Trends in Biotechnology

CellPress

Review

New Technologies for Insect-Resistant and Herbicide-Tolerant Plants

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The advent of modern molecular biology and recombinant DNA technology has resulted in a dramatic increase in the number of insect-resistant (IR) and herbicide-tolerant (HT) plant varieties, with great economic benefits for farmers. Nevertheless, the high selection pressure generated by control strategies for weed and insect populations has led to the evolution of herbicide and pesticide resistance. In the short term, the development of new techniques or the improvement of existing ones will provide further instruments to counter the appearance of resistant weeds and insects and to reduce the use of agrochemicals. In this review, we examine some of the most promising new technologies for developing IR and HT plants, such as genome editing and antisense technologies.

Trends

We provide a concise but complete overview of the present status of the technologies used for commercially available varieties for producing IR and HT plants.

We describe the limitations of current technologies.

We also describe the most promising genetic manipulation technologies, such as genome editing and antisense technologies [by means of oligodeoxynucleotides (ODNs) and double-stranded (ds)RNA] as well as the development of transgenic plants expressing proteinase inhibitors. In the near future, such technologies could contribute to a reduction in the use of agrochemicals and to meet the demand for increased crop production, perhaps resulting in the public acceptance of biotech crops.

Conventional Approaches

Each year, insect pests and weeds are estimated to lower crop productivity worldwide by an average of 28% [1]; thus, the development of IR and HT varieties is a primary goal for plant breeders. Over the centuries, farmers have selected plant varieties that are more resistant to pests; by contrast, the mass diffusion of synthetic herbicides began in 1946 with the use of 2,4-dichlorophenoxyacetic acid (2,4-D), while the first commercial HT crop obtained through conventional breeding (a triazine-resistant oilseed rape marketed in Canada, named OAC Triton) dates back to 1984 [2]. Hence, given our ability to artificially alter plant genetic material, there has been a significant boost in the creation of IR and HT plants by mean of mutagenesis and genetic engineering (GE).

Mutagenesis relies on the use of ionizing radiations, lower ultraviolet (UV) rays, chemical agents, or mobile genetic elements, to induce favorable mutations [3]. According to the United Nations Food and Agriculture Organization (FAO)/International Atomic Energy Agency (IAEA) Mutant Variety Database[i], there are 30 patented plant varieties resistant to insect pests and a few that are tolerant to herbicides that have been obtained through mutagenesis; to these can be added the multiple lines of BASF Clearfield® crops tolerant to herbicides of the imidazolinone family.

Biotech crops engineered for IR, HT, or both (stacked traits) cover most of the approximately 181.5 million ha of agricultural land occupied by GE plants worldwide [4]. The main strategies to develop HT plants via GE are based on the introduction of genes encoding enzymes [e.g., glyphosate *N*-acetyltransferase (*gat*) and glyphosate oxidase (*gox*)] degrading the herbicide into nontoxic compounds, or through the modification of plant genes encoding biochemical targets of the herbicide [e.g., 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) and aromatic amino acid (*aro*A)], or inducing the overproduction of the unmodified target protein permitting normal metabolism to occur [5]. By contrast, IR in transgenic plants is commonly

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obtained through the insertion of *Bacillus thuringiensis* (Bt) toxin genes from the soil bacterium *B. thuringiensis* encoding crystal toxins (Cry proteins) [6]. Cry proteins are solubilized in the insect midgut where intestinal proteases process the formed protoxins and cleave the C or N terminus. The activated toxins recognize binding sites on the midgut brush border membrane surface and form ion channels or pores in the epithelial membrane, leading to cell lysis and eventually death [7]. In addition, since 2011, Syngenta has been marketing the Agrisure®, Duracade™, and Viptera™ maize lines with stacked genes encoding BtCry1Ab delta-endotoxin and the vegetative insecticidal protein Vip3Aa20. Although the mode of action of Vip3A and Cry toxins is nearly identical, they recognize different membrane proteins at the surface of the midgut epithelium of susceptible insects [8]. A variant is represented by the SGK 321 transgenic cotton line, containing genes encoding a Bt toxin and a seed-expressed Bowman-Birk type trypsin proteinase inhibitor from cowpea (CpTI), approved for commercial growing in China in 1999. Protease inhibitors (PIs) in plants are naturally occurring proteins released in response to a physical injury or biological attack that inhibit the function of the proteases present in the gut tract of the insects and their larvae, involved in digestive processes. The inability to acquire essential amino acids causes the severely delayed development of the insect, making it impossible for individuals to mature and procreate [9].

However, regardless of the method by which they were implemented, control strategies have led to the development of resistant insect pests and weeds; in addition, the widespread use of pesticides has resulted in the evolution of 574 arthropod species and 238 weed species that are resistant to agrochemicals[ii,iii]. Specifically, with the cultivation of GE crops, the evolution of glyphosate resistance has been highlighted in 14 weed species and biotypes in the USA [10], while a recent study [11] demonstrated that, during 1996–2011, five out of 13 major pest species (albeit with some distinctions) had become largely immune to Bt poisons in GE corn and cotton. Furthermore, an important issue to consider is the expiration of patents on transgenic IR and HT plants, which will allow farmers to save seeds to replant freely. This is likely to result in an increase in the acreage planted under GE cropland worldwide, inasmuch as seed saving could be discouraged from the onset of resistant pests and weeds- and, overall, an increased risk of transgene escape. As an extreme effect, the need to prevent the saving of engineered seeds could lead to the revision of the international moratorium on genetic use restriction technologies (GURTs); that is, experimental methods providing specific switch mechanisms for transgene containment and intellectual property protection [12].

The described context, and the increased health and environmental concerns associated with the use of agrochemicals, are promoting both the development of new techniques and the improvement of existing methods for the production of IR and HT plants. The most promising strategies are described below.

Improvement of Traditional Methods

Gene Pyramiding

Gene pyramiding refers to the process of stacking multiple genes into a single genotype to combine desirable traits through recombinant DNA technology or conventional breeding. This approach has resulted in the so-called ‘second generation’ of GE plants. Gene stacking has been principally obtained through crosses between GE plants with different biotech traits (hybrid stacking), such as in Agrisure™ and Viptera™ maize. Other methods involve plant transformation with two or more genes harbored in a single (linked genes or multigene cassette transformation; e.g., Herculex™ maize) or in separate (co-transformation; e.g., Knockout™ maize) gene constructs, or the insertion of one or more genes into an already transgenic plant (retransformation; e.g., Bollgard™ II cotton)[iv].