PRELIMINARY STUDIES ON INDUCING HERBICIDE RESISTANCE IN SRI LANKAN RICE VARIETIES USING CHEMICAL MUTAGENS, NAN_3 AND EMS

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INTRODUCTION

Weeds are a source of biotic stresses in crop systems including rice. Weeds decrease the yield, increase production costs, and contribute to income risk in rice cultivation (Rao & Nagamani, 2007). Therefore, weed control is an essential component of profitable rice production. Rice weeds can be controlled mechanically, chemically (with herbicides) or culturally. A combined approach including herbicides is more economical and even more effective than reliance solely on mechanical and cultural control practices. Many weed species present in rice fields in Sri Lanka are difficult to control, thus application of a pre-emergence broad-spectrum herbicide, glyphosate (round-up) is recommended. Glyphosate targets monocotyledons and dicotyledonous weeds, permitting less herbicide use in terms of amount and number of application. However, glyphosate causes damages to cultivated rice as well (Labrada, 2007; Davis, Scott, Norswothy, Smith 2009).

Inducing herbicide resistance (HR) into rice is a new means to confer selectivity and enhance crop safety and production. Herbicide Resistant crops provide additional crop choice, enabling implementation of alternate weed management strategies to target specific weeds while maintaining crop sequences. Therefore, it is believed that inclusion of an HR crop in a cropping program along with a range of weed management approaches can ensure control of hard-to-control weeds (Beckie *et al*., 2006). The discovery of HR weeds in early 1970s triggered an interest on developing HR crops using crop breeding techniques. As a result, Imidazolinone-resistant rice was developed through chemically induced seed mutagenesis with Ethyl Methyl Sulfonate (EMS) (Gealy *et al*., 2003; Tan *et al*., 2005). Commercial development is underway for EMS induced glufosinate-resistant rice (Lang and Buu, 2007). The mutagen Sodium Azide (NaN_3) has also been used to produce rice mutants for the enhancement of agronomic traits (Nakata *et al*., 2008).

In Sri Lanka, there were a limited number of reports available on the induction of HR in rice varieties and at present, only a study conducted by Weerakoon *et al*. (2013) reported naturally existing inbred and traditional HR rice varieties in Sri Lanka. In the present study attempts were made to induce HR against glyphosate in 24 cultivated rice varieties (inbred and traditional) *via* mutagenesis through chemical mutagens, Sodium Azide (NaN_3) and Ethyl Methyl Sulphonate (EMS) to evaluate the mutagenic effect of these chemicals in inducing herbicide resistance. This study is an extension of the previous study on the natural herb resistance of traditional and inbred-cultivated rice varieties in Sri Lanka (Weerakoon *et al*., 2013).

METHODOLOGY

Twenty four rice varieties were selected for the study. Six traditionally cultivated varieties (*Kalu Heenati, Sudu Heenati, Suwadal, Suduru Samba, Pachchaperumal* and *Murungakayan*) and eighteen inbred cultivated varieties (Bg94-1, Bg250, Bg300, Bg304, Bg305, Bg352, Bg357, Bg358, Bg359, Bg360, Bg366, Bg379-2, Bg403, Bg406, Ld365, At362, At308, Bw364) were obtained from Rice Research Institutes (RRDI) at *Batalagoda, Ambalanthota, Bombuwela* and *Labuduwa*, Sri Lanka. These varieties were maintained in a greenhouse at the Open University of Sri Lanka. Randomized Complete Block Design (RCBD) was used in each treatment with three replicates.

Method 1 – Mutation Studies using NaN_3

The seeds of each variety were exposed to NaN_3 at 1.5 mmol l^{-1}, 3.0 mmol l^{-1} and 6.0 mmol l^{-1} concentrations for a day and allowed to germinate. Germinated rice seedlings (height *ca.*4.0cm) were immersed in Glyphosate solutions with two different concentrations, 0.25 g l^{-1} and 0.5 g l^{-1} for 4 days. Control treatment was carried out without Glyphosate. All seedlings were subsequently transferred to soil medium (sterilized mud) and allowed to grow, and eight agro-morphological characters (Table 1) were measured / observed.

Dead plants were considered as susceptible to the herbicide. The plants that survived and remained green (but did not grow) were considered as tolerant to the herbicide and the surviving plants with a substantial growth were considered as resistant to the herbicide. For each rice variety, number of resistant plants and percentage resistance was calculated. The percentage (%) of resistance was calculated using the following equation.

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

The percentage of ≥ 40% was chosen arbitrarily and considered as an indicator of glyphosate-resistance of mutated plants (Weerakoon, Somaratne, Wijeratne, Ekanayaka 2013).

Method 2 – Mutation Studies using EMS

The seeds of each rice variety were exposed to EMS at 2.5 mmol l^{-1} and 4.5 mmol l^{-1} concentrations for a day and thesame steps described in Method 1 were followed.

Statistical analysis

Descriptive statistics were performed on the data set (mean, standard deviation). The GLM (General Linear Models) was used to test the interactions of factors (rice variety, NaN_3/EMS and glyphosate concentrations) on agro-morphological characters. Since there were no significant interactions among the factors, One-way-analysis of variance (ANOVA) was performed on agro-morphological characters. All statistical analyses were carried out using SAS Version 9.2 (SAS, 2008).

RESULTS AND DISCUSSION

Compared to the previous study (Weerakoon *et al*, 2013), a higher percentage of resistant plants (a large number of varieties) resulted in 0.25 g l^{-1} glyphosate concentration. However, at 0.5 g l^{-1} glyphosate concentration caused a reduction in the percentages of resistance in rice plants and it may be due to inhibition of seed germination. Mutation study with NaN_3 revealed that four rice varieties (Bg300, Bg379-2, Bg403 and Bw364) were resistant to 0.25 g l^{-1} glyphosate concentration after mutation with NaN_3 (1.5 mmol l^{-1}) and there were three (Bg252, Bg359, Bg406) resistant varieties when mutated with 3.0 mmol l^{-1} NaN_3 (Fig 1 A and B) whereas 6.0 mmol l^{-1} concentration of NaN_3 seems to be too toxic and even the seeds had not germinated. Out of selected rice varieties, *Sudu heenati* and Bw364 showed resistance to 0.25 g l^{-1} glyphosate concentration after mutating with 2.5 mmol l^{-1} EMS and when increased, the EMS concentration up to 4.5 mmol l^{-1}, Bg300, Bg359, Bg304, Bg403, Bw364, *Suduru Samba*, *Murungakayan* were resistant to glyphosate. (Fig 2 A and B)

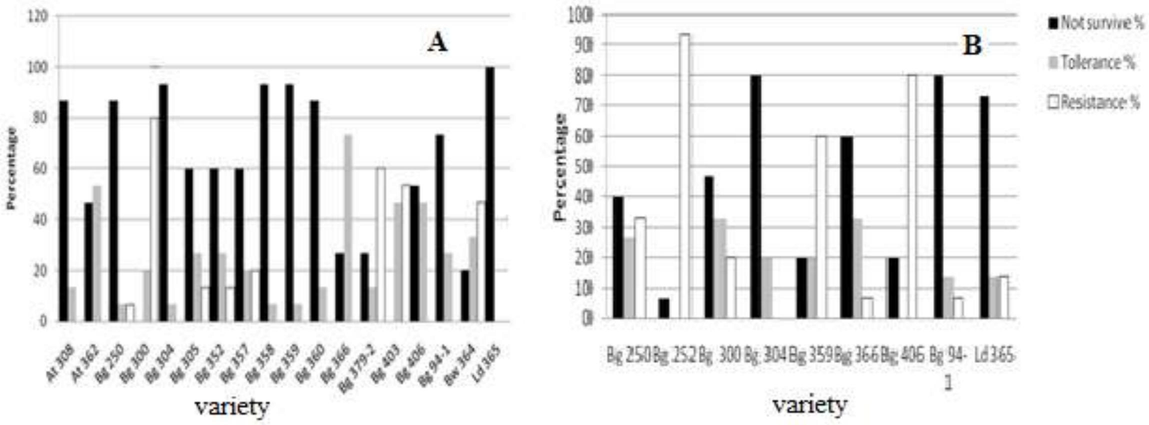

Fig 1: Glyphosate Resistance/Tolerance of rice varieties exposed to NaN_3
(A) 1.5 mmol l^{-} of NaN_3; (B) 3.0 mmol l^{-1} of NaN_3

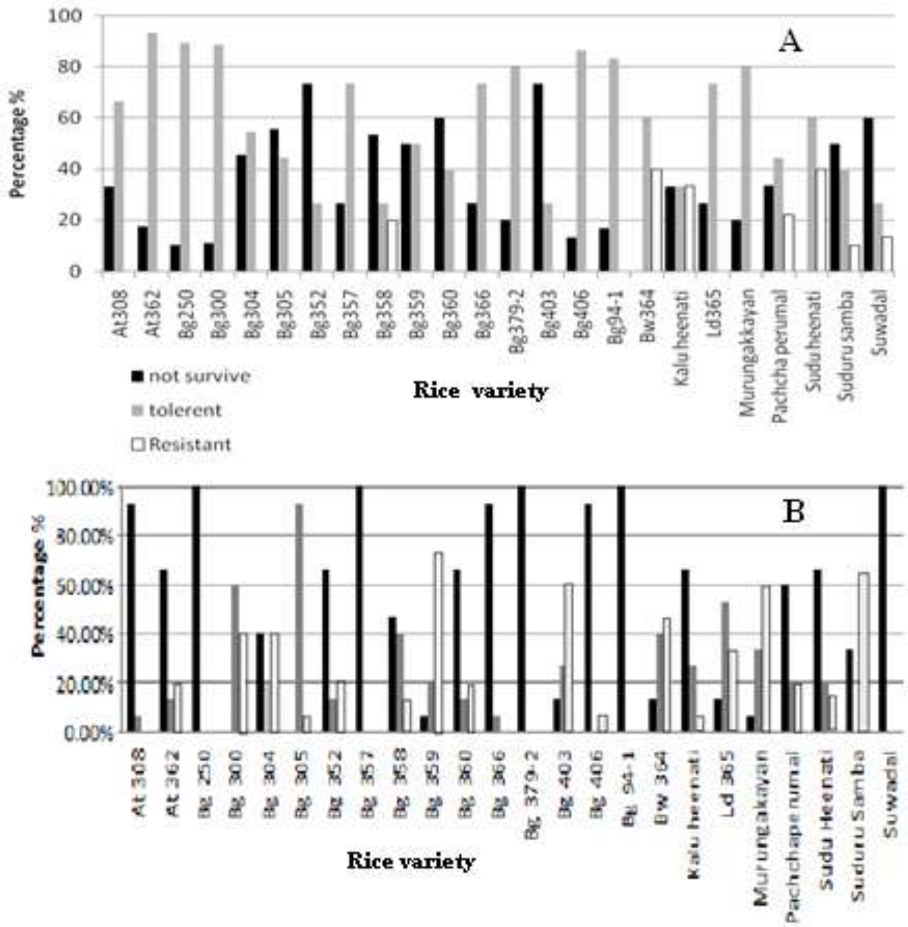

Fig 2: Glyphosate Resistance/Tolerance of rice varieties exposed to EMS.
(A) 2.5 mmol l^{-1} of EMS; (B) 4.5 mmol l^{-1} of EMS.

A combined ANOVA (results not presented) revealed that all main effects (rice variety, NaN_3/EMS and glyphosate concentrations) were not statistically significant ($p \geq 0.05$). Table 1 shows, all agro-morphological characters have no statistically significant differences between NaN_3 mediated-mutated rice plants and non-mutated rice plants under treatment at 0.25 g l^{-1} glyphosate. However, statistically significant differences were observed between EMS mediated-mutated plants and non-mutated plants under treatment at 0.25 g l^{-1} glyphosate (except germination time and flowering time). Similar observations were made by Benjavad, Talebi, Shahrokhifar (2012) with EMS mediated-mutated Malaysian rice.

CONCLUSIONS/RECOMMENDATIONS

Increasing NaN_3 and glyphosate concentrations have a negative effect on agro-morphological characters of rice varieties. Comparatively, EMS treatments resulted in higher percentage of resistance than that of NaN_3. Further, a considerable yield penalty and stunting trends in agro-morphological characters was observed in NaN_3 treated plants. Both 1.5 and 3.0 mmol l^{-1} of NaN_3 and 4.5 mmol l^{-1} of EMS seem to be most suitable chemical concentrations for mutagenic purpose in inducing HR in rice. However, the dose dependent mutagenic efficiency of NaN_3 and EMS need to be further investigated. In addition, mutated rice varieties need to be evaluated for the stability of their HR for several generations (second generation is being evaluated). Mutated rice varieties with high glyphosate resistance have a higher candidacy in rice breeding programs as well as could lead to develop HR rice varieties in future

Table 1: Summary of ANOVA performed on the agro-morphological characters with respect to different concentrations of EMS and NaN_3. The analysis includes HR resistant plants only.

Source	Sum of Squares	df	Mean Square	F	Significance
EMS treatments					
Germination time	0.03	2	0.05	0.30	NS
Height	9185.00	2	4592.50	16.67	S
Number of leaves/plant	149.43	2	74.72	15.52	S
Leaf width	267.16	2	133.58	8.18	S
Leaf length	2220.20	2	1110.10	7.80	S
Flowering time	1041.44	2	520.72	1.45	NS
Panicle length	2580.56	2	1290.28	39.48	S
Number of seeds/plant	11934.44	2	5967.22	36.89	S
NaN_3 treatments					
Germinatipn time	6.79	2	3.39	3.79	NS
Height	952.84	2	476.42	1.61	NS
Number of leaves/plant	57.37	2	28.69	3.33	NS
Leaf width	0.12	2	0.06	1.80	NS
Leaf length	701.20	2	350.60	2.70	NS
Flowering time	1999.00	2	999.50	0.58	NS
Panicle length	169.63	2	84.82	1.95	NS
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

(S = Significant at $p \leq 0.05$; NS = Not significant at $p \leq 0.05$)

REFERENCES

Beckie, H. J., Harker, K. N., Hall, L. M.,Warwick, S. I., Légère, A., Sikkema, P. H., Clayton, G.W., Thomas, A. G., Leeson, J.Y., Séguin-Swartz, G. and Simard, M. J. (2006). A decade of herbicide-resistant crops in Canada. Can. J. Pl. Sci. 86: 1243-1264.

Benjavad, T. A., Talebi, A. B. and Shahrokhifar, B. (2012). Ethyl Methane Sulphonate (EMS) Induced Mutagenesis in Malaysian Rice (cv. MR219) for lethal Dose Determination. Am. J. Pl. Sc. 3(12): 1661.

Davis, B. M., Scott, R. C., Norsworthy, J. K. and Smith. K. L. (2009). Effects of Low Rates of Glyphosate and Glufosinate on Rice AAES.Research Series. pp. 581.

Gealy, D. R., Mitten, D. I. L. and Putger, J. N. (2003). Gene flow between red rice (*Oryza sativa*) and herbicide-resistant rice (*O. sativa*). Weed Technology 17: 627-645.

Labrada, R.. (2007). Weedy and Wild Rice: Their Impact and Management. pp. 8-15. 21st Asian Pacific Weed Science Society (APWSS) Conference, Colombo, Sri Lanka.

Nakata Y., Ueno, M., Kihara, J., Ichii, M., Taketa, S. and Arase, S. (2008). Rice Blast disease and susceptibility to pests in a silicon uptake-deficient mutant *Isil* of rice. Crop protection 27: 865-868.

Lang, N. T. and Buu, B. C. (2007). Rice breeding and resistance in Clearfield rice (*Oryza sativa* L), Omonrice 15: 36-45.

Rao, A.N. and Nagamani, A. (2007).Available technologies and future research challenges for managing weeds in dry-seeded rice in India: Proceedings of the 21st Asian Pacific Weed Science Society Conf. 2 to 6th October 2007, Colombo, Sri Lanka.

Tan, S., Evans, R., Dahmer, M., Singh, B. andShaner. D.L. (2005). Imidazolinone-tolerant crops: History, current status, and future. Pest Management Science 61: 246-257.

Weerakoon,S.R.,Somaratne,S.,Wijeratne,R.D.J.,andEkanayaka,E.M.S.I.(2013).Natural Herbicide resistance(HR) to Broad-Spectrum herbicide, Glyphosate among traditional and inbred-cultivated rice(*Oryza sativa* L.) varieties in Sri Lanka. Pakistan Journal of Biological Sciences 16 (16):796-803

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