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Enhancing Jamun cv. Goma Priyanka Growth and Stress Resilience through Foliar Humic Acid and Potassium Silicate Application

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Authors' contributions

This work was carried out in collaboration among all authors. Author MKM conceptualized the study, did data collection, analysis, original draft of the manuscript, writing and drafting, wrote and prepared the draft of the manuscript, reviewed and edited the manuscript. Authors PB, JS, CKA, SBSP and IBM conceptualized the study, reviewed and edited the manuscript. Author PB supervised the work and did data curation. Author JS helped in project administration. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The present research focuses on an important knowledge deficit concerning the foliar reaction of Jamun cv. Goma Priyanka to the application of humic acid (HA) and potassium silicate (KS), emphasizing its growth and physiological functions. Humic acid promotes the production and equilibrium of phytohormones such as auxins and cytokinins, facilitating cell division, elongation, and differentiation, which in turn enhances plant development. The present investigation was carried out at the Fruit Instructional Farm of College of Horticulture and Forestry, Jhalawar, the study underscores the effectiveness of the T_{15} treatment, which combines potassium silicate and HA at elevated doses, in enhancing leaf relative humidity, chlorophyll content, stress tolerance, and overall plant vitality.

Keywords: Plant growth; humic acid; growth-promoting hormones; jamun tree.

1. INTRODUCTION

Jamun (*Syzygium cumini* Skeels), commonly known as Indian blackberry, is a tropical evergreen tree that holds notable botanical significance within the Myrtaceae family. Its distinguishing features include medium-sized, elliptical, glossy dark green leaves and clusters of fragrant white to pale yellow flowers. However, the most outstanding attribute of the Jamun tree is its fruit – oblong or oval, dark purple to black when ripe, with edible skins and a sweet-tart pulp surrounding a substantial seed (Ahmed et al., 2013). These trees predominantly flower during the spring, attracting pollinators like bees. They are naturally distributed across the Indian subcontinent and various parts of Asia, thriving in tropical and subtropical climates (Warrier, 1996). Beyond their ecological roles, Jamun trees possess significant economic and medicinal importance (Al-Wasfy, 2014).

The fruit of the Jamun tree is renowned for its rich nutritional content and remarkable medicinal properties, primarily attributed to its abundant antioxidants, particularly anthocyanins. These antioxidants play a pivotal role in neutralizing harmful free radicals within the body, potentially reducing the risk of chronic diseases such as cancer and cardiovascular disorders. Jamun has a long history in indigenous medicine, notably Ayurveda, where it is valued for its therapeutic attributes. Its anti-diabetic potential is especially noteworthy, as it contains compounds that regulate blood sugar levels, enhancing insulin sensitivity and glucose uptake by cells (Hidayatullah et al., 2018). Additionally, it exhibits anti-inflammatory and antimicrobial properties, making it suitable for treating various health conditions.

Jamun trees also have economic significance due to their durable wood, which has traditionally been employed in furniture making and construction (Sairam, 1994). Research across diverse fields, including botany, medicine, and agriculture, continues to explore the multifaceted attributes of Jamun. Botanists delve into its taxonomy, morphology, and ecological interactions, while medical researchers investigate its potential as a natural remedy, particularly in managing diabetes. In agriculture, efforts are concentrated on optimizing fruit production. This research contributes to a deeper understanding of Jamun and its potential in enhancing human health and supporting sustainable agriculture.

In a related context, humic acid, derived from the decomposition of organic matter, plays a pivotal role in agriculture as a biostimulant. It enhances plant growth and productivity by significantly affecting soil fertility and nutrient cycling. Humin, the most stable component of humic substances, remains insoluble in water due to complex polymeric structures, resisting microbial degradation (Rose et al. 2014; Naidu et al., 2013).

In foliar bio stimulation, humic acid's ability to improve nutrient uptake in fruit-bearing plants is remarkable, owing to its chelating properties (Khattab et al., 2012). It forms complexes with nutrients, making them more accessible and absorbable through leaves, influencing various physiological processes like photosynthesis and enzyme activity. Humic acid also enhances plant resilience against environmental stressors, stimulates root and shoot growth, and increases nutrient availability (Nardi et al., 2016).

Furthermore, humic acid influences the synthesis and balance of phytohormones in plants, elevating levels of growth-promoting hormones such as auxins and cytokinins. This promotes cell division, elongation, and differentiation, ultimately contributing to enhanced plant growth and development (Nardi et al. 2002). Research findings suggest that humic acid enhances mesophyll conductance, facilitating better CO_2 diffusion into chloroplasts, and thereby promoting higher rates of photosynthesis (Nikbakht et al., 2008; Zhang et al., 2013). The augmentation of mesophyll conductance results in increased carbon assimilation and heightened photosynthetic efficiency, ultimately advancing plant growth and productivity.

The current research endeavour aims to address a significant knowledge gap concerning the foliar response of Jamun cv. Goma Priyanka to humic acid (HA) application, with a focus on its growth and physiological processes. Given the inherent resilience of Jamun plants in withstanding temperature extremes, drought, and water stress, the study investigates the impact of HA treatment in an orchard situated in clayey soils within the Jhalawar district. Understanding this interaction holds implications for optimizing Jamun cultivation and its potential for enhancing agricultural practices in challenging environments.

In a parallel vein, potassium silicate, also known as K_2SiO_3, emerges as a vital component in plant science (Ma, 2004; Zargar et al., 2012). It serves as a source of potassium (K) and highly soluble silicon, finding applications in plant biology as a biostimulant. Silicon is recognized as one of the most critical elements in crop production due to its capacity to mitigate the detrimental effects of salt stress and oxidative stress (Epstein, 1999; Savvas & Ntatsi, 2015). Silicon enhances various aspects of plant physiology, including root architecture, plant development, leaf orientation, photosynthesis, and water relations (Pavlovic et al., 2021; Tubana et al., 2016). Additionally, potassium, a key plant nutrient, plays a crucial role in processes such as sugar and starch production, protein synthesis, cell division, and overall plant growth and development.

Potassium silicate's significance is underscored by its ability to improve the uptake of essential nutrients by fruit-bearing plants (Ma and Yamaji, 2006). This efficacy arises from its chelating properties, enabling the formation of complexes with nutrients that become more readily accessible and easily absorbed by plants through their leaves. Furthermore, potassium silicate exerts influence on a wide range of physiological processes in fruit plants, encompassing photosynthesis, respiration, and enzyme activity. These effects collectively lead to enhanced plant metabolism and overall growth.

Moreover, potassium silicate demonstrates a remarkable capacity to bolster the resilience of fruit plants against various environmental stressors, including drought, salinity, and extreme temperatures (Van Oosten et al., 2017). The application of potassium silicate to leaves can stimulate both root and shoot growth in these plants, thereby reinforcing their structural integrity. Notably, potassium silicate contains functional groups capable of chelating or forming complexes with essential metal ions like iron, manganese, zinc, and copper. This chelation process elevates the availability and mobility of these vital nutrients, rendering them more easily absorbable through the leaves. The outcome is improved nutrient utilization by the plant, leading to potential enhancements in growth and yield (Ma and Yamaji, 2008).

Another facet of potassium silicate's influence lies in its impact on the synthesis and equilibrium of phytohormones in plants (Zhu & Gong, 2014). It has been observed to elevate the levels of growth-promoting hormones, such as auxins and cytokinins, which govern critical processes like cell division, elongation, and differentiation (Selim et al., 2012; Singh et al., 2007). These hormonal changes contribute to enhanced plant growth and development. Additionally, research findings suggest that potassium silicate can improve mesophyll conductance, facilitating more efficient CO_2 diffusion into chloroplasts, ultimately leading to increased carbon assimilation and heightened photosynthetic efficiency (Sajadian & Hokmabadi, 2015).

Given the complex interplay of these factors, the current research seeks to address a critical knowledge gap concerning the foliar response of Jamun cv. Goma Priyanka to humic acid (HA) and potassium silicate (KS) applications. This study focuses on understanding their combined or individual effects on growth and physiological processes within an orchard situated in clayey soils within the Jhalawar district. Given the inherent resilience of Jamun plants, this research aims to shed light on the potential synergistic benefits of HA and KS treatments, providing valuable insights into optimizing Jamun cultivation practices and their broader implications for sustainable agriculture in challenging environments.

Jamun, an underutilized evergreen fruit crop, has received relatively limited attention in terms of research pertaining to its growth and developmental physiology. These plants, characterized as mesophytes, often encounter moisture deficits during their active growth phases, leading to substantial limitations in photosynthesis and, consequently, adverse effects on overall plant growth (Tang et al., 2021;). Given that Jamun plants are currently in their gestation phase, understanding how HA applications influence their growth and development assumes paramount importance.

In the context of our research, we formulated the following hypotheses:

1. The application of KS and HA holds promise for augmenting diverse growth and developmental aspects in Jamun cv. Goma Priyanka plants.
2. Anticipated outcomes of KS and HA treatments encompass the improvement of stress-related indicators, including the Membrane Stability Index (MSI), proline content, total chlorophyll content, as well as pivotal photosynthetic parameters like photosynthetic rate, Photosynthetic Active Radiation (PAR), stomatal conductance, and relative humidity percentage in Jamun cv. Goma Priyanka plants.

Through a rigorous investigation of these hypotheses, our research aims to contribute valuable insights into the optimization of Jamun cultivation practices and, more broadly, enhance our understanding of the physiological responses of this resilient fruit crop to HA applications, which can ultimately inform sustainable agricultural strategies.

2. MATERIALS AND METHODS

2.1 Plant Materials and Treatment

The research study took place in a well-established Jamun orchard characterized by a planting pattern with 6 x 6 meters spacing. This orchard is situated within the Fruit Instructional Farm, which is an integral part of the Department of Fruit Science at the College of Horticulture and Forestry located in Jhalawar.

In this study, a total of sixteen distinct treatment combinations were created, featuring both individual and combined applications of HA and KS, which included the utilization of humic acid and potassium silicate on Jamun cv. Goma Priyanka plants. These treatments were executed three times, with two plants allocated for each specific treatment in every repetition. The implementation of these treatments occurred in August in both 2021 and 2022. During the following two years, the leaf responses of the Jamun cv. Goma Priyanka plants were assessed. These assessments included a variety of growth-related and physiological factors.

Below is the specific treatment combinations used in this research explained in detail:

Table 1. Treatments detail

Sr. No.	Symbols	Treatments Detail
1.	T_0	Control
2.	T_1	Potassium silicate (1000 ppm)
3.	T_2	Potassium silicate (2000 ppm)
4.	T3	Potassium silicate (3000 ppm)
5.	T4	Humic acid (1000 ppm)
6.	T5	Humic acid (2000 ppm)
7.	T6	Humic acid (3000 ppm)
8.	T7	Potassium silicate (1000 ppm) + Humic acid (1000 ppm)
9.	T8	Potassium silicate (1000 ppm) + Humic acid (2000 ppm)
10.	T_9	Potassium silicate (1000 ppm) + Humic acid (3000 ppm)
11.	T_{10}	Potassium silicate (2000 ppm) + Humic acid (1000 ppm)
12.	T_{11}	Potassium silicate (2000 ppm) + Humic acid (2000 ppm)
13.	T_{12}	Potassium silicate (2000 ppm) + Humic acid (3000 ppm)
14.	T_{13}	Potassium silicate (3000 ppm) + Humic acid (1000 ppm)
15.	T_{14}	Potassium silicate (3000 ppm) + Humic acid (2000 ppm)
16.	T_{15}	Potassium silicate (3000 ppm) + Humic acid (3000 ppm)

2.2 Solution Formulation, Time and Method of Spray

A. Potassium Silicate: We procured potassium silicate in powder form from Aldrich in the market and prepared a stock solution with a concentration of 5000 ppm. This stock solution served as the basis for creating working solutions of potassium silicate at concentrations of 1000 ppm, 2000 ppm, and 3000 ppm for foliar application on Jamun cv. Goma Priyanka plants. The process involved the following steps:

1. **Calculating Required Volumes:** Initially, we determined the necessary volume of the stock solution and then added distilled water to achieve the desired concentrations. We used the following formula for this purpose: Volume of Stock solution (ml) = (Desired concentration / Stock solution concentration) * Final volume (ml).
2. **Preparing the Stock Solution:** To create the stock solution with a concentration of 5000 ppm, we weighed 5g of potassium silicate using an electronic balance. Subsequently, we dissolved the powder in a small amount of water and stirred it with a stirring rod to ensure complete dissolution. After the potassium silicate powder had fully dissolved, we added distilled water to reach a final volume of one litre. We thoroughly stirred the mixture until a homogeneous stock solution with a concentration of 5000 ppm of potassium silicate was achieved.
3. **Creating Working Solutions:** We prepared the working solutions of 1000 ppm, 2000 ppm, and 3000 ppm as follows:

- For the 1000 ppm solution, we added 200 ml of the stock solution to a container and then added distilled water to reach a final volume of 1000 ml.
- For the 2000 ppm solution, we added 400 ml of the stock solution to a container and supplemented it with distilled water to reach a final volume of 1000 ml.
- For the 3000 ppm solution, we added 600 ml of the stock solution to a container and added distilled water to achieve a final volume of 1000 ml.

These meticulously prepared working solutions with varying concentrations were subsequently applied to Jamun cv. Goma Priyanka plants through foliar spraying, employing different treatment combinations on August 10, 2021, with a second application on October 10, 2021. In the second year, the first spray was conducted on August 10, 2022, followed by the second spray on October 10, 2022, with the aim of achieving optimal absorption during the morning hours.

B. Humic Acid: In the course of this experimental procedure, we sourced liquid humic acid from the IIFCO market and prepared solutions with varying concentrations. To achieve these concentrations of 1000 ppm, 2000 ppm, and 3000 ppm, we meticulously dissolved 1 ml, 2 ml, and 3 ml of humic acid, respectively, in 1 litre of distilled water for each corresponding concentration.

The primary objective of this study was to apply specific concentrations of humic acid to Jamun cv. Goma Priyanka plants through foliar spraying. This application method was carried out using a Knapsack sprayer and involved different treatment combinations. The experiment commenced on August 10, 2021, with the initial application of humic acid treatments. Subsequently, a second round of foliar spraying was conducted on October 10, 2021, to continue the treatment process.

Moving into the subsequent year, the experimental protocol was replicated. The first foliar spray was administered on August 10, 2022, followed by the second application on October 10, 2022. The specific aim was to optimize the absorption of humic acid treatments, with an emphasis on conducting this process during the morning hours. Adhering to this meticulous schedule allowed us to comprehensively evaluate the effects of varying humic acid concentrations on the growth and development of Jamun cv. Goma Priyanka plants over the defined timeline.

Regarding fertilizer application, consistent nutrient levels were maintained for all Jamun plants throughout the experimental period spanning 2021 and 2022. Specifically, each individual plant received a combination of 500 grams of nitrogen (N), 200 grams of phosphorus (P), and 200 grams of potassium (K) per year in canopy area of 1.5m^2, following the recommendations of K.L. Chadha (1997).

2.3 Physiological Parameters Studied

In this scientific study, we investigated various physiological parameters in the leaves of Jamun cv. Goma Priyanka. These parameters included:

1. Relative water content (% in leaves)
2. Total chlorophyll content (mg^{-1})
3. Proline content (µmoles g^{-1})
4. Membrane stability index (%)
5. Photosynthetic Active Radiation (PAR) (mmol m^{-2}s^{-1})
6. Photosynthetic rate (µmol CO_2 m^{-2}s^{-1})
7. Stomatal conductance (mmol m^{-2}s^{-1})
8. Relative humidity in leaves (%)

During the initial year of our experimentation, we meticulously collected data on these physiological parameters in the months of October 2021, December 2021, and March 2022 for Jamun cv. Goma Priyanka. Subsequently, we carefully recorded the same physiological parameters for the second year, specifically in October 2022, December 2022, and March 2023.

2.4 Methodology Employed for the Assessment of Physiological Parameters

A. Relative Water Content (%): Relative Water Content (RWC) is a commonly assessed parameter in plant leaves, involving the use of leaf tissue. The process involves the following steps:

1. **Sample Preparation:** A composite sample of leaf discs was meticulously collected from the Jamun leaves under examination. These leaf discs represent a cross-sectional portion of the leaf tissue.
2. **Fresh Weight Determination:** The collected leaf discs were weighed to establish their initial fresh weight. This step serves as a baseline for calculating the RWC.
3. **Flotation on Water:** The leaf discs were subsequently placed on the surface of water and allowed to float for a specified duration, typically up to 4 hours. This procedure permits the leaf tissue to reach its maximum turgidity.
4. **Turgid Weight Recording:** Following the flotation period, the leaf discs were retrieved from the water, and their turgid weight was measured. This weight corresponds to the leaf tissue's fully hydrated state.
5. **Oven Drying:** After recording the turgid weight, the leaf tissue underwent oven drying. The tissue was placed in an oven set to a constant temperature, usually around 75°C. The drying process continued until the leaf tissue achieved a constant weight, indicating that all moisture had been removed.

The Relative Water Content (RWC) of the Jamun leaf tissue was computed using the following formula:

Relative water content (%) = (Fresh weight - Dry weight) / (Turgid Weight-Dry weight) ×100

B. Total Chlorophyll content (mg g^{-1}): The determination of chlorophyll content followed the established protocol outlined by Sadasivam and Manickam in 1997. To commence the process, an exact one-gram portion of mature Jamun leaves, finely chopped for uniformity, was accurately weighed. Subsequently, these leaves were meticulously pulverized in a clean mortar using a 20 ml solution of 80% acetone as the solvent. This solvent was chosen for its ability to effectively extract chlorophyll.

The resulting mixture underwent centrifugation at 5000 rotations per minute (rpm) for 5 minutes. The purpose of this step was to separate the chlorophyll-containing liquid from the solid plant material. The extracted liquid was then meticulously transferred to a 100 ml volumetric flask, ensuring precision in volume measurement.

This centrifugation and extraction process was iteratively repeated until the liquid in the flask became entirely colourless. This colour change signified the completion of a thorough extraction process, ensuring accurate and reliable quantification of chlorophyll content in the Jamun leaves. This methodology allows for the precise assessment of chlorophyll levels, providing valuable information about the photosynthetic activity and health of the plant material under investigation.

To ensure a thorough extraction, we took extra care by diligently cleaning the mortar and pestle with 80% acetone, ensuring the clarity of the leaf extract. The volume of the extract was then carefully adjusted to 100 ml using 80% acetone. After this preparation, we proceeded to measure the solution's absorbance at three distinct wavelengths: 645 nm, 663 nm, and 652 nm. Each of these absorbance values was compared against a blank solution composed solely of 80% acetone.

To determine the quantity of chlorophyll present in the extract, we employed specific equations as stipulated by the method. These equations were instrumental in converting the absorbance readings into meaningful and quantifiable chlorophyll concentration values. This meticulous approach allows for the precise and accurate assessment of chlorophyll content, facilitating a deeper understanding of the photosynthetic activity and health of the plant material under investigation. The cleanliness and precision in these extraction and measurement steps are crucial to obtaining reliable and scientifically meaningful results.

Total Chlorophyll mg/g tissue = $20.2(A_{645}) + 8.02 (A_{663}) \times V/1000 \times W$

Where, A_{645} and A_{663} are the absorbance readings at wavelengths 645 nm and 663 nm, respectively.

V=Volume of extraction solvent usually in ml.
W is the weight of the sample usually in gm.

C. Proline content in leaf ((µ moles g^{-1}): The proline estimation was carried out through a precise and systematic procedure, ensuring reliable results:

a. Initially, exactly 2 grams of leaf material were finely ground in the presence of 10 ml of sulfo salicylic acid. Following this, the resulting mixture underwent centrifugation at 4000 rpm for a duration of 15 minutes. Post-centrifugation, the supernatant was carefully separated and transferred into a 50 ml test tube.
b. To this solution, 5 ml of glacial acetic acid and 5 ml of acid ninhydrin were added. The test tube was securely sealed with polythene paper and fastened with a rubber band. Subsequently, the sealed test tube was subjected to an hour of boiling in a water bath maintained at 100°C. This boiling process was executed for both the standards and the plant samples.
c. Following the boiling step, the reaction mixture was transferred to 60 ml separating funnels. In each funnel, 20 ml of toluene was introduced, and the mixture was vigorously shaken. After thorough agitation, the funnel was set aside to allow for the separation of phases. The toluene, containing the chromophore, was then carefully extracted from the bottom of the funnel.
d. The absorbance of the extracted solution was measured at a specific wavelength, specifically at 520 nm. Using data derived from a standard curve, the proline content in the plant sample was quantified. This proline content was expressed in milligrams of proline per gram of fresh sample (Verbruggen & Hermans, 2007).

This meticulous method was employed to ensure the accurate determination of proline content in the plant sample. It provides valuable insights into the plant's responses to stress conditions and the accompanying physiological changes. The precision in this proline estimation procedure contributes to the reliability of the obtained results, furthering our understanding of the plant's stress tolerance mechanisms.

D. Membrane stability index (%): The Membrane Stability Index (MSI) serves as a vital measure in plant physiology, offering insights into the resilience and stability of cell membranes, particularly under conditions of stress. The methodology, as outlined by Sairam in 1994, entails subjecting Jamun leaf material to various treatments involving different temperature regimes, followed by the assessment of electrical conductivity as an indicator of membrane damage.

Here is an elaboration of the steps involved in the MSI determination:

a. **Sample Preparation**:

1. A total of 100 mg of leaf material was meticulously divided into two distinct sets.
2. Each of these sets was placed into separate test tubes, each containing 10 ml of double-distilled water.

b. **Temperature Treatments**:

1. **Set 1**: The first set of leaf material in the test tube was subjected to heating at 40°C for a duration of 30 minutes in a water bath.
2. **Set 2**: The second set of leaf material in the test tube was boiled at 100°C using a boiling water bath for 10 minutes.

c. **Conductivity Measurement**:

1. Subsequent to each temperature treatment, the electrical conductivity of the

solution within each respective test tube was measured.

2. In the case of **Set 1**, the electrical conductivity of the solution from the first set (subjected to 40°C) was assessed using a conductivity bridge (C1).
3. For **Set 2**, the electrical conductivity of the solution from the second set (boiled at 100°C) was measured using a separate conductivity bridge (C2).
4. The MSI is calculated by following formula:
5. MSI = [1- (C1/C2)] x100

Where C1=Electrical conductivity of the solution from the first test (heated at 40^0C).

C2 = Electrical conductivity of the solution from the second test (boiled at 100^0C).

This method enables the evaluation of membrane stability by quantifying the extent of electrical conductivity, which is indicative of membrane damage caused by varying temperature treatments. The MSI offers valuable insights into the plant's ability to maintain membrane integrity under stress conditions, aiding in the assessment of its overall stress tolerance and adaptation mechanisms.

E. Photosynthetic Active Radiation (PAR) (mmol $m^{-2}s^{-1}$): To quantitatively assess Photosynthetic Active Radiation (PAR), a meticulous and systematic approach was employed in monitoring the physiologically mature leaves of Jamun cv. Goma Priyanka plants. This methodological framework encompassed the following key components:

1. **Leaf Selection and Measurements:** To ensure representative assessments across the various treatment groups, three physiologically mature leaves were thoughtfully selected for measurement in each replication. This careful selection process aimed to capture a comprehensive view of PAR responses.
2. **Timing and Frequency of Measurements:** Observations of PAR were conducted at strategic time intervals as the plants progressed in their growth. These measurements were consistently taken during the early morning hours, specifically between 8:00 AM and 11:00 AM. This timeframe was deliberately chosen to coincide with optimal light conditions conducive to photosynthesis.
3. **Measurement Instrument:** For precise and accurate measurement of PAR, a CIRAS Portable Photosynthesis System from the USA was utilized. This advanced equipment played a pivotal role in quantifying the light energy available for the vital photosynthetic processes within the plant.
4. **Observation Periods:** The monitoring of PAR was conducted systematically at specific intervals over the course of the study. During the initial year of observation, measurements were taken in August 2021, October 2021, December 2021, and March 2022. Similarly, in the second year, observations were carried out in August 2022, October 2022, December 2022, and March 2023.

F. Photosynthetic rate (μmol CO_2 $m^{-2}s^{-1}$): The evaluation of the photosynthetic rate in Jamun cv. Goma Priyanka was conducted meticulously, involving a systematic approach to ensure the accurate measurement of this crucial physiological process. The methodology encompassed the following structured steps:

1. **Leaf Selection and Replications:** Three physiologically mature leaves were thoughtfully chosen from each plant replication within the various treatment groups. This meticulous leaf selection process was designed to provide a well-rounded and representative assessment across the diverse treatment conditions.
2. **Timing and Frequency of Measurements:** The observations of the photosynthetic rate were consistently performed during the early morning hours, specifically between 8:00 AM and 10:00 AM. This specific time window was selected to coincide with optimal light conditions, ensuring the acquisition of precise and reliable data.
3. **Measurement Instrument:** The measurements of the photosynthetic rate were executed using the advanced CIRAS PP Systems from the USA. This specialized equipment played a pivotal role in facilitating the accurate quantification of the rate at which photosynthesis occurred within the leaves under investigation.
4. **Observation Periods:** The assessment of the photosynthetic rate was conducted at predefined intervals over the duration of the study. In the inaugural year of the research, measurements were recorded in

August 2021, October 2021, December 2021, and March 2022. Likewise, during the second year, observations were carried out in August 2022, October 2022, December 2022, and March 2023.

G. Stomatal conductance (µmol $m^{-2}s^{-1}$): The assessment of stomatal conductance, which serves as a measurement of gaseous diffusion, was systematically carried out on three carefully chosen physiologically mature leaves of Jamun cv. Goma Priyanka plants. This approach was consistently followed across different treatment replications throughout the research period.

1. The observations related to stomatal conductance were conducted during the early hours of the morning, specifically from 8:00 AM to 11:00 AM. This time frame was selected to ensure optimal conditions for accurate measurements of gaseous exchange.
2. The CIRAS Portable Photosynthesis System from the USA was employed for these observations. This sophisticated system facilitated precise determinations of stomatal conductance on the selected leaves.
3. Observations were recorded at distinct intervals throughout the research duration. In the initial year of the study, measurements took place in August 2021, October 2021, December 2021, and March 2022. Subsequently, during the second year, observations were conducted in August 2022, October 2022, December 2022, and March 2023.

H. Relative humidity in leaves (%): From August 2021 to March 2023, we conducted a comprehensive assessment of relative humidity on three carefully selected physiologically mature leaves of the Jamun cv. Goma Priyanka, encompassing various treatment combinations. These measurements were diligently carried out during the early morning hours, precisely between 8:00 AM and 11:00 AM. This specific time frame was chosen to ensure ideal conditions for the accurate recording of relative humidity levels.

For these observations, we utilized the advanced CIRAS Portable Photosynthesis System from the USA. This cutting-edge equipment played a pivotal role in guaranteeing precise determinations of relative humidity on the designated leaves, contributing to the reliability of our data.

Throughout the research duration, we consistently recorded observations at predefined intervals. In the initial year of the study, measurements were conducted in August 2021, October 2021, December 2021, and March 2022. Subsequently, during the second year, our observations were repeated in August 2022, October 2022, December 2022, and March 2023. This rigorous and systematic approach allowed us to gather valuable data on relative humidity, shedding light on its variations and impact on Jamun cv. Goma Priyanka plants under different treatment conditions.

2.5 Experimental Design

In the realm of statistical analysis, our experiment spanned for two years, during which we diligently collected data at predetermined intervals. We focused on a total of sixteen distinct treatments, which involved the application of potassium silicate and humic acid to Jamun cv. Goma Priyanka plants. To ensure robustness in our findings, each treatment was replicated three times.

The experimental design followed a Randomized Block Design (Factorial), allowing the assessment of not only the individual effects of each treatment but also the potential interactions that might occur between them.

The collected data underwent thorough scrutiny, and its significance was evaluated at a 5% level of significance, ensuring that our conclusions were statistically sound. Furthermore, we conducted multiple correlation analyses to investigate the relationships between various growth and physiological variables in Jamun cv. Goma Priyanka. Our goal was to unveil the effects and identify both positive and negative correlations that may exist among these diverse attributes. Fischer's test was employed to facilitate these correlation analyses, enhancing the depth of our insights.

3. RESULTS AND DISCUSSION

3.1 Relative Water Content (%)

The combined analysis presented in Fig. 1 illustrates a substantial impact of the interactive treatments on the relative water content (RWC) in the leaves of Jamun cv. Goma Priyanka. Throughout our study, spanning from 2021 to 2023, our observations provided valuable insights into the fluctuations in RWC. Particularly noteworthy were the variations in RWC that emerged during March in the second year (2022-23).

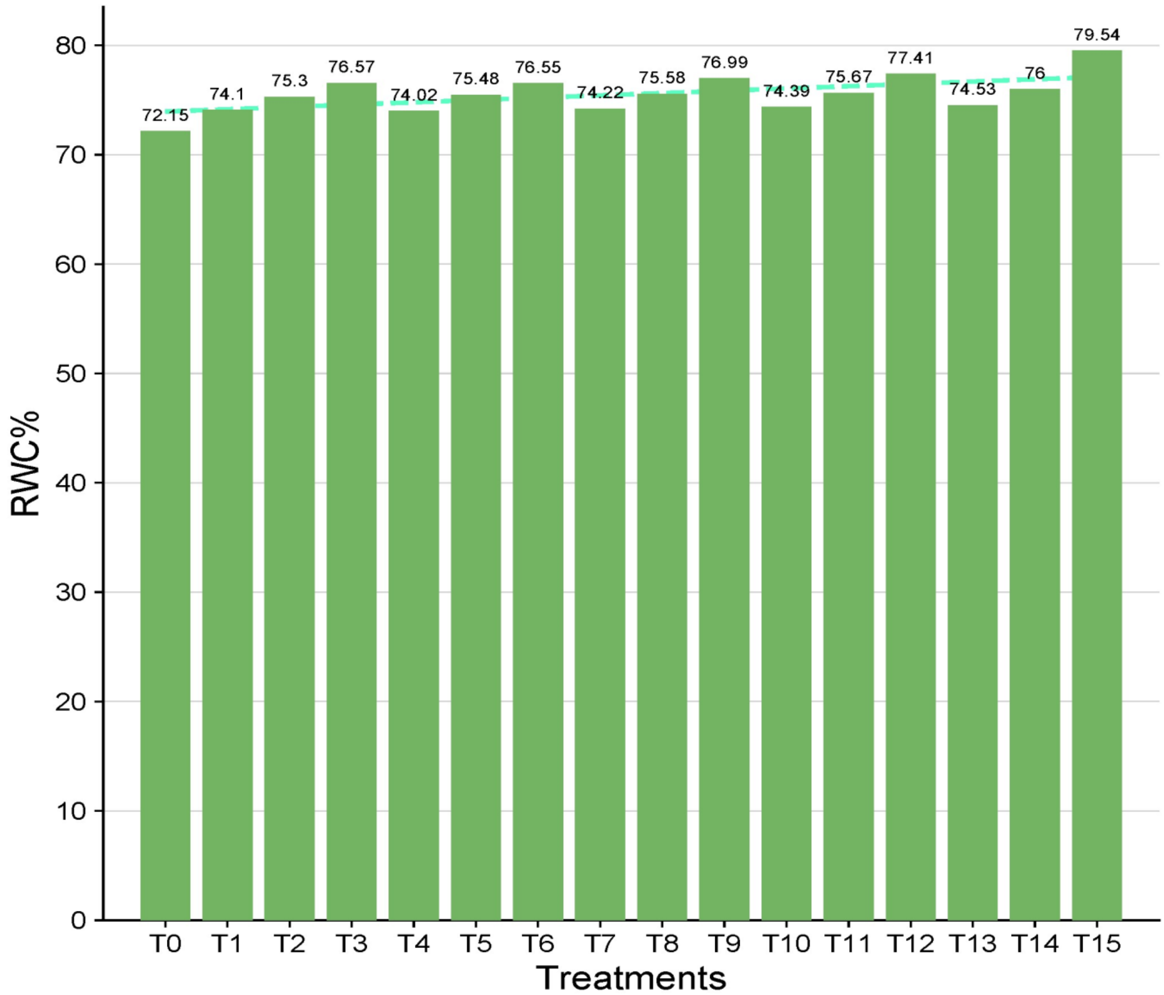

Fig. 1. Aggregate evaluation of relative water content (%) in 'Goma Priyanka' Jamun Plants responding to foliar treatment with KS and HA

Among all the treatment combinations, the T_{15} treatment, which involved the application of both KS (Potassium Silicate) at a concentration of 3000 ppm and HA (Humic Acid) at 3000 ppm, exhibited the highest RWC value, recorded at 79.54%. This particular treatment stood out as it showcased a significantly higher relative humidity percentage compared to the other treatments. Conversely, the control group, which did not receive any special treatment, displayed the lowest RWC value at 72.15%. This observation marked a substantial deviation from the RWC values observed in the other treatments.

These findings suggest a direct and tangible impact of the KS and HA treatment combination on the water content within the leaves of Jamun cv. Goma Priyanka. The higher RWC in the T_{15} treatment indicates a potential positive effect of this specific combination on the water retention capacity of the leaves. This outcome is of significance as it may reflect the plant's ability to withstand water stress and maintain adequate hydration levels, which could have implications for its overall health and stress tolerance.

3.2 Total Chlorophyll Content (mg g^{-1})

Fig. 1 presents a comprehensive analysis carried out from 2021 to 2023, emphasizing the accumulation of total chlorophyll in the leaves of Jamun cv. Goma Priyanka. This analysis provides captivating insights into the physiological responses of these plants. Particularly striking were the substantial fluctuations observed in the total chlorophyll content among the different treatments during the month of March in the second year (2022-23).

Among all the treatment levels, it was the KS_3 treatment, where potassium silicate (KS) was applied at a concentration of 3000 ppm, that stood out. In this treatment, the leaves exhibited the highest total chlorophyll content, reaching an impressive value of 1.80 mg g^{-1}. This finding highlights a significant increase in chlorophyll accumulation, signifying the positive influence of the KS3 treatment on the photosynthetic pigment content in the leaves. It is well-established that chlorophyll is essential for photosynthesis, and an elevated chlorophyll content can contribute to enhanced photosynthetic capacity, potentially leading to improved plant growth and vitality (Selim et al*.* 2012).

Conversely, during the same period, the control group, which did not receive any specialized treatment, displayed the lowest total chlorophyll content at 1.73 mg g^{-1}. This measurement notably lagged behind the chlorophyll content observed in the other treatments. This observation suggests that the absence of treatment had a limiting effect on chlorophyll accumulation in the leaves.

The variations in chlorophyll content among these treatments (Fig. 6) have profound implications for the plant's ability to harness light energy for photosynthesis. The elevated chlorophyll content in the KS_3 treatment implies a potential enhancement in photosynthetic efficiency, which, in turn, can influence overall plant productivity.

3.3 Proline Content (μmoles g^{-1})

In Fig. 5, it is evident that the overall proline content in Jamun cv. Goma Priyanka leaves were significantly influenced by the various

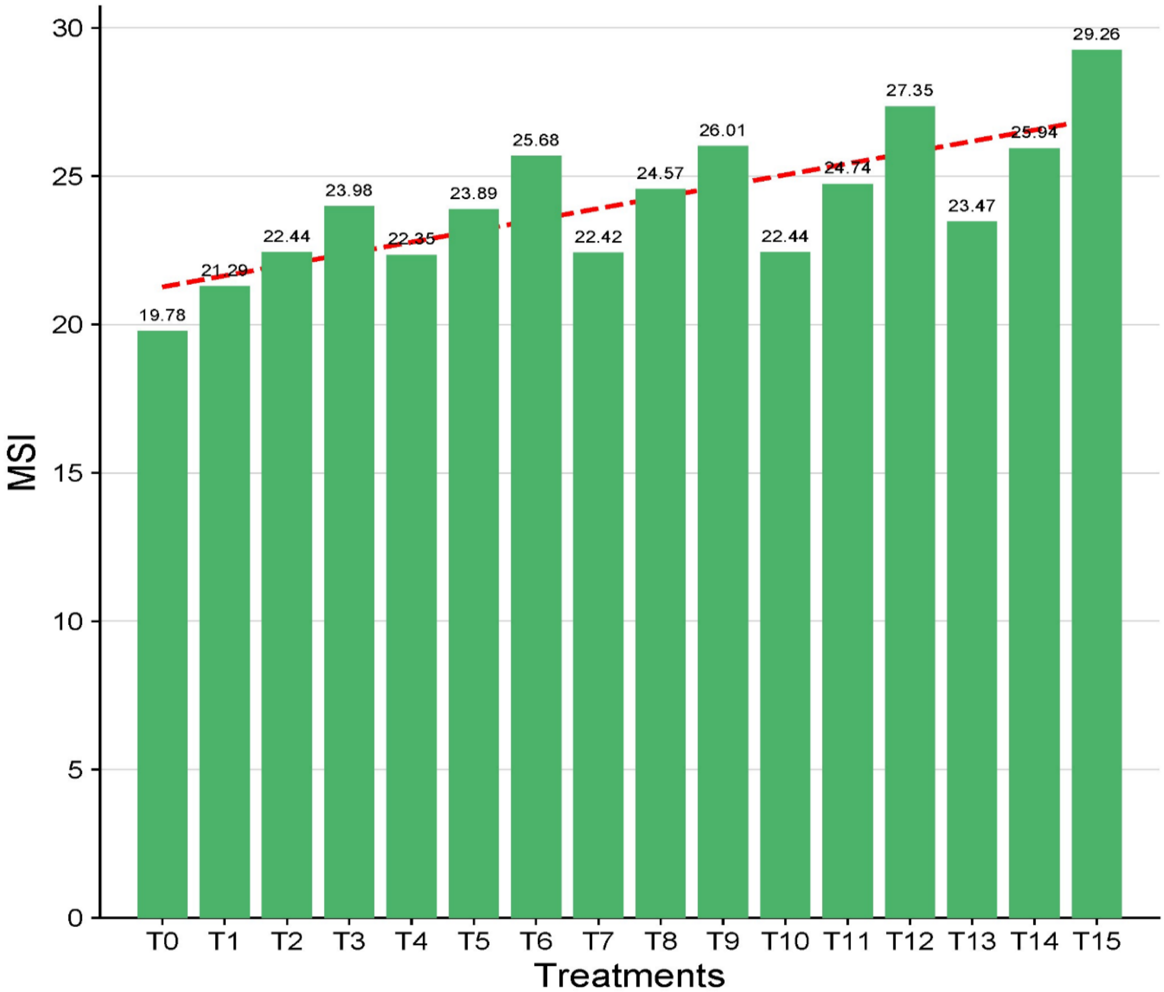

Fig. 2. Combined analysis representing Membrane Stability Index of Jamun cv. Goma Priyanka leaves in response to foliar application of KS and HA (March 2022-23)

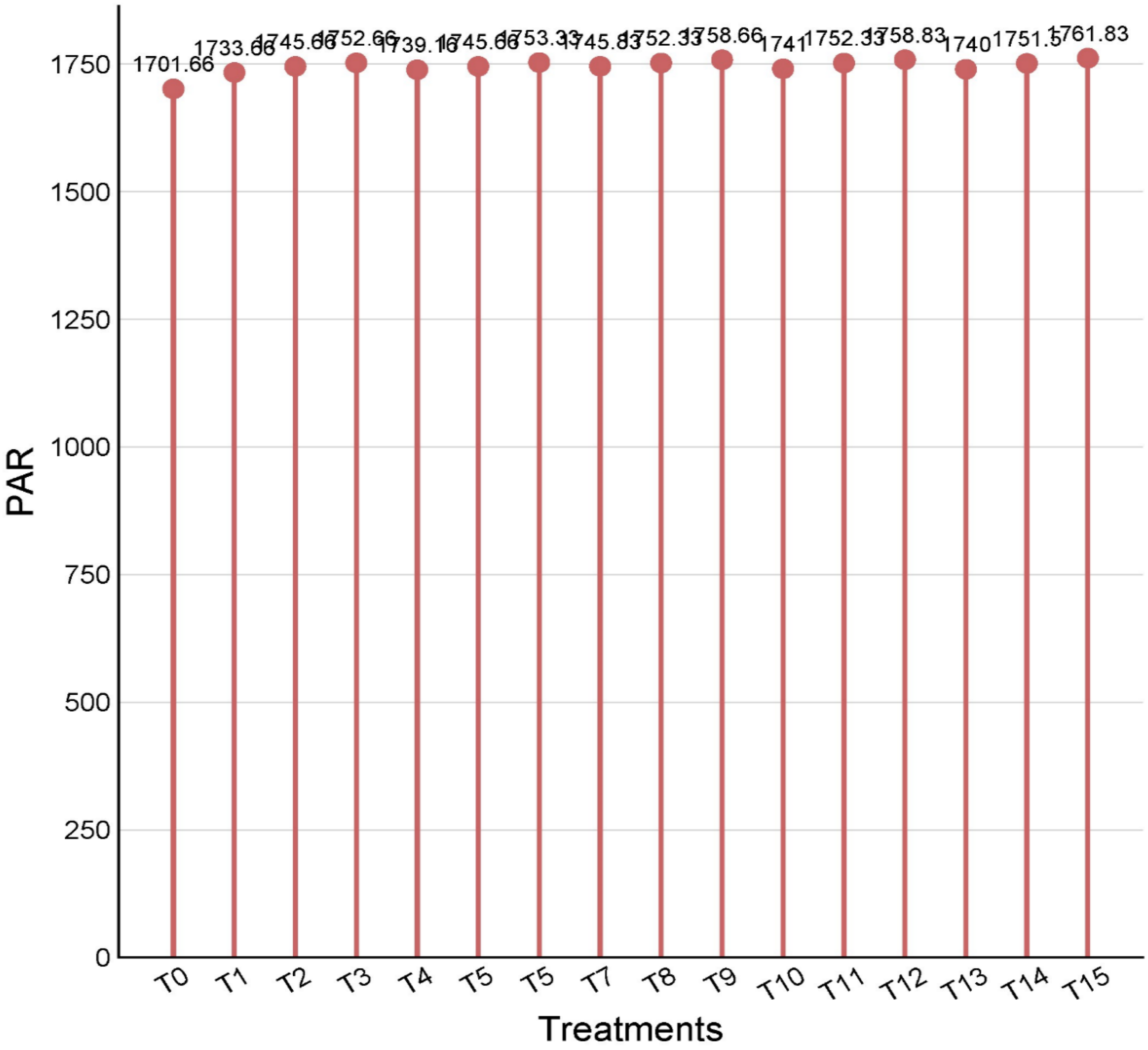

Fig. 3. Combined analysis representing Photosynthetic Active Radiation (PAR) of Jamun cv. Goma Priyanka leaves in response to foliar application of KS and HA (March 2022-23)

interaction treatments. The collective findings, spanning the entire experimental duration from 2021 to 2023, revealed intriguing patterns, particularly during March in the second year (2022-23).

During this critical period, the T_{15} treatment, which involved the simultaneous application of KS (Potassium Silicate) at 3000 ppm and HA (Humic Acid) at 3000 ppm, exhibited the lowest proline content, recorded at 39.47 µ moles g-1. Remarkably, this treatment displayed a substantial reduction in proline content compared to the other treatments. This outcome is particularly noteworthy as it suggests that the combination of KS and HA had a mitigating effect on proline synthesis in response to moisture stress conditions.

Conversely, the control group, which did not receive any specialized treatment, displayed the highest proline content, measured at 49.41 µ moles g^{-1}. This observation indicated a significant increase in proline content compared to the other treatments. Proline is known to be synthesized in plant leaves as a response to reactive oxygen species when plants experience moisture stress. It serves a crucial role as an amino acid that helps maintain osmoregulation and stabilizes the structure of plant cells.

The results of our investigation provide valuable insights into how the interaction between potassium silicate and humic acid influenced proline content. Specifically, this interaction led to a decrease in proline content compared to the control group. This finding suggests that the application of KS and HA may have a mitigating effect on moisture-induced stress in Jamun cv. Goma Priyanka plants, potentially enhancing their resilience under adverse environmental conditions.

3.4 Membrane Stability Index (%)

Fig. 6 provides clear evidence that the membrane stability index (MSI) was significantly impacted by the various interaction treatments, as our findings distinctly demonstrate. The comprehensive dataset, spanning the entirety of our experimental timeline from 2021 to 2023, has revealed captivating trends, with a particular emphasis on the observations made during the second year in March (2022-23).

During this crucial period, the T_{15} treatment group, which involved the concurrent application of KS (Potassium Silicate) at 3000 ppm and HA (Humic Acid) at 3000 ppm, displayed the highest recorded value for the membrane stability index, reaching an impressive 29.26. This outcome underscores the remarkable effectiveness of the T_{15} treatment in enhancing membrane stability when compared to the other treatments. It indicates that the combination of KS and HA had a substantial positive impact on the stability of cell membranes in the leaves of Jamun cv. Goma Priyanka.

Conversely, the control group, representing plants that did not receive any specialized treatment, exhibited the lowest membrane stability index value at 19.78. This value was comparable to that of the T_1 treatment, where KS was applied at a concentration of 1000 ppm. However, it's important to note that the T_0 (control) treatment demonstrated a significantly lower membrane stability index compared to the other treatment groups.

The membrane stability index is a crucial physiological parameter that reflects the ability of plant cell membranes to withstand stress and maintain their integrity. The observed variations in MSI among the treatments suggest that the application of potassium silicate and humic acid, especially in the T_{15} treatment, can enhance the resilience of cell membranes in Jamun cv. Goma Priyanka leaves. This improvement in membrane stability is indicative of a potential increase in the plant's ability to cope with environmental stressors, ultimately contributing to its overall health and vitality (Morozesk et al., 2020).

3.5 Photosynthetic Active Radiation (PAR) (mmol $m^{-2}s^{-1}$)

The figure illustrates significant findings derived from experiments conducted spanning the years 2021 to 2023. Particularly, these findings pertain to the period of March 2022-23, the highest recorded value of Photosynthetic Active Radiation (PAR) at 1761.83 m molm^{-2}s^{-1} in leaves of the Jamun cv. Goma Priyanka was observed in the T_{15} treatment group, characterized by the application of a combination of potassium sulphate at 3000 ppm (KS3000 ppm) and humic acid at 3000 ppm (HA3000 ppm). Interestingly, statistical analysis showed that the T_{15} treatment was comparable to the T_{14} treatment. However, T_{15} treatment demonstrated a significantly higher and superior PAR compared to all other treatment groups. Conversely, the control group exhibited the lowest PAR value at 1701.66 m molm^{-2}s^{-1}, signifying a diminished absorption of active radiation in Jamun cv. Goma Priyanka leaves when compared to the other treatment groups.

These findings underscore the pivotal role of Photosynthetic Active Radiation (PAR) in influencing the growth and development of plants. PAR denotes the specific portion of the electromagnetic spectrum that plants utilize for the process of photosynthesis. The elevated PAR levels observed in the T_{15} treatment can be attributed to the synergistic effect of enhanced potassium levels in Jamun leaves, coupled with the presence of increased potassium in conjunction with humic acid. This combination appeared to promote greater chlorophyll synthesis, subsequently enhancing the photosynthetic rate in the treated plants. Additionally, it is plausible that the cooperative action of potassium silicate and humic acid induced structural and metabolic modifications in the Jamun plants (Nada, 2020; Trevisan et al., 2010). These modifications potentially encompassed increased leaf thickness, a higher membrane stability index, and augmented enzymatic activity. These alterations likely contributed to improved PAR absorption and utilization in the treated plants.

3.6 Photosynthetic Rate

In the context of this study, the carboxylation parameter, particularly the photosynthetic rate, demonstrated significant fluctuations in reaction to the various treatment combinations investigated, as illustrated in Fig. 6.

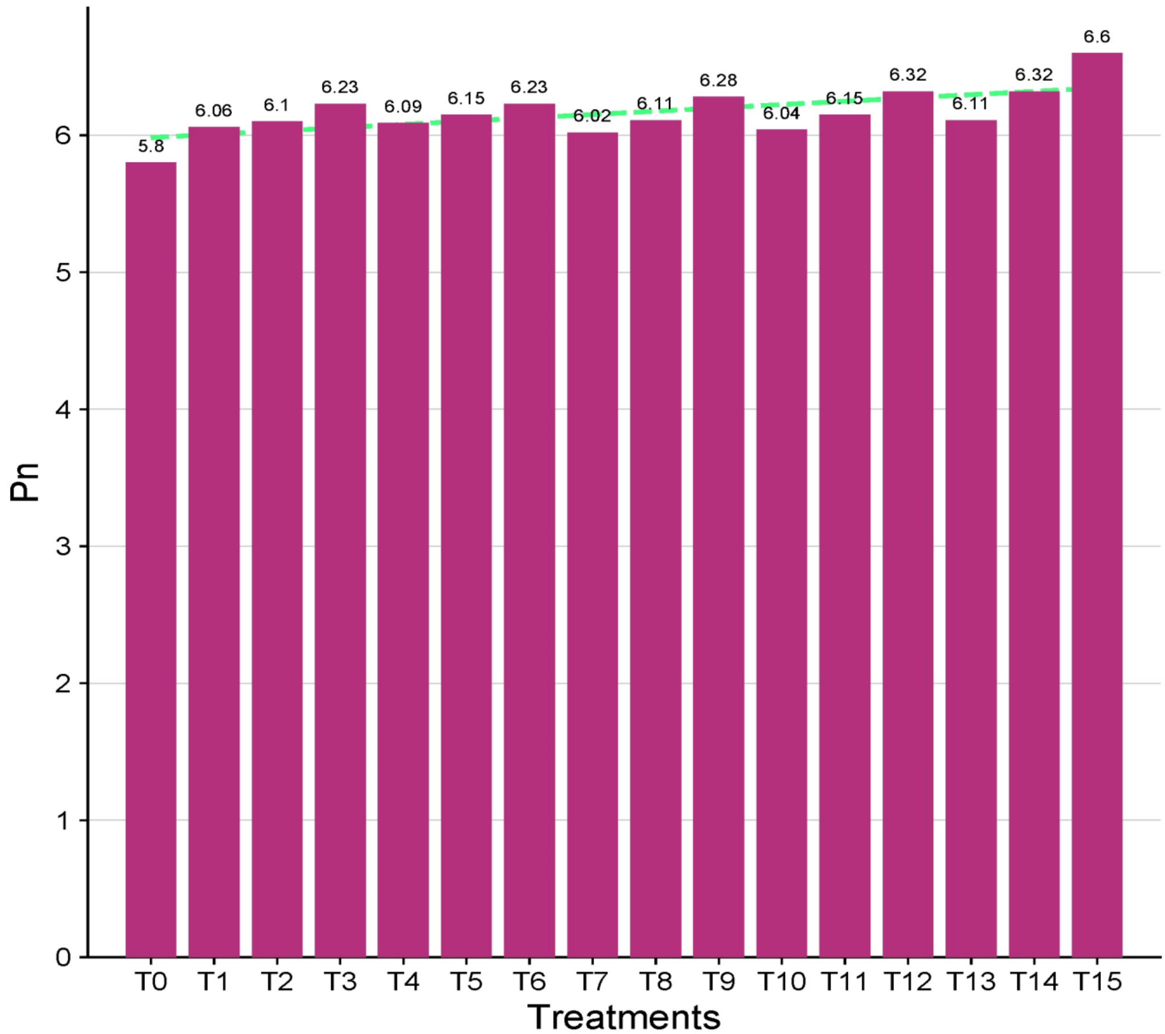

Fig. 4. Aggregate evaluation depicting the photosynthetic rate (Pn) of Jamun cv. Goma Priyanka Plants Following Foliar Application of KS and HA (2022-23)

Over the extensive experimental period spanning from 2021 to 2023, a particularly intriguing revelation emerged during March 2022-23. This period unveiled a remarkable observation: the leaves of Jamun's cv. Goma Priyanka displayed the highest recorded photosynthetic rate, registering at 5.71 µ mol m^{-2} s^{-1}, within the T_{15} treatment group. The T_{15} treatment was characterized by the application of a combined regimen involving potassium sulphate at 3000 ppm (KS 3000 ppm) and humic acid at 3000 ppm (HA 3000 ppm). This discovery carries significant physiological implications.

What sets this observation apart is the subsequent statistical analysis, which not only established the distinctiveness of the T_{15} treatment group compared to others but also demonstrated its superiority in terms of photosynthetic rate. This suggests that the specific synergy between potassium sulphate and humic acid within the T_{15} treatment exerted a pronounced and beneficial influence on the photosynthetic rate in the leaves of Jamun cv. Goma Priyanka.

Conversely, the control group exhibited the lowest recorded photosynthetic rate, measuring at 5.32 µ mol m^{-2} s^{-1}. This finding accentuates a substantial decline in photosynthetic activity when juxtaposed with the remaining treatment groups. The reduced photosynthetic rate in the control group can be attributed to the absence of the beneficial elements present in the T_{15} treatment, thus highlighting the vital physiological role played by potassium and humic acid in stimulating photosynthesis and overall plant well-being. This underscores the potential for optimizing plant productivity through the careful application of these elements in agricultural practices.

3.7 Stomatal Conductance (mmol $m^{-2}s^{-1}$)

In the realm of plant physiology, the parameter of gaseous exchange, specifically stomatal conductance, exhibited discernible responses to the various interaction treatments applied as illustrated in Fig. 6. The comprehensive findings, covering the entire experimental duration from 2021 to 2023, unveiled a noteworthy observation during March 2022-23.

Precisely, it was observed that the leaves of Jamun cv. Goma Priyanka displayed the highest recorded stomatal conductance value at 6.60 m mol m^{-2} s^{-1} within the T_{15} treatment group. The T_{15} treatment involved the application of a combination of potassium sulphate at 3000 ppm (KS3000 ppm) and humic acid at 3000 ppm (HA3000 ppm). Notably, the statistical analysis highlighted the significant superiority of the T_{15} treatment over all other treatment groups concerning stomatal conductance.

Conversely, the lowest stomatal conductance, measuring 5.80 m mol m^{-2} s^{-1}, was observed in the control group. This value was on par with the T_1 treatment group (KS1000 ppm). The T_0 treatment (Control) demonstrated a notable decrease in stomatal conductance when compared to the other treatments. This variance in stomatal conductance among the treatment groups underscores the impact of these treatments on the regulation of gaseous exchange in Jamun cv. Goma Priyanka leaves.

This physiological insight highlights the significance of potassium sulphate and humic acid, particularly in the T_{15} treatment, in influencing stomatal conductance. Stomatal conductance plays a crucial role in plant water

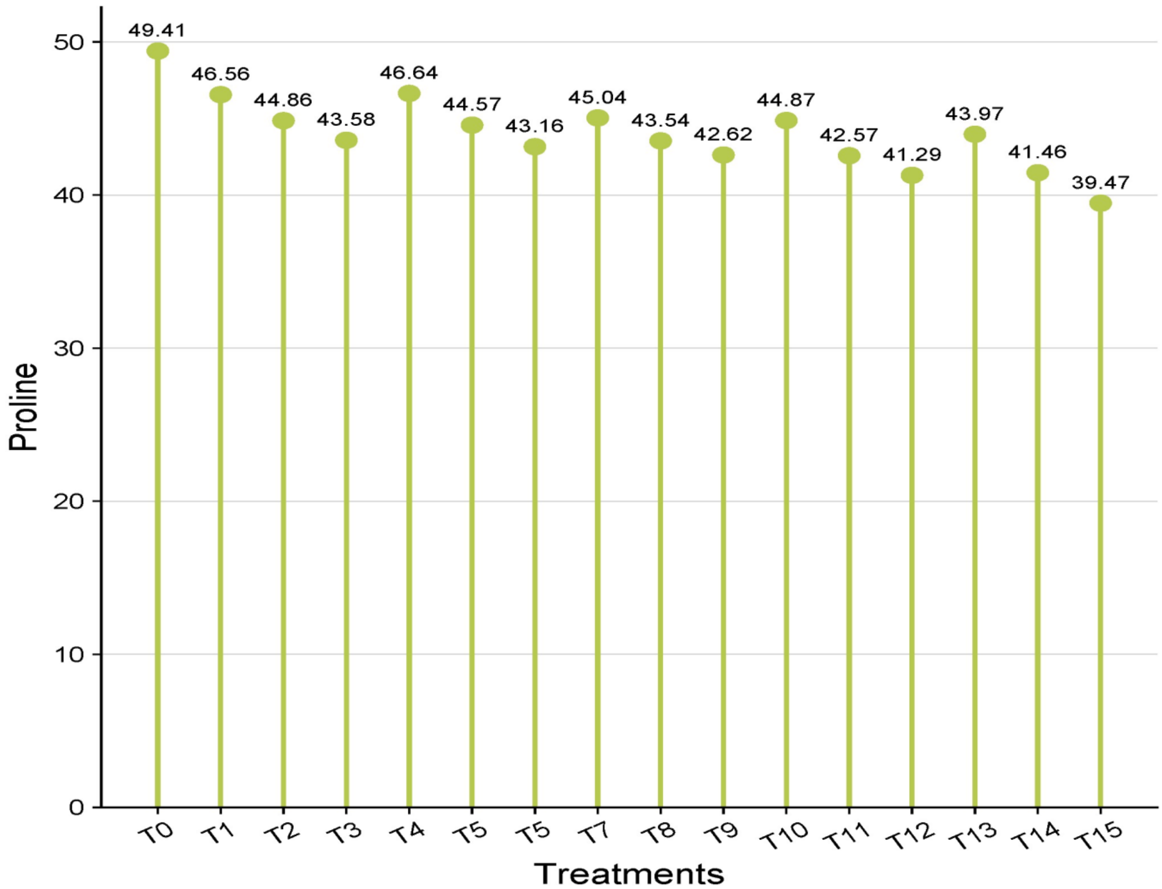

Fig. 5. Combined assessment of proline levels (μ moles g^{-1}) in Jamun Cultivar 'Goma Priyanka' Plants following Foliar Application of Potassium Silicate (KS) and Humic Acid (HA)

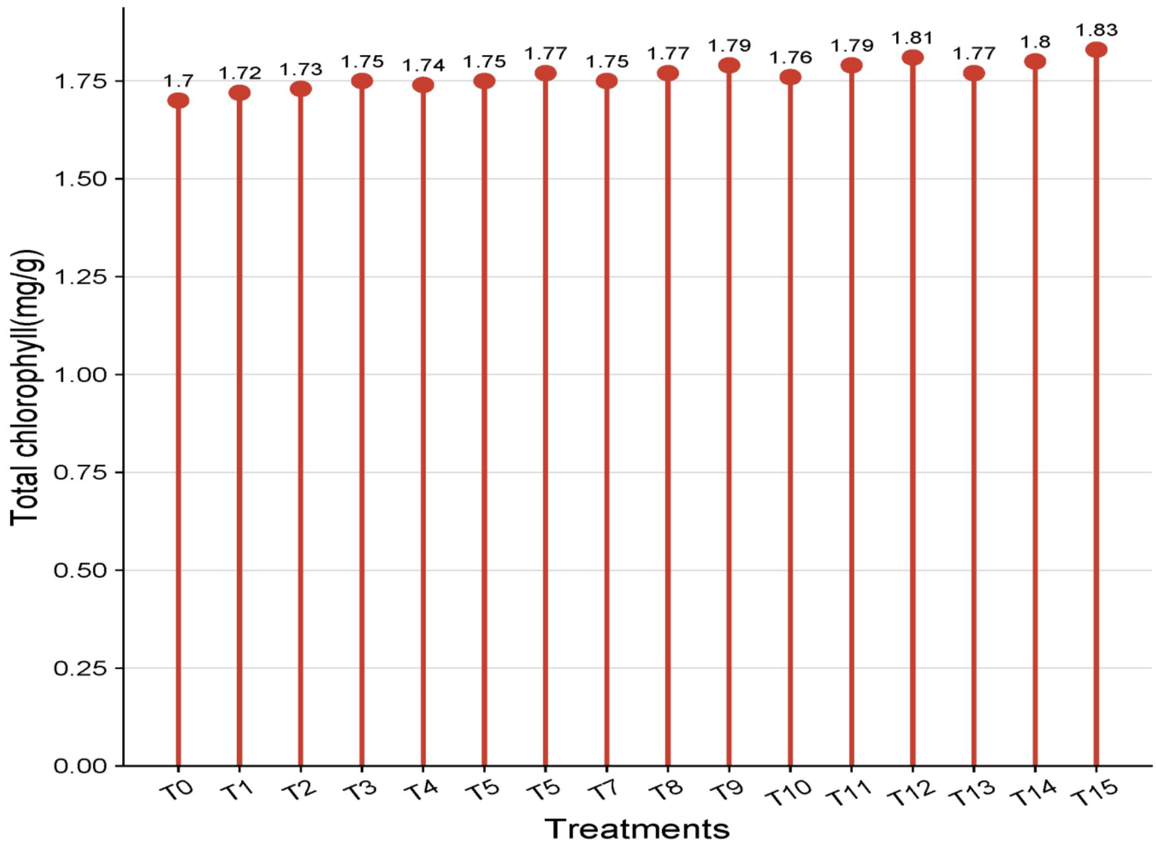

Fig. 6. Aggregate outcome of total chlorophyll content in Jamun cv. Goma Priyanka leaves in response to foliar application of Potassium silicate (KS) and Humic acid (HA)

and gas exchange processes, influencing overall plant health and productivity. Therefore, these findings underscore the potential benefits of these treatments in optimizing plant physiological processes and enhancing agricultural outcomes.

3.8 Relative Humidity in Leaves (%)

The relative humidity levels within the leaves of Jamun cv. Goma Priyanka exhibited significant variations due to the diverse interaction treatments, as depicted in Fig. 6. The comprehensive results, spanning the entire experimental timeframe from 2021 to 2023, revealed a remarkable observation during March 2022-23.

Specifically, it was observed that the highest recorded relative humidity level, reaching 2.75%, in Jamun cv. Goma Priyanka leaves were documented in the T_{15} treatment group, which involved the simultaneous application of potassium sulphate at 3000 ppm (KS3000ppm) and humic acid at 3000 ppm (HA3000 ppm). Notably, the statistical analysis highlighted that the T_{15} treatment exhibited a significantly higher relative humidity percentage compared to all other treatment groups.

Conversely, the lowest relative humidity level, measuring 2.32%, was observed in the control group. This value was markedly lower when compared to the relative humidity levels observed in the other treatment groups. Relative humidity percentage in Jamun plants serves as a pivotal indicator, reflecting the rate of gaseous diffusion, stomatal conductance, and ultimately, the efficiency of carboxylation in maintaining the plant's physiological processes under various stress conditions.

The results of this study underline the significance of the combined application of potassium silicate and humic acid, particularly at higher doses within the T_{15} treatment, in enhancing the overall relative humidity percentage within the leaves of Jamun cv. Goma Priyanka. This augmentation in relative humidity aligns with the prevailing environmental conditions in the Jhalawar district, demonstrating the potential benefits of these treatments in supporting the physiological well-being of Jamun plants. This finding has implications for optimizing plant responses to environmental stresses and promoting their overall health and productivity.

4. CONCLUSION

The interactive treatments significantly impacted Jamun's cv. Goma Priyanka leaf water content (RWC%) from 2021 to 2023, with a notable increase in RWC to 79.54% in the T_{15} treatment (KS 3000 ppm + HA 3000 ppm) compared to the control group's 72.15%. Fluctuations in chlorophyll content during March 2022-23 revealed the T_{15} treatment (KS 3000 ppm) and (HA 3000ppm) with the highest chlorophyll at 1.80 mg g^{-1}, potentially enhancing photosynthesis, while the control group had the lowest chlorophyll at 1.73 mg g^{-1}. Proline content was reduced to 39.47 µmoles g^{-1} in the T_{15} treatment, indicating moisture stress mitigation, whereas the control group showed higher proline content at 49.41 µmoles g^{-1}, suggesting stress response. The T_{15} treatment exhibited the highest membrane stability index (MSI) at 29.26, enhancing cell membrane resilience, while the control group had the lowest MSI at 19.78. Photosynthetic active radiation (PAR) in the T_{15} treatment reached 1761.83 mmol $m^{-2}s^{-1}$, promoting photosynthesis, whereas the control group had the lowest PAR at 1701.66 mmol $m^{-2}s^{-1}$. Stomatal conductance was most effective in the T_{15} treatment at 6.60 mmol $m^{-2}s^{-1}$, while the control group had the lowest conductance at 5.80 mmol $m^{-2}s^{-1}$, emphasizing treatment impacts on gaseous exchange and overall plant health. These findings collectively suggest that the T_{15} treatment, involving potassium sulphate (KS) and humic acid (HA), significantly improved water content, chlorophyll accumulation, stress resilience, membrane stability, photosynthesis, and stomatal conductance in Jamun cv. Goma Priyanka plants, highlighting its potential for enhancing plant growth and health.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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