IT Tralee Masters by Research Programme Details

Title of Project: Crop **Sal**inity **To**lerance **I**nduction Using Novel **Bio**stimulants (SALTIBIO)

Name of Principle Supervisor: Dr. Oscar Goñi

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Brief Biography of Principle Supervisor:
Dr Oscar Goñi received his Degree in Chemistry from the University of Navarra (Spain), an MSc in Biochemistry and Molecular Biology from Complutense University of Madrid (Spain) and completed his PhD in Plant Protein Biochemistry at ICTAN-CSIC (Spain) and Complutense University of Madrid (Spain). Dr Goñi has previously worked as a Postdoctoral Research Fellow in the Max Planck Institute of Plant Breeding Research (Cologne). He is a protein biochemist with experience in the purification and characterization of functional proteins, enzymology and development of protein biomarkers. Dr. Goñi currently holds the position of Postdoctoral Researcher with Shannon ABC / Brandon Bioscience and specialises in the development of enzyme activities for the production of macro-algae derived oligosaccharides and chitin/chitosan derived oligosaccharides for crop protection and yield enhancement.

Recent Research Publications:
Peer reviewed research articles

- Goñi, O., Quille, P. and O'Connell, S., 2016. Production of chitosan oligosaccharides for inclusion in a plant biostimulant. Pure and Applied Chemistry, 88(9), pp.881-889.
- Goñi, O., Fort, A., Quille, P., McKeown, P. C., Spillane, C., O'Connell, S. 2016. Comparative transcriptome analysis of two Ascophyllum nodosum extract biostimulants: Same seaweed but different. Journal of Agricultural and Food Chemistry, 64: 2980–2989
- Goñi, O., Sanchez-Ballesta, M.T., Merodio, C., Escribano, M.I. (2013). Two cold-induced family 19 glycosyl hydrolases from cherimoya (Annona cherimola) fruit: An antifungal chitinase and a cold-adapted chitinase. Phytochemistry, 95: 94-104.
- Nguyen, H.M., Schippers, J.H., Goni-Ramos, O, Christoph, M.P., Dortay, H., van der Hoorn R.A., Mueller-Roeber, B. (2013). An upstream regulator of the 26S proteasome modulates organ size in Arabidopsis thaliana. Plant Journal, 74: 25-36.
- Hervas, R., Oroz, J., Galera, A., Goñi, O., Valbuena, A., Vera, A.M., Uversky, V.M., Menendez, M., Bruix, M., Laurents, D., Carrión-Vazquez, M. (2012). Common features at the start of the neurodegeneration cascade. PLOS Biology, 10 (5): e1001335. doi:10.1371/journal.pbio.1001335.
- Goñi, O.*, Sanchez-Ballesta, M.T., Merodio, C., Escribano, M.I. (2011). A cryoprotective and cold-adapted 1,3-β-endoglucanase from cherimoya (Annona cherimola) fruit. Phytochemistry, 72: 843-853.
- Blanch, M., Goñi, O., Sanchez-Ballesta, M.T., Escribano, M.I., Merodio, C. (2011). Characterization and functionality of fructo-oligosaccharides affecting water status of strawberry fruit (Fragaria x vesca cv. Mara de Bois) during postharvest storage. Food Chemistry, 134: 912-919.
- Goñi, O.*, Fernandez-Caballero, C., Sanchez-Ballesta, M.T., Escribano, M.I, Merodio, C. (2011). Water status and quality improvement in high-CO2 treated table grapes. Food Chemistry, 128: 34-39.
- Goñi, O.*, Sanchez-Ballesta, M.T., Merodio, C., Escribano, M.I. (2010). Potent cryoprotective activity of cold and CO2-regulated cherimoya (Annona cherimola) endochitinase. Journal of Plant Physiology, 167: 1119-1129.

Edited conference proceedings

- Goñi O., Quille, P., O'Connell, S. 2015. Impact of biostimulants on plant growth and stress tolerance. Irish Plant Scientists' Association Meeting (IPSAM), Maynooth, Ireland (ORAL PRESENTATION).
- Goñi O., Quille, P., O'Connell, S. 2015. Production of chitosan oligosaccharides for inclusion in a plant biostimulant. 12th International Conference of the European Chitin Society/ 13th International Conference on Chitin and Chitosan (ICCC/EUCHIS), Münster, Germany (ORAL PRESENTATION).
- Bußwinkel F., Goñi O., Moerschbacher B.M., O'Connell, S. 2015. Expression and characterisation of a recombinant chitinase for generation of chitosan oligosaccharides for use as plant biostimulants. 12th International Conference of the European Chitin Society/ 13th International Conference on Chitin and Chitosan (ICCC/EUCHIS), Münster, Germany (POSTER).
- Bußwinkel F., Goñi O., O'Connell, S., Moerschbacher B.M. 2015. Heterologous expression of *Trichoderma virens* endochitinase I in *Pichia pastoris* and characterization towards an assessment of its biotechnological potential. 12th International Conference of the European Chitin Society /13th International Conference on Chitin and Chitosan (ICCC/EUCHIS), Münster, Germany (POSTER).
- O'Connell, S., Goñi O., Quille, P. 2015. The impact of *Ascophyllum nodosum* biostimulant compositional variation on performance in drought stressed tomato. 2nd World Congress on the use of Biostimulants in Agriculture, Florence, Italy (POSTER)
- Quille, P., Goñi O., O'Connell, S. 2015. Investigating the use of a high throughput screening tool in order to evaluate a range of biostimulants. 2nd World Congress on the use of Biostimulants in Agriculture, Florence, Italy (POSTER)
- Goñi O., Sanchez-Ballesta M.T., Merodio C., Escribano M.I. 2013. Two antifungal cold-induced glycosyl hydrolases from cherimoya (Annona cherimola Mill.) fruit: an endochitinase and a cold-adapted chitobiosidase. Irish Plant Scientists' Association Meeting (IPSAM), Galway, Ireland.

Research Project Abstract

The United Nations' and Agriculture Organization predicts that by 2050 the world will need to produce 70 percent more food than it does currently. Along with improving food storage and transport, increasing crop yields is seen as a primary solution. Salinity is one the major environmental stresses affecting crop production, particularly in arid and semi-arid areas. Most of the vegetable crops are salt sensitive, growing poorly in salinized soils due to the accumulation of toxic ions from prolonged irrigation regimes.

A meaningful approach to increase crop yield and counteract salt stress would be the use of protein hydrolysate-based biostimulants, which are gaining interest worldwide. Nowadays, more than 90% of the protein hydrolysates market in agriculture is based on products obtained through chemical hydrolysis of proteins from animal origin. The production and use of new vegetable derived-protein hydrolysates with high plant biostimulant activity has become the focus of much research interest due to their lack of plant phytotoxicity, absence of degraded or biologically inactive amino acids or compatibility in the production of food for vegetarians. The commercial partner, Deltagen UK, aims to commercialise protein hydrolysate biostimulants with superior salinity inducing tolerance.

The aim of this research is the development of an innovative system to produce protein hydrolysates from the defatted by product meals of flax, lentil and sesame seeds with the ability to biostimulate plant tolerance to salt stress. Novel protein hydrolysates will be produced using a cocktail of suitable proteases, they will be applied to tomato plants (cv. Micro-Tom) in a controlled growth room under salt stress conditions. Treatments will be assessed by comparing classic phenotypical parameters. Plant tissue will also be saved in order to assess other biochemical and molecular parameters such as stress related proteins and osmoprotectant metabolites.

Research Context (Technical Merit & Impact)

The beginning of 21st century is marked by global scarcity of water resources, environmental pollution and increased salinization of soil and water. An increasing human population and reduction in land available for cultivation are two threats for agricultural sustainability. It has been estimated that worldwide 20% of total cultivated and 33% of irrigated agricultural lands are afflicted by high salinity. **It has been projected that more than 50% of the arable land would be salinized by the year 2050**. Use of optimized farm management practices such as shifting crop rotation or better irrigation systems can ameliorate yield reduction under salinity stress. However, its implementation is often limited because of cost and availability of good water quality. Several salt-tolerant varieties have been released, the overall progress of traditional breeding has been slow and has not been successful, as only few major determinant genetic traits of salt tolerance have been identified. The utilisation of agro-food processing wastes to generate value added products is an extremely convincing argument as it makes commercial and environmental sense. In addition, it is an excellent, demonstrable example of the European circular economy in action, a key objective of the H2020 research programme, turning waste into value and ultimately food for a growing population.

Research Methodology

Three process variables will be studied in order to obtain the maximum degradation of seed proteins: incubation time, temperature and the initial concentration of meal protein. The Response Surface Methodology (RSM) will be used to reduce the cost and duration of experiments and allow for the observation of any interacting factors in the final process response. Amino acid and monosaccharide composition will be determined by sensitive high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) according previous bibliography. Molecular weight distribution of protein hydrolysates will be characterized by protein electrophoresis (SDS-PAGE) and high performance size exclusion chromatography (HPSEC). The plant trials will involve 2 separate sets of experiments under unstressed and salt-stressed conditions respectively. Experiments will be carried out in a growth room with different concentration rates of different protein hydrolysates and the tomato variety Micro-Tom will be used. This extensive factorial experiment will be assessed by fruit yield, fruit quality, chlorophyll (SPAD measurement), MDH content (cell membrane integrity) and levels of protective compounds (proline and soluble carbohydrates). The presence of stress proteins such as HSPs will be determined using immunoblotting techniques (Western blot). RT-qPCR is another advanced laboratory technique that will be employed in order to investigate gene expression of specific markers involved in salt stress tolerance.

PROJECT SCHEDULE – GANTT CHART

WP1 = Literature review and introductory course

WP2 = Production of protein hydrolysates

WP3 = Plant Trials

WP4 = Analysis of Plant Material

WP5 = Write up

WP	M1	M3	M6	M9	M12	M15	M18
1							
2							
3							
4							
5							