spectrometry, which can be cross-validated by GUIDE-seq.

Theoretically, short, neutral, and selective dsODNs are the best choice for GUIDE-seq. The ideal dsODN should not cause frame shift in coding genes. However, testing this assumption and determining the optimal length for dsODNs requires further tests with the aim of achieving integration of dsODNs in an NHEJ-dependent manner. Short indels are often introduced between the dsODN and the integrated genome sequence. The lengths of the indels may also be cell-survival dependent.

In conclusion, the optimal and extreme lengths of a dsODN remain to be determined. In rare cases, a longer dsDNA, instead of a short dsODN, may serve a special purpose. A long dsDNA containing a GFP reporter might be useful for high-throughput detection/evaluation of genome-wide DSBs. A long dsDNA containing a positive selection marker may be useful for enriching such dsDNA-integrated cells by selection. The integration efficiency of a long dsDNA could be too low to be useful.

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Forum

Recognizing Plant Defense Priming

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Defense priming conditions diverse plant species for the superinduction of defense, often resulting in enhanced pest and disease resistance and abiotic stress tolerance. Here, we propose a guideline that might assist the plant research community in a consistent assessment of defense priming in plants.

An Ecogenomic Approach for Studying Defense Priming

Ten years after publication of the seminal review ‘Priming: getting ready for battle’ [1], the importance of **defense priming** (see Glossary) as an adaptive trait for the adjustment of plant defense in unpredictable environments is well established. Defense priming has been reported for a range of plant taxa, including wild species and cultivated varieties, and from herbaceous to long-lived woody plants [2]. Defense priming is postulated to be an adaptive, low-cost defensive measure because defense responses are not, or only slightly and

Glossary

Allocation costs: fitness losses caused by allocation of metabolic resources toward defense that would otherwise have been allocated to growth and reproduction.

Defense priming: induces a physiological state (the primed state of defense) in which a plant is conditioned for the superactivation of defenses against environmental challenges.

Ecological costs: occur when fitness-relevant interactions of an organism with its natural environment are impaired.

Memory: the processes by which information about an environmental stimulus is stored and maintained for future use.

Naïve state: a state of a plant or cell in the absence of stress or stress memory.

Plant fitness: the genetic contribution of a plant to the next generation. Seed production, number of flowers, pollen quality and number, and plant growth, among others, are generally accepted proxies for plant fitness.

Priming stimulus: the trigger that initiates defense priming. The priming stimulus can be a stress itself, an indicative of an imminent stress, a chemical compound, or a beneficial organism. The priming stimulus does not, or only slightly and transiently, activate defense responses. It rather promotes the plant to a persistently primed state of enhanced defense readiness.

Transgenerational defense priming: the transmission of the primed state from a parental plant to its offspring.

Triggering stimulus: an external factor that activates a stress response.

transiently, activated by a given **priming stimulus**. Instead, defense responses are deployed in a faster, stronger, and/or more sustained manner following the perception of a later challenging signal (the **triggering stimulus**); that is, in times of stress [2,3]. Recent studies revealed that defense priming can pass down generations, indicating an epigenetic component of **transgenerational defense priming** [4].

Molecular studies of defense priming recorded changes to chromatin and the accumulation of mRNA of genes with a signaling role in defense, of signaling proteins and pattern-recognition receptors, metabolites, and other molecular components supporting a faster, stronger, and more sustained response to a triggering stress. Given that priming is often postulated to improve **plant fitness** in complex environments, the relevance of the molecular findings should ideally be tested in experiments evaluating plant performance and fitness in relevant ecological conditions. However, ecological investigations of defense priming mostly addressed the impact of a first stimulus on the interaction of plants with other community members, such as microbes, insects and con- and heterospecific plants. Many of the studies assessed just a few defensive traits, thereby ignoring the overall defensive status of **naïve** versus **primed** plants (i.e., before exposure to a triggering stress). Moreover, better plant performance under enemy pressure does not necessarily reflect defense priming, because there are additional mechanisms by which plants can adjust their defensive state to the environment. Such additional mechanisms encompass, for example, directly induced defenses, an enhanced tolerance to biotic and abiotic stress, cross-protection from viruses and microbial pathogens, or acclimation. Therefore, a methodological approach for studying defense priming should ideally integrate both molecular analyses of plant defense modulation and the ecological assessment of fitness-related costs and benefits (Figure 1).

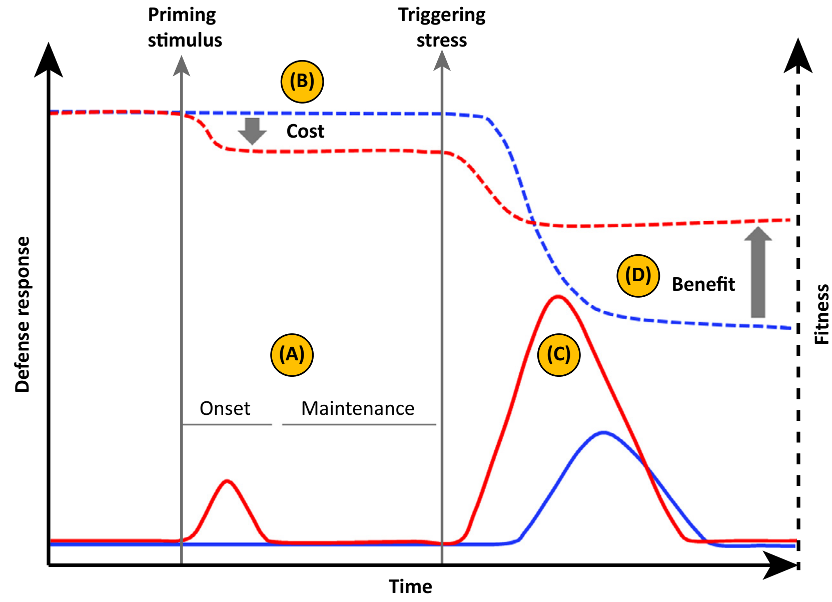

Figure 1. Scheme of the Relation between Defense Responses (Solid Lines) and Fitness (Dashed Lines) in Primed (Red) versus Unprimed (Blue) Plants. Analysis of defense priming requires a set of steps encompassing both the assessment of plant defenses and the associated cost–benefit balance. Here, we suggest some criteria that may help in deciding whether defense priming is present: (A) Memory: two sequential environmental events are required for asserting memory in the absence of molecular markers: the priming stimulus and the triggering stress. During priming and in the primed state (before the triggering stress), plant defenses are expected to be only transiently and generally faintly induced. (B) Low fitness costs: the maintenance of the primed state (before the triggering stress) has low fitness costs compared with the direct activation of defense. (C) A more robust defense response: in response to the triggering stress, primed plants mobilize cellular defenses in a faster, earlier, stronger, and/or more sustained manner than do unprimed plants. (D) Better performance: primed plants are expected to defend better against a given stressor than unprimed plants. Therefore, priming enhances plant fitness in hostile environments. Adapted from [2,6] and https://tonlab.wordpress.com/.

Key Characteristics of Defense Priming

Defense priming can be induced by chemical compounds (e.g., β-aminobutyric acid, salicylic acid, pipecolic acid, jasmonic acid, or volatile organic compounds), pathogens, insect herbivores, or environmental cues that indicate an increased probability of attack (e.g., insect eggs) [2,3]. Plant defense can also be primed by beneficial soil organisms, such as rhizobacteria and rhizofungi [5]. Depending on the nature of the priming stimulus and the stressor, priming can engage diverse mechanisms [6]. However, irrespective of the inducing and target stimulus, defense priming has characteristic key features. Here, we propose some key criteria that might help in assessing the presence of defense priming in plants (Figure 2).

Memory

When a plant is primed, the information of the priming stimulus is stored, eventually until exposure to a triggering stimulus. We refer to this effect as the **memory** in plant defense [3,7]. In Arabidopsis, several molecular markers were found to be useful for detecting the primed state. They include elevated levels of pattern-recognition receptors (e.g., FLS2 and CERK1), enhanced accumulation of the mitogen-activated protein kinases MPK3 and MPK6, augmented expression of transcription factor genes (e.g., *WRKY*s and *MYC2*), certain modifications to histones (e.g., trimethylation of lysine residue 4 in

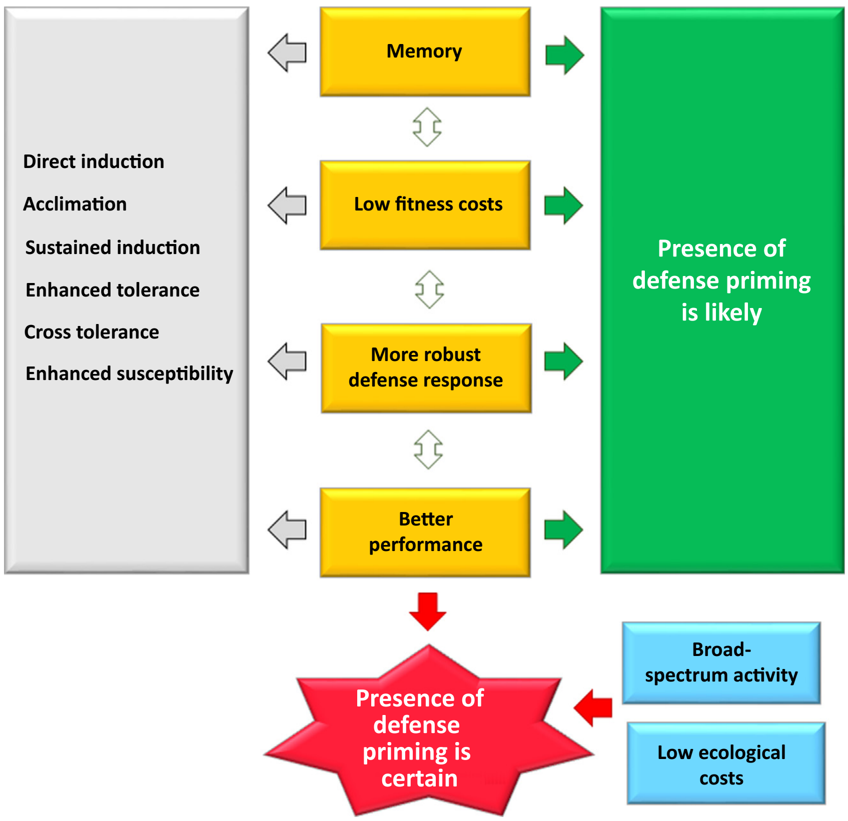

Figure 2. Flow Diagram Showing the Suggested Guideline for Analyzing the Presence of Defense Priming. The main criteria proposed for analyzing the presence of defense priming are in yellow squares. The accomplishment of such criteria (green arrows) suggest the presence of defense priming. The more criteria are accomplished, the more likely is the presence of defense priming. The gray square shows other mechanisms by which plants cope with environmental stress. These might have similarities with defense priming. These mechanisms are not exclusive and can co-occur with defense priming. Bright-blue squares show other features of priming. The presence of these characteristics suggests the presence of defense priming. However, the analyses required may be difficult, especially for non-model and slow-growing plants, and would involve experiments in natural habitats.

histone H3), and DNA hypomethylation [3]. More reliably, but also more elaborately, memory can be revealed by applying at least two sequential incidents: (i) the priming event, which primes the defense-related traits; and (ii) the challenge (the triggering stress), which activates the defense-related traits at the phenotypic level in a more robust manner in primed compared with unprimed plants [8] (Figure 1). The time span between the two events is not defined and may vary among stimuli. However, any memory effect would allow some time to pass between the perception of the priming stimulus and the triggering stress. During this time, the defense traits in question, which often are only slightly and transiently induced by the priming stimulus, would return to nearly basal levels. Recent findings revealed that at least some types of defense priming can be inherited, a phenomenon referred to as 'transgenerational priming'. Although the molecular events associated with transgenerational priming remain largely unknown, DNA demethylation has been suggested to contribute to the phenomenon [4]. However, assessing heritability of priming can be challenging, especially when working with non-model and slow-growing plants.

Low Fitness Costs

Defense priming is expected to cause an overall positive cost–benefit balance in times of stress [9]. Therefore, assessing priming would require an evaluation of the fitness consequences of activating and maintaining the primed state of enhanced defense (i.e., storage of information after priming). Although defense priming has lower costs than the direct activation of defenses, it might still incur some **allocation costs** (and/or **ecological costs**), probably because it causes physiological alterations (e.g., deposition of dormant signaling enzymes or modification to histones on defense gene promoters [3]) while shifting the plant to the alert. However, the fitness-related advantage of priming becomes obvious only upon exposure to a triggering stress, after which primed plants outperform unprimed plants (Figure 1). Thus, the benefits of priming outweigh its costs in hostile conditions [9,10]. Although this is well appreciated, surprisingly few studies have measured the fitness effects of defense priming. Allocation costs of priming can only be determined in situations that lack the potential benefits of being primed (i.e., before exposure to a triggering stress toward the end of the memory-retaining period). Useful traits for evaluating the fitness costs of defense priming include key physiological processes, such as seed production (see 'plant fitness' in the Glossary).

More Robust Defense

The molecular, biochemical, and physiological events associated with phenotypic defense are faster and/or stronger and/or activated earlier in primed versus unprimed plants (Figure 1). Primed plants often also display longer-lasting activation or attenuated repression of defense upon challenge than unprimed plants [2]. Defensive traits may include, among others, changes in defense-related signaling compounds or processes (e.g., hormones and enzymes, alterations to chromatin, or enhanced presence of pattern-recognition receptors), or actual defense responses, such as accumulation of phytoalexins, glucosinolates, phenolic compounds, reactive oxygen species, lignin, or herbivory-induced plant volatiles [6]. Given that naïve plants can be produced in the lab, it is feasible to compare defense responses in naïve-challenged versus primed-challenged plants. However, such studies are difficult for plants taken from natural habitats or in the field, where naïve plants are essentially unavailable. Nevertheless, field studies have revealed the presence of defense priming in plants in their natural habitat [11].

Better Performance

Although more robust defense is usually associated with better performance in times of stress, boosting induced defense responses does not necessarily provide an advantage. For example, negative hormonal crosstalk has been reported for induced defenses against herbivores and necrotrophic pathogens on the one hand and biotrophic pathogens on the other. Thus, an attack by insects sometimes compromises the future capacity of a plant to mount defenses against biotrophic pathogens, whereas infection by biotrophic pathogens can affect the ability of the plant to mount effective defense against later attack by insects or necrotrophic microbes [12]. These examples emphasize the importance of studying under ecologically realistic conditions whether priming influences plant fitness. It is also important to determine which plant-response variables are the most appropriate for evaluating the benefit of priming. Whereas the contribution of priming to disease and pest resistance can readily be tested by comparing the obvious damage caused by plant enemies or their performance on primed versus unprimed plants, a role in plant defense implies that the appearance of priming is associated with a gain of plant fitness. Therefore, the impact of priming on plant performance must ideally be demonstrated in terms of plant survival or reproduction. Furthermore, the benefits of defense priming frequently become evident only in ecologically realistic scenarios, in which the plant might experience resource limitation or multiple interactions with other community members.

Further Characteristics of Defense Priming

In Figure 2, we suggest a guideline with some key criteria to test the presence of defense priming in plants. Below, we describe additional characteristics that usually are associated with priming. Although informative, the study of such characteristics might be difficult, especially for non-model and slow-growing plants, and/or when requiring experiments in natural habitats.

Broad-Spectrum Activity

Given that defense priming is a state of enhanced defense readiness, which has been associated with enhanced levels of pattern-recognition receptors, priming helps defeat a broad spectrum of diseases and pests [3]. Priming enhances multiple (if not all) defense responses stimulated by a given biotic or abiotic stress and, thus, also augments the defenses of the plant against pathogens, pests, and abiotic stresses related to the priming stimulus. In the absence of a subsequent biotic or abiotic triggering stimulus, primed plants usually only marginally activate direct defense responses.

Low Ecological Costs

Although the ecological costs of defense priming are expected to be low, strong experimental evidence for this is scarce. Ecological costs can result, for example, from the deterrence of mutualists (predators and parasitoids of herbivores, symbiotic fungi or bacteria, etc.) or reduced intra- or interspecific competitive power. Therefore, the ecological costs of defense priming can only be detected in variable natural habitats with multiple interacting species.

Concluding Remarks

Defense priming is a complex phenomenon that conditions plants for enhanced defense against diverse environmental challenges. Ideally, the presence of defense priming would be supported by a phenotypic analysis of the defensive state of a plant before and after later challenge with a biotic or abiotic stress, combined with an assessment of the resulting cost–benefit balance. Such studies are only feasible in multifactorial experiments [2], including naïve, primed, naïve-and-triggered, and primed-and-triggered plants.

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