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Vegetative growth and characteristic of Alfalfa (*Medicago sativa* L.) in the first and second generation with different light intensity

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Abstract: This study aimed to measure the effect of light intensity on the vegetative growth of the first and second generation of alfalfa (*Medicago sativa* L.). Completely randomized design nested patterns was used in this research with two different light intensities and 2 different generations of the alfalfa. The alfalfa used was 2-year-old at 14th regrowth stage which was already planted in a glass dome at Forage and Pasture Laboratory. Light intensity was distinguished by using shade (shading net 65%) and without shade. Plants watering was done once a day in the afternoon, and monitoring was done every week, starting at the 1st week after defoliation until the plant age of 4 weeks. The variables observed were vegetative growth (plant height, plant length, number of branches, and number of leaves), productivity (biomass production), and nutrient content (dry matter content, organic matter, crude fat, crude fiber, and crude protein). The results of this study showed that light intensity had a significant effect ($P < 0.05$) on all of the alfalfa vegetative growth parameters and productions of fresh alfalfa, whereas differences of alfalfa generation did not have significant effect ($P > 0.05$). The research results can be concluded that 100% light intensity resulted in higher vegetative growth and fresh production performance than 35% light intensity, while the first and second generations had relatively the same vegetative growth performance. The second generation produces higher fresh production than the first generation.

Keywords: crop production, defoliation, light intensity, lucerne, nutrient, shade, photosynthesis

Introduction

Providing quality forage is an important aspect in supporting ruminant productivity. The development of quality forages such as alfalfa in tropical environments has the potential to be carried out. Alfalfa is a legume that grow well in subtropical which was first developed by Sajimin (2011) production of dry matter can reach 15.48 tons/ha/year with a crude protein content of 18.0 - 29.1%. Furthermore, alfalfa was developed by Suwignyo et al. (2016, 2017, 2019, 2020a, 2020b, 2021a, 2022, 2023). In the tropic with seed viability of 82%, fresh yield of 8 - 13.75 tons/ha/month and crude protein content of 15.3 - 32.27%, known as tropical alfalfa. Thus, the improvement of alfalfa as a quality feed ingredient has the potential to continue to be cultivated.

The development of alfalfa in Indonesia is still not widely done. The information relating to the relationship between different light intensities on the vegetative growth of alfalfa has not been widely studied. Alfalfa requires 6 - 8 hours of sunlight, while sunlight in the tropics reaches 12 hours (Suwignyo, et al., 2020a). In the controlled environment, light intensity is generally added throughout the day or 24 hours light to promote seedling growth and to achieve high production and good quality (Dong et al., 2014; Suwignyo et al., 2021b). Whereas light intensity is important in supporting productivity, except for soil quality and water availability (Suwignyo et al., 2021a; Tang et al., 2022). The light efficiency is a decisive component in plant growth and development that accumulates biomass quantity and nutrient quality.

The intensity of different light affects the plant's response. The light intensity is one of the important factors affecting morphology, photosynthetic characteristics, nutrient contents (Miao et al., 2023; Murod et al., 2025). The plants with good photosynthesis will increase the yield of plant dry weight. Suwignyo et al. (2020a) explains that the light can continuously improve the photosynthesis process of plants. Photosynthesis allows plants to convert light energy into chemical energy (Bello-Olivera et al., 2022). The ability of alfalfa to utilize nitrogen fixation and the support of light energy in the photosynthesis process allows nutrient transport to accumulate into biomass to increase (Suwignyo et al., 2021a; Suwignyo et al., 2020c; Tang et al., 2022). Therefore, this study is important to investigate the adaptability of alfalfa to light intensity on vegetative growth in different generations.

Results and discussion

Soil condition

According to the results of the analysis of soil chemical content (Table 1), it shows that the quality of soil used in growing alfalfa has a low standard based on the SNI (Standar Nasional Indonesia, 2021) soil quality assessment standat. The results of the analysis show that the soil planted with alfalfa needs to be added with organic matter to provide soil available nutrients. Jennings and Loftin (2021) explained that fertility management in alfalfa is emphasized on phosphorus, potassium, boron, and soil pH (6.5 - 7.0), and soil P and K levels should be medium to high before cultivation.

Vegetative growth

The different light intensities and generation differences have complex effects on vegetative growth, biomass production and nutrient quality of alfalfa. The growth of alfalfa for four weeks showed that vegetative growth based on height, length, number of branches of alfalfa was influenced by light intensity (Table 3, 4, 5). It shows that the average height of alfalfa is influenced by light intensity from the 2nd week to the 4th week significantly ($P < 0.05$). Then, the different light intensities showed a significant effect ($P < 0.05$) on the length of alfalfa at the age of the 2nd and 3rd weeks. While the combination of generation differences and light intensity affects the number of alfalfa leaves (Table 6). This is related to plant growth is strongly supported by biotic and abiotic factors. Avice et al. (1997) explained that legume growth is influenced by the interaction of environmental factors (air, soil temperature, solar irradiance levels), endogenous plant factors (reserve levels, number of remaining active meristems), and abiotic (light, water, nutrients). The chemical, physical and biological structure of soil and abiotic factors contribute to the enhancement of growth through the utilization of available nutrients in the soil (Gao et al., 2019; Hidayah et al., 2023; Mauri et al., 2024).

Based on the calculated number of alfalfa leaves, there are significantly different ($P < 0.05$) influenced by both light intensity differences and alfalfa generation in the 1st week and 2nd week. Although, these two factors do not affect the number of alfalfa leaves at the age of the 3rd week and the 4th week (Table 6). The increase in height, stem length and number of branches is influenced by differences in light intensity. Similarly, Suwignyo et al. (2020a) explained that differences in light duration significantly affected the height and number of branches of alfalfa. Likewise, Kurilčik et al. (2008) resulted that the duration of 8 - 16 hours of light intensity increases the length of the stem and roots. The increase in size is supported by the energy from the photosynthesis rate of plants to produce energy for the growth process by utilizing light (Miao et al., 2023). Chlorophyll-rich leaves are an important part of the light-harvesting system, and are affected by shading. Gao et al. (2019); Tang et al. (2022) described that the difference in the amount of light received by the leaves reduces the diameter of the stem in the transportation of nutrients, water and photosynthetic products thus affecting biomass production.

Biomass production

Based on the results of fresh alfalfa harvest, it shows that differences in light intensity and generation differences have a significant effect ($P <0.05$) on the alfalfa fresh yield. The results of our study (Table 7), showed that the combination of different light intensities and different generations affected the production of fresh alfalfa biomass at harvest time of 4 weeks. Alfalfa, second generation and no shade (100%), produced higher biomass production compared to first generation alfalfa with 65% shade. This is indicated by the influence of several Internal factors (plant adaptability, level of food reserves, number of active meristems), and external factors (air, soil temperature, level of sunlight, chemical structure, physics and biology of the soil, and nutrients) (Avice et al., 1997; Gao et al., 2019; Mauri et al., 2024). Biomass production is the accumulation of nutrients stored in plant cells.

Plants tend to utilize accumulated nutrients as a source of energy storage, which is reflected in dry matter and organic matter content (Suwignyo et al., 2017; Suwignyo et al., 2021; Suwignyo et al., 2021). We found that different light intensities and different generations were related to the yield parameters of height, length, number of stems and leaves of alfalfa for 4 weeks. Biomass production may be represented through the accumulation of nutrients that are utilized in the process of cell proliferation for the growth of shoots, branches, leaves, and stems (Gao et al., 2019; Suwignyo et al., 2020; Sariffudin et al., 2021; Tang et al., 2022; Mauri et al., 2024). The ability of legumes to fix nitrogen allows the plants to have quality biomass. Alvarenga et al. (2024) explained that light is a factor that contributes greatly to increasing nitrogen fixation in all substrates, both air and soil. Thus, alfalfa grown without shade is able to fix nitrogen better than those grown with shade.

Nutrient content

Based on the analyzed nutrient content of alfalfa with different light intensities and different generations, it shows that different light intensities affect the nutrient content of dry matter and organic matter of alfalfa significantly ($P < 0.05$). Although, the difference in generation did not show a significantly effect ($P> 0.05$) on the nutrient content of alfalfa at harvest age 4 weeks. Light intensity and generation differences in alfalfa affect the nutrient content of dry matter and organic matter of alfalfa. This indicates the alfalfa response to light intensity and different generation differences. Light intensity is an important factor affecting plant growth and quality, affecting morphology, photosynthetic characteristics, nutrient content (Zainia et al., 2021a; Zainib et al., 2021b; Miao et al., 2023).

The difference in light intensity received can also affect the photosynthetic ability of plants. Tang et al. (2022) explained that photosynthesis allows plants to convert light energy into chemical energy. The study results are in accordance with the results of research by Suwignyo et al. (2020a) explained that the difference in irradiation increases the content of plant organic matter, it is influenced by the rate of photosynthesis to be optimal resulting in cell components by supporting dry matter will increase then followed by an increase in organic matter content. The accumulated energy is used for growth.

The results of this study show that different generations affect the nutrient content of dry matter and organic matter. In contrast to the results of the study by Suwignyo et al. (2021a) showed that the first and second generations did not show different dry matter and organic matter contents, although the results had similar values related to crude protein content that did not differ at the same harvest age. This indicates that the interaction between different light intensities and different generations affects the accumulation of stored nutrients. Suwignyo et al. (2020c); Tang et al. (2022); Miao et al. (2023) explained that exposure to appropriate light intensity during storage after harvest defoliation is beneficial for retaining nutrients, which is an important factor in promoting the accumulation of key nutrients and improving crop quality.

Table 1. Results of soil analysis in the Forage and Pasture Science land.

Soil chemical characteristics	Quantity	SNI	Clasification
pH	7.12	6 – 7	Neutral
N total (%)	0.24	0.21 – 0.5	Medium
C/N rasio (mg100g^{-})	8.00	5 – 10	Low
P_2O_5 potential (mg/100g)	1.46	< 4	Very low
K_2O potential (mg/100g)	0.12	< 10	Very low

Source: Sample analysis at Balai Pengkalan Teknologi Pertanian (BPTP) Yogyakarta; Light Intensity.

Table 2. Results of light intensity measurements with different shade levels at different times.

Shading level (%)	Light Intensity (lux)			
	06.00 a.m	11.00 a.m	04.30 p.m	07.00 p.m
0	6596,2	7138,6	6332,2	633,56
65	4708,6	5063,4	4586,4	222,88

Table 3. The average results of alfalfa height with light intensity in different generations.

Height (cm) measured after	F	Light Intensity		Average
		35%	100%	
After 1st week (indicate the height in cm)	F1	28.08±7.36	31.98±3.73	30.02±6.04
	F2	28.28±4.80	29.60±5.10	28.94±5.35
Average		28.18±6.08	30.78±5.03	
2nd week	F1	30.81±4.62	48.03±3.62	39.42±9.69^{x}
	F2	29.07±5.13	42.02±4.94	35.54±8.25^{y}
Average		29.93±4.86^{a}	45.02±5.23^{b}	
3rd week	F1	30.07±6.81	46.74±5.56	38.40±10.46
	F2	28.56±5.63	40.47±7.83	34.51± 9.03
Average		29.31±6.16^{a}	43.60±7.37^{b}	
4th week	F1	27.37±5.55	39.16±16.25	33.26±13.31
	F2	28.10±5.64	40.88± 7.93	34.49± 9.38
Average		27.73±5.48^{a}	40.02±12.53^{b}	

^{a,b}: Different superscripts on the same line indicate significantly different (P<0,05); ^{x,y}: Different superscripts in the same column indicate significantly different (P<0,05); F: Generation; F1: first-generation breeding seeds; F2: second-generation breeding seeds.

Table 4. The average results of alfalfa length with light intensity in different generations.

Length of alfalfa (cm)	F	Light Intensity		Average
		35%	100%	
1st week	F1	29.82±7.59	31.61±3.94	30.72±5.98
	F2	30.96±5.46	31.54±6.34	31.25±5.79
Average		30.95±6.49	31.57±5.16	
2nd week	F1	38.35±8.36	48.20±4.02	43.27±8.15
	F2	43.09±8.36	47.58±7.15	45.33±7.94
Average		40.72±8.52^{a}	47.89±5.68^{b}	
3rd week	F1	42.25±10.62	49.42±5.66	45.84±9.09
	F2	39.22±6.44	47.70±7.39	43.46±8.04
Average		40.74±8.73^{a}	48.56±6.50^{b}	
4th week	F1	45.60±9.05	48.46±21.44	47.03±16.16
	F2	44.00±8.37	52.85±8.85	48.52±13.08
Average		44.80±8.56	50.25±16.14	

^{a,b}: Different superscripts on the same line indicate significantly different (P<0,05); F: Generation; F1: first-generation breeding seeds; F2: second-generation breeding seeds.

Table 5. The average results of the number of alfalfa branches with light intensity in different generations.

Number of branches	F	Light Intensity		Average
		35%	100%	
1st week	F1	16.58±4.48	19.66±3.49	18.12±4.23
	F2	16.33±5.14	22.91±7.98	19.62±7.37
Average		16.45±4.71^{a}	21.29±6.25^{b}	
2nd week	F1	28.25±8.58	43.25±11.04	35.75±12.34
	F2	30.41±8.98	53.50±16.26	41.95±17.44
Average		29.33±8.66^{a}	48.37±14.57^{b}	
3rd week	F1	36.08±14.98	44.08±6.59	40.08±12.03
	F2	33.83±6.01	51.75±10.12	42.79±12.25
Average		34.95±11.22^{a}	47.91±9.23^{b}	
4th week	F1	41.75±16.87	52.16±21.13	46.95±19.45
	F2	36.50±8.96	59.33±10.50	47.91±15.07
Average		39.12±13.48^{a}	55.75±16.73^{b}	

^{a,b}: Different superscripts on the same line indicate significantly different (P<0,05); F: Generation; F1: first-generation breeding seeds; F2: second-generation breeding seeds.

Table 6. The average results of the number of alfalfa leaves with light intensity in different generations.

Number of leaves	F	Light Intensity		Average
		35%	100%	
1st week	F1	15.66±10.41	17.33± 4.77	16.50±7.96[x]
	F2	22.58± 5.85	25.66±13.06	24.12±10.02[y]
Average		19.12± 8.98[a]	21.50±10.51[b]	
2nd week	F1	16.25± 4.84	25.00± 9.30	20.62±8.51[x]
	F2	29.58± 7.37	38.50±17.96	34.04±14.18[y]
Average		22.91± 9.14[a]	31.75±15.59[b]	
3rd week	F1	19.00± 7.41	21.08± 6.57	20.04±6.93
	F2	21.50± 5.10	23.91± 8.65	22.70±7.06
Average		20.25± 6.35	22.50± 7.65	
4th week	F1	24.08±14.24	18.66±11.02	21.37±12.76
	F2	28.00± 6.68	28.41±12.63	28.20±9.88
Average		26.04±11.06	23.54±12.61	

[a,b]: Different superscripts on the same line indicate significantly different (P<0,05); [x,y]: Different superscripts in the same column indicate significantly different (P<0,05); F: Generation; F1: first-generation breeding seeds; F2: second-generation breeding seeds.

Table 7. The fresh alfalfa yield with light intensity at different generations (tons/ha).

Alfalfa yields	F	Light Intensity		Average
		35%	100%	
Fresh alfalfa yield (tons/ha)	F1	0.67±0.00	2.67±0.91	1.67±1.23[x]
	F2	1.22±0.95	7.51±2.73	4.36±3.89[y]
Average		0.94±0.67[a]	5.08±3.21[b]	3.01±3.09

[a,b]: Different superscripts on the same line indicate significantly different (P<0,05); [x,y]: Different superscripts in the same column indicate significantly different (P<0,05); F: Generation; F1: first-generation breeding seeds; F2: second-generation breeding seeds.

Table 8. The chemical nutrient content of alfalfa with different light intensities and generations.

Nutrient content (%)	F	Light Intensity		Average
		35%	100%	
Dry matter	F1	24.95±0.38	29.83±3.72	27.39±3.56
	F2	24.14±0.44	28.37±1.38	26.25±2.49
Average		24.55±0.57[a]	29.10±2.63[b]	26.82±2.99
Organic matter	F1	90.02±0.24	90.31±0.93	90.16±0.63
	F2	89.12±0.62	90.79±0.49	89.96±1.04
Average		89.57±0.64[a]	90.55±0.71[b]	90.06±0.83
Crude protein	F1	22.32±0.97	22.24±1.04	22.28±0.90
	F2	22.18±0.66	23.05±1.01	22.61±0.89
Average		22.25±0.74	22.64±1.02	22.45±0.87
Ether extract	F1	2.73±0.11	2.80±0.57	2.76±0.37
	F2	2.47±0.62	3.15±0.73	2.81±0.71
Average		2.60±0.42	2.97±0.62	2.78±0.54
Crude fiber	F1	25.40±0.67	24.16±2.28	24.78±1.65
	F2	23.47±1.55	21.97±1.15	22.72±1.47
Average		24.44±1.50	23.07±2.01	23.75±1.84

[a,b]: Different superscripts on the same line indicate significantly different (P<0,05); [x,y]: Different superscripts in the same column indicate significantly different (P<0,05); F: Generation; F1: first-generation breeding seeds; F2: second-generation breeding seeds.

Materials and methods

Plant material and growth conditions

The study used alfalfa (*Medicago sativa* L.) developed by producing a new variety called tropical alfalfa (*Medicago sativa* L. cv Kacang Ratu BW). The study used the results of the first-generation seed and second-generation seed that were 2 year old or had 14th regrowth. To homogenize growth, the research was defoliated firstly (for about 10 cm from the soil surface). A week after defoliation, the research was obeserved for 4 weeks in a dome greenhouse with a UV polyethylene plastic roof, in the forage field of the Laboratory of Forage and Pasture Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia. located at 7°46'08.7 "S 110°23'10.3 "E. The average temperature was 26.64°C with 79.33% humidity.

The study was conducted with a 2 × 2 factorial completely randomized design in a nested design consisting of two types of factors. The first factor was light intensity, composed of 35% (65% shade) and 100% (0% shade). The second factor was the difference in generations resulting from the first and second generation of alfalfa seeds which were 2 years old or had 14th regrowth. Each alfalfa generation had three plants planted in 25 cm diameter pots with regosol soil type media arranged with a distance of 30 cm × 50 cm between pots.

The implementation of the study

The research treatments used shading levels with 0% shade (100% light intensity) and 65% shade (35% light intensity). Irradiation for 15 hours, with 12 hours of sunlight and additional irradiation with lamps for 3 hours starting from 06.00 p.m to 09.00 p.m. the lamp was installed at a height of 1.5 meters from the ground. Measurement of light intensity was carried out using a lux meter. Temperature and

humidity were measured using a thermohygrometer. These measurements were taken in the morning at 06:00 a.m, afternoon (11:00 a.m), evening (04:30 p.m), and night (07:00 p.m). Plants were watered twice a day in the morning and evening.

Plant morphology and Productivity parameters

The variables observed were vegetative growth (plant height, plant length, and number of leaves), and fresh yield. Plant height was measured from the first week after defoliation to 4 weeks. The measurements plant height was measured from the soil surface in the polybag to the highest leaf or stem. whereas, the length of the plant to the peak of the main stem was measured. The number of plant branches was counted once every week until 4 weeks. All branches on the main stem were counted. The number of plant leaves was counted once every week until 4 weeks. All leaves completely opened (trifoliate) of a plant were counted. Harvested alfalfa is sampled to be analyzed for nutrient content. Chemical analysis uses proximate analysis including dry matter content, organic matter, crude protein, crude fat, and crude fiber.

Statistical analysis

Data obtained were analyzed using Analysis of Variance (ANOVA) with a complete randomized block design (Nested Design). Statistical analysis calculations were carried out with the help of personal computer software Statical Product and Service Solution (SPSS) version 25.

Conclusion

Alfalfa with 100% light intensity produces higher vegetative growth performance and fresh production. The second generation produced higher fresh production than the first generation. Then, differences in light intensity and alfalfa generation were only able to affect the nutrient content of dry matter and organic matter.

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