

An overview of genetic transformation of glyphosate resistant gene in *Zea mays*

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Abstract: *Zea mays* is an important cereal crop through out the world used as food for human and feed for livestock. The crop yield is highly affected by biotic and abiotic stresses that include insects, pests, pathogens, weeds, drought, salinity, alkalinity, heat, cold etc. Among the biotic factors weeds caused major yield losses and affect plant growth due to nutrient, light, water, etc. competition with crop plants. The use of non-selective herbicides is highly un-safe because of the susceptibility of the corn plant to glyphosate herbicide. Therefore, there is need to develop such maize varieties and hybrids that have tolerance against glyphosate. The present review paper will contribute to understand the method of transformation of glyphosate gene in maize to develop glyphosate tolerant crop plants.

[Qamar, Z, Aaliya K, Nasir IA, Farooq AM, Tabassum B, Ali Q, Awan MF, Ali A, Tariq M and Husnain T. **An overview of genetic transformation of glyphosate resistant gene in *Zea mays***. *Nat Sci* 2015;13(3):80-90]. (ISSN: 1545-0740). <http://www.sciencepub.net/nature>. 14

Keywords: Glyphosate, *Zea mays*, molecular, gene transfer technique, biotic, abiotic

1. Introduction

Maize (*Zea mays* L.) belongs to family Poaceae. As a characteristic of grasses, it is also a monocot and an angiosperm plant (Cheng *et al.*, 1983). Maize belongs to the genus *Zea* of family Poaceae and *Zea mays* is the only specie of the genus with can be domesticated (Weatherwax, 1935). Approximately, 50% of the total human population uses maize because of its use as staple food grain by many developed and under developing countries. Beyond acting as major food grain other outlets of maize are the livestock feed and wet-milling industry. Its grain constitutes about 9.7396 % grain protein, 4.85% grain oil, 9.4392% grain crude fibre, 71.966% grain starch, 11.77% embryo while fodder contains 22.988% acid detergent fibre, 51.696% neutral detergent fibre, 28.797% fodder cellulose, 40.178% fodder dry matter, 26.845% fodder crude fibre, 10.353% fodder crude protein and 9.095% fodder moisture (Ali *et al.*, 2013; Ali *et al.*, 2014abc). Maize in Pakistan has third place among cereals after wheat and rice and accounts for 4.8% of the total cropped area which accounts for 3.5% of the value of agricultural output. Annual production of maize is 3.34 million tons, that comes from the plantation on 0.94 million hectare. In Pakistan 40-50% of the consumption of maize is on farm and only 15-20% is marketed locally while 40% is sold in the organized wholesale market (Anonymous 2012).

Genetic engineering is the process of inserting DNA from one organism into another, either the same species or a different one, by artificial means. Most widely used methods for gene transfer used in plants are "gene gun method" (involving blasting foreign piece of DNA into the plant genome) or a bacterial system. *Agrobacterium tumefaciens*, a bacterium is

found naturally in soil, can transfer some of its own DNA to plants that it has infected. Scientists learned to take advantage of this bacterial system and it is now used to transfer small pieces of targeted DNA to plants to create plants that contain the new gene(s) (Montagu *et al.*, 1980). Owing to its importance, Pakistan has joined the club of top 10 countries having commercialized biotech crops albeit introduction of Bt cotton through informal channel. And now it is in the process of approving genetically modified (GM) or biotech maize for commercial cultivation (Raineri *et al.*, 1990). Glyphosate (*N*-(phosphonomethyl) glycine) is a well-known broad-spectrum systemic herbicide used to kill weeds, especially annual broadleaf weeds and grasses, which compete with commercial crops. It was initially patented and sold by Monsanto Company in the 1970s under the tradename *Roundup*, and its US patent expired in 2000. Glyphosate is quite effective in killing a wide variety of plants, including broadleaf, grasses and woody plants. On the contrary, it has a relatively small effect on some clover species. By volume, it is one of the most widely used herbicides.

Mode of action of Glyphosate's involves the inhibition of enzyme involved in the synthesis of the aromatic amino acids: tryptophan, tyrosine and phenylalanine. It is absorbed through foliage and then it is translocated to growing points. Owing to this mode of action, its effectiveness is limited to actively growing plants. Some crops have been genetically engineered to be resistant to glyphosate (i.e. *Roundup Ready*, also created by Monsanto Company). Thus, allowing farmers to use such crops with glyphosate as a post-emergence herbicide against both broadleaf and cereal weeds. These Herbicide-tolerant (HT) crops offer farmers a chance to fight weeds while preserving

the topsoil. They give farmers the flexibility to apply herbicides only when needed, to control total input of herbicides and to use herbicides with preferred environmental characteristics. Advantages of Herbicide Tolerant Crops include excellent weed control and hence higher crop yields, reduced numbers of sprays in a season, reduced fuel use, reduced soil compaction, use of low toxicity compounds which do not remain active in the soil and ability to use no-till or conservation-till systems, with consequent benefits to soil structure and organisms (Felsot, 2000).

2. Anatomy of Maize (*Zea mays* L.)

Most maize plants consist of a stalk, growing vertically upward from the ground varying in height due to maize variety or growing environment. It is reported that a typical maize plant with 7 to 10 feet tall stalk have 16 to 22 leaves (Anonymous, 2012). Leaves are attached to the nodes and the leaf lower part wraps itself around the stalk. Typically the lowest four nodes are below ground (Kowles & Phillips, 1988). Roots develop from each of these nodes. Maize is a hermaphrodite containing both male and female parts. The male part is known as the tassel and the female floral organ is called an ear. The immature ear consists of a cob, eggs that develop into kernels after pollination, and silks. Following pollination, a male sex cell grows down each silk to a single egg and fertilization (the union of the male and female sex cells) occurs. The fertilized egg develops into a kernel and inside each kernel is a single embryo (a new plant). A vigorous maize plant may have 500 to 1000 kernels on a single ear.

2.1. Endosperm Development in Maize

Endosperm development is of great interest and importance both in agriculture, for biotechnical improvement, and in developmental biology research (Clare *et al.*, 1996; Lopes & Larkins, 1993; Olsen *et al.*, 1992). It has been extensively studied morphologically, ultra-structurally, and histochemically; however, there is a lack of information on sperm-central cell fusion and on early molecular events after fertilization. Russell explain the process of fertilization in angiosperms, he elaborated that fertilization occurs due to the two sperm cells of a pollen grain or tube: in which one fuses with the egg and the other with the central cell (Russell, 1992). Goldberg named these processes as double fertilization that results in the formation of an embryo and endosperm tissue (Goldberg *et al.*, 1994). Double fertilization in maize after pollination in the plant has been widely investigated both cytological and ultra-structurally (Diboll, 1968; Diboll & Larson, 1966; Mol *et al.*, 1994; Rhodes *et al.*, 1988) and during *in vitro* ovary culture (Schel & Kieft, 1986). Clare *et al.*, (1996) described maize endosperm development and illuminate that it can be divided into four stages.

During stage I [DAP], rapid nuclear divisions without cell wall formation occur (syncytium formation). During stage II (cellularization, 3 to 5 DAP), Cell wall formation around the single nuclei takes place, resulting in a tissue with uninucleate cells followed by stage III, which is characterized by the occurrence of mitotic divisions until 12 DAP in the centrally located cells and until 20 to 25 DAP in the peripheral tissue. Starch grains and protein bodies accumulate in the center of the tissue. This process starts during stage III at 10 DAP and continues during stage IV, when the maize kernels desiccate and cell death of the endosperm occurs. In maize, zygotes produced *in vitro* divide (Digonnet *et al.*, 1997; Kranz *et al.*, 1991; Kranz & Lörz, 1994) and can regenerate via direct, primary embryogenesis into fertile plants (Kranz & Lorz, 1993).

2.3. Regeneration in Maize

Maize is herbaceous monocot with an annual cycle. Its embryo lies embedded in the endosperm at one side. Abbe & Stein, (1954) has described eight different stages of zygotic embryo development. However, the development of somatic embryogenesis system adept of plantlet regeneration is still in its beginning. Somatic embryogenesis has been validated in immature embryos culture (Armstrong & Green, 1985; Emons & Kieft, 1991; Franz & Schel, 1991; Vasil & Vasil, 1986). Plant regeneration via somatic embryogenesis starts with one or only a few cells, this type of regeneration is important for plant production and plant biotechnology such as somaclonal propagation, multiplication and especially genetic transformation. This assemblage of techniques can be directed toward production of identical plants or to induce variability (Gordon-Kamm *et al.*, 1990). The conventional method techniques of crop improvement in agricultural system involve a search for stain of plant. It is suggested that, the possible to produce asexual embryos *in vitro*. Synthetic seeds technology is one of the important applications of somatic embryogenesis. In these first synthetic seed system, somatic embryo is encapsulation in protective alginate matrix with provides mechanical support, protection and was coated with a wax film to prevent desiccation. However, the stage life and vigor of synthetic seed is limited. An efficient seed-based protocol for the production of maize shoot meristem cultures was developed (Zhong *et al.*, 1992) and modified by several authors. The meristem culture derived from shoot tips of maize seedlings has been successfully used as a for biolistic transformation (Zhong *et al.*, 1996).

2.4. Major Problems of Maize

Maize instead of being vigorous and tall is very sensitive to weed competition at early stages of growth. Rehman, (1985) reported that weed lead to

more than 30% losses in maize yield. In Pakistan, on an average 45% reduction in maize yield due to weeds infestation has been reported (Rashid and Shahida, 1987). However, this loss may increase up to 90% due to uncontrolled weeds. So to control weeds methods such as mulching, herbicide spray or hoe weeding can be used. Chemical weed control is a better supplement to conventional methods and forms an integral part of the modern crop production. Most of the presently available herbicides provide only a narrow spectrum weed control (Karimi *et al.*, 2006). Olorunmaiye *et al.*, (2009) checked the use of pre-emergence herbicides, hoe weeding and live mulch of herbaceous cover plants in a maize/cassava intercrop. The pre-emergence herbicides gave satisfactory weed control up to 6WAP, but failed to give season-long weed control. However, better weed control was achieved when the pre-emergence herbicides were supplemented with two hoe-weedings. Patel *et al.*, (2006) observed that maximum weed control efficiency (> 98 %) was achieved with pre-emergence application of atrazine @ 0.5 kg a.i./ha in combination with pendimethalin @ 0.25 kg a.i./ha and atrazine + alachlor each applied @ 0.5 kg a.i./ha and twice hand weeding carried out at 20 and 40 DAS. Similar trend was observed in grain yield. The best cost effective method of controlling late weeds in maize is by spraying non selective herbicides.

3. Plant Transformation

Genetic transformation has become an important tool in the study of basic plant processes and in crop improvement. The development of genetic transformation techniques for the major cereal crops has been relatively slow, mainly as a consequence of their limited susceptibility to *Agrobacterium* and their poor capacity to regenerate fertile plants from protoplasts (Rhodes *et al.*, 1988). Plant genetic engineering is progressing very rapidly since the first success of introducing a foreign gene into a plant via *Agrobacterium tumefaciens* (Fraley *et al.*, 1983). Since then the number of transgenic plants produced has increased exponentially. The International Service for Acquisition of Agri-biotech Applications (ISAAA) has recently summarized that the area commercially planted with transgenic plants worldwide has increased almost 53 fold, from 1.7 million hectares in 1996 to 90 million in 2005 (Harwood *et al.*, 2005). Through genetic engineering, agronomic traits of a particular plant can be improved and furthermore, production of value added products and nutrients can also be obtained. Genetic engineering reduces the time required for introducing a novel trait into plants as compared to conventional breeding. It was postulated for oil palm; up to 80% reduction in time could be achieved for introducing a novel trait through genetic engineering. Furthermore, transgenic plants can be

used as bioreactors or bio-factories for producing novel products, such as pharmaceuticals, continuously.

3.1. Maize Transformation

Different methods and explants can be used to produce genetically modified maize. Usually freshly isolated or pre-cultured immature embryos (abbreviated as IE and PCIE, respectively) (Ishida *et al.*, 1996; Songstad *et al.*, 1996) as well as embryogenic Type I (Wan *et al.*, 1995) and Type II (Walters *et al.*, 1992) callus derived from them are used for maize transformation. Embryogenic callus derived from IEs has also been used for the production of regenerable suspension (Armstrong & Green, 1985; Fromm *et al.*, 1990; Gordon-Kamm *et al.*, 1990; V. Vasil & Vasil, 1987) and protoplast cultures (Morocz *et al.*, 1990; Rhodes *et al.*, 1988; Shillito *et al.*, 1989). It was also shown that other maize explants such as immature tassels and ears, microspores (anthers), and the base of leaves can produce regenerable cultures and can be used for transformation (Cheng *et al.*, 2004).

3.2. Maize Transformation by Micro-projectile Bombardment

Micro-projectile bombardment using DNA-coated particles has been used to transform embryogenic maize cultures, which have subsequently been regenerated into fertile transgenic plants (Fromm *et al.*, 1990; Gordon-Kamm *et al.*, 1990; Walters *et al.*, 1992). These authors used derivatives of a particular maize inbred line, A188. Particular callus cultures, the so-called type II callus, were a prerequisite for the initiation of cell cultures suitable for transformation (Fromm *et al.*, 1990; Gordon-Kamm *et al.*, 1990; Walters *et al.*, 1992). Type II callus is highly embryogenic, white or pale yellow, friable, and rapidly growing. Its establishment is very genotype dependent and is only achieved at low frequency (Armstrong & Green, 1985; Vasil & Vasil, 1986). The cell culture properties of A188 can be transmitted through genetic crosses to recalcitrant inbreds (Kamo & Hodges, 1986). Back-crossing, combined with selection in tissue culture in each generation, can lead to the development of agronomically relevant inbreds with tissue culture properties amenable to genetic transformation using micro-projectile bombardment.

3.3. Maize Transformation by Electroporation

Halluin described the transformation of regenerable maize tissues by electroporation. In many maize lines, immature zygotic embryos can give rise to embryogenic callus cultures from which plants can be regenerated (D'Halluin *et al.*, 1992). Immature zygotic embryos or embryogenic type I calli were wounded either enzymatically or mechanically and subsequently electroporated with a chimeric gene

encoding neomycin phosphotransferase (neo). Transformed embryogenic calli were selected from electroporated tissues on kanamycin-containing media and fertile transgenic maize plants were regenerated. The neo gene was transmitted to the progeny of kanamycin-resistant transformants in a Mendelian fashion. This showed that all transformants were nonchimeric, suggesting that transformation and regeneration are a single-cell event. The maize transformation procedure presented here does not require the establishment of genotype-dependent embryogenic type II callus or cell suspension cultures and facilitates the engineering of new traits into agronomically relevant maize inbred lines.

3.3.1. *Agrobacterium*-mediated Transformation

3.3.1a. The Genus of *Agrobacterium*

The genus of *Agrobacterium* has been divided into a number of species based on its disease symptomology and host range. A Radiobacter is an avirulent specie, *Agrobacterium tumefaciens* causes crown gall disease, *Agrobacterium rhizogenes* causes hairy root disease and a new specie *Agrobacterium vitis* causes galls on grapes and a few other plant species (Otten *et al.*, 1996). The host range of *Agrobacterium* is extensive. As a genus, *Agrobacterium* can transfer DNA to a remarkably broad group of organisms including numerous dicot and monocot angiosperm species and gymnosperms. In addition, *Agrobacterium* can transform fungi, including yeasts, ascomycetes and basidiomycetes (Gelvin, 2003). The most widely used specie in plant transformation is *Agrobacterium tumefaciens*. *Agrobacterium tumefaciens* is a naturally occurring soil borne pathogenic bacteria that causes crown gall disease. The crown gall disease has been shown to be due to the transfer of a specific fragment, the T-DNA (transfer DNA), from a large tumor-inducing Ti plasmid within the bacterium to the plant cell (Zambryski *et al.*, 1983).

3.3.1b. *Agrobacterium*-mediated transformation

Agrobacterium-mediated transformation is the most widely used method to transfer genes into plants. Transformation is typically done on a small excised portion of a plant known as explant. The small piece of transformed plant tissue is then regenerated into a mature plant through tissue culture techniques. The first reported plant transformation by *Agrobacterium* was in 1983 (Fraley *et al.*, 1983). Since then, major advances have been made to increase the number of plant species that can be transformed and regenerated using *Agrobacterium*. Recent research indicates that *Agrobacterium*-mediated transformation is possible in monocots such as rice (Raineri *et al.*, 1990), maize (Gould *et al.*, 1991; Ishida *et al.*, 1996) and banana (May *et al.*, 1995). This system offers several advantages, such as technical simplicity, minimal

genome rearrangements in transformants, low copy number and the ability to transfer long stretches of DNA. After transfer, T-DNA becomes integrated into the plant genome and its subsequent expression leads to the crown gall phenotype (Hood *et al.*, 1986). There are two bacterial genetic elements required for T-DNA transfer to plants. The first element is the T-DNA border sequence that consists of 25bp direct repeats flanking defining the T-DNA. The borders are the only 12 sequences required in cis for T-DNA transfer (Zambryski *et al.*, 1983). The second element consists of the virulence (vir) genes encoded by Ti Plasmid in a region outside of the T-DNA. The vir genes encode a set of proteins responsible for the excision, transfer and integration of the T-DNA into the plant genome (Montagu *et al.*, 1980).

3.3.1c. T-DNA Binary Vector System

Scientists have taken advantages of the naturally occurring transfer mechanism, and have designed DNA vectors from the tumor inducing plasmid DNA to transfer desired genes into the plants. The development of DNA vectors using *Agrobacterium tumefaciens* is based on the fact that besides the border repeats, none of the T-DNA sequence is required for the transfer and integration. This means that the T-DNA genes can be replaced by any other DNA of interest, which will be transformed into the plant genome. Also the length of T-DNA is not critical. Small (a few kb or less) as well as large T-DNAs (150kb) (Hamilton *et al.*, 1996) will be transferred by the *Agrobacterium tumefaciens* into the plant cell. It has also been found that T-DNA and vir genes do not have to be in the same plasmid for transfer of T-DNA (Hoekema *et al.*, 1984). This achievement allowed development of a binary vector system to transfer foreign DNA into plants. Two plasmids are used in binary method, i.e., the Ti plasmid containing the vir genes which oncogenes eliminated, a so called 'disarmed' plasmid or 'vir helper', and a genetically engineered T-DNA plasmid containing the desired genes. The plasmids in T-DNA binary vectors are smaller than plasmids in *Agrobacterium* and easier to manipulate in *E. coli* and *Agrobacterium*. This has allowed researchers without specialized training in microbial genetics to easily manipulate *Agrobacterium* to create transgenic plants.

3.3.1d. *Agrobacterium* mediated Maize Transformation

Miller *et al.*, 2002 has developed a highly efficient system to segregate transgenes in maize that were co-transformed using an *Agrobacterium tumefaciens* 2 T-DNA binary system. Three vector treatments were compared in his study; (1) a 2 T-DNA vector, where the selectable marker gene bar (resistance to bialaphos) and the β -glucuronidase (GUS) reporter gene are on two separate T-DNA's

contained on a single binary vector; (2) a mixed strain treatment, where bar and GUS are contained on single T-DNA vectors in two separate *Agrobacterium* strains; (3) and a single T-DNA binary vector containing both bar and GUS as control treatment. Bialaphos resistant calli were generated from 52 to 59% of inoculated immature embryos depending on treatment. A total of 93.4% of the bialaphos selected calli from the 2 T-DNA vector treatment exhibited GUS activity compared to 11.7% for the mixed strain treatment and 98.2% for the cis control vector treatment. For the 2 T-DNA vector treatment, 86.7% of the bialaphos resistant/GUS active calli produced R0 plants exhibiting both transgenic phenotypes compared to 10% for the mixed strain treatment and 99% for the single T-DNA control vector treatment. A total of 87 Liberty herbicide (contains bialaphos as the active ingredient) resistant/GUS active R0 events from the 2 T-DNA binary vector treatment were evaluated for phenotypic segregation of these traits in the R1 generation. Of these R0 events, 71.4% exhibited segregation of Liberty resistance and GUS activity in the R1 generation. A total of 64.4% of the R0 2 T-DNA vector events produced Liberty sensitive/GUS active (indicating selectable-marker-free) R1 progeny. A high frequency of phenotypic segregation was also observed using the mixed strain approach, but a low frequency of calli producing R0 plants displaying both transgenic phenotypes makes this method less efficient. Molecular analyses were then used to confirm that the observed segregation of R1 phenotypes were highly correlated to genetic segregation of the bar and GUS genes. A high efficiency system to segregate transgenes in co-transformed maize plants has now been demonstrated (Miller *et al.*, 2002).

4. Glyphosate

Glyphosate (N-(phosphonomethyl)glycine) is a broad-spectrum systemic herbicide used to kill weeds, especially annual broadleaf weeds and grasses was discovered in 1970 (Franz *et al.*, 1996). When applied at lower rates, it serves as a plant growth regulator. The most common uses include control of broadleaf weeds and grasses in : hay/pasture, soybeans, field maize; ornamentals, lawns, turf, forest plantings, greenhouses, rights-of-way. It hinders plant growth by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS; 2.5.1.26), the sixth enzyme in the shikimate biosynthetic pathway that produces the essential aromatic amino acids (tryptophan, tyrosine, and phenylalanine) and subsequently phenolics, lignins, tannins, and other phenylpropanoids. To date, companies have sought regulatory approval for nine GR crops maize (*Zea mays* L.) is one of them (AGBIOS 2008). However the herbicide may also cause damage to the crop plant (Anonymous, 2011).

4.1. Herbicide Tolerance

To avoid this damage herbicide-tolerant (HT) crops are formed. These crops allow farmers to spray broad-spectrum herbicides over the top of growing plants, controlling most weeds while leaving crops largely unharmed. HT weed management systems are simple, flexible, and forgiving, and in most years and regions, they have been very effective, at least until recent years. Good performance coupled with ease of use explains why farmers have so enthusiastically embraced HT crops. The herbicide tolerant plant can be achieved by gene transformation.

4.2. Production of Herbicide Tolerant Plant

Castle *et al.*, (2000) reported that herbicide glyphosate is effectively detoxified by N-acetylation. They screened a collection of microbial isolates and discovered enzymes exhibiting glyphosate N-acetyltransferase (GAT) activity (Castle *et al.*, 2004). From the fifth iteration and beyond, GAT enzymes conferred increasing glyphosate tolerance to *Escherichia coli*, *Arabidopsis*, tobacco, and maize. Glyphosate acetylation provides an alternative strategy for supporting glyphosate use on crops.

Fillatti *et al.*, (1987) developed an efficient transformation system for tomato by studying several factors that affect the rate of *Agrobacterium* mediated transformation of explant tissue. Cotyledons of tomato were co-cultivated with *Agrobacterium tumefaciens* harboring a binary vector with two neomycin phosphotransferase (NPT) II genes and a mutant *aroA* gene. Over 100 transgenic plants were regenerated and rooted on medium containing kanamycin and eighty percent of these plants exhibited both NPT II enzyme activity and produced the mutant *aroA* protein. Progeny of *aroA* positive plants were tolerant to glyphosate at concentrations of 0.84 kg active ingredient/ha. The tolerance phenotype segregated in a manner consistent with Mendelian inheritance.

In 1983, scientists at Monsanto and Washington University isolated the common soil bacteria, *Agrobacterium tumefaciens* strain CP4, which is highly tolerant to glyphosate because its EPSPS is less sensitive to inhibition by glyphosate than EPSPS found in plants (Watrud *et al.*, 2004). By 1986, they had successfully inserted the cp4 EPSPS gene into the plant genome and obtained GR plants. The initial GR crops were the most quickly adopted technology in the history of agriculture (Baum *et al.*, 2007). In 2007, 12 million growers in 23 countries planted 114.3 million ha of biotech crops (Baum *et al.*, 2007). Growers chose GR crops because glyphosate made weed control easier and more effective, increased profit, required less tillage, and did not restrict crop rotations. Before the introduction of GR crops, growers routinely used many different herbicides with many different modes of action, e.g., 10 different herbicide

modes-of-action in soybean. Now, many growers rely only on glyphosate (Foresman & Glasgow, 2008; Gustafson, 2008). Applying glyphosate alone over wide areas on highly variable and prolific weeds made the evolution of resistant weeds inevitable (Gower *et al.*, 2003). The first reports of GR weeds increased concerns about long-term sustainability of glyphosate and started a large effort in private and public laboratories to develop alternative options.

There are two basic strategies that have been successful in introducing glyphosate resistance into crop species: (a) expression of an insensitive form of the tar-get enzyme; and (b) detoxification of the glyphosate molecule. The strategy used in existing commercial glyphosate-tolerant crops is the former, employing a microbial (*Agrobacterium* sp. CP4) or a mutated (TIPS) form of EPSPS that is not inhibited by glypho-sate. The theoretical disadvantage of this approach is that glyphosate remains in the plant and accumulates in meristems, where it may interfere with reproductive development and may lower crop yield. Resistance to herbicides is more commonly achieved through their metabolic detoxification by native plant gene-encoded or transgene-encoded enzymes. The advantage of glyphosate detoxification is the removal of herbicidal residue, which may result in more robust tolerance and allow spraying during reproductive development.

The discovery of EPSPS as the molecular target of glyphosate in 1980 prompted extensive studies on the catalytic mechanism and the structure–function relationships of this enzyme, performed by various laboratories over the past three decades.

EPSPS catalyzes the transfer of the enolpyruvyl moiety of phosphoenolpyruvate (PEP) to the 5-hydroxyl of shikimate 3-phosphate (S3P) to produce 5-enolpyruvyl shikimate 3-phosphate (EPSP) and inorganic phosphate. This reaction forms the sixth step in the shikimate pathway leading to the synthesis of aromatic amino acids and other aromatic compounds in plants, fungi, bacteria, and apicomplexan parasites. The only enzyme known to catalyze a similar reaction is the bacterial enzyme MurA, which catalyzes the first committed step in the synthesis of the bacterial cell wall. Early kinetic characterization established that glyphosate is a reversible inhibitor of EPSPS, acting by competing with PEP for binding to the active site. Several studies on the reaction mechanism of EPSPS by different laboratories in the 1990s, using chemical and spectroscopic methods, provided evidence that the EPSPS reaction proceeds through a tetrahedral intermediate formed between S3P and the carbocation state of PEP, followed by elimination of inorganic phosphate.

Conclusions

It was concluded from above discussion that the transformation of glyphosate resistant gene may be helpful to improve resistance against herbicides in order to increase crop yield and production.

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