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Genomic distribution of EPSPS copies conferring glyphosate resistance in Palmer amaranth and kochia

Mithila Jugulam* and Andrew J. Dillon
Kansas State University, Manhattan, KS, USA

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ABSTRACT

Palmer amaranth and kochia are major problem weeds in many cropping systems in the United States. Wide acceptance of glyphosate tolerant crop technology has resulted in extensive use of glyphosate, consequently, a number of weeds including Palmer amaranth and kochia evolved resistance to glyphosate throughout the US. Within a span of 5-7 years the glyphosate resistance in these weeds has spread extensively, devastating several major crops. Understanding the mechanisms of herbicide resistance is valuable to determine the level of resistance as well as how the resistance spreads in the populations. Glyphosate resistance mechanisms in Palmer amaranth and kochia have been investigated extensively. Although resistance to glyphosate has evolved as a result of amplification of 5-enolpyruvylshikimtate-3-phosphate synthase (EPSPS), the target site of glyphosate, but the distribution and configuration of amplified copies of EPSPS gene in the genomes of these two species is different. The EPSPS gene amplification may have possibly mediated by transposons in Palmer amaranth and whereas, likely to have resulted because of unequal recombination in kochia. These findings suggest that the EPSPS amplification can occur via different mechanisms in different weeds. Evolution of glyphosate resistance as a result of EPSPS gene amplification is a threat to long-term sustainability of glyphosate-resistant crop technology.

Key words: EPSPS, Gene amplification, Glyphosate resistance, Glyphosate, Kochia, Palmer amaranth

Glyphosate is widely used for wide spectrum weed control in both cropland and non-crop land, more importantly, in Roundup Ready cropping systems. Originally, when glyphosate was introduced for weed control, it was extensively used as a non-selective herbicide, for vegetation management in non-crop areas. Upon introduction of glyphosate-resistant (GR) crops in the late 1990, combined with wide acceptance of this technology, led to accelerated use of this herbicide totaling ~128 million ha worldwide in 2012 (James 2012). Adoption to GR crop technology has made a significant contribution to global agriculture and the environment as it not only increased farm income by $32.2 billion (Brookes and Barfoot 2013) but also moderated the negative environmental impacts of mechanical weed management practices (Bonny 2011; Gardner and Nelson 2008). This was possible because, glyphosate offers a simple, effective and economic weed management option in GR crops. In addition, it provides immense value in no-till crop production systems by enabling soil and moisture conservation. However, consequence of extensive use of glyphosate resulted in intensive selection pressure. As a result, several weed populations globally have evolved resistance to glyphosate. Herbicide resistance, in particular the recent proliferation of glyphosate resistance in weed species worldwide is a major crop protection threat; nearly two dozen GR weed species have been reported in the last 15 years (Heap 2015).

Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, impairing conversion of shikimate to chorismate in the shikimate pathway (Amrhein *et al*. 1980, Duke and Powles 2008). This metabolic pathway facilitates the synthesis of aromatic amino acids: phenylalanine, tyrosine, and tryptophan. Glyphosate, acts as a competitive inhibitor of EPSPS, leading to accumulation of shikimate and plant death occurs as a result of lack of aromatic amino acid synthesis (Duke and Powles 2008).

It was hypothesized that the likelihood of weeds evolving resistance to glyphosate is negligible (Bradshaw *et al*. 1997), primarily because of its complex biochemical interactions in the shikimate pathway, and also due to the absence of known

***Corresponding author:** mithila@ksu.edu

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glyphosate metabolism in plants. Nonetheless, several cases of glyphosate resistance were confirmed throughout the world (Heap 2015). The first case of glyphosate resistance was reported in rigid ryegrass (*Lolium rigidum*) in Australia in 1996, the same year GR-crop technology was first released in the market (Baerson *et al*. 2002). Currently, there are 32 glyphosate resistant weeds in 22 different countries around the world (Heap 2015). The main factor driving this increase in resistance to glyphosate is over reliance on this single chemical option for weed control in GR minimum till production in many cropping systems (Duke and Powles 2008).

Several different mechanisms of glyphosate resistance have been reported in weeds. These mechanisms can be grouped into two broad categories: Non-target-site and target-site based. Non-target-site resistance to glyphosate involves physiological and biochemical processes that prevent the herbicide from reaching its intended site of action, while target-site based resistance involves changes to the site-of-action of glyphosate, *i.e*. EPSPS. Non-target based mechanism of glyphosate resistance, as a result of reduced translocation was documented in rigid ryegrass, common waterhemp and horseweed (Preston and Wakelin 2008), whereas sequestration or compartmentalization of glyphosate into vacuole was also reported in horseweed (Sammons and Gaines 2014). The known target-site based glyphosate resistance mechanisms include mutations (Baerson *et al.* 2002) or duplication/amplification (Gaines *et al*. 2010) of EPSPS gene. Importantly, duplication/amplification of the EPSPS appears to be the basis for glyphosate resistance in several weeds (Sammons and Gaines 2014). The mechanisms of glyphosate resistance in weeds has been comprehensively reviewed by Sammons and Gaines (2014). The purpose of this review is to discuss the mechanisms of evolution of glyphosate resistance via EPSPS gene amplification using two important weeds, Palmer amaranth and kochia as examples.

EPSPS gene amplification mechanisms

Generally, amplification of EPSPS gene causes increased expression, elevated enzyme activity, and higher protein content of the gene (Sammons and Gaines 2014). This increase in the EPSPS gene copies results in excessive amount of the enzyme production, which in turn acts like a sponge, binding and deactivating glyphosate in solution, while the remaining unbound portion of EPSPS functions normally, ensuring plant growth and development. This has become a very widespread mechanism of glyphosate resistance, and screening for elevated EPSPS copy number has become a normal procedure in determining glyphosate resistance in weeds (Sammons and Gaines 2014). Glyphosate resistance, mediated by EPSPS gene duplication has been reported in many species such as Italian ryegrass (Salas *et al*. 2011), kochia (*Kochia scoparia*) (Jugulam *et al.* 2014, Wiersma *et al.* 2014), Palmer amaranth (Gaines *et al.* 2010, *et al.* 2012, Mohseni-Moghadam *et al.* 2013), and waterhemp (Chatham 2015). This mechanism has been extensively studied, and increase in EPSPS copies correlated positively with increased level of resistance to glyphosate when compared to susceptible plants with lower EPSPS copy number (Gaines *et al.* 2010 and Jugulam *et al.* 2014). It has been found that different species require different EPSPS copy numbers to confer resistance to glyphosate. For example, Palmer amaranth from Georgia and elsewhere in the United States required 30 to 50 copies of glyphosate to survive a field dose of glyphosate (868 g ae/ha) (Gaines *et al.* 2010, 2011). Other species, such as kochia needed 3-10 copies for withstanding the same field use rate of glyphosate (Jugulam *et al*. 2014; Wiersma *et al.* 2014).

EPSPS gene amplification in GR Palmer amaranth

The first case of EPSPS gene amplification-based glyphosate resistance was reported in a Palmer amaranth population from Georgia, USA (Gaines *et a*l. 2010). In this population, there is a massive increase (>100-fold relative to glyphosate-susceptible plants) in EPSPS copies (Gaines *et al*. 2010). The copy number threshold necessary for glyphosate resistance and inheritance are most likely explained by the genetic mechanisms involved in EPSPS amplification in this species. Florescence in situ hybridization (FISH) of glyphosate-resistant Palmer amaranth (Gaines *et al*. 2010) suggests that EPSPS copies spread throughout the genome and they hypothesize that this pattern of distribution of EPSPS copies may have been facilitated via transposable elements. This hypothesis was tested in another study by Gaines *et al*. (2013). The results of this study suggest that miniature-repeat transposable elements (MITEs) are found closer to EPSPS gene. Furthermore, Activator (Ac) transposases and repetitive sequences associated with transposons were also found. Although this study does not conclusively suggest the involvement of transposable elements in EPSPS copy distribution, it provides some evidence for a possible role of these elements in gene duplication in GR Palmer amaranth. On the other hand, EPSPS gene amplification was found to be caused as a result of amplification of only one of two

EPSPS alleles (Gaines *et al.* 2013, Wiersma *et al.* 2014) in kochia. The other allele is present only in susceptible plants. No evidence of alternative splicing of the EPSPS gene has been seen in kochia, and no other genes seem to have their expression reduced as a result of elevated EPSPS copy number. Furthermore, glyphosate-resistant Palmer amaranth also show no fitness penalty in the absence of herbicide with increases in gene copy number and expression. Palmer amaranth plants with increased EPSPS copy number and expression were found to grow and reproduce similar to susceptible plants, so this trait will most likely persist in the absence of selection by glyphosate (Giacomini *et al.* 2104).

EPSPS gene amplification in GR kochia

Field-evolved GR kochia populations were first reported in western KS, USA in 2007 (Heap 2015). However, it quickly widespread throughout the US Great Plains and Canadian Provinces by 2013 (Heap 2015). The evolution of GR in kochia populations is also attributed to amplification of the EPSPS gene (Wiersma *et al.* 2014). Unlike in GR Palmer amaranth, EPSPS: acetolactate synthase (ALS) copies ranging from 3 to 9 were found in GR kochia (Jugulam *et al.* 2014, Wiersma *et al.* 2014). GR kochia populations were 3- to 11-times resistant (population level) to glyphosate compared to a glyphosate susceptible population. Similar to GR Palmer amaranth, EPSPS expression was also positively correlated with EPSPS copies (Wiersma *et al.* 2014). FISH results of GR kochia indicate that unlike in Palmer amaranth, all the amplified EPSPS copies are located on two homologous chromosomes, and these copies are aligned in tandem on these chromosomes as illustrated by fiber FISH analyses (Jugulam *et al.* 2014). Continuous variation in EPSPS copies, and a positive correlation between EPSPS expression and copies (Wiersma *et al.* 2014), suggests that the EPSPS copy number in kochia increases through an adaptive process. Furthermore, hybridization of EPSPS probes at distal ends of homologous chromosomes of kochia also suggests that duplication of EPSPS gene in GR kochia may have occurred as a result of unequal crossover, because, the gene duplication via unequal crossover most likely occurs at telomere region of chromosomes (Royle *et al.* 1988, Amarger *et al.* 1998, Ames *et al.* 2008).

The natural occurrence of EPSPS gene amplification in GR weeds is becoming prevalent in more weeds and this will be a threat to sustainable use of glyphosate in crop production. As discussed above, massive amplification of the EPSPS gene and distribution of these copies throughout the genome (Gaines *et al.* 2010), likely mediated by transposable elements (Gaines *et al.* 2013), has been found in GR Palmer amaranth. On the other hand, EPSPS copies arranged in tandem on a single chromosomes was reported from our laboratory (Jugulam *et al.* 2014). More importantly, these results trigger an intriguing question about the mechanisms of glyphosate, specifically whether EPSPS copy number increased in response to a positive selection or whether rare plants with multiple copies existed prior to selection. Therefore, uncovering molecular basis of EPSPS gene amplification mechanisms will help us understand how plants will respond to glyphosate selection resulting in evolution and spread of resistance to this important herbicide.

REFERENCES

Amarger V, Gauguier D, Yerle M, Apiou F, Pinton P, Giraudeau F, Monfouilloux S, Lathrop M, Dutrillaux B and Buard J. 1998. Analysis of distribution in the human, pig, and rat genomes points toward a general subtelomeric origin of minisatellite structures. *Genomics* **52**: 62-71.

Ames D, Murphy N, Helentjaris T, Sun N and Chandler V. 2008. Comparative analyses of human single- and multilocus tandem repeats. *Genetics* **179**: 1693-1704.

Amrhein N, Deus B, Gehrke P and Steinrücken HC. 1980. The site of the inhibition of shikimate pathway by glyphosate. *Plant Physiology* **66**: 830-834.

Baerson SR, Rodriguez DJ, Tran M, Feng Y, Biest NA and Dill GM. 2002. Glyphosate-resistant goosegrass: Identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. *Plant Physiology* **129**: 1265-1275.

Bonny S. 2011. Herbicide-tolerant transgenic soybean over 15 years of cultivation: Pesticide use, weed resistance, and some economic issues. The case of the USA. *Sustainability* **3**: 1302-1322.

Bradshaw LD, Padgette SR, Kimball SL and Wells BH. 1997. Perspectives on glyphosate resistance. *Weed Technology* **11**: 189-198.

Brookes G and Barfoot P. 2013. The global income and production effects of genetically modified (GM) crops 1996-2011. *GM Crops Food* **4**: 74-83.

Chatham LA, Bradley KW, Kruger GR, Martin JR, Micheal JR, Owen DK, Peterson DE, Mithila J and Tranel PJ. 2015. A multi-state study of the association between glyphosate resistance and EPSPS gene amplification in waterhemp (*Amaranthus tuberculatus*). *Weed Science* **63**: 569-577.

Duke SO and Powles SB. 2008. Glyphosate: A once-in-a-century herbicide. *Pest Management Science* **64**: 319-325.

Gaines TA, Ward SM, Bukun B, Preston C, Leach JE and Westra P. 2012. Interspecific hybridization transfers a previously unknown glyphosate resistance mechanism in *Amaranthus* species **5**: 29-38 DOI: 10.1111/j.1752-4571.2011.00204.x

Gaines TA, Wright AA, Molin WT, Lorentz L, Riggins CW, Tranel PJ, Beffa R, Westra P and Powles SB. 2013. Identification of genetic elements associated with EPSPS gene amplification. *PLOS One* **8**: e65819.

Gaines TA, Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ and Culpepper AS.2010. Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. *Proceedings of National Academy of Science* **107**: 1029-1034.

Gardner JG and Nelson GC. 2008. Herbicides, glyphosate-resistance and acute mammalian toxicity: simulating an environmental effect of glyphosate-resistant weeds in the USA. *Pest Management Science* **64**: 470-478.

Giacomini D, Westra P and Ward SM. 2014. Impact of genetic background in fitness cost studies: An example from glyphosate-resistant Palmer amaranth. *Weed Science* **62**: 29-37.

Heap I. 2015. The international survey of herbicide resistant weeds. Accessed September 13, 2015. Online. Internet. *Available at: www.weedscience.com.*

Hoen DR, Park KC, Elrouby N, Yu Z, Mohabir N, Cowan RK and Bureau TE. 2006. Transposon-mediated expansion and diversification of family ULP-like genes. *Molecular Biology and Evolution* **23**: 1254-1268.

James C. 2012. 2012 ISAAA report on global status of Biotech/ GM Crops. In: *International Service for the Acquisition of Agri-Biotech Applications Brief 44-2012*, Ithaca.

Jugulam M, Niehues K, Godar A, Koo DH, Danilova T, Friebe B, Sehgal S, Varanasi V, Wiersma A, Westra P, Stahlman PW and Gill BS. 2014. Tandem amplification of a chromosomal segment harboring 5-enolpyruvylshiki-mate-3-phosphate synthase locus confers glyphosate resistance in kochia *scoparia*. *Plant Physiology* **166**: 1200-1207.

Mohseni-Moghadam M, Schroeder J and Ashigh J. 2013. Mechanism of resistance and inheritance in glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) populations from New Mexcico, USA. *Weed Science* **61**: 517-525.

Preston C and Wakelin AM. 2008. Resistance to glyphosate from altered herbicide translocation patterns. *Pest Management Science* **64**: 372-376.

Ribeiro DN, Pan Z, Duke SO, Nandula VK, Baldwin BS, Shaw DR and Dayan FE. 2014. Involvement of facultative apomixes in inheritance of EPSPS gene amplification in glyphosate-resistant *Amaranthus palmeri*. *Planta* **239**: 199-212.