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Avoidance of detrimental defense responses in beneficial plant-microbe interactions

Benjamin Gourion¹ and Pascal Ratet^{2,3}

¹LIPME, Université de Toulouse, INRAE, CNRS, Castanet-Tolosan, France

²Université Paris-Saclay, CNRS, INRAE, Univ Evry, Institute of Plant Sciences Paris-Saclay (IPS2), 91405, Orsay, France.

³Institute of Plant Sciences Paris-Saclay IPS2, Paris Diderot, Sorbonne Paris-Cité, Bâtiment 630, 91405, Orsay, France.

Corresponding author: P. Ratet (pascal.ratet@cnrs.fr)

Highlights:

- Plant beneficial microorganisms can be rhizospheric, epiphytic or endophytic.
- Plant benefits include resistance to abiotic and biotic stress, as well as nutritional benefits.
- Beneficial microorganisms are generally first recognized as invaders.
- Beneficial microorganisms use multiple mechanisms to protect themselves against plant defense reactions.
- Intimate mutualistic relationships involve plant controlled active suppression of immunity.

Abstract

In the environment microbes interact with plants and provide them with benefits that include protection against biotic and abiotic stresses as well as improved nutrition. However, plants are also exposed to parasites and pathogens. To manage appropriate responses, evolution has resulted in improved tolerance of plants to beneficial microbes while keeping the ability to recognize detrimental ones and to develop defense responses. Here we review the mechanisms involved in these interactions. We also discuss how the interactions might be handled to improve crop resistance to pathogens without losing the ability to establish beneficial interactions.

Plants live in a microbial world for better or for worse

Plants are surrounded by a myriad of microorganisms both at the root level and in the aerial parts. While the vast majority of these microbes do not seem to influence the plant's health others interact intimately with the plant and are either beneficial or detrimental. These interactions have strong impacts on plant evolution [1]. Although pathogenic interactions have been and are still the focus of the very large majority of

research on plant microbe interactions [2], the study of mutualistic interactions has encountered a renewed interest notably with the development of research conducted on plant microbiota.

Beneficial microorganisms can protect plants against diseases by direct competition and niche occupation, antibiosis and plant defense priming. Microorganisms can also provide protection against abiotic stress such as desiccation, salts, heavy metals [3] and even against exposure to high temperature [4]. Finally, microorganisms can drastically improve plant nutrition. For instance, arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) or both [5] collaborate with plants to improve their phosphorus nutrition, whereas sulfur and potassium nutrition can be improved by the presence of rhizospheric microorganisms. However, one of the most spectacular nutritional benefits is obtained by nodulating plants interacting with nitrogen-fixing bacteria.

Plant defense mechanisms are made of multiples barriers

Plants release in the soil primary and secondary metabolites that contribute to shape the structure of the microbiota through their nutritional effects or their antimicrobial activity. Plant defense against microbes also includes physical barriers, such as cuticles, that can prevent infection by invaders not genetically equipped to disrupt them. Different perception systems allow the plant to detect intrusions [6]. Amongst them, plant membrane pattern recognition receptors (PRR) sense conserved microbial associated molecular patterns (MAMP) or endogenous plant molecules that are normally extracellular. Sensitivity to MAMP was recently proposed to be induced by wounding [7]. Furthermore, cytosolic nucleotide-binding leucine-rich repeat (NLR) receptors, perceive pathogen effectors inside the plant cells. This effector recognition triggers the development of intense defense reactions that can lead to effector triggered immunity (ETI), associated with local controlled cell death. Perception of pathogens by the plant cells can lead to the production of reactive oxygen species (ROS), synthesis of antimicrobial compounds, transcription of defense related genes and production of defense hormones. The latter will prime plant defense reactions distantly from the perception site to activate induced systemic resistance (ISR) [8]. In general, the more intimate the contact, the stronger the defense reactions will be.

Various types of benefit and degree of proximity require different levels of control of immunity

Beneficial interactions are not exempt from activation of defense reactions. Here are a few examples: i) when the benefit is ISR, plant defense reactions are not turned off. This is for instance the case of incompatible Bradyrhizobium mediated ISR in soybean [9], ii) local cell death typical of a strong plant response to pathogens can be used by beneficial bacteria to initiate colonization [10], iii) some plant factors have detrimental effects on their symbionts [11]. Thus, plant defense responses are not totally avoided even during beneficial interactions.

This said, interacting with beneficial microorganisms generally requires avoiding killing them. Thus, exudation of metabolites, plant driven infection programs and/or local suppression of defense responses are used by plants to influence the microbial community structure around and in their tissues. Furthermore, as the development of defense reactions against beneficial microbes would annihilate the benefit of the association, the plant immune response has to be controlled.

The level of this control depends on the degree of proximity that beneficial microbes share with their host. For instance, rhizospheric PSB associated with mycorrhizal fungus

remain *a priori* at a reasonable distance from the plant and will not induce the same type of reaction as those triggered in intracellular endomycorrhizal arbuscules [12]. More generally, the control of immunity might be less crucial (or absent) for rhizospheric microbes and epiphytes [13] than for endophytic ones (Figure 1).

Avoidance of defense reactions during Legumes-Rhizobia interactions

The association between legumes and rhizobia is one of the best described beneficial interactions during which plant innate immunity has to be tightly controlled. In this symbiosis, soil bacteria reside within the plant cells of root nodules at densities that are higher than those observed for most pathogens. In rhizobia-legume interactions the mechanisms involved in the avoidance of defense responses have been studied at various stages of the interactions and several molecular actors have been identified: amongst them are plant and bacterial factors. The environment is also a determinant for the output of the interactions.

In this legume-rhizobium interaction, two symbiotic infection processes have been described. The crack entry one seems to be essentially driven by bacteria and is proposed to be the ancestral one [14]. The other infection process is more controlled by the plant and involves an infection structure (the infection thread), initiated at the root hair. It is worth noting that rhizobia as legume endophytes also colonize root and aerial tissues [15]. For most legumes, the symbiotic infection process depends on a molecular dialogue between the host and the microsymbiont that starts with the releasing of flavonoids by the plant into the rhizosphere. These flavonoids are sensed by cytosolic rhizobial proteins and trigger the production and secretion of the so called nodulation factors (NF) that when perceived by compatible hosts activate two processes in the plant: the dedifferentiation of cortical cells that will lead to nodule primordia formation and, concomitantly, the development of infection structures.

In addition to these roles, NF can interfere with pathogen induced ROS production [16] and inhibit MAMP triggered immunity (MTI) in various plants including non-legumes [17]. Such a proposed role of NF is interesting as upon inoculation with rhizobia, plant defense reactions are activated at least transitorily in various legumes (for reviews see [18,19]).

The importance of the control of plant defense from the earliest stage of the interactions can be illustrated by the requirement of rhizobial compatible exopolysaccharides (EPS) to avoid the development of defense reactions [20], an effect likely mediated by MTI suppression [21]. EPS are recognized by specific LysM receptor kinases [22] that allow root hair infection by compatible bacteria. It is also interesting to note that divergence of MAMP in rhizobia might contribute to avoid eliciting plant defense responses [19,23].

Avoidance of ETI is also crucial to initiate a functional symbiosis. For instance, the thaumatin-like protein, Rj4, controls nodulation specificity with rhizobia in soybean as demonstrated using the CRISPR/Cas9 technology [24]. In this interaction, incompatibility is mediated by an ETI-like response [25] activated by the putative effector protein, MA20_12780 [26]. Similarly, different combinations of R gene alleles encoding resistance proteins (Rj2, NNL1) together with alleles of the NOP effector proteins control the specificity of infection in soybean/Lotus-rhizobia symbioses [27-31]. In case of incompatibility the root hair infection is blocked but a functional NF signaling allows infection by the crack entry mechanism.

These studies indicate that the tight control of infection might result in the rejection of microbes equipped to be beneficial for the plant. This negative effect of the strict control may reduce the risk for the plant to be invaded by non-beneficial microorganisms. The

interaction can however elicit ISR and help protect the plant against pathogens as exemplified by [9].

Developing organs dedicated to host microbes to avoid defense reactions

Noteworthy, Rhizobial effectors do not always have a negative impact on compatibility and can even promote the interaction with legumes. For instance, the Bradyrhizobium ErnA and Bel2-5 effectors are sufficient to trigger nodulation on Aeschynomene and soybean plants respectively [32,33]. Such situations are reminiscent of pathogen induced structures such as those triggered by *Agrobacterium tumefaciens* or nematodes. In NF and effector signaling it is interesting to note that the bacteria elicit the development of an organ in which immunity is globally down regulated compared to the roots. Such a strategy might be seen as a way to locally increase tolerance to beneficial microorganisms without altering the overall plant capacity to resist pathogens.

Indeed, later during the interaction, the rhizobia are released into the plant cells where they do not trigger defense responses possibly through down regulation of PRRs [34] even though no rhizobial MAMP active on its host has been identified so far. In addition, in *M. truncatula*, down-regulation of resistance genes in the developing nodule using miRNA, produced a suitable niche for rhizobia [35].

Although plant defense reactions are not visible, functional nodules are still able to develop defense responses and cannot be easily colonized by disarmed pathogens [36]. In *M. truncatula*, using Tnt1 insertion mutants [37], four genes required to avoid triggering defense responses specifically against rhizobia were identified. Those genes prevent the development of what looks like a hypersensitive response. Using proteomics and CRISPR/Cas9 technologies the mechanisms controlling immunity in nodules are starting to be deciphered [38,39] including in soybean [40]. Reminiscent of the cell specific defense program of the root recently described [41], these studies show the existence of an organ specific plant driven control of immunity, triggered by the symbiotic program.

Beyond one to one genotype interactions

Transcriptomic approaches using combinations of plant ecotypes and beneficial strains indicate that signaling might be genotype dependent for both partners [42-46] and highlight that the mechanisms mediating plant responses to mutualists overlap with defense responses. These studies also show the plasticity of the interactions and the multiple ways used by the two partners to adapt to symbiotic life. This implies that studying beneficial interactions *in natura* will need to address highly complex (a)biotic interactions. Indeed roots of a given plant are exposed to multiple microenvironments in which both biotic and abiotic conditions can be drastically different (Figure 2). This may require a tissue or cell specific response from the plant to adapt to all these microenvironments. In the future, in order to better understand these complex situations, we will need to extend the use of the high throughput technologies such as NGS, including single cell transcriptomics, in order to study the establishment/specificity of complex interactions. Such studies and their application may help improving beneficial interactions for the development of a more friendly agriculture in diverse environments.

Beyond the rhizobia/legume associations

The legume-rhizobial interaction described above has been intensively studied and the multiple aspects of immunity control of this symbiosis probably reflect what is or will be observed in other beneficial interactions [8]. There is for example multiple evidence of induction of defense reactions and molecular dialogues between plants and mycorrhizal

fungi during the establishment of this beneficial interaction (reviewed in [47]). Beneficial fungal effectors might be the main tools used by mutualistic fungi to control the interaction [48] but fungi also express ROS scavenging enzymes [49] and plants probably downregulate immunity [50] during these interactions. Another example of defense response avoidance is described for *Bacillus subtilis* that antagonizes the plant pathogen *Erwinia carotovora* and hides its flg22 MAMP with the lantibiotic subtilomycin in order to colonize the plant without triggering defense responses [51].

Biotechnological approaches to improve beneficial interactions; are they opening the door to pathogens?

One may hope that by comparing plant responses to various beneficial microbes we could design plants that better interact with friends. However the complexity of the response (reviewed in [47]) indicates that plants adapt their response to each of the multitude of organisms they encounter. As many signals and responses are common between mutualistic organisms and pathogens, altering one element may reveal new susceptibilities in the plant. For example, several genes that are required for beneficial interactions confer to the plant higher susceptibility to some pathogens [52,53]. This may explain the multiple loss of nodulation observed in the legume clade [54]. In contrast, other symbiotic genes contribute to resistance against pathogens [53,55,56]. The lesson from these examples is that developing plants better interacting with beneficial microbes might result ultimately with plants more susceptible to pathogens. One challenging possibility to avoid this problem is to create plants with an organ dedicated to the beneficial interaction. Such an organ could be inspired from the legume nodule or from the mucilage producing aerial roots of some maize landraces [57].

Conversely, selecting for improved pathogen-resistant plants may result in genotypes with reduced capacity to interact with beneficial partners [58,59]. Breeders should thus also integrate more broadly the evaluation of the interaction with mutualists and even with the microbiome in their selection programs against pathogens. This can be done for example under conditions of reduced (or absence of) fertilizers in which the interactions with beneficial microbes are favored and efficient.

Another possibility to improve the interaction relies on the use of microorganisms with better beneficial performances. This can be achieved by selecting *in situ* such beneficials [60] which can be facilitated by new technologies [61]. Alternatively targeted engineering or experimental evolution approaches can be used to obtain efficient beneficial microorganisms [62,63].

Conflicts of interest

The authors have no conflicts of interest to declare.

CRedit author statement

Pascal Ratet is the corresponding author.

Benjamin Gourion and Pascal Ratet conceptualized, organized and wrote the review. B. Gourion designed the figures.

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Legends to figures:

Figure 1: Relationships between control of plant immunity and types of interactions. Beneficial microbes are found in the plant vicinity in the soil, or on the plant surfaces, in the aerial part or on the roots, or within the tissues. Those microbes confer different advantages to the plants and, depending on their locations, the interactions require different types of control of the plant immunity.

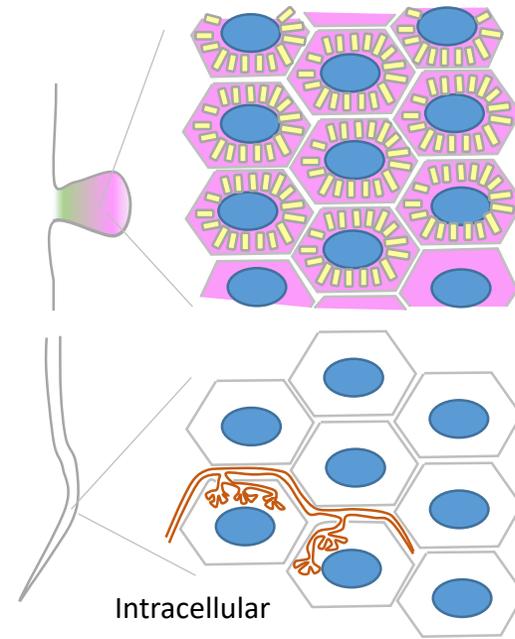
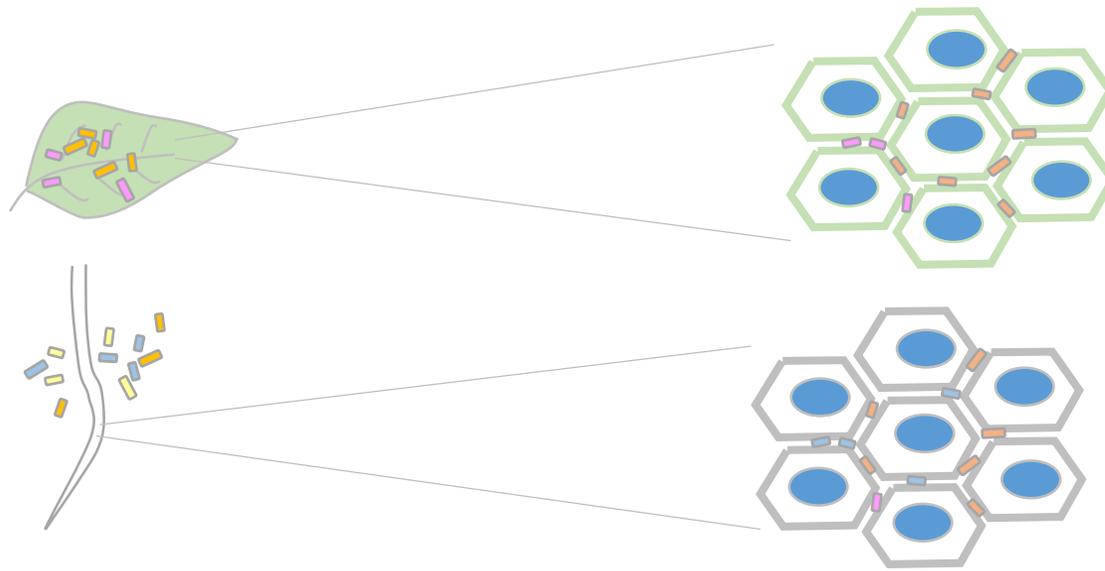
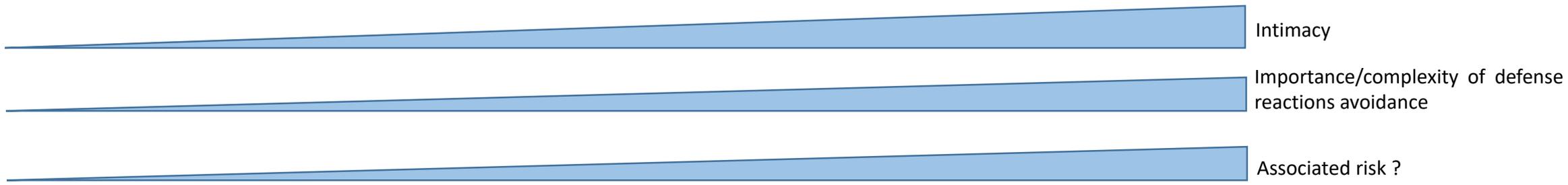
Figure 2: Reaching higher complexity in the study of plant-beneficial microbes. A, in classical studies, plant microbe interactions are characterized with model systems involving one host genotype and one microbial genotype in one environment. B, a higher complexity is reached when studying one host or one microbial genotype with either different genotypes of microbes or hosts. Characterizing the effect of the environment also increases studies complexity. C, D and E new technologies like high throughput next generation sequencing should help characterizing more complex systems, deciphering both the genotype x genotype and complex environmental effects on the plant microbe interactions.

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Colonization: Rhizospheric & Epiphytic

Apoplastic-extracellular

Intracellular

- Immunity:**
- Suppression of immunity?
 - Control through:
 - antimicrobials
 - chemoattractant
 - feeding

- Microbial mediated control of defense:
- Interference with phytohormones
 - translocated & secreted effector

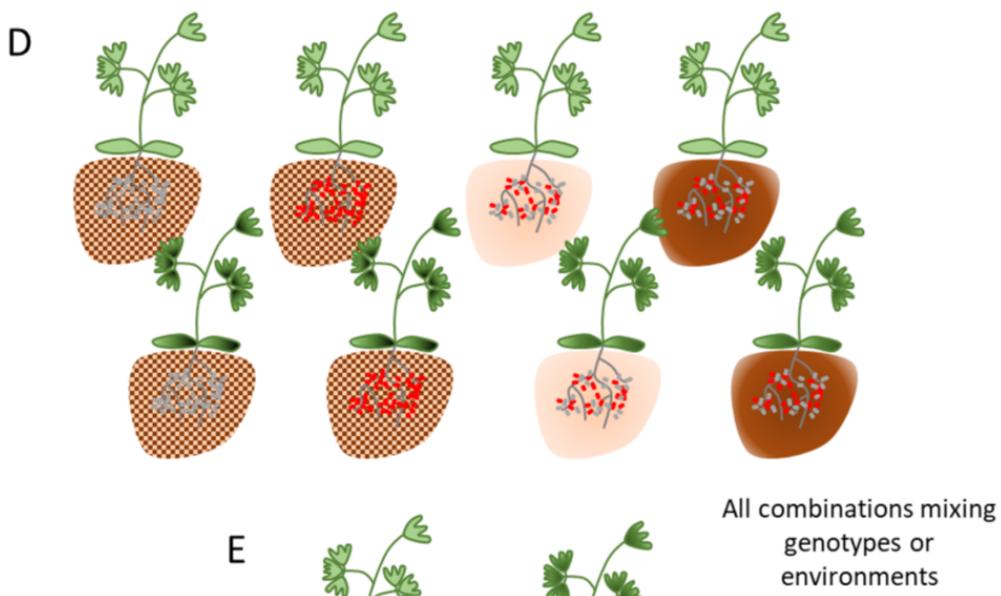
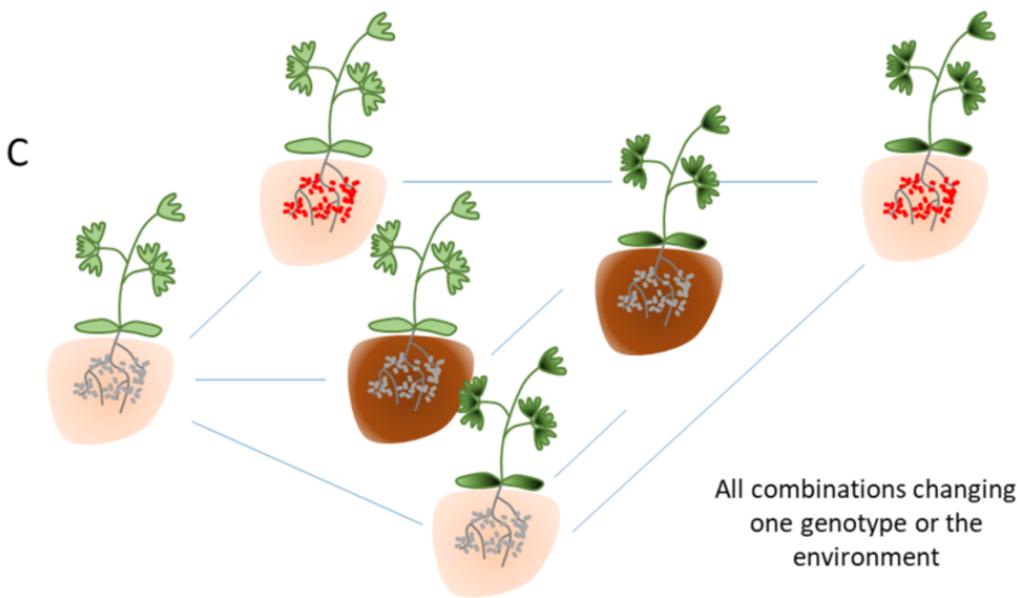
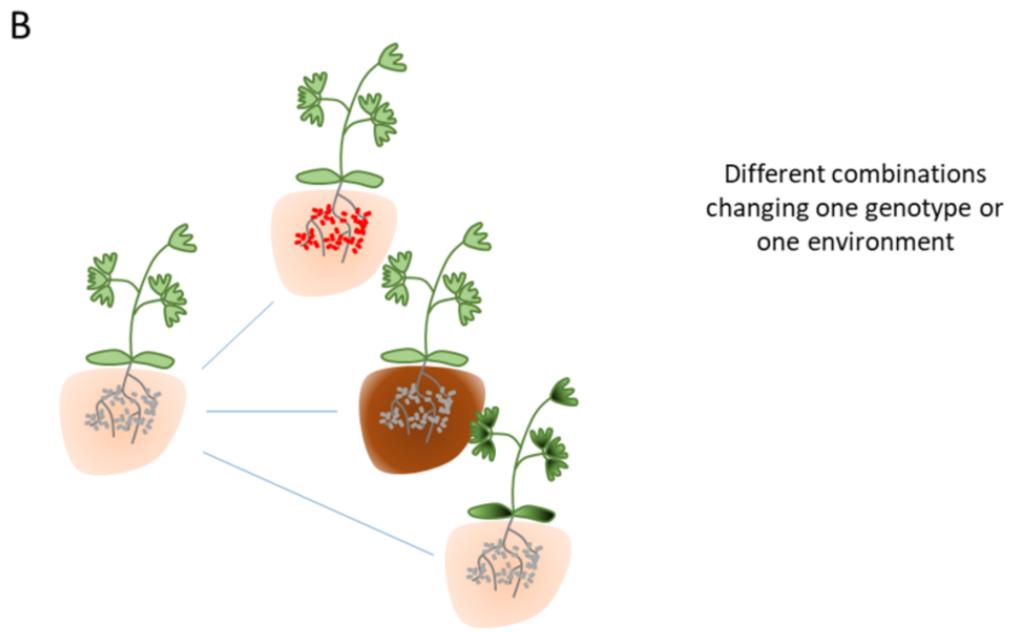
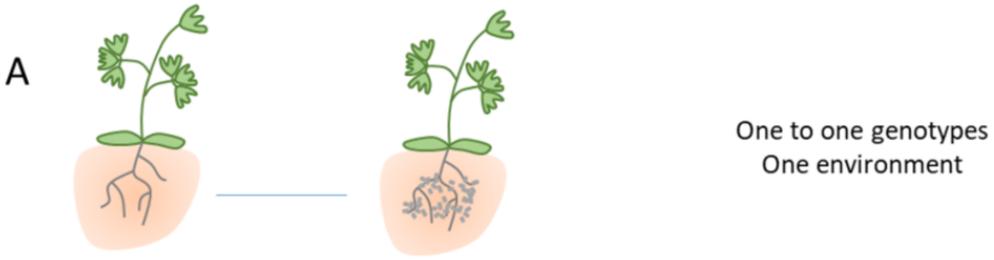
- Plant driven suppression of immunity
- Microbial mediated control of defense

Benefits:

Nutrient solubilisation, abiotic stress
Niche occupancy/competition

+/-ISR

Nutrition
+/-ISR



Homogeneous
biotic and
abiotic
conditions

Heterogeneous
biotic and
abiotic
conditions

Increased complexity