Chapter 11

Natural Resistance of Sri Lankan Rice (*Oryza sativa* L.) Varieties to Broad-Spectrum Herbicides (Glyphosate and Glufosinate)

Shyama R. Weerakoon, Seneviratnage Somaratne,
E. M. Sachini I. Ekanayaka and Sachithri Munasighe

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http://dx.doi.org/10.5772/intechopen.76991

Abstract

Since studies on herbicide-resistant rice (HRR) are limited in Sri Lanka, the present study conducted to screen the naturally existing glyphosate and glufosinate resistance in traditional and inbred rice varieties. Six traditional varieties and nineteen inbred lines were selected for the study. Complete randomized design with three pots with 10 replicates for each herbicide concentration was employed. Optimal concentrations of glyphosate (0.5 gl^{-1}) and glufosinate (0.05 gl^{-1}) were applied at 3–4 leaf stages. Varieties ≥50% survival percentage was considered as resistant to respective herbicides. Twelve varieties showed resistance (≥50%) at 0.5 gl^{-1} glyphosate concentration. Survived plants were monitored and agro-morphological and yield characters/parameters were measured. Fifteen varieties were to glufosinate at 0.05 gl^{-1}. Even though no significant differences ($p > 0.05$) were observed in growth parameters across control and treated plants, there was a yield penalty. Nine varieties (At362, Bg352, Bg359, Bg366, Bg369, Bg379-2, Bg403, Bg454, and Pachchaperumal) indicated moderate resistance to both glyphosate and glufosinate. The emerged HRRs indicated varying responses of agro-morphological and yield characters across the type of herbicide and the variety. Glyphosate reduced the growth parameters and yield penalty compared to glufosinate treated varieties. These HRR varieties have a higher potential in rice breeding programs and in developing HR rice varieties in future.

Keywords: glyphosate, glufosinate, herbicide resistance, *Oryza sativa*

1. Introduction

Rice, one of the most important grains, fulfills the carbohydrate requirement of people in the tropical countries and to a lesser extent in subtemperate areas. The cultivated rice belongs to the grass family Gramineae (Poaceae) under the tribe-Oryzeae of the subfamily Pooideae [1]. However, the genus *Oriza* has been divided into several sections and placed *O. sativa* under Series Sativa in Section Sativae [2]. *O. sativa,* an indigenous rice species in Asia, is a diploid species consisting 24 chromosomes. The genomic formula of *O. sativa* is AA [2]. The species *O. sativa* is an annual grass, with round, hollow, jointed culms, rather flat, sessile leaf blades, and a terminal panicle, under favorable conditions. As the other members in the tribe Oryzeae, rice is well-adapted to aquatic and swampy habitats [3].

Rice is cultivated on about 156 million hectares of land to produce about 696 million tons annually in Asian countries which account for 90% of the world's total rice production [4]. There is a growing trend of increasing rice consumptions since 2000s, which surpasses the production. On an annual basis, global rice demand keeps increasing by *ca.* 8 million tons implying that during next 10 years, the rice production need to increase to 80 million tons which is double the present production [5].

The increasing world population especially in tropical countries where rice serves as the staple food, one billion people per year demands an additional rice production (100 million MT) [6]. In future, it is apparent that rice production will continue to grow rapidly as increasing populations attempt to secure food supplies. In order to obtain a good yield in rice, farmers are required to overcome several biotic and abiotic stresses. Among biotic stresses, weeds stand out as the major threat to rice cultivation, which reduce the yield qualitatively and quantitatively. Over the past few decades, climate change has induced transformations in the weed flora of arable ecosystems and the changes in the climate have also influenced weeds indirectly by enforcing adaptations to agronomic practice [7]. Therefore, it is imperative to develop effective weed control strategies while maintaining crop yield [8]. Globally, *ca.*10% loss of rice yield is attributed to weed and specific quantity is more or less closer to 46 million tons (based on 1987 world rough rice production). Depending on the predominant weed flora and on the control methods practiced by farmers, loss of yield caused by weeds varies across countries in the world. In Sri Lanka, a country considered self-sufficient in rice, weeds are the major biotic stress in rice production and account for 30–40% of yield losses [9]. Thus, there is a need to take timely and appropriate measures to preserve the country's rice production.

Rice weeds are the major barriers to rice production because they possess the ability to compete for CO_2, space, moisture, sunlight and nutrients. Under certain conditions, crops fail to successfully compete with weeds [10]. Weed flora varies spatially due to type of rice culture, soil type, hydrology, tillage, cultural practices and irrigation pattern and so on. Approximately, 134 weed species belonging to 32 taxonomic families were identified in rice fields in Sri Lanka, and they were categorized as grasses, sedges and broad leaves [11].

Rice weeds adversely affected on final yield in number of ways. Weed increases the cost of production of rice. The cost of rice weed control, including herbicides, cultural and mechanical practices, and hand weeding, is estimated to be about 5% of world rice production and amount to US$3.5 billion annually. When the10% loss of rough rice grain yield is added to this cost, the world's total estimated cost for rice weeds and their control amounts to 15% of total annual production [12].

Weeds indirectly limit production and act as a host of plant harboring pathogens and pests that adversely affect rice. Furthermore, weeds intervene rice harvesting and increase harvest costs through direct interference with the harvesting operation and by causing lodging. Contamination of rough rice by the seeds of the weeds reduces the grain quality and market value for example weed red rice (*Oryza sativa f. spontanea*) has a pigmented layer that shatters easily and readily contaminates rough rice. Removing all traces of the pigmented layer requires intense milling and results in decreased grain quality and lower milling rates [12]. The drudgery of weeding and labor shortages have made rice farming unattractive. In most tropical countries, farmers spend more time on weeding, by hand or with simple tools, than on any other farming task. Hand weeding of one (01) ha. of rice requires from 100 to 780 labor-hours per crop, depending on the rice culture. Due to these adverse effects, there is a need to improve the present weed control practices.

Herbicides are chemical substances used to kill plants which are often placed under the group of chemicals known as pesticides that prevent, destroy, repel, or mitigate any pest [13]. Herbicides, in general, are classified using different criteria such as activity, timing of application, method of application, mechanism of action and chemical family. Based on the time of application there are three main categories of herbicides recommended for rice. "Pre-plant herbicides" are applied before the crop is planted in order to eliminate weeds that have germinated before planting or were left from following (e.g., glyphosate, glufosinate). "Pre-emergence herbicides" are applied after the crop has been planted but before weeds emerge (butachlor, pretilachloroxadiazon, pendimethalin, oxadiargyl) and "Post-emergence herbicides" are applied after weeds have emerged (bisparybacbispyribac, pentagon, 2,4-D). These herbicides are either broad-spectrum (nonselective) or narrow-spectrum (selective). Some of the most common modes of actions are auxin mimics, mitosis inhibitors, photosynthesis inhibitors, amino acid synthesis inhibitors and lipid biosynthesis inhibitors.

The usage of pre-emergent, broad spectrum herbicide in controlling weeds in rice cultivation has become a popular method among the farmers since it minimizes cost, labor and time. Glyphosate and glufosinate are the most commonly used broad-spectrum herbicides (BSHs) in rice fields and glyphosate usage is comparatively higher. Glyphosate (*N*-(phosphonomethyl) glycine) or $C_3H_8NO_5P$ is a broad spectrum, nonselective systemic herbicide. It is effective in killing all plant types including grasses, perennials and woody plants. Glyphosate is a versatile herbicide used by farmers, land managers and gardeners to simply, safely and effectively control unwanted vegetation. Initially glyphosate was patented and sold by Monsanto

Company in the 1970s under the trade name Roundup and after that glyphosate-based products have become the most commonly used herbicides in the U.S. [14]. This widespread adoption is the result of glyphosate's ability to control a broad spectrum of weeds, its extensive economic and environmental benefits and its strong safety profile. Glyphosate is currently undergoing registration review by the US Environmental Protection Agency (EPA or the Agency) and it is essential that farmers, land managers and gardeners retain access to this important tool for weed control.

As an herbicide, glyphosate is activated by absorbing into the plant mainly though leaves and also through soft stalk tissue. Subsequently, glyphosate is transported throughout the plant where it acts on various enzyme systems inhibiting amino acid metabolism (shikimic acid pathway). Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate-synthase, 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 [15]. The shikimate pathway is found in all microorganisms and crop plants. This pathway is essential for the biosynthesis of chorismate, the precursor for aromatic amino acids and aromatic secondary metabolites [16] (Priestman *et al*., 2005). Glyphosate is reported to be causing a significant damage to rice yield with a reduction of yield up to 80% by blocking the shikimate pathway of crop plant [17].

Glufosinate is converted within the plant cell into the phytotoxin named as phosphinothricin (PT). As a structural analogue of glutamic acid, PT inhibits glutamine synthatase—GS (E.C.6.3.1.2.), competitively and irreversibly [18, 19]. GS is an essential ammonia assimilation enzyme found in plants. Inhibition of GS causes a rapid, toxic accumulation of intercellular ammonia resulting in metabolic disruption and inhibition of photosystem I and photosystem II in treated plants [18, 19] (Senseman, 2007; Hensley, 2009). Over 40 monocotyledonous and more than 150 dicotyledonous species are sensitive to PT [20].

In relation to herbicide, usage of the terms "tolerance" and "resistance" are inconsistent among the general public and even weed scientists. Among the members of the weed science community, tolerance and resistance are used interchangeably. Further, herbicide manufacturers/seed companies that develop and/or market HR crop cultivars/varieties generally refer to these as herbicide-tolerant. The present study recognizes the definition of herbicide tolerance and resistance established by the Weed Science Society of America (WSSA) [21].

The official Weed Science Society of America [21] defines herbicide resistance as "the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In a plant, resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis". Herbicide tolerance is defined by WSSA as "tolerance is the inherent ability of a species to survive and reproduce after herbicide treatment. This implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant" [21]. Resistance may occur in plants as the result of random and frequent mutation. Through selection, where the herbicide is the selection pressure, susceptible plants are killed while herbicide resistant plants survive.

During the last two decades, considerable effort has been made to breed HR crops and it was expected to relieve the constraints imposed by different combinations of chemicals, overcome problems associated with herbicide residues, expand the range of compounds available for selective use in-crop, simplify the crop management and extend the useful life span of the current nonselective herbicides [22].

Rice cultivars resistant to glufosinate [23], sulfonylureas, imidazolinones and glyphosate have already been developed and are being field-tested, mostly in the USA but also in South America and Japan [24–26]. The main reason for developing HR rice is to attain control of weed species that fail to control rice weeds selectively [27]. In addition, introduction of HR rice improves current cropping systems, with more efficient weed control measures and could reduce the amount of land required to satisfy the global rice needs and fulfill the increase in the future demand of rice. Particularly, HR rice provides the farmer with new efficient chemical options for weed control, for instance, glyphosate and glufosinate target both monocotyledonous and dicotyledonous weeds, which probably allow less herbicide use in terms of amount and number of applications. In relation to HR, both herbicides were post-emergence, which means that doses can be adjusted to actual weed infestation, and spraying can be performed within a wider time frame due to their high efficacy and crop tolerance. Therefore, HR could result in adequate control of hard-to-kill weeds. In addition, weed populations already resistant to currently used herbicides could be controlled with these broad-spectrum herbicides [28].

Many studies focused on optimization of weed management in HR have been conducted with rice resistant to either glufosinate or imidazolinone. Almost complete control of weedy rice and other grasses, including *Echinochloa crus-galli* (L.) Beauv. was achieved in glufosinate-resistant rice in Arkansas (USA) by sequential applications of glufosinate. Initial studies on weed control in Imidazolinone resistant rice (IMI rice) were conducted with imazethapyr, an herbicide proven effective against weedy rice and other rice weeds when applied as a soil or foliar treatment. Imidazolinone resistant rice varieties carrying an insensitive target acetolactatesynthase (ALS) enzyme, which is the target site of these herbicides, were developed through anther culture and backcrossing without exposure to mutagens or genetic transformation [29]. Further, imidazolinone-tolerant rice variety was engineered through mutation of the rice variety AS3510 with EMS. The resulted M2 plants were sprayed with imazethapyr. A single surviving plant was identified, and the progeny of this rice plant showed tolerance to several AHAS-inhibiting herbicides [30]. This mutant line was referred to as 93AS3510, and subsequently two imidazolinone-tolerant rice varieties, CL121 and CL141 were developed with this tolerance trait and were first marketed in the USA in 2001 [31, 32].

Even with such achievements, inadequate weed and pest management practices led to creation of a yield gap in the rice production. Literature on the subject revealed that studies have focused on the importance of controlling weeds including hard-to-control *Echinochloa* spp.

and *Eleusine* spp. [8]. In addition, herbicide resistant (HR) conspecific weeds such as weedy rice with varying dormancy patterns have become more abundant in rice fields in Sri Lanka throughout the cropping season. As a result, Sri Lankan farmers tend to use pre- and postemergent herbicides in land preparation specially to control weedy rice. These pre- and postemergent herbicides include selective and nonselective (broad spectrum) herbicides. As far as selective herbicide usage is considered, the number of application and their amount to control common weeds such as: *Cyperus iria* L. (family: Cyperaceae), *Echinochloa* sp. (family: Gramineae), *Monochoria vaginalis* (family: Pontederiaceae) and weedy rice (*Oryza sativa f. spontanea*; family: Poaceae) has been increased considerably leading to sever threats to the rice growing environment [33]. Thus, it is critically important to evaluate the possibility of applying commonly used broad-spectrum herbicides; glyphosate and glufosinate as postemergent herbicides along with herbicide resistant technology to eliminate hard-to-control weeds. Thus, the objective of the present study was to evaluate the herbicide resistance of Sri Lankan traditional and inbred cultivated rice varieties to pre-emergent herbicide—glyphosate and glufosinate.

2. Methodology

Seeds of twenty-five rice (*Oryza sativa* L.) varieties (**Table 1**) were collected from the Rice Research and Development Institute (RRDI) of Sri Lanka. These lines were maintained in a plant house at the Open University of Sri Lanka, located in low country wet zone of the Western province, with an average temperature of 28–32°C and 65–70% relative humidity.

The selected seeds were pre-soaked overnight and allowed to germinate. One week old seedlings were planted in pots (with 23 cm diameter) filled with puddle soil (5.5 kg per pot) and excess plantlets were thinned out 1 week after planting [34] leaving 10 plants per pot. Fertilizer application and other crop management practices were performed according to the recommendations of the Department of Agriculture, Sri Lanka.

Glyphosate (0.5 gl^{-1}) and glufosinate (0.05 gl^{-1}) [35] were applied at 3–4 leaf stage (Department of Agriculture, Sri Lanka) of plants separately. The research design used was complete randomized design (CRD) with three pots (10 replicates in each pot) for each treatment and nontreated plants served as the control.

The total number of plants and the number of surviving plants were counted for each variety and percentage resistance (PR) was calculated as follows: plants with ≥50% resistance to herbicides were arbitrary considered as resistant varieties [36].

$$PR\ (\%) = \left[\frac{Number\ of\ survivng\ seedlings\ in\ a\ variety}{Total\ number\ of\ seedlings\ grown\ in\ the\ same\ variety}\right] \times 100$$

Agro-morphological characters of resistant plant were measured/evaluated in 2 weeks after sawing and the yield parameters, respectively, by application of respective herbicide.

Selection number	Name	Age (month)	Attributes
1	Bg94-1	3 ½	High yield WP
2	Bg250	2 ½	
3	Bg300	3	Resistant to GM-1, BL, BB, Bph
4	Bg304	3	Resistant to GM 1&2, BL, BB, Bph
5	Bg305	3	Resistant to GM-1 and 11, BPH, BL and BLB
6	Bg352	3 ½	Resistant to BL, BB & GM-1, Bph
7	Bg357	3 ½	Resistant to GM-1& 2, BL, BB, Bph
8	Bg359		Resistant to GM 1 & 2, BL, BB, Bph
9	Bg360	3 ½	Resistant to GM-1, GM-2, BL, Bph
10	At362	3 ½	
11	Bw364	3 ½	
12	Ld3 65	3 ½	Resistant to iron toxicity
13	Bg366	3 ½	
14	Bg369	3 ½	
15	Bg379-2	4 ½	Resistant to Bph and BB
16	Bg403	4	Resistant to BB, BL and Bph
17	Bg450	4 ½	Resistant GM-I
18	Bg454	4 ½	
19	H4	4	Resistant to BL
20	*Kaluheenati*	4	Moderately tolerant Gr. 2
21	*Kuruluthuda*	4	
22	*Suwadal*	5	
23	*Rathhal*	5	
24	*Madel*	5	
25	*Pachchaperumal*	3 ½	

BB: bacterial leaf blight; BL: rice blast disease; GM-1: biotype one of rice gall midge; GM-2: biotype two of rice gall midge; Bph: brown plant hopper; PS: photo period sensitivity.

Source: Jeyawardena *et al.*, 2010, RRDI, Batalagoda, Department of Agriculture, Sri Lanka

Table 1. List of chosen Sri Lankan rice varieties form the results of a previous for the study on natural herbicide resistance.

3. Results and discussion

Evaluation of natural resistance to glyphosate and glufosinate among rice varieties.

The results obtained from the screening for glufosinate resistant and glyphosate resistant varieties revealed that some of the selected traditional rice varieties and inbred lines possess the ability to resist the detrimental effects of those broad-spectrum herbicides (**Figure 1**). Two rice varieties (*Rathal*—2% and Bg305—1%) were found to be lethal to 0.05 gl^{-1} glufosinate concentration whereas no such varieties were observed under the application of 0.5 gl^{-1} glyphosate. Fifteen rice varieties (At362—90%, Bg250—83%, Bg300—96%, Bg352—100%, Bg357—53%, Bg359—100%, Bg360—96%, Bg366—73%, Bg369—83%, Bg379/2—93%, Bg403—100%, Bg450—57%, Bg454—97%, Bg94/1—73%, *Pachchaperumal*—53%) showed natural resistance under glufosinate application and 12 rice varieties (At362—75%, Bg352—50%, Bg359—55%, Bg366—65%, Bg369—60%, Bg379/2—65%, Bg403—60%, Bg454—55%, Ld365—70%, *Kaluheenati*—55%, *Kuruluthuda*—55%, *Pachchaperumal*—70%) were able to survive under glyphosate application. Results indicated that nine varieties (At362, Bg352, Bg359, Bg366, Bg369, Bg379-2, Bg403, Bg454 and *Pachchaperumal*) were resistant for both glyphosate and glufosinate (**Figure 1**).

Very limited studies have been conducted regarding the natural or induced herbicide resistance in Sri Lankan rice varieties [36] and findings of Sri Lankan rice varieties which are able to resist broad-spectrum herbicides (BSHs) such as glufosinate and glyphosate have hardly been recorded. In this study, nine rice varieties with the ability to resistant the application of concentrations, 0.05 gl^{-1}glufosinate and 0.5 gl^{-1} glyphosate have been identified. Among these varieties, only two red grain rice varieties *(Pachchaperumal* and At362) are included indicating that most of the cultivated traditional rice varieties, except *Pachchaperumal* did not possess the ability to resist both glufosinate and glyphosate as observed in inbred rice varieties. However, further studies are required to confirm such findings. Sri Lankans do admire red grain rice such as *Kuruluthud*a, *Kaluheenati* and *Rathal* due to their high nutritive qualities (**Table 1**), and it is important to note that such varieties need to be developed as BSHs resistant varieties in future.

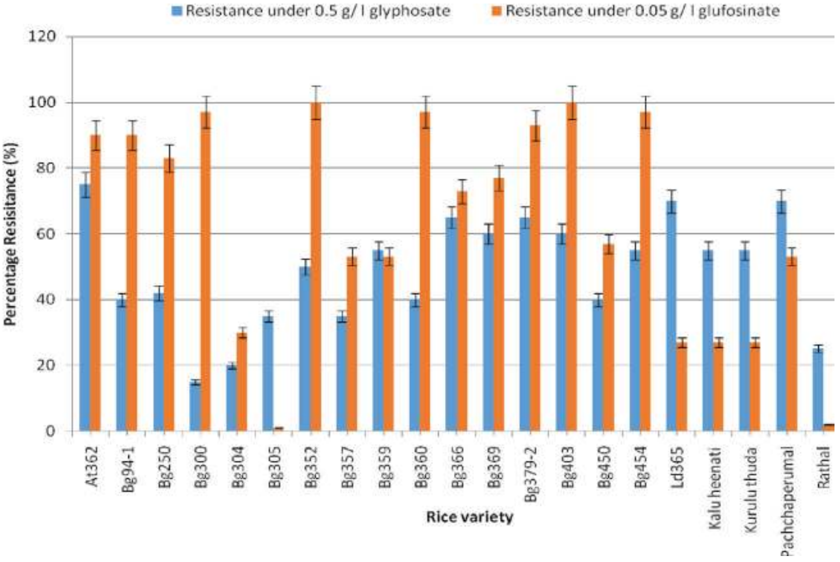

Figure 1. Comparison between natural resistances in selected rice (*Oryza sativa* L.) varieties to glyphosate and glufosinate.

On the other hand, according to the results of the study, relatively high survival percentage toward both BSHs was reported by inbred rice varieties which possess many valuable attributes other than glyphosate- and glufosinate-resistance such as resistant to GM-1, GM-2, BL, BB, Bph and have high yield potential (**Table 1**). These rice varieties could be incorporated in rice breeding programs to strengthen the sustainable cultivation.

Comparison table of plant height and yield parameters of glyphosate treated and untreated rice varieties are shown in **Table 2**. Though rice plant showed considerable HR in general, growth

Rice variety	Plant height (cm)	1000-grain weight (g)	Yield/plant (g)
Control			
At362	66.33 (1.20)	25.00 (0.44)	23.43 (2.69)
Bg359	62.00 (2.08)	22.46 (0.32)	12.38 (0.46)
Bg366	46.67 (2.73)	23.17 (0.28)	5.34 (0.60)
Bg369	34.33 (1.67)		
Bg379-2	64.00 (0.58)	25.67 (0.44)	5.22 (0.70)
Bg403	67.00 (1.15)	21.39 (0.38)	15.00 (1.09)
Bg454	52.00 (2.08)		
Bw364	58.67 (2.33)	19.23 (2.25)	12.51 (2.65)
Ld365	59.00 (2.08)	13.26 (0.29)	4.04 (0.77)
Kaluheenati	73.33 (0.88)	22.89 (1.51)	4.69 (0.75)
Kuruluthuda	70.00 (1.15)		
Pachchaperumal	70.33 (1.20)	31.64 (0.38)	11.31 (1.02)
Treated			
At362	50.33 (0.88)	16.86 (0.32)	12.61 (3.25)
Bg359	52.00 (3.06)	16.37 (0.29)	5.38 (0.94)
Bg366	27.67 (3.67)	18.49 (0.90)	3.19 (0.36)
Bg369	48.67 (2.33)		
Bg379-2	48.67 (3.48)	15.27 (1.41)	1.84 (0.40)
Bg403	49.00 (2.65)	17.56 (0.38)	6.50 (1.45)
Bg454	45.33 (1.45)		
Ld365	48.00 (2.00)	9.85 (0.20)	1.90 (0.38)
Kaluheenati	59.83 (3.68)	14.37 (0.59)	3.11 (0.50)
Kuruluthuda	64.33 (2.33)		
Pachchaperumal	61.00 (3.12)	22.94 (1.34)	3.29 (0.37)

Table 2. Summary of the parametric variables; plant height, 1000-grain weight and yield per plant control and treated with glyphosate (0.5 g/l).

retardation is indicated by the decrease in plant height resulting stunting of glyphosate treated plants. Similarly, the yield parameters such as 1000-grain weight yield per plant also showed apparent decrease in treated plants. However, yield per plant of the treated At362 indicated comparatively less reduction of yield. These findings led to conclude that though most of the rice varieties included in the study were resistant to the glyphosate, there was a considerable yield penalty. Similarly, the response of rice varieties included in the study to glufosinate are summarized in **Table 3** and according to the table, a general trend of decreasing plant height that is stunting growth and yield parameters specially yield per plant was observed. Comparatively, glufosinate treated At362 variety indicated low reduction in yield per plant (**Table 3**).

Rice variety	**Plant height (cm)**	**1000-grain weight (g)**	**Yield/plant (g)**
Control			
At362	66.33 (1.20)	25.00 (0.44)	84.57 (0.59)
Bg359	62.00 (2.08)	22.46 (0.32)	75.37 (0.64)
Bg366	46.67 (2.73)	23.17 (0.28)	72.27 (0.50)
Bg369	34.33 (1.67)		
Bg379-2	64.00 (0.58)	25.67 (0.44)	68.70 (0.29)
Bg403	67.00 (1.15)	21.39 (0.38)	82.43 (0.67)
Bg454	52.00 (2.08)		
Bw364	62.33 (1.45)	24.21 (0.42)	85.17 (0.49)
Ld365	59.00 (2.08)	13.26 (0.29)	81.27 (0.50)
Kaluheenati	73.33 (0.88)	22.89 (1.51)	70.93 (0.54)
Kuruluthuda	70.00 (1.15)		
Pachchapermal	70.33 (1.20)	31.64 (0.38)	80.23 (1.13
Treated			
At362	50.33 (0.88)	16.86 (0.32)	82.87 (0.24)
Bg359	52.00 (3.06)	16.37 (0.29)	69.60 (0.38)
Bg366	27.67 (3.67)	18.49 (0.90)	64.27 (0.62)
Bg369	48.67 (2.33)		
Bg379-2	48.67 (3.48)	15.27 (1.41)	65.20 (0.59)
Bg403	49.00 (2.65)	17.56 (0.38)	80.57 (0.46)
Bg454	45.33 (1.45)		
Bw364	55.00 (3.40)	14.26 (0.55)	70.33 (2.50)
Ld365	48.00 (2.00)	9.85 (0.20)	74.97 (2.98
Kaluheenati	59.83 (3.68)	14.37 (0.59)	68.50 (0.36)
Kuruluthuda	64.33 (2.33)		
Pachchaperumal	61.00 (3.12)	22.94 (1.34)	70.63 (0.30

Table 3. Summary of the parametric variables; plant height, 1000-grain weight and yield per plant of control and treated with glufosinate (0.05 gl^{-1}).

The nonparametric variables of treated and untreated rice varieties with glyphosate and glufosinate are shown in **Tables 4** and **5**, respectively. According to the tables, it is evident that there was no discernible different in growth parameters; however, yield parameters were considerably varied between the herbicide treated plants.

Rice variety	Number of tillers/ plant	Number of leaves/ plant	Number of panicles/ plant	Number of seeds/ panicle
Control				
At362	1	1	2	15
Bg359	0	3	1	14
Bg366	1	2	1	10
Bg369	1	2		
Bg379	1	2	1	10
Bg403	1	5	2	20
Bg454	1	3		
Bw364	1	1	1	15
Ld365	1	5	2	20
Kaluheenati	1	3	2	6
Kuruluthuda	1	3		
Pachchaperumal	1	3	1	15
Treated				
At362	0	2	3	34
Bg359	1	4	2	10
Bg366	2	1	1	5
Bg369	1	2		
Bg379	1	4	1	9
Bg403	1	3	3	20
Bg454	1	2		
Bw364	1	1	2	22
Ld365	0	2	1	15
Kaluheenati	0	1	1	11
Kuruluthuda	1	4		
Pachchaperumal	0	2	2	10

Table 4. Summary of nonparametric variables; number of tillers per plant, number of leaves per plant, number of panicles per plant and number of seeds per panicle of control and treated with glyphosate (0.5 gl^{-1}).

Rice variety	Number of tillers/ plant	Number of leaves/ plant	Number of panicles	Number of seeds/ panicle
Control				
At362	1	1	2	15
Bg359	0	3	1	14
Bg366	1	2	1	10
Bg369	1	2		
Bg379	1	2	1	10
Bg403	1	5	2	20
Bg454	1	3		
Bw364	1	1	1	15
Kaluheenati	1	3	2	6
Ld365	1	5	2	20
Kuruluthuda	1	3		
Pachchaperumal	1	3	1	15
Treated				
At362	0	2	3	34
Bg359	1	4	2	10
Bg366	2	1	1	5
Bg369	1	2		
Bg379	1	4	1	9
Bg403	1	3	3	20
Bg454	1	2		
Bw364	1	1	2	22
Kaluheenati	0	1	1	11
Kuruluthuda	1	4		
Ld365	0	2	1	15
Pachchaperumal	0	2	2	10

Table 5. Summary of nonparametric variables; number of tillers per plant, number of leaves per plant, number of panicles per plant and number of seeds per panicle of control and treated with glufosinate (0.05 gl^{-1}).

3.1. Effect of glufosinate and glyphosate on agro-morphological characters of HR resistant rice varieties

The results of the study suggest that several specific growth parameters of certain glufosinate-resistant varieties at 0.05 gl^{-1}showed no significant difference ($p > 0.05$) compared to control plants. For instance, plant height of Bg379-2 (**Table 3**), leaf blade width of Bg366, and leaf length of Bg352 at 0.05 $gl^{-1}$of glufosinate application showed no significant difference ($p > 0.05$) (data not given). In addition, the control plants and the plants resistant to 0.05 gl^{-1}glufosinate, number of leaves per plant and number of tillers per plant (**Table 5**) were not statistically significant.

Analysis of the variance of yield parameters indicated no significant difference for number of the seeds per panicle at 0.05 gl^{-1} glufosinate application except Bg454, Bg369 and *Kuruluthuda*. Almost all varieties indicated significant differences ($p \leq 0.05$) for flag leaf length, flag leaf width at0.05 gl^{-1}glufosinate application predicting the possibility of glufosinate (at 0.05 gl^{-1}) to cause reduction in flag leaf quality even when applied at 3–4 leaf stage of the plant (data not given). Varieties such as Bg360, Bg357, Bg369, Bg379-2, Bg450, Bg403, Bg250 and Bg 454 reported insignificant differences for thousand seed weight character at 0.05 gl^{-1}. Significant yield reduction was observed for Bg362, Bg359, Bg94-1, Bg358, Bg300 and At362 at 0.05 gl^{-1}.

After application of 0.05 gl^{-1} concentration of glufosinate, injuries were identified (**Figure 2**) as rapid chlorosis (**Figure 2B**) of treated leaves followed by wilting (**Figure 2D**), necrosis (**Figure 2C**) and ultimate death of susceptible plants. Similar symptoms have been reported for different rice varieties [19, 34, 35] and for wheat [37] (Deeds *et al.*, 2006). In addition, brown color lesions (**Figure 2A**) were also observed on leaves, and browning of leaf tips (**Figure 2E**) commonly occurred on all varieties. The injuries were significantly higher after 1 week from herbicide application. Severe chlorosis was observed in rice leaves depending on the susceptibility of the varieties within 3–6 days after herbicide treatment. Within 2 weeks after herbicide application, the observable symptoms were disappeared even in the varieties which were exposed to the highest concentration of glufosinate. Previous studies have been shown that rupture and contortion of inter-venal mesophyll cells with concomitant disorganization of bundle sheath cells herbicide treated plants [38, 39].

Comparatively, the glyphosate treated rice plants indicated that all the yield parameters (number of panicle/plant, number of seeds/panicle and 1000 grain weight) were significant differ from the controls (**Tables 2** and **4**).

After application of 0.5 gl^{-1} glyphosate concentration, a number of visual injuries were observed in individuals of varieties (**Figure 3**). The injuries were promptly observable after 1 week of herbicide application. Among these injuries, general chlorosis in the upper part of the leaves was most abundant. Comparatively, severe chlorosis was observed in rice leaves that depend on the resistance of the varieties within 3–6 days after herbicide treatment. In susceptible varieties, leaf wilting leads to plant death. Newly emerged leaves of survived varieties remained in green color; however, the young emerging leaves which were subjected to treatment were often tightly curled inwardly. Multiple shoots arising from internodes of main stem (**Figure 3A**) were observed, and the secondary shoots and flag leaves were wrinkled or curled in *Kaluheenati* and *Pachchaperumal*. At the booting stage, all the leaves of the variety *Kuruluthuda* were curled and leaf discoloration had occurred. The plants remained in the same stage and indicated no maturity until the harvesting stage (**Figure 3B**). Malformation of inflorescences was also observed in certain varieties at the reproductive stage. The inflorescence of Bg369 and Bg454 was found aborted inside the flag leaf sheath and unable to emerge as a panicle (**Figure 3C**). Meanwhile, panicles of certain varieties were yet to appear in full due

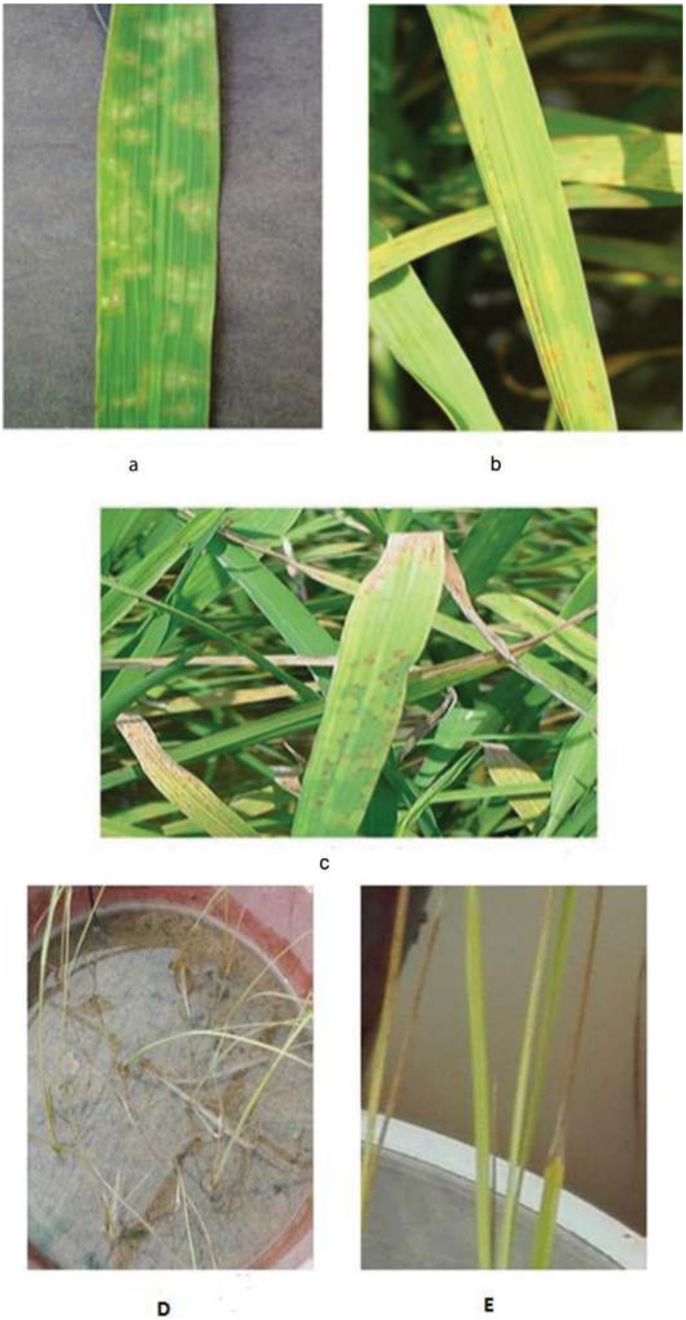

Figure 2. Visual injuries caused by glufosinate: (A) brown color lesions on leaf blade, (B) severe chlorosis on leaf blade after glufosinate treatment, (C) necrotic areas of leaf blade, (D) wilting of susceptible plants, and (E) browning of leaf tips.

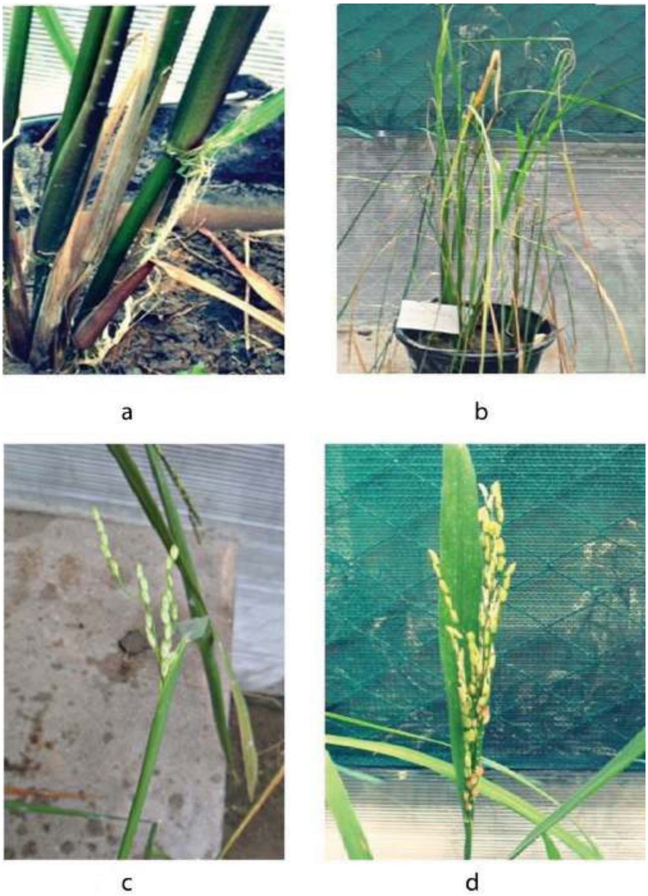

Figure 3. Visual injuries caused by glyphosate: (A) multiple shoots and roots that sprouted from the internodes, (B) leaf curling and discoloration, (C) fused panicle to flag leaf, and (D) bleached lemma and Palea.

to the fusion of the flag leaf at the maturity stage. Malformation of inflorescence and developing grains with only bleached lemma and palea were commonly found in Bg366 (**Figure 3D**). In the variety Bg379-2, distorted and crescent-shape spikelet were observed.

4. Conclusions

The rice varieties such as At362, Bg359, Bg366, Bg369, Bg379-2, Bg403, Bg454 and *Pachchaperumal* were resistant to both glyphosate (0.5 gl^{-1}) and glufosinate (0.05 gl^{-1}) applications. Even though the herbicide resistant varieties emerged from the screening, the responses of agro-morphological and yield characters varied across the type of herbicide and the variety. Glyphosate substantially reduced the growth parameters as well as yield compared to glufosinate treated varieties. As far as yield is concerned, there was a significant yield penalty in HR rice varieties. These broad-spectrum HR rice varieties have a higher potential to be utilized in rice breeding programs to breed new HR varieties and can be used to develop HR rice in future.

Acknowledgements

The research grants provided by the National Research Council, Sri Lanka (Grant No. NRC 12-037) and the Faculty of Natural Sciences, The Open University of Sri Lanka are greatly acknowledged. The assistance provided by Ms. Deshani Lakshika is highly appreciated.

Author details

Shyama R. Weerakoon*, Seneviratnage Somaratne, E. M. Sachini I. Ekanayaka and
Sachithri Munasighe

*Address all correspondence to: shyamaweerakoon@gmail.com

Department of Botany, The Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka

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