differences.

Based on Figure 38, plants treated with a nutrient concentration of 0% still have the lowest results, including 100-grain weight, and significant with other nutrient concentration treatments. The weight of 100 grains increased under the 50% nutrient concentration treatment and continued to increase significantly when 100% nutrient concentration was applied.

Treatment with 100% concentration gave the highest value of 100-grain weight, but it was not significant with 100-grain weight at 150% treatment. The difference in weight between the 100% and 150% treatments was only 0.08 g. The results of the different varieties revealed that the weight of 100 grains for the Johanna variety was significantly higher than that of the Martina variety. The difference was 1.33 g.

Table 26. Analysis of variance (ANOVA) for 100-grain weight of soybeans as affected by nutrient concentration and variety

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	105.57	3	35.19	132.29	0.0001
Variety (V)	6.41	1	6.41	24.09	0.0001
N x V	0.31	3	0.10	0.39	0.766
Error	4.26	16	0.27		
Total	116.54	23			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

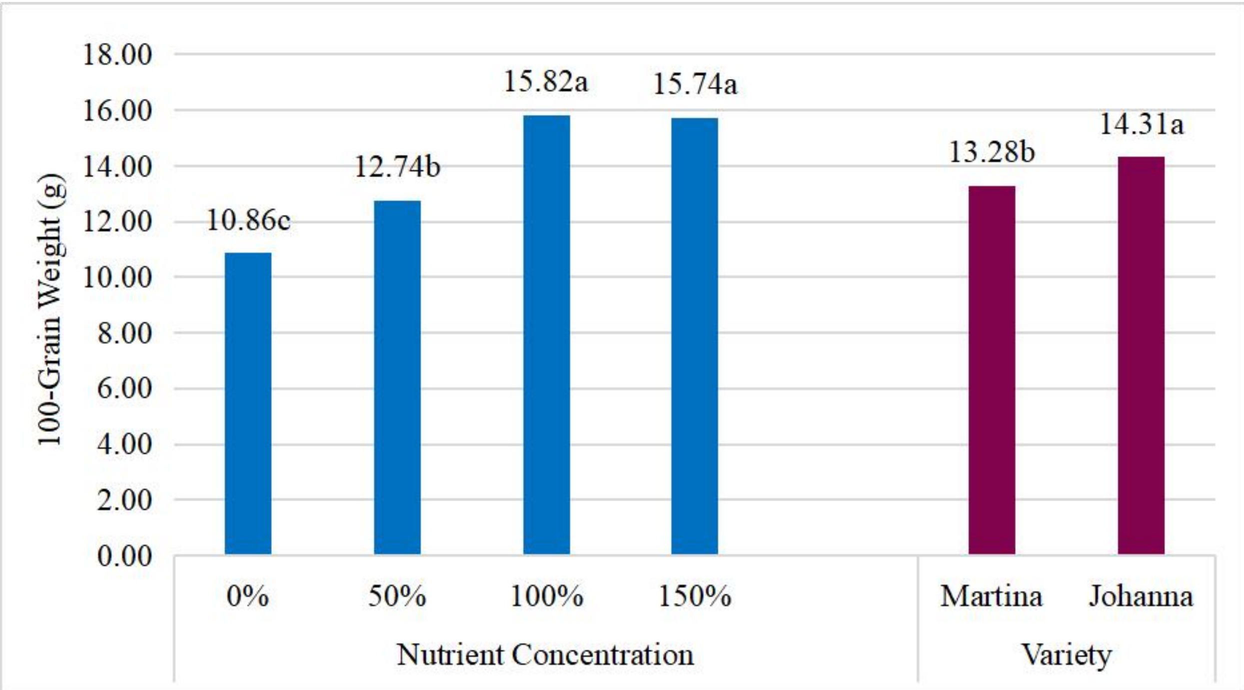


Figure 38. Effect of nutrient concentration and variety on the 100-grain weight of soybeans grown using soilless culture. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

4.3.1.9 Grain yield/pot (g)

The other finding was the grain yield/pot (g). Records were taken from six soybean plants per pot. According to the ANOVA table in Table 27, only the nutrient concentration treatment significantly differed in grain yield/pot. Different varieties were insignificant, and even the interaction of the two factors was not significant.

Grain yield/pot for soybeans with 0% treatment was the lowest and increased in plants with 50% nutrient concentration (Figure 39). Grain yield continued to increase when 100% nutrient concentration was given and increased again when the nutrient was applied at 150%. From the 0% treatment up to 150%, the increase in yield was as much as 26.73 g. All the nutrient concentration treatments were significant at $p < 0.05$ on grain yield. For the variety treatments, both the Martina and Johanna varieties gave comparable grain yield/pot results and were not significantly different. The difference in total yield between the Martina and Johanna varieties was only 0.69 g (Figure 39).

Table 27. Analysis of variance (ANOVA) for grain yield of soybeans as affected by nutrient concentration and variety

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	2647.44	3	882.48	44.87	0.0001
Variety (V)	2.92	1	2.92	0.15	0.705
N x V	9.57	3	3.19	0.16	0.920
Error	314.66	16	19.67		
Total	2974.58	23			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

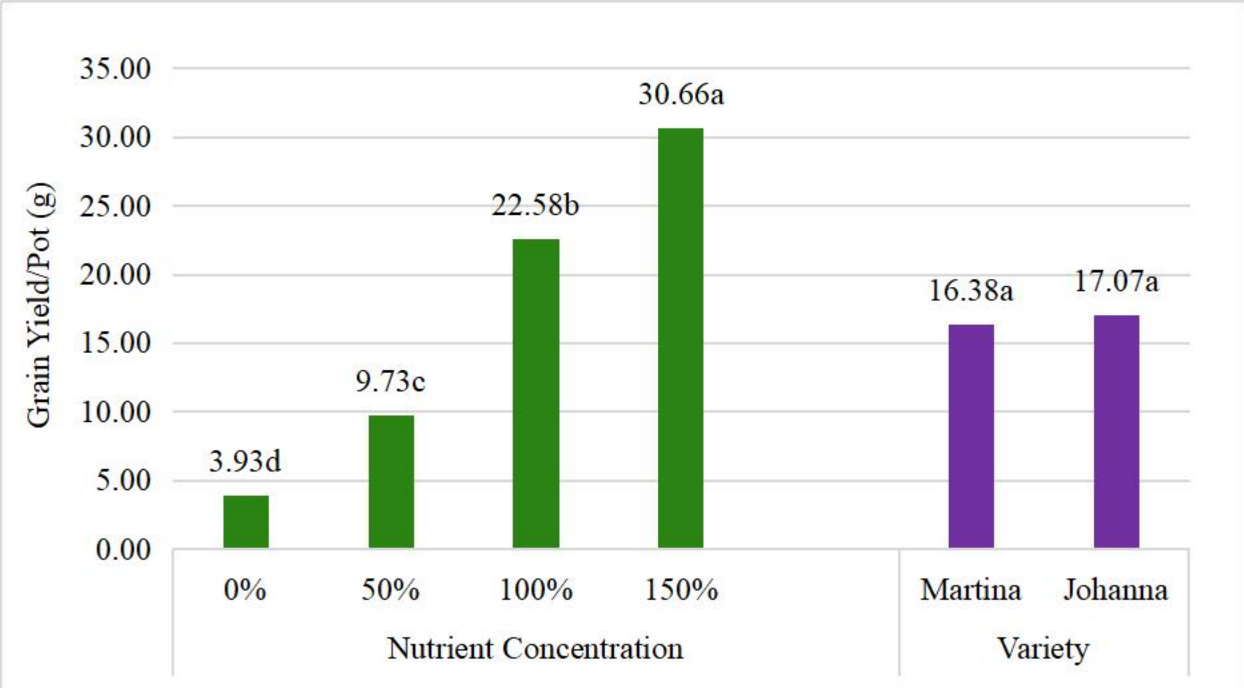


Figure 39. Effect of nutrient concentration and variety on grain yield of soybeans grown using soilless culture. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

4.3.1.10 Protein content (%) and lipid content (%)

The chemical composition of soybeans, such as protein content (%), revealed that both tested factors significantly affected the protein content of soybeans grown on a soilless substrate under this controlled environment condition. The ANOVA table below (Table 28) shows the obtained results. Besides, according to Table 28, both factors significantly influenced protein content.

The results of the interaction effects are to be discussed because nutrient concentration and variety showed a significant impact. According to the interaction effect in Figure 40, the Johanna variety has greater protein content than the Martina variety at each tested nutrient concentration. At a nutrient concentration of 50%, both varieties had the highest protein content, 46.59% (Johanna) and 46.15% (Martina), respectively. However, the protein content of both varieties decreased by 3.49% (Johanna) and 3.70% (Martina) when the nutrient concentration was supplied at 100%. At a nutrient concentration of 150%, the protein content of the Johanna variety was greater by 1.81% compared with the protein content at 100% treatment. However, the protein content of the Martina variety under the 150% nutrient concentration was almost the same as that of the 100% treatment, which had 42.47% and 42.45% protein content, respectively.

Table 28. Analysis of variance (ANOVA) for protein content of soybeans as affected by nutrient concentration and variety

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	42.19	3	14.06	40.33	0.0001
Variety (V)	8.28	1	8.28	23.76	0.0001
N x V	3.61	3	1.20	3.45	0.042
Error	5.58	16	0.35		
Total	59.66	23			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

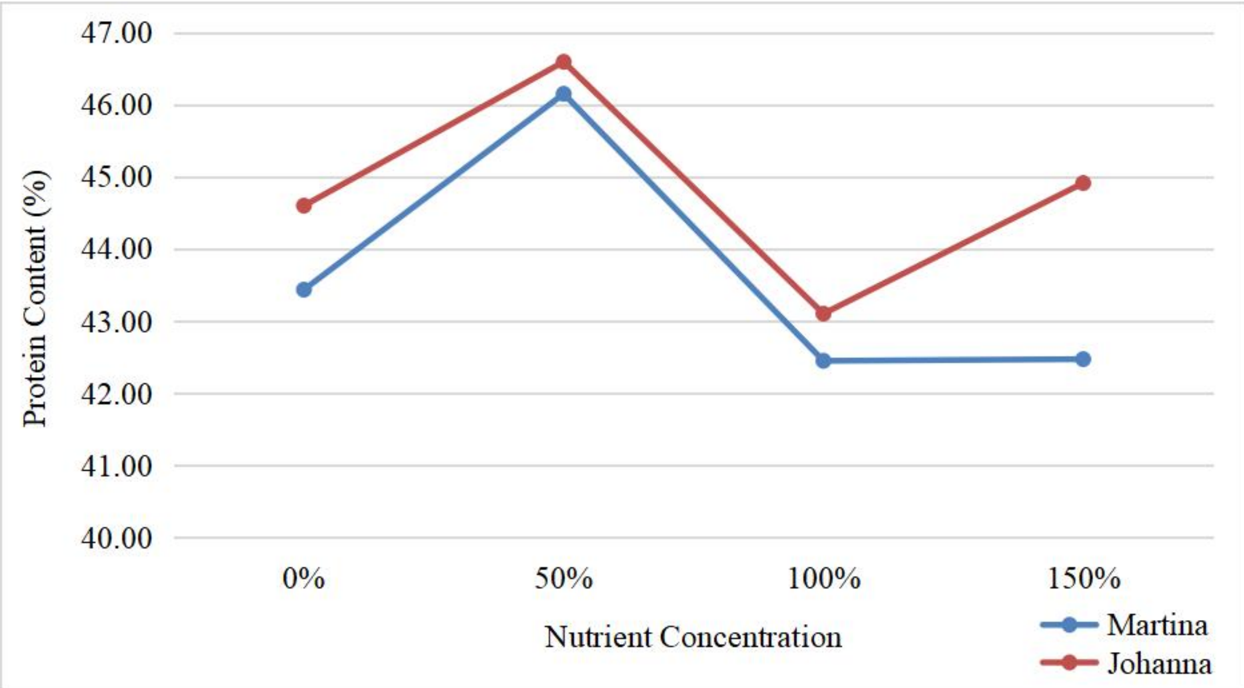


Figure 40. Interaction effect of nutrient concentration and variety on the protein content of soybeans grown under soilless conditions

The study's results on lipid content also showed that all main factors and interaction effects were significant, as shown in the ANOVA table in Table 29. In the treatment without nutrients (0%), the variety of Martina had a greater lipid content than Johanna. The percentages of lipid content, respectively, were 14.27% and 13.67% for Martina and Johanna (Figure 41). However, the lipid content at the 50% nutrient concentration treatment for both varieties was similar to that of the 0% treatment but had the lowest value compared to other treatments. The lipid content under the 50% nutrient was 8.04% (Martina) and 9.40% (Johanna). At 100% nutrient concentration, the lipid content increased again and was higher than the 50% nutrient treatment, but the Martina variety (14.98%) was higher than Johanna (13.84%). The lipid content in the Martina variety's 150% nutrient concentration treatment continued to increase slightly from the 100% treatment, which was 14.98%. However, the lipid content of the Johanna variety slightly decreased in the 150% nutrient treatment, which was 12.51%.

Table 29. Analysis of variance (ANOVA) for lipid content of soybeans as affected by nutrient concentration and variety

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	131.29	3	43.76	226.61	0.0001
Variety (V)	3.85	1	3.85	19.93	0.0001
N x V	13.48	3	4.49	23.27	0.0001
Error	3.09	16	0.19		
Total	151.70	23			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

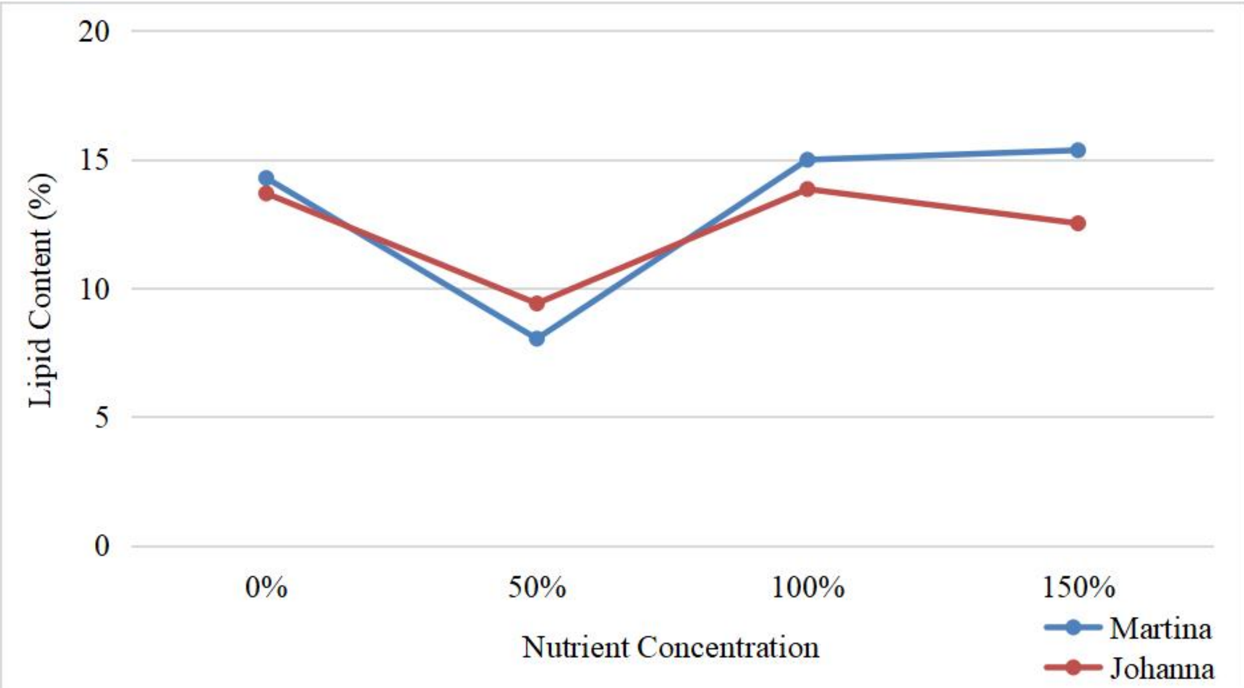


Figure 41. Interaction effect of nutrient concentration and variety on the lipid content of soybeans grown under soilless conditions

4.3.1.11 Protein yield (g) and lipid yield (g)

Only the main factor of nutrient concentration was significantly different at $p < 0.05$ on both protein yield/pot (Table 30) and lipid yield/pot (Table 31). Protein yield/pot increased as the concentration of nutrients increased from 0 to 150% (Figure 42). The percentage of increase in protein yield from 0 to 150% was as much as 87%. All the treatments of 0%, 50%, 100%, and

150% showed a significant effect at $p < 0.05$. Meanwhile, the results for lipid yield also showed an increasing trend from 0 to 150% (Figure 42). However, the lipid yield of soybeans treated with 0% and 50% did not significantly differ.

Both tested soybean varieties had almost similar protein and lipid yields and did not differ significantly ($p < 0.05$) (Figure 42). The Johanna variety was only 7% higher in protein yield than the Martina variety. The variety of Martina produced a lipid yield of 6.9% higher than the Johanna variety.

Table 30. Analysis of variance (ANOVA) for protein yield of soybeans as affected by nutrient concentration and variety

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	13.63	3	4.54	40.80	0.0001
Variety (V)	0.05	1	0.05	0.45	0.511
N x V	0.11	3	0.04	0.32	0.809
Error	1.78	16	0.11		
Total	15.57	23			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

Table 31. Analysis of variance (ANOVA) for lipid yield of soybeans as affected by nutrient concentration and variety

Source	Sum of Squares	df	Mean Square	F	Sig.
Nutrient concentration (N)	58.94	3	19.65	53.49	0.0001
Variety (V)	0.16	1	0.16	0.43	0.523
N x V	0.53	3	0.18	0.48	0.699
Error	5.88	16	0.37		
Total	65.50	23			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

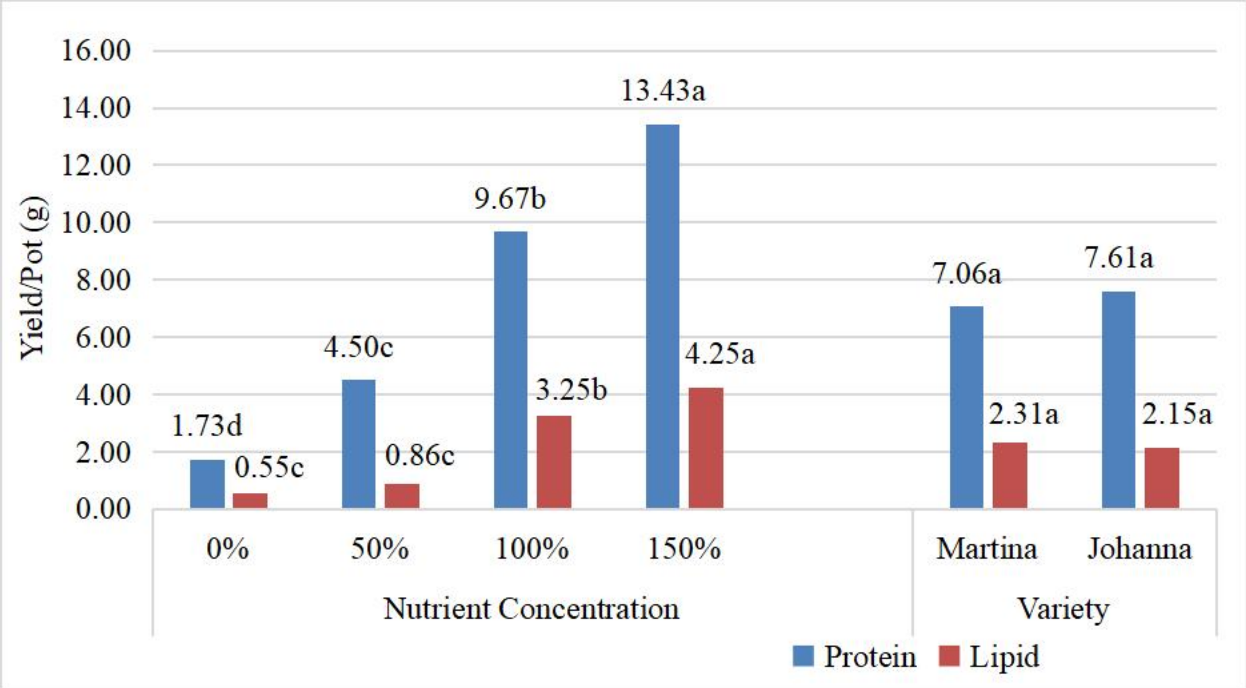


Figure 42. Effect of nutrient concentration and variety on protein and lipid yield of soybeans grown using soilless culture. Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

4.3.2 Discussion

4.3.2.1 Week number affected plant height and number of leaves

Plant height and number of leaves were significantly increased with an increasing number of weeks at the early growth stage of soybeans grown in a controlled environment using a soilless culture (Figures 27 and 30). These are in line with studies done by PARADISO et al. (2014) on soybean plants and PURBA (2021) on lettuce. It can be explained that every plant in excellent condition will continue to grow with increasing time. The plant is the most actively growing, especially in the vegetative stage, including the increase in plant height and number of leaves. For example, according to the vegetative growth standard for soybean in stages V2, V3, V4, V5, and V6, the plant height increment is around 15-20 cm, 18-23 cm, 23-25 cm, 25-31 cm, and 31-36 cm, respectively (ENDRES and KANDEL 2021). However, results from the study found that the plant height of soybeans was higher (Figure 27) compared to the standard range. This difference is probably due to several factors, and among them are the differences in cultivation system, environmental condition, and variety used.

4.3.2.2 Nutrient concentration affected number of leaves and leaf area

There was only a significant increase in the number of leaves from a nutrient concentration of 50 to 100%, but there was no significant difference in nutrient concentration between 0 and 50% (Figure 29). This is probably due to the soybean seeds being large and the storage compounds in the cotyledons providing the nutrients required for early plant growth (OHYAMA et al. 2017). This can explain why soybeans with 0% nutrients can also produce the same number of leaves as those with 50% nutrients, even if no nutrients are supplied. However, the findings of this study differ from the findings of the survey by HATA and FUTAMURA (2020), where the number of trifoliate leaves has increased with increasing nutrient concentration from 0 to 50%. This difference is probably due to the study by HATA and FUTAMURA (2020) using *Rhizobium* sp. inoculant for soybean cultivation by mixing rhizobia into the growing medium (silica sand). Therefore, apart from the nutrient source from the additional application, the inoculated *Rhizobium* also helps to increase and encourage growth and productivity by increasing the efficiency of nodulation and nitrogen fixation by soybean plants (KYEI-BOAHEN et al. 2002). Thus, the plant obtains a sufficient supply of nutrients, especially a supply of nitrogen, which is very necessary at the vegetative stage. The number of leaves was also insignificant between 100% and 150% nutrient concentration. This means that the soybean plant did not respond to additional nutrients to increase the number of leaves, where only 100% nutrient concentration is sufficient to produce the maximum number of leaves in the early development stages of two soybean varieties (Martina and Johanna).

Furthermore, the number of leaves and leaf area of soybeans grew in a pattern that was almost similar when they were exposed to different nutrient concentrations. However, the leaf area increased significantly from 0 to 150% nutrient concentrations (Figure 32). It is the same as the findings reported by HADDAD and ABAHRI (2022) for the faba bean legume plant. They found that there was an increase in leaf area with an increase in nutrient solution concentration for all three varieties of tested faba beans. Since plants at higher nutrient concentrations allocate a larger fraction of carbohydrates to shoot growth than those at lower concentrations, plants at high nutrient concentrations could produce a wider leaf area. It was shown by KANG and VAN IERSEL (2004) through a study on salvia plants that there was a significant increasing trend in plant leaf area ratio (LAR) at different nutrient concentrations (up to 1x strength). LAR is calculated based on the leaf area divided by the total dry weight. Because plant LAR indicates how much leaf area a plant produces per gramme of dry matter, a high LAR suggests that a plant is efficient at producing leaf area.

4.3.2.3 Nutrient concentration and variety interacted on plant height

Nutrient concentration and variety have a significant interaction with plant height (Figure 28). The variety of Martina was much higher than Johanna at nutrient concentrations of 0% and 100%. Both varieties gave almost a similar response to the plant height when supplied with 50%

nutrients. When the nutrient concentration was increased to 150%, the Johanna variety was taller than Martina, where the difference was only 2 cm. This clearly shows that the determination of the nutrient concentration for a plant does not only depend on the type of plant but also needs to consider the variety used because each variety has a different response to nutrients.

4.3.2.4 Variety and week number interacted on number of leaves and SPAD reading

Variety Martina and Johanna also interacted with the week number on the number of leaves (Figure 30) and SPAD reading (Figure 31). The interaction trend for both varieties on the number of leaves increased from Week 1 to Week 5. However, the Johanna variety produced a more significant number of leaves than the Martina variety in Week 1 and Week 4. In Week 2 and Week 3, Johanna and Martina have the same number of leaves. In Week 5, the leaf number of the Martina variety increased and was higher than that of the Johanna variety. As explained earlier, the growth of a plant in the early stages, including the formation of leaves, will increase with increasing time.

As for SPAD reading, both the Martina and Johanna varieties showed an increment in SPAD reading value only from Week 1 to Week 3 (Figure 31). It showed that the Johanna variety continued to give a higher SPAD reading than Martina in Week 1, Week 2, and Week 3. Meanwhile, in Week 4 and Week 5, the SPAD reading for the Johanna variety decreased and was lower than for Martina. The SPAD reading is leaf green colour intensity, which shows the chlorophyll content in leaves and stems. Plants use chlorophyll to produce food through photosynthesis (PALTA 1990, BUTTERY and BUZZELL 1977). The chlorophyll content is directly proportional to the rate of photosynthesis, which increases from the youngest leaf to the leaf, which can be described as "photosynthetically mature". After reaching a maximum value, chlorophyll content and photosynthesis rate decreased (SESTAK 1963). This is in line with the findings of this study, which showed that the Johanna variety had a high photosynthesis rate in the early stages of plant growth and decreased in the last two weeks of its vegetative stage. However, this is contrary to the Martina variety, which was more productive in producing food during the two weeks before its vegetative stage ended.

4.3.2.5 Nutrient concentration and variety interactions regarding root length

In our study, the response of root length to tested soybean varieties showed that the Martina variety had deeper roots than the Johanna variety when treated with 0% nutrient concentration. At other nutrient concentrations (50%, 100%, and 150%), the roots of the Johanna variety were more profound than those of the Martina variety. However, at a 150% nutrient concentration, the root length of the Martina variety was slightly shorter than at a 100%

concentration, but the opposite effect was found for the Johanna variety. This showed that soybean root length was influenced by nutrient concentration and the type of variety. Although both nutrient concentration and variety factors influenced root length, the findings in this study showed that the roots of both varieties were the deepest at a low nutrient concentration of 50%. Based on the data collected by BALLIU et al. (2021) in their review article on environmental and cultivation factors that affect the morphology, architecture, and performance of root systems in soilless-grown plants, they found that there was an increase in the elongation of vertical or deep roots for various plants grown in soilless culture systems when there was limited nitrogen supply. When plants are deprived of nutrients, their root morphology changes, and their root surface area expands (MARZEC and MELZER 2018).

Therefore, deeper roots in plants with low nutrient supply were due to the nature of the root itself, which functions as a vital organ that gives physical anchoring, nutrient absorption, water, stress prevention mechanisms, and particular signals to the aerial part of the plant (VIVES-PERIS et al. 2020). Thus, the roots assist the plant in obtaining the necessary nutrients and are extending to get enough nutrients to meet its growth needs.

4.3.2.6 Nutrient concentration and variety affected on root weight, shoot weight and shoot:root ratio

The effect of nutrient concentration on root weight also showed that low nutrients gave the highest root weight, and the trend shown was almost the same as the trend shown on root length. This finding was further strengthened by the statement by MARZEC and MELZER (2018) that when plants encounter nutrient deficiency, it causes an increase in root surface to improve the ability of the roots to uptake nutrients. In contrast to the shoot weight, when the nutrient concentration was raised, the shoot weight increased dramatically. This finding was in line with a study by KANG and VAN IERSEL (2004) on salvia plants (*Salvia splendens*) that found that the shoot dry weight of salvia increased significantly with increasing nutrient solution concentrations from 12.5 to 100 to 200% concentrations. They used a source of fertilizer from the Hoagland solution that is also available on the market. The shoot weight was determined based on the weight of all the above-ground parts of the plant. The shoot weight increased with increasing nutrient concentration, possibly due to the role of the fertilizer itself, which supplies food sources through uptake by the roots to support plant growth. The more fertilizer is supplied until a certain level, the more nutrients required can be absorbed by the plant so that the plant can grow actively, especially during its vegetative stage (JONES 1982).

Similar to shoot weight, the shoot:root ratio also showed an increase with increasing nutrient concentration from 0 to 150% in our study. A similar result was reported by Kang and VAN IERSEL (2004), who found that nutrient concentration greatly affected the shoot:root ratio of salvia. They found that there was an increase in the shoot:root ratio when the nutrient concentration increased. An increase in shoot:root ratio with increasing fertilizer concentration is common (MAK and YEH 2001, SATTELMACHER et al. 1990). Deficiencies of water or nutrients can strongly decrease the shoot:root ratio of plants (FINDENEGG 1990). Shoot:root ratio is a measurement of the amount of plant tissue with growth function (shoots) compared to the amount of plant tissue with supportive functions (roots) (HARRIS 1992). A high value of shoot:root ratio in a plant shows a greater proportion of shoots compared to roots, and plants with a higher proportion of shoots are better able to capture light energy and grow larger (FINDENEGG 1990).

Shoot and root weight also depends on the variety. The Martina variety had a higher weight and significance than the Johanna variety which contributed to the higher shoot:root ratio in the Martina variety. The difference in shoot and root growth is due to the nature of the growth of the variety itself. Although the Johanna variety had lower above- and below-ground growth, the yield component was not affected and was comparable to the Martina variety. This is discussed in the sub-topic below.

4.3.2.7 Nutrient concentration affected on number of grains/pods, grain weight/pod and grain yield/pot

The main concern of producers in soybean cultivation is grain yield. In our study, different nutrient concentration rates significantly affected the number of grains/pods, grain weight/pod, and grain yield/pot. The Martina and Johanna varieties have an average number of grains/pods of 2 grains, regardless of how many nutrients were supplied. A similar result was reported by ETONE EPIE et al. (2022), who found that the number of grains/pods was insignificant when different concentrations of nitrogen fertilizers were applied to several soybean varieties. However, their study was on field planting. Our results also found that when no nutrient (0%) was supplied, both varieties only had 1 grain/pod. When the grain weight/pod was measured, the grain weight rose as the nutrient concentration increased, but the weight of the grain/pod for 100% and 150% concentrations was not significant. At a nutrient concentration of 50%, even though it had 2 grains/pod, the weight of the grain/pod was lighter compared to other nutrient concentrations.

The number of grains/pods and grain weight/pod affected both soybean varieties' grain yield/pot. Due to the high grain weight/pod and the high pod number/plants on soybeans with high nutrient concentrations, the grain yield/pot also showed the same increasing trend. The result of the study was the same as reported by MORSHED et al. (2008), who found no significant difference in the yield of grain/plant on soybeans planted in the field when the nitrogen fertilizer was increased to a certain level. In their field experiment studies, GAI et al. (2017) also found that soybean grain yield was significantly different when different nitrogen rates were applied, with the highest yield obtained at the rate of 50%. They tested up to a fertilizer rate of 75%. For soybean cultivation using a soilless system in a controlled condition, VALDEZ et al. (2002) reported that pod yield/plant of snap bean (*Phaseolus vulgaris*) increased with increasing nutrient levels from 25 to 150%, and pod yield/plant decreased at a nutrient level of 200%. They used the nutrient solution 'Enshi-shoho,' which is widely used in Japan. This may be due to the greater uptake of crop and influencing the crop growth and yield components that effectively assimilated partitioning of photosynthesis from source to sink in the post-flowering stage and resulted in the highest grain yield (SHIVAKUMAR 2001, DESTA 1986).

As mentioned earlier, increasing nutrients in a planting system, whether using soil or without soil, increases nutrient uptake by plants, but only at certain nutrient levels. After a certain level, the excess of nutrients occurred, which means that the plants did not absorb the nutrients and became toxic; consequently, the plant production decreased. In our study, the results increased until the maximum nutrient concentration (150%), and the probability that the yield will decrease after that rate is high.

4.3.2.8 Nutrient concentration and variety affected on number of pods/plants and 100-grain weight

The pod number increased significantly with the increase in nutrient concentration, up to 150% in our study. A study done by SMITH et al. (2002) in a controlled environment and soilless culture cultivation system (mixture of sand and vermiculite at a rate of 50:50) for common bean (*Phaseolus vulgaris*) plants also found that there was an increase in pod number when the fertilizer concentration was increased from low (10%), medium (50%), and high (100%). As described in subtopic 4.3.2.7 above, there was also a rise in grain yield/pot at a nutrient concentration of 150%. The highest number of pods/plants at this nutrient concentration was one of the contributing factors. Nevertheless, 100-grain weight only increased to 100% nutrient concentration and was insignificant with the 150% treatment. This means that to reach the maximum 100-grain weight, the optimal nutrient solution for tested soybean varieties was as much as 100%.

The result by OLJIRRA and TEMESGEN (2019) differs from the findings of our study, in which the 100-grain weight of soybean varieties (Dhidhessa, Ethio-Yugoslavia, and Wello) planted in the field did not show significant differences when supplied with different nutrient levels, where the 100-grain weight obtained was in the range of 16.02 to 16.42 g. They used blended NPS fertilizer sources. Similarly, another study conducted by ETONE EPIE et al. (2022) in the field of other soybean varieties found that the weight of 100 grains did not show a significant difference when different concentrations of nitrogen fertilizer were used. This difference in finding may be due to differences in environmental conditions, planting systems, and nutrient management, which play an integral part in determining the growth of plants and subsequently affect yield, including 100-grain weight.

However, OLJIRRA and TEMESGEN (2019) and ETONE EPIE et al. (2022) found significant differences in 100-grain yield at different varieties. Their findings were similar to the conclusions of our study. Besides soybeans, 100-grain weights were strongly controlled genetically in field beans (*Vicia faba*) and chickpeas (*Cicer arietinum*) (JAWAHAR et al. 2017, TANAKA et al. 1979). The 100-grain weight is an essential agronomical characteristic, and genes related to it have been targeted to enhance the soybean grain's quality (QI et al. 2020). In addition, a 100-grain weight is vital because the grain size of a soybean variety can be determined (CHOI et al. 2021). This supports the results of our study when the 100-grain weight of the Johanna variety was higher and had a more prominent grain size than Martina.

The result of our study also found that the variety affected not only the 100-grain weight but also the number of pods/plants, as reported by AGEGN et al. (2022), the pod number of soybean field planting was influenced not only by the nutrient level but also by the variety. This indicates that the traits of the two tested soybean varieties, Martina and Johanna, are not only influenced by cultivation management but also controlled by genetic factors.

4.3.2.9 Nutrient concentration and variety interacted on protein and lipid content

Our study results on protein and lipid content proved that both contents were affected by the interaction between nutrient concentration and variety. As for protein content, both varieties showed almost the same trend at nutrient concentrations of 0%, 50%, and 100%, with the Johanna variety containing a higher protein content than Martina. This means the Johanna variety accumulated more protein in seeds than the Martina variety. This may be because, in terms of size, Johanna's variety was larger than Martina's. The large soybean grain can produce more protein content. This was found by CHOI et al. (2021) that the average total protein content of soybean grain dropped in the order of large (39.63%) > medium (39.31%) size. Therefore, the grain size of the Johanna variety can be categorized as large, and the grain size of the Martina variety can be categorized as medium.

In our study, both varieties also had the highest protein content when applied with a low nutrient concentration of 50%. Protein content decreased when nutrients were increased to 100 and 150% for both varieties. This is supported by findings from RAY et al. (2006), which found that when a significant amount of nitrogen fertilizer was supplied at rates of 290, 310, and 360 kg/ha on the Sharkey clay soil, the protein concentration of irrigated soybeans decreased by 2.7%. A significant effect of nutrients was also reported by JARECKI and BOBRECKA-JAMBO (2015), who found that the nitrogen fertilizer supplied at 25 kg/ha significantly boosted the seed's protein content compared to the control. In our study, the total protein content of the unfertilized (0%) and 100% treatments was approximately the same, with a difference of only 1.5% for Johanna and 0.98% for Martina. In agreement with findings by PURCELL et al. (2004) in their study on silt loam soil, they found that when 112 kg N/ha was supplied during the R2 stage of soybean growth, there were no differences in the amount of seed protein and oil concentrations compared to the soybean planted without fertilizer.

Our findings also had a similar trend to those of KAUR et al. (2017). The soybean variety 'Pioneer 49T80' was planted in field conditions on soil clay texture when it showed the highest protein content was on low nitrogen fertilizer and decreased after the fertilizer rate was increased and was almost the same as the protein content without fertilization treatment. However, they reported no significant difference in protein content when the soybean variety was planted in soil with a silty loam texture. According to JURGONSKI (1997), soybean seeds are rich in protein and lipid content for soybeans grown in a controlled environment compared to seeds grown in field planting. Although most of the studies on soybeans indicated that the protein content increased with lower nutrient concentrations, some studies on wheat crops reported that the protein content increased with higher fertilizer rates up to a certain level, such as 160 kg/ha for planting in fields (ESER et al. 2020, PEPÓ 2010). However, according to WAN et al. (2023), the relationship between protein content and fertilizer generally followed a quadratic function in which the content increased initially and then declined with increases in nutrient level.

The opposite trend from protein content was shown for lipid content in our study. Both varieties showed the lowest lipid content at 50% nutrient; the lipid content of the Johanna variety exceeds the Martina variety at this rate. At other nutrient concentrations, the Martina variety had the highest lipid content. The response of these two varieties to lipid content differed from the response to protein content, probably because the smaller-sized variety usually has a higher lipid content. According to CHOI et al. (2021), grain size and lipid content were categorized as follows which was medium (17.32%) > large (16.93%). Meanwhile, the low lipid content under low nutrients was supported by the findings of another study in which the lipid content of soybeans grown on clay soil showed a low lipid content at a nutrient level of 45 kg N/ha and increased with increasing nutrient levels (KAUR et al. 2017). Surprisingly, in our study, the lipid content at 0% was higher than that at nutrient 50%. This finding is the same as SZOSTAK et al. (2020), who reported that the lipid content of the soybean variety without nutrients was higher than with 30 kg/ha of nitrogen. Based on some research results, most showed that the lipid content became low when the protein content was increased. CHOI et al. (2021) discovered that there was a significant but inverse correlation ($r = -0.714$, $p < 0.0001$) between the total lipid and protein levels of soybeans. This meant that soybeans with greater protein contents also had lower lipid contents, and contrarily. This inverse relationship was expected to result from the pleiotropic effects of minor and major genes related to protein and lipid content (PATHAN et al. 2013). Therefore, protein and lipid content strongly influence each other in soybean plants, which are influenced not only by the environment or meteorological conditions but also by nutrient management and variety.

4.3.2.10 Nutrient concentration affected on protein and lipid yield

Our findings also revealed that only nutrient concentration significantly affects protein and lipid yield. Varieties Johanna and Martina have comparable protein and lipid yields. Protein and lipid yields were calculated based on protein and lipid content multiplied by grain yield/pot. Protein and lipid yield was increased from 0% nutrient concentration to 150% nutrient concentration in this study. Although the protein content was higher in low nutrient (50%), but the low grain yield/pot in this treatment caused the protein yield to be low. Similarly to lipid yield, lipid yield was the highest at a high nutrient concentration (150%) due to lipid content, and grain yield was high at the treatment. Protein and lipid yield are important in soybean production in addition to grain yield because they consider the total nutritional value of soybean crops. It is important, particularly in the production of soybean-based secondary products for human or livestock consumption.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The performance of the soybean variety ES Gladiator grown in the open field revealed that it was not influenced by nitrogen (N) nutrition but was strongly influenced by the weed canopy. The presented outcomes in Experimental Research 1 suggest that the variety which was cultivated in black forest soil did not require additional N supplies from mineral fertilizer since all the recorded parameters were not statistically significant. The results on the influence of weed canopy can be concluded that weed canopy under hand weeded had a positive effect on grain, protein, and lipid yields, with all these parameters measured being the highest and almost doubled compared to control (weedy). However, the weed canopy under hand weeded treatment was not significant compared to the control in affecting protein and lipid content. For all treatments, the protein content had the opposite effect as the lipid content.

Meanwhile, the performance of the soybean varieties Martina and Johanna at the germination stage, which was 12 days after planting in a controlled environment, as presented through outcomes in Experimental Research 2 showed that the Martina variety was significantly higher than the Johanna variety on germination rate, seed vigour, and seed viability. However, variety interacted with temperature in influencing germination rate. Both varieties have comparable germination rates at temperatures of 15 °C and 25 °C and were higher compared to the germination rate at high temperatures (35 °C). Variety also interacted with temperature and germination period (days) in influencing the total seedling length. The longest total seedling length was found in the Martina variety at a temperature of 25 °C on the ninth day. Both varieties were more viable at low temperatures (15 °C) and suboptimal temperatures (25 °C), while none survived at high temperatures (35 °C). Therefore, both of these soybean varieties were also tolerant to the low temperatures.

In Experimental Research 3, the Martina and Johanna varieties, grown in a controlled environment using the soilless substrate of expanded clay aggregate, showed good vegetative growth. The nutrient solution of Advance Hydroponics of Holland at different rates influenced plant growth, including plant height, leaf number, and leaf area. The Johanna varieties produced more leaves and higher chlorophyll content, while Martina had larger leaf sizes. Both varieties required additional nutrients between 100% and 150% for optimal early growth. Meanwhile, the effects of different nutrient solutions on shoot and root growth, grain yield, and chemical composition of the Martina and Johanna varieties were that the application of nutrient concentration at 50% produced the deepest root and the highest protein content for both varieties. However, the application of 100% nutrient concentration gave a higher 100-grain weight for the Johanna variety. The application of nutrient concentration at 150% was good for shoot growth since it gave the highest shoot weight and shoot:root ratio for both varieties, with Martina being higher than Johanna. At 150% nutrient concentration, it also produced the highest number of pods/plants for the Johanna variety and the highest grain yield/pot for both varieties. Meanwhile, the Martina variety had the greatest lipid content at 150% concentration. Protein and lipid yields were also higher for both varieties at a nutrient concentration of 150%.

Therefore, it can be concluded that the use of nutrient concentrations of Advance Hydroponics of Holland (Dutch Formula) between 100% and 150% gave a positive effect. It can be used for planting Martina and Johanna varieties in a controlled environment using an expanded clay aggregate substrate. This is based on this cultivation technique that produced a high 100-grain weight and grain yield as well as high protein and lipid yields for both varieties.

Thus, all the information obtained from all three experimental studies will indirectly contribute to the determination of the appropriate cultivation practices for the planting of soybeans using the ES Gladiator variety for field conditions and the Martina and Johanna varieties for controlled environment conditions. These findings also provide specific information for the development of new planting technologies for these varieties in both cultivation systems.

5.2 Recommendations for future research

Based on the findings of this study, some recommendations for future research are as follows:

- ✧ This study examined the effect of nitrogen fertilizer on the ES Gladiator soybean variety grown in a field. Therefore, it is suggested for future research investigate the effects of other macronutrient elements such as phosphorus (P) and potassium (K) on this variety in the same soil type so that a complete NPK fertilizer recommendation can be made.
- ✧ This study also investigated the influence of temperature on the early stages of growth, including germination and seedling development of the soybean varieties Martina and Johanna. Thus, other factors that influence the growth of this soybean variety, such as water requirements, can be investigated, as water requirements are crucial, particularly in the early stages of plant growth.

- ✧ The effect of nutrient concentration in a controlled environment was tested on Martina and Johanna varieties, and the appropriate rate can also be recommended. However, further research on other crucial agronomic practices, such as the requirements for light, water, and different types of soilless substrates, can also be done to boost the yield and chemical composition of soybeans.
- ✧ This study examined the effect of variety, nutrient requirements, and weed control on the growth, yield, and nutritional composition (protein and lipid content) of soybeans. It is also possible to investigate the effect of these factors on biochemical content or secondary metabolites such as isoflavones, which function as antioxidants and anti-inflammatory agents.

CHAPTER 6

NEW SCIENTIFIC RESULTS

1. This study proved that the use of nitrogen fertilizer at an amount of 200 kg N/ha did not have a positive effect on the number of pods, grain yield, protein content, protein yield, and lipid yield of ES Gladiator varieties grown in black forest soil.

2. The weed canopy under hand weeded treatment had the highest grain, protein, and lipid yields and almost doubled compared to the control (weedy), but was not significant compared to the control in affecting the protein and lipid content of the ES Gladiator variety.

3. The germination study revealed that the Martina variety outperformed Johanna in germination rate, seed vigour, and viability. Both varieties had high germination rates at 15 °C and 25 °C, with the Martina variety having the longest total seedling length at a temperature of 25 °C on the ninth day.

4. The study in a controlled environment using a soilless substrate of expanded clay aggregate revealed that the Martina variety had the highest plant height at 0% and 100% nutrient concentrations, while the Johanna variety had the lowest height. Martina leaves were larger, but Johanna produced more leaves and had a higher chlorophyll content.

5. The study in a controlled environment also revealed that the SPAD reading for both Martina and Johanna varieties increased until Week 3 (30 days after planting), decreased at Week 4, and was maintained at Week 5.

6. The use of a 50% nutrient concentration of Advance Hydroponics of Holland in a controlled environment using a soilless substrate of clay mineral aggregate produced the deepest root and the highest protein content for both the Martina and Johanna varieties.

7. The use of nutrient concentrations of Advance Hydroponics of Holland between 100 and 150% gave a positive effect for both the Johanna and Martina varieties planted in a controlled environment based on high pod number, better 100-grain weight and grain yield per pot, high lipid content, as well as high protein and lipid yields per pot.

CHAPTER 7

SUMMARY

Soybean is the most economically important bean in the world. It is among the richest and cheapest sources of protein. Soybean is currently planted over an area of 136.14 million ha worldwide, with a global production of 388.01 million metric tonnes in 2022/23. The production of soybeans is anticipated to rise by 11% in 2023/24. The cultivation system widely used for soybeans is a conventional system that is influenced by environmental and agronomic factors. Nutrient and weed management are among the most important agronomic factors in conventional planting. Soybean can produce nitrogen (N) through the biological N-fixation process, which requires additional mineral N when there is insufficient supply from the N-fixation process. Meanwhile, improper weed management causes competition between plants and weeds for agricultural inputs such as nutrients and water. Due to various problems such as the reduction of agricultural area, low soil fertility, pest and disease infestation, and unpredictable climatic conditions, causing conventional cultivation systems cannot be done properly in some regions. Therefore, there is an alternative cultivation system that can be implemented in a controlled environment using a soilless cultivation system. Cultivation of soybeans in a controlled environment using soilless culture is still not much explored. Therefore, three experiments were carried out to study the effects of several agronomic and environmental factors on soybeans, including experiments in a field, in a laboratory, and in a controlled growth chamber.

The first study involved a field experiment conducted at the Hungarian University of Agriculture and Life Sciences (MATE), Gödöllő, to achieve the first objective, which is to evaluate the effect of N nutrition and weed canopy on yield formation and the chemical composition of soybeans grown in the field. The ES Gladiator variety was used in this study. The treatments of this experiment comprise two N nutrition rates (0 and 200 kg N/ha) and three weed canopy treatments (weedy, hand weeded, and mechanically weeded). This experiment was designed as a split plot with four replicates with N as a main plot and variety as a subplot. The results of the study found that the N nutrition treatment showed the number of pods, grain yield, protein content, protein and lipid yields were the highest under the treatment of 200 kg N/ha. However, there was no significant effect on all the parameters. The results on the influence of weed canopy showed that hand weeded treatments were significant compared to weedy and mechanically weeded treatments on grain, protein, and lipid yield. However, the weed canopy under hand weeded was not significant compared to control in affecting protein and lipid content. Protein content showed the opposite effect compared to lipid yield under different N nutrient and

weed canopy treatments. Therefore, from this experiment, the additional N from fertilizer is not necessary for the ES Gladiator variety, while hand weeded control is the best for weed control.

The second experiment aims to investigate the influence of temperature and variety in a controlled growth chamber on the germination rate, seedling length, and viability of soybeans. This study was done in the Crop Production Laboratory, MATE, Gödöllő. Two soybean varieties (Martina and Johanna) were used in these experiments and were germinated in three units of plant growth chambers set up with varying temperatures (15, 25, and 35 °C). The results of the study found that Martina had a higher and more significant germination rate, seed vigour, and seed viability than the Johanna variety. Both varieties have comparable germination percentages at 15 °C and 25 °C. The total seedling length was the longest found in the Martina variety at a temperature of 25 °C on the ninth day. Therefore, both varieties are also more viable and tolerant at low temperatures (15 °C).

The third experiment was the cultivation of the Martina and Johanna varieties in a controlled growth chamber to achieve the third objective, which is to study the response of growth, yield components, yield, and chemical compositions to the nutrient concentration of two soybean varieties. This experiment was also conducted at MATE, Gödöllő. The tested varieties were supplied with different nutrient concentrations (0, 50, 100, and 150%). The liquid fertilizer of Advance Hydroponics of Holland and the soilless substrate of expanded clay aggregate were used. The results found that the Martina variety had the highest plant height at 0% and 100% nutrient concentrations. The results of the study also found that the application of nutrient concentration at 50% produced the deepest root and the highest protein content for both varieties, but Johanna had a higher protein content than Martina. However, the application of 100% nutrient concentration was better in the 100-grain weight of the Johanna variety. The application of nutrient concentration at 150% gave the highest shoot:root ratio, lipid content, protein, and lipid yields for both varieties, with Martina being higher than Johanna. At 150%, it also produced the highest number of pods/plants for the Johanna variety and the highest grain yield/pot for both varieties. Therefore, a nutrient concentration between 100 and 150% can be used for planting the Martina and Johanna varieties in a controlled environment using a substrate of expanded clay aggregate.

Thus, the information obtained from these studies will contribute to determining the appropriate cultivation practices for the ES Gladiator variety for field planting and the Martina and Johanna varieties for controlled environmental conditions. These findings can also provide particular information for developing new planting technologies for these soybean varieties in both planting systems.

APPENDICES

A1: Bibliography

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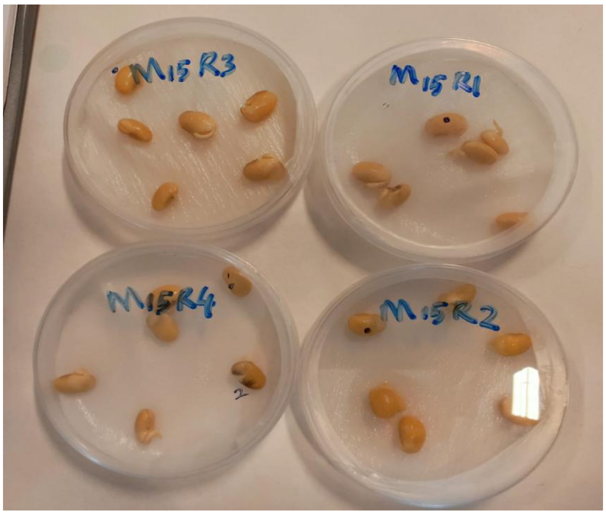
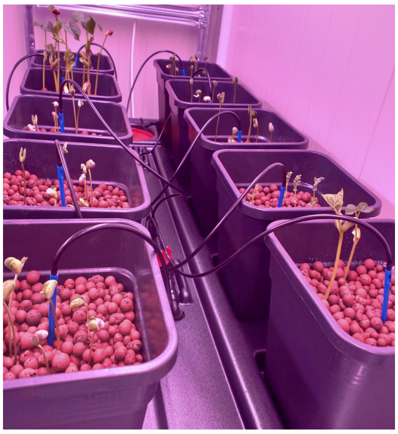
A2: Photos

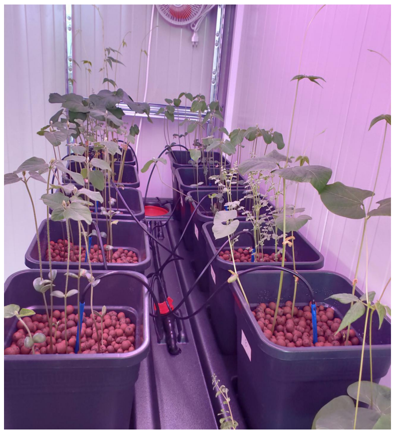
1) Working photos during the field experiments (Experimental Research 1)

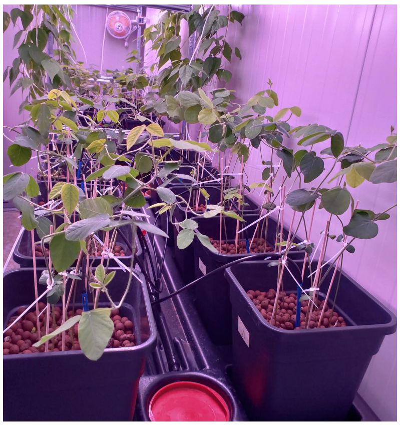
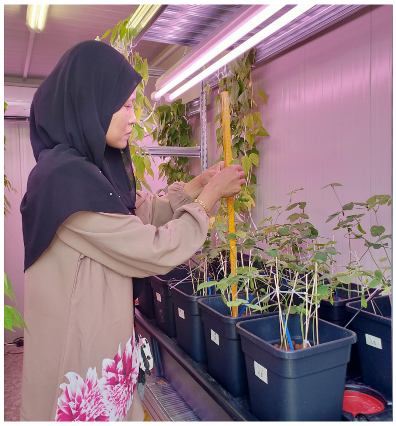
2) Working photos during the controlled environment experiments (Experimental Research 2)



3) Working photos during the controlled environment experiments (Experimental Research 3)



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LIST OF PUBLICATIONS RELATED TO THE TOPIC OF THE DISSERTATION

1. Abd Ghani, R., Omar, S., Jolánkai, M., Tarnawa, Á., Khalid, N., Kassai, M.K., & Kende, Z., (2023). Response of shoot and root growth, yield and chemical composition to nutrient concentrations in soybean varieties grown under soilless and controlled environment conditions. *Agriculture*, 13(10), 1925. IF: 3.6. https://doi.org/10.3390/agriculture13101925
2. Abd Ghani, R., Omar, S., Jolánkai, M., Tarnawa, Á., Kende, Z., Khalid, N., Gyuricza, C., & Kassai., M.K. (2023). Soilless culture applications for early development of soybean crop (*Glycine max* L. Merr). *Agriculture*, 13(9), 1713. IF: 3.6. https://doi.org/10.3390/agriculture13091713
3. Abd Ghani, R., Jolánkai, M., Omar, S., Khalid, N., & Tarnawa, Á. (2023). Influence of temperature and variety on seeds germination and seedlings emergence of soybean (*Glycine max* L. Merr) at different germination times. *Acta Agraria Debreceniensis*. 2023(2). https://doi.org/10.34101/actaagrar/2/13171
4. Abd Ghani, R., Kende, Z., Tarnawa, Á. Omar, S., Kassai, M.K., Jolánkai, M., & Khalid, N. (2023). Nitrogen nutrition and weed management effects on yield and chemical composition of soybean (*Glycine max* L. Merr). *Proceeding of The Youth Science Forum,* 8 June 2023, Keszthely, Hungary, 241-246.
5. Abd Ghani, R., Kende, Z., Tarnawa, Á. Omar, S., Kassai, M.K., & Jolánkai, M. (2021).The effect of nitrogen application and various means of weed control on grain yield, protein and lipid content in soybean cultivation. *Acta Alimentaria*, 50(4), 527-547. IF: 1.2. http://doi.org/10.1556/066.2021.00095
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LIST OF PUBLICATIONS NOT RELATED TO THE TOPIC OF THE DISSERTATION

1. Omar, S., Abd Ghani, R., Khalid, N., Jolánkai, M., Tarnawa, Á., Percze, A., Mikó, P.P., & Kende, Z. (2023). Effects of seed quality and hybrid type on maize germination and yield in Hungary. *Agriculture*, 13(9), 1836. IF: 3.6. https://doi.org/10.3390/agriculture13091836
2. Khalid, N., Tarnawa, Á., Balla, I., Omar, S., Abd Ghani, R., Jolánkai, M., & Kende, Z. (2023). Combination effect of temperature and salinity stress on germination of different maize (*Zea mays* L.) varieties. *Agriculture*, 13(10), 1932. IF: 3.6. https://doi.org/10.3390/agriculture13101932
3. Omar, S., Abd Ghani, R., Tarnawa, Á., Kende, Z., Kassai, M.K., & Jolánkai, M. (2023). Impact of N Supply on Some Leaf Characteristics of Maize Crop. *Columella,* 10(1), 15-25. https://doi.org/10.18380/SZIE.COLUM.2023.10.1.15
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5. Omar, S., Abd Ghani, R., Khaeim, H., Sghaier, A.H., & Jolánkai, M. (2022). The effect of N fertilization on yield and quality of maize (*Zea may* L.). *Acta Alimentaria*. 51(2), 249–258. IF: 1.2. https://doi.org/10.1556/066.2022.00022
6. Omar, S., Tarnawa, Á., Kende, Z., Abd Ghani, R., Kassai, M.K., & Jolánkai, M. (2022). Germination Characteristics of Different Maize Inbred Hybrids and Their Parental Lines.

Cereal Research Communication, 50, 1229–1236. IF: 1.24.
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LIST OF CONDUCTED CONFERENCE PRESENTATION

1. Abd Ghani, R., Kende, Z., Tarnawa, Á. Omar, S., Kassai, M.K., Jolánkai, M., & Khalid, N. (2023). Nitrogen nutrition and weed management effects on yield and chemical composition of soybean (*Glycine max* L. Merr). Oral presented in *Youth Science Forum,* 8 June 2023, Keszthely, Hungary.
2. Omar, S., Abd Ghani, R., Khalid, N., & Jolánkai, M. (2023). Evaluation of maize inbred lines and hybrids for agronomic characteristics, yield, and grain quality. Oral presented in *Youth Science Forum,* 8 June 2023, Keszthely, Hungary.
3. Jolánkai, M., Abd Ghani, R., Omar, S., Kende, Z., Kassai, M.K., & Tarnawa, Á. (2021). Water footprint of protein yield of field crop species based on evapotranspiration patterns. Oral presented in *First National Interdisciplinary Climate Change Conference* (HUPCC), Online conference. 12 - 15 April 2021.