The new biostimulant KIEM® improves heat stress tolerance during cucumber (*Cucumis sativus* L.) seed germination

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INTRODUCTION

Crops can be subjected to multiple abiotic stresses during their life cycle and these factors may have a significant impact on plant growth and final productivity. So far, different approaches have been employed to enhance plant stress tolerance, however some treatments can be particularly time-consuming (e.g., conventional breeding) and others not accepted by all countries in the world (e.g., plant genetic modification). Priming could represent an alternative tool to prepare plants to counteract abiotic stress conditions more successfully (1). Plants can be pretreated at different developmental stages (e.g. vegetative or reproductive stage), however in the last few years the attention has been focused on seed priming. This approach consists in a pre-sowing treatment of seeds with synthetic or natural compounds, including biostimulants, aimed to increase uniformity and vigor of seedlings and to enhance the tolerance of plants to different abiotic stresses. The priming treatment at seed stage leads to a reduction of application costs (a single treatment instead than multiple treatments) and often to a prolonged potential protection (2).
The aim of this study was to evaluate the potential priming effects of KIEM® (Green Has Italia S.p.A), a new biostimulant based on lignin derivatives (lignosulphonates) and containing plant-derived amino acids and molybdenum on cucumber (*Cucumis sativus* L. cv. Piccolo di Parigi) seed germination and plant development under heat stress conditions.

MATERIALS AND METHODS

Cucumber (*C. sativus cv. Piccolo di Parigi*) seeds

KIEM® treatment

final dosage of 2 mL kg^{-1}

28 °C optimal temperature

35°C heat stress

- ✓ **Morphological analysis**
  - Safranin staining
  - Primary root length
  - Number of secondary roots
  - Number of leaves/plant
  - Number of flowers
- ✓ **Biochemical analysis**
  - Spectrophotometric assays
  - Quantitative real time PCR (qPCR)
- ✓ **Transcriptional analysis**

RESULTS AND DISCUSSION

✓ MORPHOLOGICAL ANALYSIS RESULTS

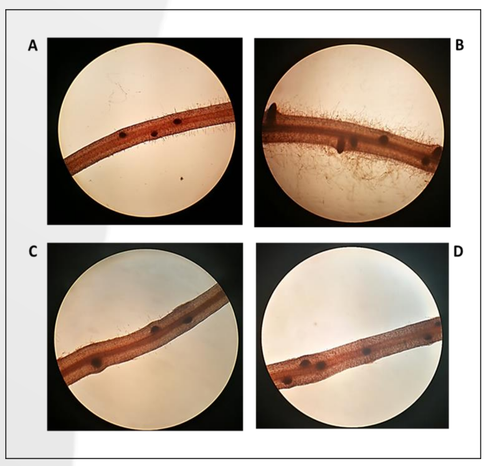

Fig. 1 Root Safranin staining: A) 28°C Control; B) 28°C Treated; C) 35°C Control; D) 35°C Treated. Measurements at 48h after seed imbibition

Seedlings

Tab.1. Germination percentage, fresh weight and number of root primordia at 48h after seed imbibition. Values are expressed as a mean (±SD). *Significant differences with respect to control (ANOVA, Tukey's post-hoc test, p<0,05)

Seed treatment	Germination %	Fresh weight (g)	Number of lateral root primordia (Mean±SD)
28°C Control	98±3,1	1,55±0,06	22,13 ± 3,16
28°C Treated	96 ±2,2	1,46±0,04	22,27 ± 3,22
35°C Control	96 ±2,4	1,00±0,05	10,4 ± 2,5
35°C Treated	98±1,9	0,91±0,11	12,93 ± 2,25*

Plants

Tab.2. Some morphological parameters measured on 40-day old plants grown from untreated and KIEM-treated seeds pre-incubated at 28 and 35°C. Values represent the mean (±SD). *Significant differences (ANOVA, Tukey's post-hoc test, *p<0,05*). The total number of flowers is measured on all plants

Seed treatment	Number of leaves per plant (±SD)	Total number of flowers (10 plants)	Primary root length (cm) (±SD)
28°C Control	2,9 ± 0,31	29	26,7 ±4
28°C Treated	2,5 ± 0,52	45	33,1 ±6,47*
35°C Control	2,9 ± 0,56	24	29 ±6,18
35°C Treated	3,9 ± 0,99*	31	37,7 ±13,23

At 48 h, both control and KIEM®-treated seeds incubated at 28°C and 35°C showed similar fresh weight and germination percentage (Tab.1). However, significant results were recorded with regard to lateral root primordia between treated and control seedlings germinated at 35°C (Fig. 1, Tab. 1).
An increase in root length at 28°C and number of leaves and flowers at 35°C was observed on plants grown from seeds treated with KIEM® , indicating a positive effect of this new biostimulant at later stages of plant development (Tab. 2).

✓ BIOCHEMICAL AND TRANSCRIPTIONAL ANALYSIS RESULTS

Oxidative stress

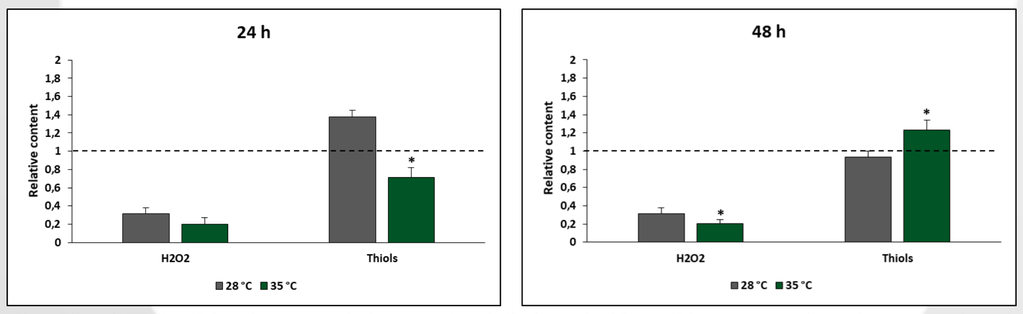

Fig. 2. Effect of KIEM® on H_2O_2 and non-protein thiol content at 24 and 48h after seed imbibition. Values are expressed as a relative content obtained by comparing KIEM®-treated samples with the corresponding untreated controls. Bars represent the mean ± SD of 3 biological replicates. Asterisks (*) indicate significant differences between treatments at the two different temperatures (ANOVA, Tukey's post-hoc test, *p<0,05*).

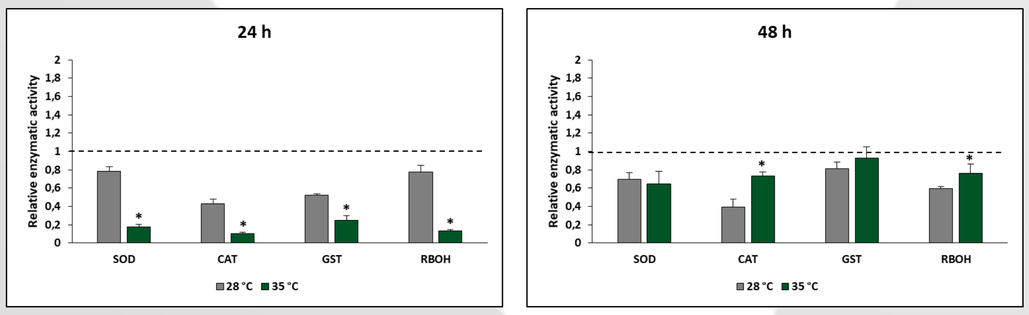

Fig 3. Effect of KIEM® on enzymatic activities of SOD, CAT, GST and RBOH at 24 and 48 h after seed imbibition. Values are expressed as a relative enzymatic activity obtained by comparing KIEM®-treated samples with the corresponding untreated controls. Bars represent the mean ± SD of 3 biological replicates. Asterisks (*) indicate significant differences between treatments at the two different temperatures (ANOVA, Tukey's post-hoc test, *p<0,05*)

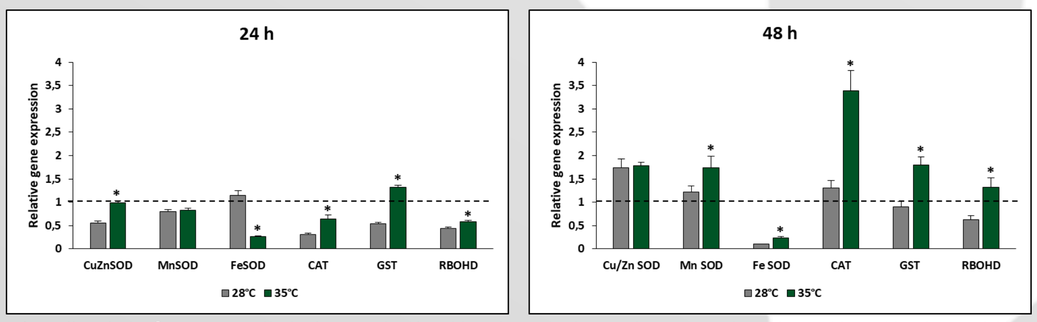

Fig 4. Effect of KIEM® on expression levels of genes coding for ROS producing (*RBOHD*) and scavenging (*CuZnSOD*, *MnSOD*, *FeSOD*, *CAT2* and *GST*) enzymes after 24 and 48h from seed imbibition. Values are expressed as a relative gene expression obtained by comparing KIEM®-treated samples with the corresponding untreated controls. Bars represent the mean ± SD of 3 biological replicates. Asterisks (*) indicate significant differences between treatments at the two different temperatures (ANOVA, Tukey's post-hoc test, *p<0,05*)

Germination process

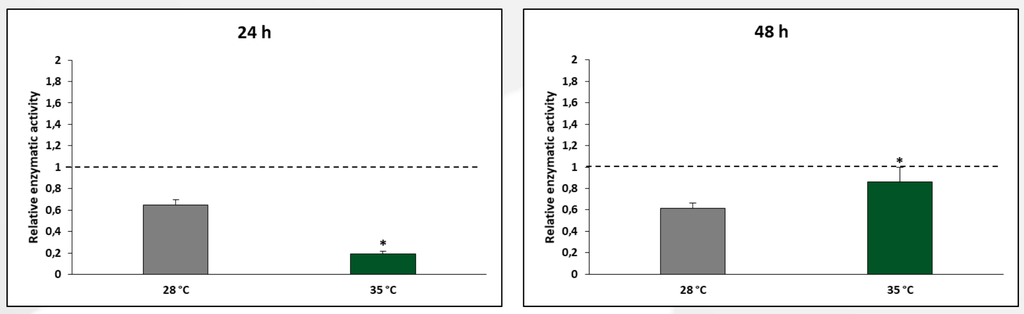

Fig. 5. Effect of KIEM® on ICL enzymatic activity at 24 and 48 h after seed imbibition. Values are expressed as a relative enzymatic activity obtained by comparing KIEM®-treated samples with the corresponding untreated controls. Bars represent the mean ± SD of 3 biological replicates. Asterisks (*) indicate significant differences between treatments at the two different temperatures (ANOVA, Tukey's post-hoc test, *p<0,05*)

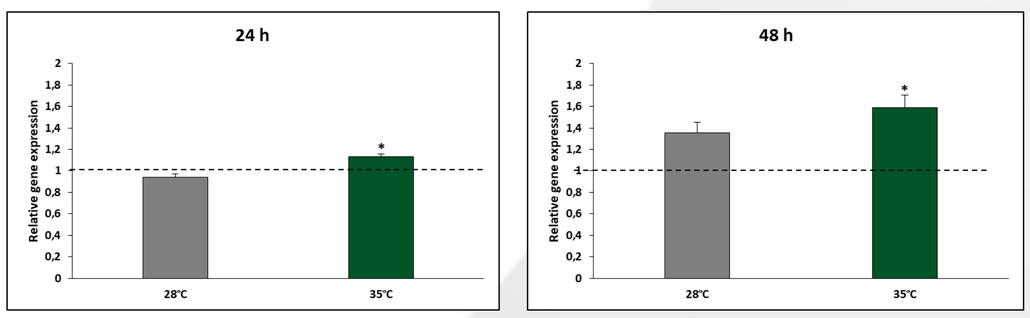

Fig. 6. Effect of KIEM® on expression levels of *ICL* after 24 and 48h from seed imbibition. Values are expressed as a relative enzymatic activity obtained by comparing KIEM®-treated samples with the corresponding untreated controls. Bars represent the mean ± SD of 3 biological replicates. Asterisks (*) indicate significant differences between treatments at the two different temperatures (ANOVA, Tukey's post-hoc test, *p<0,05*)

Oxidative stress

A strong decrease in H_2O_2 content was observed at 24h after imbibition in seeds treated with KIEM® and incubated at both temperatures, but in particular at 35°C (Fig. 2). The lower levels of H_2O_2 were correlated to the activity of the ROS producing enzyme (RBOH), to the other scavenging enzymes (superoxide dismutase, SOD, catalase, CAT, and glutathione-S-transferase, GST) and to the amount of non-protein thiols, important antioxidant molecules. Similar results were obtained at 48h, although a slight increase in the enzymatic activity was observed (Fig. 2). With regard to gene expression, the trend observed for enzymatic activities was maintained at 24h (Fig. 4), whereas at 48h and at 35°C, a significant increase in the transcription level was recorded for almost all genes, suggesting a stronger action of KIEM® in heat stress conditions (Fig. 4).

Germination process

The isocitrate lyase (ICL) activity, a key enzyme of the glyoxylate cycle, resulted lower in seed treated with KIEM® at 24h after imbibition, at both incubation temperatures (Fig. 5). An opposite trend was observed for gene expression: an increased up-regulation was recorded at 48h and at 35°C (Fig. 6).

CONCLUSIONS

Taken together our results suggest that KIEM® is able to improve heat tolerance in cucumber by:
- acting as a priming agent by initially slowing-down the germination process (preventing a rapid seed rehydration) and enhancing a subsequent recovery at later developmental stages (improved plant growth)
- preventing the production of reactive oxygen species and oxidative stress damages

REFERENCES

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2) Savvides, A., Ali, S., Tester, M., and Fotopoulos, V. 2016. Chemical priming of plants against multiple abiotic stresses: mission possible? *Trends Plant Sci*. 21, 329-340.