June 2020

Towards seed protection using biocontrol strategies

Bailly C. (Sorbonne Université, IPBS), Bousquet A. (RAGT), Braun V. (Syngenta), Buitink J. (INRAE, IRHS), Desbois-Vimont C. (Limagrain, Vilmorin & Co), Durand-Tardif M. (GIS-BV), Fougereux J.-A. (FNAMS), Gaillard A. (MAS Seeds), Gouleau A. (GEVES), Grappin P. (Institut Agro, IRHS), Hily Y. (Vegepolys Valley), Lejard F. (Bayer), Malausa T. (INRAE, Institut Sophia Agrobiotech), Marchi M. (INRAE, IRHS), Multeau C. (INRAE, Département SPE), Orgeur G. (GEVES), Piovan R. (GIS-BV), Poisson A.-S. (GEVES), Profizi C. (Agrauxine), Rajjou L. (AgroParisTech, IJPB), Reboud X. (INRAE, Agroécologie), Szambien M. (GIS-BV)

The French "Consortium public-privé Recherche-Développement-Innovation sur le Biocontrôle" and the "Groupement d'Intérêt Scientifique Biotechnologies Vertes" (GIS BV), both public-private groups, organized a scientific workshop on "Seeds and biocontrol: solutions for tomorrow's agricultures" on October 2nd, 2019 in Paris. Seed biocontrol is an emerging research topic that could meet economic issues and environmental and societal expectations to reduce the use of chemical farm inputs and adapt agriculture practices to climate change. Seventy-eight scientists, including forty-five from the private sector joined this workshop in order to debate on the most promising research areas to address a successful implementation of biocontrol solutions. They unanimously agreed that effective biological control solutions will result from multidisciplinary approaches and require innovative in plant breeding developments.

This article describes the current strengths of French research in an international context around seed and biocontrol and highlights the research priorities between the public and the private sectors, the upmost being transdisciplinarity for the benefit of future agricultures.

Introduction

The French private seed sector represents 3,3 Billion euros of Annual Turnover, almost 12 000 employees and 400 Million Euros invested in R&D in 2018/2019. The seed, as propagating material, is the first input in cropping system. Seed quality, usually defined by germination, sanitary and genetic quality, is therefore a priority for growers. Seed- and soil-borne pathogens, nematodes and insect pests can limit or inhibit germination and seedling emergence, thus impacting crop yield, and can also play a major role in epidemic of bioagressors. To secure seed quality and high yield, plant protection products are commonly used during seed production and on seeds after harvest before sowing. The development of biocontrol products in the last decades can be a key lever in seed and plantlet protection. According to the French legislation, biocontrol refers to a set of methods relying on natural mechanisms to protect plant against bioagressors, in the framework of integrated pest management. These methods are based on the action of macroorganisms, microorganisms, semiochemicals or natural substances (article L253-6 of the Code Rural et de la Pêche Maritime). Mostly defined as minor uses, seed treatments are not widely supported for authorizations. Only three biocontrol seed treatments are currently recorded in the French list of biocontrol products[1] and they are authorized only for a few crops (ANSES, 2020/03/26).

[1] According to articles L253-5 and L253-7 of the Code Rural et de la Pêche Maritime (dated February 26, 2020): VOTIVO (Bacillus firmus I-1582) on sugar beet and forage beet seeds, INTEGRAL PRO (Bacillus amyloliquefaciens MBI 600) on OilSeedRape and field mustard or "Navette", against fungi other than Pythiaceae and CERALL (Pseudomonas chlororaphis MA342) on rye and wheat seeds against fungi other than Pythiaceae.

Plant-variety improvement through partial and complete resistance selection exists to cope with many diseases, however very few traits of resistance to seed or soil-borne diseases have been characterized to date. Agronomic practices (i.e. rotations, tillage...), are also used but not always sufficient to protect seeds and seedlings. This bottleneck leads actors from research and technical institutes to explore alternative solutions considering seed as a material to protect and as a vector for innovation.

A number of research areas have been identified in this study to contribute to the expansion of biocontrol use for seed protection. They are summarized here below:

- Seed biology and seed's biotic environment to identify new biocontrol methods and to optimize their efficacy within various seed development stages and pedoclimatic conditions.
- Role of the mother plant in enhancing seed protection (seed microbiota, seed and seedling defense gene priming) through vertical transmission of biocontrol agents to the seed and role of the treated seed in vectorizing biocontrol strategies to protect mature crops (defense elicitation, endophytes...).
- Combination between genotype and biocontrol products, especially microbial inoculants known to establish molecular dialogues, to adapt varieties to biocontrol products and vice versa.
- Adaptation of seed treatment formulation to biocontrol products to make them compatible with seed processes.
- Development and use of new technologies, like sensors and other phenotyping tools, which could play a key role in the management of seed protection with biocontrol products and foliar applications after sowing.
- Evaluation of the protection mechanism sustainability and of biocontrol application efficacy.

At the forefront of science

Seeds allow plant multiplication in most agrosystems. The development and dispersal of seeds as well as their transition to seedlings represent the most critical stages of a plant life cycle. The formation of mature seed starts after the plant pollination and goes through the development of three seed tissues: the embryo, the endosperm and the seed coat. Besides the deposition of storage reserves, seed maturation is characterized by the acquisition of functional traits that allow germination and seedling establishment until favourable conditions.

Seed vigour is a concept used in the seed industry to define the seed performances in the field, namely rate and uniformity of seed germination and seedling growth, emergence ability under unfavourable environmental conditions and retention of these characteristics during storage (Rajjou et al., 2012). These characteristics are acquired during seed development and influenced by genotype x environment interactions. The production of highly vigorous seeds is a key leverage to improve and stabilize yield. It is also admitted that rapid germination limits pathogen damages. However, germination phenology is likely to be largely affected by biotic and abiotic stresses, at any stage of seed life from development to germination. The development of biocontrol solutions as key success factor to increase seed protection, needs in-depth and multidisciplinary knowledge relative to seed biology and seed interactions with the environment.

Innate seed resistance

Seeds have well-established passive physical and chemical defence mechanisms that protect them from decay-inducing organisms and phytophagous pests. For instance, they constitutively produce antimicrobial compounds or can have reinforced cell-walls. In the dry quiescent state, the seed cannot activate defence responses, so the expression of defence-related components is a programmed mechanism activated during seed maturation, while seeds are still attached to the mother-plant. It allows to install passive barriers as a preemptive strategy to protect the embryos from pathogen attack. These mechanisms are also known to be activated in dormant seeds that go through hydration cycles in the soil (Bolingue et al. 2010, Fuerst et al., 2018). The activation of these passive defence mechanisms is influenced both by genetic and environmental factors on the mother plant during seed production. Indeed, there exists a large natural variation in the level of phytochemical compounds in seeds throughout species, and elicitation of defence responses in a seed can occur when the mother plant is under attack from pathogens. In addition, the environmental conditions experienced by the mother plants during seed development have a direct effect on seed dormancy and vigour at harvest. A better knowledge of these mechanisms and their activation represent an interesting lever to increase seed innate resistance during seed production or seedling establishment, or for engineering biocontrol products

Immune priming during seed multiplication

Plant defense is triggered by exposure to microorganisms and their derived molecules. In the immune response that is orchestrated by plant regulators, defense genes and antimicrobial molecules are generally induced locally and in whole-plant, commonly referring to the local acquired resistance (LAR), induced systemic resistance (ISR) and systemic acquired resistance (SAR) respectively (Pieterse et Van Wees, 2015). Interestingly, the sensitivity of their defense systems could also be enhanced avoiding a direct induction of defense genes (Pieterse et al., 2014). This “alert stratus”, also called immune priming (Martinez-Medina et al., 2016), involves a low fitness costs (van Hulten et al., 2006) and maintains an immunological memory that can be transmitted to the next progeny during seed formation. Noticeably, epigenetic modifications induced by priming stimulation, i.e. chromatin remodeling or DNA methylation levels, have been documented by several studies (Jaskiewicz et al., 2011) in a way that defense gene promoters become easier to activate (Conrath et al., 2015) in case of subsequent stresses (Bruce et al., 2007; van den Burg and Takken, 2009). Epigenetic modifications are one of the mechanisms that enable plants to acquire stress memory that can even be inherited by subsequent generations as illustrated by Molinier et al. (2006). A better understanding of these mechanisms transmitted to the next progeny during seed development will open opportunities to improve pest management of crops during seed production.

Seed microbiota

Dry seeds represent a stressful environment. Microorganisms associated to seeds, also called seed microbiota, have developed biological mechanisms in order to colonize them (N’Guyen et al., 2019). They are considered as the primary inoculum for plants and may have an impact on plant’s possibilities to deal with biotic and abiotic stresses (Wassermann et al., 2019). So, a promising lever to improve seed health and seedling establishment is to act on this primary inoculum in order to limit the transmission of seed-borne pathogens and to increase plant protection abilities against soil-borne pathogens. Seed microbiota result from distinct way of contamination, with microorganisms vertically transmitted from the mother plant and horizontally transmitted from the environment. Geographic site, soil type, soil microbiome and plant genotype impact seed microbiota composition (Klaedtke et al., 2016; Pérez-Jaramillo et al., 2019). Seed microbiota optimization could be considered using multi-strains microbial inoculum also called Synthetic communities (SynComs) applied to the mother-plant or at the surface of the seed. As the robustness of beneficial microbial trait expression is increased by redundancy (*i.e* by multiple strains) and dominance (*i.e* whenever one strain has the respective trait), communities’ efficiency will depend on SynCom’s design. The use of native, locally adapted plant associated microbes for plant field protection has been highlighted in several studies (Vannier et al., 2019). These results raise research questions about the adequacy of the SynCom composition with the plant and the local environment, and only little is known today. Moreover, due to the influence of plant genotype and environment on seed microbiota, breeding strategies that include microbial beneficial traits and agronomic practices impact also need to be considered.

Crop genotype and biocontrol product

Each plant species is able to recruit specific rhizosphere microbial assemblages and genotypes of the same species may differ in their rhizosphere microbiome composition (Pérez-Jaramillo et al., 2016), which may affect the successful establishment of introduced biocontrol microorganisms. When colonizing roots and/or internal tissues, a biochemical dialog appears between the biocontrol agent and the crop. The plant itself, by its exudates or rootlets architecture, can favor the beneficial agent development. Hence, considering the interaction biocontrol x genotype x cultural practice will be a success key in the development of both biocontrol solutions and crop varieties. In the target environment, objectives will be to develop (i) varieties favouring the attraction and establishment of biocontrol agents as well as their elicitation effect on constitutive and inducible defences, and (ii) biocontrol solutions combining as many beneficial effects as possible: biocide for pathogens, competing for space and resource with pathogens, enhancing the response of the plant towards abiotic stresses – concomitant biostimulation effect, etc. . This will need an in-depth knowledge on molecular dialogues and genetic determinisms which promote the interaction of plants with beneficial microbes, on biological mechanisms mediating constitutive and inducible defences activation in response to biocontrol and on the conditions of successful establishment and action of biocontrol agents.

Seed technology

Seed technology gathers techniques that improve seed quality. In particular, seed treatment reduces damages due to seed transmitted pathogens and damping-off diseases that can drastically damage germination and impact final crop yield. While chemicals are often small organic or inorganic molecules for which seed treatment processes and specific formulations have been developed, biocontrol products are biological agents or biomolecules that need specific formulations to guarantee their stability and efficacy (Rocha et al., 2019).

Selection and production of microorganisms and bio-based molecules

Until now biocontrol products applied to seeds were previously homologated for foliar application. These products have been historically hampered by the variation in efficacy of the microbial strains employed, partly explained by the empirical selection of biocontrol agents (Barret et al., 2016). Most of microorganisms used were isolated from soil and rhizosphere of wild and cultivated plants (Harman, 2000; Hökeberg et al., 1997) whereas microorganisms sourced from seeds seemed underutilized.

In the current context, it appears crucial to characterize novel treatment solutions adapted to seeds, either to cope with pathogens or to positively influence seedling establishment. Special attention should be brought on the influence of (i) plant genotype, (ii) seed physiological quality during maturation and at harvest, and (iii) environmental factors on seed microbiota structure. On the one hand and interestingly, decrease of microbiota diversity (including soil-borne pathogens) is often observed during germination and seedling establishment (Barret et al., 2015). The best would be to keep only the beneficial ones. On the other hand, bio-based molecules (i.e. polyphenols, polysaccharides, proteins/peptides, nucleic acids, organic acids and hormones) coming from seed exudates at imbibition and/or seed coat interfere with the dynamics of microbial communities. Interdisciplinary research and development are essential to optimize microbes (single or communities) and/or biomolecules to display a variety of physico-chemical properties adapted to seeds.

The use of micoorganisms for biocontrol can be oriented towards several kinds of products like:

- Spores (when the microorganisms can sporulate)
- Microorganisms in a vegetative form
- Molecules (metabolites, peptides, small RNA…) produced by microorganisms
- Mix of microorganisms and bio-based molecules.

In the fermentation processes a production of a large biomass is often at the expense of the desired activity. Overcoming the trade-off between the cost of production and the expected product efficiency is a real industrial challenge that can only be solved by mobilizing front science technologies like high volume of data processing and machine learning processes. It must be underlined that production chain optimization need quantitation of the final activity that is not easy to reach with biocontrol products. For this correlation with a marker that can be traced during the fermentation is even more challenging.

Stability and conservation of biocontrol solutions applied on seeds

Several challenges appear when biocontrol solutions are applied on seeds.

First when choosing the biocontrol solution to apply on a given seed lot.

The "Classical" supply chain responds to several constraints at the scale of the seed lot (genetic, sanitary, germination) and at the scale of the whole seed process (seed processing, quality requirements, number and diversity of crops and varieties, requiring several production lines...). At the top, comes the correct association of a compatible biocontrol product and a plant genotype, or concordant supply chains of both seeds and biocontrol products in suitable quantity and quality (stability, concentration, nature of impurities, delivery time...). There are also regulatory constraints, like the country of seed origin, or the diversity of legislation according to countries (authorization of sowing of treated seeds of a given crop).

Second during application on seeds.

In most of cases, seed applications are done with water-based slurries. Extemporaneous incorporation of biocontrol agent in the seed application slurry a few minutes before seed application can limit the physiological activation of microorganisms and increase their survival at seed surface. The ideal situation would be to apply microorganisms without having to dehydrate and rehydrate them, like by:

- Endophytic colonization during seed development on the mother plant,
- Incorporation during wet processes like priming,
- Coating in lipid phase (similar to pet food pellets application of palatability agents),
- Injection of microorganisms in large enough seeds (corn, sunflower…), process that exist at laboratory scale (Reichenberger and al., 2017),
- Extemporaneous mixing of the microorganisms with the seeds at sowing.

In addition, biocontrol agents and substances need to be compatible with the other compounds of the seed application (fungicides, insecticides, pigments…) and their associated coformulants (preservatives...). The slurry mixing technology can be considered only for spore seed coating; new mild coating technologies should be developed for surface coating allowing oxygenation to preserve living microorganisms. Application processes contains or are followed by a drying step, which can also lead to microorganisms death (several logs of mortality) and denature some complex substances. All these parameters imply to develop a biocontrol formulation compatible with seed treatment and different from other uses (plant or soil applications).

Third during seed logistics between application on seeds and sowing.

Challenges are adequate storage before and during shipment (including delays to perform Quality Control tests just after application or validate phytosanitary checks to cross countries boarders). Once seeds are by the final client, the conditions of storage (usually between 0 to 18 months) directly impact the biocontrol efficacy.

Evaluation of biocontrol products: from their effectiveness to their environmental impact

- **Evaluation methods and tools allow the characterisation of products and their integration into cropping systems.**

Existing evaluation methods have been developed for chemicals in conventional crop production systems based on their spectrum of action, effectiveness and mode of action. Given the diversity, selectivity and the nature of potential biocontrol products, the current evaluation methods are not appropriate. The development and deployment of biocontrol products for seed require new tool development for the assessment of their efficacy, their environmental impact or the quality of the final agricultural product. This assessment is essential to ensure the proper integration of these solutions into cropping systems.

Methods and tools for the determination of the efficacy

Screening and characterisation of the effect of biocontrol products are long and costly processes that are mainly based on trials under optimal and controlled growth conditions. However, effectiveness in the laboratory and/or greenhouse does not always translate into success in the field (Parnell et al., 2016). The variability in the effectiveness of these products could result from variations in environmental conditions, the physiological state of the plants, or the crop practices. According to Rocha and colleagues (2019), this inconstancy field performance can be one of the main restraints for the wide application of seed coating with plant beneficial microbes. Contrary to conventional pesticides, efficacy of biocontrol products is not based on the eradication of bioaggressors but rather on the installation of a biological balance in the plant ecosystem. Thus, although they have a very positive image and a major interest in pesticide reduction, biocontrol products do not seem to offer enough guarantees as to their effectiveness, which limits their use by farmers. In order to accelerate the availability of effective biocontrol products, methods and tools for evaluating the effectiveness of these alternatives to pesticides must be developed, adapted or optimised to provide an adequate experimental framework.
Currently, robustness and plasticity of biocontrol solutions checked in numerous real representative conditions are highly recommended in biocontrol seed treatment development, with associated statistical analysis that will help concluding. Environmental parameters measurements can help to identify best conditions for expression of biocontrol seed treatment efficacy. Either direct (speed of beneficial agent root colonisation, % of contaminated plantlets, severity of symptoms, grow out level in the field…) or indirect aspects (final crop yield, elicitation markers quantification…) can be quantified to check this success. But research efforts are still necessary for better understanding of the key parameters of field efficacy related to the diversity and nature of these biocontrol products and their mode of action (Nicot et al., 2011). The study of efficacy cannot be carried out without the help of high-performance measurement, calculation and analysis tools, and their development depend on the understanding of the biological mechanisms and ecological interactions, as well as the implementation of the digital and new technologies for relevant decision-support tools, sensors and phenotyping tools. It is thanks to such development that the methodologies used, whether under controlled conditions or in the field, will be better adapted to determine in the end the field efficacy of biocontrol products.

Potential impact of biocontrol seed treatments on the performance (reliability and detection threshold) of standard methods used to detect pathogens on seeds

To a large extent, seeds traded around the world have received treatment, which can be a physical treatment, chemical or biological treatment. All these different treatments can have consequences regarding to the methods of detection of pathogens in seed lots (Sérandat *et al.*, 2019). It is important for seed trade to be able to define if pests can be detected on treated seeds and to know if the result is due to the efficiency of treatment or if the treatment affects the performance of the method for detecting the pest. With most non-chemical seed treatments, pathogens may still be detectable even though they are non-viable. So, additional measures to determine the viability of any pest detected after treatment is required. An evaluation of the effectiveness of these biocontrol treatment methods remains essential.

To reach this goal, the capacity of transmission of the pathogen from seed to plant (pathosystem) is being evaluated and the treatment effectiveness is being assessed. Viability and damage potential of the pathogens can be evaluated using the existing detection methods (grow out, culture on media…) but there is also a need to develop new complementary methods (vital staining, bio test...). Current detection methods have mainly been developed and validated for non-treated seeds. Due to the high diversity of treatments, it could be difficult to validate the methods for each type of treatment and so it is necessary either to review and adapt current methods or develop and validate new ones. In our globalized market context, the presence of pathogens on seeds needs to be detected and their damage potential needs to be assessed.

Evaluation of the sustainability of biocontrol product

Evaluation of the risk of release into the environment

Biocontrol products are composed of microbial agents and/or complex mixture of molecules naturally present in the environment. They are therefore generally considered as a safe and environmentally friendly alternative to conventional pesticides. Whether one agrees or not with this assumption, all agree that further studies are needed to provide scientific evidences on the environmental fate and behaviour of biocontrol products in order to ensure environment protection, to support the sustainability of these innovative products or to encourage and accelerate the placing on the market of new biocontrol products. Indeed, placing a Plant Protection Product on the European market, biocontrol or not, requires an environmental risk assessment (Regulation EC 414/1991) by authorities involving extensive testing and a significant amount of data to be provided by the applicants. Better knowledge and understanding of potential adverse effects are crucial for the determination of appropriate data requirements as well as for the development of reliable, relevant and reproducible assessment methods.

Evaluation of the sustainability of the crop protection

According to the official French definition, biocontrol is based on managing the balance of pest populations through natural mechanisms rather than on their eradication. Under the principles of agroecology, biocontrol products must be used in combination with other leverages to ensure effective and sustainable crop protection and not be part of a dynamic of substitution of chemical plant protection products. However, care must be taken not to rule out the risks of circumvention of natural mechanisms by phytopathogenic agents that may occur with the massive and repeated use of biocontrol products. Indeed, it is important to bear in mind that natural does not mean sustainable, depending on the use made of it. It is therefore essential to improve our knowledge of potential resistance to biocontrol products and to develop tools and methods to assess and control this risk.

Conclusion

New biocontrol solutions, crop varieties and their combination are important levers to reach sustainable seed and crop protection strategies. Biology and ecology of three parties have now to be put in balance when developing biocontrol methods and crop varieties : the plant genotype, the biocontrol agent at the strain level for microorganisms and their environment at the scale of soil and rhizosphere for seed protection.

In France, research community on biocontrol started to jointly address those questions by transdisciplinary approaches however a few numbers of initiatives are specifically centered on seed protection. The scientific workshop organized in Paris on October 2019 was a first step to establish close connections between research communities focusing on seeds and biocontrol and will lead to new partnerships.

It is noteworthy that the successful integration of biocontrol strategies in technical routes will rely on the design of new cropping systems combining several levers, such as biocontrol or genetic, to reach an equilibrium between yield and sustainability. Some biostimulation strategies were already successfully implemented in technical routes. For example, commercialization of Myc (Mycorrhizal) and Nod (Nodulation) factors, both involved in the establishment of microbial (fungal and bacterial respectively) root symbiosis, allowed sustainable yield increasing by enhancing plant capacities to fix nitrogen without adding chemical fertilizers. Nod factors are lipochitooligosaccharide (LCO) able to promote germination and plant growth of both legumes and non-legumes when applied as a seed treatment. Seed-applied LCO technology for crops are already available (Smith et al., 2015). Developing optimal associations between biocontrol and biostimulation strategies in innovative cropping systems will lead to an agriculture less and less dependent of chemical inputs. Furthermore, in upcoming years, innovative cropping systems will more and more lean on precision agriculture. Therefore, bridges have also to be built with research communities on digital farming and cropping systems.

Annex 1. List of French academic laboratories involved in biocontrol research community (with potential application for seed protection)

Unit	City	Plant	Biocontrol strategies
Agroécologie	Dijon	Agricultural crops	Microorganisms against phytopathogenic fungi
BGPI	Montpellier	Agricultural crops	Plant defense inducers, epidemiology and evolution of phytopathogens (and microbial agents)
BIOGER	Grignon	Agricultural crops	Phytopathogenic microorganisms and microbial substances against phytopathogenic microorganisms
I2BC	Gif s/ Yvette	Agricultural crops	Microorganisms against phytophatogenic microorganisms
IBPS	Paris	Model species and Agricultural crops	Natural substances enhancing seed vigour
IGEPP	Rennes	Agricultural crops	Natural substances against phytopathogenic nematods
IJPB	Versailles	Model species and Agricultural crops	Plant defense inducers, beneficial microorganisms, stimulation of seeds
IPME	Montpellier	Agricultural crops	Bacteria against phytopathogenic nematods
IPS2	Saclay	Model species	Microorganisms Phytopathogenic fungi on cereals
IRHS	Angers	Agricultural crops	Plant defense inducers, seed vectorization of microbial biocontrol agents (bacteria, fungi)
ISA	Sophia-Antipolis	Agricultural crops	Microorganisms against phytopathogenic oomycetes
LEM	Lyon	Agricultural crops	Microorganisms against phytopathogenic bacteria and fungi
LRSV	Toulouse	Model species	Microorganisms and natural substances against phytopathogens
LSTM	Montpellier	Mediterranean and tropical plants	Microorganisms against phytopathogenic bacteria and fungi
MCAM	Paris	Agricultural crops	Bacterial and fungi endophytes against phytopathogenic microorganisms
LUBEM	Plouzané	Agricultural crops	Microorganisms against phytopathogenic fungi on cereals
PV	Avignon	Agricultural crops	Microorganisms against phytophatogenic microorganisms
ProBioGEM	Lille	Agricultural crops	Microorganisms and microbial substances against phytopathogenic microorganisms
RIBP	Reims	Agricultural crops	Plant defense inducers and bacteria against phytopathogenic microorganisms

Annex 2. List of French academic laboratories involved in seed protection research community

Unit	City	Plant	Topic
AGIR – AGroecologie, Innovations, teRritoires	Toulouse	Agricultural crops	Seed germination and seedling emergence
IBPS – Institut de Biologie Paris-Seine	Paris	Model species and Agricultural crops	Seed physiology, dormancy, germination
IJPB - Institut Jean-Pierre Bourgin	Versailles	Model species and Agricultural crops	Seed physiology and seed filling, storage and germination vigour, seed priming
IRHS – Institut de Recherche en Horticulture et Semences	Angers	Agricultural crops	Seed and seedling physiology (maturation, conservation, emergence)

Annex 3. References

1. Babin A, Nawrot-Esposito MP, Gallet A, Gatti JL, Poirié M. 2019. Adverse effects of Bacillus thuringiensis bioinsecticide on non-target Drosophila species. Preprint 10.1101/541847.
2. Barret M, Briand M, Bonneau S, Préveaux A, Valière S, Bouchez O, Hunault G, Simoneau P, Jacques MA. 2015. Emergence shapes the structure of the seed microbiota. Appl. Environ. Microbiol., 81(4): 1257-1266.
3. Barret, M., Guimbaud, J. F., Darrasse, A., & Jacques, M. A. (2016). Plant microbiota affects seed transmission of phytopathogenic microorganisms. Molecular plant pathology, 17(6), 791.
4. Bolingue W, Rosnoblet C, Leprince O, Ly Vu B, Aubry C, Buitink J. 2010. The MtSNF4b subunit of the sucrose non-fermenting-related kinase complex connects after-ripening and constitutive defense responses in seeds of Medicago truncatula. Plant J. 61: 792–803
5. Bruce TJA, Matthes MC, Napier JA, Pickett JA. 2007. Stressful “memories” of plants: evidence and possible mechanisms. Plant Sci. 173: 603–608
6. van den Burg HA, Takken FLW. 2009. Does chromatin remodeling mark systemic acquired resistance? Trends Plant Sci. 14: 286–294
7. Code Rural et de la Pêche Maritime. 2018. Article L253-6
8. Conrath U, Beckers GJM, Langenbach CJG, Jaskiewicz MR. 2015. Priming for enhanced defense. Annu. Rev. Phytopathol. 53:97–119
9. Fuerst EP, James MS, Pollard AT, Okubara PA. 2018. Defense Enzyme Responses in Dormant Wild Oat and Wheat Caryopses Challenged with a Seed Decay Pathogen. Front Plant Sci. 8: 2259
10. Harman, G. E. (2000). Changes in perception derived from research on Trichoderma harzianum T− 22 (Myths and dogmas of biocontrol). Plant Dis, 84, 377-393.
11. Hökeberg, M., Gerhardson, B., & Johnsson, L. (1997). Biological control of cereal seed-borne diseases by seed bacterization with greenhouse-selected bacteria. European Journal of Plant Pathology, 103(1), 25-33.
12. van Hulten M, Pelser M, van Loon LC, Pieterse CM, Ton J. 2006. Costs and benefits of priming for defense in Arabidopsis. PNAS 103: 5602–5607
13. Jaskiewicz M, Conrath U, Peterhänsel C. 2011. Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. EMBO Rep. 12: 50–55
14. Klaedtke S, Jacques MA, Raggi L, Préveaux A, Bonneau S, Negri V, Chable V, Barret, M. 2016. Terroir is a key driver of seed-associated microbial assemblages. Environ. Microbiol. 18: 1792-1804.
15. Martinez-Medina A, Flors V, Heil M, Mauch-Mani B, Pieterse CMJ, Pozo MJ, Ton J, van Dam NM, Conrath U. 2016. Recognizing plant defense priming. Trends in Plant Sci. 21: 818–822.
16. Molinier J, Ries G, Zipfel C, Hohn B. 2006. Transgeneration memory of stress in plants. Nature 442: 1046–1049
17. N'Guyen, G., Raulo, R., Brisach, C. A., Iacomi, B., Pelletier, S., Renou, J. P., ... & Mouchès, C. (2019). Responses to hydric stress in the seed-borne Necrotrophic fungus Alternaria brassicicola. Frontiers in microbiology, 10, 1969.
18. Nicot P, Blum B, Kohl J, Ruocco M. 2011. Perspectives for future research-and-development projects on biological control of plant pests and diseases, in Classical and Augmentative Biological Control Against Diseases and Pests: Critical Status Analysis and Review of Factors Influencing Their Success, ed. P. Nicot (Zürich: IOB-International Organisation for Biological and Integrated Control of Noxious Animals and Plants), 68–70.
19. Parnell JJ, Berka R, Young HA, Sturino JM, Kang Y, Barnhart DM and DiLeo MV. 2016. From the Lab to the Farm: An Industrial Perspective of Plant Beneficial Microorganisms. Front. Plant Sci. 7: 1110.
20. Pérez-Jaramillo JE, de Hollander M, Ramírez CA, Mendes R, Raaijmakers JM, Carrión VJ. 2019. Deciphering rhizosphere microbiome assembly of wild and modern common bean (Phaseolus vulgaris) in native and agricultural soils from Colombia. Microbiome. 7(1):114.
21. Pérez-Jaramillo JE, Mendes R, Raaijmakers, JM. 2016. Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol. Biol. 90: 635–644.
22. Pieterse C, Van Wees SC. 2015. Induced disease resistance. In: Lugtenberg B, ed.Principles of plant–microbe interactions. Cham, Switzerland: Springer International Publishing. 123–133.

23. Pieterse CM, Zamioudis C, Berendsen RL, Weller DM, Van Wees SC, Bakker PA. 2014. Induced Systemic Resistance by Beneficial Microbes. Annu. Rev. Phytopathol. 52(1): 347-375.
24. Rajjou, L., Duval, M., Gallardo, K., Catusse, J., Bally, J., Job, C., Job, D. 2012. Seed germination and vigor. Annu. Rev. Plant Biol. 63: 507-533.
25. Reichenberger G., Pfaffenbichler N., Mitter B., Riesing J., Brader G. 2017. WO2017162758A1 Patent. Seed injection.
26. Rocha I, Ma Y, Souza-Alonso P, Vosátka M, Freitas H, Oliveira RS. 2019. Seed Coating: A Tool for Delivering Beneficial Microbes to Agricultural Crops. Front. Plant Sci. 10: 1357.
27. Sérandat I, Orgeur G, Grimault V. 2019. How to adapt methods to evaluate seed health of treated seeds. Communication presented at ISTA congress, Hyderabad, India.
28. Smith, R. S., Habib, A., & Kosanke, J. (2015). *U.S. Patent No. 8,992,653*. Washington, DC: U.S. Patent and Trademark Office.
29. Vannier N, Agler M, Hacquard S. 2019. Microbiota-mediated disease resistance in plants. PLoS pathogens. 15(6), e1007740.
30. Wassermann B, Adam E, Cernava T, Berg G. 2019. Understanding the Indigenous Seed Microbiota to Design Bacterial Seed Treatments (eds) Seed Endophytes. Springer, Cham.

9 782957 301607