

PROBLEMS & PARADIGMS

Prospects & Overviews

Symbiont effector-guided mapping of proteins in plant networks to improve crop climate stress resilience

Symbiont effectors inform highly interconnected plant protein networks and provide an untapped resource for crop climate resilience strategies

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Abstract

There is an urgent need for novel protection strategies to sustainably secure crop production under changing climates. Studying microbial effectors, defined as microbe-derived proteins that alter signalling inside plant cells, has advanced our understanding of plant immunity and microbial plant colonisation strategies. Our understanding of effectors in the establishment and beneficial outcome of plant symbioses is less well known. Combining functional and comparative interaction assays uncovered specific symbiont effector targets in highly interconnected plant signalling networks and revealed the potential of effectors in beneficially modulating plant traits. The diverse functionality of symbiont effectors differs from the paradigmatic immuno-suppressive function of pathogen effectors. These effectors provide solutions for improving crop resilience against climate stress by their evolution-driven specification in host protein targeting and modulation. Symbiont effectors represent stringent tools not only to identify genetic targets for crop breeding, but to serve as applicable agents in crop management strategies under changing environments.

KEYWORDS

AlphaFold, climate change stress, effector, hormone signalling, host reprogramming, protein networks, symbiosis

INTRODUCTION

Plants depend on beneficial symbioses for their performance in natural ecosystems and agricultural fields. Nutrient supply is often considered as the canonical driver and outcome of beneficial symbioses where symbionts receive sugars and lipids, while they supply plants with phosphorus, nitrogen, iron and mineral nutrients.^[1,2] However, it

is not clear, how developmental and environmental signals are integrated in this exchange while phytohormones often have important regulatory functions in these interactions.^[2] Whereas the molecular mechanisms for increased host stress tolerance and fitness are generally unknown, beneficial effects of microbes often involve changes in plant hormone metabolism and signalling.^[3] This is not further surprising as phytohormones are instrumental in every aspect of plant life from germination,^[4,5] to vegetative development, reproduction and senescence.^[6] In addition, hormones coordinate developmental plant plasticity as an integral part of biotic and abiotic stress

Abbreviations: ABA, abscisic acid; AMF, arbuscular mycorrhizal fungi; AUX, auxin; cPEP, complementary peptides; ETI, effector-triggered immunity; JA, jasmonic acid; PTI, pattern-triggered immunity; SA, salicylic acid; Si, *Serendipita indica*.

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adaptation deriving from pathogen attack,^[7] nutrient shortage (e.g., nitrate availability),^[8,9] drought^[10,11], salinity^[12] and heat.^[13] This multifunctionality of phytohormones originates from their frequently overlapping activities, which adds to the flexibility and robustness of plant adaptations to environmental stress.^[14] Effectors, small, secreted proteins from microbes, are known to modulate a vast range of phytohormones during plant-microbe interaction.^[15]

Climate change is a central cause for biotic and abiotic stress for crops and, thus, severely compromises crop growth and yield.^[16] At a global scale, the consequences are manifold and range from the emergence of new diseases, higher pest infestation, shifting of weed flora and reduced crop growth periods to altered soil-microbe communities and their activities.^[17] These impacts are particularly pronounced in more vulnerable cropping systems in developing countries.^[18] As sessile organisms, plants rely on beneficial interactions to cope with changing environments. In addition to promoting plant growth, symbiotic microbes can stimulate phenotypic plasticity and fitness of plants which enhances host tolerances against various stresses.^[19,20] Some plant-growth-promoting bacteria and fungi improve defence against a broad range of pathogens and insect herbivores by eliciting systemic resistance and local resistance.^[21–23] This ability of symbionts to enhance plant fitness and resilience is driven by plant-symbiont co-evolution. Plants faced extreme environments, including climate with high CO₂ concentrations and irregular precipitation in the course of land colonisation more than 450 Mio. years ago. To succeed the transition from water to land, plants were highly dependent on beneficial symbioses with fungi.^[24–26] Ever since, co-evolution with beneficial microbes is a functional concept of plants to withstand environmental challenges.^[27,28] Consistent with this, elevated CO₂ was shown to positively affect beneficial symbionts like mycorrhizal fungi. Such findings indicate symbioses as an important factor in influencing plant robustness and adaptation to changing climates.^[29] Plants share common symbiosis signalling components for the establishment of both arbuscular mycorrhizal (AM) and root-nodule symbioses. Early signal transduction upon perception of symbionts is mediated by at least seven shared mechanisms.^[30] Despite morphological differences between AM and root-nodule symbioses, both genetically control colonisation and lead to transcriptional activation of a common set of symbiosis genes (*SYM* genes).^[31] It indicates that plant interactions with mutualistic microbes have co-evolved over time. Additionally, microbe-plant relationships in roots are formed through specific interaction types and a common architecture exists between nodulation and mycorrhizal interactions.^[32] Thus, plant-symbiont co-evolution points to promising solutions for enhanced crop fitness under changing climates. It is unclear, however, how symbionts regulate the adjustment of host signalling to confer beneficial effects and to what extent this is linked to the establishment of symbiosis. The establishment of beneficial symbioses are based on a tight molecular communication between both partners. Recent studies, for instance, demonstrated the significance of lipid exchange between AM fungi (AMF) and most ancient plant lineages. This lipid exchange essentially supports the formation of arbuscules, a fungal interface in plant cells that facilitates cross-species communication and nutrient exchange.^[33] It was

revealed that lipids in arbuscule-containing cells are transferred to AMF, following upregulation of lipid synthesis and metabolism pathways for example by *WRINKLED* genes in the host cells. Without these lipid-synthesis-regulating genes symbiosis with AMF was strongly reduced.^[33]

Interestingly, many conserved signalling and recognition mechanisms as well as transcriptional and cellular responses highly overlap between beneficial and pathogenic microbes.^[34] This is not further surprising as the lifestyle of fungi is ranging from mutualism (beneficial for both partners) to commensalism (beneficial for the microbe but no effect for the host) to parasitism (host is harmed on the benefit of the fungus). Among others, factors such as partner combination, developmental stage, physiology, habitat conditions and stress situations determine if a mutualistic interaction can shift to commensalism or even parasitism.^[35] Most critical for the symbiotic outcome are external circumstances such as temperature, moisture and nutrient availability.^[36] Overall, it suggests that the establishment of symbioses (in terms of colonisation) is not necessarily dependent on the regulation of beneficial effects. It raises the question about the molecular basis of mutualism in plant-microbe symbioses and if it can uncover solutions to enhance crop fitness under climate stress. The intimate interaction of beneficial symbionts with plants relies on the reprogramming of cellular processes in the host.^[37,38] Similar to pathogens, symbionts use secreted proteins (effectors) to modulate hosts.^[39] Functional analyses of pathogen effectors have greatly advanced our knowledge about the organisation and regulation of the plant immune system.^[40] Considering the importance of symbionts for environmental adaptation of plants,^[28] symbiont effectors can be instrumental in the identification of plant proteins and pathways with key functions in climate stress resilience. In this article, we will give an outlook on our current knowledge about beneficial effector functions and possible applications of symbiont effectors to sustain crop production.

MICROBIAL EFFECTORS IN PLANT COLONISATION

During co-evolution with plants, microbes have developed sophisticated strategies for the targeted alteration of plant signalling to prepare host cells for colonisation. At the initial interaction stages, all microbes are confronted with plant immunity.^[41] Plants perceive microbe-associated molecular patterns (MAMPs), for example flg22, a 22-amino acid conserved peptide in bacterial flagellin,^[42] to activate highly effective layers of plant immunity in leaves and roots,^[43,44] which is referred to as pattern-triggered immunity (PTI). Plants use pattern recognition receptors (PRRs) for the specific recognition of a large number of different MAMPs from diverse microbes such as bacteria, fungi and oomycetes.^[45,46] The activated cellular immune signalling aims at establishing an antimicrobial micro-environment and includes intracellular reactive oxygen and calcium bursts, kinase cascade activation and transcriptional reprogramming.^[47]

To overcome PTI, pathogens secrete proteins that were later defined as effectors. Suppression of plant innate immunity was first

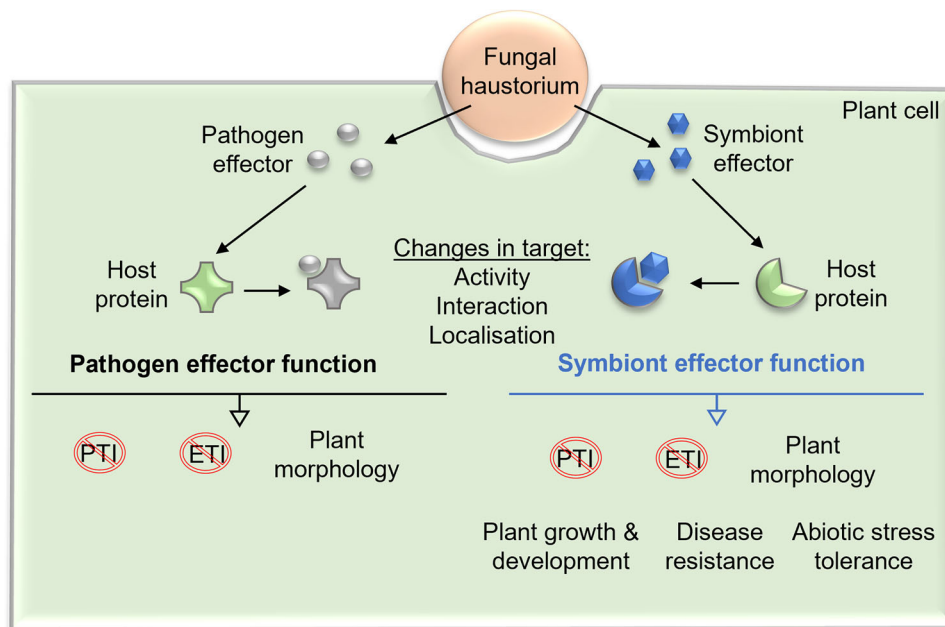


FIGURE 1 Effector proteins from pathogenic and beneficial fungi are often translocated into plant cells, where they modulate target localisation, interaction and activity.^[40] While microbes utilise effectors to suppress host PTI and ETI and affect plant morphology,^[48] symbiont effectors additionally have possible functions that increase plant growth, biotic and abiotic stress resilience.^[85]

described as principal effector function of plant pathogenic bacteria by affecting post-translational modification of host proteins (e.g., ubiquitination, phosphorylation) and RNA homeostasis.^[48] Similarly, pathogenic fungi and oomycetes have an arsenal of several hundred effector proteins to channel every step of plant infection.^[40] Global effectome studies revealed that effectors from pathogens of different kingdoms and different life-cycle strategies manipulate common host target proteins. This convergent targeting of host protein networks helped to identify core immunity network components.^[49,50] In response plants developed an additional immunity layer called effector-triggered immunity (ETI), which utilizes nucleotide-binding domain leucine-rich repeat (NLR) receptors to detect effectors.^[51] Even though, PTI and ETI require different receptors they share some signalling components, and ultimately unite in similar downstream responses with distinct amplitudes and dynamics.^[52] Recent studies even indicate components previously thought to be related to ETI to function in PTI responses and suggest convergence points of defence signalling cascades.^[53] Current concepts consider ETI to be essential in the restoration and potentiation of PTI to launch a more robust overall plant immune response.^[54]

Pathogen effectors from model pathogens (e.g., *Pseudomonas syringae*) have been instrumental for our understanding of the regulation and interconnection of PTI and ETI.^[48] In addition to the immunity-interfering activity, pathogen effectors were later recognised for altering plant behaviour and morphology during pathogen infection.^[48] This functional versatility seems to be even more elaborated for symbiont-derived effectors (Figure 1).

EFFECTORS ARE REQUIRED IN BENEFICIAL SYMBIOSIS

Pathogens and beneficial symbionts follow, in general, highly similar colonisation and cellular interaction strategies.^[55] The suppression of PTI is therefore equally important to them. The outcome is, however, detrimentally different. In contrast to diseases caused by pathogens, symbionts enhance host fitness.

Therefore, fungal symbioses are of great value for agriculture, by promoting plant health, growth, and development. Fungal effectors play an important role in complex plant-symbiont crosstalk and were previously defined as proteins that modulate plant physiology to facilitate fungal colonisation and growth within the host.^[56] The relevance of common symbioses genes^[31] suggests that nodulating rhizobacteria and arbuscular mycorrhiza employ effectors with overlapping host targets.

Si (*Serendipita indica* formerly *Piriformospora indica*) is an endophytic root colonising fungus that establishes symbioses with a broad spectrum of monocotyledonous as well as dicotyledonous plants. The fungus forms inter- and intracellular hyphae in root cells that eventually differentiate into chlamydozoospores.^[57] The biotrophic growth phase is occasionally followed by a cell death-associated colonisation phase; possibly to turn cells into vessels for spore deposition.^[43,58] *Si* is confronted with a root immune system and does not necessarily evade detection but suppresses PTI to colonise host roots.^[43] As a result of this intimate colonisation, *Si* transfers various beneficial effects to monocots and dicots. In addition to improved growth and development, colonised plants show increased abiotic stress tolerance

as well as local and systemic disease resistance.^[59,60] Growth and developmental effects might be partly attributed to improved nutrient acquisition. The fungus was shown to employ a high affinity phosphate transporter, which is involved in P transfer to the host.^[61] A more recent study revealed that *Si* adapts host gene expression depending on phosphate limitation but does not stimulate phosphate uptake.^[62] Additionally, a high-affinity sulphur transporter of *Si* was reported to improve maize growth by compensating for low sulphur availability and *Si* colonisation allowed maize to allocate resources away from sulphur assimilation.^[63] A fungal high-affinity iron transporter has a functional impact on improved performance of rice. Rice interacting with *Si* with functional Fe transporter under iron limiting conditions was larger, contained more chlorophyll and had higher biomass.^[64] These findings indicate that promotion of growth and developmental effects of symbionts have great agricultural potential and cannot be solely explained by an improved nutrient supply but involve an alteration of plant signalling and physiological processes.

Limitation of nutrients is one of many abiotic stresses plants have to cope with in nature. Other abiotic stress conditions include drought, heat, salinity and cold, often occurring in different combinations. Plants activate signalling pathways that for example involve a variety of molecules ranging from reactive oxygen species (ROS) to hormones.^[65] Independent of biotic (e.g., pathogens, herbivores) or abiotic stress, stimuli often result in a fast Ca²⁺ influx into the cytoplasm recognised by Ca²⁺ sensors which transfer signals through hormone pathways.^[66] Hormone signalling in response to stresses causes various adaptational changes in all plant cell regulating processes, like translational modifications, transcription, activation and deactivation of signalling pathways.^[12,67,68] Especially, abscisic acid (ABA) and jasmonic acid (JA) signalling are coordinated by Ca²⁺ signalling and were shown as convergent points between biotic and abiotic stress regulation.^[69] The symbiont *Si* is known to affect host signalling pathways related to stress responses including Ca²⁺ signalling and ROS production.^[70,71]

Correspondingly, *Si* mediated abiotic stress tolerance and disease resistance to microbial pathogens and insects^[72] is associated with host signalling and subsequent transcriptional reprogramming. Improved salt-tolerance observed in barley was connected to an increase of the antioxidative capacity of host plants, while increasing barley resistance to fungal diseases. Elevated antioxidative capacity resulted in an overall increase in grain yield.^[59,71] Consistently, *Si* stimulated antioxidant enzymes and expression of drought-related genes to confer drought tolerance in Chinese cabbage leaves and promoted root and shoot growth and lateral root formation.^[73] The fungus was reported to regulate transcription of genes to mediate cold tolerance under freezing stress (members of freezing responsive *WRKY*, *ERF*, *bHLH*, *HSF*, *MYB* and *NAC* transcription factor families),^[74] drought stress (e.g., *DREB2a*, *CBL1*, *ANACO72* and *RD29a*)^[73,75] and salt stress response (*HKT1*, *KAT1*, *KAT2* functional in regulation of Na⁺ and K⁺ homeostasis).^[76] In addition, local and systemic resistance to fungal and viral pathogens is based on *Si*-mediated adaptation of genetic and physiological host programs.^[59,77,78] As a result, *Si* symbioses lead to a better plant performance, including root proliferation, crop growth and productivity under changing environmental conditions with great potential for improvement of agriculture.^[77,79] Even though, effector

proteins of symbionts are known to promote host interactions,^[80,81] how exactly symbiont effectors are involved in these stress regulations is mostly unknown. The studies indicate, however, a diversity of *Si*-mediated benefits that have an obvious dependency on host reprogramming in common. It suggests a direct involvement of symbiont effectors in launching beneficial effects (Figure 1). Beneficial microbes rely on effectors with highly variable functions for host colonisation.^[82] Irrespective of beneficial or pathogenic lifestyles, previous effector analyses centred around their function in immunity suppression and host colonisation^[48,50,82,83] by altering host protein stability, disturbing protein complexes and relocalization of their targets.^[39] The mutualistic fungus *Laccaria bicolor* was among the first to reveal its dependency on an effector (MiSSP7) for colonisation.^[80,84] Likewise, effector targeting of the PTI backbone mitogen-activated kinases was identified for rhizobia to suppress host defence.^[81]

Despite various indications, a potential function of symbiont effectors in the activation of beneficial effects is still underinvestigated. Rhizobia, for instance, use effectors not only to promote nodule formation but to stimulate other symbiosis-related activities.^[81] To receive a more comprehensive overview of effector functions in plant benefits we analysed effector proteins of *Si* and their targets in comparative and functional effectome studies.^[85] *Si* possesses a diverse repertoire of effectors^[86,87] and *Si* effector Dld1 (PIIN_05872) was identified to affect metal ion homeostasis and oxidative stress^[88]; traits of known relevance for beneficial plant effects.^[77,89] Our comparative effectome analysis identified *Si* effectors that shared targets with pathogen effectors and were most likely involved in PTI modification at initial cell colonisation. The majority of symbiotic effectors, however, had exclusive targets^[85] which may indicate *Si* effector-host protein interactions involved in the activation of beneficial pathways. A subsequent annotation analyses revealed an abundant function of *Si* effectors in interacting with hormone pathways.^[85] The data suggested a function of effectors on plant hormones to regulate beneficial host traits. It will be interesting to find out in future, to what extent effectors of symbionts with a broad host range have conserved targets and/or functions (e.g., in hormone pathways) across their different host plants. This knowledge will be critical for exploiting effector-based mechanisms in future biotechnological application strategies.

SYMBIONT EFFECTORS IDENTIFY BENEFICIAL GENETIC TRAITS IN HIGHLY INTERCONNECTED PLANT NETWORKS

Plant hormones are principal regulators in all aspects of plant development and integrate environmental stress and developmental processes.^[90] Phytohormone signalling and regulation of plant stress response and growth is highly dependent on vast crosstalk and pathways regulating each other.^[8,10,69,91] In plant immunity salicylic acid (SA) and JA are key hormones in regulation of defence signalling against pathogens.^[92] Additionally, hormones primarily known for abiotic stress signalling (e.g., ABA) or developmental function (auxin, cytokinin) also affect immunity. Signalling pathways are interconnected in complex networks for rapid adaptation to the biotic

environment.^[22] ABA interacts with the SA and JA signalling pathways to mitigate effects of abiotic stress versus pathogen defence. Apart from initial Ca²⁺ signalling, transduction of these pathways are often carried out via GTP-binding proteins. They comprise a large group of proteins with a high variation of structure and function.^[69] Thus, hormones are attractive points of attack for both pathogens and symbionts to modulate plants for colonisation, disease, or beneficial effects.^[15,93-95]

Symbiotic fungi and other beneficial microbes developed mechanisms for targeting defence- and growth-related hormones.^[15,40] *L. bicolor*, for instance, promotes mutualism though blocking JA action via the interaction of the effector MiSSP7 and the *Populus* host protein JAZ6.^[84] In addition to modulation of hormones for defence suppression during initial colonisation, hormones were found to be involved in conferring beneficial effects in symbioses as reported for arbuscular mycorrhizal fungi.^[96] Consistent with this, cytokinin (CK) signalling is an important factor for rhizobia nodulation^[97] and auxin (AUX) production by root-associated microbes is necessary for regulating plant growth.^[98]

Si interferes with the synthesis and signalling of various plant hormones.^[77,94,99] *Si* colonisation success involves ethylene (ET), JA and gibberellin (GA) signalling.^[43,100-102] Earlier studies revealed *Si* effector PIIN_08944 to play an important role in its ability to colonise *Arabidopsis* by interfering with SA signalling.^[103] Nevertheless, plant hormone modulation is not only recruited to establish colonisation, but to confer benefits and balancing *Si* benefits as reported for example for ET.^[94,104] AUX signalling modulation and production by *Si* is important for altering root development and involved in growth promoting effects.^[105] Besides changing hormone signalling, *Si* increases hormone levels/hormone production in hosts like AUX, ABA, SA and GA in addition to betaine, glycine, and proline. Increased levels of these hormones and molecules were shown to improve plant resistance to environmental stresses, like salinity.^[79] Proline and glycine betaine are osmolytes involved osmotic balance during stress.^[106]

The vast crosstalk of the hormone network makes it generally difficult to decipher the contribution of individual proteins or specific interactions to phenotypic traits.^[69,91,107] In an effort to disentangle hormone networks and to identify principal regulators, a recent extensive network-study based on protein-protein interactions revealed hormone pathway contacts that represent potential crosstalk points.^[108] In our comparative effectome analysis, we employed the same protein-protein interaction platform as well as effector target networks of plant pathogens in *Arabidopsis*.^[85] More than 80% of *Si* effectors either directly interacted with proteins connected to hormone signalling pathways, or targeted proteins interacting with proteins involved in particular hormone pathways.^[85] This pronounced hormone targeting effect was further evaluated in comparison to pathogens. Symbiont and pathogens both significantly interact with hormone related proteins, as the frequency of effector interactions with hormone network proteins was higher than the simulation-based random expectation. However, *Si* targeted these pathways more frequently and more extensively. It indicated that modulation of hormones is not only involved in colonisation strategies but supports the

hypothesis that *Si* effectors recruit specific proteins of the hormone network to confer beneficial traits.^[85]

Functional assays confirmed that in addition to targeting hormone related proteins symbiotic effectors alter hormone signalling pathways in planta. Hormone marker assays in protoplasts identified extensive and highly specific hormone signalling modulation by *Si* effectors. Interestingly, hormone effects translated into individual increases in primary root growth and/or hypocotyl length in *Si* effector-expressing *Arabidopsis* plants.^[85] These assays uncovered previously unknown, highly specific function of *Si* effectors in hormone signalling to confer host benefits independent of improved nutrient transfer. It further indicates the suitability of effectors as tools to disclose molecular mechanisms and genetic determinants operating in the establishment of symbiont-mediated plant benefits. Considering that they are the result of plant symbiont co-evolution, symbiont effectors have a high potential to identify regulatory principles of plant stress resilience and, thus plant fitness under environmental challenges.

STRATEGIES TO IDENTIFY AND USE THE GENETIC POTENTIAL OF SYMBIONT EFFECTORS

Uncovering the underlying symbioses-associated genetic resources requires a deeper understanding of symbiont effector targets and the functional context of these targets within interconnected protein networks. Network mapping based on systematic protein-protein interaction analyses has given a profound insight into the organisation of the highly entangled plant hormone signalling network.^[108] Applying such a systems level approach we generated a symbiont-host interactome map and discovered symbiont effectors that target specific host proteins often associated with the hormone network.^[85] Such effector-guided approach can be very effective in assigning previously unknown functions to plant proteins and help in the identification of pathway contacts and pathway connecting points conferring host benefits (Figure 2). More importantly, it can reveal effectors with beneficial effects such as growth promotion.^[85] These effector targets represent possibly new host protein candidates that improve plant fitness under environmental stress.^[85]

In addition to experimentally demanding protein-protein or comprehensive interactome studies, recent advances in in silico prediction of protein structures have opened-up exciting opportunities for hypothesis-driven functional analyses. AlphaFold2 and RoseTTaFold are currently the most advanced software tools to predict protein structures based on artificial intelligence trained on multiple sequence alignments.^[109,110] AlphaFold-Multimer is an extension of this system that predicts structures of protein complexes developed by DeepMind^[111] and was used, for instance, to screen ~1800 pathogen small, secreted proteins for interaction with six defence related hydrolases.^[112] Utilising the AlphaFold-Multimer prediction model allows to screen previously identified effector candidates^[85] for interaction partners in plants. While these tools cannot substitute wet bench experiments, they are highly suitable to screen larger sets of effectors from different organisms for putative interactors in

Highly complex, partially annotated plant protein networks

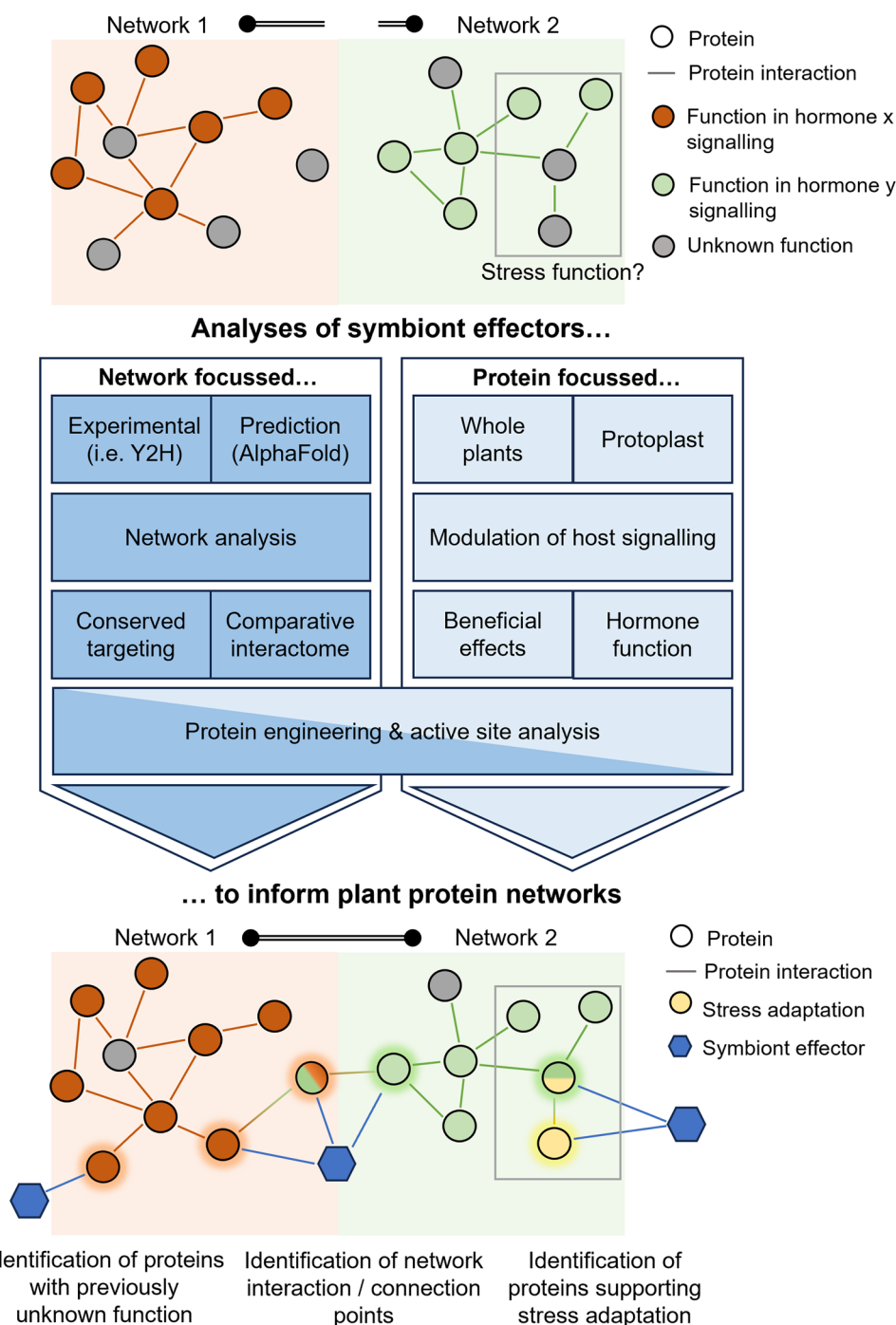


FIGURE 2 Symbiont effector analysis informs highly complex plant hormone networks and assigns previously unknown functions to host proteins.^[85] Protein networks involved in hormone and stress signalling can be studied with a diversity of effector analysis approaches focusing on interactions, networks and protein functions. Combination of methods allows protein engineering and active site analyses resulting in informed plant networks regarding protein function, network connection points and stress signalling.

different plants. This is especially relevant for systems where respective analytic platforms (e.g., comprehensive yeast-two-hybrid libraries) are not available and it can assist in the identification of proteins involved in improving plant stress resilience and development under changing climates. These tools can elucidate conserved targets of ben-

eficial microbes that are particularly valuable for future application of symbionts in agriculture or generation of crops with advantageous traits.

An important aspect of AlphaFold-Multimer is the ability to predict active sites for protein interaction.^[112] In addition to expand our

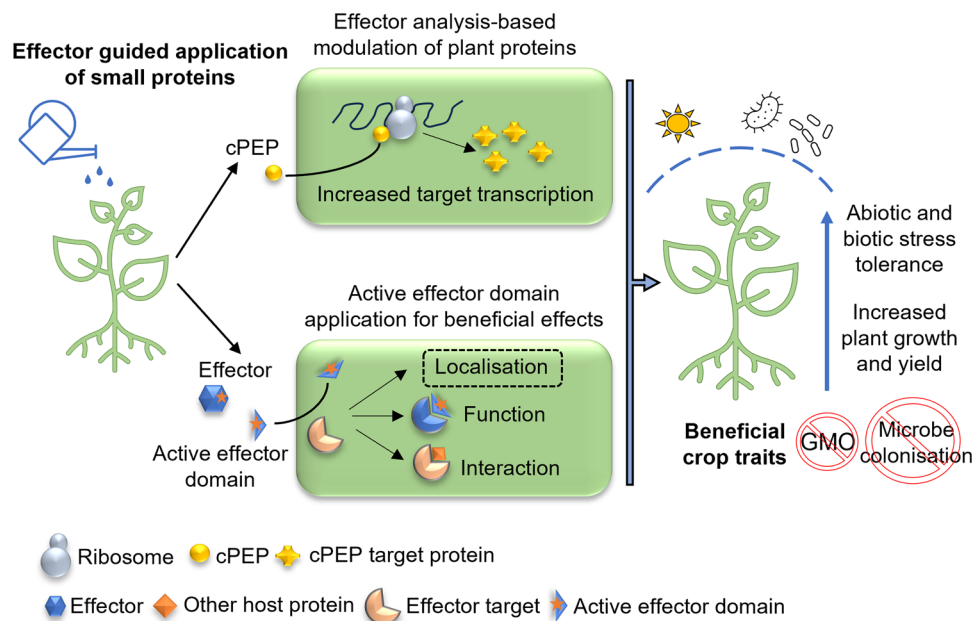


FIGURE 3 Application of small proteins introduces a system for symbiont effector analysis-informed crop stress adaption and overall fitness improvement. Complementary protein (cPEP) application increases, for example, target transcription and improves crop fitness,^[113] which can be informed based on symbiont effector targets with beneficial stress tolerance or growth function. The application of effectors represents a potential strategy to improve plant fitness and effectiveness of fertilizers to address environmental challenges and protect crops. These approaches are GMO free and do not require microbial colonisation of crops.

understanding of effector targeting and target modulation it can help to develop active peptide versions of effectors or effector targets. In this respect, a recent technical study suggested simple spraying or watering of plants with complementary peptides (cPEP) from any gene to increase target protein abundance resulting for example in improved growth and heat stress tolerance without genetic modification.^[113] The cPEP method is based on findings, that short peptides produced in planta interact with their nascent RNA sequences.^[113,114] Ormanecy et al. (2023) showed that peptides with 5–40 amino acids in size increased target activity with strong specificity. cPEPs, when externally applied, were able to modulate mRNA translation by increasing ribosomal recruitment at translation initiation sites. For this function, RPL19 (AT4G17560) was necessary, which is assumed to increase stability of inter-ribosomal subunit bridges.^[113] The cPEP approach could become highly valuable in protecting crops more flexibly and immediate against rapidly changing climates and new emerging diseases and pests. Apart from the identification of possible cPEP targets for crop improvement it may also be possible to apply effectors directly to induce beneficial signalling pathway modulation and favourable plant phenotypes (Figure 3). Fungal effectors are small, secreted proteins and plant uptake is possibly unspecific or depending on protein structure.^[115] In the latter case AlphaFold2^[110] may help to define structures that would facilitate plant uptake. Our recently identified effector proteins as well as fungal effectors in general are very variable in size, sequence, and function.^[85,116] Functional domains of effector peptides could be transferred into plants by spray-application. While plant uptake of cPEPs is based on a currently unknown mechanism, cPEPs must have some hydrophilic properties to facilitate solubiliza-

tion and between 5 to 40 amino acids in size. Functional cPEP might be applied by drainage or spraying leaves or seedlings.^[113] Therefore, small functional domains of effector proteins could be designed, that are possibly taken up and confer beneficial effects in plants similar to their native function during *S. indica* interaction. We showed previously that expression of single effectors in Arabidopsis was sufficient to improve seedling growth.^[85] The application of single or a combination of effectors could represent a strategy to improve the effectiveness of biological fertilizers or to flexibly address multiple environmental challenges to protect crops (Figure 3). The feasibility of this approach in large-scale agricultural settings will certainly depend on an understanding of effective protein treatment concentrations, the efficiency of uptake mechanisms and economic factors such as peptide production costs.

CONCLUSION

Effector analyses were shown as useful tool to identify plant immunity and defence pathways and the mechanistic processes of pathogen microbe colonisation.^[48] Additionally, these studies aided in the development of plant disease protection products.^[117] Symbiont effector analyses regarding their function in conferring beneficial effects to plants was previously neglected, even though numerous studies identified signalling modulation by symbionts as necessary for increased plant stress tolerance.^[77,94] However, the investigation of plant signalling networks can be exceptionally challenging because of vast pathway crosstalks and overlapping functions.^[10,91]

In this review and our previously published study^[85] we show not only, that symbiont effector analyses reveal molecular mechanisms regulating the establishment and beneficial outcome of plant-symbioses, but also several potential applications of symbiont effectors. Studies of effectors derived from beneficial microbes are able to inform highly complex and interconnected signalling networks.^[85] Effector network analysis is capable of identifying signalling network connection points and assign new, beneficial functions to host proteins.^[85]

Based on this identification of previously unknown functions of plant proteins in hormone signalling and plant stress resilience, new application points for crop improvement are available. Using in silico methods like AlphaFold-Multimer^[111] extensive studies of beneficial effectors are promising to investigate signalling pathways for increased plant stress tolerance, growth and yield. Modulation of the identified symbiont effector targets as well as direct application of effectors in previously demonstrated GMO-free approaches like cPEP^[113] may be important tools for improved crop stress tolerance in the future. These techniques are highly valuable in drastically changing environments for improved, greatly flexible crop production and protection products to ensure food security for a growing world population.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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