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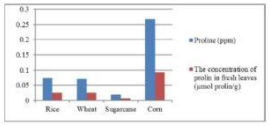

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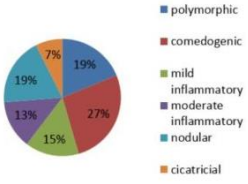

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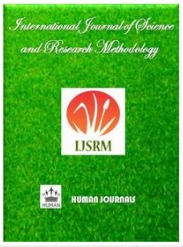

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Analysis of Proline Concentration in Several Poaceae Family Plant Groups

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ABSTRACT

A ninhydrin test was conducted to evaluate the difference in the concentration of proline across some groups of plants from Poaceae family. The experiment was conducted from October-November 2018 at the Environment Laboratory of Universitas Khairun in Ternate. Fresh leaves from rice, wheat, corn and sugarcane plants were collected as the samples. The research data were analyzed descriptively. The highest proline concentration (0.268 ppm) was found in corn, followed by rice (0.074 ppm), wheat (0.071 ppm), and sugarcane (0.020 ppm). In a similar way, a higher concentration of proline was observed in corn's fresh leaves (0.092 μ mol prolin/g) compared to rice (0.026 μmol prolin/g), wheat (0.025 μ mol prolin/g), and sugar cane (0.007 μ mol prolin/g). It can be concluded that type of plants has no correlation to proline concentration.

I. INTRODUCTION

Poaceae is an angiosperm plant family which contains around 500 genera and 3,000 species. These plants have a cosmopolitan distribution but are mostly found in tropical areas and north temperate zone with enough rainfall to form grasslands Arsyad [1]. In addition to its ubiquity and multitude, Poaceae also gives humans important benefits, such as providing food and medicine for human use Solikin [12]. Poaceae or commonly referred to collectively as grass (Liliopsida) includes rice (*Oryza sativa*), corn (*Zea mays*), and sugarcane (*Saccharum officinarum*) Suhono and LIPI Team [13].

Plants from Poaceae family obtain their energy through photosynthesis. During photosynthesis in autotroph organisms, light energy is transformed into energy-rich organic compounds. The chlorophyll inside in the green plants absorb and use sunlight to synthesize oxygen and carbohydrate from CO_2 and water Pertamawati [10]. Based on its photosynthesis characteristics, Poaceae family members fall into two categories, namely C3 and C4 plants.

C3 plants are adaptive to the atmospheric CO_2levels but not resistant to heat or drought. In fact, these plants require a lot of water to grow. The C3 plants have a Photosynthetic Carbon Reduction (PCR) trajectory or cycle called Calvin cycle which can produce organic acids containing 3C atoms. The photosynthesis process occurs in mesophyll tissues. Inside the C3 plants, *Rubisco* combines CO_2 with RuBP (RuBP is a substrate used for the formation of carbohydrates) and binds O_2at the same time to assist the photorespiration process. If the CO_2in the atmosphere is increased, the competition between $CO_2$and O_2 will provide more benefits to CO_2, so that photorespiration can be inhibited and assimilation can be accelerated.

The photorespiration activity of C3 plants has an impact on their net photosynthetic yield. Since respiration depends on light, C3 plants lose more CO_2, resulting in a lower net photosynthesis rate compared to that of C4 plants. Drought stress in plants can slower down the rate of cell turgor pressure and inhibit the diffusion of water vapor and CO_2. As a result, the plants growth rate and crop production decreases. Plants included in this group are rice and wheat Miyao [7].

On the other hand, C4 plants can stand heat and drought. In other words, these plants have high water-use efficiency. C4 plants contain PEP, an enzyme which is more exclusive to CO_2, so it cannot bind O_2. Therefore, there is no competition between $CO_2$and O_2. This early association takes place in mesophyll cells. CO_2binding to PEP is then transferred to bundle

sheath cells, a layer of cells around xylem and phloem, and bound to *RuBP*. Due to high CO_2levels, O_2has no opportunity to react to *RuBP* so that photorespiration becomes very small. PEP has tied a strong binding knot with CO_2, so the photosynthesis reaction to CO_2 is very high. As the CO_2 level increases, assimilation rates decline gradually in C4 plants. Plants that belong to this category include sugarcane and corn (Campbell [2].

C3 and C4 plants have different properties in terms of heat or drought resistance. C4 plants are more adaptable to heat, while C3 need much water to survive. Corn, rice, wheat, and sugarcane have been reported to contain proline, a widely distributed compound which can protect cells from a lower concentration of water. However, there has been no study conducted to examine the concentration of proline in these four groups of plants. The main objective of this study, thus, was to describe the comparison of proline levels between some groups of plants from Poaceae family.

II. METHODS

This study was conducted from October-November 2018 at the Environment Laboratory of Universitas Khairun in Ternate. Samples used for the experiment were extracted from rice, wheat, sugarcane, and corn fresh leaves. To examine the levels of proline accumulated in each of the plants, a ninhydrin test Konstantinova et al., [5], was conducted with the following procedures. The fresh leaves (the second leaves near the tip of the stem) (0,5 g) were ground in the mortar and added with 10 ml of sulfosalicylic solution (3%). The result was filtered using Whatman no.1 filter paper.

The filtrate (2ml) was added with 2 ml of ninhydrin acid and 2 ml of glacial acetic acid in a reaction tube at 100ºC for an hour. The reaction was ended by inserting the tube into a glass cup filled with ice. The ninhydrin acid solution was prepared by heating thus dissolving 1.25 g of ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid. This mixture was extracted with 4 ml of toluene, then shaken out with vortex for 15-20 seconds, so that two separate layers of liquid were formed. The red toluene containing proline is usually located at the top. The upper solution was sucked using a pipette and measured using a spectrophotometer. The absorbance level of the solution was read at a wavelength of 520 nm. The proline level was determined based on the readings for the proline standard curve. Data analysis was descriptively and quantitatively performed by revealing the concentration of level in rice, wheat, sugarcane, and corn (Poaceae family).

III. RESULTS AND DISCUSSION

A. Results

The results of the analysis of proline levels in rice, wheat, sugarcane, and corn are presented in Table 1.

Table 1. The Concentration of Pure Proline (ppm) at absorption of 520 nm

No	The concentration of proline (ppm)	Absorption 520 nm		
		Replicate 1	Replicate 2	Average
1	0.1	0.052	0.052	0.052
2	0.3	0.073	0.073	0.073
3	0.5	0.097	0.097	0.097
4	0.7	0.117	0.117	0.117
5	1.0	0.146	0.146	0.146
6	3.0	0.390	0.390	0.390
7	5.0	0.586	0.586	0.586
8	7.0	0.803	0.803	0.803

The concentration of proline (ppm) contained in rice, wheat, sugarcane, and corn fresh leaves are shown in Table 2.

Table 2. The Concentration of Proline in Fresh Leaves

No	Plant Species	Proline (ppm)	The concentration of prolin in fresh leaves (μmol prolin/g)
1	Rice	0.074	0.026
2	Wheat	0.071	0.025
3	Sugarcane	0.020	0.007
4	Corn	0.268	0.092

The tables above were converted into a diagram to make it easier to understand the difference in the proline concentration.

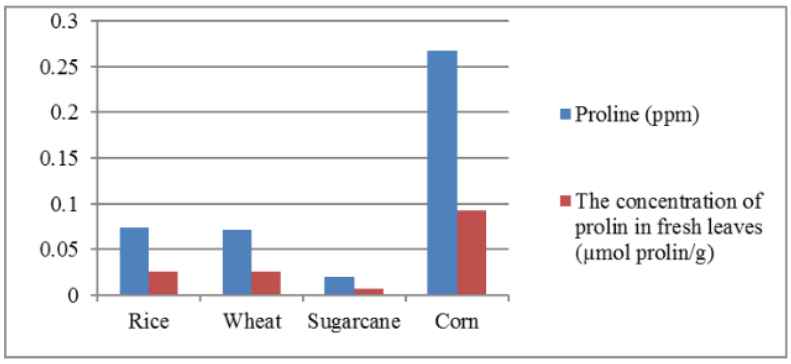

Figure 1. The Proline Concentration Diagram

Figure 1 shows that the highest concentration of proline can be observed in corn, followed by rice (0.074 ppm), wheat (0.071 ppm), and sugarcane (0.020 ppm). Similarly, a higher concentration of proline was observed in corn's fresh leaves (0.092 µ mol prolin/g) compared to rice (0.026 μ mol prolin/g), wheat (0.025 μ mol prolin/g), and sugar cane (0.007 μmol prolin/g).

B. DISCUSSION

The results indicate that corn contains the highest proline concentration compared to rice, wheat and sugarcane. Corn and sugarcane are C4 plants, while rice and wheat are C3 plants. According to Novenda and Nugroho [8], C4 plants do not usually produce much proline because of their resistance to hot conditions, while C3 plants need proline to adapt to water stress. Findings of this research, however, are not in line with this theory. The high level of proline produced by corn in this study can be explained as a self-defense form to adapt to the environment changes. As suggested by Sinay [11], certain plants can develop an ability to accumulate non-toxic compounds such as proline to protect their cells from damage when the water potential is reduced (drought tolerance). Proline levels are a common indicator used to show plant tolerance ability. Proline is a compound that is able to maintain cell turgor. It can interact with the membrane system, regulating the balance of cytosolic acidity with a comparison of NADH / NAD + which serves as an energy source to help cells deal with stress Konstantinova *et al*., [5].

Based on the photosynthesis type, corn and sugar cane belong to the C4 plant's category that can adapt to hot, dry, and humid conditions. Therefore, compared to the C3 groups, they can

grow better even in bad environment Perkasa *et, al.,* [9]. The negative effect of O_2on C3 net photosynthetic yield (the Warburg effect) results from the competition between $O_2$and CO_2in contributing to the work of the photosynthetic carboxylase enzyme called Ribulose Biphosphate Carboxylase (RuBP carboxylase = Rubisco). This enzyme also serves as a catalyst in an oxidation reaction where Ribulose bisphosphate (RuBP) is transformed to PGA (PhosphoGlycerate Acid) and phosphoglycolic acid.

Proline is a solute formed by plants to survive in drought conditions as a mechanism to maintain turgor, so plasmolysis does not occur. The results of an osmotic regulation appear in the form of turgor pressure should be maintained above zero. Otherwise, the process of cell division cannot continue and the wilt that can harm cells cannot be avoided Hendrati, Rachmawati and Pamuji [4].

It has been reported that proline is synthesized at the roots. Effendi and Azrai [3] state that proline in the primary root of corn plays an important role in regulating the cells' osmotic pressure. This pressure sustains water absorption which stimulates root length growth in drought stress conditions. In line with Lawlor [6], proline is a distinctive biochemical compound or osmotic metabolite which is widely synthesized and accumulated in various plant tissues, especially in leaves.

IV. CONCLUSIONS

The results of this research showed that corn contained the highest concentration of proline (0.268 ppm), followed by rice (0.074 ppm), wheat (0.071 ppm), and sugarcane (0.020 ppm). In a similar way, corn fresh leaves also reported the highest concentration of proline (0.092 μmol prolin/g), while the lowest was observed in sugarcane (0.007 μ mol prolin/g). Rice and wheat fresh leaves contained 0.026 μmol prolin/g and 0.025 μ mol prolin/g proline, respectively.

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