

Les diffusions de fréquences sonores conçues pour cibler les déshydrines induisent la tolérance au stress hydrique des semis de *Pisum sativum*

*Diffusions of sound frequencies designed to target dehydrins induce hydric stress tolerance in *Pisum sativum* seedlings*

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INTRODUCTION :

Dehydrins are part of a family of proteins that provide resistance to environmental stresses in plants, such as drought, heat or cold. In the face of current and future climate challenges, these proteins have a key role to play. The aim of this study is to regulate their synthesis, using sound sequences designed on a patented model⁴ that has been the subject of preliminary studies^{1,2,3}, and more specifically **dehydrin (DHD) cognate in pea**.

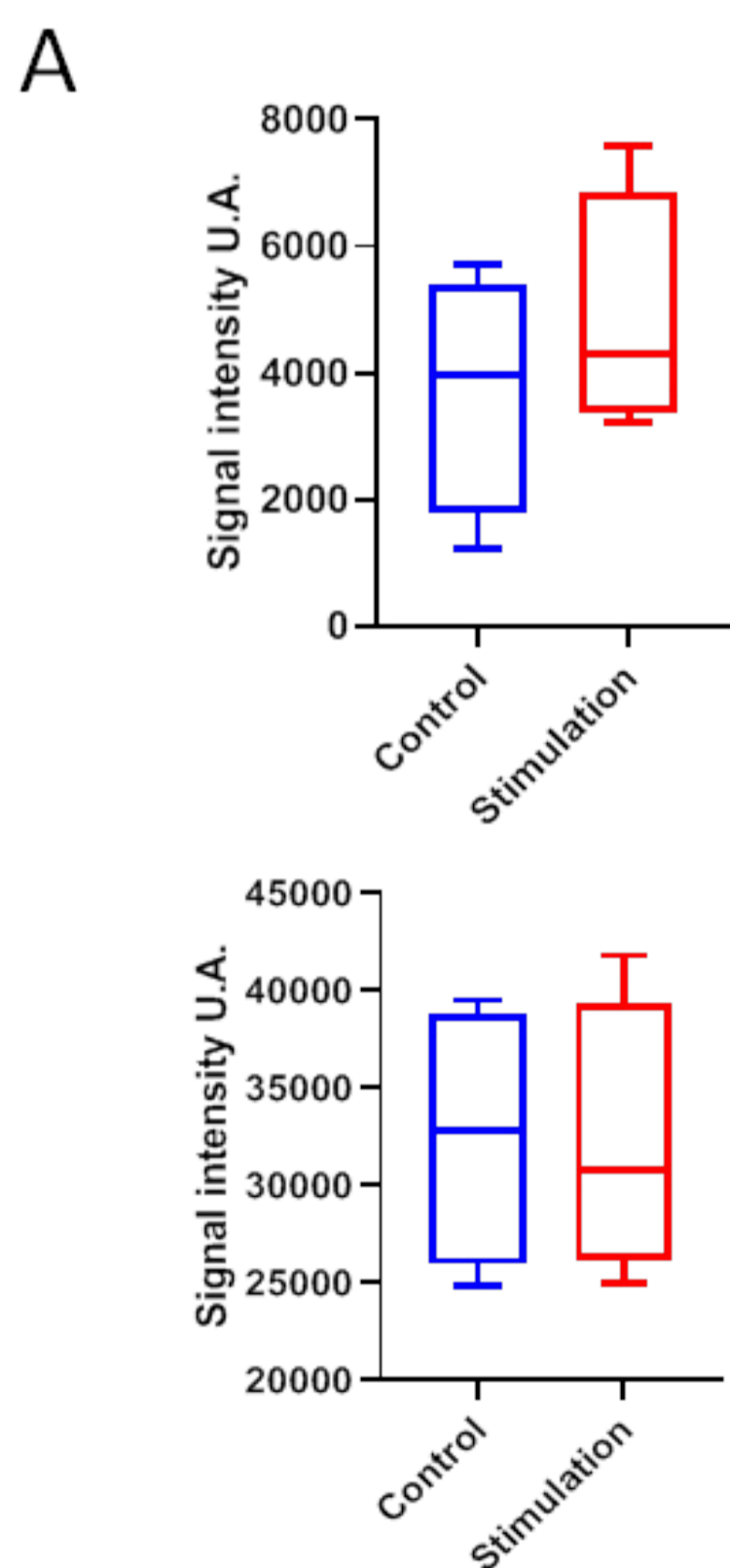
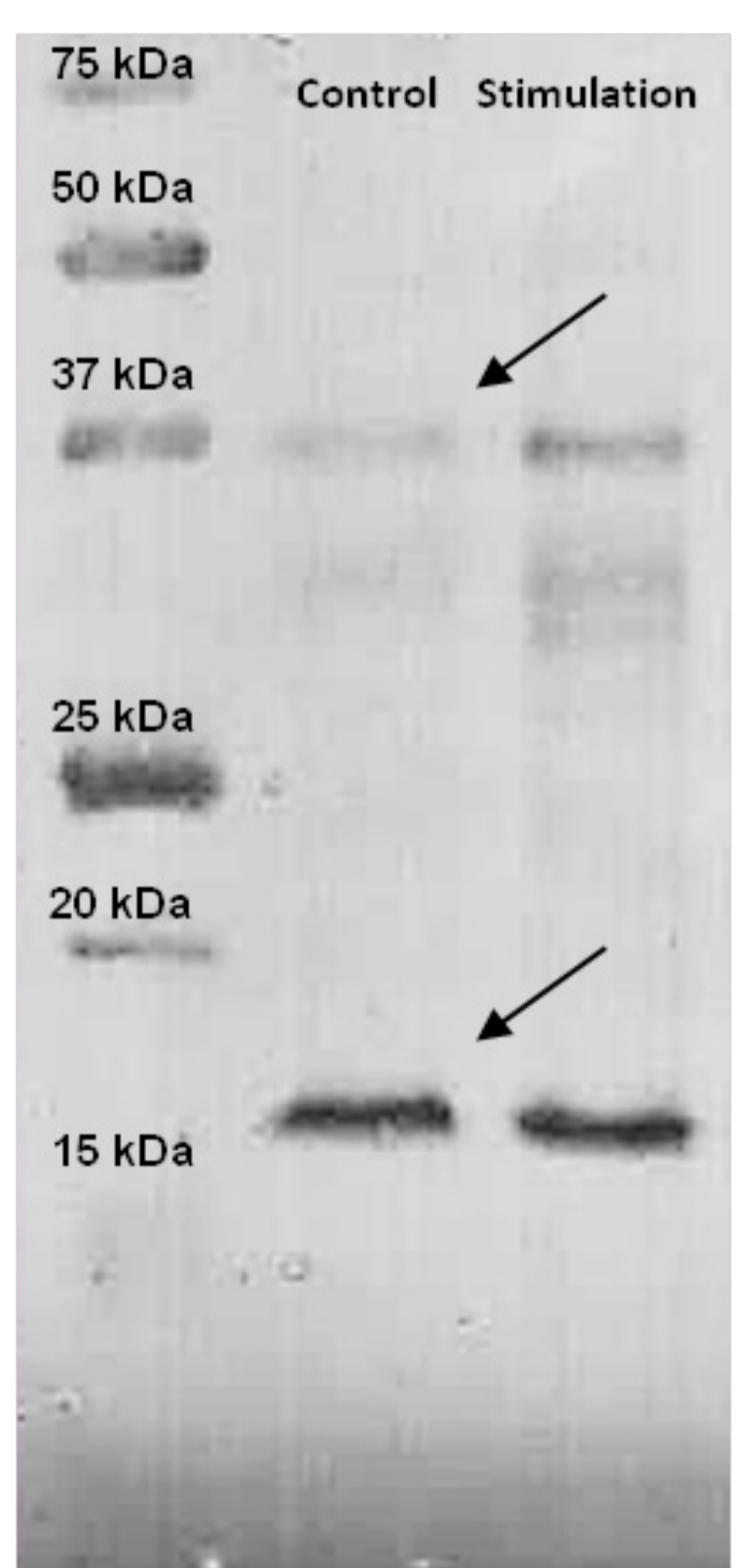
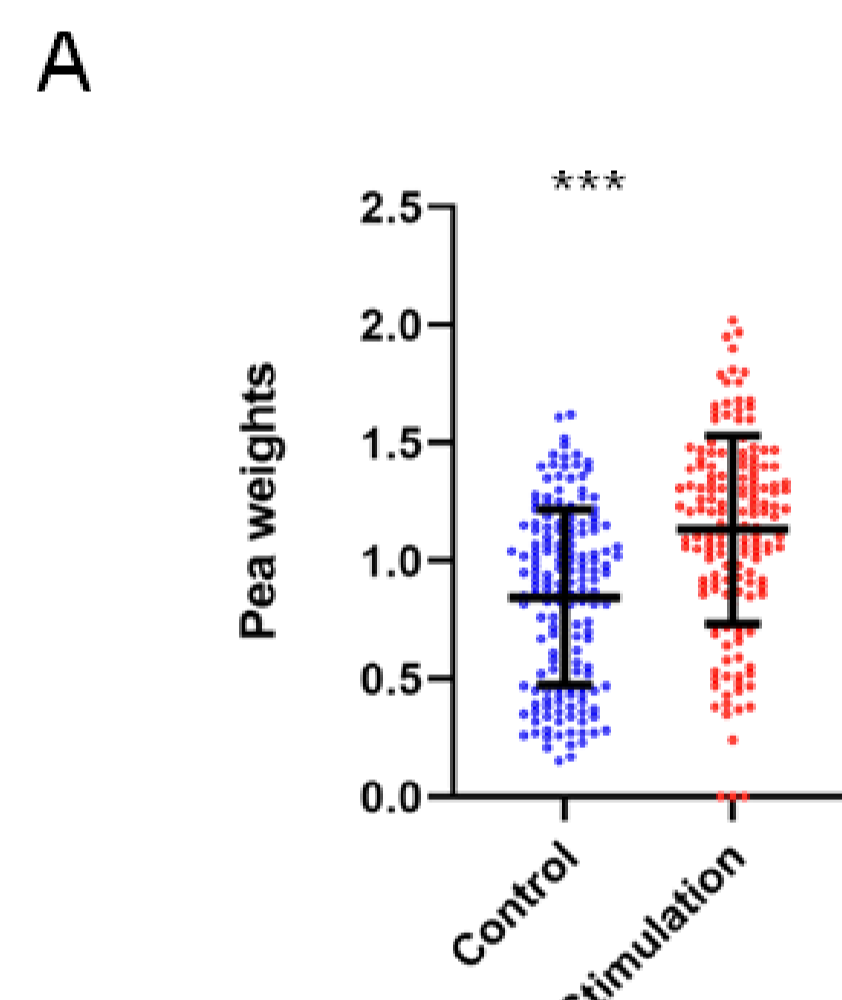
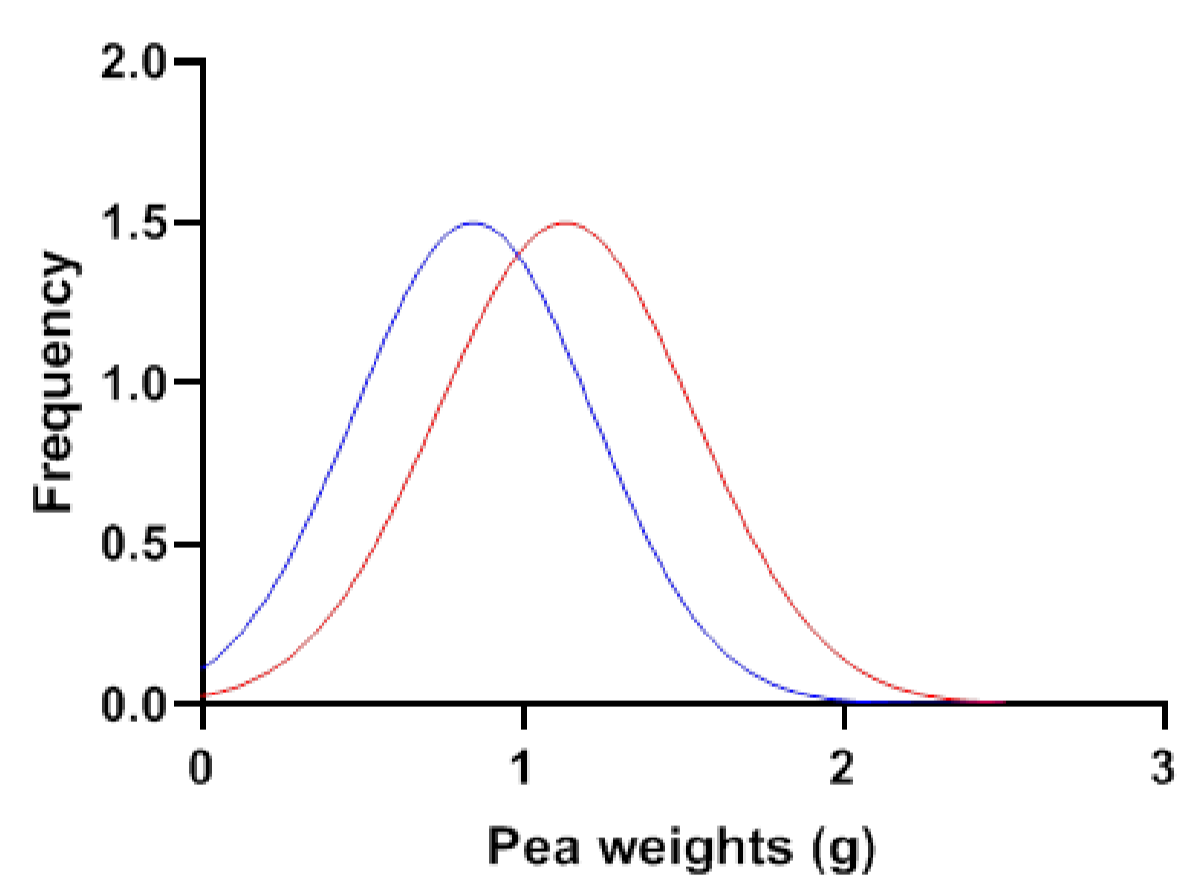
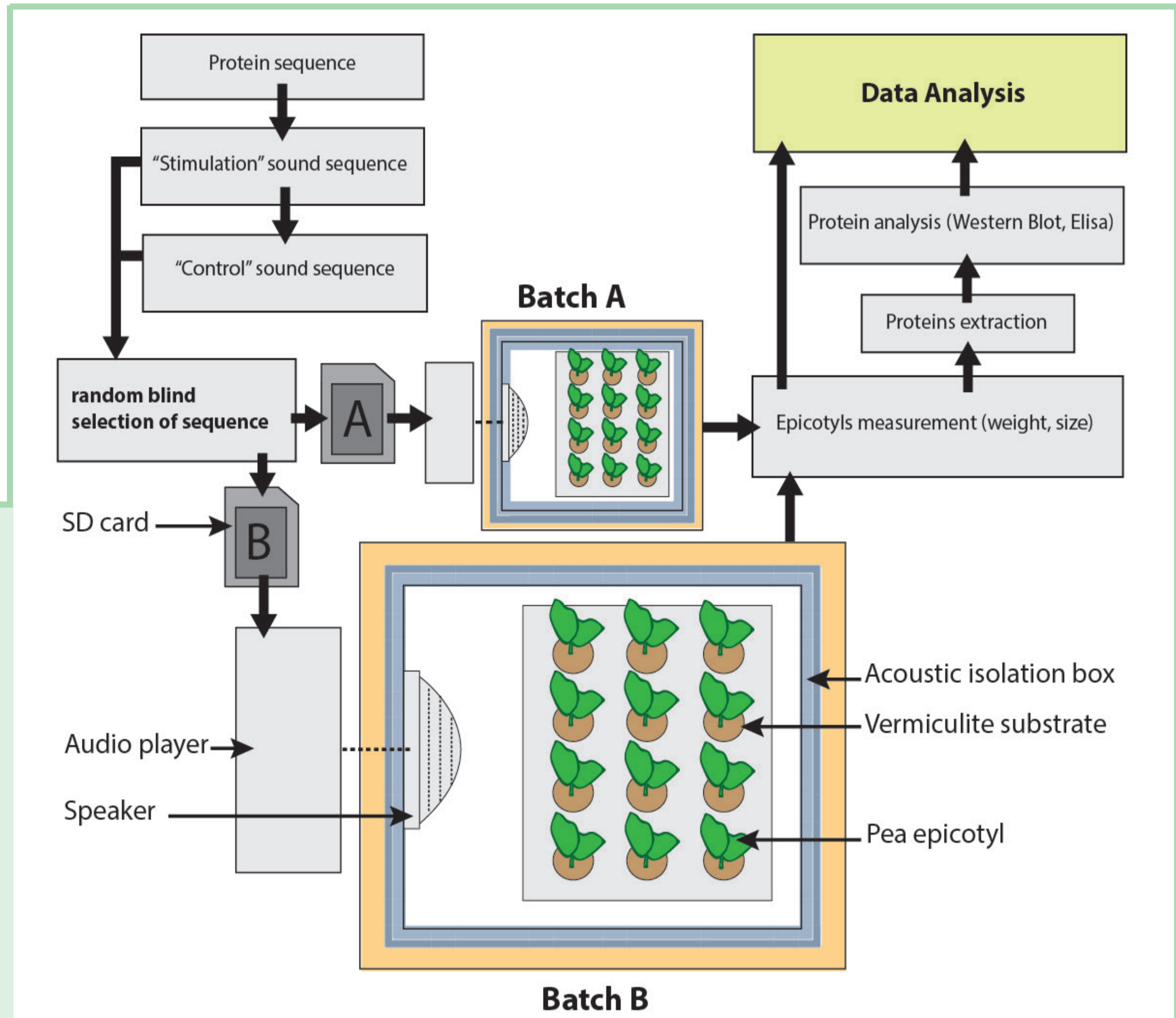
The aim is to assess the response of pea seedlings under conditions of salt stress when they are exposed to sound sequences produced to stimulate DHD synthesis. This model could be used to assess the potential of this method to meet needs in agriculture and also in biology⁵. This study was carried out blindly at each stage of the protocol, from the conduct of the experiment to the measurement and processing of the results.

EQUIPMENT AND METHOD :

Two batches were grown at the same time, in two separate, acoustically isolated rooms. The batches were also placed in sound-isolation boxes. The pea seeds were sown in a water-soaked vermiculite substrate. During the experiment, the substrate was not watered in order to simulate increasing water stress.

The sound sequences were broadcast for **5 minutes each day**. The test and control sequences were broadcast at the same time and at the same intensity. The epicotyls were harvested after **8 days of culture**. They were weighed and measured directly after harvesting.

Total proteins were extracted and purified, then dehydrin cognate was assayed by **Western blot**.



RESULTS :

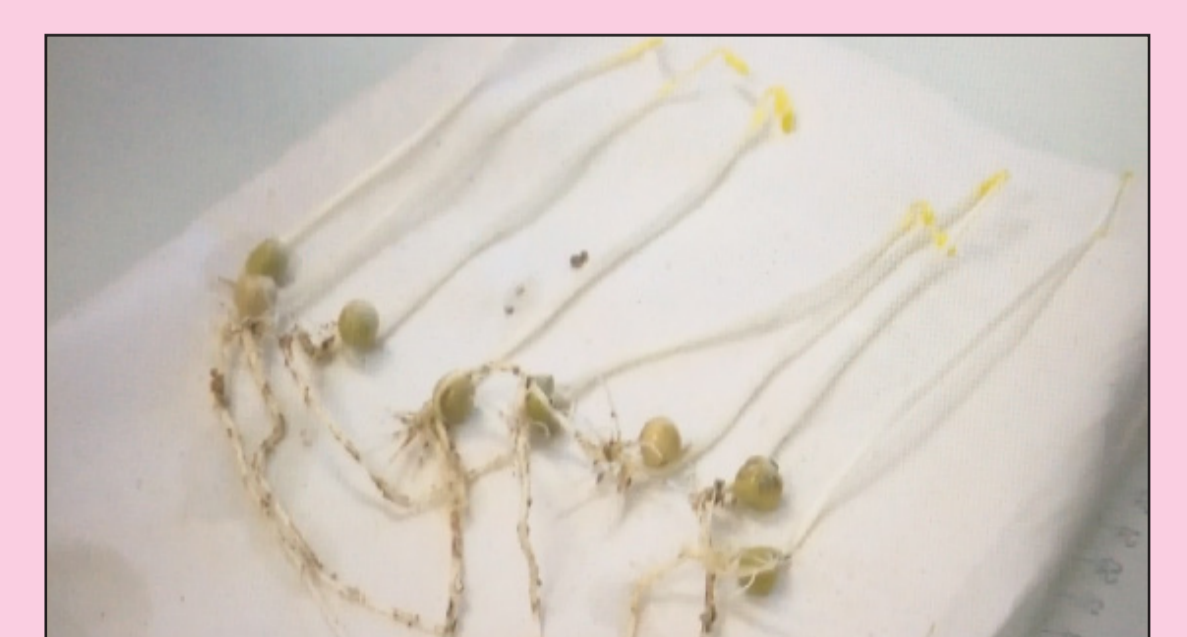
Epicotyl weights: Average epicotyl weights were significantly higher for the batch that received the sequence designed to stimulate DHD synthesis (**Stimulation population (1.13 g +/- 0.39 g)**) compared with the **Control population (0.84 g +/- 0.37 g)**. The data presented are the results of 3 different experiments with a minimum of **340 epicotyls for each condition**.

Protein Amount: Our results also suggest an increase in the size of the band located around **37 kDa** in the case of the stimulation sequence, compared with control peas subjected to the same water stress. This band corresponds to the molecular weight of the target dehydrin, cognate dehydrin. Another band around **15 kDa** showed no significant difference between the two batches. This band seems to correspond to the basal level of dehydrins in the young shoot, and does not seem to be affected by the sound sequence.

DISCUSSION:

- These results suggest **an increase in the amount of DHD of 37 kDa** in peas exposed to the sound sequence compared with control peas.
- **An increase in epicotyl fresh weight** was induced when they were exposed to an acoustic sequence generated from a fragment of the cognate DHD.

Taken together, our results suggest that exposure to a sequence of acoustic frequencies correlated with a specific DHD amino acid sequence could act as a positive modulating factor in pea adaptation to water stress. Using a specific sequence correlated with DHD cognate, we obtained a specific response by acoustic stimulation.



CONCLUSION AND PERSPECTIVES:

Given the recent data showing that an inducible DHD promoter is over-expressed under abiotic stress, and our results showing the increase in DHD levels under specific acoustic stimuli, we could hypothesize that the diffusion of these sequences acts in a specific way in synergy with water stress signalling pathways. These preliminary results could be extended by precise monitoring of protein and gene expression in other living organisms such as bacteria, fungi and mammalian cells. To decipher the mechanisms involved in living organisms during environmental disturbances triggered by acoustic waves we continue our research through the GenodiCY program funded by France Relance under 2 complementary approaches :

- Develop a set of shared collaborative tools (dedicated not only to structural but also to frequency homologies) enabling the deployment of analysis of the relationships of full proteome frequency homologies (plant and animal models).
- Demonstrate and optimize the impact of acoustic waves on the adaptation of plant cells (lettuce protoplasts) to abiotic parameters (drought and water stress).

These encouraging results on the potential of the approach developed here open up prospects for applications in the field to support crops in unstable climatic conditions, directly through the regulation of dehydrins in the case of long periods of water shortage, and also through the regulation of other proteins involved in plant resistance. This is why this approach is already being considered as a future means of action in agriculture⁶, and has been deployed in the field for several years now in disease management⁷.

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