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RESEARCH ARTICLE

Enhancing leaf nutrient status and stress tolerance in jackfruit plants (*Artocarpus heterophyllus* L. cv. Chandra) through the application of a thiourea plant bioregulator

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Abstract

Given the shifting climate, jackfruit is among the best minor fruit crops that can withstand drought. The effect of thiourea on leaf nutrient status and stress tolerance in jackfruit plants was investigated in a present experiment. The research comprised 9 different treatments of thiourea (0.25 %, 0.50 %, 0.75 %, 1.00 %, 1.25 %, 1.50 %, 1.75 % and 2.00 %) set out in randomized block design (RBD) with three replications. The foliar application of thiourea had a significant effect on the stress parameters. Among the different treatments, treatment T_8 was recorded to be significantly superior to the other treatments. The minimum proline content (0.339 µmol. g^{1}) was found in treatment T_8 (Thiourea 2.00 %) and other parameters such as stomatal conductance (7.58 mmol m^{-2} s^{-1}), relative water content (RWC) (82.48 %), chlorophyll content (2.23 mg g^{-1}), N content (1.59 %), P content (0.279 %), K content (0.572 %) and S content (0.218 %) were greatest in treatment T_8 (Thiourea 2.00 %) at the time of final observation in March. Overall T_8 (Thiourea 2.00 %) was superior to the other treatments for improving the growth and development parameters of jackfruit plants and in improving stress parameters.

Keywords: bioregulator; jackfruit (*Artocarpus heterophyllus* L.); proline; stomatal conductance; thiourea

Introduction

Jackfruit (*Artocarpus heterophyllus* L.) is an important fruit tree in tropical and subtropical regions, especially in Southeast Asia (1, 2). The basic chromosome number is 14. It is native to the Western Ghats of India. It is viewed as a "poor man's fruit" in the eastern and southern parts of India (3).

It is an evergreen tree with male and female inflorescence appearing separately on the same plant. This species has a special kind of bearing habit known as a cauliflorous bearing, where the female flowers are borne on the trunk of the tree (4). In eastern India, tender fruit is popular as a vegetable. The rind is rich in protein and the principle protein has been extracted as "jacalin." (5, 6). The fruit of the jackfruit tree is the largest eatable fruit in the world weighing about 20 kg and larger fruits of about 50 kg (7, 8). It consists of bulbs, seeds, tasty golden flesh and a rind (9). The maximum length of the fruit is 90 cm and its weight varies from 2 kg to 36 kg. 15-20 % of the fruit's weight is made up of meat, which can be consumed raw or prepared. 8 to 15 % of the weight of a single fruit is made up of its seeds, which typically number between 100 and 500. Typically measuring 2-4 cm in length and 1-2 cm in diameter, the seeds are edible despite their poor digestion in human digestive systems (10, 11). According to 3rd advance estimate of the Ministry of Agriculture and Farmers Welfare (2021-22), the total area of jackfruit cultivation in India is 1.88 lakh hectares and its production is 19.46 lakh metric tonnes (12). Jackfruit is considered as a species that is important for research because of its wider potential for use in nutrition and the enhancement of people's income when grown in agroforestry and home garden systems. It has proven valuable when introduced to other parts of the world where it is widely cultivated in suitable climates (13). A study found that mature jackfruit contains more vitamins and minerals than apples, bananas, avocados and apricots (14). Jackfruit has a low calorie level; 100 g of jackfruit only has 94 calories. (15). In tropical and subtropical areas, jackfruit has emerged as the most popular and rapidly growing fruit tree due to its low labor costs and environmental requirements (16). Thiourea, also called as thiocarbamide, is a nitrogen and sulphur containing compound. Thiourea includes 42.1 % sulphur and 36.8 % nitrogen. The plant leaves absorb about half of the foliar thiourea, with the remaining portion remaining on the ground (17). Many crops grew more when thiourea was applied exogenously because it contained biologically significant functional groups (18). Currently, with the increasing dryness in the atmosphere, the normal growth and progress of the plant are strongly affected. Minor crops are becoming more important since climate change is negatively harming major crops ability for production. Because they have a comparable amount of nutritional value as major crops, these minor crops can be cultivated alongside major crops to meet the growing demand for nutrition security. Given the shifting climate, jackfruit is among the best minor fruit crops. A crop that can withstand drought. It has several uses, including in the timber industry, which creates job opportunities. Many studies discover that growing plants in their early stages might be challenging in Rajasthan's harsh climate.

Thiourea has been reported to be beneficial for improving the stress resistance in plants and subsequent growth and development. Hence, its impact on the growth and development of jackfruit (*Artocarpus heterophyllus* L.) cv. Chandra has been investigated.

Materials and Methods

The College of Horticulture and Forestry Jhalawar in southeastern India (latitude 23° N 24° N and longitude 75° E 76° E) conducted a field experiment on the jackfruit cultivar “Chandra” for the 2022-2023 year. Their soil was black cotton (vertisols) with a pH between 6.5 to 7.35, 0.55 % organic carbon and nitrogen, phosphorus and potassium accessible at 350.55, 46.51 and 322.34 kg/ha respectively. During the summer months their temperature is typically between 43 and 48 °C and during winter it ranges from 1 to 2.6 °C. On average the region receives 954.7 mm of precipitation annually (Fig. 1). Paradoxically, the rains are heavy during the rainy season, which actually starts in July and August and continues until the first week of September. High humidity levels reaching up to 98 % characterize the entire month of July and August in the rainy season. The high humidity level occurs throughout the year in the region of Jhalawar, except for the months of April, May and June.

Plant material

The experimental orchard for the jackfruit cultivar "Chandra" was established using an 8 x 8 m planting pattern through a square planting strategy. Plants of the jackfruit cultivar (cv.) "Chandra" were selected from the Fruit Research Farm at the College of Horticulture and Forestry, Jhalrapatan, Jhalawar, India. The plants were one year old and had uniform size, growth and vigor. Of the recently established jackfruit, a total of 27 plants were selected for the study.

Doses and time of application of thiourea

Thiourea (treatments) was used at rates of T_0-0.25 %, T_1-0.50 %, T_3-0.75 %, T_4-1.00 %, T_5-1.25 %, T_6-1.50 %, T_7-1.75 % and T_8-2.00 %. The treatments were applied in the form of foliar spray. Foliar spray applied during the 2nd week of June, August and October (2022) after the initial (base) growth and development parameters of the plants were recorded.

Method analysis

Stress tolerance-related parameters

Proline content (μg g^{-1}): The proline content was calculated (19). 5 g of leaves were homogenized in 10 mL of 3 % sulphosalicylic acid for measurement purposes. Whatman No. 2 filter paper was used to filter the homogenate. Then, 2 mL of the supernatant was combined with 2 mL of acid ninhydrin (which contained 1.25 gm of ninhydrin, 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid). The mixture was cooled after an hour in a hot water bath. The blend was then mixed with 4 mL of toluene. After separating the toluene layer from the brilliant red colour (chromophore layer), a peak at 520 nm was obtained. Proline was utilized to create a standard graph with a range of 0-80 μg.

The amount of proline was calculated by using the following formula:

$$\text{Proline content (μ mole/g tissue} = \frac{\mu g \frac{\text{proline}}{\text{mL}} \times \text{mL toluene} \times 5}{115.5 \times \text{g sample}}$$

Chlorophyll content (mg/g): The chlorophyll content was assessed (20). The following are the specifics of the method used: mature leaves that had been neatly chopped weighed 1 g and 20 mL of 80 % acetone were used to smash them in a sanitized mortar. After centrifuging the sample for 5 min at 5000 rpm, the supernatant was poured into a 100 mL volumetric flask. The centrifuging procedure was repeated until a colourless residue appeared. To obtain the clear leaf extract, the mortar and pestle were methodically washed with 80 % acetone. Then, 80 % acetone was used to bring the volume to 100 mL. The volume of chlorophyll present in the extract was premeditated using the following equation:

$$\text{Total chlorophyll (mg/g tissue)} = 20.2\,(A645) + 8.02\,(A663) \times V/1000 \times W$$

where,

A = precise wavelength absorbance

V = extraction of the final volume of chlorophyll in 80 % acetone

W = extracted tissue fresh weight

Stomatal conductance (mmol m^{-2}s^{-1}): The stomatal conductance, measured as the amount of gas diffusion, was obtained in the early morning hours (8.00-11.00) am by using the CIRAS portable photosynthesis system (USA) in the month of March-2023, which was the end of the experiment.

Relative water content (RWC) (%): The relative water content was measured (21). The fresh weight was obtained by weighing leaf pieces from the stressed plants. The leaf material was placed in Petri dishes with water immediately after it had been weighed. To obtain the turgid weight, the leaf material was surface blotted and weighed after 12 hrs. After oven drying at 80 °C, the dry weight of the leaf units was measured. The RWC was calculated using the following formula:

$$\text{RWC \%} = \frac{\text{Initial weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Leaf nutrient analysis:

Nitrogen (%): Leaves positioned 7 or 8 from the apex of terminal growth were selected for nutritional analysis. Since the nutrient concentrations of the young leaves are unstable due to their stage of ongoing growth, old and mature leaves were chosen. The leaf's midrib and other leftover parts were thrown away, but the leaf's central half was collected (22). The nitrogen content in the leaves was determined by Kjeldahl and calorimetric (23).

Phosphorus (%): The phosphorus content in leaves was estimated from Tri-acid extract by ammonium vandate and ammonium molybdate in HNO_3 medium resulted in a yellow colour and the absorbance at 470 nm was recorded with a spectrophotometer (24).

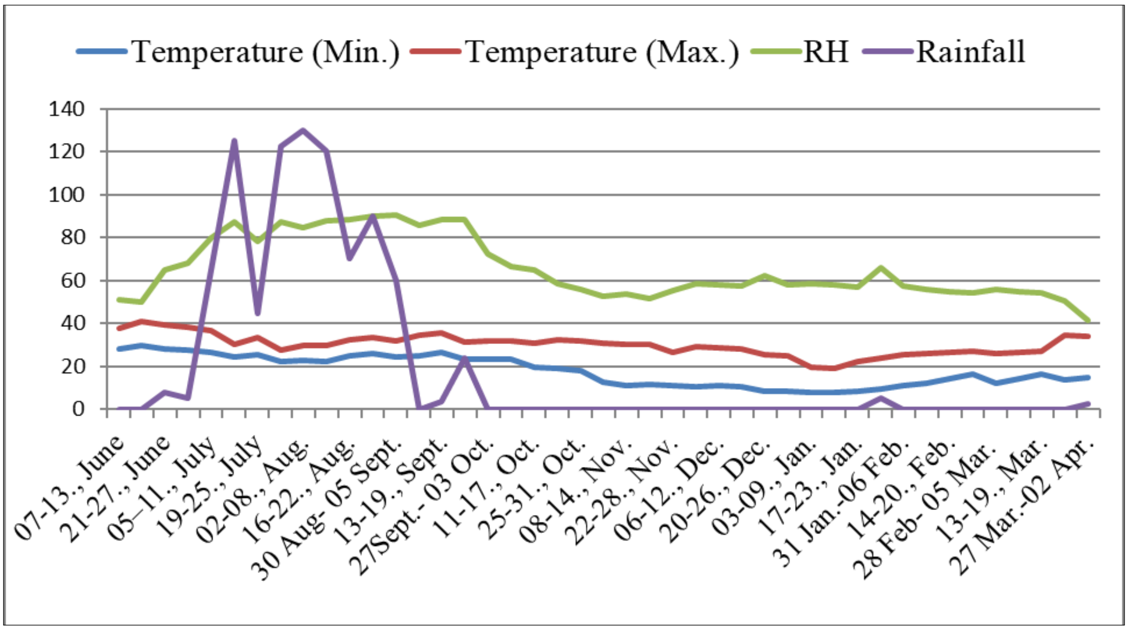

Fig. 1. Mean 7 days meteorological data recorded for the period of experimentation (from June 2022 to March 2023) Jhalawar, India

Potassium (%): The potassium content in the leaves was estimated from Tri-acid extract by using a flame photometer (24).

Sulphur (%): Total sulphur was calculated from diacid extract by the turbidimetric method by using a spectrophotometer (at a wavelength of 420 nm) (25).

Statistical analysis

The analysis of variance approach method was used to examine the experiment's data for the jackfruit cultivar "Chandra" in the year 2023 (26). The significance of the treatments was evaluated at the 5 % level of significance using the F test. The crucial difference (CD) was calculated to assess the importance of the variations among the various treatments combinations.

Results and Discussion

Effects of thiourea plant bioregulators on the leaf nutrient parameters of jackfruit plants

The data with respect to the effect of thiourea on N content (%), P content (%), K content (%) and S content (%) in leaves are presented in Table 1.

For the different treatments of thiourea at different levels, the maximum N content (%) (1.59 %) was recorded in T_8 (Thiourea 2.00 %) and this treatment was found at par with T_7 (Thiourea 1.75 %) (1.55 %). The minimum N content (%) (1.36 %) was observed in T_0 (Control) at the time of final observation of the experiment during March.

Similarly maximum increase in P content was recorded in treatment T_8(Thiourea 2.00 %) (0.279 %) and was found at par with T_7 (Thiourea 1.75 %) (0.266 %). The minimum P content (%) (0.221 %) was observed in T_0 (Control) at the time of final observation of the experiment during March.

For the different treatments of thiourea, treatment T_8 (Thiourea 2.00 %) (0.572 %) recorded maximum increase in K content and was found at par with T_7 (Thiourea 1.75 %) (0.561 %) and treatment T_6 (Thiourea 1.50 %) (0.553 %). The minimum K content (%) (0.529 %) was observed in T_0 (Control) at the time of final observation of the experiment during March.

The maximum S content (%) (0.218 %) was recorded in T_8 (Thiourea 2.00 %) and the T_8 treatment was found at par with T_7 (Thiourea 1.75 %) (0.199 %). The minimum S content (%) (0.156 %) was observed in T_0 (Control).

The application of thiourea resulted in a notable increase in the P content. This may perhaps be explained by the fact that a greater N_2 content makes the plant alkaline and an alkaline environment encourages the activity of some enzymes that break down the organic P in the soil, increasing the amount of available P. As a result, P is taken up by the roots and subsequently taken up by the leaves (27). Similarly, thiourea increased the K content of the leaves. This may possibly be the consequence of improved assimilate transport, which increases the K content in leaves (28, 29).

Effect of a thiourea plant bioregulator on stress tolerance in jackfruit plants

The data presented in the Table 2. represent the impact of different concentrations of thiourea on stress parameters.

The minimum proline content (0.339 µmol/g) was recorded in treatment T_8 (Thiourea 2.00 %). T_8 (Thiourea 2 %) treatment was found significantly superior over other treatments except treatment T_7 (Thiourea 1.75 %) (0.343 µmol/g). Maximum increase at the time of final observation during March in proline content (0.385 µmol/g) was noted in T_0 (Control) treatment.

There was significant difference among the various treatments in respect to chlorophyll content. It was observed that the maximum chlorophyll content (2.23 mg/g) was there in T_8(Thiourea 2.00 %) treatment. This treatment was found at par with treatment T_7 (Thiourea 1.75 %) (2.15 mg/g) and T_6 (Thiourea 1.50 %) (2.11mg/g). The minimum increase at the time of final observation during March in chlorophyll content (1.91 mg/g) was noted in T_0 (Control) treatment.

The data revealed that there was significant difference among the various treatments in respect to stomatal conductance. It was noted that the maximum stomatal conductance (7.58 mmol m^{-2} s^{-1}) was recorded in T_8(Thiourea 2. %) treatment at the termination of the experiment during March and was found at par with treatment T_7 (Thiourea 1.75 %) (7.37 mmol

m^{-2} s^{-1}). The minimum stomatal conductance (6.80 mmol m^{-2} s^{-1}) at the time of final observation during March was noted in T_0 (Control) treatment.

The data revealed that there was significant difference among the various treatments in respect to relative water content. It was seen that the maximum RWC (82.48 %) was recorded in T_8 (Thiourea 2.00 %) treatment at the termination of the experiment during March and was found at par with treatment T_7 (Thiourea 1.75 %) (79.61 %). The minimum increase (71.42 %) at the time of final observation during March in the relative water content was noted in T_0 (Control) treatment.

The plants grew more quickly and had higher levels of chlorophyll and RWC when exogenous bioregulators were applied during the growth phase. This allowed the plants to use water more effectively (30, 31). Thus, after thiourea was applied, the RWC in the leaves increased. When applied under stress, thiourea increased stomatal conductance, chlorophyll content and osmolyte accumulation. This may be related to an upsurge in the photosynthetic rate, which deliberates resistance in contrast to temperature abnormalities through physiological controls (32-35).

Table 1. Effect of thiourea on N, P, K and S contents (%) in jackfruit leaves

Treatments	N content	P content	K content	S content
T_0 (Control)	1.36±0.023	0.221±0.018	0.529±0.001	0.156±0.006
T_1 (Thiourea 0.25 %)	1.37±0.009	0.224±0.001	0.531±0.003	0.158±0.007
T_2 (Thiourea 0.50 %)	1.40±0.030	0.228±0.002	0.533±0.012	0.161±0.001
T_3 (Thiourea 0.75 %)	1.39±0.006	0.233±0.002	0.537±0.001	0.166±0.012
T_4 (Thiourea 1.00 %)	1.45±0.030	0.239±0.005	0.541±0.009	0.171±0.006
T_5 (Thiourea 1.25 %)	1.48±0.012	0.247±0.006	0.546±0.001	0.178±0.001
T_6 (Thiourea 1.50 %)	1.50±0.012	0.256±0.007	0.553±0.015	0.187±0.006
T_7 (Thiourea 1.75 %)	1.55±0.015	0.266±0.007	0.561±0.032	0.199±0.003
T_8 (Thiourea 2.00 %)	1.59±0.015	0.279±0.007	0.572±0.002	0.218±0.010
SEm (±)	0.02	0.003	0.009	0.003
CD (5 %)	0.05	0.025	0.022	0.026

Note: N-Nitrogen, P-Phosphorus, K-Potassium, S-Sulfur

Table 2. Effect of thiourea on the proline content (µmol. g^{-1}), chlorophyll content (mg g^{-1}), stomatal conductance (mmol $m^{-2}s^{-1}$) and RWC (%) in jackfruit leaves

Treatments	Proline content	Chlorophyll content	Stomatal Conductance	RWC
T_0 (Control)	0.520±0.006	1.91±0.036	5.25±0.095	60.88±1.735
T_1 (Thiourea 0.25 %)	0.379±0.001	1.94±0.001	7.10±0.058	74.73±0.665
T_2 (Thiourea 0.50 %)	0.370±0.007	2.02±0.050	6.98±0.027	72.95±1.666
T_3 (Thiourea 0.75 %)	0.362±0.007	1.98±0.027	7.15±0.089	75.52±1.116
T_4 (Thiourea 1.00 %)	0.355±0.007	2.06±0.047	7.20±0.125	75.56±0.387
T_5 (Thiourea 1.25 %)	0.349±0.015	2.04±0.058	7.19±0.146	73.87±0.559
T_6 (Thiourea 1.50 %)	0.345±0.015	2.11±0.036	7.20±0.036	76.95±0.525
T_7 (Thiourea 1.75 %)	0.343±0.013	2.15±0.071	7.37±0.033	79.61±0.994
T_8 (Thiourea 2.00 %)	0.339±0.009	2.23±0.046	8.55±0.164	82.48±942
SEm (±)	0.003	0.06	0.10	1.08
CD (5 %)	0.005	0.14	0.31	3.22

Conclusion

On the basis of the results achieved after the field experiment, T_8 (Thiourea 2.00 %) was found to be significantly superior to the other treatments with respect to leaf nutrient parameters; N, P, K and S. Thiourea contains greater N_2 content that made the plant alkaline which encouraged the activity of enzymes that increased the amount of available P and K content in leaves. It increased stomatal conductance, chlorophyll content and RWC due to an upsurge in the photosynthetic rate, which deliberates resistance in contrast to temperature abnormalities through physiological controls. These findings highlight the potential of thiourea as an effective strategy to enhance plant resilience to growth, leaf nutrient and stress and promote sustainable agricultural practices.

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Authors' contributions

AT experimented, collected data, performed statistical analysis and drafted whole manuscript. JS advised and supervised the full research work critically. PB conceived of the study and participated in its design and coordination. PB helped in data collection and statistical analysis. JS assisted in manuscript editing and conducted review of the article. JS reviewed the manuscript and offered critical feedback. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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