Screening Herbicide Resistance in Selected Sri Lankan Cultivated Rice (*Oryza sativa* L) Varieties Through Induced Mutagenesis

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Abstract

Herbicide resistant (HR)of rice has the potential to improve efficiency of weed management practices, reduction in rice production cost, increase productivity and reduce usage of herbicides. However, HR in Sri Lankan rice varieties has so far not been evaluated or developed. Sri Lankan rice varieties may harbor HR for possible incorporation in rice-breeding programs and mutagenesis may activate new genes conferring HR. The chemical mutagen, Sodium azide (NaN_3) (1.5 mmol/l, 3.0 mmol/l and 6.0 mmol/l concentrations) was used to induce HR in eighteen cultivated rice varieties against pre-emergent broad-spectrum herbicide, Glyphosate. Randomized Complete Block Design (RCBD) was used in the experiment and there were five replicates and three blocks in each treatment. For each rice variety, percentage resistance for Glyphosate concentrations (0.25 g/l and 0.5 g/l) was noted. The plants with ≥ 40% resistance were considered as resistant to Glyphosate. Morphological characters, Days to seed germination, Days taken to flower, Plant height at 12 weeks after sowing (WAS), number of leaves at 12WAS, leaf length. width, length of panicle and number of seeds/panicle of the herbicide-treated resistant plants and un-treated resistant plants were recorded. Statistical analyses were carried out using SAS 6.12. Five cultivated rice varieties, Bg406, Bg352, Bg379-2, Bg300, Bw364 showed resistance against Glyphosate at 0.25 g / l concentration. The results showed that NaN_3 could be used to produce Glyphosate resistant rice varieties from existing cultivated rice varieties (or new improved varieties). The mutant rice varieties were greatly differ from their parental varieties having increased days taken to flower and seed germination, reduced plant height, number of leaves/plant, leaf-length, leaf-width panicle-length and number of seeds/panicle., and imposing a mutagenic penalty on the yield of the crop as whole. The higher the NaN_3 concentration used for mutagenesis, the greater the Glyphosate resistance. However, further studies are being carried out to confirm the stability of Glyphosate resistance among these mutated rice varieties.

Key words: Glyphosate, Herbicide Resistance, *Oryza sativa*, Sodium azide, Sri Lanka.

Introduction

Weeds are the major biotic constraint to increased rice production worldwide. Therefore weed control is an essential component of profitable crop production and it can be controlled mechanically, chemically or by crop rotation. Most farmers rely on a blend of these methods. Application of high concentrations of broad-spectrum systemic post-emergence herbicide, Glyphosate is necessary to control rice weeds present in rice fields of South East Asian countries including Sri Lanka and this intern cause damages to the cultivated rice as well (Davis *et al.* 2009). Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase, an enzyme involved in the shikimic acid pathway of plants. Glyphosate can cause a significant damage to rice yield with a reduction of yield up to 80% (Davis et al. 2009). Breeding HR into rice is a new means to confer selectivity and enhance crop safety and production (Guttieri et al. 1996). Over the last two decades, mutational techniques have become one of the most important tools available to progressive rice- breeding programs. Imidazolinone-resistant rice was developed through chemically induced seed mutagenesis with ethyl methyl sulfonate (EMS) and "Clear field" rice variety was also developed through EMS mutagen against herbicides Imidazolinone and Imazapyr (Lang and Buu 2007). HR crops can bring significant benefit to farmers, consumers and the environment. Farmers get benefited from the excellent broad-spectrum weed control provided by such herbicides and from substantially lower costs of growing some HR crops. HR crops provide additional crop choice, enabling implementation of alternate weed management tactics to target specific weeds while maintaining crop sequences. Therefore, inclusion of an HR crop in a cropping program along with a range of weed management tactics can ensure to control hard-to-control weeds. In Sri Lanka there were no research efforts on the development of HR rice varieties until now. Therefore, selected eighteen cultivated rice varieties (Bg, At, Bw, Ld series) with no natural HR (previously screened for HR against Glyphosate), a study was conducted to induce HR against Glyphosate *via* mutagenesis using the chemical mutagen, Sodium azide (NaN_3).

Methodology

Materials - Rice varieties: Eighteen different rice varieties (Bg94-1, Bg250, Bg300, Bg304, Bg305, Bg352, Bg357, Bg358, Bg359, Bg360, Bg366, Bg379-2, Bg403, Bg406, Ld365, At362, At308, Bw364) were collected from RRDI at Batalagoda, Ambalanthota, Bombuwela and Labuduwa for the study. (These varieties were previously screened for Glyphosate resistance at 0.25g/l and 0.5g/l (360g/l Glyphosate) concentrations and found they were susceptible). These lines were maintained in a greenhouse at The Open University, Nawala.

Mutation Studies using NaN_3; The seeds of each developed-cultivated rice variety were exposed to the chemical mutagen Sodium azide (NaN_3) at 1.5 mmol/l, 3.0 mmol/l and 6.0 mmol/l concentrations for one day. Then the seeds were allowed to germinate and the rice seedlings (height - 4 cm) were immersed in Glyphosate solution with two different concentrations, 0.25g/l and 0.5g/l (360g/l glyphosate) for 4 days. The control treatment was without Glyphosate. Randomized Complete Block Design (RCBD) was used in the experiment and there were five replicates used for each treatment and three blocks in each treatment combination. All the seedlings were then transferred to soil medium (sterilized mud from a paddy field), allowed to grow and observations were taken for 12 weeks. Dead plants were considered as susceptible to the herbicide and surviving plants with a substantial growth were considered as resistant to the herbicide. For each rice variety, percentage resistance was noted as follows.

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

The plants with $\geq 40\%$ resistance were considered as resistant to Glyphosate. Morphological characters, Days to seed germination, Days to flowering, Plant height at 12 weeks after sawing (WAS), Number of leaves at 12 WAS, leaf length and width, length of panicle and number of seeds in the panicle of the resistant plants with herbicide treatment and the controls without herbicide treatment were also recorded. The statistical analyses were carried out using SAS Version 6.12. (SAS 1989).

Results and Discussion

Out of eighteen rice varieties tested, five cultivated rice varieties, Bg406, Bg352, Bg379-2, Bg300, Bw364 showed resistance against Glyphosate at 0.25g/l concentration. All the resistant varieties showed a percentage of resistance $\geq 56\%$. At 0.5g/l Glyphosate concentration, majority of rice plants did not survive.

A combined ANOVA (results not given) revealed that all main effects (rice variety, NaN_3 and Glyphosate concentration) were not statistically significant ($p \geq 0.05$) across the agro morphological characters. Further, in relation to all agro-morphological characters observed, there were no statistically significant differences between NaN_3 mediated-mutated were observed in rice plants compared to non-mutated rice plants under treatment of different Glyphosate concentrations. However, an increase in number of days taken to seed germination and flowering was observed for rice plants mutated with higher concentration of NaN_3 (Table 1).b Decreasing trends were observed in plant height, number of leaves/plant, leaf length, leaf width and panicle length, in the mutated rice plant along the increasing Glyphosate concentrations compared to the parental lines. Apparently, number of seed/panicle indicated a considerable reduction across increasing Glyphosate concentrations.

The results of the study showed that NaN_3 could be used to produce the Glyphosate resistant rice varieties from the existing cultivated rice varieties. The mutant rice varieties were greatly differ from their parent

Table 1: Summary of the morphological and yield characters of parental varieties and Glyphosate resistant rice produced by different concentrations of NaN_3.

Glyphosate concentration (g/l)	Sodium aside Concentration (mmol/l)	Variety	No. of days taken to flowering	No. of days taken to germinate	Height (cm)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Panicle length (cm)	No. of seeds/panicle
Mutated line										
0.25	1.5	Bg 406	NA	3 (0)	19.0 (2.3)	6.0 (1.4)	12.0 (0)	0.6 (0)	NA	NA
0.25	1.5	Bw 364	74.5 (2.1)	3 (0)	57.0 (12.7)	12.5 (0.7)	19.3 (6.0)	0.8 (0.1)	12.0 (5.7)	14.5 (10.7)
0.25	3	Bg 352	38.0 (53.7)	3 (0)	32.3 (18.0)	11.0 (1.4)	19.5 (13.4)	0.6 (0.3)	1.3 (1.8)	2.0 (2.8)
0.25	3	Bg 406	NA)	5 (0)	33.5 (6.4)	13.0 (2.8)	16.0 (1.4)	0.7 (0.1)	NA	NA
0.25	6	Bg 304	NA	5 (0)	56.5 (5.0)	6.0 (1.4)	44.0 (2.8)	1.0 (0.1)	NA	NA
0.5	3	Bg 366	NA	4 (0)	19.0 (2.3)	4.0 (0)	13.0 (6.0)	0.6 (0)	NA	NA
Parental line										
control	control	Bg 304	47.5 (0.7)	2 (0)	59.0 (1.4)	11.0 (1.4)	47.5 (2.1)	1.1 (0.1)	29.5 (3.6)	50.0 (1.4)
control	control	Bg 352	55.5 (2.1)	2 (0)	67.5 (3.5)	14.0 (1.4)	51.5 (2.1)	1.4 (0.1)	29.5 (3.6)	57.5 (2.1)
control	control	Bg 366	60.5 (2.1)	3 (0)	66.5 (0.7)	13.0 (1.4)	47.5 (6.4)	1.2 (0.2)	35.0 (1.4)	65.0 (0)
control	control	Bg 406	69.5 (0.7)	3 (0)	73.0 (5.7)	14.5 (0.7)	52.0 (5.7)	1.2 (0.3)	33.5 (6.4)	64.0 (4.2)
control	control	Bw 364	57.5 (2.1)	3 (0)	64.5 (5.0)	11.5 (0.7)	55.0 (2.8)	1.4 (0.2)	34.0 (1.4)	58.5 (6.4)

Mean values and standard error of mean is given within parenthesis. NA = Not analyzed

varieties having increased number of days taken for flowering and germination, reduced plant height, leaf length, leaf width, panicle length and number of seeds/panicle. This imposes a mutagenic penalty on the yield of the crop as whole. Similar observations have made and reported by Awan et al. (1980) and Young Seop et al. (2009) with NaN_3 showing a decreased germination rate and seedling height with the increasing NaN_3 concentration in *Oryza sativa* L. ssp. *Japonica* and few other varieties. The higher the NaN_3 concentration used in the mutation, the greater the Glyphosate tolerance. The mutated rice varieties with high Glyphosate resistance have higher potential to incorporate in rice breeding programs as well as could lead to develop herbicide resistant rice varieties in future. However, in order to confirm the stability of herbicide resistance among Glyphosate resistant rice varieties, further studies are being carried out currently for the next 3-4 generations.

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