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BIOTECHNOLOGICAL APPLICATIONS IN WEED MANAGEMENT IN AGRICULTURAL SYSTEMS

Jorge Arturo Zegarra Flores1
jzegarraf@ucsm.edu.pe
https://orcid.org/0000-0002-5430-4283
Unversidad Catolica de Santa María Arequipa Peru

Marco Alexis Vera Zúñiga2
mavera@unap.edu.pe
https://orcid.org/0000-0002-2014-2845
Universidad Nacional del Altiplano Puno Peru

Fredy Grimaldo Calizaya Llatasi2
fcalizaya@unap.edu.pe
https://orcid.org/0000-0002-8042-852X
Universidad Nacional del Altiplano Puno Perú

Carmen Gisela Mindani Caceres2
cmindani@epg.unap.edu.pe
https://orcid.org/0000-0003-2765-4357
Universidad Nacional del Altiplano Puno Perú

Ada Luz Flores Ortega2
Adaflores1624@gmail.com
https://orcid.org/0000-0003-0565-9720
Universidad Nacional del Altipano Puno Perú

Ernesto Javier Chura Yupanqui2
echuray@unap.edu.pe
https://orcid.org/0000-0003-4227-220X
Univeridd Nacional del Altiplano Puno Perú

Abstract
Weeds represent a major problem in crop production, and their management in modern agriculture is crucial to avoid yield losses and ensure food security. Intensive agricultural practices, climate change and natural disasters affect population dynamics and this requires a change in weed management protocols. Emerging innovative trends in the field of biotechnology in weed management offer sustainable hope for the future; biotechnological tools that can contribute to

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weed management are herbicide-resistant crops, improved biocontrol agents, development of transgenic allelopathy in crops and characterization of weeds using molecular systems.

Keywords: Biotechnology. Weed control. Allelopathic crops. Biocontrol, Genetic silencing

INTRODUCTION

The production of food for a growing world population is and has been a topic of debate and concern, since the second century AD, Tertullian, raised the issue, followed in the eighteenth century by Malthus and more recently Paul Ehrlich, so it is expected that by the year 2050, the population could reach 9.15 billion. Therefore, an increase of 2.25 million is expected over the next 30 years, lower than the increase of 3.2 million that materialized between 1970 and 2010. This slowdown will have an impact on world agriculture, increasing per capita food consumption, which has improved to 2,770 kcal/person/day, however, this picture is not uniform as about 2.3 billion people are living in countries with less than 2,500 kcal/person/day (Alexandratos, s.f.).

From the early 1950s to the early 1980s, a new herbicide with a different mechanism of action was marketed every 2.5 to 3 years, however, a herbicide with new mechanisms of action has not been introduced since the 1980s. Herbicides with new modes of action are necessary to manage to evolve weed resistance to existing herbicides. New commercial products have probably remained inactive because of the reduced herbicide market due to glyphosate-resistant crops (Duke, 2012).

So weed management is essential for agricultural production and the environment and will play an important role in the future. As a challenge, weeds will continue to evolve and persist and sustainable management will have to be found.

Among the biotic stresses, weeds caused the highest potential loss (34%), with insects (18%) and pathogens (16%). The efficacy of protection was higher in cash crops than in food crops (Oerke, 2006).

Recent advances in agricultural biotechnology provide new options and alternative approaches to address these problems. At present, four main areas within the weed field offer opportunities for the application of biotechnology:

1. Development of herbicide-resistant crops.
2. Improvement of biocontrol agents.
3. Development of transgenic allelopathy in crops.
4. Characterization of weeds using molecular systems

DEVELOPMENT OF HERBICIDE-RESISTANT CROPS

Increases in crop productivity achieved in recent decades have resulted from genetic improvements and advances in agricultural technology, the latter of which have included better control of weeds, diseases and insects through the use of chemicals, as well as improved mechanization, and increased water and nitrogen supplies, and planting densities. The introduction of genetic engineering in weed control refers to those practices that lead to the development of improved varieties, so crop resistance to herbicides, which was one of the first genetic approaches to be

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applied; studies had shown that resistance to a herbicide was a dominant trait exhibiting the simple Mendelian inheritance pattern of a mutation in a gene; the agronomic importance of herbicides has been a driving force behind the development of herbicide-resistant plants (Mazur & Falco, 1989).

Herbicide-resistant crops (HRC) have been widely used for the last five decades, so herbicide resistance is defined as the ability of a plant population to tolerate herbicides at higher doses than the wild type of this plant. We know that an ideal herbicide should be able to kill weeds without affecting crop plants, however, available herbicides cannot select between weeds and crop plants. Therefore, crop resistance to herbicides has been developed through increased selectivity (Charudattan & Dinoor, 2000).

Hatzios (1987) indicates that there are five major mechanisms of herbicide resistance:

1. Target site resistance. It is the result of a modification of the herbicide binding site (usually an enzyme), which prevents a herbicide from binding effectively. If the herbicide cannot bind to the enzyme, then it does not inhibit the enzyme and the plant survives.
2. Enhanced metabolism. Occurs when the plant can degrade the herbicide before it can affect the plant.
3. Decreased absorption and/or translocation. May cause resistance because the movement of molecules is restricted and the herbicide does not reach its site of action in sufficient concentration to cause death.
4. Sequestration of a herbicide in vacuoles or cell walls can keep the herbicide distant from the site of action resulting in resistance.
5. Gene amplification/overexpression, the most recently identified herbicide, causes resistance by increasing the production of the target enzyme, diluting the herbicide relative to the site of action.

Techniques involved in the development of herbicide-resistant crops

The herbicide-resistant crop can be developed through traditional plant breeding and biotechnology. However, the latest biotechnological techniques are more prevalent than the former. These techniques include a) in vitro mutant selection in cells or tissue, b) somatic hybridization, c) microspore and seed mutagenesis, and d) cloned gene transfer into susceptible plants.

Traditional plant breeding

In traditional inter- and intraspecific breeding, variability in herbicide tolerance of crops and weeds is used. The success rate is very limited in this method due to sexuality, incompatibility, time, labor costs and slowness in the process (Beversdorf et al., 1980), In the first case of herbicide resistance cultivars, using conventional plant breeding techniques, when crossing a Brassica napus L. (canola) cultivar with a biotype resistant to the triazine Brassica rapa L., the modified cultivars showed lower yields, related to triazine resistance.

Biotechnological techniques

The different techniques in biotechnological methods are:

a. In vitro mutant selection at the cellular or tissue level

Plant cells are exposed to herbicides to select for resistance. Herbicide-tolerant mutants are selected. Rabin and Carlson (1978), sprayed immature leaves of mutagenized haploid tobacco plants with the herbicides bentazon and phenmedipham, after herbicide treatment and leaf expansion, the small green islands that appeared on the yellow leaves were cut off and placed on a known culture medium, this method is also known as the "green island technique". However, plants regenerated from these cultures retained only a small level of 13 to 21% resistance to the herbicides bentazon and phenmedipham

b. Somatic hybridization

The lack of sexual compatibility between crop plants and weeds was a major factor limiting the application of classical breeding techniques in genetic manipulations for the development of selected herbicide-tolerant crops. The problem can be avoided by using somatic cell genetic procedures such as fusion protoplast. Protoplasts are plant cells devoid of their cell walls. Under appropriate conditions, protoplasts from different plants can be induced to fuse in culture, combining their genetic information to create a new hybrid, one that could never have happened in nature. Recombinant protoplasts can be induced to reform their cell walls, proliferate, form callus and regenerate (Datta & Pilli, s.f.).

The first work for developing the feasibility of protoplast fusion to transfer atrazine resistance from botanically related resistant biotypes of black nightshade (Solanum nigrum L.) to the potato crop. However, although the goal was to achieve a crop with only one desirable trait of the weed, i.e. atrazine resistance, the hybrid formed resembled the weed more than the potato crop (Binding et al., 1982).

c. Microspores (gametophytes) and seed mutagenesis

Development of seed mutants with the application of a mutagen (ethyl methanesulfonate/EMS). However, several backcrosses of the isolated resistant mutant lines will likely be required to eliminate undesirable mutations. Wheat mutants with enhanced resistance to terbutryn seedlings (2-tert-butylamino-4-ethylamino-6-methylthio-s-triazine) and tomato mutants with enhanced resistance to diphenamid (N, N-dimethyl-2,2-diphenylacetamide) were developed by this method (Pinthus et al., 1972).

d. Transfer of cloned genes to susceptible plants

The direct transfer of cloned genes into sensitive plant cells is seen as a more innovative approach to genetically engineering crops for herbicide tolerance. There are different techniques.

1. **Vector engineering technique.** Hatzios (1987), states that, at present, several vectors can be used to transfer DNA from one plant to another. The most promising and widely used is a Ti plasmid from Agrobacterium tumefaciens. This bacterium has the unique ability to enter a plant cell and insert a small part of its plasmid DNA into the chromosomes of the host plant causing a plant disease known as crown gall, Caplan et al. (1983). By recombination of DNA, the gene

to be transferred is spliced into the Ti plastid of Agrobacterium tumefaciens. The engineered bacterium, which carries the recombinant DNA in its plasmid, is then introduced into excised leaf disc or plant cells grown in culture. The bacterium enters the cells and the plasmid inserts a part of its DNA into the chromosomes of the plant cells, thus introducing the cloned gene into the plant genome.

2. **Gene gun technique.** This tool facilitates gene transfer, developed in 1986 at Cornell University, USA. The gene gun fires DNA segments into the cell at high speed and some DNA segments are incorporated into the plant genome (Datta & Pilli, s. f.).

3. **Transposable elements (TE).** Döring (1985), determines that biochemical and genetic analysis of plant transposons have shown that they can induce unstable mutations and also that the transposon structure can be directly altered. The TE studied in plants is the Ac-Ds system, which operates in maize. After insertion, transposons can give rise to a variety of chromosomes with changes at the insertion site. Recent reports of successful transfer of the Ac element from maize into tobacco and tomato suggest that this element may apply to a wide variety of monocot and dicot systems for mutagenesis and subsequent isolation of TE-tagged genes involved in herbicide resistances.

4. **Direct gene transfer or electroporation.** Direct gene transfer (electroporation) describes the phenomenon of the uptake of genes into plant cells (protoplasts) through the naked plasma membrane independent of a biological vector and their functional integration of these genes into the host genome. This makes use of the fact that DNA can pass through plasma membranes and that protoplasts can be totipotent. The procedure requires only one gene under the control of plant expression signals (signals that function in plant cells) and protoplasts. However, it also has its limitations because it requires that totipotent protoplasts are not available for each desired plant species, especially cereals (Potrykus et al., 1985).

Strategies developed for herbicide resistance through genetic engineering

The different strategies developed for the resistance of crops to herbicides through genetic engineering are as follows:

1. **Modification of the herbicide action target.** To exert their phytotoxic action, most herbicides must bind to a receptor, which is usually a protein or enzyme involved in plant metabolism. Resistance to such herbicides develops if the protein or enzyme can be modified to functionally discriminate between the herbicide and the normal substrate for the binding site (Hatzios, 1987a).

   This approach depends on the identification in molecular terms of the biochemical site of action of the herbicide in the plant cell. Some herbicides have been shown to disrupt amino acid biosynthetic pathways and others interfere with photosynthesis. Glyphosate is currently the most widely used non-selective herbicide. Glyphosate has been shown to act by inhibiting 5-

enolpyruvylshikimate-3-phosphate synthetase (EPSP), a key enzyme in aromatic amino acid biosynthesis in bacteria and plants (Botterman & Leemans, 1988).

2. **Metabolic detoxification.** The selective activity of most commercial herbicides depends on their differential metabolism by tolerant and susceptible plant species (Hatzios, 1987b). Tolerant plants can produce phytotoxins that inactivate the herbicide molecule by enzymatic conversion. Maize, for example, is tolerant to atrazine due to its ability to metabolize this herbicide by conjugating it with glutathione tripeptide (GSH). The reaction is enzymatic, being catalyzed by GSH-sulfotransferase. Most of the currently available maize hybrids possess a dominant gene for atrazine-specific GSH-sulfotransferase biosynthesis.

3. **Alteration in translocation.** Resistant or tolerant plants survive by preventing the herbicide from reaching its site of action (target site). Use of radiolabeled herbicides has demonstrated modified translocation and vascular compartmentalization as the cause of tolerance, such as that observed in cucumber (Cucumis sativus L.) to atrazine herbicide and soybean to metribuzin, which showed that translocation of atrazine and metribuzin herbicide were limited to leaf veins and reached insufficient amounts of each herbicide to mesophyll cells containing the site of action (chloroplasts) of these photosynthesis-inhibiting herbicides (Hatzios, 1987b).

4. **d. Production of antibodies against the active ingredient.** Increased target enzyme activity is another mechanism that can confer resistance to selected herbicides. Such a mechanism has been demonstrated with glyphosate-resistant mutants.

Development of resistance in crops against various herbicides

Glyphosate

Glyphosate (Round Up) is a non-selective systemic herbicide that inhibits 5-enolpyruvylshikimate-3-phosphate synthetase (EPSP), which uses phosphoenol pyruvate (PEP) and shikamate-3-phosphate as substrates. EPSP is essential for the shikamate acid pathway for the production of aromatic amino acids. Glyphosate interferes with the binding of PEP to the active site of EPSP synthetase. According to the study by (Busi et al., 2013), glyphosate-resistant crops account for 80% of the 180 million hectares of transgenic crops grown annually worldwide(Datta & Pilli, s.f.).

Genetic engineering work to develop glyphosate-resistant crops has focused on three strategies:

1. EPSPS gene overexpression: an EPSP overexpression gene was detected in Petunia sp. The Petunia gene was isolated in introduced into other plants through Agrobacterium-mediated gene transfer. Transgenic plants can tolerate glyphosate 2-4 times greater than that required to kill wild-type plants.
2. Introduction of a metabolic detoxification gene: glyphosate oxidoreductase (GOX), the gene was cloned from Ochrobactrum anthropi and has been used together with the cp4 gene to confer glyphosate resistance in several commercially available crops such as soybean, maize, canola and cotton.

3. Altered EPSP synthase enzyme with decreased affinity for glyphosate: EPSP synthase is modified with amino acid substitution of proline for serine by the aroA gene of Salmonella typhimurium causing decreased affinity for glyphosate without affecting the kinetics of the enzyme. For example, glyphosate-resistant transgenic tobacco (Stalker et al., s. f.).

Glufosinate

Glufosinate is a key herbicide for managing glyphosate resistant weeds, mainly because it is a broad spectrum herbicide, and there are transgenic crops resistant to glufosinate. Its use has increased during the last decade, however, the area treated with this herbicide is much smaller compared to glyphosate, mainly because it provides variable field performance, among the factors are environmental conditions, application technology and weed speciess (Takano & Dayan, s.f.).

Bialaphos is a pro-herbicide that plants convert to glufosinate or its ammonium salt L-phosphinothricin (PPT) which is an active ingredient in several non-selective herbicides such as Basta. The herbicide acts by inhibiting the ammonia assimilation enzyme glutamine synthetase (GS) (Datta & Pilli, s.f.).

Different strategies were developed to develop resistance to PPT in transgenic crops:

1. Detoxify PPT: the Bar gene (resistance to Bialaphos) was isolated from Streptomyces sp, which encodes a phosphonothricin acetyl transferase (PAT) that converts the herbicide molecule into a non-toxic acetylated form. For example, phosphonothricin-resistant tobacco, potato, tomato (Datta & Pilli, s.f.).
2. Gene overexpression: an alfalfa gene overexpressing the enzyme glutamine synthetase (GS) was integrated into the tobacco plant with the help of cauliflower mosaic virus 35S and *Agrobacterium tumefaciens* mediated gene transfer. The transgenic tobacco showed resistance to glusinate through overproduction of GS (Donn et al., 1984).

Sulfonylurea

Sulfonylurea herbicides inhibit acetolactate synthase (ALS) in plants involved in the biosynthesis of each branched amino acids such as leucine, isoleucine and valine. Mutant genes that exhibit resistance to sulfonylureas are found in some microorganisms and plants such as *Arabidopsis thaliana* (Haughn & Somerville, 1987). These mutant ALS genes can be isolated and transferred into crop plants to impart resistance. For example, sulfonylurea-resistant cotton, soybean, canola and rice.

Bromoxynil

Bromixinil is a nitrile herbicide that inhibits PSII by hindering electron transfer. A gene (bxn) encoding the enzyme bromoxynyl nitrilase (BXN) isolated from the soil bacterium Klebsiella ozaenae or *Agrobacterium sp*. transforms bromoxynil to nontoxic 3,5-dibromo-4-hydroxybenzoic acid, imparting resistance to bromixinil. For example, broboxynil-resistant cotton and canola (Datta & Pilli, s. f.).

Table 1. Crops modified to generate herbicide resistance

Herbicide	Resistance gene	Gene source	Crop

Clorosulfuron	ALS gene mutagenesis	*Nicotiana tabacum*	Tobacco, tomato, potato, corn
Sulfonamide	Sul, DHPS (Dihydroteroate synthase)	Plasmidio de amplio rango	
Norflurazon	Carotenoid biosynthesis	*Erwinia uredovora*	Cotton
Dalapon	Dehalogenase	*Pseudomonas putida*	Tobacco
2,4-D	Monooxygenase	Alcaligenes eutrophus	Tobacco, cotton
Phenmedipham	Carbamate hydroxylase	Arthrobacter oxidans	Tobacco

Source: (Datta & Pilli, s.f.)

Advantages and limitations of herbicide-resistant crops

In the first 21 years of commercialization of herbicide-resistant crops (1996 to 2016), they have produced significant benefits agronomically, environmentally, economically and socially to farmers and probably perhaps to consumers (ISAAA 2017).

In 2017, cumulative biotech crop acreage (since 1996) increased to a record 2.3 billion hectares. Global hectares increased from 185.1 million hectares to 189.8 million hectares, an increase of 3% equivalent to 4.7 million hectares. This global area is equivalent to almost 20% of China's total land area (956 million hectares) and more than 7 times the land area of the United Kingdom (ISAAA 2017).

Of the total number of 24 countries planting biotech crops, 19 are developing countries and 5 countries are industrialized. The top ten countries, grew by more than 1 million hectares each in 2017, which was released by the United States, which grew 75 million hectares (40% of the global total, higher than 1% in 2016), Brazil with 50.2 million hectares (26%), Argentina with 23.6 million hectares (12%), Canada with 13. 1 million hectares (7%), India with 11.4 million hectares (6%), Paraguay with 3.0 million hectares (2%), China with 8 million hectares (1%), South Africa with 2.7 million hectares (1%) and Bolivia with 1.3 million hectares. Another 14 countries grew a total of approximately 3.7 million hectares in 2017 (ISAAA 2017).

a. Efficiency

The main benefit of herbicide-resistant crops is the elimination of any injury caused by the previously non-selective herbicide, in addition to improved weed control resulting in higher yields, lower costs, fewer application restrictions and environmental improvement (Green, 2012).

b. Increased profitability and yields

Weeds compete for water, nutrients and sunlight and are the largest pest complex in crop production. Herbicide-resistant crops help growers meet an ever-increasing demand for food, fiber and fuel. Yield increases in general depend on how effectively the grower has previously controlled weeds. No herbicide-resistant GM crop directly increases yields.

c. Lower production costs

Los cultivos resistentes a herbicidas han estado disponibles momentos en el que la cantidad de trabajadores agrícolas estaban disminuyendo y las malezas se vuelven ampliamente resistente a los herbicidas selectivos y son difíciles de controlar. El glifosato era el herbicida ideal para cultivos resistentes a herbicidas, su aplicación foliar controla todas las malezas-300 especies de malezas-en una amplia gama de etapas de crecimiento sin restricciones. Los productores podrían aplicar un solo herbicida en lugar de muchos otros, para el control de malezas de hoja ancha y angosta. La capacidad de usar glifosato en cultivos resistentes, ha hecho que el manejo de malas hierbas sea fácil, efectivo y eficiente mejorando la productividad agrícola.

d. Beneficios ambientales

The potential impact of herbicide-resistant crops on the environment clearly shows that cropping systems reduce soil erosion. The widespread use of reduced tillage, also known as conservation tillage, which maintains a soil covered with crop residues, has been allowed to have many positive environmental effects such as reduced soil erosion, reduced water pollution from nutrient and sediment runoff, protection against wind erosion, improved habitat for birds, mammals and microorganisms, and reduced fossil fuel use and carbon dioxide emissions. For example, conservation tillage can reduce soil loss by more than 90 percent and phosphorus movement by more than 90 percent 70% (Green, 2012).

Resistant crops do not increase herbicide use; since 1996, the amount of herbicides decreased in corn, soybean, cotton and canola crops by 204 million kg, a very similar amount by which insecticides have been reduced with insect-resistant transgenic crops (Green, 2012).

Environmental impact of herbicide use change on resistant crops (mostly glyphosate resistant) worldwide from 1996 to 2009.

Crop	Change in herbicide use (million kg)	Change in herbicide use (%)	Change in environmental impact (%)
Soya HR	-40.9	*-2.2*	-16
Maíz HR	-140.3	-9.2	-10.5
HR Cotton	-8.9	-4.0	-6.9
HR Canola	-14.0	-16.2	-23.2

Source: (Green, 2012)

Environmental impact is determined by the environmental impact quotient (EIQ), an indicator of a wide range of environmental impacts, including the amount and toxicity of pesticides used, leaching, runoff, and potential exposure to workers and consumers.

Among the constraints is resistance, at least 23 weed species have developed resistance to glyphosate, exerting unprecedented selection pressure on weeds and ultimately leading to widespread weed evolution, contamination of organic crops by genetic pollution, and the development of super weeds through gene flow.

IMPROVEMENT OF BIOCONTROL AGENTS

Initially, the term biocontrol was defined as the use of parasites and predators to control pests including weeds, however, Harris (1991) indicated that the term biocontrol can be extended to all non-chemical methods of control, including the use of products derived from microorganisms (Triolet et al., 2020).

Bacteria, fungi and viruses have been used for weed control, however, the use of the latter has been very limited. The application of bioherbicides in weed management is rapidly gaining acceptance among environmentalists, farmers and consumers, given the negative consumer perception of food products grown with synthetic chemical herbicides (Verma et al., 2020). The study of actual microbial herbicides, by definition, began to be studied in the middle of the last century, and has developed rapidly with the advancement of the study of phytotoxins, for the development of new types of bioherbicides. The study and separation of phytotoxins can be divided into three types: bacterial, fungal, actinomycete. The pathogens that produce phytotoxins as a microbial herbicide must meet: 1. be reproduced by biological techniques, 2. grow rapidly within a defined time, 3. adapt to industrial production. 4. Suitable for packaging and transport (Li et al., 2003).

Bacterial bioherbicides

Bacteria as biocontrol agents multiply rapidly, possess genetics that are rapidly modified and require readily available nutrients for growth. The genera Pseudomonas and Xanthomonas have exerted the best control (Banowetz et al., 2008). *Pseudomonas: Pseudomonas fluorescens* strain D7 and P. fluorencens BRG100 have suppressed the growth of *Bromus tectorum* and *Setaria viridis*, respectively. Imaizumi et al. (1997) reported the use of *Xanthomonas campestris* cv:*poae* JTP482 and X. *campestris* LVA-987 as biocontrol agents of *Poa annua* and *Conyza canadensis*, respectively. The mechanism of operation of these bacteria used as bioherbicides is their production of metabolites that alter their germination and plant growth.

Fungal bioherbicides

The use of bioherbicides containing fungal active ingredients or natural fungal molecules is one of the possible solutions to reduce the use of chemicals. A dozen fungus-based bioherbicides are on the market in the United States and Canada, while countries such as China and South Africa have one, and none are available in Europe. The active ingredients of these bioherbicides are live fungi, but so far there is no product based on fungal molecules on the market (Triolet et al., 2020).

One of the most successful examples of classical biological weed control is the introduction of a rust fungus, Puccinia chodrillina, in Australia to control the weed "skeleton weed" (Chodrilla juncea), the success of the biocontrol project has resulted in a 1:100 cost-benefit ratio in Australia (Imaizumi et al., 1997).

The use of fungi as herbicides has been used as biocontrol agents in the control of weeds. The use of fungi of the genus *Colletotrichum* in commercial formulations is reported in the control of certain weeds. (Morthensen et al.) analyzed the fungal pathogen, *Colletotruchum gloeosporioides* (Penz.) Sacc. F. sp, mallows and C. *gloeosporioide f. sp. aeschynomene* as an effective agent for the control of Malva pusilla and *Aeschynomene virginica*, respectively, which resulted in excellent control of Malva pusilla and Aeschynomene virginica, respectively.

Viral bioherbicides

The application of viruses as bioherbicides in agricultural crop management has been established. Studies have shown that tobacco green mosaic virus has the ability to reduce the growth and phenology of Solanum vivarium. In addition, the use of Araujia mosaic virus in the effective control of the weed Araujia hortorum was reported. Another virus of interest being considered as a bioherbicide in the control of Impatiens glandulifera is tobacco rattle virus. The report by Kazinczi et al. also showed the efficacy and potential of Obuda pepper virus and Cucumber mosaic virus in the control of Solanum nigrum. These viruses resulted in significant reduction of weed populations. The result of the application of these viral bioherbicides is the prevention of losses and increase of yields and quality of agricultural products (Verma et al., 2020).

WEED CONTROL WITH ALLELOPATHY

The term allelopathy, proposed by Molisch en (1937), derives from two Greek roots, allelon= reciprocal and pathos= suffering, taken literally, would mean the detrimental effect between two plants. However, Molisch, with great vision, defined allelopathy as the biochemical interaction, harmful or beneficial, between all types of plants, and included in microorganisms (Zuñiga, 2008). Allelopathy can be defined as the "chemical warfare" between different plant species, both crops and weeds produce phytotoxins that could be allelochemicals that provide an advantage in plant-plant competition (John Wiley & Sons, Inc, 2000).

According to the definition given by the International Allelopathy Society in 1996, allelopathy includes "any process involving secondary metabolites produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including both positive and negative effects" (Macías et al., 2007).

Allelopathic compounds are natural products that may be direct metabolites, by-products of other metabolic steps, or breakdown products of other compounds or biomass. The compounds are usually toxic to the plant that produces them if they are not stored in a non-toxic form or released before they accumulate to levels that are toxic within the plant. In some cases, even when toxins are released from the plant, they may accumulate in the immediate environment and become toxic to the plant that produced them. Allelopathic products come in many forms, from water-soluble to volatile, simple to complex, short-lived to persistent. The most common allelopathic compounds belong to the chemical groups: tannins, phenolic acids, terpenes and alkaloids.

Allelopathic products are released from the plant in a variety of forms. They can be washed from green leaves, leached from dried leaves, volatilized from leaves, exuded from roots, or released from plant debris during decomposition. Even flowers, fruits and seeds can be sources of allelopathic toxins. There are also cases in which the products do not present toxicity, a situation that changes when they are altered when released into the environment, either by natural chemical degradation or by their conversion into toxic products by the activity of certain microorganisms (Gliessman, 2002).

In natural ecosystems, allelopathy can help to explain some important phenomena:

- The dominance of a single species or group of species over others.
- Successional change and replacement of species, or the maintenance of a delayed state in the successional process.
- The reduced productivity of the ecosystem, and
- The characteristic pattern of distribution of plant species in the environment.
- Allelopathy is a form of amensalism, an association between organisms in which one is inhibited or destroyed and the other is unaffected by the release into the environment of secondary metabolites called allelochemicals. Allelopathic interactions between plants have been known since the 4th century BC, but only in recent years have they received adequate attention from the international scientific community and from farmers. Today, in modern agriculture, allelopathy plays a key role in maintaining the sustainability of agroecosystems through the adoption of environmentally friendly strategies such as crop rotation, cover or smother crops, intercropping, incorporation of crop residues, mulching and bioherbicides. Crops that exhibit allelopathic properties are numerous: they include tree and herbaceous species as well as many weeds (Scavo & Alessia, 2018).

Current status of the use of allelopathy for weed management

Both crops and weeds produce phytotoxins that could be allelochemicals that provide an advantage in plant-plant competition. Allelopathy can be used in several ways in weed management: allelopathic cover or smother crops, allelopathic companion crops, allelopathic mulching or incorporation of phytotoxic crop residues, production of allelopathic crop cultivars with weed suppression potential, and use of allelochemicals as sources of natural herbicides (John Wiley & Sons, Inc, 2000).

a. Allelopathic cover or smothering crops

Cover crops can cause the accumulation of one or more allelochemicals in the rhizosphere. After cover crop dewatering, the crop of interest is planted through cover crop residues, provided the crop is resistant to the accumulated allelochemicals, which had accumulated in the soil, or were released by degradation of cover crop residues, the allelochemicals may act to suppress weed emergence and/or growth.

A recent example is the use of *Sorghum Sudanese* as a cover crop to inhibit weed establishment, followed by no-tillage planting of large seed crops such as soybeans that are relatively insensitive to allelochemicals. This method is effective but is not economically competitive with synthetic herbicides. Research to produce crops with significant allelopathic properties has so far failed to produce a commercial product.

The allelopathic effects of sudex [Sorghum bicolor (L.) Moench x *Sorghum sudanese* (P.) Stapf. CV. FFR 201] on weeds and plant species were also evaluated. Allelopathic potential was measured by radicle elongation of species used as indicator plants, which decreased with increasing sudex age (Weston et al., 1989).

Many others have considered allelopathic suppression of weeds by various cover crops: such as rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.), buckwheat (*Fagopyrum esculentum Moench*), black mustard [*Brassica nigra* (L.) Koch], or *sorghum-sudangrass* hybrids [Sorghum bicolor (L.) Moench × S. sudanense (Piper) Stapf] can be very effective in reducing weed populations (Table 1) (Weston, 1996).

Table 3. Cover crops used for weed control, with allelochemical compounds identified.

Common name	Scientific name	Allelochemicals	References
Black mustard	*Brassica nigra* (L.) Koch	Allyl isothiocyanate and other water-soluble inhibitors	Bell and Muller, 1973; Muller, 1969
Clover (red, white)	*Trifolium* spp.; *Melilotus* spp.	Isoflavonoids, phenolics	Rice, 1984
Oats	*Avena saliva* L.	Phenolic acids, scopoletin	Rice, 1984; Guenzi and McCalla, 1966
Rye	*Secale cereale*	Phenolic acids, benzoxazinone	Barnes and Putnam, 1987; Mwaja et al. 1995; Nair et al., 1990; Shilling et al., 1985.
Wheat	*Triticum aestivum* L.	Phenolic acids, simple acids	Shilling et al., 1985; Guenzi and McCalla, 1966
Sorghum	*Sorghum spp.*	Phenolic acids, durrin, sorgoleone	Einhellig et al., 1993; Forney and Foy, 1985;
Buckwheat	*Fagopyrum esculentum* Moench	Fatty acids	Tsuzuki et al., 1987

Source: (Weston, 1996)

b. Intercropping

Growing several crops at the same time in the same field is an important strategy to increase the efficiency of input use (water, fertilizer and water) and improve crop yield and economic profitability. Recent studies have explored the effectiveness of intercropping with allelopathic crops as a good alternative to chemical weed control.

Intercropping of forage legumes in corn helped control Striga hermonthica Del. (Benth). Infestation of Cyperus rotundus L. in cotton crop was significantly reduced with intercropping of sesame (Sesamum indicum L.), soybean and sorghum in alternate rows (Farooq et al., 2020).

The intercropping of corn and cowpea in alternating rows helped reduce the weeds *Echinocloa colona* (L.) Link, Portulaca oleracea L., and Dactyloctenium aegyptium (L.) Willd. by approximately 50 percent, as well as improve soil use efficiency (Saudy, 2015). In another study, the intercropping of legume cover crops with wheat was evaluated for weed control compared to wheat alone, four forage legume species (Medicago lupulina, Medicago sativa, *Trifolium pratense* and *Trifolium repens*) were planted in six organic wheat fields. The dynamics of legume cover crops were very different between species and crop periods, they helped to suppress weeds in addition to reducing weed density in the following crop, red clover was the most effective intercrop for weed suppression in the organic wheat crop (Amossé et al., 2013).

c. Allelopathic plant residues

In most cases, specific parts of the crop are used for consumption, while the remaining portions of the plant are fed to animals, discarded or incorporated into the soil as organic matter. For example, wheat, corn and rice are grain crops that are consumed as food, while other parts of the plant are fed to animals or left in the field (Jabran et al., 2015). For example, barley, rye and triticale residues left in a corn field were evaluated for their allelopathic effect against E. *crus-galli* and *Setaria verticilata* (L.) P. *Beauvc* (Dhima et al., 2006). Allelopathic mulches decreased the occurrence of S. *verticilata* (67%) and E. *cruz-galli* (27-80%) compared to the control treatment. Corn plants did not receive any damaging effect and corn grain yield was increased by 45% in plots applied with barley mulch.

In another study, tomato seedlings were transplanted on three different types of mulches from cover crops hairy vetch (*Vicia villosa* Roth.), subterranean clover (*Trifolium subterraneum* L.), oats (*Avena sativa* L.), and the results indicated that all mulches suppressed weeds in density and aerial biomass compared to the conventional system (on average -80% and -35%, respectively). Oats were the best mulch for weed control, but also affected the marketable tomato yield (-15% compared to the conventional treatment). *Amaranthus retroflexus* L. and *Chenopodium album* L. are typical weeds associated with the conventional treatment. Legume cover crops, in particular hairy vetch, gave the best marketable tomato yield 28% higher than the conventional system, which shows us that winter cover crops converted to dead mulch could be successfully used in integrated weed programs to reduce weed infestation in tomato crops (Campiglia et al., 2010).

d. Inclusion of allelopathic crops in rotation

Crop rotation is the sequence or arrangement of crops planted in a field to maintain soil productivity and sustainability, certain changes in crop sequence can reduce pest infestation. Crop rotation alone decreases weed infestation in crop fields while improving effectiveness when combined with other methods (Amossé et al., 2013). Allelopathic plants have been shown to insert the soil with allelochemicals, which suppress weeds in the next crop, recently (Tabaglio et al., 2008) determined the inclusion of rye in rotation with a corn crop, the purpose was to suppress weeds such as P. *oleracea* and A. *retroflexus* in the following corn crop, through the exudation of allelochemicals 2,4-dihydroxy-1,4 (2H)-benzoxazin-3-one (DIBOA) and benzoxazolin-2 (3H)-one (BOA). In conclusion, elelopathic crops included in the rotation help suppress weeds in the following crop through exudation of allelochemicals.

Development of herbicides from allelochemicals and their derivatives

Herbicides with new modes of action are much needed due to increasing herbicide resistance in weeds against all major herbicide groups. Additionally, weed management in organic production systems presents a major challenge (Farooq et al., 2020). Natural compounds from different microorganisms and crops with herbicidal action have been identified and can be classified into two main groups: phenolics and terpenoids (Anaya, 2006). These natural phytotoxins offer a great opportunity to be used directly as natural herbicides and to develop new herbicides with different modes of action.

The toxicity of allelopathic compounds depends on several factors including cultivar, plant part, extract concentration, donor plant growth and environmental conditions.

The biotechnological future in germplasm selection to improve allelopathic potential

The importance of cultivars with weed suppressive ability is increasing due to weed resistance to herbicides. In this scenario, it is important to develop cultivars with high allelopathic potential to control weeds and reduce herbicide use. Allelopathic trait variability can be used to develop cultivars with higher weed suppressive ability, for example, rice produced from two inbred lines, one with allelopathic gene and the second with restorer gene had strong suppressive ability against E. cruz-galli.

Marker-assisted selection can help develop crops with allelopathic potential. For example, in the study of molecular markers associated with wheat allelopathy developed by (Wu et al., 2003), Using molecular markers, restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and microsatellite markers (SSR) analysis was performed and identified two major QTLs on chromosome 2B associated with wheat allelopathy. Linkage analysis of genetic markers and QTLs can improve genetic gains for allelopathic activity through marker-assisted selection in wheat breeding. The development of allelopathic wheat cultivars could reduce the over-reliance of weed control on synthetic herbicides (Wu et al., 2003).

The stimulant effect of low doses of toxicants is known as hormesis, this definition does not establish whether it is beneficial or harmful to the organism, only that it is a stimulant, and it was first used in 1943, for the stimulant effect of low doses of an extract of red cedar (*Juniperus virginiana* L.) on certain fungi (Abbas et al., 2017).

The hormesis of allelochemicals can play an important role in sustainable weed management. The dose-response effect of allelochemicals (inhibition at higher concentrations and growth enhancement at lower concentrations) can be used in the production of weed-inhibiting crops. For example, the application of hormetic doses to crop plants can produce a herbicidal effect on weeds at their sensitive growth stage (Farooq et al., 2020).

WEED CHARACTERIZATION USING MOLECULAR SYSTEMATICS

Molecular systematics is the use of molecular genetics to study the evolution of relationships between individuals and species. The use of molecular systematics is necessary because of limited numbers of characters with morphological data, misinterpretation of characteristic changes, complicated classical taxonomy, and phenotypic versus genotypic differences are not elucidated (Datta & Pilli, s. f.).

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Among the advantages of molecular systematics, it is possible to obtain phylogenetically reliable characters from any genome of the organism, less possibility of obtaining misinterpreted data and providing data to differentiate between two similar specimens.

Weed species of the genus Amaranthus have increased in frequency and severity in recent years, their identification is difficult due to similar morphological characteristics among species and variation within species. To address this concern, a molecular marker identification system was developed using amplified ribosomal DNA (rDNA) restriction enzyme analysis that allowed the identification of eight species (Wetzel et al., 1999).

The study of the morphological and molecular characterization of Echinochloa spp in rice (Ruiz-Santaella et al., 2006) determined that correct species identification is important both agronomically and economically because Echinochloa ssp, an aggressive weed species, is difficult to control. The limited use of available herbicides to control weeds in rice fields showed the need to establish methods to discriminate genotypes and establish taxonomic relationships within Echinochloa. In this regard, DNA-based molecular markers are particularly useful for identifying genetic diversity within plant species using the RAPD markers (Ruiz-Santaella et al., 2006).

GENETIC ENGINEERING IN WEED MANAGEMENT

The advent of new genetic technologies such as gene silencing and gene drive are tools for weed management that are gaining significant momentum. These technologies promise new ways to develop sustainable weed control options; gene silencing can switch off genes that control adaptation (e.g. growth, herbicide resistance) and gene drive can be used to disseminate modified traits and engineer wild populations with a reduced competitive ability (Kumaran et al., 2020).

CONCLUSIONS

The possible biotechnological applications for weed management are quite promising, the use of genetic engineering techniques for the generation of crop resistance to herbicides is one of the applications that have been successful in recent years, as well as the use of bioherbicides formulated from natural products that have a greater degradative capacity, the advance in our knowledge of the interactions of plants and phytopathogenic organisms at the molecular level increases the understanding of the role of phytotoxins in crops creating allelopathies for weed control in the field. New genetic technologies such as isolation and gene drive show us a pathway for a sustainable increase in crop production and environmental friendliness.

After exploring different aspects of the biotechnological approach to weed management, it can be concluded that its biotechnological application offers a powerful new tool that has been used and updated and that can be integrated into Integrated Weed Management economically and effectively.

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