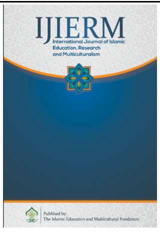

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EFFECT OF SEEDLING AGES AND GENERATIONS ON THE MORPHOLOGY AND PRODUCTIVITY OF TROPICAL ALFALFA (*MEDICAGO SATIVA L. CV KACANG RATU BW*)

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Abstrak

Produktivitas ternak ruminansia sangat bergantung pada ketersediaan pakan hijauan berkualitas yang memenuhi kebutuhannya. Alfalfa memiliki rasa yang lezat dan kaya akan nutrisi, mineral, dan vitamin yang dibutuhkan ternak. Penelitian ini bertujuan untuk mengetahui pengaruh umur bibit dan generasi alfalfa tropis terhadap morfologi dan produktivitas alfalfa tropis (Medicago sativa L. cv Kacang Ratu BW). Rancangan penelitian yang digunakan adalah Rancangan Acak Lengkap (RAL) 2 x 2 dengan dua faktorial dan tiga kali ulangan. Evaluasi pengaruh umur bibit (1 bulan dan 2 bulan) dan generasi (F2 dan F3) terhadap pertumbuhan tanaman (tinggi tanaman, cabang, dan daun tanaman) dan produktivitas dicatat. Perbedaan antar perlakuan dilanjutkan dengan analisis menggunakan Uji Jarak Berganda Duncan. Hasil penelitian menunjukkan bahwa umur bibit 2 bulan menghasilkan produktivitas tanaman tertinggi ($P<0{,}05$). Di antara generasi yang diuji, F3 muncul sebagai produktivitas tanaman teratas ($P<0{,}05$). Produktivitas tanaman terdiri dari tinggi tanaman, jumlah cabang, jumlah daun. Hasil penelitian menyimpulkan bahwa penanaman bibit alfalfa pada umur dua bulan memberikan pengaruh yang signifikan terhadap tinggi tanaman dan jumlah daun, sehingga menghasilkan pertumbuhan yang lebih baik secara keseluruhan. Namun, pengaruh generasi, khususnya F3, mempengaruhi tinggi tanaman. Jumlah cabang tidak memberikan pengaruh yang signifikan terhadap umur bibit maupun generasi.

Kata Kunci: *Generasi, Morfologi, Produktivitas, Stres, Alfalfa Tropis, Vegetatif.*

Abstract

The productivity of ruminant livestock is highly dependent on the availability of quality forage feed that meets their needs. Alfalfa is palatable and rich in nutrients, minerals and vitamins that livestock may

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require. This study aimed to investigate the effect of age of tropical alfalfa seedlings and generations on the morphology and productivity of tropical alfalfa (*Medicago sativa* L. cv Kacang Ratu BW). The research design is *Completely Randomized Design* using 2 × 2 with two factorial and three replications. Evaluation of the effect of seedling ages (1 month and 2 months) and generations (F2 and F3) on plant growth (height of plants, branches, and leaves of plants) and productivity were recorded. The differences between treatments were continued, analysis with *Duncan's Multiple Range Test* is used. The result showed that seedling ages in 2 months produced the highest plant productivity (P<0,05). Among the generations tested, F3 emerged as the top plant productivity (P<0,05). Plants productivity consisted of height of plants, number of branches, number of leaves. The study concluded that planting alfalfa seedlings at two months old significantly influences plant height and leaf count, resulting in better overall growth. However, the influence of generation, specifically F3, affects plant height. The number of branches does not significantly affect either seedling age or generation.

Keywords: *generations, morphology, productivity, stress, tropical alfalfa, vegetative.*

INTRODUCTION

Forage plays a key role in promoting sustainable growth and production among ruminant animals as the main nutrient; however, there are some typical problems that challenge forage availability namely water shortage, animal excrement pollution, and greenhouse gasses emissions (Lou et al., 2021). To tackle forage shortages, particularly during the dry season, the cultivation of drought-resistant legumes is imperative (Ayele et al., 2021; Notenbaert et al., 2021; Tulu et al., 2023). Moreover, in hot climates, decreased rainfall negatively affects forage quality, leading to a decrease in nutrient content. As a result, the nutritional needs of livestock are not met the dietary requirement of livestock (Pérez-Prieto et al., 2018). Farmers heavily depend on natural forages growing in the grassland without engaging in additional forage planting and cultivation.

Conversely, a significant challenge will appear particularly the dry season poses is the scarcity of forage, which, over time, can adversely affect the amount of forage produced leads to livestock productivity, particularly causing weight loss in livestock due to insufficient forage (Sulfiar et al., 2020). Addressing the challenges posed by the dry season requires strategic planning and sustainable practices. Introducing drought-resistant forage species can be beneficial (Guo et al., 2019). In 2007, the introduction of alfalfa imported seed in Ciawi, Indonesia, marked a pivotal moment contributing in tackling this forage shortage. Especially tropical alfalfa, it is notably considered as forage that offers high-quality nutrients such as protein, fibers, minerals, and vitamins, making them suitable for livestock feed known as the "Queen of Forage".

This forage also produces high quality seeds if treated properly (Barros et al., 2019; Suwignyo et al., 2020; Suwignyo et al., 2021). Correspondingly, the chlorophyll content of alfalfa is four times higher compared to other leguminous plants (Suwignyo et al., 2014). On a practical level, Farmers cultivate alfalfa on 30 million hectares globally to satisfy the needs of the livestock and dairy industries (Barros et al., 2019). This signifies the prominents needs of Alfalfa stock in the world especially Indonesia. Like other forage, Alfalfa also faces challenges posed by water shortage on the early growth of Alfalfa seedlings continuing to be a considerable hindrance to Alfalfa cultivation (Radović et al., 2009; Guo et al., 2019). Furthermore, the plant did not produce viable seeds, limiting its production due to scarcity of fertile seeds (Suwignyo et al., 2023). Consequently, it is crucial to employ effective methods to enhance alfalfa's productivity, especially during its initial developmental stages, ensuring the long-term sustainability of alfalfa production (Slathia et al., 2012; Roy et al., 2021).

There are several key methodologies that may enhance alfalfa productivity namely seedling age and its generation. The age of the seedlings significantly impacts their ability to adapt when transplanted in the soil. If the seedling age is older (± 2 months) they often struggle more when transferred to a new environment. They tend to adapt more slowly, exhibit uneven growth, develop shallow roots, and experience incomplete growth, leading to stress. On the other hand, younger seedlings adapt more quickly to the new environment and develop deeper roots, making the plants more resistant to toppling, more drought-tolerant, and more effective in water and nutrient utilization from deeper soil layers. The quality of planted seedlings is linked to their age during the nursery phase. Stronger plant roots contribute to increased environmental stress and improve overall plant health and productivity (Grossnickle & MacDonald, 2018). Young seedlings are especially prone to diseases and damage from herbicides. However, as they progress in their growth, they develop increased resistance to seedling disease and become less vulnerable to herbicide-related harm. Hence, it is crucial to be able to identify each growth stage accurately to ensure that treatments are applied at the appropriate times (Undersander et al., 1997).

LITERATURE REVIEW

Another important factor to develop new varieties of major crops is through plant breeding that is more tolerant to drought stress (Joshi, 2017). Phenotypic variations and genetic diversity associated with drought tolerance in alfalfa accessions has been investigated by (Zhang et al., 2018). Additionally, several studies have explored the molecular or proteomic analysis of alfalfa under drought conditions (Kang et al., 2016; Arshad et al., 2017; Li & Su, 2017; El-Ramady et al., 2020), including during seed germination (Ma et al., 2017; El-Ramady et al., 2020). To support alfalfa tropic productivity by its generation, there are several generations of alfalfa tropic that have been investigated so far, namely F1 and F2, it resulted that the second generation exhibited better growing ability (Suwignyo et al., 2021). F3 is then devices as superior generations which exhibit traits such as higher productivity, improved disease resistance, forage quality-traits (Julier et al., 2000; Javaid et al., 2020).

These traits increase yield potential that can determine the potential of alfalfa tropic for fodder. Genetic diversity in F3 is shaped by crossing previous two parents. Pure parent lines (homozygous) produce uniform F1 hybrids, leading to the F2 generation (Kirk et al., 2012). The F2 generation is the generation with the maximum recombination or gene exchange, making it the best choice for plant breeding selection activities. This selection activity is expected to result in high-yielding varieties. The F3 generation has a wider recombination or gene exchange or variety due to selection in the F3 generation caused by selection (Zečević et al., 2011).

In Indonesia, very little research has been done on alfalfa tropic, especially its productivity. Several research that has been conducted were held by Bambang Suwignyo aiming to discover tropical alfalfa since 2010.

Based Suwignyo et al. (2023) found that tropical alfalfa has positive result, it thrives at temperatures up to 48°C while optimal growth for subtropics occurs at around 20°C, the average growth rate of tropical alfalfa after defoliation is about 1.4 to 1.6 cm per day and during the first two weeks post-defoliation, the growth rate can reach 2 to 5 cm per day and lastly, supplementation with up to 6% lower cholesterol levels and boost blood HDL levels (Suwignyo et al., 2021; Suwignyo et al., 2023).

This denotes that Alfalfa research in Indonesia is still limited especially after the emergence of tropical alfalfa (Kacang Ratu BW) developed by Bambang Suwignyo brings hope for its development in tropical regions, especially Indonesia. However, while these findings provide a strong basis for tropical alfalfa development in Indonesia, there are still significant knowledge gaps related to specific factors that affect its productivity, such as the impact of seedling age and generation on morphology and yield. This study aims to address these gaps by systematically evaluating how seedling age and generation affect the growth, survival and productivity of tropical alfalfa under tropical conditions. With this approach, this research is expected to provide new insights that support the development of tropical alfalfa, especially varieties suitable for tropical conditions such as in Indonesia.

Specifically, this study aims to determine the most optimal seedling age to maximize alfalfa growth, survival and yield in tropical environments. In addition, this study also evaluated the performance of different generations of alfalfa (F2, F3) in terms of plant growth (plant height, branches, and plant leaves) and productivity recorded. Furthermore, the study sought to identify key morphological and physiological traits that contribute to the superior performance of alfalfa across different generations. With this approach, the results of the study are expected to make a significant contribution towards improving alfalfa productivity in tropical regions. This research is expected to contribute to the development of tropical alfalfa in Indonesia, especially its productivity based on the generation and the seedling age.

RESEARCH METHODS

This research was conducted for 2 months from October to November 2023 at the Livestock Feed and Pasture Research Area at Gadjah Mada University. The equipment used in this research included an oven with a temperature of 55°C, a digital scale with a sensitivity of 0.1 kg, staples, newspapers, silica discs, hoses, sprayers, hoes, pots, scissors, a long ruler, and writing tools. The materials used were alfalfa seedlings aged 1 and 2 months, and alfalfa plants from generations F2 and F3.

This research was conducted using a completely randomized factorial design of 2x2 patterns with the first factor being seedling age consisting of 1 month and 2 months, and the second factor being generations 2 and 3. The research was carried out on an area of 100 m² divided into 5 plots measuring 4 x 9 m each. The bed size used was 1 x 4 m with a distance of 50 cm between beds. Regosol soil type was used. The seedling age treatments consisted of U1 (1 month) and U2 (2 months). The generation treatments consisted of F2 (Generation 2) and F3 (Generation 3).

The research data were analyzed using analysis of variance (ANOVA) according to the Completely Randomized Design factorial experiment (Astuti, 1980). As there were significant differences in the results, Duncan's Multiple Range Test (DRMT) was continued. All statistical analysis calculations were performed using the Statistical Product and Service Solution (SPSS) version 27.0 software

RESULTS AND DISCUSSION

The age of seedlings and generations influence both the morphology and productivity of the plant species Kacang Ratu BW (KRBW) or *Medicago sativa* cv Kacang Ratu BW, commonly known as tropical alfalfa. It can be investigated by its plants' height, number of leaves and branches produced.

Amount of height

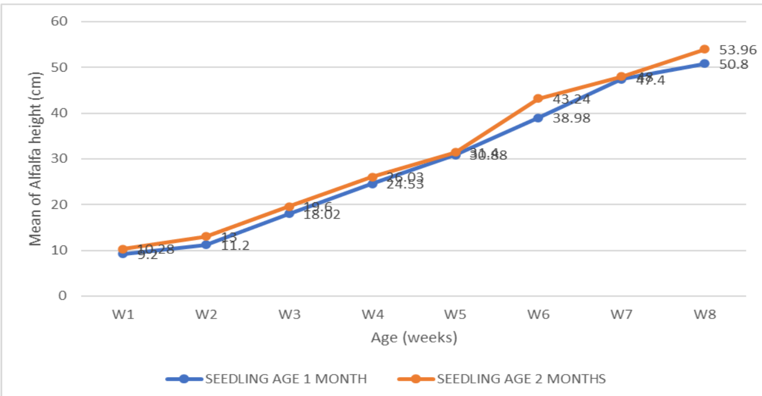

Figure 1. Average growth of tropical alfalfa height from the first week to the eight weeks for seedling age

Figure 1 depicts a clear and consistent upward trend is evident from week 1 to week 8 across both seedlings' ages. Interestingly, an intriguing convergence occurred at weeks 5 and 7, where seedlings aged 1 month and 2 months reached similar growth points. However, it is essential to highlight that the most substantial increase was noted in seedlings aged 2 months during the sixth week, persisting and peaking at 53.96 cm, the conclusion of the 8-week period, while seedlings aged 1 month reached a height of 50.80 cm (Figure 1).

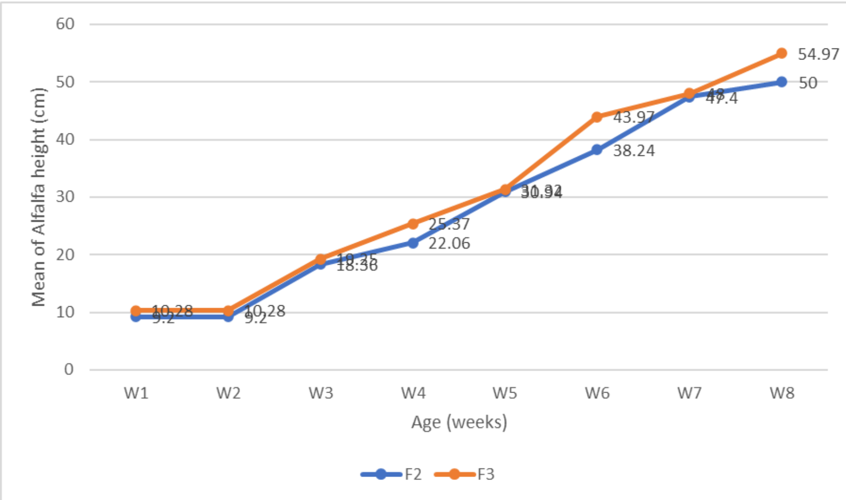

Figure 2. Average growth of tropical alfalfa height from the first week to the eight weeks for generations

Figure 2 shows a constant pattern of growth in the initial stages, with progression continuing steadily until reaching notable milestones in week 5 and 7. Particularly, the growth of generation F3, which reached 43.97 cm by week 6 and peaked at 54.97 cm thereafter. In contrast, generation F2 stood at 50 cm at the same point in time. This suggests that F3 generation is only slightly better than its parent, F2 generation (Average growth of tropical alfalfa height from the first week to the eight weeks for generations can be seen in Figure 2). The average height of tropical alfalfa over 6 weeks can be seen in Table 1.

Table 1. Average height of alfalfa plants over 6 weeks.

Parameters	**Plant height (cm)**		**Average**
	Generation (F2)	**Generation (F3)**	
SA (1-month)	36.18±3.70	41.75±4.40	38.97±4.84$_b$

SA (2-months)	40.30±3.43	46.17±2.54	43.24±4.20$_a$
Average	38.24±7.13^q	43.96±3.47$_p$	

$_{a,\ b:}$ Different superscripts within the same column indicate significant difference (P<0,05)

$_{p,\ q:}$ Different superscripts within the same row indicate significant differences (P<0,05)

SA: Seedling age

Table 1 shows the statistical analysis result of both seedling age and generation comparison. From this table, it is evident that the age of 61 days after sowing significantly influences the plant height compared to 30 days after sowing (P<0.05), which is 43.24±4.20. Research states that alfalfa seedling age significantly affects plant height 35 days after sowing. Factors such as seedling age, light availability, and genetic background significantly impact growth rates and overall height (De Frutos et al., 2024). Another finding finds that age and growth conditions, including soil quality and water availability, are critical determinants of seedling height (Collet & Le Moguedec, 2007). Therefore, seedling age cannot be considered as the only primary factors that determine height.

However, the results of this study which showed that seedling age significantly affected the height of tropical alfalfa plants-60 days after sowing produced taller plants. Research find that alfalfa roots developed well in the first month; as a result, alfalfa height is better in the second month as it has a strong root system already to impede water uptake from the soil.

Besides the seedling age, the generation of alfalfa also contributes to the plant height. This research found that generation F3 is significantly higher compared to generation F2 (P<0.05), which is 43.96±3.47 and 38.24±7.13 respectively. This is in line with what Adrianto et al. (2021) has stated that F2 offspring have higher diversity and new traits that can emerge that are not possessed by their parents through gene interaction for example height. Alfalfa F3 generation can have higher height compared to its parent, because it is derived from genotype variations between generations F2 and F1. The success of selection programs for forage yields and its components primarily relies on the significant genetic variability observed in morphological traits and forage yield. The heritability of the chosen traits, the correlation between different characteristics, and the intensity of selection applied are also crucial factors (Abdel-Galil, 2007; Veronesi et al., 2010; Bakheit et al., 2011, Annicchiarico, 2015; Seiam & El-Nahrawy, 2020). Thus, generation can significantly affect the height of alfalfa plants, indicated by new generations F3 being taller than F2.

In conclusion, these two factors: seedling age and generation can significantly affect the height of tropical alfalfa seedling. Older seedling age (2 months) have a better growth compared to younger one (1 month) as it has well developed roots to absorb water and nutrients from soil. In addition, generation is also significant as the latest generation (F3) can have better traits compared to its parents (F1 and F2) including having a better growth of height.

Amount of leaves

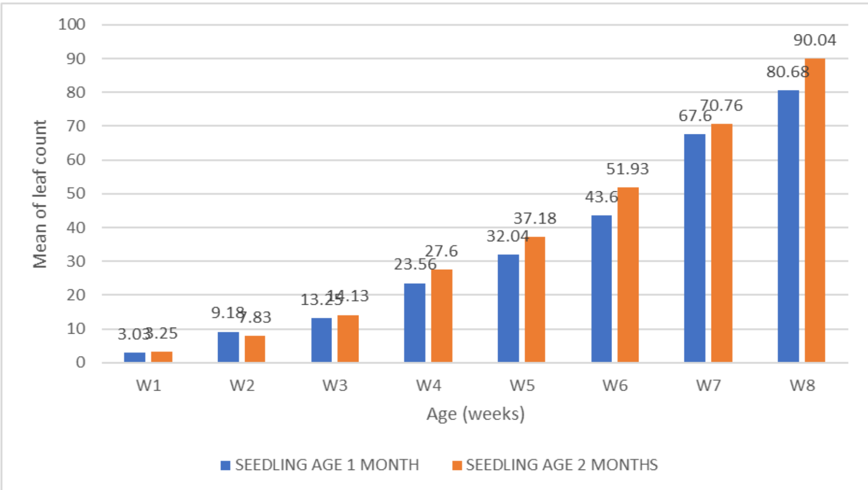

Figure 3. The Average of tropical alfalfa leaves over eight weeks for seedling age

Figure 3 shows the progress of seedling growth throughout times, from week 1 to week 8. This figure reveals a significant increase in all measured parameters. Substantially, seedlings aged 2 months had a slower growth week 1 to week 2, yet surpassed seedling age 1 month in growth by week 3, accelerating thereafter. By week 3, seedlings aged 2 months had reached a leaf count of 14.13 and continuously had more outstanding growth than seedling age 1 month until week 8. At week 8, it reached 90.04, surpassing the leaf count of seedlings aged 1 month, which stood at 80.68 (Figure 3).

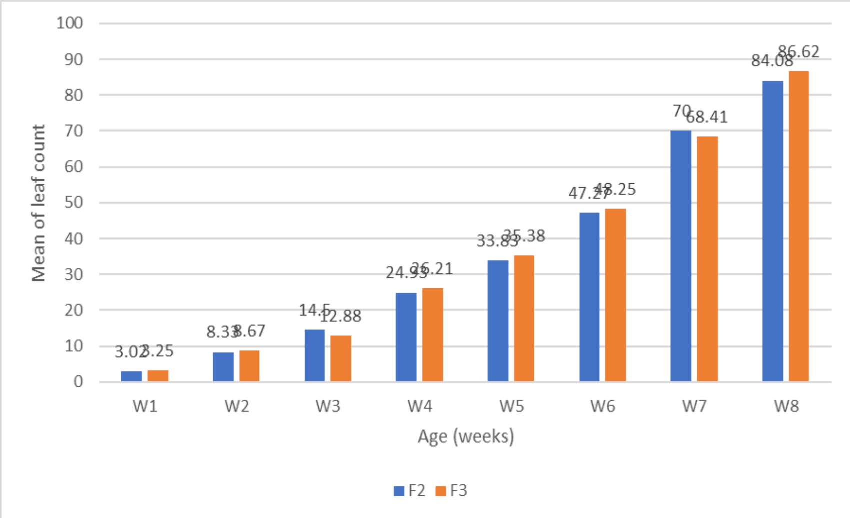

Figure 4. The Average of tropical alfalfa leaves over eight weeks for seedling age

Figure 4 depicts an upward trajectory for both generation F2 and F3 over the observed period. Notably, in week 3 and 7, generation F2 spotted higher values than F3. However, in the other weeks depicted on the figure, generation F3 always had more leaves compared to its parent, F2 (The average of tropical alfalfa leaves over eight weeks for generations can be seen in Figure 4). The average amount of leaves of tropical alfalfa over 6 weeks can be seen in Table 2.

Tabel 2. Average number of leaves of tropical alfalfa over 6 weeks

Parameters	Plant height (cm)		Average
	Generation (F2)	Generation (F3)	
SA (1-month)	43,92±7,64	43,25±6,72	43,58±6,87[b]
SA (2-months)	50,58±5,73	53,25±2,32	51,92±4,39[a]
Average	47,25±6,68[ns]	48,25±4,52[ns]	

a, b: Different superscripts within the same column indicate significant differences (P<0,05)

ns: No significant difference

SA: Seedling age

Table 2 shows that 2-month-old seedlings significantly result in more leaves compared to 1-month-old seedlings ($P<0.05$) at 51.92±4.39 and 43,58±6,87 respectively. This aligns with who stated that older seedlings are more likely to adapt to their environment, thus growing well and producing an optimal number of leaves. Photosynthesis and root system are the reasons why older seedlings grow more leaves; the plant has more stomata to be able to absorb CO_2 for photosynthesis, and it has a well-developed root system enabling better absorption from soil.

Another potential reason why younger seedlings (1 months) can not grow as well as older seedlings (2 months) is because of stress encountered as the result of immediate planting, termed as planting stress. These seedlings are not yet fully integrated into the hydrological cycle where water moves from the soil to the plant's roots, traversing through the plant and into the atmosphere. This planting stress can result in restricted root growth, thereby hindering photosynthesis due to water stress stemming from inadequate root development. As photosynthesis is a crucial process that is sensitive to water shortages and high temperatures (Xu et al., 2021) seedlings should be old enough in order to have a better root growth. Photosynthesis relies on light intensity that captured by plant leaves effects depends on leaf area at the field (Tardieu, 2013). In short, this research result supports the idea that older seedling has a better morphological performance or growth especially its leaves in those 61 days after sowing seedling has a more leaves count compared to 30 days after sowing seedling.

Based on generation, the morphology alfalfa tropical generation F3 (48.25 leaves) does not significantly affect the leaf count compared to generation F2 (47.25 leaves) as the statistics show that P value is bigger than 0.05 ($P>0.05$). This research on alfalfa tropical morphology, especially leaves based on the generation, has rarely been conducted in the research. However, this is essential because leaves are regarded as a vital functional unit of a plant including alfalfa and significantly contribute to yield formation as well as showing a positive correlation with forage yield (Jia et al., 2022). Min et al. (2022) states that leaves are crucial for an agronomic trait in which it will increase its nutrient accumulation. This insignificant difference between generations is actually acceptable because there are some other factors that affect the growth of alfalfa leaves. For example, that genetics is not the only factor that affects the plant but also its environment as in the formula: P= G + E + G x E (interaction effect). In addition, that there was a positive correlation observed between leaf traits measured over three years, indicating their dependence on environmental changes. This variation may stem from hereditary differences influenced by a particular climate or environment.

Additionally, Previous research studies *Medicago truncatula* about miR156-targeted MsSPL08 in leaf development and abiotic stress response in alfalfa, states that the growth and development of plant leaves are controlled by transcriptional regulators such as SPL of various biological process, such as phase change, leaf development, branching, stem growth, and response to stress. Several developmental processes and abiotic stress responses are regulated by these transcriptional regulators.

In addition, previous research found that some environmental factors, particularly drought or salt stress may affect plants' leaf resistance. Lignin, for example, is synthesised in various plant leaves during the drought and salt stress. For instance, maize leaves showed a significant increase in lignin content under both severe and moderate drought stress, making lignin a useful indicator of drought stress in maize. Similarly, chickpeas accumulated more lignin inter primary roots when subjected to drought stress. Drought stress is also rooted to generic traits eliminating bad traits from the parent can address abiotic problems such as making plants more drought-resistant, tolerant to temperature, pests, and resulting in increased number of leaves (Lamers et al., 2020). The amount of lignin (a component of the cell wall) promotes and strengthens plant resistance to abiotic stress biosynthesis in alfalfa (Yang et al., 2024).

Thus, generation of Alfalfa has a correlation to its morphology growth, yet is not the primary factor that can influence the development of the plant, especially its leaves. Some other potential factors that affect the Alfalfa morphology are environmental changes, transcriptional regulators, drought stress and salt stress that led to lignin production. These factors work separately but integratively to influence the development of Alfalfa leaves.

Amount of branch

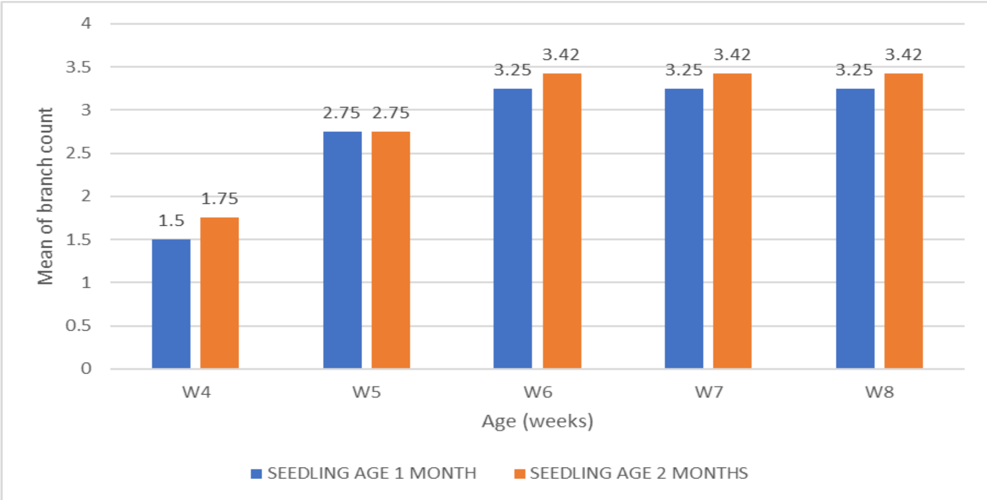

Figure 5. The average number of tropical alfalfa branches

Figure 5 delineates that seedling age 2 months has relatively better performance in branch growth compared to seedling age 1 month. However, by week 5, both seedlings aged 1 month and 2 months were at the same point, at 2.75 (Figure 6).

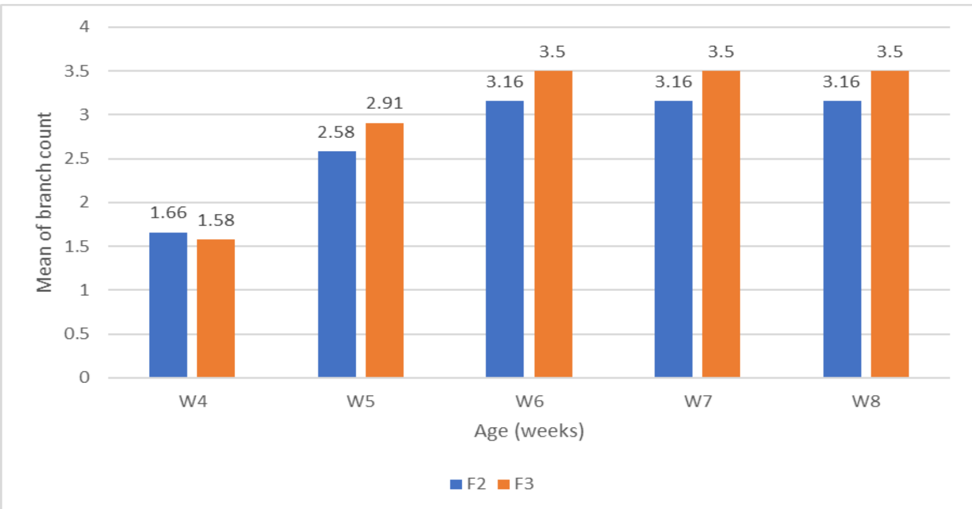

Figure 6. The average number of tropical alfalfa branches

Figure 6 indicates tropical alfalfa branch growth based on generation. Generation F3 exhibits a constant increase throughout the weeks (week 4 - week 8) despite that during week 4, generation F2 is instead higher in performance compared to its next generation (The average number of tropical alfalfa branch generations can be seen in Figure 6. The average amount of branches of tropical alfalfa over 4 weeks can be seen in Table 3.

Tabel 3. Average number of branches of tropical alfalfa over 4 weeks

Parameters	**Plant height (cm)**		**Average**
	Generation (F2)	**Generation (F3)**	
SA (1-month)	1,50±0,55	1,50±0,55	1,50±0,52^{ns}
SA (2-months)	1,83±0,98	1,67±0,82	1,75±0,87^{ns}
Average	1,66±0,76^{ns}	1,58±0,68^{ns}	

^{ns} : No significant difference
SA : Seedling age

As can be seen from table 3, the performance on branch growth based on seedling age and generation seem to be insignificant. The age of seedling does not significantly influence the number of branches growth as both branches reach almost the same branch count: 1,75±0,87 (1 month) and 1,50±0,52 (2 months) despite a slight increase of branch number of the 2-month-old seedling. The *P* value is more than 0.05 (P>0.05). Apart from seedling age, generation also influences morphological growth of alfalfa seedling. This research, however, finds that both generation F2 and F3 have relatively the same performance in terms of branch growth during the 4-week planting period. From the branch count, F2 seems to be slightly more outstanding than his other breeding, F3.

Rarely have research on seedling age and generation effect on branches count been conducted. Branch number is a crucial morphological trait that could improve forage yield in alfalfa cultivated in arid environments (Jia et al., 2022). Most research on branching, branch count, and branch growth on Alfalfa plant and Alfalfa seedling revolve around the fertilisers effect on branch, the light availability on branch, transcriptional regulators availability on branching, environment on branch (Buhaerah & Kuruseng,2016; Susanto & Baskorowati, 2018; Lamers et al., 2020), root system influences the spatial distribution of roots in the soil and their efficiency in water and nutrient uptake (Berntson et al., 1997), morphological plasticity in response to the physicality of soil (Toca et al., 2022).

Another factor that may contribute to the insignificant difference between generation and seedling age on branch count and growth is the condition during the research. This research was held during October, November, and December in Indonesia during the rainy season. This allowed the seedling to have a greater amount of water intake that can support their growth. Nevertheless, not only the plant that received plenty of water but pests also manage to grow quite well that it almost competes with the height of the seedling especially in week 4. Weed infestation has a direct impact on the production of desirable species (Lavarello Herbin et al., 2020) by competing for essential resources such as nutrients, water, light, and space, ultimately reducing their productivity.

CONCLUSION

The study concluded that the seedling at two months old significanly affects alfalfa tropical height and leaf count, resulting in better overall growth. However, generations F3, affects plant height. The number of branches does not significantly influence either seedling age or generations.

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