The Use of Biotechnology in Weed Science

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The most notable application of biotechnology in weed science has been the development and successful commercialization of herbicide resistant crops. Various single genes conferring resistance to non-selective herbicides have been identified, ranging from mutations in target site genes, different isoforms of target site genes from bacteria, and genes for herbicide metabolism from bacteria (Tan et al., 2006). These genes have been inserted into crop species and have been in widespread use globally since 1995. In addition to herbicide resistant crops, biotechnology has many other uses in weed science research, some of which have now been utilized and others with great future potential. This paper will review research highlights in weed science research related to biotechnology, in particular genomics and molecular biology, and offer recommendations for future uses of biotechnology in weed science.

A major area of research related to herbicide-resistant crops has been assessing the risk of pollen-mediated gene flow to related weed species and to neighboring fields of the same crop. The perceived risk is that a weed could become resistant to a herbicide by this mechanism. Crop to crop gene flow has been studied under commercial conditions in Australian canola (Brassica napus) (Rieger et al., 2002), and in US Central Great Plains bread wheat (Triticum aestivum) (Gaines et al., 2007). Hybridization between wheat and Aegilops cylindrica has been shown to occur in the US Central Great Plains and in the US Pacific Northwest, and backcrosses have been shown to form a bridge for gene transfer from wheat to jointed goatgrass (Gaines et al., 2008; Gandhi et al., 2006; Hanson et al., 2005; Morrison et al., 2002a; Morrison et al., 2002b; Perez-Jones et al., 2006; Zemetra et al., 1998). An effective use of molecular biology in the area of gene flow, and also for gene flow between weed populations, has been sequence analysis to confirm hybrids at the molecular level. Such tools have been applied to studies of hybridization in Lolium rigidum (Busi et al., 2008; Busi et al., 2011), Amaranthus species (Franssen et al., 2001; Gaines et al., 2012; Trucco et al., 2005; Trucco et al., 2009; Wassom & Tranel, 2005; Wetzel et al., 1999), and between bread wheat and Aegilops cylindrica (Gaines et al., 2008; Gandhi et al., 2006; Perez-Jones et al., 2006).

Herbicide resistance due to target site mutations can be rapidly and economically genotyped using several different methods. Specific mutations in herbicide target site genes can be genotyped in a very high throughput fashion using pyrosequencing, a PCR-based technique with real-time detection of base pair addition known as sequencing by synthesis (Petersen et al., 2010). Another technique known as derived cleaved amplified polymorphic sequence (dCAPS) has been developed and is useful for genotyping known target site mutations (Kaundun & Windass, 2006). Such assays can be developed for any single point mutation of interest; for example, once non-target site resistance (NTSR) genes are characterized, such assays could also be developed to rapidly diagnose weed populations for NTSR.

Another use of biotechnology in weed science research is cell culture. Cell culture of Chlamydomonas reinhardtii (algae) has been used to study herbicide resistance evolution (Lagator et al., 2012). This study system offers great promise to provide further insights into the early stages of resistance evolution. Cell culture has also been used to select for and identify herbicide resistance with the goal of producing herbicide-resistant crops, mostly for glyphosate resistance (e.g., Murata et al., 1998; Papanikou et al., 2004; Widholm et al., 2001), although no commercial crops have yet resulted from these approaches.

Gene expression studies provide excellent insight into the molecular basis of many phenotypes. Technologies now available for studying gene expression range include quantitative real-time PCR on cDNA,

microarrays, and RNA-sequencing using available high-throughput sequencing technology (Lister et al., 2009a). Increased EPSPS gene expression due to amplification of the EPSPS gene has been measured in glyphosate-resistant Amaranthus palmeri, first by genomic DNA Southern blots and then by quantitative PCR on genomic DNA and cDNA (Gaines et al., 2010). Many applications of microarrays have been used in weed science research. Microarrays can be used to study the mode of action of new herbicides with unknown modes of action, by first building a library of gene expression responses with known modes of action, and then comparing with the unknown mode of action gene expression (Duke, 2012). Herbicide resistance mechanisms due to changes in gene expression can also be studied using microarrays, either with available microarrays from model species for heterologous hybridization, or with custom-developed microarrays. Arabidopsis microarrays have been used to study glyphosate resistance in Conyza canadensis (Yuan et al., 2010). Custom-developed microarrays from ESTs have been used to study multiple biological traits in Euphorbia esula, including dormancy regulation and stress responses (e.g., Anderson et al., 2007; Dogramaci et al., 2010; Foley et al., 2010; Foley et al., 2012; Horvath et al., 2008), and candidate genes have been evaluated for biological effect in the model plant Arabidopsis (Horvath et al., 2010). Partial sequences have been obtained of the Conyza canadensis transcriptome (Peng et al., 2010) and the Amaranthus tuberculatus genome and transcriptome (Lee et al., 2009; Riggins et al., 2010) using Roche 454 pyrosequencing technology; these studies represent significant advances in weed science genomics and will provide valuable resources for future research.

The newest technology for studying gene expression uses available next-generation sequencing platforms, including Roche 454, Illumina, SOLiD, and PacBio, to sequence RNA from populations of interest (Lister et al., 2009a). The different platforms have different advantages, which should be considered when designing a sequencing experiment. Platforms with longer base pair reads (e.g., Roche 454) have advantages for developing a reference sequence, and platforms with shorter base pair reads (e.g., Illumina) have advantages for quantifying gene expression. Additionally, proper experimental design is critical for successful differential expression experiments, including adequate biological replication and population structure. Complex genomes can now be sequenced de novo, such as the 2.25 gigabase Giant Panda (Ailuropoda melanoleuca) genome sequenced using only Illumina short reads (Li et al., 2010). Experimental design is also critical to successfully assemble a de novo genome; in the case of the Giant Panda genome, the research team used paired-end reads on inserts with five different sizes to build scaffolds for the assembly, rather than only sequencing one insert size (Li et al., 2010). This approach enabled a rapid and good quality initial assembly with only de novo sequencing, and without requiring genomic libraries. In addition to the nucleotide sequence of the genome, it is now also possible to identify genome-wide patterns of methylation in the DNA, critical for understanding gene regulation due to changes in the epigenome (Lister et al., 2009b). Both de novo genome sequencing and epigenome sequencing will certainly have useful applications in weed science. The costs for next-generation sequencing are rapidly decreasing, and in the future genomics research will be used in weed science to study both herbicide resistance evolution along with weediness and invasiveness traits (Stewart et al., 2009; Tranel & Horvath, 2009).

Another technique using high-throughput sequencing technology is known as deep sequencing, in which alleles of a gene of interest are first amplified from many individuals and/or populations, and then sequenced (e.g., with Illumina technology) to identify rare sequence polymorphisms (mutations) (Lister et al., 2009a). This approach is useful to identify rare alleles that may be of interest for industrial purposes, such as new herbicide resistance traits, or for identifying important adaptive alleles. For example, herbicide detoxification genes including cytochrome P450 or glutathione-S-transferase genes from many biological sources could be deep sequenced to identify previously unknown variants, followed by cloning and expression to identify useful alleles. A further technique that has successfully been applied to develop glyphosate metabolism in crops is known as directed evolution (Castle et al., 2004). This involves taking the sequences of a gene from diverse sources, shuffling the different alleles to develop new combinations, and then screening in a high-throughput fashion to identify more effective variants. This procedure can continue until a novel version of a gene is optimized; it requires the ability to conduct very high-throughput screening of enzyme activity, and to clone and express the gene of interest (Johannes & Zhao, 2006).

The future of biotechnology research in weed science holds many exciting possibilities. Improved understanding of the molecular basis of herbicide resistance mechanisms will allow the development of new, more precise diagnostic tools, enabling faster and better informed recommendations for identifying and managing herbicide resistant weed populations. Various sequencing techniques can be used to study weediness traits and improve our understanding of the biology of weeds, and to identify new mode of action targets. The potential exists to use genomics-level understanding to develop new weed control technology and approaches with new molecular targets, and to design custom inhibitors to interfere with specific plant molecular processes, including gene expression regulation (Duke, 2012). Plant defense mechanisms could be repressed, and compounds designed to interfere with herbicide resistance mechanisms could be developed. To complement weed control technology, crops could be developed with greater competitive ability and/or the ability to produce allelopathic compounds to interfere with competing weeds.

With greater understanding from basic research, the traits and genes underlying weediness and invasiveness can be explored (Stewart et al., 2009; Tranel & Horvath, 2009). In addition to specific genes, weed scientists can investigate the involvement of gene expression regulation mechanisms including transposable elements and modifications to the epigenome, such as DNA methylation and histone modification. Advances in plant genomics have shed light on adaptive evolution, including periods of rapid adaptation such as crop domestication, and this information can help weed science studies characterize why weeds are invasive, competitive, and highly fecund. As the cost of sequencing continues to fall, the weed science research community may wish to develop genomics resources for several important species (Stewart et al., 2009). Initial approaches can include sequencing expressed genes in transcriptomes and developing genetic maps for key weed species.

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