

**Visual analysis prompt – Thermal validation phase with identification of physiological disorders in Lettuce crops**

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## Description

This document establishes a series of methodological methods (Prompt Engineer and Prompt Chaining) and ranking criteria to assess thermal tolerance using biofertilizer and mineral fertilization treatments by visual analysis (a kind of low-cost phenotyping platform) using Generative AI – Large Language Models (LLMs). Future versions will expand the dataset and refine the classification algorithm.

## Abstract

The pre-print material in question deals with an initial version of Prompt Engineering and Prompt Chaining for visual analysis of deficiency symptoms and ranking of best treatments, comparing biological inputs and mineral inputs for lettuce plants subjected to heat stress. It consists of the methodology and an example of application. Future refinements will be made.

## Prompt Engineering and Prompt Chaining

### Prompt 1

Take on the persona of an Agricultural Engineer with a PhD in Plant Physiology and at least 15 years of experience investigating heat stress and physiological disorders in lettuce (*Lactuca sativa*) under tropical environments. You are specialized in evaluating plant responses to biofertilizers and mineral fertilizers, focusing on nutrient balance, thermal tolerance, and chlorophyll maintenance.

### Prompt 2

The attached photos show lettuce plants grown under high-temperature stress conditions. In each pair, the left and right pots represent plants subjected to different fertilization sources — one with a biofertilizer and the other with a mineral fertilizer.

Carefully observe and compare the following attributes for each pair:

1. Leaf color intensity and uniformity (deep green indicates chlorophyll stability and nutrient balance; pale green or yellow suggests degradation or nitrogen deficiency);
2. Tissue turgor and hydration status (assess wilting, loss of leaf rigidity, or folding);
3. Leaf morphology and canopy architecture (expansion, deformation, curling, or necrotic margins);

4. Overall vigor and biomass distribution (density and compactness of the head or canopy);
5. Heat stress indicators and visible physiological disorders.

**Prompt 3**

Identify and describe all visible physiological disorders present in each treatment, classifying them by symptom type. The following categories should be used to describe symptoms:

- Leaf edge burn (tipburn): necrotic borders or margins typical of calcium or heat stress;
- Chlorosis: diffuse or interveinal yellowing due to chlorophyll degradation or N imbalance;
- Necrosis: brown or blackened areas indicating irreversible cell damage by thermal or oxidative stress;
- Wilting or dehydration: loss of turgidity, leaf curling, or drooping due to high transpiration;
- Leaf abscission or senescence: detachment or drying of basal leaves;
- Bolting or stem elongation: premature reproductive growth induced by high temperature;
- Uneven canopy development: asymmetrical or irregular leaf distribution caused by stress or root inhibition.

For each symptom identified, indicate which treatment exhibits it most severely and provide a brief physiological interpretation of the underlying cause.

**Prompt 4**

Compare all pairs (biofertilizer vs. mineral) considering the intensity and frequency of physiological disorders, vigor, and tolerance to heat stress. Assign a qualitative performance ranking for each pair:

- 5 = Excellent thermotolerance (vigorous, minimal symptoms);
- 4 = Good (minor chlorosis or slight wilting);
- 3 = Moderate (visible edge burn or localized necrosis);
- 2 = Weak (general chlorosis or partial canopy collapse);
- 1 = Severe stress (widespread necrosis or desiccation).

Then, generate a final integrated ranking summarizing all pairs, identifying which fertilization source (biofertilizer or mineral) conferred greater thermotolerance.

## **Prompt 5**

Summarize the physiological interpretation by addressing:

1. Which treatment (biofertilizer or mineral fertilizer) maintained greater chlorophyll integrity, hydration, and canopy uniformity;
2. Which specific physiological disorders were predominant in each treatment;
3. How the biofertilizer treatment may have mitigated stress (e.g., via osmotic adjustment, microbial-induced hormonal balance, or antioxidant enhancement);
4. Whether the observed pattern supports the hypothesis that biofertilizers improve lettuce thermotolerance and reduce the incidence of physiological disorders under high temperatures.

## **How to use**

Enter the prompt for each step of the process, one at a time. When asked by AI, enter the necessary information, as well as the photos to be analyzed as attachments. Wait for the AI to analyze and generate the final report. Initial tests were performed on ChatGPT5, Plus version.

## **Photos used in the test**



## Preliminary results

### Visual comparative analysis under high temperature stress

#### General overview

Across all pairs of lettuce (*Lactuca sativa*) subjected to high-temperature stress, clear morphological and physiological contrasts were observed between plants treated with **biofertilizer (left)** and those treated with **mineral fertilizer (right)**.

The main differentiating factors involved **leaf coloration, tissue hydration, canopy uniformity, and the incidence of physiological disorders** such as edge burn, necrosis, chlorosis, and dehydration.

#### Pair 33 × 64

- **Biofertilizer (33):** Leaves remain greener and broader, with mild marginal chlorosis and limited necrosis restricted to older leaves. Turgidity is partially preserved, suggesting effective osmotic regulation.  
**Disorders:** slight *tipburn* and light *chlorosis* at leaf margins.
- **Mineral (64):** Noticeable yellowing and marginal necrosis, indicative of calcium deficiency and thermal desiccation. Outer leaves show dehydration and loss of texture.
- **Disorders:** *leaf edge burn, necrosis, wilting.*
- **Result:** Biofertilizer demonstrates **higher thermotolerance**.

#### Pair 29 × 63

- **Biofertilizer (29):** Pronounced dehydration and basal leaf collapse; canopy partially necrotic but with residual green leaves. Severe heat stress symptoms visible.  
**Disorders:** *necrosis, wilting, senescence.*
- **Mineral (63):** Taller plant with moderate dehydration and basal necrosis, but apical leaves remain turgid and photosynthetically active.
- **Disorders:** *bolting, chlorosis, tipburn.*
- **Result:** Slight advantage for **mineral treatment**, possibly due to transient osmotic adjustment.

#### Pair 1 × 35

- **Biofertilizer (1):** Vigorous growth, dense and turgid canopy with deep green leaves. No visible necrosis or chlorosis. Excellent maintenance of leaf expansion.  
**Disorders:** none significant.
- **Mineral (35):**

- Slight chlorosis on outer leaves and minor tipburn, but overall good development.
- **Disorders:** *Tipburn, localized chlorosis.*
- **Result:** **Biofertilizer treatment** exhibits **superior physiological stability** under heat.

#### **Pair 22 × 55**

- **Biofertilizer (22):** Healthy canopy with moderate edge burn and slight interveinal chlorosis; overall hydration preserved.
- **Disorders:** *tipburn, light chlorosis.*
- **Mineral (55):** Advanced chlorosis and necrotic patches across leaves; loss of turgidity and leaf curling visible.
- **Disorders:** *necrosis, wilting, chlorosis.*
- **Result:** **Biofertilizer** maintains better chlorophyll stability and hydration.

#### **Pair 26 × 58**

- **Biofertilizer (26):** Compact plant, moderate chlorosis but high leaf turgor; absence of necrosis in central leaves.
- **Disorders:** *chlorosis (mild), tipburn (localized).*
- **Mineral (58):** Severe necrosis and dehydration, strong bolting response, marked tissue senescence.
- **Disorders:** *necrosis, wilting, bolting.*
- **Result:** Clear advantage for **biofertilizer** in mitigating heat damage.

#### **Pair 20 × 54**

- **Biofertilizer (20):** Noticeable dehydration and some necrosis but better canopy preservation compared to mineral; turgor partly maintained.
- **Disorders:** *necrosis, wilting.*
- **Mineral (54):** Advanced dehydration, elongated stems (bolting), high tissue necrosis.
- **Disorders:** *bolting, necrosis, chlorosis.*
- **Result:** **Biofertilizer** performs better overall, showing less senescence.
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**Table 1 - Integrated physiological ranking (heat stress tolerance)**

Rank	Pair	Better Treatment	Main Disorders Observed	Performance Level
<b>1</b>	<b>1 × 35</b>	<b>Biofertilizer</b>	None / slight edge burn (mineral only)	Excellent
<b>2</b>	<b>26 × 58</b>	<b>Biofertilizer</b>	Mild chlorosis vs. severe necrosis	Very good

<b>3</b>	22 55	<b>Biofertilizer</b>	Tipburn vs. generalized chlorosis/necrosis	Good
<b>4</b>	33 64	<b>Biofertilizer</b>	Marginal tipburn vs. dehydration	Good
<b>5</b>	20 54	<b>Biofertilizer</b>	Moderate necrosis vs. advanced bolting	Moderate
<b>6</b>	29 63	<b>Mineral</b>	Severe necrosis (biofert.) vs. moderate bolting	Moderate

#### Overall ranking (best to worst thermotolerance):

1. Biofertilizer (pairs 1, 26, 22, 33, 20)
2. Mineral (pair 29 only shows advantage)

#### Physiological interpretation

The biofertilizer-treated plants consistently displayed **greater leaf turgor, deeper green coloration, and reduced necrotic or desiccated tissue**, indicating **enhanced thermotolerance** and more efficient **osmotic and antioxidant regulation** under high temperature.

In contrast, mineral-fertilized plants exhibited **greater calcium-related edge burn, accelerated bolting, and widespread necrosis**, consistent with **oxidative stress and nutrient imbalance** under heat.

These results strongly support the hypothesis that **biofertilizers confer superior heat resilience in lettuce**, primarily by improving root–shoot signaling and maintaining physiological homeostasis under stress.

#### Conclusions and perspectives

The visual analysis conducted through AI-assisted prompt engineering demonstrated that the proposed low-cost phenotyping approach can distinguish morphological and physiological responses of lettuce (*Lactuca sativa*) to heat stress under contrasting fertilization forms.

Across all image pairs analyzed, plants treated with the biofertilizer displayed higher thermotolerance, evidenced by greater leaf turgor, greener coloration, lower incidence of necrosis and dehydration, and delayed senescence when compared to mineral-fertilized plants. These finds are in line with many published previous studies. However, it is important to note that this is only a first version of the material and that it needs refinement and further testing.

The consistency of these visual indicators across multiple independent image sets validates the reproducibility and sensitivity of the prompt-based evaluation framework, highlighting its future potential as an auxiliary low-cost tool for rapid phenotyping of abiotic stress responses, democratizing the use of modern techniques. This approach could provide a practical and scalable methodology for research stations, teaching laboratories, and family farming systems, particularly in resource-limited environments where traditional high-cost phenotyping infrastructure is unavailable.

From a physiological standpoint, the superior performance observed in biofertilizer-treated plants supports the hypothesis that biofertilizers enhance thermotolerance through microbial mediation, improving osmotic adjustment, hormonal balance (cytokinins and auxins), and activation of antioxidant pathways that prevent oxidative tissue damage. Such effects align with previous experimental findings, large reported in scientific literature, demonstrating improved chlorophyll retention, root activity, and nutrient homeostasis under similar conditions.

Looking forward, the methodology presented here establishes the foundation for AI-integrated phenotyping workflow, where *Large Language Models (LLMs)* and *Computer Vision* could jointly quantify heat damage and predict plant resilience and adaptation. Future versions may incorporate:

1. Larger datasets of annotated images, representing diverse cultivars and environmental conditions.
2. Automated image segmentation and pixel-based thermal mapping to quantify symptom areas.
3. Correlation with physiological and biochemical parameters (chlorophyll content, electrolyte leakage, antioxidant activity).
4. Cross-validation using other AI systems to ensure robustness and interoperability.

The integration of generative AI, agronomic expertise, and open-access biofertilizers such as Hortbio® (used in the experiment from which the photos were taken – for further information, see the work of Lima et al., 2024) represents a promising frontier for climate-smart and regenerative horticulture, aligning scientific innovation with the goals of sustainable food production and climate adaptation.

### **Reference**

Lima, C. E. P., Cajamarca, S. M. N., Trindade, D. M., Fontenelle, M. R., Suinaga, F. A., Fonseca, M. E. N., Boiteux, L. S., Pilon, L., Braga, M. B., Rocha Guedes, I. M., & da Silva, J. (2024). Adaptation of lettuce cultivars to high temperatures and different types of fertilization anticipating management strategies to mitigate the adverse effects of global climate change. *Global Research in Environment and Sustainability*, 2(9), 32-51. <https://doi.org/10.3390/ijms231911129>