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Comment

Phytovaccines, a New Paradigm for Crop Protection: Towards a Sustainable Agriculture

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Abstract

In this comment, we propose to incorporate the concepts "phytovaccines" to refer to bioproducts containing defense elicitors, and "phytovaccination" to the process of applying such phytovaccines to activate plants' innate immune system. We bring support to these concepts based on the traditional process of human vaccination. We posit that plant defense elicitors are to plants what antigens are for humans, and that the elicitor-induced innate immune system in plants is to some extent similar to the immunization process triggered by an antigen in humans-saving the differences between both kinds of organisms. Both defense responses share many features, including an immunization agent (elicitor/antigen), the recognition of such agent by a receptor that initiates a cascade of reactions tending to activate a systemic response, that has a long-term effect and can passively be transmitted across the placenta to newborn infants in the case of humans, and to following generations of plants agamically propagated. We further compare phytovaccination to transgenic plants, as two valid biotechnological tools for crop protection, highlighting the benefits of the former. We encourage the academic and agricultural community in general and the plant-pathogen interaction community in particular, to incorporate the use of phytovaccines and phytovaccination into the biocontrol vocabulary.

Keywords: phytovaccines, phytovaccination, elicitors, induced plant protection, plant defense responses

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1 INTRODUCTION

Vaccination is a common biotechnological procedure used to provide humans (and animals in general, but hereafter we will refer to humans' vaccines) resistance against pathogens causing diseases. The term "vaccination" has traditionally been used for humans, not plants, which is probably due to that plants do not exhibit an immune system based on the formation of antibodies. However, plants do share some typical features observed in the vaccination procedure and the effects achieved in humans.

In this context, we hypothesized and verified the idea that plant defense elicitors are to plants what antigens are for humans, and that the elicitor-induced innate immune system in plants exerts a similar effect to the immunization process triggered by an antigen in humans-putting aside the differences between both kinds of organisms. Moreover, based on the evidence that plants that were previously treated with an elicitor can activate an effective local and systemic protection against the subsequent attack of one or more pathogens. Therefore, we propose the innovative idea that biological supplies (also referred to as bioproducts in this article) based on plant elicitors should also be considered as "phytovaccines", and the process by which plant defenses get induced by the treatment with an elicitor, as "phytovaccination".

Therefore, this comment aims to encourage the academic and agricultural community in general, and the plant-pathogen interaction community in particular, to integrate and use the specific concepts "phytovaccines" for bioproducts based on defense elicitors, and "phytovaccination" for the process of applying phytovaccines to activate plants' innate immune system. Arguments about this issue and its comparison with the use of transgenic plants as another biotechnological tool for crop protection are also discussed.

2 COMMENT

2.1 Origin of the Term "Vaccination"

Edward Jenner is regarded as the founder of immunology for his innovative contribution to the aeradication of a disease that devastated mankind for centuries, such as smallpox[1]. However, he was neither the first to suggest that infection with cowpox conferred specific immunity to smallpox nor the first to attempt cowpox inoculation for this purpose[2]. During his early years as a surgeon and apothecary apprentice, he had repeatedly heard the tales that dairymaids were naturally protected from smallpox after having suffered from cowpox[3]. Since then, and inspired by his interest in science and nature, Edward Jenner assumed it was possible to provide people with an effective defense against smallpox disease[4]. With that idea in mind, in 1796, Jenner inoculated an 8-year-old boy, James Phipps, with tissues obtained from the fresh cowpox lesions of a young dairymaid, Sarah Nelms. Two months later, Jenner inoculated the boy again, but this time with exudates of a fresh smallpox lesion, and no disease was observed thereafter, indicating a complete protection against the disease[5]. Jenner called this new procedure "vaccination", a word derived from the Latin word for cow "vacca", and the cowpox "vaccinia". Although his achievement was discredited during Jenner's lifetime, in the coming years vaccination spread rapidly in England, and it was accepted in most of the European countries by 1800[5]. Almost 100 years after Jenner's first "vaccination", the work of Louis Pasteur on the rabies vaccine led to a period of development of new vaccines. By the middle of the twentieth century, vaccines for many different diseases (such as diphtheria, pertussis, and typhoid) were developed using inactivated pathogens or toxoid products[6]. Since then, with the introduction of comprehensive vaccine programmes as a major public health tool from the 1950s onwards, the success of vaccines has been so remarkable that in 1980 the World Health Assembly declared the world free of naturally occurring smallpox, realizing the brilliant prediction made by Jenner nearly two centuries ago.

2.2 Biotechnology and the Way to the Modern Concept of Vaccination

Currently, vaccination is the best biotechnological strategy used to prevent infectious diseases in humans. Interestingly, however, even when the procedure was successful, Edward Jenner had no idea why and how this simple procedure could provide protection against the disease.

In the middle 1960s, biotechnology applied to medicine witnessed such a colossal leap that meant a major milestone in the history of mankind. Antibiotics and vaccines were widely available to cure and prevent lethal infection diseases. Over time the mechanism underlying the process of immunization by vaccination was unveiled giving rise to the "modern concept of vaccination", which implied the use of molecules that induce the immune system to produce antibodies, known as "antigens" and such process as "cell-mediated immunity"[7]. Since then, the idea of vaccination was strongly linked to the production of antibodies, and the historical, intuitive, and classical idea of vaccination initially conceived by Jenner, evolved into a rational and modern concept, with ample scientific support.

2.3 The Beginnings of Plant Biotechnology and the Evolution Toward "Plant Vaccination"

Whilst medical biotechnology flourished, obtaining unprecedented achievements and provided important biotechnological products, biotechnology applied to agriculture was taking its first steps. Thus, it was not until the 1960s that the "green revolution" saw its first breakthroughs, marking a profound turning point in agriculture: the beginning of a new era of "plant biotechnology"[8]. The hallmark of this new era began with plant breeders obtaining a new rice cultivar by crossing selected line carrying a mutation in a gene called semi-dwarf1, which exhibited a shorter plant stem length, than with another Peta (tall), resulting in a semidwarf cultivar IR8[9] that rendered record yields across Asia. This technology, applied in traditional plant breeding-dominated the scene until another milestone occurred during the 1980s, when biologists transferred bacterial genes from *Agrobacterium tumefaciens* into plant cells[10], giving rise to the era of modern plant biotechnology. Since then, plant biotechnology has experienced exponential development.

Although the concept of plant biotechnology includes all the activities involving the use of almost any technological procedure to manipulate plants, it has lately been orientated mainly to the genetic engineering of plants using recombinant DNA[11]. The advances achieved in this direction are such that nowadays it is relatively easy to transform plants, and genetically reprogram them to do almost anything one would tell them to do, without any implicit ethical limitation. The huge potential of this technology encouraged breeders, plant biotechnologists and biotech companies to obtain a variety of genetically modified plants to meet the demands of a fast-growing global community. Plants tolerant to herbicides, biotic and abiotic stresses, with improved wood properties for the biofuel and paper industries, plants with special nutritious or pharmaceutical traits, or for phytoremediation are currently available[12]. The success of this strategy has convinced consumers of this biotechnology that the only way to provide plants with protection against disease was through genetic reprogramming.

Paradoxically, whilst it is feasible and fairly easy today to obtain transgenic plants that are resistant to specific diseases, this is not the case for animals and humans. Conversely, protecting humans from disease through vaccination remains easier, simpler, and more rational, and without any ethical considerations. The latter poses the question, why don't we promote plant vaccination as a strategy to protect crops? This question is relevant because obtaining stable transformants suitable for delivery as a commercially profitable product requires, in addition to a substantial investment of time, effort and money, a lengthy and tedious legal process, and the resulting transgenic products are not exempt to negative environmental impact. This scenario motivated us to envision an eco-friendlier technology. At the end of this comment article, we compare plants' genetic engineering technology with plant vaccination, as two potential biotechnological tools to achieve crop protection.

The idea of plant vaccination has been insinuated as earlier as 1986 when Schmeck (1986) reported that they could provide plants hereditary resistance against a viral infection when genetically transformed-an achievement that inevitably remains the vaccination carried out by Jenner with a boy, but this time with a plant. However, if we consider the meaning of the word vaccination as it is understood nowadays, it cannot be accepted that plants expressing a defense gene are "vaccinated" since the immune system might not be activated. Likewise, neither the use of chemical antimicrobials nor the production of animal antibodies using plants, known as plantibodies[13], should be confused with the proposed concept of plant vaccination because they do not imply the activation of the plant immune system. As time passed, the concept of plant vaccination became more accurate. Recently, the term "green vaccination" as a strategy to provide plant protection by activating their innate immune system has been proposed by Luna (2016)[14], suggesting further that this technology may contribute as a tool for a "second green revolution".

In the same conceptual frame as Luna (2016)[14], but unlike the concept of "green vaccines" that refers to the expression of antigens via plant nuclear or chloroplast genomes[15], we propose to redefine the term "plant protection" as "phytovaccination" or "plant vaccination". Some detractors might argue that these concepts are not the most suitable for plants because they do not have an immune system based on the production of antibodies exhibited by humans, mammals and other animals. However, in support of the concept of "phytovaccination", we can argue that plants do exhibit a defense response mechanism, and it shares more similar features with the one triggered upon vaccination in humans than we may preliminarily imagine.

3 THE PARALLELISM BETWEEN VACCINATION AND PHYTOVACCINATION

With the aim to support the conceptual similarities between vaccination and phytovaccination a comparison of both processes is presented in Table 1 in which the reactions and effects that vaccination and phytovaccination induce in humans and in plants, respectively. In Table 1 we summarize the main features shared between vaccines/vaccination and phytovaccines/phytovaccination.

3.1 Immunization Agents and Types of (Phyto) Vaccines

The essential component of most vaccines is one or more antigens that are responsible to induce the immune responses providing by this means suitable protection against the intruder; most antigens are proteins, although there are also effective polysaccharide antigens. Traditional vaccines are classified depending on whether they use active, inactivated or attenuated strains of the pathogens[6]. On the other hand, the elicitors, the main components of phytovaccines, should be compared to the antigens provided by inactivated pathogens. Likewise, the vaccine antigens, numerous types of defense elicitors have been reported, including proteins, peptides, glycoproteins, lipids, oligosaccharides, and volatile organic compounds, among other kinds of molecules that were either isolated and purified from bacteria, fungi, and oomycetes[16-22] and even synthetic ones[23].

In our plant biotechnology laboratory, different kinds of elicitors of microbial, plant and synthetic origin were isolated and characterized in the last years. Two elicitors were obtained from strawberry leaves, the Fragarin[24] and the *Ellagitanin HeT*[25] and the elicitor *Acremonium strictum* Elicitor Subtilisin (AsES) that was purified from the avirulent fungus *Acremonium strictum*[26-32]. We have also investigated the two synthetic brassinosteroids (e.g., EP24 and BB16) with both plant defense and plant growth stimulation capacities[33-35], and fungal-derived extracts, which are a complex mixture of elicitors-the latter may be comparable those vaccines made with whole inactivated pathogens[36,37].

Table 1. Main Features Shared between Vaccines and Phytovaccines

Features	Vaccines*	Phytovaccines
Immunization agent	Antigen	Elicitor
Types of (phyto) vaccines	Whole inactivated pathogens	Biological supply containing one or more elicitors
	Components of a pathogen	Biological supply containing extracts of inactivated microbes
Recognition	Pattern recognition receptors	Transmembrane pattern recognition Receptors
Triggered mechanism	Immunization	Priming
Systemic response	Yes	Yes
Local response	No	Yes
Long-term effect	Yes	Yes
Passive protection	Yes	Yes

*The types of vaccines considered here were only the traditional ones. Modern vaccines such as RNA-based ones were not included.

Just to mention, we have also induced innate defenses in strawberry plants through a soft mechanical stimulus[38], similar to what was previously observed by Benikhlef et al.[39] in the model plant *Arabidopsis thaliana.* This may constitute an interesting alternative to induce immunity in plants opening the chance to be used in a large-scale crop production, something unthinkable with human or animal vaccination procedures.

3.2 Recognition of Antigen/Elicitor and Defense-Triggered Mechanisms

It has been well established that when injecting a vaccine into muscle, the antigen is captured by dendritic cells, which are activated through pattern recognition receptors (PRRs), and then trafficked to the draining lymph node. Then, the antigens are presented to the T cells inducing a signaling cascade that finishes with the maturation of the antibodies that recognize the antigens and the activation of the adaptative immune response[6].

When a microorganism is recognized by a plant, and if the microorganism is a pathogen that evades the plant constitutive barriers, the particular molecular patterns of the pathogen are detected by mean of plant specific trans-membrane PRRs[40]. This specific recognition leads to the activation of a concerted cascade of molecular and biochemical events that prepare plants to fight against the invaders, including the production of reactive oxygen species, calcium influx from extracellular spaces and changes of its free cytosolic concentrations, cell-wall strengthening by callose and lignin depositions, production of phytoalexins, protein phosphorylation, activation of mitogen-activated protein kinase signaling pathways and induction of gene transcription[41].

3.3 Systemic Defense Response

In humans, the short-lived plasma cells produced are responsible for the secretion of specific antibodies synthetized against the vaccine antigen, producing a rapid rise in serum antibody levels over the next 2 weeks. While antibodies are systemically distributed, B cells mediate immune memory[6].

On the contrary, in plants does exist an initial local defensive response that provides initial protection against pathogens, and is generally followed by a systemic defense, known as a systemic acquired response (SAR). SAR, unlike human antibodies-based immunization, depends on phytohormones such as salicylic acid, jasmonic acid and ethylene[42-44], which confer protection in distal non-infected tissues.

3.4 Immune Memory

Another feature shared between vaccines and phytovaccines is their capacity to induce "immune memory". The immune system of an individual who has been vaccinated against a specific pathogen can more rapidly and more robustly mount a protective immune response through B cells[6]. In plants, this feature is known as "priming", and is defined as "a physiological status of plants leading to faster and stronger activation of defense responses to subsequent biotic and abiotic stresses"[17]. The latter was thoroughly reviewed elsewhere[45-47]. Unlike humans, priming in plants is governed by chromatin modifications due to histones methylation and acetylation, which cause the upregulation of transcription factors after stress exposure[48], leading to the fast defense response.

A relative drawback we may argue is that, due to the lack of a humoral immunity system, priming in plants does not last as much as the immune memory in humans that, depending on the vaccine, can last several years[49]. However, priming can be maintained long after the initial stimulus[14,47], keeping plants protected for two or three months depending on the plant species and the elicitor used[50,51]. If we think of seasonal agriculture crops or even fruits that will be harvested at the end of the productive cycle, it is not indispensable that priming lasts too long as immune memory does in humans. Moreover, some vaccines (as is the case for diphtheria, tetanus, pertussis and polio vaccines) need one or more booster doses to maintain the level of antibodies above the protective threshold against those pathogens that show a short incubation period that enable a new immune response to develop[52]. Similarly, whether the priming effect decays before the harvest, such booster doses of a phytovaccine would activate the immune system rendering plants protected from potential pathogens[53,54].

3.5 Passive Protection

Another interesting feature shared between vaccines and phytovaccines that we would like to highlight is passive protection. In the former case, it refers to the transference of maternal antibodies across the placenta that can provide newborn infants with protection against a wide variety of pathogens, at least for a few months after birth (as is the case for pertussis, tetanus and influenza vaccines), and in the latter case, plant priming can also be transmitted to the following generations, showing adaptive transgenerational plasticity in response to biotic and abiotic stresses[14,55-63].

These examples of long-lasting maintenance of the primed state demonstrate that plant immune memory may provide a potential source for future eco-friendly protective strategies. For instance, in agricultural practice, plant vaccination (plants pre-treated with elicitor-based supplies in the absence of virulent pathogens) would yield a defense response that could provide partial or even total protection to subsequent pathogen challenges.

As vaccines have transformed public health, especially since national immunization programs were properly established and coordinated in the 1960s, we expect that phytovaccines would transform the agriculture industry, particularly for crop protection and plant sanity. We believe that we are at the onset of a fourth "green revolution", orientated to increase the awareness of environmental protection, and the increasing willingness of consumers worldwide to have access to healthier foods with low or even no impact on human and animal health. Altogether, these issues have increased the demand for biological supplies leading to sustainable agriculture. According to the Agricultural Biologicals Market Report recently delivered by Markets and Markets (2022)[64], the market for biological supplies is estimated at USD 12.9 billion in 2022 and projected to reach USD 24.6 billion by 2027 by growing at a compound annual growth rate of 13.7%.

4 COMPARISON BETWEEN PHYTOVACCINATION AND GENETIC TRANSFORMATION AS BIOTECHNOLOGICAL TECHNIQUES FOR CROP PROTECTION

It is well known how genetic transformation has contributed to agriculture as a tool for crop protection. In the last years, many genetic modified crops and foods were poured onto the market, and a comprehensive list of such bioengineered organisms worldwide was prepared by the U.S. Department of Agriculture and is available for consultation. However, if we compare the technologies underlying plant transformation and phytovaccination, and the implications they imply, we would find the former is much more advantageous than the latter one for many reasons. For example, since transgenic plants usually express a unique or a group of genes whose activities are directed to solve a single problem, phytovaccination provides a wider mechanism of protection; defense elicitors usually activate a multitude of reactions that bring about a broad range of protection against biotic and abiotic stresses). Another aspect that deserves special attention is how each of them impacts the environment. Transgenic plants require the use of auxiliary agrochemicals (i.e., glyphosate, gluphosinate), besides the inherent risk of gene drift/escape to cohabitant species that cannot be properly controlled. Phytovaccines, on the other hand, is made up of natural components (biological elicitors), and thus, are biodegradable, safe for farmers and consumers, and there is no risk of gene escapes. Moreover, the way phytovaccines are applied and the application methods are compatible with those used for agrochemicals, such as spray, dust, granule, gases (vapors), fog, bait, rub, or dip. They do not require mechanical infiltration or wounding as vaccines in humans, and the effect is systemic and requires minimal amounts of elicitor to activate the system, contributing further to environment protection.

5 CONCLUSION

Vaccines, and phytovaccines, are biological products that can be used to safely induce an immune response, either in humans or plants, conferring further protection against infection and/or disease on subsequent exposure to a pathogen. Although the mechanisms underlying the activation of the immune system when applying both kinds of products are unquestionably different, this commentary aims to point out some of the similarities found between them, claiming therefore to incorporate the use of the terms "phytovaccines" and "phytovaccination" based on the ultimate effects achieved rather than the mechanism and molecular players involved. We support our claim in the following facts: both vaccines and phytovaccines activate the innate immune system in humans or plants, respectively, upon recognition of specific molecules (elicitor/antigen), and both induce systemic and long-lasting protection against pathogens, as well as immune memory and passive protection. Therefore, we would like to extend the concept of vaccination to plants, posing the use of phytovaccination or plant vaccination when plant protection is achieved by activation of the plant immune system.

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Conflicts of Interest

All authors declared no conflict of interest.

Author Contribution

Tomas-Grau RH and Díaz-Ricci JC wrote the manuscript. Martos GG and Castagnaro AP critically reviewed the article. All authors approved the final version of the manuscript.

Abbreviation List

AsES, *Acremonium strictum* Elicitor Subtilisin
PRRs, Pattern recognition receptors
SAR, Systemic acquired response

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