Second regrowth phase generative characteristics of alfalfa *(Medicago sativa* L.) with addition of lighting duration and dolomites

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**Abstract.** This study determines generative growth characteristics of alfalfa in second regrowth with the addition of dolomite and extended photoperiod. This study used a completely randomized design tested via Least Significant Difference. Dolomite was added at 0, 6 and 12 ton/ha. Photoperiod was varied at 12, 14 and 16 hours. The results conclude that extended photoperiod promoted alfalfa growth. Lengthier exposure to lighting promoted faster flowering and greater proportions of pod emergence and pod ripening. The addition of dolomite levels did not affect generative growth, except in the percentage of pods per plants. The addition of 16-hour lighting duration improved the second regrowth stage of alfalfa as compared to 14 and 12-hour photoperiods. The result yielded 61.63% seed purity and 13.33% seed viability.

1. **Introduction**

Feed plays an important role in the livestock business. Ruminant feed in the form of forage should be consistent in quantity and quality. One potential forage plant to be developed is alfalfa *(Medicago sativa* L.). The chemical composition of the feed includes 16.0-29.1% crude protein [1], [2]. They are long day plants, flowering well in areas with greater than 14 hours of sunshine [3]. Alfalfa is a fertile plant with potential for development in tropical countries like Indonesia [4].

The challenge of developing alfalfa plants lies in cultivation. An equatorial country, Indonesia’s experiences approximately equal lengths of daytime and night time (12 hours). Different soil conditions (environment) and maintenance management add to the challenge of cultivating alfalfa in Indonesia. Soil fertility can be increased by adding dolomite as a source of calcium and magnesium [5]. Dolomite is a fertilizer derived from secondary mineral deposits which contain elements of calcium and magnesium, with the chemical formula CaMg (CO$_3$) [6].

Potential cultivation efforts include increasing the length of lighting, conducting defoliation, and adding dolomite to the soil of alfalfa plants. The lengthened photoperiod with white lights can increase the productivity of chrysanthemum *(Chrysanthemum morifolium)* [7]. It encourages crop productivity seen in healthier growth, longer life and flower induction. Lighting colors, such as white neon lights, also increase the productivity of kailan *(Brassica oleracea)* and pakcoy *(Brassica rapa)* plants [6], [8]. Vegetative growth and kailan crop production increase with treatment under white fluorescent lamps. These lights produce better results than red, blue, or yellow fluorescent lights, although fluorescent lights are not as effective compared to exposure to natural sunlight [6].
To increase the productivity of alfalfa plants, one solution is cutting/ defoliation. Bag states that this method can optimize production, quality, and continuity of alfalfa production [9]. Cutting management depends on the stage of plant development, height, and defoliation interval. Alfalfa can be grown for up to 10 years with cutting every 30-60 days. This research was informed by previous research on the development and cultivation of alfalfa seed production, and characteristics of Alfalfa's generative growth pattern. The goal was to determine generative growth characteristics of alfalfa in the second regrowth stage, with the addition of light and different dolomite concentrations.

2. Material and methods
The research was conducted at the Laboratory Greenhouse for Animal Feed and Pasture, Department of Animal Nutrition and Food, Faculty of Animal Husbandry, Gadjah Mada University, Yogyakarta. The tools used in the study include polybags (45 x 45 cm), plastic mulch, scales, bamboo, raffia rope, hoes, saws, machetes, knives, scissors, portray, buckets, dipper, sprayer, cables, cool daylight fluorescent lights (36 watts), thermometer, switch timer, roller meter, stationery, label paper, ruler, camera, petri dish. The ingredients used were alfalfa (Medicago sativa L.) seeds, dolomite, leaf fertilizer, water, and regosol soil from the Laboratory of Feed Forages and Pasture Science, Faculty of Animal Science at Universitas Gadjah Mada, Yogyakarta, Indonesia.

This study used a completely randomized design, following the Least Significant Difference (LSD) test at the 5% significance level. It consisted of 2 treatments. The first, dolomite lime level (D), consisted of 3 levels: D0 = 0 ton ha\(^{-1}\) (control); D1 = 6 ton ha\(^{-1}\); D2 = 12 ton ha\(^{-1}\). The second was the extension of photoperiod, consisting of 3 levels: C0 = 12 hours (control); C1 = 14 hours; C2 = 16 hours per day.

The planting media was soil collected from the farmland of the Forage and Pasture Science Laboratory, Faculty of Animal Science at Universitas Gadjah Mada, Yogyakarta, Indonesia. The soil was mixed with leaf fertilizer in a ratio of 1: 1, then added into a polybag/ each polybag contained 3 plants. The artificial sunlight was a lamp with a 36-watt cool daylight fluorescent lamp, installed 1 meter above the plant. Curtains were used to separate each treatment.

Watering was done twice a day (6am and 5pm). Alfalfa was watered until the entire surface of the soil was moist. In the morning, curtains that divided each treatment were opened. They were closed in the afternoon (6pm). Environmental temperature was measured three times a day, morning (7am), noon (12pm) and evening (6pm).

3. Results and discussion
3.1. General research conditions
Observations at the study site showed temperatures ranging from 21-34.5 °C, with an average temperature of 27.47 °C. Alfalfa requires temperatures ranging from 22-27 °C [3]. The temperature at the study site was less optimal for alfalfa growth. The soil before the study had a carbon content of 5.32-7.69%, nitrogen content of 0.48-0.64%, potential P\(_2\)O\(_5\) 210-250 mg/100g, potential K\(_2\)O 37-48 mg/100g. The results of the soil composition in the study are presented in Table 1.

3.2. First flower
The results show the effects of lighting duration and dolomite on the first flower in the 2nd regrowth stage (Table 2). The results of the analysis show that additional lighting treatment promoted first flower significantly (P < 0.05). The 16 hours photoperiod accelerated the first flower, at 18.83 days. Dolomite doses and interactions between treatments showed no significant differences (P > 0.05).

The first flower in the first generation of alfalfa was day 48 after planting [4]. Flowering was influenced by 3 factors: photoperiod, phytochrome, and plant maturity. Photoperiod is the ratio between the duration of sun exposure from day to night. The farther from the equator, will be the greater the difference between the length of day and night. Phytochrome is a type of protein pigment with components that can absorb light. Phytochrome plays an important role in plants during the day [9].
Table 1. The quality of sample soils.

| Sample soil | Parameter       | C-organic (%) | N-content (%) | Potential P<sub>2</sub>O<sub>5</sub> (mg/100g) | Potential K<sub>2</sub>O (mg/100g) |
|-------------|----------------|---------------|---------------|--------------------------------|--------------------------------|
| C0D0        |                | 7.69          | 0.64          | 239                            | 39                            |
| C0D1        |                | 6.41          | 0.48          | 221                            | 37                            |
| C0D2        |                | 6.22          | 0.51          | 230                            | 39                            |
| C1D0        |                | 6.94          | 0.55          | 250                            | 41                            |
| C1D1        |                | 7.09          | 0.61          | 249                            | 37                            |
| C1D2        |                | 7.54          | 0.59          | 224                            | 43                            |
| C2D0        |                | 5.96          | 0.51          | 212                            | 39                            |
| C2D1        |                | 7.46          | 0.55          | 210                            | 41                            |
| C2D2        |                | 5.32          | 0.48          | 214                            | 48                            |

Table 2. First flower in 2<sup>nd</sup> regrowth of alfalfa (days).

| Lighting duration | Dolomite dosage | Average |
|-------------------|-----------------|---------|
|                   | D0 (0 ton/ha)   | D1 (6 ton/ha) | D2 (12 ton/ha) |         |
| C0 (12 hour)      | 26.50±6.19      | 23.66±8.38   | 22.00±0.00     | 24.87±6.31<sup>a</sup> |
| C1 (14 hour)      | 22.75±2.62      | 24.75±1.25   | 22.75±2.75     | 23.41±2.31<sup>a</sup> |
| C2 (16 hour)      | 20.25±5.37      | 17.00±2.75   | 19.25±3.09     | 18.83±3.80<sup>b</sup> |
| Average           | 23.16±5.23      | 21.63±5.51   | 21.11±3.10     | -       |

<sup>a,b</sup> different superscripts in the same row or column shows significant differences (P<0.05). Mark (-) indicates there was no interaction.

3.3. The percentage of flowering plants

The analysis shows that the duration of lighting treatment significantly increased (P < 0.05) the percentage of flowering plants (Table 3). The highest percentage of flowering plants (83.33%) occurred with 16 hours treatment (C2), followed by 14 and 12 hours of lighting. Treatment with dolomite and interactions between treatments showed no significant differences.

Table 3. Percentage of flowering plants (%).

| Lighting duration | Dolomite dosage | Average |
|-------------------|-----------------|---------|
|                   | D0 (0 ton/ha)   | D1 (6 ton/ha) | D2 (12 ton/ha) |         |
| C0 (12 hour)      | 33.33±00.00     | 33.00±27.21  | 16.66±33.33    | 27.77±23.92<sup>a</sup> |
| C1 (14 hour)      | 74.99±16.67     | 49.99±19.24  | 66.66±00.00    | 63.88±17.16<sup>b</sup> |
| C2 (16 hour)      | 91.66±16.67     | 91.66±16.67  | 66.66±00.00    | 83.33±17.41<sup>c</sup> |
| Average           | 66.66±28.42     | 58.33±32.17  | 49.99±30.14    | -       |

<sup>a,b,c</sup> superscripts in the same row or column show significant differences (P<0.05). Mark (-) indicates there was no interaction.

This study observed a greater percentage of flowering plants as a result of lighting treatment than a previous study [4]. The study reported the percentage of flowering plants in the regrowth phase was
77.78% (16 hours), 66.88% (14 hours), and 2.78% (12 hours). In the regrowth phase, the perennial alfalfa plant already has roots. Since alfalfa stores carbohydrates in its roots, those carbohydrate reserves can be used in its regrowth phase.

The greater the length of photoperiod, will be the greater the percentage of flowering plants. The duration of lighting determines the length of the internode. Short duration of lighting results in short internodes and causes flowering to be obstructed. Artificial lighting can be used to control the flowering time of plants with the aim of increasing crop yields [9].

3.4. First pod set
The results in Table 4 demonstrate that the duration of lighting treatment resulted in significantly different instances of first flower (P < 0.05) compared to the initial group. Dolomite dose treatment and interaction between treatments showed no significant differences (P > 0.05). This indicates that photoperiod affects the first flower in alfalfa. Plants that flower faster will also produce pods faster. In the regrowth phase, the average time needed for alfalfa to produce pods is 5 days after flowering. Previous research found that pods were produced 7-14 days after flowering [4]. Podding relies on pollination: without pollination, the flowers will fall and fail to bear fruit [5].

| Lighting duration | Dolomite dose | Average |
|-------------------|---------------|---------|
|                   | D0 (0 ton/ha) | D1 (6 ton/ha) | D2 (12 ton/ha) |
| C0 (12 hour)      | 30.00±0.00    | 33.00±4.58   | 28.50±3.53     | 30.85±3.67* |
| C1 (14 hour)      | 27.00±1.82    | 30.00±2.44   | 28.50±2.88     | 28.50±2.54a |
| C2 (16 hour)      | 25.25±4.27    | 23.50±1.73   | 23.75±2.87     | 24.16±2.94b |
| Average           | 26.90±3.24    | 28.45±3.88   | 26.60±3.59     | -           |

3.5. Podding plants
The data in Table 5 show the duration of lighting treatment and interaction between treatments resulted in significantly different pod set (P < 0.05). Dolomite dose treatment did not significantly affect pod set (P > 0.05). Longer photoperiod resulted in a higher percentage of plants with pod set. The number of flowering plants shows a positive correlation with the number of podding plants. Not all flowering plants will set pods.

| Lighting duration | Dolomite dose | Average |
|-------------------|---------------|---------|
|                   | D0 (0 ton/ha) | D1 (6 ton/ha) | D2 (12 ton/ha) |
| C0 (12 hour)      | 16.66±19.24c  | 24.99±16.66c | 16.66±33.33c   | 19.44±22.28a |
| C1 (14 hour)      | 74.99±16.67b  | 33.33±0.00ab | 58.32±16.66ab  | 55.55±21.71b |
| C2 (16 hour)      | 91.66±16.67b  | 91.66±16.67b | 58.32±16.66a   | 80.55±22.28c |
| Average           | 61.10±37.15   | 49.99±33.33  | 44.44±29.58    | -            |

The average percentage of podding plants decreases 6.48% from the percentage of flowering plants. That is, 93.52% of flowering plants will produce pods. There are 3 reasons for failure of pod set: 1) lack
of pollination due to the loss of stamens and pollen; 2) lack of pollination because pollen is weak or unsuitable; 3) the fall of flowers [5].

The percentage of podding plants in this study was greater than the percentage reported in a previous study [4]. The study found the average percentages of podding plants were 50% (16 hours), 44.44% (14 hours) and 2.78% (12 hours). Differences occur because this study analyses second regrowth phase while the previous study followed the initial growth phase from seeds.

3.6. Number of pods per plant
The results in Table 6 show the duration of lighting treatment and dolomite dose resulted in significantly different proportions of pod set plants (P < 0.05). The results showed a positive correlation between the number of flowering plants, the number of podding plants, and the number of pods in each individual. The results of a previous study showed the highest average number of pods in C2D2 treatment was 252.22 pods [4]. In contrast, this study found the highest average number of pods in C2D2 treatment was 180.25.

Table 6. Number of pods.

| Lighting duration (D0) | Dolomite dose (D1) | Dolomite dose (D2) | Average |
|------------------------|-------------------|--------------------|---------|
| 12 hour                | 0.00±0.00         | 0.00±0.00          | 42.00±0.00 | 42.00±0.00 |
| 14 hour                | 0.00±0.00         | 0.00±0.00          | 0.00±0.00 | 0.00±0.00 |
| 16 hour                | 38.66±2.08        | 39.66±2.08         | 40.00±2.00 | 39.44±1.87 |
| Average                | 38.66±2.08        | 39.66±2.08         | 40.50±1.91 | -         |

a,b different superscript on the same row or column showed significant differences (P<0.05). Mark (-) indicates there was no interaction.

Calcium and magnesium soil abundance can stimulate cell turgor and chlorophyll formation, increasing the photosynthetic process and its products. Conversely, calcium deficiency inhibits root system growth, and magnesium deficiency inhibits the enzymatic action of the Citric Acid Cycle. Similarly, phosphorus deficiency can inhibit root and generative growth [10][11], Administration of dolomite can alleviate this problem by increasing the availability of phosphorus in the soil.

3.7. First time ripe pods
The results of the observation in Table 7 showed that alfalfa pods first ripened i37 days after defoliation, i.e. with the previous treatment of 16 hours photoperiod and without the addition of dolomite (C2D0).

Table 7. First pod ripening (days).

| Lighting duration (D0) | Dolomite dose (D1) | Dolomite dose (D2) | Average |
|------------------------|-------------------|--------------------|---------|
| 12 hour                | 0.00±0.00         | 42.00±0.00         | 42.00±0.00 |
| 14 hour                | 0.00±0.00         | 0.00±0.00          | 0.00±0.00 |
| 16 hour                | 38.66±2.08        | 40.00±2.00         | 39.44±1.87 |
| Average                | 38.66±2.08        | 39.66±2.08         | 40.50±1.91 | -         |

Mark (-) indicates there was no interaction.
There were no ripe pods for plants treated with 14 hours of lighting (C1), nor 12 hours of lighting (C0). Only one plant was observed with ripe pods, i.e. without addition of dolomite at 12 ton/ha (C0D2). All treatments showed non-significant differences (P > 0.05) in the percentage of ripe pods, likely due to alfalfa pods requiring longer time to mature. Alfalfa blooms about 7 weeks each period, producing pods and ripe pods 3 to 5 weeks after flowering [12]. Crops that grow faster will also more quickly produce pods that will also ripen faster. In the second regrowth phase, the average first ripening was 12.34 days after first podding.

3.8. Percentage ripe pods plants
The percentage of ripe pods plants was 42 days after defoliation are presented in Table 8. The results show that differences in lighting duration resulted in significantly different percentages of plants with ripe pods (P < 0.05). Dolomite dose treatment and interaction between treatments show no significant differences (P > 0.05).

Table 8. Percentage of ripe pods plants (%).

| Lighting duration | Dolomite dose | Average |
|-------------------|---------------|---------|
|                   | D0 (0 ton/ha) | D1 (6 ton/ha) | D2 (12 ton/ha) |       |
| C0 (12 hour)      | 0.00±0.00     | 0.00±0.00    | 8.33±16.66     | 2.77±9.62<sup>a</sup> |
| C1 (14 hour)      | 0.00±0.00     | 0.00±0.00    | 0.00±0.00      | 0.00±0.00<sup>a</sup> |
| C2 (16 hour)      | 33.33±27.21   | 24.99±16.66  | 24.99±16.66    | 27.77±19.24<sup>b</sup> |
| Average           | 11.11±21.70   | 8.33±15.07   | 11.11±16.41    | -     |

<sup>a,b</sup> superscripts in the same row or column show significant differences (P<0.05). Mark (-) indicates there was no interaction.

Results indicate not all pod set plants will ripen (only 51.86%). The number of unripe pods may be due to the pods requiring a longer time to mature. Alfalfa pods ripen at 3 to 5 weeks after flowering. A fruit is considered mature if the maximum size and growth rate of dry weight has reached zero. Mature pods are characterized by a change in color from green to brown, due to the disappearance of chlorophyll during the maturing process [5], [12].

3.9. Test of purity, damage, and viability of seeds
Factors that affect seed quality include seed purity, damage and viability. Seed quality determines the quantity of seeds available for cultivation in a particular area (able 9).

Table 9. Percentage (%) purity, damage, and viability of alfalfa seed.

| Parameter          | F1 alfalfa | Regrowth |
|--------------------|------------|----------|
|                    | 1<sup>*</sup> | 1 | 2         |
| Seed purity        | -          | - | 61.63     |
| Seed damage        | -          | - | 33.69     |
| Seed viability     | 67.00      | - | 13.33     |

<sup>*</sup>Suwignyo et al., 2017

3.9.1. Seed purity. Seed purity test results showed that the purity of alfalfa seeds was 61.63%. Overall seed weight was 1.4446 g, foreign body weight was 0.0674 g, and seed weight was 0.4868 g. A purity test is the first test administered to determine the quality of seed. The pure seeds are then used for other tests. Seed testing is important, especially when buying seeds or using seeds that have been stored for a
long time. Information on the results of seed testing allows the user to determine the requirements for planting a set of seeds [13][14].

3.9.2. Seed damage. The calculation result shows that 33.69% of the samples were damaged seeds. Seed damage is affected by the harvesting and storage process. A good harvest should occur when the seeds are mature. When harvested during the maturation process, seed moisture content is between 25% to 30%. This causes the seeds to become easily damaged. Damage to seeds can be prevented by drying the seeds immediately after harvesting. After drying, the seeds need to be packed with the right packaging methods and materials to maintain the quality of the seeds during storage. Consideration for the method of storing seeds helps to maintain seed quality [15].

3.9.3. Seed viability. The results of the viability test were 13.33%, which means that the survival of alfalfa seeds that can germinate in this study is low. As a comparison, the growth test of alfalfa seed [4] with regosol soil media showed that the percentage of F1 alfalfa seed growth was 67.00%. Germination includes water absorption, rapid O2 uptake, food reserve hydrolysis, and new tissue synthesis. Factors that influence seed germination are 1) Environment (water, temperature, gas, light, exogenous chemical compounds); 2) Seed maturity [5].

4. Conclusion
This study concludes that regrowth affects the generative growth of alfalfa plants. The longer the photoperiod will be the faster the first time of flowering, pod set, and ripening. Longer light treatments increase the percentage of flowering, pod set, and ripening in alfalfa plants. Dolomite addition does not affect generative growth, except in the percentage of pods per plants. The result yielded a seed purity of 61.63% and seed viability of 13.33%.

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