Vol. 14, No. 3, pp. 790-803, August 2026 DOI: https://doi.org/10.23960/jat.v14i3.12836

Jurnal Agrotek Tropika

P-ISSN: 2337-4993
E-ISSN: 2620-3138

Journal homepage: https://jurnal.fp.unila.ac.id/index.php/JA

ANALYSIS OF LEAF AREA INDEX AND RELATIVE WATER CONTENT OF WAXY CORN LEAVES INDUCED BY ACTINOMYCETES BIOSTIMULANT

ANALISIS INDEKS LUAS DAUN DAN KADAR AIR RELATIF DAUN TANAMAN JAGUNG PULUT YANG DIINDUKSI BIOSTIMULAN *ACTINOMYCETES*

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ARTICLE HISTORY:

Received: 04 May 2026
Peer Review: 02 June 2026
Accepted: 21 August 2026

KEYWORDS:

Actinomycetes, bio stimulant, corn, leaf area index, relative water content

ABSTRACT

Waxy corn (Zea mays L. var. ceratina) is one of the important food crops in Gorontalo whose productivity is still limited. Low soil fertility and drought often become obstacles during its growth period. Indicators of leaf area index and leaf relative water content can be used as indicators of plant growth physiology. The purpose of this study was to examine the administration of Actinomycetes biostimulant with different doses on the leaf area index and relative water content of waxy corn leaves. Field experiments were conducted with a randomized block design, one factor with three replications. The doses of Actinomycetes Biostimulant used were: 0, 5, 10 grams/10 kg soil in a polybag. Observed variables: Leaf Area Index (LAI) at growth intervals of 14, 21, 28, 35, and 42 days after planting, and leaf relative water content (RWC) at the age of 35 and 42 days after planting (DAP). Quantitative descriptive was conducted for data analysis. This study revealed that Actinomycetes biostimulant of 10 grams /10 kg soil in a polybag resulted in the highest leaf area index value of 4.00 cm^2 m^{-1} at the age of 35 - 42 DAP and the highest relative leaf water content at 35 DAP of 66.52 %, while at 42 DAP, the highest RWC was 71.65 % in the control treatment. This shows that the Actinomycetes biostimulant with different doses can provide different LAI and RWC.

KATA KUNCI:

Actinomycetes, biostimulan, indeks luas daun, jagung, kadar air relatif

ABSTRAK

Jagung ketan (*Zea mays* L. var. ceratina) merupakan salah satu tanaman pangan penting di Gorontalo yang produktivitasnya masih terbatas. Kesuburan tanah yang rendah dan kekeringan sering menjadi kendala pada masa pertumbuhannya. Indikator indeks luas daun dan kadar air relatif daun dapat digunakan sebagai indikator fisiologi pertumbuhan tanaman. Tujuan penelitian ini untuk mengkaji pemberian biostimulan Actinomycetes dengan dosis yang berbeda terhadap indeks luas daun dan kadar air relatif daun jagung pulut. Percobaan lapang telah dilakukan dengan desain rancangan acak kelompok, satu faktor yang diulang sebanyak tiga kali. Dosis Biostimulant *Actinomycetes* yang digunakan adalah: 0, 5, 10 gram/10 kg tanah perpolybag. Variabel yang diamati: Indeks Luas Daun (ILD) pada interval masa pertumbuhan 14, 21, 28, 35, dan 42 hari setelah tanam, dan kandungan air relatif daun (KARD) pada umur 35 dan 42 hari setelah tanam (HST). Deskriptif kuantitatif dilakukan untuk analisis data. Penelitian ini mengungkapkan bahwa biostimulant *Actinomycetes* sebesar 10 gram /10 kg tanah perpolybag menghasilkan nilai indeks luas daun tertinggi sebesar 4,00 m^2 m^{-2} pada umur tanaman 35 - 42 HST dan kadar air relatif daun tertinggi pada 35 HST sebesar 66, 52 %, sedangkan saat 42 HST, KARD tertinggi sebesar 71, 65 % pada perlakuan kontrol. Hal ini menunjukkan bahwa biostimulant *Actinomycetes* dengan dosis yang berbeda dapat memberikan ILD dan KARD yang berbeda pula.

1. INTRODUCTION

Waxy corn (*Zea mays* L. var. ceratina) is known as a local corn cultivar that has adapted well to certain environmental conditions, with the main characteristics of the dominance of amylopectin content in its starch structure. The composition has made the seeds have a softer and stickier texture after cooking. In Gorontalo, waxy corn is not only used as a traditional food source, but also has an important role in supporting the lives of people in the countryside. For many farmers, this plant can be used as a main source of income and indicates the use of local genetic resources that have important value (Kandowangko *et al.*, 2025). However, the productivity level of waxy corn at the farmer level is still low where the condition is due to several argonomial limitations, which are specifically related to the limited availability of water on dry land that is the main location for the cultivation of the crop. In addition, in the use of monoculture systems in a continuous way, it can cause a gradual decrease in soil fertility and can increase the vulnerability of plants to several abiotic problems (Rotasouw *et al*., 2020; Lopes *et al*., 2025).

In the Gorontalo region, drought is often considered a considerable environmental challenge because it can slow down crop development and can reduce corn yields. In the vegetative phase, insufficient water availability can cause the process of cell division to be hampered and tissue enlargement to be limited, so that in the formation of vegetative structures cannot take place optimally, causing the area of leaves formed to be small with plant growth more inclined to be less than optimal. In dry conditions like this, the physiological response of plants is generally indicated by symptoms that are easy to observe, for example leaves that curl with withering (Chen *et al*., 2023). From a psychological point of view, where a lack of water can lower the pressure of the actual turgor of the cell, it is very important in maintaining the structural stability of the plant tissue. When turgor decreases, the stomata in general can close so that the entry of carbon dioxide into the mesophile becomes limited, which ultimately decreases the efficiency of the photosynthesis process (Arsa *et al.*, 2023).

The implication of drought stress can be examined through alterations in important physiological indicators especially the Leaf area index and relative water content. These two parameters are typically used to measure how efficiently plants capture light and transform it into biochemical energy via photosynthesis while also indicating their capacity to sustain water balance within their tissues (Umam *et al*., 2023; Munir *et al.*, 2024). A lower in LAI reflects a decline in leaf surface site which is crucial for photosynthesis. Likewise a lower RWC indicates an imbalance in the plant's internal water condition which can disrupt metabolic activities and negatively affect overall plant performance (Soedradjad & Soeparjono, 2022).

A biological approach that utilizes plant growth-promoting microorganisms has been broadly examined as an alternative options to improve plant adaptability to environmental stress. Microorganisms living in the rhizosphere have an important role function in enhancing the efficiency of water and nutrient absorption also as supporting plant adjustment to abiotic stress conditions (Santari & Hatta, 2023). Their activity also contributes to enhancing soil quality and boosting nutrient availability especially in marginal lands (Hariyanti *et al.*, 2021).

Actinomycetes are considered one of the microbial groups with significant capacity to promote plant growth. These filamentous bacteria classified within the phylum Actinobacteria are well known for their ability to persist and adjust in arid environments. Their capability to colonize the rhizosphere makes them especially suitable for use in dryland agricultural systems (Latif *et al*., 2023). Beyond that Actinomycetes are possess the ability to produce multiple extracellular enzymes and bioactive compounds that are important in decomposing organic matter and enhancing nutrient availability for plant absorptions (Chukwuneme *et al*., 2020). Previous studies have reported that Actinomycetes isolates from the Gorontalo karst ecosystem possess several characteristics that

support plant growth. Streptomyces albus strain RzPK-05, for example, is capable of producing indole-3-acetic acid (IAA), solubilizing phosphate, and suppressing plant pathogens. These traits can improve nutrient availability and uptake, promote root growth, and enhance plant vigor. As a result, the isolate has the potential to support leaf development and maintain plant water status, thereby improving the physiological performance of waxy maize (Retnowati *et al*., 2024; Matalauni *et al*., 2025).

This study was conducted with the aim of determining the leaf area, leaf area index and relative water content of waxy corn leaves given Actinomycetes biostimulant at various doses and observed at different growth times.

2. MATERIAL AND METHOD

2.1 Research Location and Timeline

Research activities were executed at dual locations, centered in the Biology Laboratory under the auspices of the Faculty of Mathematics and Natural Sciences at Gorontalo State University. Corn planting was carried out in an experimental garden using polybags. The location is in Wumialo Village, Kota Tengah District, Gorontalo City, Gorontalo Province, Indonesia. The research period was 6 months, from July to December 2025.

2.2 Materials and Equipment

The equipment used included: an autoclave, laminar air flow meter, spectrophotometer, incubator, drying oven, analytical balance, drying oven, shovel, scissors, labels, ruler, measuring tape, petri dish, micropipette, loop needle, centrifuge, Bunsen burner, test tube, glass funnel, cuvette, mortar, stationery, and 35 x 35 cm plastic bags. Materials used: waxy corn seeds (*Zea mays* var. ceratina) and Actinomycetes isolate, Streptomyces albus strain RzPK-05, originating from the *Hulonthalangi* karst ecosystem, Gorontalo. Starch Casein Agar (SCA) media, Agar Powder (AP), 100% acetone, Actinomycetes Agar media, rice as a propagation medium, soil as a planting medium, distilled water, aluminum foil, sterilization materials, 100% acetone.

2.3 Research Procedures

2.3.1. Preparation of Plant Materials and Experimental Soil Media

The plant materials used were local Gorontalo corn seeds, waxy corn (*Zea mays* L. var. ceratina). The soil media used was garden soil. Prior to the experiment, an initial soil chemical analysis was conducted using a composite soil sample. The initial soil sample was analyzed at the Gorontalo Agricultural Research and Modernization Agency (BRMP) laboratory and analyzed using an XRF (X-Ray Fluorescence) tool in the Materials Laboratory, Faculty of Mathematics and Natural Sciences, Gorontalo State University.

2.3.2 Isolate Preparation and Propagation

Actinomycete isolates were purified on starch-casein agar and incubated at 28–30°C for 7–14 days until optimal colony growth occurred. Next, the isolates were propagated on rice media that had been thoroughly washed, parboiled, and sterilized at 121°C for 15 minutes. After the media cooled, the isolates were aseptically inoculated and incubated at 30°C for 10–14 days until uniform mycelial growth occurred (El Karkouri *et al*. 2019).

2.3.3 Experimental Design

This study used a randomized block design (RBD) with a single treatment factor, namely the application of actinomycete microbial biostimulants. The treatment consisted of three levels: P0 = Control (no biostimulant), PA1 = 5 g actinomycetes, and PA2 = 10 g actinomycetes. Each treatment was replicated three times, and plants were observed at 14, 21, 28, 35, and 42 days after planting. This resulted in 45 experimental units.

2.3.4 Planting and Plant Maintenance

Each 35 x 35 cm polybag was filled with 10 kg of soil. A 5-7 cm hole was dug in the soil. Next, 0, 5, and 10 grams of actinomycete inoculant with a viability of 6.5 x 10^5 CFU/g were applied to the planting hole, depending on the treatment. Three glutinous corn seeds were then planted per polybag, placed on top of the inoculant, and then covered with soil. When preparing the planting medium, 10 grams of NPK fertilizer (20-20-20) per polyethylene bag was mixed into the growing medium as a base fertilizer. No additional fertilization was applied during the experiment.

The polybags were arranged approximately 25 cm apart, following a randomized block design. Of the three seeds planted, only two plants were maintained per polybag. Watering was carried out regularly every day, according to plant needs and adjusted to environmental conditions such as rainfall, air temperature, and humidity of the growing medium. To control grasshoppers (*Locusta migratoria*) and armyworms (*Spodoptera frugiperda*) attacking the experimental plants, Emacel 30 EC insecticide was sprayed at a dose of 1-2 mL/L of water on the 15th and 30th days after planting. The plants were maintained until they reached their maximum vegetative stage with regular watering and weed control.

2.3.5. Determination of Leaf area Index

Morphophysiological observations were carried out up to 42 days after planting. The leaf area was calculated based on the formula: leaf length × width × 0.75 (Yang *et al*., 2022), and the reported values represent the average of two consecutive observation periods (Ratnaningsih *et al*., 2025).

The Average Leaf Area Index is the ratio of leaf area to land area on average over a seven-day period, calculated using the formula:

$$\text{LAI} = \frac{LA_2 + LA_1}{2} \times \frac{1}{P}\ cm^2\ \ m^{-2} \quad (1)$$

Note: LA = Leaf area (cm^2); t = Time (days); P = Land area (m^2); Index 1 and 2 refer to samples from two consecutive times.

2.3.6. Determination of Relative Water Content of Leaves

Relative water content was assessed by comparing fresh weight, turgid weight, and dry weight to reflect the hydration condition of plant tissue (Jahan *et al*., 2023). Leaf samples used to measure relative water content were taken from the third and fourth leaves from the top of the plant, which were fully developed. The leaves were then weighed using an analytical balance to obtain their fresh weight. The samples were then soaked in distilled water for 24 hours and then reweighed to obtain their turgid weight. The leaf samples were then oven-dried at 70°C to constant weight, and their dry weight was measured. Relative Water Content (RWC) was calculated using the following formula (Meriem *et al*., 2020):

[(fresh weight − dry weight) / (turgid weight − dry weight)] × 100% (2)

2.4 Data Analysis

Leaf area index (ILD) data were analyzed using two-way ANOVA and DMRT (5%), while relative water content (RWC) and leaf area data were analyzed descriptively.

3. RESULT AND DISCUSSION

3.1 Soil Analysis

The results of the analysis of the soil samples used in this study are shown in the following Table 1 and Table 2. Based on the data in Tables 1 and 2, there is a high phosphorus (P) content, and XRF test results indicate that available P was undetectable. Soil analysis conducted in the BRPM laboratory used Bray extractant, which measures phosphorus content. The fact that total P was not detected by XRF testing indicates that the soil is highly weathered and poor in phosphate minerals. The limiting factor in the soil medium used is P fixation by Fe and Al, which is considered very critical. Fe (17.59%) and Al (14.20%) content are very high. At slightly acidic pH (e.g., 5.5), Al^{3+} and Fe^{3+} ions actively form strong bonds with phosphate ions ($H_2PO_4^-$), forming Al-P and Fe-P compounds, which precipitate (are insoluble). Therefore, available P is 0 or undetectable.

Another constraint is Al and Mn toxicity. This soil condition is caused by the acidic pH, which dissolves Al^{3+} and Mn^{2+} into the soil solution. A total Mn content of 0.40% indicates a high potential for Mn toxicity. Low K Availability: Although the total K content is 3.25% (quite high in XRF), K-bearing minerals (such as feldspar or illite) are incompletely weathered, resulting in very low available K. The silicon concentration (Si 48 %) is dominated by quartz (inert), meaning the soil has a low Cation Exchange Capacity (CEC) and water-holding capacity, compounded by low organic carbon.

Table 1. Initial soil chemical properties of the experimental soil

Parameter	Status
Phosphorus content	High
Potassium content	Low
Soil pH	Slightly acidic
Organic carbon content	Low

Table 2. Results of soil analysis using XRF (X-Ray Fluorescence)

Element Number	Element Symbol	Element Name	Concentration (%)
14	Si	Silicon	48,13
26	Fe	Ferrum	17,59
13	Al	Aluminum	14,20
20	Ca	Calcium	7,93
19	K	Kalium	3,25
12	Mg	Magnesium	3,0
11	Na	Natrium	3,0
22	Ti	Titanium	1,81
25	Mn	Mangan	0,40
17	Cl	Chlorin	0,21
24	Cr	Chromium	0,05
29	Cu	Cuprum	0,04
30	Zn	Zink	0,04

Table 3. The Average Leaf Area of Waxy Maize Following Actinomycetes Application

Treatment	Mean leaf area of waxy maixe following actinomycetes application (Cm^2)				
	14	21	28	35	42
PA0	31.35	34.60	74.15	305.36	162.64
PA1	36.59	50.30	72.74	332.17	150.19
PA2	37.59	58.08	86.35	327.83	176.95

Table 4. The average Leaf Area Index (LAI) of waxy maize recorded between 14 and 42 days after planting (DAP) following treatment with Actinomycetes.

Treatment	Mean Leaf Area Index $m^2 m^{-2}$			
	14 - 21	21 - 28	28 - 35	35- 42
PA0	1,38	0,82	2,86	1,80
PA1	1,82	0,93	3,05	1,85
PA2	1,99	1,09	4,00	2,19

3.2 Leaf area

Leaf area is an important morphological characteristic that reflects the growth performance of plants during the vegetative stage. An increase in leaf area indicates active leaf development and a greater capacity for light interception, which supports photosynthetic activity and biomass production. Leaf area increased during the vegetative stage and declined at the end of the observation period. Actinomycetes application tended to promote greater leaf development than the control treatment, indicating improved vegetative growth. This response may be associated with the ability of Actinomycetes to enhance nutrient availability and produce plant growth-promoting compounds that support leaf development (Retnowati *et al*., 2024; Matalauni *et al*., 2025).

3.3 Leaf Area Index (LAI)

LAI is a morpho-physiological parameter that represents the portion between total leaf area and the ground area inhabited by the plant as its growing medium. This indicator is commonly used in plant physiology to evaluate how efficiently leaves capture and utilize sunlight as the primary energy source for photosynthesis. LAI values indicate the capacity of the plant canopy to intercept photosynthetically active radiation which is vital for carbon assimilation and biomass formation. Plants with higher LAI values generally have a greater ability to utilize light energy efficiently leading to enhanced photosynthetic performance (Khan *et al*., 2025). In turn promotes increased production of photosynthates that are distributed to support vegetative growth as well as the development of generative organs (Kalogeropoulos *et al*., 2024; Fu *et al*., 2025).

Several studies have reported that higher LAI values are closely linked to better light interception or increased biomass production and enhanced yield potential in maize and an increase in leaf surface area allows plants to utilize solar radiation more efficiently (Kalogeropoulos *et al*.,2024). This aligns with the result from Fu *et al*.,(2025) who showed that a larger leaf area contributes to higher carbon fixation rates resulting in greater biomass accumulation. Therefore in this study LAI was used as a physiological parameter to evaluate the response of waxy maize to Actinomycetes-based biostimulant application (Retnowati *et al*.,2024) and (Matalauni *et al*.,2025).

The changes in LAI of waxy maize throughout the study period are presented in Table 1. The results show that LAI values in all treatments consistently increased from 14 to 42 days after planting. This pattern reflects active vegetative growth indicated by the expansion of leaf area resulting from cell division and elongation in leaf tissues. Leaf development during the vegetative phase is closely associated with the activity of meristematic tissues which continuously undergo differentiation and cell separation (Yang *et al*.,2022). This process supports the formation of new leaf

tissues thereby improving the plant's photosynthetic capacity and contributing to biomass accumulation (Fu *et al*., 2025).

Differences in LAI values among treatments are also presented in the graph in Figure 1. The graph shows a consistent increase in LAI values across all treatments as the plants developed over time. Differences in curve patterns among treatments indicate variation in the rate of leaf area development which is influenced by the application of biostimulants such as actinomycetes. The dynamics of leaf area development have also been reported by Kalogeropoulos *et al*.,(2024) who stated that changes in leaf area can serve as an indicator of plant physiological responses to various cultivation treatments.

Two-way ANOVA revealed that observation time significantly affected LAI ($p < 0.001$), while Actinomycetes concentration also had a significant effect on LAI ($p = 0.024$). However, the interaction between observation time and Actinomycetes concentration was not significant ($p = 0.800$). These results indicate that leaf area development was strongly influenced by plant age and was enhanced by Actinomycetes application. Duncan's test further showed that PA2 produced the highest mean LAI and differed significantly from the control treatment.

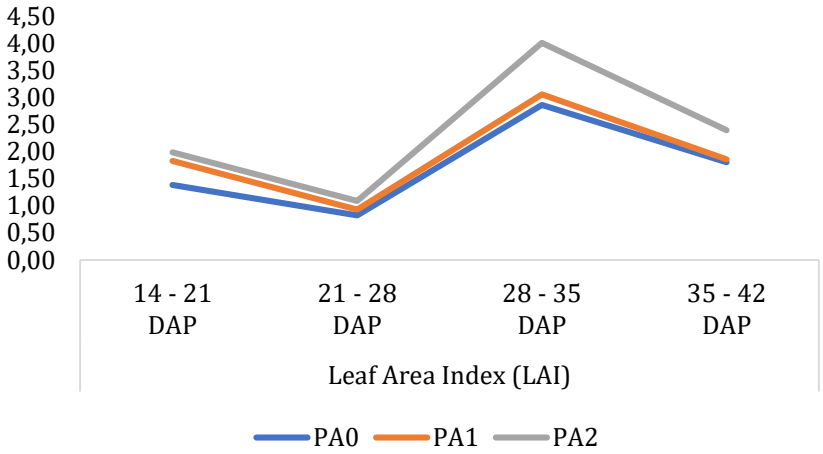

Figure 1. Graph of changes in the leaf area index (LAI) of waxy corn under various *Actinomycetes* treatments.

Table 5. Two-way ANOVA for the effect of observation time and Actinomycetes concentration on leaf area index (LAI)

Source of Variation	df	F-value	p-value
Observation time	3	28.895	<0.001**
Actinomycetes concentration	2	4.394	0.024*
Time × Concentration	6	0.503	0.800ns
Error	24	-	-

Note: ** significant at $p < 0.01$; * significant at $p < 0.05$; ns = not significant.

Table 6. Duncan's Multiple Range Test (DMRT) for mean leaf area index (LAI).

Treatment	Mean LAI	Group
PA0	0.5725	a
PA1	0.6375	ab
PA2	0.7883	b

Note: Means followed by the same letter are not significantly different at the 5% level according to DMRT.

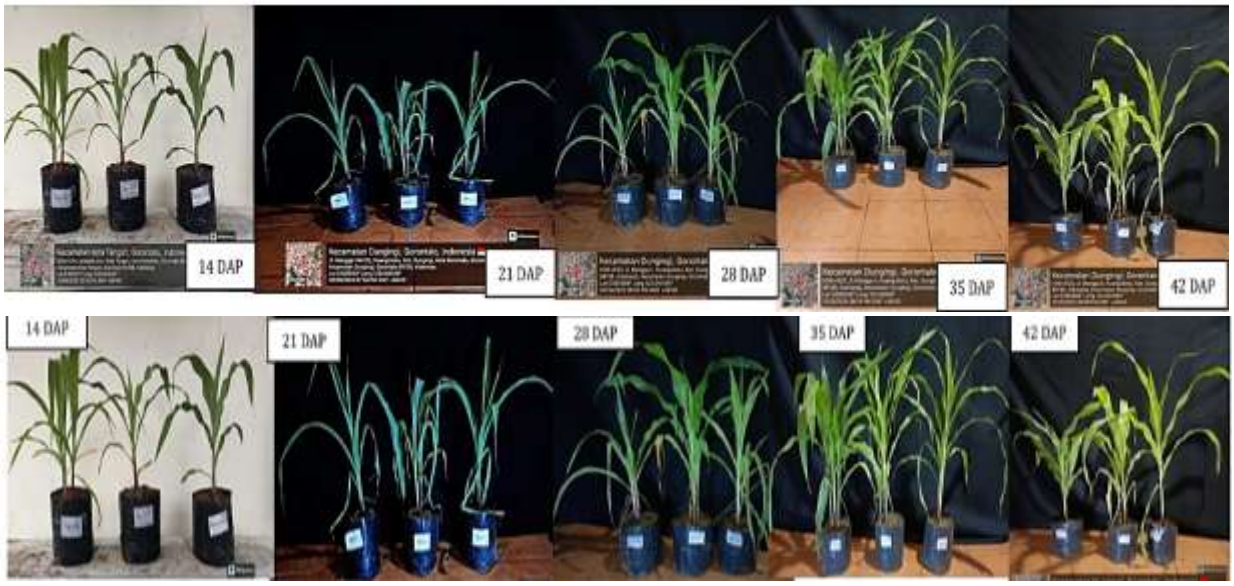

Figure 2. Growth of waxy maize plants at several vegetative stages: at 14, 21, 28, 35, and 42 days following planting (DAP).

During the initial observation period (14–21 days after planting), treatment PA2 exhibited the highest LAI value of 1.99, followed by PA1 with 1.82 and PA0 with 1.38. This variation suggests that although microbial inoculation had been applied, the plant response to rhizospheric microorganisms was not yet fully optimized at this early growth stage. At this phase, plants are still undergoing physiological adjustment to microbial interactions occurring in the root zone. A significant portion of photosynthates is allocated to the roots in the form of organic exudates, which serve as an energy source for microbial proliferation. Consequently, the allocation of assimilates for leaf expansion remains limited, resulting in relatively lower LAI values and minimal differences among treatments. This phenomenon aligns with findings indicating that, during early microbial colonization, competition for assimilates occurs between plants and microorganisms (Bouremani *et al*., 2023) Furthermore, Basu *et al*., (2021) and Napieraj *et al*., (2023) reported that the beneficial physiological effects of plant–microbe interactions typically become evident only after microbial populations reach a stable equilibrium. Morphologically, this condition is reflected in the limited number and size of leaves, as the plants are still in the early vegetative phase (Yang *et al*., 2022).

In the subsequent observation period (21–28 days after planting), LAI values exhibited dynamic changes across all treatments. PA2 remained the highest at 1.09, followed by PA1 at 0.93 and PA0 at 0.82. Although there was a decline compared to the previous period, the relative ranking among treatments remained consistent, with PA2 outperforming the others. This trend indicates that Actinomycetes populations had begun to stabilize and gradually exert a measurable influence on plant growth. At this stage, rhizospheric microorganisms are known to produce secondary metabolites, including Phytohormones such as auxins, cytokinins, and gibberellins regulate plant growth contribute to leaf development and expansion. Although the LAI values did not increase linearly, microbial activity continued to support plant physiological processes. Compounds with biological activity synthesized by these microorganisms function as plant growth stimulators (Du Jardin *et al*., 2020; Franzoni *et al*., 2022). Studies by Retnowati *et al*., (2024) and Matalauni *et al*., (2025) further demonstrated that Actinomycetes application enhances plant growth through physiological stimulation. Additionally, Boukelloul *et al.*, (2024). emphasized that Actinobacteria possess the ability to synthesize bioactive compounds that promote plant development. Morphological improvements at this stage are evident from the increase in both leaf number and size.

During the 28–35 days after planting interval, a substantial increase in LAI was observed across all treatments. PA2 recorded the highest value at 4.00, followed by PA1 at 3.05 and PA0 at 2.86. These results indicate that the effect of Actinomycetes-based biostimulants becomes more pronounced during the advanced vegetative stage. The elevated LAI in PA2 suggests that microbial activity in the rhizosphere had reached optimal functionality, significantly enhancing leaf area development. Actinomycetes are known for their ability to solubilize bound phosphate in the soil, thereby improving nutrient availability for plants (Du Jardin *et al.*, 2020; Rouphael & Colla 2020; AbdElgawad *et al.*, 2020). Increased nutrient accessibility directly supports vegetative growth, particularly leaf expansion. Findings from, Retnowati *et al*., (2024) and (Matalauni *et al*.,2025) also confirm that Actinomycetes improve nutrient use efficiency in plants. Visually, this phase is characterized by a marked increase in leaf size and number, indicating that the plants have entered an advanced vegetative stage with enhanced photosynthetic capacity (Fu *et al*., 2025).

In the final observation period (35–42 days after planting), LAI values in PA2 (2.19) remained higher than those in PA1 (1.85) and PA0 (1.80). Although a decline was observed compared to the previous interval, PA2 continued to demonstrate superior performance, suggesting a cumulative effect of Actinomycetes application on leaf development. The reduction in LAI at this stage can be attributed to the plant's transition from vegetative to generative growth, during which assimilates are increasingly redirected toward reproductive organ formation. Despite this shift, the existing leaf area continues to play a crucial role in supporting photosynthesis for grain filling. This observation is consistent with studies by Chukwuneme *et al*., (2020), Warrad *et al*., (2020) and Nozari *et al*., (2021) which reported that Streptomyces application enhances vegetative biomass in maize. Moreover Fu *et al*.,(2025) highlighted the importance of optimal leaf area during the late vegetative phase in ensuring sufficient assimilate supply for reproductive development.

Overall, the LAI dynamics observed in this study reflect three major growth phases: the initial adaptation phase, the active vegetative growth phase, and the peak leaf development phase. In the early stage, plants are still adjusting to microbial presence, resulting in suboptimal leaf growth. This is followed by a phase of accelerated leaf expansion driven by intensified microbial metabolic activity and plant–microbe interactions. Finally, during the late vegetative stage, leaf area reaches its maximum, supporting efficient photosynthesis. These findings indicate that Actinomycetes-based biostimulants function progressively as physiological enhancers that promote vegetative growth in waxy maize(Rouphael & Colla 2020; Santoyo *et al.*, 2021).

3.4 Leaf Relative Water Content (RWC)

Leaf Relative Water Content or RWC is a physiological parameter commonly used to indicate the level of water status in plant tissues at the time of measurement. This parameter represents the ratio between the actual water content within the cells and the maximum water-holding capacity of leaf tissues. Therefore RWC is commonly function as measurement of a plant's ability to maintain internal water balance during its growth. Physiologically RWC is primarily influenced by the hydric balance through the roots and loss of water via transpiration via the leaf stomata. The equilibrium between water uptake by roots and transpiration loss is the main factor controlling plant tissue hydration status (Zhou *et al*., 2021) When tissue hydration is maintained and the cellular turgor pressure stays stable allowing normal cell expansion also maintaining membrane integrity and sustaining photosynthetic activity (Gupta *et al.*,2020).

Table 7. Mean Relative Water Content (RWC) of leaves in waxy maize measured at 35 and 42 days after planting (DAP) following *Actinomycetes* induction.

Treatment	Mean Leaf Relative Water Content (%)		Deference
	35 DAP	42 DAP	
PA0	54,66	71,65	16,99
PA1	51,14	71,87	20,73
PA2	66,52	67,94	1,42

The results shown in Table 7 indicate that RWC values varied among treatments throughout the observation period. At 35 days after planting which PA2 produced the highest RWC value of 66.52% while the control and PA1 treatments recorded 54.66% and 51.14% respectively. These differences suggest that applying an Actinomycetes-based biostimulant at 10 g per plant improve the plant's capacity to retain water content in leaf tissues during the vegetative phase. The increased ability to maintain tissue hydration reflects the role of rhizosphere microorganisms in improving water use efficiency in plants. That drought-tolerant rhizosphere microorganisms can enhance the stability of plant tissue hydration by increasing water uptake through the root system (Agunbiade *et al*., 2024).

The physiology of *Actinomycetes* in the rhizosphere, which is indicative of the plant's capacity to maintain its internal water balance, could be linked to the increase in RWC seen in the treatment PA2. The first notable action that occurs in this regard involves the production Microorganisms produce ACC (*1-aminocyclopropane-1-carboxylic acid*) deaminase that breaks down ACC, lowering ethylene levels associated with stress responses is reduced allowing plants to maintain a more regulated physiological response under stress conditions. It has been shown that microorganisms producing ACC deaminase can improve plant tolerance to environmental stress by lowering ethylene levels (Ojuederie & Babalola, 2023). Reduced ethylene concentrations also help delay premature leaf senescence and maintain proper stomatal function thereby supporting cell turgor pressure and ensuring stable metabolic activity (Shaikhaldein *et al.*, 2021). Beyond hormonal effects Actinomycetes are also recognized for producing exopolysaccharides that help improve soil structure within the rhizosphere. These compounds promote the aggregation of soil particles thus improve the soil's water holding capacity. Exopolysaccharides synthesized by soil microorganisms are microscopic soil organisms known to significantly make the stabilization of soil structure and the enhancement of its water retention properties. As the soil's capacity to save water improves moisture conditions in the root zone become more consistent ensuring a steady supply of water for plants throughout the vegetative growth stage (Peng *et al.*,2025)

In addition actinomycetes activity can induce osmoregulatory reactions in plants through the collection of osmoprotectants like proline and soluble sugars. Bioactive compounds function as osmoprotectants that help maintain water potential balance within plant cells (Khati *et al*., 2025) Earlier evidence suggests that the accumulation of proline has major function in maintaining cellular osmotic balance and improving plant tolerance under conditions of water deficit. Furthermore proline functions as a protective compound that helps preserve protein stability and shields cell membranes from damage induced by environmental stress (Khan *et al*.,2019). Rhizosphere microorganisms can also enhance the plant's antioxidant defense system. Several researches reveal that microorganism based biostimulants could enhance the performance of antioxidant enzymes like catalase and others superoxide dismutase. The role of these antioxidant enzymes is very important for combating cellular harm caused by oxidative stress, which usually happens due to lack of water. Enhanced enzymatic performance of antioxidants can increase plant resistance against oxidative stress, and provide protection to cellular components in adverse conditions of environment (Dewi *et al*., 2024). Water retention capacity of plant tissues can be increased due to enhanced enzymatic performance.

At 42 days after planting the RWC value in the control treatment namely 71.65% was slightly higher than in PA2 which is 67.94%. This condition may be linked to the high LAI observed in PA2 which reached 2,19. As leaf area expands the surface area for evapotranspiration also increases leading to greater water demand in the plants. Greater leaf area can lead to increased transpiration rates due to the expanded evaporative surface (El-Saadony *et al*.,2024). Moreover rhizosphere microorganisms are known to induce genes responsible for encoding aquaporin proteins in both root and leaf tissues. Aquaporins act as channel proteins that enable the movement of water across cell membranes. Increased aquaporin expression enhances the efficiency of water movement from the roots to the aboveground plant parts thereby improving water distribution within plant tissues. (Kesavardhini *et al.*,2025)

The relationship between LAI and RWC in this study reflects a physiological balance between leaf area development and the plant's ability to regulate internal water availability. An increase in leaf area without proper water regulation can result in reduced tissue hydration. An imbalance between leaf area and water availability can reduce the hydration status of plant tissues (Khan *et al.*,2025). Overall, keeping RWC stable during the vegetative phase is important for supporting photosynthesis efficiency as well as encouraging biomass accumulation and generative organ development at the next stage of growth. Plants that are able to maintain tissue hydration consistently generally indicate better vegetative performance and have higher yield potential. (Gupta *et al*., 2020).

4. CONCLUSIONS

The research results show that the physiological performance of waxy corn plants, including leaf area index and relative leaf water content, varies across plant growth phases. The application of *Actinomycetes* biostimulants at varying doses impacts plant physiological changes. This opens up opportunities for the use of *Actinomycetes* biostimulants in sustainable corn cultivation systems on dryland land.

5. ACKNOWLEDGMENTS

The authors express their sincere gratitude for the financial support provided by the Directorate of Research and Community Service, Ministry of Higher Education and Science. This research was conducted as part of the Regular Basic Research program 2025. The implementation of this research is based on Contract Number 082/C3/DT.05.00.PL/2025 and Decree of the Rector of Gorontalo State University Number 987/UN47/HK.02/2025 stipulated on May 28, 2025. The authors express their deep gratitude to the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Gorontalo State University for providing facilities to support this research. The research team also declares that there is no conflict of interest related to this work.

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