GHI_18_120, a new biostimulant based on hydrolysable and condensed tannins, improves root growth in tomato cultivated under salt-stress conditions

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INTRODUCTION & AIM

Biostimulants are increasingly studied as new generation products able to counteract abiotic stresses. Salt stress is one of the most important limiting factors, affecting plant productivity and having negative effects on seed germination, plant vigour and crop yield (Abiala et al., 2018).
Tannins are known to be used for different purposes, such as leather treatment or wine and beer clarification, but little is known about their use in agricolture. GHI_18_120, a new biostimulant based on hydrolysable and condensed tannins extracted by hot water from wood, tested in two years on different horticultural crops, showed a positive effect on roots and therefore higher yield in presence of multi-stress environmental conditions.
The aim of this research was to study the effects of GHI_18_120 on tomato (*Solanum lycopersicum* Mill. cv. Heinz1706) grown in greenhouse under standard and salt stress conditions.
To study its mode of action, a multidisciplinary approach, including biometric measurements and transcriptomics, was used.

MATERIALS AND METHODS

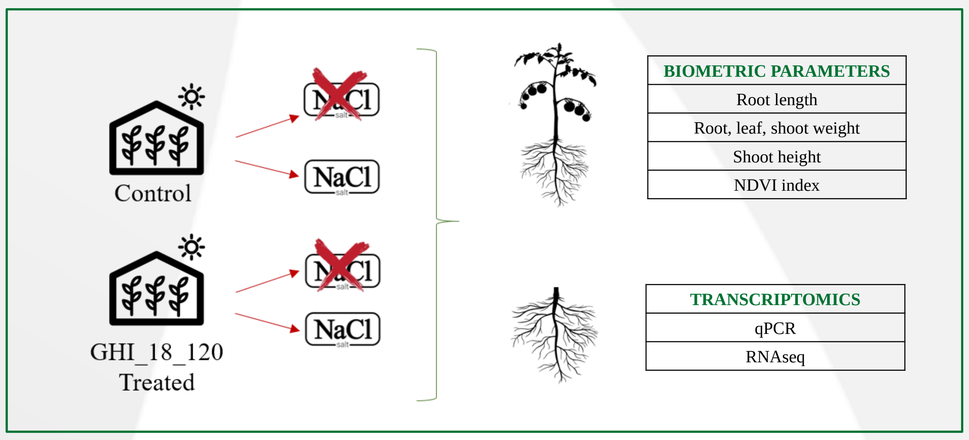

Fig.1 *In vivo* trial work-flow. Tomato plants grown in green house were treated with water (Control) and GHI_18_120 1ml/l (Treated) and subjected to standard and salt stress conditions (100 mM NaCl). Biometric parameters were collected on plants and transcriptomic analyses were performed on roots, the main target of GHI_18_120.

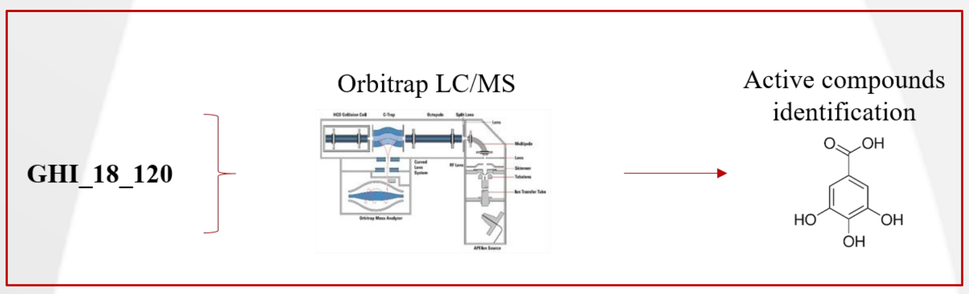

Fig.2 Biostimulant chemical characterization work-flow. After liquid/liquid extraction, the biostimulant was analyzed using an Orbitrap LC/MS instrument (Thermo Fisher). The main active compounds, involved in plant growth and root development, were identified.

In vivo trials

GHI_18_120 was tested on plants. *In vivo* trials were performed in green house on tomato cv. Heinz 1706. Plants were treated 4 times, once a week. Half of the plants were stressed by adding a 100 mM NaCl solution, starting just after the first treatment (priming). Roots and leaves were collected for biometric and transcriptomic analyses 24 h after the second and the fourth treatment (Fig. 1). Imaging analysis (Root System Analyzer®) was also performed on roots to evaluate total root number.

GHI_18_120 chemical characterization

After methanol (50% v/v) extraction, GHI_18_120 was analyzed using an Orbitrap LC/MS (Thermo Fisher) in order to identify the main active compounds involved in root development and salt stress response (Fig. 2). A semi-quantitative analysis was performed. The most interesting components will be further quantified by using external standards.

RESULTS

✓ *In vivo* trials

Biometric parameters

Root fresh weight (FW) was significantly increased by the treatment in both optimal and salt stress conditions.
In particular, the values obtained following the GHI_18_120 treatment on stressed plants were comparable to the control in optimal conditions, suggesting a potential stress mitigation action of this biostimulant (Fig. 3A). On the contrary, **root length** seemed not to be affected by the treatment (Fig. 3B).

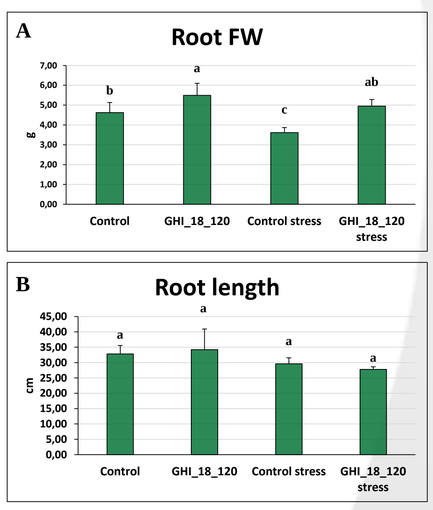

Fig. 3. Root fresh weight (A) and length (B) of control and treated plants, grown under standard and salt stress conditions. Material was collected after 4 weekly treatments.

Root imaging

Root imaging analysis (Root System Analyzer®) provided the root total number and allows to visualize the root architecture (Fig. 4). Plants treated with GHI_18_120 showed a statistical significant increase in the number of roots, in particular under optimal conditions (Table 1).
The high standard deviation values recorded in this experiment are mainly due to the variability, typical of crop plants.

	Total root №	SD	Tuckey's post-hoc test
Control	31.2	7.1	ab
GHI_18_120	36	10.5	a
Control stress	17.2	11.9	b
GHI_18_120 stress	17.7	11.7	b

Table 1 – Total root number

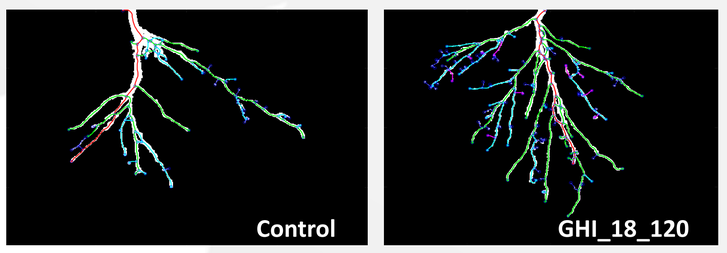

Fig. 4. Root System Analyzer® output. This imaging software highlights roots of different orders with different colours, allowing to count and classify them (red: primary root; green: second order; blue: third order). GHI_18_120 treated plants (right) show an higher number of lateral roots in comparison to the control (left).

Transcriptomic analysis

RNAseq, performed on roots of plants grown under standard and salt stress conditions collected 24 h after the fourth treatment, showed interesting results about gene regulation in treated plants in comparison to the control. Plants treated with GHI_18_120 showed the up-regulation of more than 280 genes involved mainly in abiotic stress response (63%), root growth (18.5%) and other functions (18.5%) (Fig. 5). Based on these transcriptional data, treated plants appeared to be more prone to respond to abiotic stress stimuli and show a more developed root system.
Moreover, several down-regulated genes are involved in phosphate availability. These genes are normally up-regulated in presence of phosphate starvation, while the transcripts rapidly decrease when Pi-starved tomato plants are resupplied with Pi.

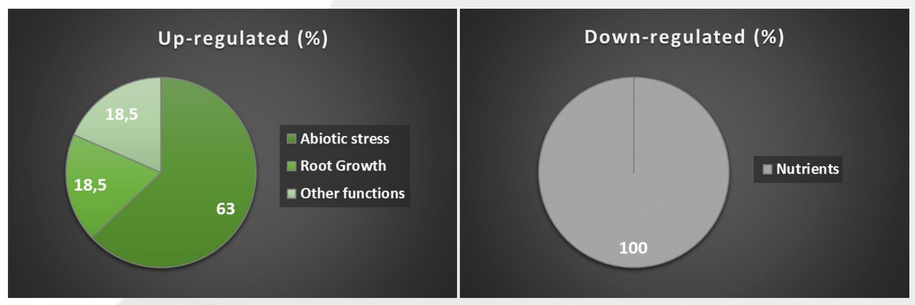

Fig. 5. RNAseq results. The most significantly up-regulated genes (left) are involved in abiotic stress response (63%), root growth (18.5%) and other functions (18.5%). The totality of the most significantly down-regulated genes (right) are involved in the increase of nutrients availability

✓ GHI_18_120 chemical characterization

Compound	%Area
Gallic acid	7.1
Ellagic acid	3
Digalloylglucose	4.18
Valoneic acid dilactone	1.27
Eriodictyol	0.96
Phloionic acid	0.73
1,3,6-trigalloylglucose	5.7
1,2,3,6-tetra-O-galloyl-β-glucose	3.14

Table 2 – Main active compounds identified in GHI_18_120

LC/MS analysis on GHI_18_120 allowed the identification of several active components (Table 2). The major part of the identified compounds are tannin building blocks, also known to be involved in root and plant development (i.e. gallic acid). Other interesting molecules are eriodictyol, involved in root length improvement and phloionic acid, a product of wood hydrolysis.

CONCLUSIONS

Based on biometric and transcriptomic results, tomato plants treated with GHI_18_120 resulted more active in the response against salt stress and showed an enhanced root development. Moreover, the gene expression data confirmed a decrease of oxidative damage and absence of phosphate starvation. The chemical characterization of GHI_18_120 identified active components whose effects on plants can be correlated to the morphological observations. In conclusion, the overall results provide insights on the mechanism of action of GHI_18_120 and on its application in agriculture as a valid tool to improve root growth, especially in soils subjected to salt stress.

REFERENCES

Abiala, M. A., Abdelrahman, M., Burritt, D. J., & Tran, L. S. P. (2018). Salt stress tolerance mechanisms and potential applications of legumes for sustainable reclamation of salt-degraded soils. *Land degradation & development*, *29* (10), 3812-3822