Physiological Response of Two Amaranth Varieties (*Amaranthus tricolor* L.) to Urea Applications

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ABSTRACT

Amaranth, a highly nutritious leaf vegetable, requires adequate nutrients, especially nitrogen fertilizers, to grow well and produce an optimal yield. Urea is commonly used to promote the growth of amaranth plants. This study examined the physiological responses of two varieties of amaranth to the application of urea fertilizer. This study examined the physiological responses of two varieties of amaranth to the application of urea fertilizer. This investigation was conducted in July - August 2023 in Sukosari Village, Jumantono, Karanganyar. The study utilized a completely randomized design (CRD) with two treatment variables. The first variable consisted of a variety of amaranth, including green (B1) and red (B2) varieties. The second variable in the study was the dosages of urea, including 0, 50, 100, 150, 200, 250, and 300 kg.ha-1. The study observed no correlation between various varieties of amaranth and the dose of urea applied in terms of plant growth and physiological activities. There was no discernible impact on the growth or physiological activity of both varieties of amaranth when urea fertilizer was applied. Both varieties of amaranth showed similar physiological responses to urea fertilization. However, the effect of adding urea fertilizer was to enhance the vegetative growth of amaranth plants.

Keywords: Amaranth; Analysis; Fertilizer; Growth; Nitrogen

INTRODUCTION

Amaranth (*Amaranthus sp.*) originated in Tropical America and then spread to various parts of the country. Amaranth was originally an ornamental plant but is now known as a popular vegetable high in protein and vitamins. Amaranth is a plant rich in protein and a valuable source of vitamins A, B, C, and fiber. Additionally, the leaves of amaranth contain significant amounts of oxalic acid. Furthermore, amaranth also includes minerals, iron, magnesium, phosphorus, calcium, and charcoal hydrate in the form of cellulose (Suarjana et al., 2019). The two most consumed and cultivated amaranth types in Indonesia are green and red, with similar nutritional content. Amaranth contains all the nutrients such as carbohydrates, proteins, fats, vitamins A, B1, B3, B9, C, and minerals such as calcium, sodium, and potassium (Mahmud et al., 2018). The high nutritional content of amaranth makes it one of the sources of leaf vegetables that are beneficial to the body, so it is widely cultivated both commercially and at the household level (Ghufron et al., 2023).

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The cultivation of amaranth plants is quite simple, with factors that must be considered, such as the availability of nutrients necessary for plant growth. The availability of nutrients is crucial in increasing plant growth and yield. The nutritional needs of plants in cultivation activities are met through fertilization (Nurrudin et al., 2020). During its growth and development, amaranth plants depend highly on nitrogen availability. Amaranth is a leaf vegetable, where the leaves and stems are consumed, so it requires a large amount of nitrogen during its growth and development. Nitrogen is a vital element that serves an indispensable function in plant vegetative stages through its provision of assimilation, which is utilized in the development of organ structures (including roots, stems, and leaves) and metabolic processes (Annisa et al., 2018; Rahmani et al., 2020).

Nitrogen requirements during cultivation are met through fertilization. A nitrogen fertilizer that is widely applied in vegetable cultivation is urea. The high nitrogen content in urea fertilizer supports plant growth and development. Urea fertilization produces greener, fresher, and more lush plant leaves. Nitrogen plays a role in the production of chlorophyll, the green colour found in leaves. The high chlorophyll level will increase the photosynthesis process and encourage the formation of vegetative parts like plant height, count of tillers, count of branches, and others (Kogoya et al., 2018). Fertilization with chemical fertilizers (inorganic) with high nutrient content can meet the nutrient needs of amaranth, such as N from urea fertilizer, which has a nitrogen content of 46%, which is important for vegetative growth (Ramadhanty et al., 2024). Nitrogen is a crucial element for plants, serving as the main nutrient. It is a fundamental component of proteins, which are made up of amino acids. These proteins catalyze chemical reactions, transport electrons, and produce chlorophyll. Chlorophyll is essential for photosynthesis, which occurs in various important parts of the plant's body. Nitrogen imparts plants with their characteristic dark green hue, facilitating the proliferation and maturation of foliage, stems, and other non-reproductive components.

Furthermore, nitrogen also promotes the development of roots. Nitrogen is important in numerous physiological processes that impact a plant's growth and productivity (Zayed et al., 2023). Efforts have been made in previous research to increase the yield of amaranth plants. The height of the plant, leaf area, and count of leaves are the main focus of various nitrogen fertilizer applications during plant growth and development. According to Gorung et al. (2022), a 200 kg.ha^{-1} urea dose can enhance amaranth growth, including plant height, leaf count, and total weight. Budiyani et al. (2022) found that the application of urea fertilization significantly interacted with green amaranth varieties to improve the total fresh weight of plants by up to 75.18%. Nevertheless, further investigation is required to thoroughly examine the physiological reaction of amaranth plants when exposed to nitrogen fertilizer during their growth and development. This study examined the physiological responses of two amaranth varieties to urea fertilizer.

MATERIALS AND METHOD

Study Area

The experiment was conducted at the experimental area of the Faculty of Agriculture, Sebelas Maret University in Sukosari Village, Jumantono District, Karanganyar Regency, from July to August 2023, with an altitude of 177.7 meters above sea level at the coordinates of -7°37'50.496"S and

110°56'55.116"E. Laboratory analysis was performed at the Laboratory of Plant Physiology and Biotechnology, as well as the Laboratory of Ecology and Crop Production Management, Faculty of Agriculture, Sebelas Maret University.

Experimental Design

The experiment was arranged in a two-factor factorial completely randomized design (CRD), consisting of amaranth varieties and urea doses as factors. The amaranth varieties used were certified seeds widely cultivated and composed of two amaranth plants: the Maestro variety of green amaranth and the Mira variety of red amaranth. The doses of urea consisted of 7 levels, namely 0, 50, 100, 150, 200, 250, and 300 $kg.ha^{-1}$ based on nitrogen fertilization recommendations of 100 $kg.ha^{-1}$ to 200 $kg.ha^{-1}$ (Gorung et al., 2022; Hendra et al., 2021). From each treatment, 14 combined treatments were obtained, which were then made into three replications to obtain 42 experimental units.

Research Practices

The research stages included land preparation, seed germination, transplanting, fertilization, watering, pest control, and harvesting. The seeds were germinated in seedling trays for 14 days. Amaranth seedlings were transferred from the seedling tray and planted in the planting media in polybags with one seedling per polybag (Annisa et al., 2018). 25 cm x 30 cm polybags were placed atop gallons for planting. The distance between the gallons was 30 cm x 30 cm. The planting medium was soil mixed with dolomite at 0.09 g/polybags. Amaranth plants require a pH of 6-7 during their growth, so adding dolomite increases the pH of the soil to reach a neutral reaction. The dolomite application increases soil pH because dolomite is used as a substitute for cations such as Al^{3+}, which can acidify the soil. An increase in base cations, neutralising soil pH balances a decrease in the concentration of Al^{3+} dissolved in the soil (Iswara & Nuraini, 2022). KCL and TSP fertilizers were applied after seven days after transplanting (DAT) at a dose of 100 $kg.ha^{-1}$ and 50 $kg.ha^{-1}$, respectively, while urea fertilizer application was given 7 and 14 days after transplanting (DAT) with a dose ratio 50:50 for each treatment dose. Watering was done twice daily while handling plant-disrupting organisms was done manually.

Observation

The research observations included several variables, namely plant growth (plant fresh weight, leaf area index, specific leaf weight, and specific leaf area) and plant physiological activity (photosynthetic rate, transpiration rate, stomatal conductance, and leaf chlorophyll content). Plant fresh weight (g) was obtained by weighing all plant parts from each treatment carried out at harvest time, namely at the age of 28 DAT. Weighing was carried out in the condition of plants cleaned from the soil using analytical scales (accuracy ± 0.01). Leaf area index, specific leaf weight, and specific leaf area were calculated using the equation from (Gardner et al., 1991) as follows:

$$LAI = \frac{LA}{A} \tag{1}$$

LAI = leaf area index, LA = total leaf area, and A = area. Specific leaf weight was calculated with the following equation:

$$SLW = \frac{Lw}{LA} \quad (2)$$

SLW = specific leaf weight, Lw = fresh leaf weight, and LA = leaf area, while the specific leaf area was calculated using the following equation:

$$SLA = \frac{LA}{Lw} \quad (3)$$

SLA = Specific Leaf Area, LA = leaf area, and Lw = leaf weight.

Photosynthetic rate, transpiration rate, and stomatal conductance were measured using a Portable Plant Photosynthetic Meter (Model NY-1020, Nanbei instrument limited) on the third fully opened leaf between 10:00 a.m. and 1:00 p.m. under sunny conditions (not cloudy) (Boy et al., 2022). The chlorophyll content of leaves was determined utilizing the Arnon (1949) method (Rahayu et al., 2021) by pulverizing 0.5 grams of fully opened amaranth leaves in a mortar, followed by the addition of 10 ml of 80% acetone. Subsequently, the solution was passed through a piece of filter paper. The filtrate was introduced into the cuvette to the designated limit to determine the absorbance. A spectrophotometer measured the absorbance at two specific wavelengths (λ) of 645 and 663 nm. The calculation for chlorophyll content was as follows:

Chlorophyll a = (12.7 x A663 – 2.69 x A645) x (10 ml/1000 x 0.5 g) (4)

Chlorophyll b = (22.9 x A645 – 4.68 x A663) x (10 ml/1000 x 0.5 g) (5)

Total Chlorophyll = (20.2 x A645 + 8.02 x A663) x (10 ml/1000 x 0.5 g) (6)

Stomatal openings and stomata density were quantified using the replica method (Wolf et al., 1979). First, the surface of the leaf samples on each side was cleaned using tissue to remove dust, dirt, and debris. The lower leaf surface (abaxial) that had been cleaned was smeared with a cuticle and left for approximately 10–15 minutes until the cuticle dried. The latex spread that had dried was peeled off / taken slowly directly, or the latex spread that had dried was plastered with tape and flattened, then the tape was taken and attached to the glass object, flattened, and labeled according to the treatment. The number of stomata and the width of stomatal openings were observed using a binocular microscope with weak (100x) and strong (400x) magnification and documented using an Optilab microscope camera (Prastika et al., 2023).

Data Analysis

The data were analyzed using analysis of variance and then tested using the Duncan Multiple Range Test (DMRT) at a significance level of $\alpha = 5\%$. The analysis was conducted to determine the presence of a highly significant effect on the variables observed.

RESULTS AND DISCUSSION

The results showed no significant interaction ($p > 0.05$) between urea doses and amaranth varieties for all observed variables. Doses of urea and amaranth varieties showed results that were not significantly different ($p > 0.05$) between treatments. These results indicate that the doses of urea given to the two amaranth varieties have not been able to increase amaranth growth significantly. In general, adding urea as a nitrogen source spurs the vegetative growth of amaranth plants. Nitrogen content in the growing medium is too low (Table 1), so adding urea fertilizer as the sole nitrogen source in amaranth cultivation has not met the nitrogen requirements needed to obtain maximum

Table 1. Soil Chemical Analysis Results

Soil Chemical	Results	Units	Marking *
pH	5.97	-	Slightly acidic
Potential K	7.18	mgK_2O/100g	Extremely low
Available P	1.77	ppm	Extremely low
CEC	32.08	cmol(+)/kg	High
Organic C	3.64	%	High
Total N	0.40	%	Extremely low
Texture			
Loam	8.63	%	Loam
Silt	48.36	%	
Sand	43.01	%	

Source: Soil analysis results of BSIP Environmental Agriculture, Pati, and Soil Laboratory, UGM. Marking based on Petunjuk Teknis Analisa Tanah, Tanaman, Air dan Pupuk, Balai Penelitian Tanah (2009).

Table 2. Fresh Weight (g), Leaf Area Index, Specific Leaf Weight and Specific Leaf Area

Treatment	Fresh Weight (g)	Leaf Area Index	Specific Leaf Weight ($g.cm^{-2}$)	Specific Leaf Area ($cm^2.g^{-1}$)
Amaranth				
Green	4.453 a	0.40 a	0.021 a	985.830 a
Red	3.874 a	0.31 a	0.021 a	521.201 a
Urea ($kg.ha^{1}$)				
0	2.688 a	0.27 a	0.019 a	614.908 a
50	3.653 a	0.25 a	0.021 a	531.856 a
100	4.216 a	0.38 a	0.018 a	516.265 a
150	3.966 a	0.38 a	0.018 a	619.698 a
200	5.234 a	0.37 a	0.019 a	574.551 a
250	3.551 a	0.28 a	0.025 a	554.878 a
300	5.836 a	0.56 a	0.024 a	589.788 a
Interaction	-	-	-	-

Remarks: Mean values associated with the same letters are not significantly different according to Duncan's Multiple Range Test at 0.05 level of significance.

yields. Prakoso et al. (2018) stated that the same N content in the growing media used affects the total N content so that the N absorbed during the addition of urea fertilizer is thought to be not appropriately accumulated, which results in the utilization of nitrogen by plants not being optimal. In addition, the nitrogen in urea fertilizer is susceptible to rapid loss via evaporation and leaching due to its rapid release, whereas nitrogen is mobile. Without sufficient N, plant growth will be better; however, plant growth and development will only be optimal if plant needs are fulfilled (Faqih et al., 2019; Rahayu et al., 2021). Green amaranth produced a higher fresh weight of stalks; the red amaranth exhibited a lower leaf area index and a specific leaf area than the other amaranth varieties. However, the specific leaf weight of both amaranth varieties showed similar results, with no significant effect observed ($p > 0.05$) (Table 2). It has been observed that plant genetics play significant roles in the superior growth of green amaranth over red amaranth. Plant growth is influenced by a variety of factors, including environmental conditions, genetics, and cultivation techniques. The use of various varieties is the application of genetic factors. Genetics of a variety will significantly affect the potential yield. Crop management factors include determining the amount and timing of fertilizer application. Genetic influences and environmental conditions can cause plants to have different phenotypes. Each different gene in each variety is expressed in different characters. Environmental factors continue to influence the appearance of genes, so similar plants are often obtained but with different characteristics (Apriliani, 2022).

In the treatment of urea doses given, the highest average fresh weight of stems and leaf area index were obtained from the addition of the urea dose of 300 kg.ha^{-1}; the greatest specific leaf weight was observed at urea doses of 250 kg.ha^{-1}; while the highest specific leaf area was observed at urea doses of 150 kg.ha^{-1}. Nevertheless, these values showed no substantial variation across all growth variables. The interaction between urea fertilizer and other soil nutrients affects fertilizer's effectiveness in increasing plant growth. The different doses of urea used without being followed by differences in K and P fertilizers are thought to result in an imbalance of nutrients that amaranth plants need, so they cannot increase growth optimally. KCL and TSP fertilizers were applied at the same doses to all plants based on amaranth fertilizer recommendations with a ratio of N: P: K of 100:100:50 kg/ha, so it is better if the increase in N dose is also followed by an increase in K and P fertilizers so that the nutritional needs of plants can be met. According to Satriawi et al. (2020), N is a nutrient that plays a significant role in photosynthesis. If the photosynthesis process runs well, photosynthesis will increase, increasing plant growth and fresh weight. The K nutrient element is a nutrient that plays a role in opening and closing the stomata, so if the plant lacks the K element, it will not be able to carry out the photosynthesis process optimally and result in a decrease in the fresh weight produced by the plant (Marian & Tuhuteru, 2019). Nutrient P is a nutrient that helps activate enzymes in the photosynthesis process so that the photosynthesis process can run well. The fresh weight obtained was similar, presumably due to the condition of amaranth plants, which mostly have thin and broad leaves, so even though the plants have many leaves and leaf areas, it does not affect the fresh weight of amaranth plants. The thinness of these leaves is also an indicator that the leaves have low water content and low mesophyll tissue. Leaf tissue is mesophyll. Leaf mesophyll tissue is the most active part of photosynthesis, and mesophyll forms plant assimilates. Therefore, the fresh weight produced is also low even though it has many leaves (Prakoso et al., 2018; Wahyuningtyas et al., 2022).

The whole weight of plant components not harvested is known as the fresh weight of stover. Green amaranth had a higher average fresh weight than red amaranth, although the differences were not statistically significant ($p>0.05$). Green amaranth has longer and more roots than red amaranth (Telaumbanua et al., 2023). The increase in fresh weight of stover is correlated with the increase in LAI. The addition of urea doses causes sufficient nitrogen requirements for plant growth, thus increasing the leaf area index of plants (Manurung & Nurchayati, 2020). A measure expressing the relationship between the total area of a plant's leaves and the ground acres it occupies is called the leaf area index, also referred to as LAI. The higher the LAI, the more photosynthesis the plant can carry out, which means that the leaf surface available for photosynthesis is more comprehensive in producing assimilates for plant growth. The increase in leaf area is caused by nitrogen released by urea fertilizer, which increases plants' vegetative growth, such as roots, stems, and leaves (Mondal et al., 2019). If nitrogen nutrient requirements are met, plants perform photosynthesis, producing organic compounds that are converted into energy during respiration. Plants then put this energy to use for their growth, which manifests in an increase in the number of leaves, leaf area, and the overall mass of plants (Apriliani, 2022). The LAI increase indicates plants' response to the addition of urea doses the increase in urea dose results in greater availability of nitrogen required by plants for growth. The leaves' ability to capture sunlight increases with a greater leaf area so that the photosynthesis process and the energy produced are also greater, ultimately increasing the plant's total dry weight (Lutfiah & Pratiwi, 2021).

Specific leaf weight and a specific leaf area are test parameters used to measure the thickness of plant leaves. After analyzing the variance, the specific leaf weight and specific leaf area did not show any significant variations across different amaranth varieties and urea doses. The average specific leaf weight in both varieties gave the same result of 0.021 $g.cm^{-2}$, while the urea doses produced an average specific leaf weight of 0.18 to 0.25 $g.cm^{-2}$ (Table 2).

Plants' growth and metabolism depend entirely on the nitrogen supply. Nitrogen is a crucial mineral that stimulates the development of plant structures such as stems, leaves, and roots. It also plays a vital role in producing various biomolecules, including proteins, amino acids, nucleic acids, coenzymes, and chlorophyll. Chlorophyll is particularly significant due to its crucial function in photosynthesis (Mengesha, 2021). Larger applications of urea can increase the nitrogen content available to plants that can be used in their growth. Plants absorb nitrogen to produce more vegetative parts, particularly leaves. Nitrogen is crucial in the formation of amino acids, the fundamental building blocks of proteins, so the greater the absorption of nitrogen by plants will increase the formation of the number and size of plant cells (Handayani et al., 2020; Kogoya et al., 2018; Yadav et al., 2022; Zaman et al., 2022).

Leaf thickness measurements determine the condition of the leaves produced during plant growth. The specific leaf weight (SLW) and the specific leaf area (SLA) have an inverse relationship. The specific leaf area (SLA) and/or specific leaf weight (SLW) of thick leaves exhibit lower values, while thin leaves have higher and/or lower values, respectively (Dahu, 2022). Low specific leaf weight values indicate that plants are lighter tolerant than plants with high specific leaf weight values. Plants

Table 3. Photosynthetic Rate, Transpiration Rate, Stomatal Conductance, Number of Stomata and Stomatal Opening Width

Treatment	Photosynthetic Rate ($\mu mol.m^{-2}.s^{-1}$)	Transpiration Rate ($\mu mol.m^{-2}.s^{-1}$)	Stomatal Conductance ($\mu mol.m^{-2}.s^{-1}$)	Number of Stomata	Stomatal Opening Width (µm)
Amaranth					
Green	16.894 a	0.249 a	7.762 a	27.714 a	45.343 b
Red	10.929 a	0.238 a	7.482 a	26.048 a	34.352 a
Urea ($kg.ha^{-1}$)					
0	10.973 a	0.203 a	6.256 a	30.167 a	59.867 b
50	10.363 a	0.263 a	8.120 a	33.000 a	40.133 ab
100	6.588 a	0.352 a	10.990 a	31.000 a	40.500 ab
150	19.612 a	0.286 a	8.673 a	22.500 a	31.233 a
200	20.966 a	0.181 a	5.628 a	22.167 a	35.967 a
250	20.117 a	0.205 a	6.896 a	20.167 a	34.133 a
300	8.760 a	0.214 a	6.788 a	29.167 a	37.100 a
Interaction	-	-	-	-	-

Remarks: Mean values associated with the same letters are not significantly different according to Dun can's Multiple Range Test at 0.05 level of significance.

with a small specific leaf weight usually have wider leaves with thin leaf thickness. Palisade cells that are formed are few and have a short cell size. Greater light capture is possible in wide and thin leaves, resulting in the transmission of captured light to the lower area of the leaf, which can take place quickly so that the photosynthesis process runs more optimally (Saputri et al., 2019; Sumardi et al., 2019). Leaf thickness is related to the rate of photosynthesis because leaf thickness describes the photosynthetic organelle unit. Thick leaves have more chloroplasts per unit leaf area, resulting in a greater ability to intercept light and decompose CO_2 than thin leaves (Sitompul & Guritno, 1995). In this study, leaf thickness measured through a specific leaf area and a specific leaf weight did not show significantly different results ($p > 0.05$).

Plant physiological activity can be known from several test parameters, including photosynthesis rate, transpiration rate, stomatal conductance, the number of stomata, and stomatal opening width. According to the analysis of variance (refer to Table 3), there was no interaction effect ($p > 0.05$) of amaranth varieties and urea doses on all plant physiological activities. Green amaranth's physiological activity is not significantly different ($p > 0.05$) from red amaranth except in the width of stomatal openings. The treatment of urea doses showed no significant effect ($p > 0.05$) on all plant physiological activities.

Leaves are an essential component for plants' growth and productivity because they are where photosynthetic activities take place, namely the physiological activity of food synthesis for plant growth, so the area and number of plant leaves affect photosynthetic activities. The greater number of leaves will increase the photosynthetic reaction as a result of the more food produced. Otherwise, the small number of leaves will cause a low rate of photosynthesis in plants (Wahyudi et al., 2018). The wider the plant leaves, the higher the rate of photosynthesis, which is because the wider the leaf surface can absorb sunlight for photosynthesis (Yuniansyah et al., 2022).

The availability of nitrogen affects the rate of plant photosynthesis. Plant nutrients that are sufficiently available will allow the leaves to meet their needs for photosynthesis. Lack of nitrogen will affect photosynthesis because nitrogen is an important ingredient of chlorophyll, so if its availability is limited, chlorophyll cannot be formed, which results in inhibition of the photosynthesis process (Gardner et al., 1991). Sitompul & Guritno (1995) state that the speed of plastochron formation (the time needed to develop each new leaf) and filochron emergence (the time required for each new leaf to form) affect the formation of plant leaves. Temperature, light intensity, and nitrogen (N) availability are the main factors that influence leaf growth. In this study, the average photosynthetic rate of green amaranth was higher than that of red amaranth, but the chlorophyll content of red amaranth was higher than that of green amaranth. The higher chlorophyll content in red amaranth is thought to be due to fluctuations in light intensity at the time of measurement. When exposed to intense light, photosynthesis is generally a stable process. Conversely, the photosynthesis process experiences a reduction in intensity under low-light conditions due to the equilibrium between the carbon dioxide assimilated during photosynthesis and the amount released during respiration (Lupitasari & Kusumaningtyas, 2020). In addition, variations in photosynthetic rate are also influenced by the tools' accuracy. A plant photosynthetic meter can sometimes be used when the light conditions are slightly dim, but sometimes, it must be used when the light conditions are hot. One leaf sample was measured twice or three times because the tool used could not measure all variables properly, so it was necessary to re-measure to obtain results from the observation variables.

The process of losing water from above-ground plant tissues in the form of water vapor is known as transpiration. This process occurs primarily via the lenticels, cuticle, and stomata; however, it is primarily facilitated by the stomata (Silaen, 2021). There are many factors that affect the transpiration rate, both internal and external. Internal factors refer to characteristics such as leaf count, leaf thickness, leaf surface area, the formation of a waxy coating, density of hairs on the leaf surface, and the quantity of stomatal. External factors are sunlight intensity, temperature, humidity, and wind. The transpiration rate occurs simultaneously with the photosynthesis process when the stomata on the leaves open to take CO_2 and the roots absorb water in the soil, more than 20% of which will be released by plants in the form of water vapor into the air (Anggraini et al., 2021).

The increasing doses of urea fertilizer lead to a higher number of leaves. Urea fertilization causes the availability of nitrogen nutrients in sufficient quantities to produce an environment that supports plant growth and development. The provision of increasingly large doses of urea can increase the nitrogen content in plants so that the accumulation of available nitrogen can be used for the formation of vegetative parts of the plant optimally, especially the formation of leaves (Handayani et al., 2020; Kogoya et al., 2018; Yadav et al., 2022; Zaman et al., 2022). The total overall area of the leaves exhibits a linear relationship with the number of leaves, meaning that it increases in direct proportion to the increase in leaf count. The cause of this phenomenon is the existence of a direct correlation between the number of leaves and the surface area of each leaf. Consequently, this leads to an increased rate of transpiration. This is because a greater leaf area is directly associated with a higher number of stomata on the leaves, which in turn impacts the rate at which plants release water vapor through transpiration. (Maylani et al., 2020). Leaf thickness affects the transpiration rate of plants. Leaf thickness negatively correlates with a transpiration rate, which shows an inverse ratio between leaf thickness and a transpiration rate. The lower the leaf thickness, the higher the transpiration rate. On the other hand, if a plant's leaf is thicker, its transpiration rate will be lower (Dacosta & Daningsih, 2022). The width of stomatal openings is directly proportional to the stomatal conductance and the transpiration rate. The greater width increases conductance and transpiration. The turgor pressure of the guard cells influences the opening and closing of the stomata. In high soil moisture conditions, the turgor pressure of the stomatal guard cells increases, which causes the opening of the stomata so that the stomatal conductance is maximized. Conversely, in low soil moisture conditions, the turgor pressure of the guard cells will decrease, resulting in stomatal closure and a decrease in stomatal conductance. In high soil moisture conditions, the turgor pressure of the stomatal guard cells increases, which causes the opening of the stomata so that the stomatal conductance is maximized. Conversely, in low soil moisture conditions, the guard cell turgor pressure will decrease, resulting in stomatal closure so that stomatal conductance decreases (Boy et al., 2022). The high nitrogen supply will increase stomatal density (Zhu et al., 2020), increasing the plant transpiration rate (Cechin et al., 2022).

Chlorophyll, or leaf green pigment, is vital for plant photosynthesis. Plants have two different kinds of chlorophyll, chlorophyll a, and chlorophyll b, which function like photosynthetic photoreceptors that capture sunlight for synthesizing carbohydrates from CO_2 and water, becoming energy sources during plant growth and development. The photosynthetic ability of each plant varies based on internal and external factors, including the amount of chlorophyll present (Siswanti & Riesty, 2021). This study found that variations in amaranth and urea application had no interaction effect on leaf chlorophyll content ($p>0.05$). The results indicate a notable disparity in leaf chlorophyll content based on the treatments. Red amaranth varieties exhibited significantly ($p<0.05$) greater amounts of chlorophyll a, b, and total chlorophyll retrieved from green varieties. The application of 150 $kg.ha^{-1}$ of urea led to a greater concentration of chlorophyll a, significantly different ($p<0.05$) from

Table 4. Chlorophyll Content of Leaves

Treatment	Chlorophyll a ($mg.g^{-1}$)	Chlorophyll b ($mg.g^{-1}$)	Total Chlorophyll ($mg.g^{-1}$)
Amaranth			
Green	0.467 a	0.220 a	0.687 a
Red	0.607 b	0.411 b	1.017 b
Urea ($kg.ha^{-1}$)			
0	0.492 ab	0.262 ab	0.754 ab
50	0.415 a	0.194 a	0.609 a
100	0.561 bc	0.318 abc	0.879 bc
150	0.620 c	0.347 bc	0.966 bc
200	0.544 bc	0.306 abc	0.850 bc
250	0.557 bc	0.352 bc	0.908 bc
300	0.571 bc	0.429 c	1.000 c
Interaction	-	-	-

Remarks: Mean values associated with the same letters are not significantly different according to Duncan's Multiple Range Test at 0.05 level of significance.

the treatment retrieved from 50 kg.ha^{-1}. Conversely, the treatment of 300 kg.ha^{-1} treatment yielded the greatest quantity of chlorophyll b and total chlorophyll, both of which were significantly distinct ($p<0.05$) from 50 kg.ha^{-1} treatment (Table 4).

According to Nugroho et al. (2023), the quantity of chlorophyll a, chlorophyll b, and total chlorophyll, which is present within the microgreen of red amaranth, is greater than that within the microgreen of green amaranth. Chlorophyll is crucial for plant development, particularly in terms of the color of red and green amaranth. Red amaranth contains more chlorophyll than green amaranth because it has a greater capacity to protect itself from the harmful effects of light. Red amaranth enhances the ability of photosynthetic organs to protect themselves from damage caused by intense light, both at the level of the photosystem and overall photodegradation. Photoprotection is a process that protects chlorophyll and photosynthetic membranes from damage caused by light. This is accomplished by synthesizing crucial botanical pigments, including anthocyanins, betalains, betacyanins, and carotenoids (Landi et al., 2013). Carotenoids are pigments that aid photosynthesis and protect photosynthetic apparatus. Their concentration increases by up to 115% with higher nitrogen supply compared to low nitrogen conditions, according to Cechin et al. (2022).

In addition, plant age, leaf morphology, and genetics can also affect the amount of chlorophyll in plants. Physiological stages and leaf age affect the chlorophyll content of a plant. Plant species of the same age can contain different chemicals with different genomes, leading to different metabolisms depending on the number of substrates and metabolic enzymes. The leaf area can indicate leaf chlorophyll content. A wider leaf surface indicates more chlorophyll. The greater amount of chlorophyll will increase the photosynthesis process in plants, so production will also increase (Wahyuningtyas et al., 2022; Wijaya et al., 2020). The leaf surface area helps absorb light for photosynthesis in low light conditions. Leaves that have a wide leaf area morphology will be able to capture light optimally. Then, the thickness of the leaf can also affect the amount of chlorophyll in it. Thin leaf morphology usually languishes more when picked, so the decrease in chlorophyll content is easier. The existence of adaptation factors or the dominance of other pigments in the leaves can also cause differences in chlorophyll levels in plants (Dharmadewi, 2020).

Nitrogen is a fundamental element in the chlorophyll molecule, so an increase in nitrogen supply leads to higher chlorophyll concentration in plants (Cechin et al., 2022). In amaranth leaves, chlorophyll is essential for producing nutrients for growth and development. Leaf chlorophyll content increases with nitrogen fertilizer doses (Di Mola et al., 2020). Implementing 300 kg.ha^{-1} of urea led to the highest overall chlorophyll content, while no notable differences were observed among varying urea doses. Plants that lack nitrogen can experience several negative impacts, such as leaves falling off easily, stunted growth, and reduced production. Additionally, plants with low chlorophyll content may struggle to absorb light, decreasing photosynthate production (Utami, 2020).

CONCLUSION

This study examines the physiological responses of amaranth plants to the application of urea fertilizer. The research shows no interaction between amaranth varieties and urea doses regarding plant growth and physiological activity. The physiological response of both amaranth varieties to urea fertilization is the same. In general, adding urea fertilizer stimulates the vegetative growth of amaranth plants, as indicated by the total chlorophyll content.

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AUTHOR'S CONTRIBUTIONS

FCWS conceptualized, conceived and designed the study, performed the experiments, analysed the data, wrote the manuscript, and reviewed and approved the final version. EP conceived and designed the study contributed to the data validation and final review of the manuscript, oversaw the study's conduct, and reviewed and approved the final version. MR conceived and designed the study contributed to the manuscript's data validation and final review, oversaw the study's conduct, and reviewed and approved the final version. ATS conceived and designed the study.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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