ASSESSMENT OF GLUFOSINATE RESISTANCE IN SELECTED SRI LANKAN RICE (*Oryza sativa L.)* VARIETIES

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Introduction

Infestation of weeds in rice fields during the cropping season requires pre- and post-emergence herbicides as a mean of controlling those weeds. In many countries broad-spectrum herbicides (BSH), imidazolinone, glyphosate and glufosinate, are being used to control weeds in rice fields during land preparation [1]. Broad spectrum herbicides target both monocotyledonous and dicotyledonous weeds leading the less number of herbicide applications. Application of glyphosate in rice fields has been banned recently in Sri Lanka. Therefore, the number of selective herbicide application and their amount used control common weeds such as: *Cyperus iria* L. (family Cyperaceae), *Echinochla spp*. (family Graminae), *Monchoria* vaginalis (family Pontederiaceae) and weedy rice have been increased considerably leading to sever environmental threats. In this context, glufosinate seems to be an alternative herbicide to glyphosate, as glufosinate is a non-selective herbicide for rice weeds.

One of the major drawbacks of BSH is causing damage to the cultivated rice through off- target movements and reducing the yield up to 80% [2]. The introduction of herbicide resistant (HR) crops brought a novel strategy for controlling weeds, allowing growers to use one product to control a wide range of weeds without sustaining crop injuries from substantially lower costs. Herbicide resistance can be induced in crops occupying many ways such as mutational techniques, transgenic techniques and *etc*. A considerable number of studies have been carried out on the use of BSH in controlling rice weeds as post emergent herbicide with minimal effect on the cultivated rice. Since the national Bio-safety Framework has prohibited the production or consumption of transgenic crops in Sri Lanka and due to the lack of commercialized, induced HR rice varieties via conventional breeding techniques, it is worthwhile to screen the natural herbicide resistance in Sri Lankan rice varieties.

Most of the studies has reported the use of advanced techniques such as gene transferring, hybridization etc. in developing HR crop varieties. Thus, there is an insufficiency of studies related to screening and developing HR varieties. Previous studies have shown that there is a wider variation of natural resistance to glyphosate in commercially cultivated traditional and inbred rice varieties in Sri Lanka [3] and up to date a detailed study on natural glufosinate resistance in Sri Lankan rice varieties has not being reported. Therefore, the present study was aimed to screen the naturally existing glufosinate resistance in Sri Lankan rice varieties as a preliminary case study.

Materials and Methods

Seed Material:

Twenty-five rice varieties including seven traditional varieties ('*Pachchaperumal*', '*KuruluThuda*', '*Rathal*', '*KaluHeenati*', '*RathSuwadal*', '*Handiran*', and '*Ma Wee*') and eighteen inbred lines (Bg352, Bg360, Bg359, Ld365, Bg366, Bg357, Bg94-1, Bg369, Bg379-2, Bg450, Bg403, Bg250, Bg454, Bg358, Bg300, Bg304, Bg305, At362) collected from Rice Research and Development Institute (RRDI) at Batalagoda, Ambalantota and Labuduwa were used for the study. These lines were maintained in a plant house at the Open University of Sri Lanka, located in low country wet zone of western province, with an average temperature of 40-42^{o}C and 65-70% relative humidity.

Evaluation of naturally existing HR resistance among rice varieties:

The selected seeds were pre-soaked overnight and allowed to germinate. One week old seedlings were planted in pots filled with puddle soil (5.5 kg per pot) and excess plantlets were thinned out after one week. Fertilizer application and crop practices were performed according to the recommendations of the Agriculture Department of Sri Lanka. Three different concentration of glufosinate as 0.27 kg ha^{-1}, 0.30 kg ha^{-1} and 0.33 kg ha^{-1}[2] were applied at 3-4 leaf stage [4]. Complete Randomized Design (CRD) was followed with three pots in each 10 replicates for each treatment and non-treated plants served as control.

The total number of plants and the number of surviving plants after glufosinate application were counted for each variety and percentage (%) of resistance was calculated as follows.

$$\text{Percentage resistance (\%)} = \frac{\text{Number of resistant seedlings in a variety}}{\text{Total number of seedlings grown in the same variety}} \times 100\%$$

Varieties with ≥ 50% resistance to glufosinate treatment were considered as glufosinate-resistant [3]. Variables such as plant height, number of leaves, number of tillers in every two weeks after sawing and the yield parameters were obtained.

Determination of the Recovery of Visual Quality and Chlorophyll content:

A hand-held chlorophyll content meter (model SPAD-502, Minolta, Japan) was used to assess the chlorophyll status and to correlate it with the visual quality in treated and non-treated rice varieties. Visual colour rating was performed using the leaf colour chart issued by the International Rice Research Institute (IRRI). Three mature leaves were selected per pot and three SPAD measurements were taken per plant. SPAD readings and visual colour ratings were taken in 2-day interval before and after the glufosinate treatment for a total of six sampling dates. Sampling was done at the same time of the day (around 10.00 am- 11.30 am) under the light intensity of 833-1228 µmol m^{-2} S^{-1} and subjecting to the temperature range of 40-42 ^{0}C and 65-70% relative humidity.

Spectrometric Analysis of Leaf Chlorophyll content in glufosinate treated Rice:

Leaves of all glufosinate treated and non-treated rice varieties were excised from the plants, avoiding mechanical injuries. Fresh, clean leaf samples weighing 10 mg were then cut into small pieces and preserved them in cold distilled water to prevent excess loss of moisture [5]. Three replicates were prepared from three different plants of the same variety.

Rice leaf tissues were incubated in 2 ml of 80% buffered acetone (pH 7.8) in dark, with a temperature of 4^0C and tubes were shaken occasionally to accelerate pigment extraction [5]. After a considerable time the extract liquid was filtered using Whatman No.1 filter papers, leaf pieces were removed and the volume was made up to 2 ml, and transferred to 3 ml sealed quartz-glass cuvettes with 1 cm path length.

Dual beam recording UV visible spectrophotometer was used to measure absorption of the extract at the wavelength of 663 nm and 645 nm. The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were then calculated.

$$\text{Chlorophyll a} = 12.27A_{663} - 2.59A_{645}$$

$$\text{Chlorophyll b} = 22.9A_{645} - 4.67A_{663}$$

$$\text{Total chlorophyll} = 20.31A_{645} + 8.05A_{663}$$

Results and Discussion

Evaluation of naturally existing HR resistance among rice varieties:

Results from the screening for glufosinate resistant varieties reveal that some of the selected traditional rice varieties and inbred lines possess the ability to resist the detrimental effects of glufosinate (Figure 1). The lowest concentration of glufosinate, i.e. 0.27 kg ha^{-1} was found to cause minimal damage to selected rice varieties, and only two cultivars (*Rathal*–13% and Bg305–17%) were found to be susceptible to 0.27 kg ha^{-1} glufosinate concentration. Most of the varieties (At362- 90%, Bg250- 83%, Bg300- 96%, Bg352- 100%, Bg357- 53%, Bg358- 53%, Bg359- 100%, Bg360- 96%, Bg366- 73% , Bg369- 83% , Bg379/2- 93%, Bg403- 100% , Bg450- 57% , Bg454- 97% , Bg94/1 – 73% , *Ma Wee*- 100%, *Pachchaperumal*- 53%) were able to survive under the application of 0.30 kg ha^{-1} glufosinate concentration and only six cultivars *(Ma Wee* – 77%, Bg366 - 57%, Bg379/2 – 53%, Bg403 – 73%, Bg454 – 77%, Bg94/1 – 57%) were able to survive when 0.33 kg ha^{-1} glufosinate concentration was applied (Figure 1).

Determination of the Recovery of Visual Quality and Chlorophyll content:

Glufosinate injuries included rapid chlorosis of treated leaves followed by necrosis and ultimate death of susceptible plants. Similar symptoms have been reported for different rice varieties [4] and for wheat. In addition, brown colour lesions were also observed on leaves, and browning of leaf tip was commonly occurred on all varieties. The injuries were significantly higher after one week from herbicide application. Severe chlorosis was observed in rice leaves depending on the susceptibility of the cultivar within 3-6 days after herbicide treatment. Within two weeks after herbicide application the observable symptoms were disappeared even in the cultivars which were exposed to the highest concentration. Previous study has shown rupture and contortion of interveinal mesophyll cells with concomitant disorganization of bundle sheath cells.

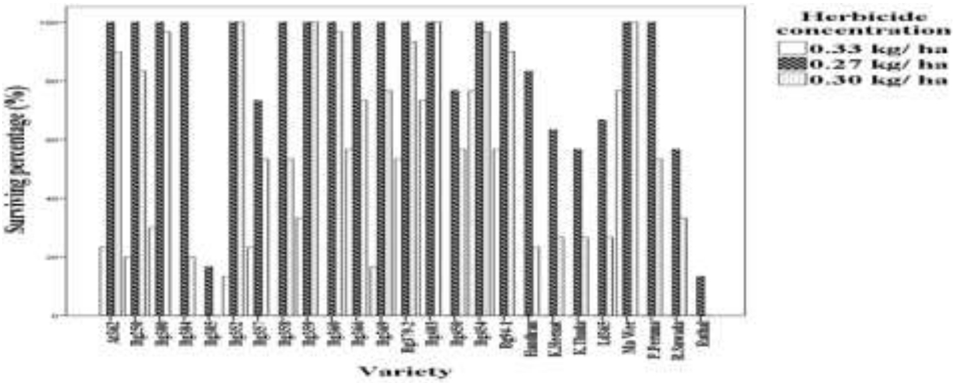

Figure 1: Herbicide resistance of rice varieties exposed to glufosinate, 0.27 kg ha^{-1}, 0.30 kg ha^{-1}, 0.33 kg ha^{-1} (Survival % ≥50% - Resistant, survival % <50% - Susceptible)

Since glufosinate is not readily translocate within the plant, resistant varieties were able to produce new, symptoms free leaves. Previous study also reported the same [2, 4]. According to the visual rating results, SPAD readings and chlorophyll concentration values, all the resistant varieties were able to recover from chlorosis within two weeks after treatment. Irrespective of the herbicide treatment, chlorophyll content of all recovered plants exhibited a significant difference when compared to that of control plants (Figure 2).

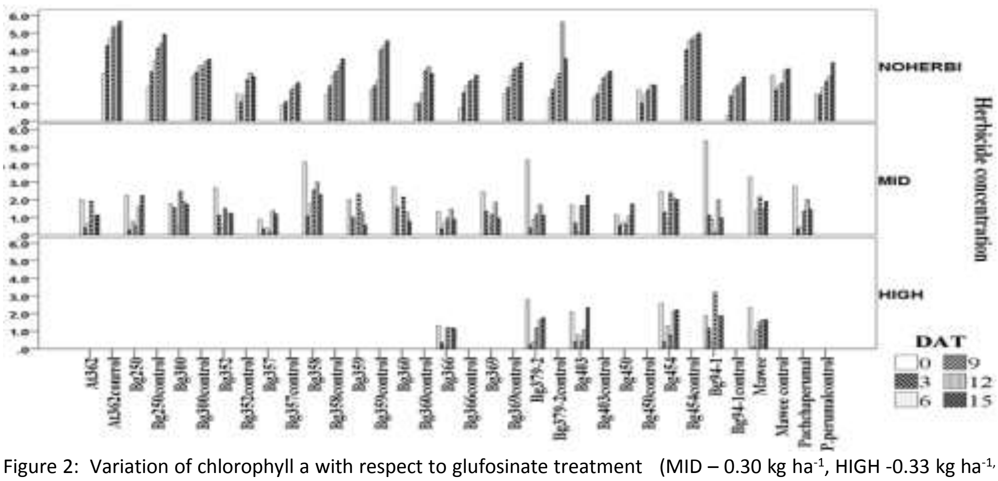

Figure 2: Variation of chlorophyll a with respect to glufosinate treatment (MID – 0.30 kg ha^{-1}, HIGH -0.33 kg ha^{-1}, NOHERBI - control)

Variation in growth parameters of glufosinate resistant rice varieties:

The comparison of plant height, leaf blade length, leaf blade width, number of tillers and number of leaves of treated plants after eight weeks of glufosinate application revealed a considerable reduction in growth parameters. A previous [4] has reported only 5% canopy height reduction due to glufosinate and 50% reduction due to glyphosate when applied at 3-4 leaf stage. However, in the present study canopy height reduction of resistant varieties due to glufosinate application at 3-4 leaf stage has been reported as 50%.

Variation in yield parameters of glufosinate resistant rice varieties:
According to ANOVA, yield characters apart from the heading date did not show a significant difference in glufosinate treated and non-treated plants.

Conclusions and Recommendation

Increasing glufosinate concentration has a negative impact on agro-morphological characters such as plant height, leaf blade length and leaf blade width and on the heading date of selected rice varieties. Even though glufosinate injuries were rapid and severe, almost all resistant varieties have the ability to recover those visual injuries even at the highest concentration of 0.33 kg ha^{-1}. A yield penalty did not occur in rice plants when glufosinate was applied at 3-4 leaf stage. Therefore, the widely recommended glufosinate concentration of 0.30 kg ha^{-1} for rice fields seem to be more suitable for application to minimize off-target damages of rice.

Six rice varieties (Bg366, Bg94-1, Bg379-2, Bg403, Bg454, and '*Ma Wee*',) have shown resistance at the highest concentration (0.33 kg ha^{-1}) of glufosinate. These could be used in a breeding programme to breed HR varieties.

Acknowledgements

The research grant provided by the Faculty of Natural Sciences, The Open University of Sri Lanka.

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