CELL BIOLOGY & DEVELOPMENT
Volume 3, Number 1, June 2019 E-ISSN: 2580-4499
Pages: 30-39 DOI: 10.13057/cellbioldev/v030105

Variations in growth, proline content, and nitrate reductase activity of *Canna edulis* at different water availability

WINTANG NUGRAHENI, SOLICHATUN♥, NITA ETIKAWATI
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57 126, Central Java, Indonesia. Tel./fax.: +62-271-663375, ♥email: solichatun@staff.uns.ac.id

Manuscript received: 3 April 2019. Revision accepted: 25 May 2019.

Abstract. *Nugraheni W, Solichatun, Etikawati N. 2019. Variations in growth, proline content, and nitrate reductase activity of* Canna edulis *at different water availability. Cell Biol Dev 3: 30-39.* Information on the physiological characteristics of Indian shot (*Canna edulis* Ker Gawl.), especially regarding the effect of water availability in its cultivation, is still limited. This study aims to determine the effect of variations in water availability on growth, proline content, and nitrate reductase activity in two intraspecies variations of *C. edulis*. Information on the physiological characteristics of *C. edulis* can be used as a basis for plant breeding efforts to optimize their cultivation. The study was conducted using a completely randomized design (CRD) with one factor, particularly water availability (A1=100% FC, A2=75% FC, A3=50% FC), with 3 replications for each intraspecies variation. The treatment of variations in water availability was given by watering once a day for 3 months. The data obtained for each intraspecies variation were analyzed by analysis of variance (ANOVA). Suppose there was a significant difference between the treatments for variations in water availability. In that case, it is followed by Duncan's Multiple Range Test (DMRT) at a 5% level. In contrast, the data obtained on both intraspecies variations were analyzed by *t*-test to compare the response of the two intraspecies variations towards variations in water availability. The research results on each intraspecies variation of *C. edulis* showed that the treatment of variations in water availability affected growth, proline content, and nitrate reductase activity. In the variables of the number of leaves, leaf area, respiration rate, plant height, gross plant weight, and plant dry weight, the optimal growth is at 100% FC water availability. Proline content and nitrate reductase activity were influenced by variations in water availability, with the highest proline accumulation at 50% FC water availability, while the highest nitrate reductase activity was at 100% FC water availability. Both intraspecies variations of *C. edulis* plants have the same growth response, proline content, and nitrate reductase activity towards variations in water availability.

Keywords: *Canna edulis*, growth, nitrate reductase activity, proline content, water availability

INTRODUCTION

Indian shot (*Canna edulis* Ker Gawl.) is a tuber-producing plant that has the potential as a substitute for wheat flour. In addition, it can also be used as an alternative food source and a source of bioethanol raw materials (Plantus 2007; Grehenson 2009). Tubers of *C. edulis* Ker. contains 80% carbohydrates and 18% water content and has a brownish White color with a smooth texture. High carbohydrate content indicates that *C. edulis* tubers can be used as raw materials for glucose production and ethanol fermentation (Putri and Sukandar 2008).

The *C. edulis* is one of the non-rice food ingredients with high nutritional value, especially the content of calcium, phosphorus, and carbohydrates. The nutritional content of *C. edulis* per 100 g completely consists of calories= 95.00 cal; protein= 1.00 g; fat= 0.11 g; carbohydrates= 22.60 g; calcium= 21.00 g; phosphorus= 70.00 g; iron= 1.90 mg; vitamin B1= 0.10 mg; vitamin C= 10.00 mg; water= 75.00 g; edible part= 65.00% (Directorate of Nutrition, Ministry of Health of RI 2007).

The *C. edulis* has not been widely cultivated and has a high potential to be cultivated (Grehenson 2009). The *C. edulis* can grow easily, either cultivated or wild (Putri and Sukandar 2008), and has tolerance to shade (Grehenson 2009). Efforts for plant cultivation include plant breeding efforts. One of the basics in plant breeding efforts is to know the physiological characteristics of *C. edulis*. Given the important value of *C. edulis*, which has potential as a substitute for wheat flour, alternative food sources, and bioethanol raw materials, a scientific study of this plant needs to be carried out so that *C. edulis* can be cultivated optimally.

The *C. edulis* cultivation can be done intensively by knowing the physiological characteristics of the plant. One of the most important aspects of plant cultivation is water because it functions as a solvent for plant nutrients in the soil and plays a role in the translocation of nutrients and photosynthesis in the plant body. The available water in the soil ranges from very low (drought) to waterlogged conditions (Gardner et al. 1991).

Water requirements for plants are influenced by several factors, including the type of plant, its type and development, soil moisture content, and weather conditions (Fitter and Hay 1998). Lack of water in plants occurs due to insufficient water availability in the media and excessive transpiration or a combination of these two factors. In the field, even though there is enough water in the country, plants can experience stress

(lack of water). this happens if the absorption rate cannot compensate for water loss through transpiration (Sasli 2004).

Low water availability will affect all plant metabolic processes so that it can reduce its growth. The mechanism of plant adaptation to overcome low water availability is by regulating cell osmosis. In that mechanism, the synthesis and accumulation of organic compounds can reduce the osmotic potential of the cell. Therefore, the level of osmoprotectant compounds (such as proline) in plants can be used as a differentiator of the tolerance level of plants to water stress (Mathius et al. 2004).

Low water availability also interferes with nutrient uptake of nitrogen, thus reducing nitrate reductase activity (Foyer et al. 1998). Nitrate reductase activity can be used as a selection criterion for high-yielding plants in plant breeding programs because it positively correlates with plant growth and production (Delita et al. 2008). The nitrate reductase enzyme is useful for converting nitrate to nitrite, which then, after going through a series of other enzymes works. This nitrite will be converted into amino acids (Loveless 1991; Alnopri 2004; Alnopri et al. 2004; Komariah et al. 2004).

Based on the explanation, it is necessary to conduct a study as scientific evidence to determine the physiological character of *C. edulis*, especially related to the effect of water availability on growth, proline content, and nitrate reductase activity in this plant, so that it can be used as Information in optimizing its cultivation.

This study aims to determine: (i) Variations in plant growth of *C. edulis* at different water availability. (ii) The proline content of *C. edulis* at different water availability. (iii) Nitrate reductase activity of *C. edulis* at different water availability.

MATERIALS AND METHODS

Materials

The main ingredients needed are *C. edulis* tubers obtained from Boyolali, Central Java, Indonesia. A Plant Assimilation Analyzer (PAA) and a UV-VIS spectrophotometer are the main tools needed.

Experimental design

This study used a completely randomized design (CRD) with one factor, particularly the level of water availability (50%, 75%, and 100% the field capacity), with 3 replications for each intraspecies variation.

Procedure

Plant preparation and care

Preparation of growing media: (i) Soil and compost that have been dried are mixed with a ratio of soil: compost = 2:1. (ii) Three (3) kg of soil-compost mixture was taken and then put in 5 L polybags.

Determination of field capacity: (i) Each prepared media was weighed (initial weight). (ii) The media is doused with water until it is saturated, then left until the water from the media stops dripping. (iii) The weight of each medium after water administration was weighed (final weight). (iv) 100% field capacity was determined by subtracting the final weight of each medium from the initial weight of each medium. (v) Field capacities of 50% and 75% were determined based on the average field capacity of 100% obtained.

Seed preparation and treatment: (i) Tubers of *C. edulis* measuring ± 20 g with one shoot were prepared, then planted on the media provided. (ii) Treatment of water availability was given after the seedlings were one week old. Treatment of water availability was given by watering using water once a day (according to the level of water availability being tested). (iii) The addition of water given to the growing media was calculated based on the amount of evapotranspiration that occurred and was carried out by weighing.

HarvestPlants were harvested after being treated for 3 months. Harvested plants were removed from polybags and then cleaned for soil debris.

Growth analysis

The number of leaves: The number of leaves was calculated at the end of the treatment by counting the total number of leaves on each plant other than this still budding.

Leaf area: Leaf area was calculated at the end of treatment by gravimetric method. The leaf whose area was to be measured was made a replica on a piece of paper with known area and weight.

The formula calculated the leaf area:

$$LD = \frac{Wr}{Wt} \times LK$$

Notes:
Wr: Leaf replica weight (g)
Wt: Total paper weight (g)
LK: Total paper area (cm^2)
LD: Leaf area (cm^2) (Sitompul and Guritno 1995).

Respiration rate: Measurement of respiration rate was carried out by calculating the amount of CO_2 produced by plants using the Plant Assimilation Analyzer (PAA) according to the procedure of the tool (Horiba Plant Assimilation Analyzer ASSA-1610) as follows:

(i) The PAA appliance was turned on for 1 hour before use. (ii) Five polybags were put into the sample holder in the growth chamber, 1 container containing 1 polybag, and 1 container was left for measurement control. (iii) The volume of gas entering each sample was set to 2 L/min. (iv) Calibration was carried out to measure N_2 + CO_2 gas levels using the zero, span, and means buttons. (v) The zero button was used to measure the volume of gas that came out every 0.5 L/min on a

scale of 0 to measure the gas content of N_2. (vi) The span button measured the gas content of $N_2 + CO_2$. (vii) The means button read CO_2 levels directly ($CO_2 + N_2$) - $N_2 = CO_2$. (viii) The volume of gas escaping at 0.5 L/min was adjusted for each sample. Finally, (ix) CO_2 levels were measured by reading on a scale of ppm CO_2/L/minute.

Respiration rate = sample CO_2 - control CO_2
Respiration rate = CO_2/L/min

Plant height: Plant height was measured from the tip of the highest leaf of the plant to the understock at ground level (Hendriyani and Setiari 2009).

The gross weight of plants: Gross weight of plants was calculated by weighing the harvested plants that had been cleaned from the soil (Hendriyani and Setiari 2009).

Plant dry weight: Harvested crops cleaned of soil residue were put in paper bags ready to be oven (temperature 60ºC) for 4-5 days until a constant weight was reached. The constant weight achieved after the oven was the dry weight of the plant (Hendriyani and Setiari 2009).

Measurement of proline content

Proline accumulation was measured using the Ninhydrin method (Bates et al. in Umebese et al. 2009). (i) Materials in the form of fresh leaves (2nd leaf from the tip of the plant) as much as 0.5 g were ground in a mortar with 10 mL of 3% sulfosalicylic solution. (ii) The results of the leaf collision were then filtered with Whatman filter paper no.1. A total of 2 mL of the filtrate was reacted with 2 mL of ninhydrin acid and 2 mL of glacial acetic acid in a test tube at 100ºC for 1 hour. The reaction was ended by inserting the test tube into a beaker containing ice. (iii) An acid solution of ninhydrin prepared by heating 1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid until dissolved. (iv) The mixture was extracted with 4 mL of toluene, then shaken with a vortex for 15-20 seconds to form two separate liquid layers. The red toluene containing proline was located at the top. (v) The upper solution was aspirated using a pipette to measure the proline content with a spectrophotometer, and the absorbance was read at a wavelength of 520 nm. (vi) The proline content was determined based on reading the pure proline standard solution.

Measurement of nitrate reductase activity

(i) Fresh leaves (2nd leaf from the tip of the plant) were washed with distilled water until clean; then, the leaf bones were cleaned to obtain leaf blades. (ii) Leaf blades weighing 500 mg were cut into thin strips of about 1 mm using a sharp knife. The leaf pieces were put into a 5 mL phosphate buffer solution in a dark tube. After soaking for 24 hours, the buffer solution was replaced with a new one. (iii) Phosphate buffer was made from a mixture of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 (Manan 2008). (iv) A 0.1 mL of $NaNO_3$ was added with a micropipette, and the time was recorded as the start of incubation for 2 hours. (v) A dye reagent consisting of 0.2 mL of a 0.02% N-naphthylenediamine solution and 0.2 mL of 1% sulphanilamide in 3 N HCl was prepared. After being incubated for 2 hours, 0.1 mL of incubation liquid was taken from a dark tube and put into a test tube containing a dye reagent, then waited for a pink color to occur as a sign that nitrate was reduced to nitrite by the enzyme nitrate reductase. One test tube was not given the filtrate and was used as a blank. (vi) After the color change occurred, 2.5 mL of distilled water was added, then transferred to a cuvette to measure the absorbance in a spectrophotometer at 540 nm.

Nitrate reductase activity was expressed in micromoles nitrate/g tissue material per hour using the following formula (Indradewa et al. 2004):

$$\text{ANR} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times 50 \times \frac{100}{\text{BB}} \times \frac{1}{\text{W}} \times \frac{1}{1000}$$

Where:
Standard absorbance: 0.0142
BB: Plant gross weight
W: Incubation time (hours)

Data analysis

The data obtained for each intraspecies variation in the form of quantitative data, including the number of leaves, leaf area, respiration rate, plant height, gross plant weight, plant dry weight, proline content, and nitrate reductase activity, were analyzed by analysis of variance (ANOVA). If there was a significant difference in water availability, the treatment was continued with DMRT (Duncan's Multiple Range Test) at a 5% level. The data obtained on both intraspecies variations were analyzed by t-test to compare the responses of the two intraspecies variations to variations in water availability (Santoso 2001).

RESULTS AND DISCUSSION

Water is necessary for plants to grow and develop (Noggle and Fritz 1983). Because water is important for plant growth and development, the availability of water in the soil must meet the needs of plants. If water availability is reduced, it will impact productivity (Gardner et al. 1991).

Plants that suffer from water stress are generally smaller than plants that grow normally. Therefore, water stress affects all aspects of plant growth. In this case, water stress affects plants' physiological and biochemical processes and causes anatomical and morphological modifications to plants (Islami and Utomo 1995).

Growth

Growth is expressed as an irreversible and restricted increase in size in living cells accompanied by metabolic processes that include the synthesis of macromolecules such as nucleic acids, proteins, lipids, and polysaccharides. Measurement of growth can be carried out in various ways, for example, by measuring plant height, leaf size (length, width, and surface area), gross weight, and dry

weight of plants or separate parts such as roots, stems, leaves and fruit, the number of cells in the plant tissues and organs, as well as the concentration of specific compounds (e.g., nucleic acids, dissolved nitrogen, etc.) in tissues and organs (Noggle and Fritz 1983).

The growth variables in this study included the number of leaves, leaf area, respiration rate, plant height, gross plant weight, and plant dry weight.

Number of leaves

Leaves are organs that are often observed in plants as growth parameters. The number of leaves will affect the results of photosynthesis which will be circulated to all parts of the plant because it is related to the light received by the leaves (Islami and Utomo 1995). Gardner et al. (1991) stated that genetic and environmental factors influence the number and size of leaves.

Leaf development is highly sensitive to environmental changes such as water availability. In addition, leaves are generally a place of carbohydrate synthesis for plants, so leaf observation is necessary as an indicator of growth and as supporting data to explain the growth process (Sitompul and Guritno 1995).

The results of the study on the number of leaves of *C. edulis* can be seen in Table 1.

The analysis of variance above shows significant differences in the number of leaves in the treatment of variations in water availability, both the Red and White varieties of *C. edulis*. Variations in water availability in this study included 100%, 75%, and 50% FC. Plants achieved the largest number of leaves with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

In the 100% FC water treatment, plants can use the more available water, thus allowing photosynthesis to exceed respiration. In addition, the availability of sufficient water can maintain cell turgor so that cell enlargement can occur. Cell turgor pressure pushes the plant cell wall and causes the cell to enlarge (Salisbury and Ross 1995).

According to Fitter and Hay (1998), water affects cell growth; the lower the water availability, the lower the turgor pressure. Those results in a decrease in the growth rate; the number of leaves produced is low. Lack of water or drought causes stomata to close and inhibits CO_2 absorption, thus reducing the rate of photosynthesis.

The lower number of leaves in the water treatment of 75% and 50% FC was thought to be due to the lack of available water in the soil, which would reduce the turgor pressure of the plant so that it interfered with the plant's metabolism, including photosynthesis. Photosynthesis will be hampered if water, the main ingredient, is unavailable, so the photosynthesis results will also decrease. Those decrease in photosynthesis is caused by the closing of stomata so that CO_2 fixation is inhibited (Lawlor 2002). Photosynthate produced will also be hampered in its circulation to all parts of the plant.

Based on the results of the t-test, it can be seen that there is no significant difference in the number of leaves between the Red and White varieties of *C. edulis*. Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

The presence of many photosynthates, one of which is used to increase meristematic activity in leaf primordial formation. Hidayat (1995) stated that the increase in the number of leaves was thought to be due to increased division of primordial leaf cells and stem tip cell differentiation. Leaves as a means of photosynthesis can play an optimal role if it is supported by the availability of water, light, and sufficient nutrients.

Leaf area

The photosynthesis process can occur in other parts of the plant, but the leaf is generally seen as the main photosynthetic producing organ. Therefore, observation of leaves is highly necessary for addition to supporting data to explain the growth process that occurs in the formation of plant biomass and an indicator of growth (Sitompul and Guritno 1995).

Observation of leaves can be based on their function as light receivers and photosynthetic tools. On this basis, leaf area is used as the main parameter because the rate of photosynthesis per unit plant, in most cases, is determined largely by leaf area (Sitompul and Guritno 1995).

The results on the leaf area of *C. edulis* can be seen in Table 2. Based on the results of the analysis of variance above, it can be seen that there are significant differences in leaf area in variations in water availability, both Red and White varieties of *C. edulis*. Variations in water availability in this study included 100%, 75%, and 50% FC. Plants achieved the largest leaf area with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

Table 1. The average number of leaves of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	4.6^{b}	4^{b}	2.3^{a}
White	5.6^{c}	4.3^{b}	2.6^{a}

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

Table 2. The average leaf area (cm^2) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	217^{c}	195^{b}	175^{a}
White	238^{c}	216^{b}	191^{a}

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

The greatest effect of lack of moisture in early vegetative development is a reduction in leaf area. Even the slightest lack of water during vegetative development can reduce the leaf dilation rate and leaf area index at the next stage of development (Gardner et al. 1991).

The reduction in leaf area at the water treatment level of 75% and 50% FC is considered to be closely related to plant adaptation to reduce transpiration and prevent evaporation of too much water from the plant body, a mechanism to reduce water use and prevent further damage due to water stress. Gardner et al. (1991) stated that the reduction in total leaf area is a strongly effective mechanism of plant adaptation to environmental stresses to prevent water evaporation.

Inhibition of cell division and enlargement due to water stress also affected the small leaf area produced by plants with 75% and 50% FC water treatment. Inhibition of cell enlargement occurs due to a decrease in cell turgor pressure. The cell turgor pressure pushes the plant cell walls and causes the cells to enlarge so that the decrease in turgor pressure causes the plant parts that are formed to be smaller than normal. The growth rate of plant cells and the efficiency of physiological processes reach the highest level when the cells are at maximum turgor.

The largest leaf area achieved in plants with 100% water treatment indicates the optimal level of water availability to support metabolic processes in plants so that sufficient energy is available for cell growth. In addition, the availability of sufficient water can maintain cell turgor so that cell enlargement can occur. Cell turgor pressure pushes the plant cell wall and causes the cell to enlarge. Harwati's study (2007) results show that tobacco plants with sufficient water availability have higher leaf area values. Based on the results of the *t*-test, it can be seen that there is no significant difference in leaf area between the Red and White varieties of *C. edulis*. Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

The leaf area is the measure of crown development and is very sensitive to water stress. Water stress results in decreased leaf formation and expansion, increased leaf senescence and shedding, or both. Leaf expansion is more sensitive to water stress than stomata closure (Nugraha 2008).

Cell growth is highly sensitive to water stress. Inhibition of cell enlargement occurs due to decreased cell turgor, forming small plant parts. The effect of lack of water during the vegetative development stage is the development of smaller leaves. During vegetative development, the slightest lack of water can reduce the rate of leaf dilation and leaf area at the next stage of development (Islami and Utomo 1995).

Respiration rate

Besides the process of photosynthesis, plants also carry out the process of respiration. Respiration is a process of disassembling energy from stored chemical energy to carry out life processes such as the formation of organic substances, activities in absorption (osmosis), accumulation of salts, protoplasm flow, and cell division, and other activities. There are two types of respiration, including aerobic respiration and anaerobic respiration. Aerobic respiration is a combustion reaction of carbon organic matter with O_2, which produces CO_2 and H_2O as well as the energy needed for growth. The respiration rate can be determined by measuring the volume of CO_2 released (Dwijoseputro 1994).

The results of the study on the respiration rate of *C. edulis* can be seen in Table 3. Based on the results of the analysis of variance above, it can be seen that there are significant differences in the rate of respiration in variations in water availability, both for the Red and White varieties of *C. edulis*. Variations in water availability in this study included 100%, 75%, and 50% FC. Plants achieved the highest respiration rate with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

In the 100% FC water treatment, the plants were in a condition with more water, allowing a higher respiration rate. The availability of sufficient water can maintain cell turgor so that the stomata are always open. Those are related to the diffusion of O_2 from the atmosphere.

Anggarwulan and Solichatun (2001) stated that the availability of O_2 will affect respiration, considering its role as the final electron acceptor. In a thick tissue with a low surface-to-volume ratio, oxygen diffusion from the atmosphere decreases so that the respiration rate is low. In conditions of more available water, plants can increase their respiration rate so that the growth process also increases. In the respiration process, energy and carbon skeletons are obtained from the oxidation of photoassimilate, which is required for growth.

The lower respiration rate in the water treatment of 75% and 50% FC is considered to be due to the lack of available water in the soil, which will reduce the turgor pressure of the plant, causing the stomata to close and decreasing the diffusion of O_2 from the atmosphere. Based on the results of the t-test shows there is no significant difference in the respiration rate between the Red and White varieties of *C. edulis*. Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

Table 3. Average respiration rate (ppm/L/min) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	36.3^{b}	26^{a}	20^{a}
While	39^{b}	30^{ab}	25^{a}

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

Plant height

The increase in plant height is a change or increase in the volume (size) of the stem because of cell enlargement to one dimension (vertical), particularly in the longitudinal direction so that the plant grows taller (Salisbury and Ross 1995).

Plant height is the most frequently observed plant size indicator of growth and is a parameter used to measure environmental influences or treatments applied. That treatment is done because plant height is the most easily seen growth measure. Therefore, plant height is sensitive to environmental influences as a parameter measuring environmental influences (Sitompul and Guritno 1995).

The results of research on plant height in *C. edulis* can be seen in Table 4. The analysis of the variance above shows significant differences in plant height in variations of water availability in both Red and White varieties of *C. edulis*. Variations in water availability in this study included 100%, 75%, and 50% FC. Plants achieved the largest plant height with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

In the 100% FC water treatment, the plants were in conditions of sufficient water availability, so that plant growth was optimal. The availability of sufficient water can maintain cell turgor so that cell enlargement can occur. Cell turgor pressure pushes the plant cell wall and causes cell enlargement.

Gardner et al. (1991) stated that cell growth is a process in the plant body that is sensitive to water shortages. The value of the meristem tissue water potential during the day often causes a decrease in turgor pressure below that required for cell development. Those reduce protein synthesis, cell wall, and cell development, resulting in smaller growth. Inhibition of cell enlargement due to decreased cell turgor results in smaller plant parts.

The plant height achieved with 75% and 50% FC treatments was lower than 100% FC. Those were presumably related to the inhibition of cell enlargement due to decreased cell turgor pressure. The cell turgor pressure pushes the plant cell walls and causes the cells to enlarge so that the decrease in turgor pressure causes the plant parts that are formed to be smaller than normal. Besides, turgor pressure also affects the opening and closing of stomata, thus reducing the supply of CO_2. The stomata opening usually decreases when the leaf water potential decreases. Those decrease in the opening is due to an increase in the abscisic acid content produced by the leaf mesophyll (Goldsworthy and Fisher 1992). Umebese et al. (2009) stated that treatment of low water availability in spinach and tomato plants decreased plant height.

The research of Peng and Weyers (1994) on the leaves of the *Commelina commenis* L. stated that ABA levels in stomata guard cells affected the opening and closing of stomata. Water stress also reduces the translocation of nutrients and photosynthate in the plant body. Based on the results of the t-test shows there is no significant difference in plant height between the Red and White varieties of *C. edulis*. Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

Plant gross weight

Plant biomass is the most commonly used parameter to describe and study plant growth. Those are based on plant weight which is relatively easy to measure and is an integration of all events experienced by plants, so that parameter is the most representative growth indicator if the main goal is to obtain an overview of the overall appearance of a plant or a particular organ (Sitompul and Guritno 1995).

The gross weight of the plant is obtained by harvesting and weighing it immediately before too much water has evaporated from the material. In species with rapid and abundant leaf development, the rate of photosynthesis will increase, which will then increase the overall plant (Salisbury and Ross 1995).

The study's gross weight of plants in *C. edulis* can be seen in Table 5. The analysis of the variance above shows significant differences in plants' gross weight in water availability variations, both for the Red and White varieties of *C. edulis*. Variations in water availability in this study included 100%, 75%, and 50% FC. The largest gross weight was achieved on plants with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

The high value of plant gross weight in 100% FC water treatment is considered to be due to sufficient water for photosynthesis associated with turgor pressure in the tissue. The availability of water will increase photosynthesis. The photosynthesis results will be translocated throughout the plant body tissue through the phloem. The energy from photosynthesis will activate the growth of shoots so that the number of branches increases. Increasing the number of branches will increase the number of leaves. The increasing number of leaves causes the gross weight of the plant also to increase (Lakitan 1995).

Table 4. Average plant height (cm) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	42.7[c]	33[b]	26.7[a]
White	51.7[c]	38.3[b]	28.7[a]

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

Table 5. Average plant gross weight (g) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	95.7[c]	74.3[b]	55.3[a]
White	116.7[c]	84[b]	65[a]

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

The lower value of plant gross weight at 75% and 50% FC water treatment was considered to be due to a lack of water availability for plants, causing inhibition of plant vegetative growth due to a decrease in turgor pressure in plant tissues. Gardner et al. (1991) stated that cell growth is a process in the plant body that is sensitive to water shortages. Therefore, lack of water availability for plants causes a decrease in turgor pressure below that required for cell development. Those cause a reduction in protein synthesis, cell wall, and cell development, resulting in smaller growth so that the gross weight of the resulting plant is low. Jumin (2002) also added that the inhibition of plant vegetative growth in stressed conditions was caused by inhibition of cell division and protein synthesis used for plant growth.

Based on the results of the t-test, it can be seen that there is no significant difference in plant gross weight between the Red and White varieties of *C. edulis*. Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

The plant's gross weight shows the plant's metabolic activity and is influenced by the water content of the tissue, nutrients, and metabolic products. Therefore, the value of a plant's gross weight also shows the amount of water in plant tissues or organs other than organic matter (Salisbury and Ross 1995).

Plant dry weight

The plant dry weight results from drying the gross weight to remove the plant's water content. Drying is intended to remove all the water content of the material and stop metabolic activity (Lakitan 1995).

Gardner et al. (1991) stated that the dry weight yield of plants is a balance between CO_2 uptake (photosynthesis) and CO_2 expenditure (respiration). Photosynthesis increases plant dry weight due to CO_2 uptake, while respiration catabolism causes CO_2 release and reduces plant dry weight.

The main components of plant dry weight are polysaccharides and lignin in the cell wall, plus cytoplasmic components such as proteins, lipids, amino acids, and organic acids (Salisbury and Ross 1995). The plant's dry weight is about 25% of the gross weight. Plant carbon comes from CO_2 gas in the atmosphere, which is bound in the form of carbon through photosynthesis. These compounds are then used to form other compounds needed to form plant cell structures and support other metabolic activities or are accumulated by certain organ cells (Sitompul and Guritno 1995).

The study's results on plant dry weight in *C. edulis* are shown in Table 6. Based on the analysis of variance above, it shows there are significant differences in plant dry weight in variations in water availability, both Red and White varieties of *C. edulis*. Variations in water availability in this study included 100%, 75%, and 50% FC. Plants achieved the largest plant dry weight with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

Table 6. Average plant dry weight (g) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	13^{c}	11.3^{b}	8.6^{a}
White	16^{c}	12.7^{b}	10^{a}

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

The increase in dry weight in 100% FC water treatment occurred due to the availability of sufficient water to increase the rate of photosynthesis, which will produce photosynthate, the end product of metabolism. The total dry weight of crop yields results from the accumulation of net CO_2 assimilation yields throughout the growing season. Plant photosynthesis utilization, among others, is for forming body structure and food reserves. Photosynthesis fixes CO_2 for use in the production of hexose, and then the hexose is utilized in plant respiration.

The end product of the photosynthesis process is carbohydrates. Carbohydrates are the basic building blocks of organic matter in plant cells, such as structural, metabolic, and important food reserves. Plant cell parts such as cytoplasm, cell nucleus, and cell wall are composed of these organic materials. Those process results in the accumulation of dry weight (Salisbury and Ross 1995).

Water stress can reduce the rate of photosynthesis which will gradually reduce the formation of body structure and food reserves, thereby reducing dry weight; Those are shown in the water treatment of 75% and 50% FC. The reduced rate of photosynthesis due to water stress also occurs because the leaves formed in those conditions experience inhibition of cell enlargement, resulting in leaves that are formed having a smaller size when compared to plants that grow normally. this means that light absorption decreases so that the photosynthetic ability also decreases (Gardner et al. 1991). Research by Hamim et al. (1996) and Hanum et al. (2007) showed a decrease in plant dry weight in several soybean varieties with low water availability.

Water is one of the raw materials in the photosynthesis process, and the effect of reducing water in the leaves on the rate of photosynthesis generally occurs indirectly. The effect of water content in the soil will cause a reduction in the rate of photosynthesis; Those could be explained as follow:

Reduced diffusion capacity of the stomata due to closing of the stomata. The closing of the stomata causes the diffusion of CO_2 from the atmosphere to the leaves to stop. As a result, (i) photosynthesis cannot occur and, in the long term, will interfere with other physiological processes, so plant growth is inhibited (Fitter and Hay 1998). (ii) Decreased hydration of the chloroplasts and other parts of the protoplasm, thereby reducing the effectiveness of the photosynthesis mechanism. (iii) There is an accumulation of sugar that inhibits the process of further photosynthesis (Heddy 1987).

Based on the results of the *t*-test, it can be seen that there is no significant difference in plant dry weight between the Red and White varieties of *C. edulis.* Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

Dry weight accumulation is generally used to characterize growth because it usually has the greatest economic importance. However, the gross weight of the plant is less useful because the number fluctuates depending on the moisture state of the plant (Gardner et al. 1991).

Proline content

One of the organisms' most common responses to water-deprivation treatments is the accumulation of compatible osmolytes. Osmolites compatible are neutral organic compounds with an active osmotic ability, which protects plants during stressful conditions (Chutipaijit et al. 2009). In addition, the accumulation of compatible osmolytes can reduce the water potential in the cells (Taylor 1996; Mathius et al. 2001).

The amino acid proline is the most widely distributed osmolyte compatible. Proline synthesized during water shortage could provide organic nitrogen, which is useful in cell recovery. Proline degradation in mitochondria is directly related to the electron transport system in the respiratory system and ATP production (Elthon and Stewart 1981), besides improving the energy status of cells recovering from water shortage conditions (Lawlor 2002).

The results of research on proline content in *C. edulis* can be seen in Table 7. The analysis of the variance above shows significant differences in proline content in variations in water availability, both Red and White varieties of *C. edulis.* Variations in water availability in this study included 100%, 75%, and 50% FC. Plants achieved the highest proline content with a water treatment level of 50% FC and the smaller at a water treatment level of 75% and 100% FC in both varieties of *C. edulis*.

The high accumulation of proline in 50% FC water treatment is considered to be because proline in plants with low water availability is synthesized as a consequence of cell osmotic regulation by increasing levels of dissolved compounds in cells so that the intracellular osmotic potential is lower or at least comparable to the osmotic potential of the medium around the cells. Some research showed an increase in proline content under conditions of low water availability, including spinach and tomato plants (Umebese et al. 2009) and corn plants (Heidari and Moaveni 2009).

Mathius et al. (2001) stated a positive correlation between proline accumulation and plant adaptation to drought stress. The accumulation of compatible osmolytes can reduce the water potential in the cell, thus allowing additional water uptake from the environment and protecting the mechanism from the effects of water deprivation. According to Rodriguez et al. (1997), an osmotic adjustment in plants can help deal with water stress.

Proline accumulation is a common response of plants to water stress (Darusman et al. 1991; Hamim et al. 1996; Mathius et al. 2001; Hamim et al. 2008; Ganesh et al. 2009; Umebese et al. 2009). Proline can act as an osmolyte compatible, a membrane and enzyme protective agent, a temporary transit site for organic nitrogen, and a free radical scavenging agent (Hare et al. 1999). Widyatmoko (2005) stated that proline accumulation is a plant's effort to maintain cell turgidity.

Proline content that was not too high in plants treated with 75% and 100% FC water was because there was still sufficient water available for plants so that plants did not have to accumulate osmolyte-compatible compounds that could reduce the water potential in the cells, which could allow additional water uptake from the environment. For example, Mathius et al. (2001) showed low proline levels in oil palm (*Elaeis guineensis* Jacq.) with sufficient water availability.

The t-test result shows no significant difference in proline content between the Red and White varieties of *C. edulis.* Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

Proline in plants is synthesized due to cell osmotic regulation caused by low water availability. That condition will spur some plants to increase their respiration rate to produce ATP, which is used to activate cells under stress as well as dissolved osmotic substances that can reduce the osmotic potential of cells, thus increasing cell water uptake which will simultaneously increase turgidity and activity (Hare et al. 1999).

Nitrate reductase activity

The enzyme nitrate reductase is useful for converting nitrate to nitrite. After going through a series of other enzymes, that nitrite will be converted into amino acids and proteins involved in metabolism. The activity of the nitrate reductase enzyme in mature plant leaves is related to the yield of the plant so that the level of nitrate reductase enzyme activity can be used as a selection criterion to select the genotype of a plant with high yields (Loveless 1991; Alnopri 2004; Alnopri et al. 2004; Komariah et al. 2004). Therefore, the positive correlation of nitrate reductase in the growth phase will impact high yields (Delita et al. 2008).

Nitrate ions absorbed from the soil must be reduced back to ammonium ions before their nitrogen components can be recombined into amino acids and other organic nitrogen compounds. The reduction of nitrate to ammonium in plants occurs in 2 stages (Noogle and Fritz 1983):

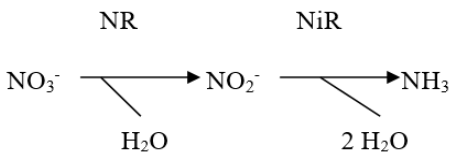

Table 7. The average proline content (mol/gram fresh leaf weight) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	0.72[a]	0.86[a]	1.51[b]
White	0.73[a]	0.91[a]	1.93[b]

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

Table 8. Average levels of ANR (mole nitrate/gram tissue material per hour) of *C. edulis* varieties of Red and White on variations in water availability

Plant varieties	Field capacity		
	100%	75%	50%
Red	0.68[b]	0.60[a]	0.55[a]
White	0.66[b]	0.59[a]	0.54[a]

Notes: a-b: Numbers followed by the same letter indicate no significant difference at the 95% confidence level

The results of the study on ANR levels in *C. edulis* can be seen in Table 8. Based on the results of the analysis of variance above, it can be seen that there are significant differences in ANR levels in variations in water availability, both Red and White varieties of *C. edulis.* Water treatment variations in this study included 100%, 75%, and 50% FC. Plants achieved the highest ANR levels with a water treatment level of 100% FC and the smaller at a water treatment level of 75% and 50% FC in both varieties of *C. edulis*.

The high ANR in plants with 100% FC water treatment is considered to be due to the availability of abundant water in the soil so that nitrogen transport from the soil to the plant body runs smoothly. Alnopri (2004) and Komariah et al. (2004) stated that the increased availability of nitrate would accelerate nitrogen synthesis so that the activity of nitrate reductase increases. According to Indradewa et al. (2004), plants that obtain stagnant water will increase ANR because plants absorb nitrate from the soil, so ANR in leaf shoots will increase when nitrate is available.

The low levels of ANR at water availability levels of 50% and 75% FC may be inhibited nutrient transport in the soil due to reduced transpiration, causing low nitrate reductase activity in plants. For example, Brandao and Sodek (2009) stated that reduced absorption of nitrogen nutrients from the soil caused a decrease in nitrate movement to the leaves, resulting in low nitrate reductase activity.

Chen and Sung's (1983) study on soybean nodules showed inhibition of nitrate reductase activity in nodules due to water stress. Likewise, the study by Umebese et al. (2009) showed decreased nitrate reductase activity in spinach and tomato plants under water shortage conditions. Foyer et al. (1998) stated that lack of water resulted in disruption of nutrient absorption. It reduced nitrate supply to the leaves, disrupting nitrate reductase activity. Based on the results of the *t*-test, it can be seen that there is no significant difference in ANR levels between the Red and White varieties of *C. edulis*. Those indicate that the two varieties of *C. edulis* have the same response to variations in water availability.

Nitrate reductase is one of the most sensitive plant enzymes studied (Alnopri et al. 2004). Nitrate reductase has been studied intensively because its activity often affects the rate of protein synthesis in plants that absorb nitrate as the main nitrogen source. The activity of nitrate reductase is influenced by several factors, including the rate of synthesis and the rate of an overhaul by protein-destroying enzymes. In addition, inhibitors and activators also influence it in cells (Salisbury and Ross 1995).

The research carried out above shows that: (i) Variations in water availability affect the growth of *C. edulis*. In the variables of the number of leaves, leaf area, respiration rate, plant height, gross plant weight, and plant dry weight, optimal growth was achieved by giving 100% FC water treatment and decreased at 75% and 50% FC water availability. (ii) Variations in water availability affected the proline content of *C. edulis*. The highest proline accumulation was produced by plants with 50% FC water treatment and decreased at 75% and 100% FC water availability. (iii) Variations in water availability affected the nitrate reductase activity of *C. edulis*. The highest ANR accumulation was produced by plants with 100% FC water treatment and decreased at 75% and 50% FC water availability.

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