TAILORING CROP NUTRITION TO FIGHT WEEDS

or

PHOSPHITE IN CROPS MENU TO BAN WEEDS

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Increasing crop productivity in an environmental-friendly way is one of the major challenges humankind is facing in the context of food security with a constantly growing population. In this scenario, ensuring adequate levels of soil nutrients and controlling aggressive weeds constitute two main problems that farmers have to dealing with. Classical methods for weed control consisted in onerous practices, such as hand weeding, or environmental aggressive approaches like tilling cultivation. The discovery of the first herbicide, the 2,4-dichlorophenoxyacetic acid, in 1940 (1) represented a breakthrough that greatly contributed to improve weed control with the subsequent increase in crop yields. In addition, the application of herbicides led to a significant reduction of tillage operations that had a direct beneficial consequence from the ecological point of view because of the decreasing in CO_2 emissions associated with farming activities (2). Later on, the commercialization of Roundup, an herbicide based on glyphosate, in the 70´s provided an unprecedented powerful tool since it was the first non-selective herbicide that eradicated most weeds germinating in soil. All these characteristics made the Roundup the most successful and used herbicide in the history of agriculture. Nowadays, however, herbicides have become victims of their own success. Their massive employment has promoted the appearance of many herbicide-resistant weeds in crop fields. It has been reported the existence of more than 254 herbicide-resistant weed species, of which 41 are glyphosate resistant (3). This situation is boosting the necessity of constant research to find new molecules and strategies to control weed spreading. In this issue of PNAS, Pandeya and colleagues (4) report the development of an original and efficient strategy for weed control, alternative to the utilization of classical herbicides. They demonstrate that selective fertilization of transgenic plants expressing a bacterial phosphite dehydrogenase (PTXD) with phosphite (Phi) provides an efficient way to restrain weed growth. *ptxD*-transgenic plants are able to convert Phi into orthophosphate (Pi, the metabolizable form of phosphorus (P)), outcompeting with different monocot and dicot weed species in both artificial substrates and natural soils from agricultural fields.

Phosphorus is an essential nutrient for plant growth and development. Most plants absorb and assimilate P as inorganic Pi that, because of its chemical properties, is immobile hampering its accessibility for plants (5). Furthermore, around 70% of arable lands have suboptimal availability of this ion, making soil amending with P fertilizers an essential practice to ensure high crop yields. The application of P fertilizers, however, has certain limitations that have to be considered. Soil microbiota competes with plants for this essential resource, converting P into insoluble inorganic forms or to unavailable organic forms. In fact, 80% of P fertilizers applied is lost by the action of these microorganisms

(6). From an ecological point of view, since Pi is metabolizable by algae, excessive applications of P fertilizers promote the bloom of these organisms in aquatic ecosystems causing eutrophication (7). More relevant, recent estimations indicate that the reserves of Pi in the earth are scarce and that, with the amounts that are being currently utilized, they will last only 50 to 200 years (8). Several laboratories have focused on developing plants with increased Pi-use efficiency or new fertilization strategies. In 2012, López-Arredondo and Herrera-Estrella proposed an original biotechnological approach to overcome the dependence of modern agriculture on Pi as a source of P. They overexpressed *ptxD*, a gene from *Pseudomonas stutzeri* encoding a Phi-specific oxidoreductase that oxidizes Phi to generate Pi, in Arabidopsis and tobacco (9). Data obtained clearly demonstrated that, under greenhouse conditions, *ptxD*-overexpressing plants were able to efficiently metabolize Phi into Pi. Interestingly, these plants required 30-50% less P when supplemented in the form of Phi instead of Pi to produce similar biomass (9). The application of Phi as fertilizer provides several advantages. For instance, it has higher solubility and lower reactivity with soil components than Pi, and, besides, soil microorganisms are unable to exploit it as source of P. Furthermore, Phi is not metabolizable by algae, thus, it can be predicted that its implementation as fertilizer will reduce the impact of Pi-based fertilizers in aquatic bodies. Plants, although able to absorb and metabolize Phi, cannot mobilize it either. Indeed, it has been shown that Phi negatively affects the growth of several plant species by interfering with Pi sensing systems (9). Based on this inhibitory activity, it was suggested that a potential additional advantage of the *ptxD*-Phi system could be its employment as a pre- and post-emergence weed control agent (9, 10). Moreover, the inhibitory activity of Phi has also been used to develop a novel selectable system for transgenic plants of different species, including Arabidopsis, tobacco, cotton and maize (11–14).

The paper by Pandeya and colleagues (4) represents a relevant step towards the validation of the *ptxD*-Phi system as a new strategy to overcome the problem caused by herbicide-resistant weeds in crop fields. As a first step, authors demonstrate that transgenic cotton plants expressing the bacterial *ptxD* gene are able to utilize Phi as a source of P. Transgenic plants fertilized with Phi displayed similar biomass as wild-type plants supplemented with Pi, confirming the adequacy of changing the P source. Then, authors performed various competition experiments with *ptxD*-transgenic cotton plants and different weeds to evaluate the efficiency of the *ptxD*-Phi system. In the first experiment, both a broadleaf and a grass weed were germinated together with transgenic cottons in a non-sterile inert substrate supplemented with Pi or Phi. The results indicated that Phi supplementation severely impaired the growth of both weeds species, while transgenic cotton plants showed a significantly increased biomass production compared to those supplemented with Pi. Similar results were obtained in competition experiments using natural soils from different locations that contained natural occurring seeds of weeds. At this point, the main issue that remained to be determined was whether the *ptxD*-Phi system was also able to restrain the growth of a glyphosate-resistant weed such as *Amaranthus palmeri*, which has well known negative effects in the production of numerous crops (15). Data presented by Pandeya and colleagues (4) confirm that, in fact, Phi fertilization schemes severely inhibit the growth of *A. palmeri*, either when carried out on inert artificial soil or non-sterile natural soil. Authors propose that, the deleterious consequences of Phi on weed growth mainly relies on its capacity to inhibit P uptake. Additionally, they suggest that the competitive advantage of *ptxD*-transgenic plants allows them to outgrow the weeds, increasing the inhibitory effect of shading.

In summary, the results reported in the paper of Pandeya and coworkers (4) provide unbiased evidence demonstrating that the *ptxD*-Phi system constitutes a powerful control technology to suppress the growth of both broadleaf and grass weeds, including glyphosate-resistant species. These results, together with the already known benefits of substituting Pi by Phi as fertilizer (see above), make the *ptxD*-Phi system a highly promising strategy, not only to increase agricultural production, but also to significantly reduce the impact of this activity in the environment (Fig 1). As stressed by authors in their work, a highly remarkably asset of this technology is the low probability that Phi-resistant weeds could develop since it would imply the acquisition of a completely new enzymatic activity to metabolize Phi that would require several mutations in preexisting dehydrogenases. Moreover, in contrast to Pi, Phi can be obtained from different sources, such as from the recycling of waste products from industrial processes in which sodium hypophosphite is employed to reduce metal ions in chemical-plating (i.e. nickel plating for decorative purposes) (5). Finally, it is worth mentioning that Phi does not represents any risk for human or animal health, and is already being extensively used as an efficient fungicide in agriculture (16). Nonetheless, it has been proposed that the use of Phi would impose a selective pressure to microorganisms unable to utilize it as a source of P, which could significantly modify the soil microflora with unpredictable effects on other members of the ecosystems (17). Taken into consideration the predicted expansive implementation of the *ptxD*-Phi technology in the next future, further studies are required to determine the impact and consequences that the application of Phi may have at different levels on the environment and, besides, on the development and reproduction of crops.

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Figure 1. The *ptxD*-Phi system represents a very effective technology to suppress weed growth while providing adequate crop nutrition and reducing environmental problems.

Pi-based fertilization allows weeds to compete with crops, such as cotton, for this P source, limiting their growth. In addition, a significant amount of Pi is also metabolized by soil microorganisms, avoiding its accessibility to crops, or ends up in the water bodies promoting algae blooms and the subsequent eutrophication. When using the *ptxD*-Phi technology, the ability of transgenic crops to utilize Phi as P source and the inhibitory effect of Phi on weed development, including on those herbicide-resistant such as *A. palmeri*, boost crop growing. Furthermore, because of the low capability of soil microbiota to metabolize Phi, its availability for crops is highly increased. Similarly, algae cannot metabolize Phi either, decreasing the risk of eutrophication of water bodies.

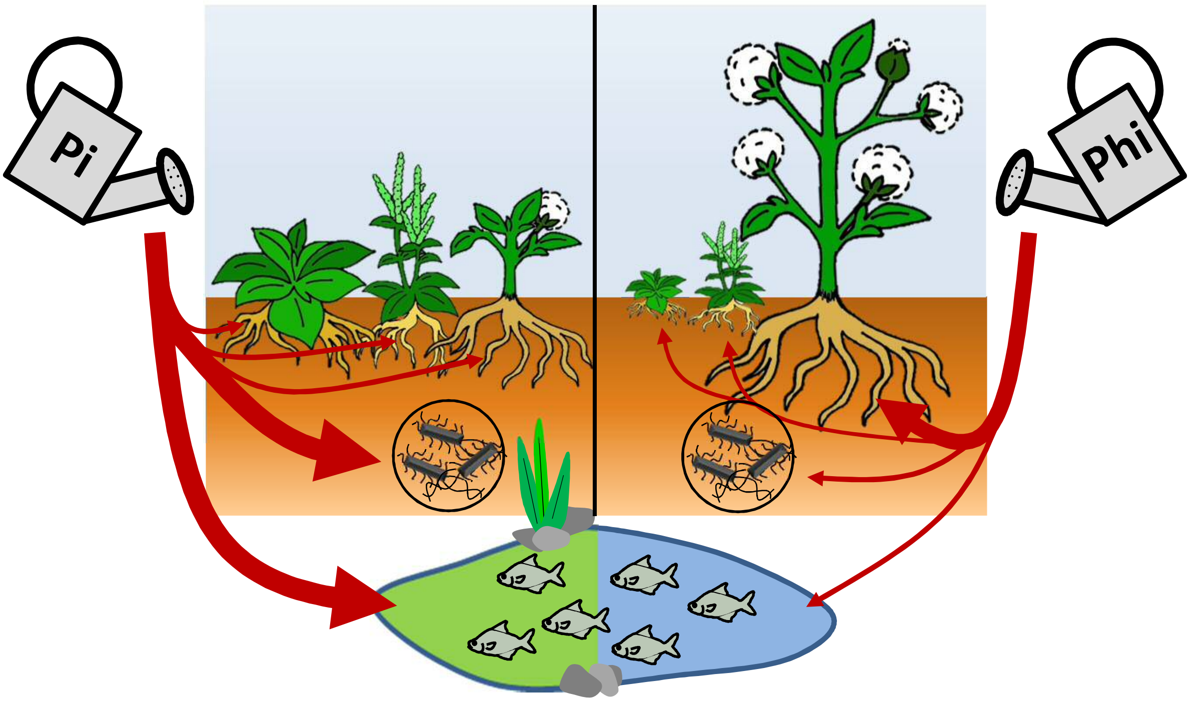

Figure 1