



Glyphosate resistance in rice: An overview

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Date of receipt : 09.11.2020

Date of acceptance : 05.05.2021

ABSTRACT

Productivity of rice is influenced by a number of biotic and abiotic factors. Among these, weed accounts as a major constraint for rice production. It competes with the associated crops for water, soil nutrients, space, and light resulting in drastic reduction in crop yield. Manual and mechanical weeding incurs huge cost and often becomes impracticable, especially in areas with heavy weed infestation. However, non-selective herbicide particularly glyphosate proved to be a feasible solution to eradicate weed infestation, but its off target movement to rice negatively influences plant growth, survival and seed yield. Therefore, development of herbicide resistance in rice turns to be the major focus in rice breeding. In the present study, the authors presented a detailed review of the weed management along with mode of action, weed resistance, genetic basis of glyphosate resistance, scope for exploring natural resistance, screening methodology and possible ways for development of glyphosate resistance in rice to confer selectivity and enhance crop safety and production.

Key words: Genetics, glyphosate resistance, mechanism, mode of action, natural resistance, rice breeding

INTRODUCTION

Rice (*Oryza sativa* L., AA type genome, genome size 430 Mb) is an important annual cereal crop which provides foods nearly two-third of the world population. It is evident that rice production need to be increased to keep pace with the ever-increasing population. Among several biotic stresses, weed stands to be one of the major impediments in rice production (30-40% reduction in yield). Weed management by intercultural operations incur a huge cost towards cultivation (Prasad et al., 2019). Now-a-days the pre-emergence (alachlor, butachlor, pendimethalin and oxyfluorfen) and post emergence (propaquizafop, quizalofop ethyl, fenoxaprop-p-ethyl and imazethapyr) weedicides are often used to control the menace of weed infestation in rice fields. Outcrossing between rice and weedy grasses is generally low and such a chance event can be alleviated by application of herbicides at least at 4-5 leaf-bearing stage (14 DAT) to make the field

free from weeds sufficiently ahead of flowering. Glyphosate has been used as the most effective herbicide to control weeds in rice cultivation areas in the world. It is a systemic broad-spectrum post emergence herbicide for weed control in glyphosate resistant crops (Senseman, 2007). Besides, it is widely used to indiscriminately kill annual and perennial weeds in heavily infested farmyards and such candidate herbicide is not potentially fit for application in rice fields as off target movements of glyphosate adversely affect growth of the rice resulting drastic reduction in yield. In this context, exploring natural herbicide resistance (NHR) to glyphosate in cultivated rice can be a novel approach to enhance selectivity and crop safety. Studies on natural resistance to weedicides in rice germplasm are limited (Nayak et al., 2020). Therefore, it is worthwhile to screen the available vast collection of rice germplasm resources for natural resistance to glyphosate.

USE OF HERBICIDES TOWARDS WEED MANAGEMENT

Weed is the major biotic stress in rice production leading to a yield loss in the range of 30- 40% (Abeysekara, 2011). They compete with crop plants in the field and negatively influence the crop yield quality and quantity along with survival of the plants. Weed control using herbicides is the most popular method among farmers and it allows economically viable weed control providing cost-effective method in the production of agricultural crops (Juraimi et al., 2013). In this context, herbicide-resistant rice has the potential to improve the efficiency of weed management. The common methods used to manage weeds include cultural, mechanical, biological, and chemical means. However, an integrated approach using a variety of methods in combination is reported to provide most successful weed management in rice fields (Tu et al., 2001).

MODE OF ACTION OF GLYPHOSATE ON WEEDS

In plants, glyphosate disrupts the shikimic acid pathway through inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. The resulting deficiency in EPSP production leads to reductions in aromatic amino acids and other crucial plant metabolites (Duke and Powles, 2009) that are vital for protein synthesis and plant growth. Action of glyphosate begins immediately as soon as it coats the leaves, but it requires 4 to 20 days to completely kill the plants. The herbicide works the best when air temperature is above 15.5°C and plants receive no rainfall or water for at least two days. It affects net CO₂ assimilation rate, transpiration rate, stomatal conductance, and internal CO₂ concentration in plants. Depending on the amount of damage sustained, plants may recover from glyphosate injury. Physiological injuries following glyphosate application in rice includes multiple shoots and roots from the internodes, leaf curling and discoloration, reduced exertion of panicles from flag leaf, and malformed inflorescence (non-emergence of panicle, aborted panicle, bleached lemma and palea).

WEED RESISTANCE TO GLYPHOSATE: A RISK FACTOR

The dreaded risk for commercialization of glyphosate resistant transgenic rice is the possibility of transfer of genes for herbicidal action to wild and weedy relatives leading to increased weed invasiveness and drastic reduction in yield (Kumar et al., 2008). Gene transfer between cultivated and wild or weedy rice is reported to form hybrids. The weedy rice hybrids could have more fitness ability for survival than cultivated rice even after glyphosate treatment. Besides, this may serve as source of gene flow owing its cross compatibility with related graminaceous weeds though at lower rate. The likelihood of such gene transfer is especially high in India owing to sympatrical distribution of the cultivated rice and its relatives and overlapping of their flowering period. Besides, indiscriminate use of glyphosate is likely to contribute evolutionary pressure for spontaneous induction of herbicide resistance in weed species. In fact, the resistant weeds are reported to be frequently evolved by modifying amino acid composition on the substrate binding site of EPSPS under high glyphosate selection pressure (Fartyal et al., 2018).

GENETIC BASIS AND MECHANISM FOR GLYPHOSATE RESISTANCE IN RICE

Herbicide resistance is the inherited ability of a plant to survive the herbicide application at a dose normally lethal to the normal population of the same species, while herbicide tolerance is the inherent ability of a species to survive and reproduce after herbicide treatment at normal concentration. Resistant weeds can often survive application of herbicide at rates that are much higher than the recommended dose. Possible mechanisms include over-expression of target enzymes, mutational change to altered target enzymes (mutant form) for reduced affinity to glyphosate, detoxification (metabolic degradation) of the herbicide and its reduced translocation and increased sequestration (partitioning to vacuole) to safe guard cells from harmful effects. Usually herbicides kill the weeds by either inhibiting or degrading the functional enzymes involved in metabolic pathway related to growth and photosynthesis. Glyphosate is

a structural analogue of phosphoenolpyruvate (PEP) and it blocks the Shikimic acid pathway by inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), the sixth enzyme in the shikimate biosynthesis pathway, resulting biosynthesis of essential aromatic amino acids (tryptophan, tyrosine and phenyl alanine) and subsequently phenolics, lignins, tannins and other phenyl-propanoids. Induced mutational variants for EPSPS gene leading to amino acid substitution at multiple active sites using site directed mutagenesis, and transgenics containing glyphosate resistant gene from bacterial source or other crop plants have been reported to offer glyphosate resistance in rice (Salas et al., 2012). Zhai et al. (2020) explored glyphosate stress-responsive microRNAs, lncRNAs, and mRNAs in rice using genome-wide high-throughput sequencing. Besides, spontaneous/natural mutation of EPSPS gene in local land races during the long span of domestication, and glyphosate resistant gene flow from weedy/red rice can not be ruled out. *Oryza spontanea*- the weedy rice, was in fact resulted from natural hybridization of *Oryza sativa* with either *Oryza nivara* or *Oryza rufipogon* (Tripathi et al., 2011).

SCOPE OF NATURAL TOLERANCE TO GLYPHOSATE IN RICE

Recently, more attention has recently been given to the naturally occurring glyphosate-sensitive EPSPS enzymes, as newer mutations providing increased glyphosate tolerance have been identified (Gherekhlou et al., 2017; Tian et al., 2015). Overexpression of improved mutant version of EPSPS gene has been reported to show increased field level glyphosate tolerance and higher grain yield in rice (Achary et al., 2020). Besides, 12 varieties (Bg359, At362, Bw364, Ld365, Bg366, Bg369, Bg379-2, Bg403, Bg454, Pachcha peruma, Kalu heenat and Kurulu thuda) had shown natural resistance to optimum concentration (0.5 g l⁻¹) of glyphosate (Ekanayaka et al., 2017) which might be due to spontaneous variation arose during the process of long period of domestication. Among these, At 362 and Ld 365 revealed survival percentage $\geq 70\%$ while Bg 300 was shown to be most sensitive to Glyphosate with very low survival percentage (15%). Similarly, in an another report

cv. Zambales was shown to be the most tolerant to glyphosate herbicide with highest survival rate and low severity index followed by Kalipao, Innaano and Kilay (among a set of 100 genotypes) which have moderate survival rate and low severity rate upon spaying of the crop with glyphosate based herbicide (locally called as 'Round-up') at 14 DAT (Palanog et al., 2015). This allows the use of such herbicide for weed control without effects on rice itself. Hence, there is scope for exploitation of natural variation for glyphosate tolerance in rice.

SCREENING TECHNIQUES TOWARDS GLYPHOSATE TOLERANCE IN RICE

A suitable glyphosate concentration can be determined using few rice varieties at varying glyphosate concentrations (0.25 g l⁻¹, 0.5 g l⁻¹, 1 g l⁻¹, 1.5 g l⁻¹, 2 g l⁻¹, 2.5 g l⁻¹ and 3 g l⁻¹). LD₅₀ based on survival percentage was worked out to be 0.5g l⁻¹ and any variety with $\geq 50\%$ survival percentage was arbitrary considered as resistant to glyphosate application (Ekanayaka et al., 2017). Subsequent to the application of glyphosate, the dead plants may be considered as susceptible to the herbicide and surviving plants with a substantial growth as resistant to the herbicide. A vast collection of rice germplasm resources can be screened at LD₅₀ concentration for natural resistance to glyphosate and the resulting glyphosate tolerant test genotypes may be assessed for growth parameters, agronomic traits and yield penalty. Besides, status of built in endogenous herbicide resistance in elite germplasm lines may be elucidated based on quantification of shikimic acid using HPLC (Vargas et al., 2014) and using cDNA sequence variation in OsEPSPS gene compared to wild type rice varieties (sensitive to glyphosate). EPSPS gene-specific forward (5'-ATGGCGGCGACCATGGCGTC-3') and reverse (5'-TCAGTTCCTGACGAAAGTGCTTAGA-3') primers (Achary et al., 2020) can be used for the purpose using PCR analysis. Ekanayaka et al.(2016) used herbicide resistance specific E11M32 primer pair (3'-TGT AAA ACG ACG GCC AGT GAC TGC GTA CCA ATT CAC-5' and 3'-GAT GAG TCC TGA GTA AAA C-5') for AFLP (amplified fragment length polymorphism) analysis for identification of glyphosate resistance induced in rice callus.

INDUCING GLYPHOSATE RESISTANCE IN RICE

Off target movements of glyphosate causes damage to the cultivated rice by reducing the yield up to 80% (Labrada, 2007). Thus, there is a need in developing crop plants which are not affected by the broad spectrum herbicides. Inducing herbicide resistance in rice is a new means to confer selectivity and enhance crop safety and production. Differential tolerance to sub-lethal rates of glyphosate exists among rice (*Oryza sativa*) cultivars (Meier, 2011). Lakshika et al. (2019) were able to identify natural as well as induced glufosinate-resistance in selected Sri Lankan rice (*Oryza sativa* L.) varieties. Rice varieties e.g., Bg94-1, Bg403, Bg454, Bg379-2 and Ma Wee had shown the said herbicide resistance without any significant yield penalty. EMS mutated variants of these varieties even showed increased resistance to Glufosinate compared their status of natural resistance. Ekanayaka et al. (2017) developed 14 glyphosate resistant Sri Lankan rice varieties following mutagenesis with EMS (4.5 mmol⁻¹ for 12 h). Besides, Ekanayaka et al. (2016) reported induction of glyphosate (0.2%) resistance following in vitro mutagenesis of rice variety cv. Bg 250 with 0.1-0.2% EMS for two hours in liquid MS medium. EMS treated calli were exposed to glyphosate (0.2%) followed by test of viability using Tetrazolium test (1% Tetrazolium chloride) as per Towill and Mazur (1975). Calli that turned red indicated viable calli (glyphosate resistant) as compared to non-mutated calli which developed no colour.

Most significant level of glyphosate resistance in rice has been achieved by the process of genetic engineering which primarily focused on three strategies: overproduction of EPSP synthase, introduction of a metabolic detoxification gene and introduction of an altered EPSP synthase enzyme with decreased affinity for glyphosate (Dill, 2005). The first commercial success of glyphosate transgenics was reported in soybean in 1996 by Monsanto (Reis et al., 2006). Transgenic rice expressing

a codon-modified synthetic CP4-EPSPS was reported to confer tolerance to broad-spectrum herbicide, glyphosate (Chhapekar et al., 2014). Fartyal et al. (2018) developed transgenic rice by co-expression of glyphosate tolerant mutant gene (OsmEPSPS) that over-express proline and serine; and glyphosate detoxifying genes igrA resulting in over all higher glyphosate tolerance than using either of the genes alone. However, the introduction of transgenic herbicide resistant crops in developing countries and their impacts is a controversial issue owing to various bio-safety issues.

However, it is worthwhile to focus on non-GMO approach using classical plant breeding as the herbicide resistance can be developed in rice using classical mutation breeding and in vitro mutagenesis to make stable resistant lines instead of a plant modified by genetic engineering. Once, the glyphosate resistant donors/mutants are established, traditional back cross breeding can be effectively employed for development of herbicide resistant introgression lines. Rangel et al. (2010) reported the possibility of transferring the in-built imidazolinone tolerance allele from mutant 93AS3510 to the recurrent parents 'BRS 7 Taim' and 'BRS Pelota'(Rangel et al., 2010) to develop the herbicide resistance in irrigated rice.

FUTURE PERSPECTIVE

Glyphosate resistant transgenic rice breeding lines are available, but their commercialization in India waits bio-safety clearance in terms of possibility of gene flow to non-target plants (weeds), rigorous herbicide bioassay tests and environmental pollution. However, there exists genetic variation for tolerance to glyphosate in rice especially among local land races. The natural herbicide tolerant (NHT) rice once identified, it may serve as valuable material for elucidation of in-built endogenous herbicide resistance using cDNA sequence variation. Besides, such NHT elite genotypes would pave the pathway for developing herbicide resistant high yielding breeding lines in future which eventually reduce the cost of cultivation towards weeding.

ACKNOWLEDGEMENT

Authors extend their sincere thanks and gratitude to the Vice Chancellor, Orissa University of Agriculture and Technology, Bhubaneswar for extending help and keen support to undertake the review.

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