INDUCED SYSTEMIC RESISTANCE: A BIO-ECOLOGICAL TOOL?

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Introduction

Progress in our knowledge of the genetic and molecular bases controlling plant defence mechanisms against pathogens and pests has enabled us to verify the existence of great similarities between plant defence mechanisms and the innate immune system, not mediated by antibodies, of animals. Both these defence systems are based on the recognition of characteristic pathogen molecules by membrane or intracellular receptors, determining the activation of inducible defence barriers. In plants, this recognition can be species-specific, as occurs in basal and non-host resistance, or crop or variety-specific, as in gene-to-gene resistance (Nürnberger and Brunner, 2002). Besides these inducible defence barriers, plants have different pre-existing defence barriers which play a significant role in basal and non-host resistance.

It has been known for over 50 years that most inducible defence mechanisms are characterised by being systemic. In other words, they are not only activated in the tissue where the pathogen/pest is recognised, but also in the rest of the plant which has not been exposed to infection. This systemic response protects the plant from subsequent pathogen/pest attacks. This property is potentially useful in farming, and has awakened the interest of many public and private research groups in studying the molecular and genetic basis of induced resistance. The ultimate objective of this study was to identify and develop chemical products (inducers) capable of activating pathogen/pest resistance in plant species of agricultural interest.

This presentation described the principal characteristics of innate plant immunity and induced resistance mechanisms, and the potential agricultural applications of this field of research.

Innate plant immunity

The ability to distinguish between what is one's own and what is not is shared by all living beings, and of key importance for activation of the innate immune system to prevent pathogenic infection. This discrimination function is performed by a family of receptor proteins, with possibly the same evolutive origin, which are capable of recognising pathogen components. The latter were originally referred to as elicitors, and have more recently become generically known as MAMPs/PAMPs (Microbe Associated Molecular Patterns/Pathogen-Associated Molecular Patterns). MAMP/PAMP recognition by these receptor proteins leads to the activation of a defence response known as PTI (PAMP

Trigger Immunity, Jones and Dangl, 2006). Elicitors/MAMPs are usually well preserved molecules which are essential for the pathogen's physiology and life cycle; they are characteristic of certain groups (not only a race) of pathogens. They include the lipopolysaccharides (LPS) present in the cell wall of gram negative bacteria, the peptidoglycane (PGN) of the cell wall of gram positive bacteria, flagellin (Fgl22), which is a structural protein found in the bacterial flagellum, EF-Tu bacterial protein, fragments of non-methylated bacterial DNA and lipopeptides and components of the cell wall (quitine, glycanes and mannanes) of fungi (Nürberger and Brunner, 2002; Bittel and Robatzek 2007). Given the functional significance of MAMPs, their variability is lower than that of other pathogen molecules, leading to a high degree of preservation and explaining why these molecules have been seen by plants and animals throughout history as the "fingerprints" of different pathogenic groups.

Several receptor proteins (e.g. FLS2, BRI1, EF-TuR) involved in the recognition of these elic¡tors/MAMPs have been identified in plants (Nürberger and Brunner, 2002, Bittel and Robatzek 2007). Pathogen recognition by these receptor proteins causes a series of cell changes which lead to defence gene expression activation. This elicitor/MAMP recognition mechanism does not appear to depend on signal transduction routes mediated by ethylene (ET), salicylic acid (SA) and jasmonic acid (JA) hormones.

Inducible resistance mechanisms

Several types of inducible systemic resistance have been characterised in plants, including: 1) systemic acquired resistance (SAR), which is activated after plants are infected by necrosis-producing pathogens (Ryals et al., 1996); 2) induced systemic resistance (ISR), which is activated after root colonisation by certain bacterial strains from the rhizosphere (Pietersen and Van Loon, 2004); 3) wound induced resistance (WIR), which can be activated by the wounds caused in plant tissue by insects or by mechanical damage (Kessler and Baldwin, 2002); and 4) the aforementioned PTI (Jones and Dangl, 2006).

- Systemic acquired resistance (SAR)

SAR is locally and systemically activated after a plant is infected by necrosis-producing pathogens (viruses, bacteria or fungi). SAR is a broad spectrum resistance, affecting not only the activating (e.g. TMV) but also other pathogens (e.g. other viruses, bacteria and fungi). It has been found that SAR is long-lasting (active for days or weeks) in both natural and laboratory conditions, which makes it very attractive from an agronomic perspective. SAR activation is accompanies by endogenous, local and systemic increase in SA. This accumulation of SA activates a series of regulatory proteins, such as NPR1/NIM, and transcriptional factors (e.g. TGAs) which control the expression of defence genes encoding PR (pathogenesis related) proteins. The exogenous application of SA on a plant's surface is capable of inducing a defence response similar to that activated in SAR. It is activated after necrosis caused by a pathogen, providing resistance against a second infection. The systemic signal of SAR is unknown.

Structural analogues of SA (INA and BION®) have been identified, which are also capable of activating SAR when applied to the surface of plants of agricultural interest. Some of these SAR-inducers have been marketed with unequal success, depending on the plant species and its physiological conditions.

- Induced systemic resistance (ISR)

ISR is activated by certain bacterial strains in the soil (rhizobacteria) which are capable of colonising plant roots. Like SAR, ISR is a broad spectrum systemic resistance (it can protect against bacteria, fungi and some viruses) which lasts for a long time in laboratory and field conditions. ISR activation does not depend on a local and systemic endogenous increase in SA, but on the routes regulated by ethylene (ET) and jasmonic acid (JA) hormones; it is not operative in plants with blocked ET and JA routes. Like SAR, ISR depends on the NPR1/NIM1 regulatory protein although, after this resistance is activated, the defence genes expressed are different. The systemic signal which activates ISR in a plant after its roots are colonised by bacteria is as yet unknown.

Different bacterial strains have been identified in the rhizosphere which are capable of activating ISR in different species of agricultural interest. The potential utility of these strains as ISR inducers in the field is under development.

- Wound induced resistance (WIR)

This resistance is activated after the plant is attacked by damaging/wounding insects, but it can also be activated by mechanical damage. WIR is systemic and long-lasting but not broad spectrum, as it primarily protects against other feeding insects. In the *Arabidopsis thaliana* plant model, it has recently been described that WIR can confer resistance against necrotrophic fungi such as *Botrytis cinerea* (J.P. Métraux et al.)

WIR activation depends on the routes regulated by the JA hormone, and possibly other transduction routes which have not yet been characterised. Unlike SAR and ISR, one of the systemic signals of WIR is known; it is a peptide called systemine. Likewise, a systemine cell receptor which is an RLK (receptor–like kinase) has been identified. The potential utility of WIR in the field is yet to be determined.

- PAMP/elicitor trigger immunity (PTI)

PTI is activated after MAMPs/PAMPs or elicitors are recognised by specific receptors. This recognition leads to activation of a defence response characterised by callose accumulation, strengthening of the cell wall and activation of defence gene expression.

In the last few years, the study of the potential utility of elicitors as resistance inducers has generated great interest in the public and private scientific community. A significant number of elicitors have been identified and the inducing activity of may has been verified in model plants and controlled laboratory conditions. In a few cases (e.g. Messenger®), these compounds have been marketed and used in different plant species.

Knowledge of the molecular bases of PTI could help to develop new strategies with great potential for farming, by the activation and rational use of plants' basal defence mechanisms, which are broad spectrum and efficient in protecting against the pathogens to which plants are exposed.

- Chemical induced resistance (CIR)

This resistance is activated in a plant after it is treated with an inorganic or organic chemical. There are a series of plant protective agents on the market which are capable of protecting plants against infections. They include compounds classified as fungicides/bactericides, although their activity has not been shown in vitro against pathogens controlling when they are applied to plants. The potential of some of these compounds as resistance inducers requires better characterisation.

Plant Response Biotech S.L.

Plant Response is a firm in the green and white biotechnology sector; this technology provides multiple tools for ensuring more efficient and sustainable agriculture. Biological pest control and the development of new substances favouring crop productivity (biostimulants) are two of the areas of most interest for investigators and industry, in which much development is expected in the next few years, especially in Europe and Spain.

The objective of Plant Response is to search for alternative solutions to the classic problems affecting the farming/forestry sector, by applying the basic knowledge generated in the last few decades in relation to the genomics and molecular biology of plants and associated organisms. This objective is divided into to main areas: crop protection against diseases caused by bacteria and fungi, and the use of lignocellulose farming waste to make biofuel.

Plant Response arose from an academic setting combining the technological tradition of the Polytechnic University of Madris with considerable involvement in basic research related to the molecular biology of plants and microorganisms. At Plant Response, we believe that plants have the response to what these new technological strategies should be. One of these new strategies is the use for agricultural purposes of plants' basal defence mechanisms, which have been providing them with long-lasting, broad spectrum immunity along the evolution. In the last few years, we have found that plants, through specific cell receptors, are capable of recognising superficial pathogenic molecules/structures which are generically known as MAMPs (microbe-associated molecular patterns) or elicitors.

In sum, the objectives of Plant Response when responding to market needs can be summarised as:

- To help the farming sector to successfully produce healthier foodstuffs.
- To optimise energy production from biomass.
- To reduce the impact of agriculture on the environment, optimising production with pest control.
- To help companies to develop R&D and innovation plans in the field.

Plant Response is well positioned on the market thanks to the following competitive advantages:

1. Management team expertise and know-how: Plant Response has highly qualified personnel comprising investigators experienced in the business areas of interest, thanks to their excellence in university research and permanent contact with the business sector by means of different collaboration agreements.
2. Facilities, equipment and technology: facilities among the most advanced in Spain, ideal for the development of research using the latest technology.

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