HERBICIDE TOLERANCE GENES DERIVED FROM BACTERIA

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ABSTRACT

Nowadays, gene transformation methods play an important role in plant improvement. Herbicides are an integral part of modern day agriculture as they facilitate efficient crop management. Most of the herbicides target specific enzymes involved in metabolic pathways that are vital for plant growth and survival. In plant biotechnology applications, the use of herbicide tolerance genes have created new approaches with the obtained herbicide tolerant plants. This review describes herbicide tolerance; concerning of herbicide tolerant transgenic crops with the genes derived from bacteria.

Key words: herbicide; herbicide resistant plant, herbicide tolerance genes, dehalogenases.

INTRODUCTION

Herbicides are chemicals that used to mitigate or kill unwanted organisms. Herbicides usually act by disrupting or blocking some of the biochemical pathways in the plant cells (Chaudhry, 2011). Although herbicides are an inexpensive way of controlling unwanted organisms, they are also potential risks for human health and environment. In the environment, the persistence of herbicides depends upon their chemical and physical properties, dose, type of soil and its moisture content. On the other hand, as a result of using herbicide, many weeds have become resistant to these herbicide. Other concerns associated with herbicides are costs, the requirement for additional equipment for precision application, and questions related to proper disposal of unused herbicides (Cobb and Reade, 2010). However, herbicide tolerance technologies allow ecological and cost-effective benefits for farmers. In these methods, herbicide tolerance is achieved by incorporating into plant a gene which is obtained from bacteria. Herbicide tolerance technologies have been applied in many plants such as, tobacco (De Block et al., 1987; Stalker et al., 1988; Streber et al., 1994; Jo et al., 2004), tomato (De Block et al., 1987), potato (De Block et al., 1987), cotton (Bayley et al., 1992) and ryegrass (Christopher et al., 1991). Herbicide tolerance has consistently been the dominant traits for plants from 1996 to 2011. Herbicide tolerance applied by 16.7 million farmers from 29 countries in 160 million hectares in 2010. In addition, a sustained increase of 8% or 12% million hectares can be seen over 2010 as shown in Figure 1. (James, 2011).

General advantages of herbicide tolerance crops include broader spectrum of weeds controlled, reduced crop injury, less herbicide carry-over, price reduction for "conventional herbicides", use of herbicides that are more environmentally friendly, new mode of action for resistance management and weed management flexibility. On the other hand, the disadvantages consist of yield performance, single selection pressure and weed resistance, shifts in weed species, gene escape, gene flow and contamination of organic crops and marketing and food labeling in global markets (Madsen and Sandoe, 2005).

In crop production, the use of bacterial genes has created new approaches for herbicide tolerance. This review describes herbicide action mechanism, herbicide tolerance and genes derived from bacteria for herbicide tolerance.

Herbicide action mechanism: Herbicide tolerance plants have the ability to break down the herbicide to non-active compounds. There are several mechanisms that are responsible for natural herbicide tolerance in plants, prominent ones being insensitivity to the target site and breakdown of the toxic herbicide to non-toxic by-products. Both of these mechanisms have been simulated in genetically engineered crops either by over expressing the target enzymes or by engineering foreign proteins that can rapidly detoxify the herbicides (Freyssinet, 2003). Mechanism of herbicide action will be discussed in understanding the mechanism through which herbicides will be selectively inhibited (Hilton et al., 1963; Tsafarlis, 1996).

ALS (Acetolactate synthase) Inhibitory: Acetolactate synthase (ALS) catalyzes the first step in the biosynthesis of the branched chain amino acids valine, leucine and isoleucine that is carried out in the chloroplast (Schulze-Siebert et al., 1984). In plants, tolerance for ALS inhibitors has been demonstrated by mutation of one or several of their amino acid residues in ALS enzymes. As a result, enzymes are still active but insensitive inhibiting action of the herbicides. Herbicide tolerance crops with mutated ALS have been achieved by using chemical
mutagenesis or soma clonal variation mutated-ALS encoding genes with recombinant DNA technologies (Whitcomb, 1999; Green, 2007). ALS is specifically inhibited by four different classes of herbicides namely Sulfonylureas (nicosulfuron), Imidazolinones (imazaquin), Triazolopyrimidine sulfonamides and Pyrimidinyl oxobenzoic acids. Each class of herbicide has a unique mode of inhibiting ALS. They can generally be considered as growth inhibitors that act faster than glyphosate. Inhibition of ALS depletes the branch chain amino acids resulting in death of the susceptible species (Dhingra and Daniell, 2004).

Figure 1. Most frequent transgenic traits in commercial plantings (James, 2011)

Resistance to ALS inhibitors has been conferred by engineering insensitive mutant genes like the csr1-1 mutant ALS gene from Arabidopsis thaliana that has been successfully engineered in oilseed rape and flax (Miki et al., 1990; McHughen and Holm, 1995). Interestingly, most ALS mutants are insensitive to only specific herbicides; therefore the strategy has been to use hybrid ALS mutants that can code for resistance to multiple herbicides. A chimeric ALS mutant derived from csr1-1 and imr1 mutant genes from A. thaliana resulted in conferring high-level tolerance to two different types of herbicides in transgenic tobacco (Hattori et al., 1992). There is one mutant gene that confers resistance to multiple classes of herbicides that specifically inhibit ALS. This mutant gene has been tested in transgenic tobacco and the plants were found to be tolerant to all the ALS specific herbicides tested (Hattori et al., 1995). A similar mutant raised in cotton was used for conferring tolerance to multiple herbicides (Rajasekaran et al., 1996).

Bromoxynil: The herbicide bromoxynil acts as a photosynthetic electron transport inhibitor (Sanders and Pallett, 1985). It is not well tolerated by dicots but in wheat fields it efficiently eliminates broad leaf weeds. A nitrilase-encoding gene bxn that uses bromoxynil as a substrate was isolated from Klebsiella ozaenae (Stalker and McBride, 1987; Stalker et al., 1988). The enzyme detoxifies bromoxynil to non-toxic benzoic acid. The bxn gene has been successfully expressed in Nicotiana tabacum, Gossypium hirsutum, Solanum tuberosum and Brassica napus where a high level of tolerance was observed (Freyssinet et al., 1989). 3,5-bromo-4-hydroxybenzonitrile derived esters are cyano groups that consist of active substances of herbicides. Bromoxynil nitrilase is a bromoxynil-degrading enzyme that was identified in bacterial isolates of Klebsiella pneumonia var. ozaenae. Bromoxynil-degrading enzymes degrade the cyano group of bromoxynil into a carboxyl group. Therefore, herbicide becomes inactive. Natural function of bromoxynil nitrilase is contribution to the converting of aldoxime compound secreted by plants into soils.

Glufosinate: Glufosinate inhibits glutamine synthetase and nitrogen metabolism in plants (Leason et al., 1982; Tachibana et al., 1986; Cox, 1996). Glufosinate is an ammonium salt (D,L-homoalanine-4-yl-methyl phosphonic acid). In prokaryotes and eukaryotes, glutamine synthase plays an important role in the metabolism of nitrogen. The condensation of ammonium ions and glutamic acid into glutamine degrades by glutamine synthase enzyme. The first plant to be engineered for glufosinate tolerance was tobacco where expression of the bar gene conferred efficient tolerance to glufosinate without any deleterious effect on flowering or seed set (De Block et al., 1987). Glutamine synthetase (GS) is the first enzyme in the glutamine synthesis pathway that is instrumental in the assimilation of inorganic nitrogen into organic compounds. The enzyme helps in the utilization of ammonia produced by nitrite reductase and recycles the ammonia produced by photospiration. Higher plants have a cytosolic and chloroplastic form of the enzyme (Lara et al., 1984). Glutamine synthetase is inhibited by naturally occurring peptides that are variations of substituted glutamate. There are three major compounds that inhibit glutamine
synthetase namely, methionine sulfoximine (MSO), phosphinothricin (glufosinate) and tabtoxine-β-lactam. Inhibition of glutamine synthetase activity leads to rapid accumulation of ammonia, cessation of photosynthesis, disruption of chloroplast structure and vesiculation of the stroma (Devine et al., 1993).

Glufosinate tolerance has been achieved through the insertion of the phosphinothricin-N-acetyltransferase (pat) gene derived from the homologous gene from Streptomyces viridochromogenes. The pat gene catalyzes the acetylation of L-glufosinate to N-acetyl-L-glufosinate (Cox, 1996).

Dalapon: The herbicide containing 2,2-dichloropropionic acid was introduced and marketed as ‘Dalapon’ by the Dow Chemical company in 1953. A 2,2 dichloropropionic acid (2,2DCPA) or Dalapon have been widely used as herbicides (Ashton and Crafts, 1981). Dalapon is an aliphatic acid herbicide, a colorless liquid with high water solubility. Extensive use of Dalapon may results in leaching of the herbicide into ground water and cause pollution since it was extremely water soluble (EPA, 1988; Christian and Thompson, 1990). Dalapon kills only certain organisms as a selective systemic herbicide (Tate et al., 2000). This herbicide used in many studies such as Saccharum officinarum, Beta vulgaris, Maize, Solanum tuberosum, Asparagus officinalis (Tseng et al., 2004; Barroso et al., 2010). Dalapon had been used around the world for many years and it was applied predominantly as an industrial/non-crop herbicide (Barroso et al., 2010). For control of various grasses, Dalapon was used in some agricultural crops. Dalapon is absorbed by the roots and leaves and then translocate via both the symplastic and apoplastic systems throughout the plants via phloem and xylem. It is not metabolise in young plant tissues. Acid form of Dalapon is able to form a hydrogen bond with amide group of the protein molecule. Therefore, this mechanism may be the cause of the phototoxic action in blocking plant enzyme activity (Barroso et al., 2010).

Trichloroacetic acid (TCA): Trichloroacetic acid (TCA) is a toxic chemical has been demonstrated to be phytotoxic (Berg et al., 2000), used as a potent herbicide (Ashton and Crafts, 1973). TCA used as a sodium salt or in the form of ester or amide derivatives against perennial grasses (Norkorpi and Frank, 1995), and woody plant species (Aberg, 1992). TCA have been shown to cause inhibition of several enzymes, interference with lipid and carbohydrate metabolisms, inhibition of plant growth, induction of leaf chlorosis, production of leaf necrosis and changes in the properties of the surface wax (Ashton and Crafts, 1973). TCA often causes inhibition of the growth of shoots and roots and causes formative effects especially in the shoot apex (Monaco et al., 2002). Exposure of TCA on pine seedling via roots and foliage has been shown to decrease in transpiration rate and in the total chloroplast area (Sutinen et al., 1997).

TCA is absorbed by leaf and roots, then it is translocated throughout the plant from the roots but only a small amount is translocated from leaf. It is primarily translocated via the apoplastic system. It has been suggested that TCA modifies sulfhydryl or amino groups of enzymes or induces conformational changes in enzymes. Its rapid contact prevents symplastic movement. This herbicide is degraded slowly by higher plants (Monako et al., 2002).

Monochloroacetic acid (MCA): Monochloroacetic acid (MCA) is a colorless crystalline substance with a molecular weight 94.50 (Toshina et al., 2004). This toxic chemical has been demonstrated to be phytotoxic (Frank et al., 1994), used as herbicide and in synthesis of various organic compounds such as carboxymethylcellulose, phenoxyacetic acid, thioglycolic acid, glycine and indigoid dye (Bhat et al., 1990; Toshina et al., 2004). MCA is known to be a contact herbicide with broad spectrum against broad leafed weeds, and the exposure via spraying (leafs) may be considered as most sensitive (Munn et al., 2005). MCA is taken up by plant cells due to its relatively small dissociation constant (Frank et al., 1994). MCA has been shown to cause a decrease in the transpiration rate and in the total chloroplast area, and it’s shown the effects of interference of photosynthesis (Sutinen et al., 1997).

Spontaneous microbial degradation of MCA in surface water has been reported (Hashimoto et al., 1998). Many bacteria strains that possess inducible dehalogenases have ability to cleave off carbon-halogen bond in chloroaacetate were isolated from soil (Hardman and Slater, 1981; Ismail et al., 2008).

Herbicide tolerance: Herbicide tolerance is defined as the inherited capability of a plant to survive and reproduce following an exposure a dosage of herbicide normally fatal to the wild type by The Weed Science Society of America (WSSA). The International Survey of Herbicide Resistant Weeds records 367 resistant biotypes of 200 species (115 dicots and 85 monocots) over 570,000 fields in 59 countries (Heap, 2011). Herbicide tolerance in plants have been studied widely (Buchanan-Wollaston et al., 1992; Naested et al., 1999; Duke et al., 2007; Duke and Stephen, 2009; Owen 2010). There are many genes from bacteria were isolated to use for herbicide tolerance crops. Bacterial genes that degrade toxic compounds have been used for selectable markers in plant transformation studies. Tolerance genes for antibiotics such as kanamycin (Gurel and Gozkirrmizi, 2000), hygromycin and more recently genes that provide the plant insensitive to herbicides have been used to produce herbicide tolerance plants (Table 1). An alternative herbicide tolerance strategy has involved introduction of mutated genes coding for the protein that
is the target for herbicide. These mutations provide gene products insensitive to herbicide action. For example, introduction of a mutated bacterial gene for the target enzyme of Glyphosate, 5-enolpyruvylshikimate -3-phosphate synthase give an increased tolerance to glyphosate action (Comai et al., 1985). In higher plants, it has been found that herbicide tolerance is furnished by transformed mark enzymes, herbicide-metabolizing enzymes and over-production of target enzymes. Herbicide tolerance for sulfonylurea, imidazolinone, glycosphosphate and nortflurazon herbicides were achieved by over-production of an unmodified and a modified target enzymes (Inui and Ohkawa, 2005).

Table 1. Herbicide tolerance genes derived from bacteria

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Source of Tolerance gene</th>
<th>Transfer in plant</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td><em>Salmonella typhimurium</em></td>
<td><em>Brassica campestris</em></td>
<td>Thompson et al., 1987</td>
</tr>
<tr>
<td>Glufosinate</td>
<td><em>Streptomyces hygroscopius</em></td>
<td><em>Glycine max</em></td>
<td>Piente et al., 2000</td>
</tr>
<tr>
<td>Bromoxynil</td>
<td><em>Klebsiella ozaeae</em></td>
<td><em>Nicotiana tabacum</em></td>
<td>Leroux et al., 1990</td>
</tr>
<tr>
<td>2,4-Dichlorophenoxyacetic acid</td>
<td><em>Acaligenes utrophus</em></td>
<td><em>Nicotiana tabacum, Gossypium hirsutum</em></td>
<td>Streber and Willmitzer, 1989; Bayley et al., 1992</td>
</tr>
<tr>
<td>Paraquat</td>
<td><em>Ochrobactrum anthropic</em></td>
<td><em>Nicotiana tabacum</em></td>
<td>Jo et al., 2004</td>
</tr>
<tr>
<td>Phenmedipham</td>
<td><em>Arthrobacter oxidens</em></td>
<td><em>Nicotiana tabacum</em></td>
<td>Streber et al., 1994</td>
</tr>
<tr>
<td>Norflurazon</td>
<td><em>Cyanobacteria</em></td>
<td><em>Nicotiana tabacum</em></td>
<td>Wagner et al., 2002</td>
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</table>

Dehalogenase gene for herbicide tolerance: Enzymes involved in the conversion of organo halogen compounds have potential applications in environmental technology and the chemical industry (Mowafy et al., 2010; Kurihara, 2011). Dehalogenases initiate the breakdown of halogenated organic compounds by cleaving the carbon–halogen bond with the inversion of the configuration of the chiral carbon (Huyop and Nemati, 2010; O’Hagan and Schmidberger, 2010).

*Rhizobium* sp. RC1 was originally isolated by Skinner and his colleagues at Nottingham University (Berry et al., 1979). Allison et al., (1983) and Leigh et al., (1988) reported that *Rhizobium* sp. RC1 was a fast growing, Gram-negative bacteria that has the ability to produce three kinds of dehalogenases, D,E, and L. All three dehalogenase genes were then isolated by Cairns et al., 1996 and Stringfellow et al., (1997) and these dehalogenases were then characterised (Huyop et al., 2004; Huyop et al., 2008a; Huyop et al., 2008b).

Transgenic plants that contain dehalogenase gene have been previously studied (Buchanan-Wollaston et al., 1992 and Naested et al., 1999). For example, the *dhIA* gene can be expressed in *Arabidopsis* as a negative marker gene derived from *Xanthobacter autotrophicus*. *dhIA* gene encodes dehalogenase that can hydrolyze 1,2-dichloroethane (DCE) into cytotoxic halogenated alcohol and inorganic halide (Naested et al., 1999). On the other hand, Buchanan-Wollastan et al., (1992) reported that transgenic *Nicotiana plumbaginifolia* plants expressed bacterial *dehI* that resist to Dalapon. Table 2. showed a summary of dehalogenase gene expressed in plants.

Table 2. Different selection system used in plant transformation

<table>
<thead>
<tr>
<th>Gene</th>
<th>Transgenic plant</th>
<th>Selection agent</th>
<th>Source of gene</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>dhIA</em></td>
<td><em>Oryza sativa</em></td>
<td>1,2 dichloroethane</td>
<td><em>Xanthobacter autotrophicus</em></td>
<td>Moore and Srivastava, 2008</td>
</tr>
<tr>
<td><em>dhIA</em></td>
<td><em>Arabidopsis thaliana</em></td>
<td>1,2 dichloroethane</td>
<td><em>Xanthobacter autotrophicus</em></td>
<td>Naested et al., 1999</td>
</tr>
<tr>
<td><em>dehI</em></td>
<td><em>Nicotiana plumbaginifolia</em></td>
<td>2,2-Dichloropropionionic acid (Dalapon)</td>
<td><em>Pseudomonas putida</em></td>
<td>Buchanan-Wollastan et al., 1992</td>
</tr>
</tbody>
</table>

A future prospect of *dehE* gene in generating herbicide resistant plant: DehE from *Rhizobium* sp. RC1 is non-stereospecific dehalogenase that can act on D-2-chloropropionate (D-2-CP), L-2-chloropropionate (L-2-CP), 2,2-dichloropropionate (2,2-DCP), trichloroacetate (TCA), dichloroacetate (DCA) and monochloroacetate (MCA). Therefore, since *dehE* gene has been isolated and characterized it can then be used as a selection marker for plant transformation system. In this review, it is proposed that *dehE* gene can be ligated into pCambia1301 vector for plant transformation. It was hoped that *dehE* from *Rhizobium* sp. RC1 will be expressed in the plant system and thus allowing the development of herbicide tolerance plant to Dalapon.

Conclusion: Plant biotechnology has many opportunities to improve production of crops with many advantages. One example, is the development of built-in herbicide resistance crops. Conventional methods for herbicide proceeds at slow rate due to long maturation times and

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the slow growth rate of plants. However, through genetically modified plants for herbicide resistance has great potential to provide important development in crop growth and quality products with high yield of product. Genetically modified crops with commercially beneficial traits such as herbicide and insect resistance have been established successfully. For herbicide tolerance crops, genetically transformed plants will bring plant biotechnology into a new era of herbicide tolerance. Further development of gene transfer technologies will allow for assessment of specific herbicide resistance genes in genetically modified crop. These technologies will improve to form commercially applicable methods.

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