IJBPAS, December, 2014, 3(12): 2825-2835

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

VARIATION OF HERBICIDE RESISTANT CLEARFIELD® RICE AND WEEDY RICE VARIANTS IN MALAYSIA

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ABSTRACT

Weedy rice (*Oryza sativa*) is a major problem in South-east Asian rice growing countries. Unfortunately, there was no effective measure to control weedy rice until the introduction of herbicide resistant Clearfield® rice. The objective of this study was to determine the variability among Clearfield® rice varieties and weedy rice variants commonly found in Malaysia in terms of seed size and vegetative growth at different growth stages. Treatments included 2 Clearfield® rice varieties namely MR220-CL1 (CL1) and MR220-CL2 (CL2) and 4 locally found weedy rice variants namely Variant 1 (V1), Variant 2 (V2), Variant 3 (V3) and Variant 4 (V4). Clearfield® rice varieties were procured from MARDI Seberang Perai. The experiment was arranged in completely randomized design (CRD) with four replications. Data were collected on seed morphology and vegetative growth. Weedy rice showed more variation compared to Clearfield® rice in terms of seed size, plant stature and tillering ability. Seeds of weedy rice were easy to shatters and had low spikelet fertility. Weedy rice seeds were found shorter but wider compared to Clearfield® rice seeds. Except for weedy rice variant V2, all other Clearfield® rice and weedy rice seeds were found slender in terms of length:width ratio. Weedy rice was much taller and had more tillering ability as compared with Clearfield® rice. All these characteristic of weedy rice offer major advantages against commercial rice and thus, weedy rice is more competitive than Clearfield® rice when grown under same agro-ecological conditions.

Keywords: Clearfield® Rice, Weedy Rice, Seed Morphology, Plant Height, Tillering Ability

INTRODUCTION

Weedy rice, weedy forms of *Oryza sativa*, is considered to be a pest in rice growing areas, and is particularly a problem in direct seeded rice systems. Weedy rice is an emerging problem in South-east Asian countries including Malaysia, the Philippines and Vietnam. It has been speculated to be progenies of wild rice with cultivated rice or degradation of cultivated rice **(Chin *et al.*, 2007)**. Weedy rice is taller, has fewer tillers and competes for sunlight, water and nutrients with cultivated rice that can cause reduction in yields **(Chin *et al*, 2007)**. **Shivrain *et al* (2008)** have confirmed that most of the applied nitrogen (N) fertilizers are lost to weedy rice by up to 60%, while the yield loss could be as high as 80%. In extreme cases, rice yield loss due to weedy rice infestation can go for up to 100% **(Saito, 2010)**.

There are three possibilities from where weedy rice comes from. Firstly, weedy rice evolves from wild rice. Second hypothesis states that weedy rice comes from escaped commercial rice and successfully adapts to its natural surroundings. The third hypothesis is that the weedy rice is hybrids of wild rice and commercial rice that produces progenies that is highly competitive **(Kane & Baack, 2007)**. Most of the wild rice and commercial rice have the same AA genotype as it is commonly found in the Asian region **(Kumar *et al.*, 2007)**. Due to the high similarities between weedy rice and cultivated rice, there has been no effective technique to control the former. This is due to the seeds shatters easily and longer dormancy period of weedy rice seeds in the soil. Some practices in controlling weedy rice are by eliminating the panicles, manual weeding and spraying of herbicides.

The introduction of genetically modified herbicide resistant rice in recent years has overcome the problem of weedy rice in field. One of the herbicide resistant rice introduced is Clearfield® rice. Clearfield® is tolerant to imidazolinone based herbicides. This herbicide work by inhibiting certain branch-chain based amino acids such as leucine, isoleucine and valine (**Croughan, 2003**). These amino acids are important for cell growth. The Clearfield® rice was first developed in Louisiana State University Agriculture Centre, USA. It is not considered transgenic rice because it was chemically induced for the seeds to be mutated. In Malaysia, the development of hybridization of Clearfield® rice with locally commercialized rice was started in 2003 at MARDI (Malaysian Agriculture Research and Development Institute) Station in Penang **(Azmi, *et al.*, 2008)**. Two popular varieties MR219 and MR220 were hybridized with Clearfield® rice Line 1770 introduced from USA. The DNA analysis shows that MR220-CL1 and MR220-CL2 have genetic similarity of about 98.5% and 92.5% respectively **(Sudianto *et al*, 2013)**. The MR220-CL1 and MR220-CL2 varieties were introduced to Malaysian farmers in 2010 **(Azmi *et al*, 2012)**. The Clearfield® rice production in Malaysia can be up to 7 metric tons per hectare **(Sudianto *et al*, 2013)**. However, there is a risk in planting the Clearfield® rice. The weedy rice was found to be a perfect plant for gene introgression because of its sexual compatibility **(Lu and Snow, 2005)**. Factors that influenced the gene flow are variation in flowering times, outcrossing rates, population sizes, distances between populations, wind speed and humidity **(Lu and Snow, 2005)**. Gene flow from herbicide resistant rice to wild and weedy relatives has been widely studied in the United States, Europe, and China with the natural hybridization rate can range from 1% to 52% **(Lang and Buu, 2007)**. If the Clearfield® rice gene incidentally transfers to the weedy rice, this will cause the production of herbicide resistant weedy rice and thus creating a super weed.

Therefore, the objective of this study is to determine the variability of locally found weedy rice variants in terms of width and length of the seeds, plant height and tillering ability using Clearfield® rice as comparisons. This is because weedy rice and Clearfield® are known to have different morphology. The difference in the morphology can help researches find ways to control weedy rice in the fields and to reduce chances of crossbreed between Clearfield® rice and weedy rice.

MATERIALS AND METHODS

Seed Categorization of Weedy Rice Variants

Seeds of four weedy rice variants were collected from different locations of Kuala Selangor, Selangor and Kuala Rompin, Pahang, Malaysia. Seeds were oven dried at 40˚C for 72 hours followed by storing in a refrigerator at -20˚C. Seed classification was referred to **IRRI (2002)**, which differentiates seeds based on type of panicles, awn, awn color, panicle threshability and spikelet fertility. Type of panicles, awn and awn color were observed directly. Panicle threshability were calculated based on the number of the rice seeds shattered after firmly grasp and pull the hand over the panicle. Spikelet fertility was calculated based on the number of fertile spikelets per panicle.

Comparing Seed and Plant Morphology of Clearfield® Rice and Weedy Rice Variants

The field study was conducted in the rice fields of *Lembaga Kemajuan Pengeluaran Pertanian* (LKPP) Padi, Pahang, Malaysia during May-September 2012 at coordinate of 2° 48` 0`` N. 103° 28` 17`` E. The local climate was hot, humid and tropic. Treatments included 2 Clearfield® rice varieties namely MR220-CL1 (CL1) and MR220-CL2 (CL2) and 4 locally found weedy rice variants namely Variant 1 (V1), Variant 2 (V2), Variant 3 (V3) and Variant 4 (V4). Clearfield® rice varieties were procured from MARDI Seberang Perai. The experiment was arranged in completely randomized design (CRD) with four replications.

The seeds were planted in trays, and 2-week old seedlings were transplanted in the field. Urea fertilizer was applied at 10 days after transplanting (DAT) with a rate of 100 kg per hectare. Mix fertilizers (17.5:15.5:10) were applied at 4th and 10th week after transplanting with a rate of 200 kg per hectare. Herbicides bispyribac sodium (Nominee®) at the rate of 1 L per hectare and pyrazosulfuron-ethyl (Basmin®) at the rate of 140 g per hectare were sprayed 1 week before transplanting to control weeds. Insecticides Imidacloprid (Confidor®) at the rate of 250 mL per hectare and Fipronil (Regent®) at the rate of 250 mL per hectare were applied at 2nd, 5th and 9th week after transplanting. Plant height and tillering ability of both Clearfield® rice and weedy rice variants were observed at 1st, 4th and 10th week after transplanting. For each replication, ten sample plants for each weedy rice variants and five sample plants for every Clearfield® rice varieties were randomly taken. Plant height was measured from ground level to the highest point of the leaves using measuring tape, and the tillers were counted manually. The agronomic traits were classified according to the **IRRI standards (2002)**. Seeds of Clearfield® rice varieties and weedy rice variants were collected at maturity for studying seed morphology. Ten seeds were randomly collected from each replication for morphology study. The length of the seeds was measured from the top to bottom of the seeds. The width was measured at the widest girth of the seeds. All the measurements were expressed in mm. The measurements were done using QuickPHOTO MICRO 2.3.

Statistical Analysis

Collected data were analyzed following ANOVA technique with SAS 9.1 software. The significant differences among means were compared with least significant difference (LSD) test, and significant difference was considered at $p \leq 0.05$. PROC CORR was used to find correlation between height and tillering ability. PROC SORT was used to differentiate between the weedy rice variants and Clearfield® rice varieties.

RESULTS AND DISCUSSION

Categorization of Weedy Rice Seeds

Seed characteristics of different weedy rice variants have been presented in **Table 1**. In this study, four variants of weedy rice were collected; of which, three variants (V1, V2 and V3) had the compact type panicle and one variant (V4) had open type panicle. Compact type panicles are close together while open type panicles are open. Among the weedy rice variants, only V3 was awn type, while others were awnless. Awn type rice seeds have awns at the base of the seeds. Thus, those weedy rice variants were labeled as Long grain close panicle (V1), Round grain close panicle (V2), Awn grain close panicle (V3) and Open panicle (V4). For shattering characteristics of the seeds, V1, V2 and V4 were easy to shatter compared to V3. Seeds that have high thre shability tend to become a problem because seeds can easily fall to the ground and become dormant in the soil and germinate in the next season. In terms of spikelet fertility, V2 was highly sterile while V1 was fertile. Both V3 and V4 were considered partly sterile. Low fertility is still a problem because even though only a few seeds are fertile, the seed can remain dormant and germinate when the condition is congenial. Rice seeds have different levels of dormancy and can remain in that state even for a couple of years **(Burgos *et al*, 2011)**. Therefore, weedy rice infestations are very hard to remove once it has settled in.

Seed Length and Width of Clearfield® Rice and Weedy Rice Variants

Table 2 shows seed length, width and length/width ratio of Clearfield® rice and weedy rice variants. Result shows that Clearfield® rice seeds were significantly longer than weedy rice seeds. Seed length of CL1 and CL2 were statistically similar. Among the weedy rice variants, V1, V3 and V4 produced seeds of statistically similar length, while V2 produced seeds shorter than those of others. A significant variation with respect to seed width among Clearfield® rice and weedy rice variants was observed **(Table 2)**. Weedy rice variants V2 and V4 produced seeds of highest width, on the other hand seed width of both the Clearfield® rice varieties were the lowest and statistically similar. As shown in **Table 2**, the standard deviations for length and width for Clearfield® rice varieties were much smaller compared to those of weedy rice variants. The larger standard deviations for weedy rice variants indicate a higher variation among the population. For the seed length to width ratio, all Clearfield® rice varieties showed significantly higher values compared to weedy rice variants. Weedy rice variants V1 and V3 showed significantly higher width/length ratio compared to V2 and V4. Among the weedy rice variants, seeds of only V2 had the width/length ratio of below 3.0. According to IRRI classifications (2002), rice seeds that have ratio of width and length above 3.0 are considered slender, and most cultivated rice are included in this category. Seeds of different weedy rice variants and Clearfield® rice varieties have been shown in **Figure 1**.

Comparison of Vegetative Growth Between Clearfield® Rice and Weedy Rice Variants

Plant height and tillering ability of Clearfield® rice and weedy rice variants at different growth stages have been shown in **Table 3**. Results show that plant height differed significantly among the Clearfield® rice and weedy rice variants at all the sampling dates. Plant height of Clearfield® rice was significantly lower than that of weedy rice irrespective of growth stages. All the weedy rice variants produced statistically similar plant height at all the sampling dates. Plant height of weedy rice variants ranged between 26.65 and 29.05 cm, 80.16 and 81.06 cm, and 139.68 and 143.11 cm respectively at 7, 28 and 70 DAT. At 7 and 70 DAT, both the Clearfield® rice varieties attained statistically similar plant height. At 28 DAT, on the other hand, plant height of CL1 was > 6 cm higher than that of CL2 (Table 3). Therefore, weedy rice can be easily identified from Clearfield® rice fields as it was taller compared to weedy rice throughout the life cycle. From the IRRI standard classification on rice agronomic traits, plant height for weedy rice was intermediate and for the Clearfield® rice was semi dwarf. **Anwar *et al.* (2010)** revealed that plant height is one of the important characteristics closely associated with rice-weed competition. The taller rice has advantages in term of light competition compared to the short rice **(Kwon *et al*, 1992)**. **Ahmed *et al.* (2012)** also observed that most of the commercial rice varieties are semi dwarf while weedy rice variants are usually taller. Thus, weedy rice gets advantages in terms of light competition compared to the commercial rice varieties of shorter stature **(Kwon *et al*, 1992)**.

Results show that tillering ability differed significantly among the Clearfield® rice and weedy rice variants at all the sampling dates **(Table 3)**. At the early growth stage (7 DAT), Clearfield® rice variety CL1 showed the maximum tillering ability among all the Clearfield® rice and weedy rice variants closely followed by CL2. No significant variation in terms of tillering abilty was recorded among weedy rice variants at 7 DAT. At mid growth stage (28 DAT), weedy rice variant V2 produced maximum number of tillers of >32 statistically followed by V1(>29) and V4 (>25). While, Clearfield® rice variety CL1 showed the poorest tillering ability (19.10) at 28 DAT. As observed at the late growth stage (70 DAT), weedy rice out competed Clearfield® rice in terms of tillering ability. All the weedy rice variants showed statistically tillering ability which ranged between 31.22 and 34.74. Similarly, both the Clearfield® rice varieties produced statistically similar number of tillers at 70 DAT **(Table 3)**. Tillering ability of rice is an important characteristic that determines the competitive ability. Higher tillering ability results in a higher survival of plant in the field as more space is colonized **(Rathore *et al*, 2013)**. Weedy rice with high tillering ability can colonizes space more efficiently, uptake more nutrients and water from soil and ultimately out-competes cultivated rice with low tillering ability **(Sánchez-Olguín *et al*, 2007)**. As revealed by **(Burgos *et al.* (2006)**, compared to commercial rice, weedy rice is known to have higher nitrogen uptake efficiency which results in higher biomass production.

Correlation Between Height and Number of Tillers

The correlation between plant height and number of tillers/hill of different weedy rice variants and Clearfield® rice varieties have been presented in **Table 4**. Correlation study shows a positive correlation between plant height and number of tillers/hill for all the weedy rice variants and Clearfield® rice varieties. However, a higher correlation was observed for weedy rice variants compared to Clearfield® rice varieties. The positive correlation between number plant height and tillering ability indicates that both the traits were exhibited simultaneously throughout the growing period. In short, taller plant produces more tillers compared to shorter plants.

CONCLUSIONS

Weedy rice has more variation compared to Clearfield® rice in terms of seed size, plant stature and tillering ability. Seeds of weedy rice are easy to shatters and have low spikelet fertility. Weedy rice seeds are usually shorter but wider compared to Clearfield® rice seeds. Except for one weedy rice variant (V2), all other Clearfield® rice and weedy rice seeds were found slender in terms of length width ratio. Weedy rice is much taller and has more tillering ability as compared with Clearfield® rice. All these characteristic of weedy rice offer major advantages against commercial rice and thus, weedy rice is more competitive than cultivated Clearfield® rice when grown in the same area and given the same conditions. Therefore, weedy rice can be easily distinguished from cultivated Clearfield® rice based on their plant stature, and removal of weedy rice from the rice field during the early growth stages can be done to avoid competition and yield losses. Hand plucking of the weedy rice panicles before maturity can be very effective at the late growth stages to prevent weed seed rain and enrichment of soil seed bank **(Anwar *et al.*, 2013)**.

ACKNOWLEDGEMENT

I would like to commemorate Lembaga Kemajuan Perusahaan Pertanian (LKPP) Padi Sdn Bhd for their cooperation in giving manpower and resources to make this research a success and Universiti Putra Malaysia for granting RUGS for the funding of the project.

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Table 1: Classification of Weedy Rice Based on IRRI Rice Evaluation System (2002)

Variants	Panicle	Awn	Awn color	Panicle threshability	Spikelet fertility
V1	Compact	Awnless	Awnless	Easy	Fertile
V2	Compact	Awnless	Awnless	Easy	Highly sterile
V3	Compact	Awn	Straw	Loose	Partly sterile
V4	Open	Awnless	Awnless	Easy	Partly sterile

Table 2: Seed Characteristics of Different Clearfield® Rice and Weed Rice Variants

Rice	Variants	Length(L) (mm)	Width(W) (mm)	Ratio (L/W)
Clearfield® rice	CL1	10.3320±0.450 a	2.41050±0.195 bc	4.3109±0.372 a
	CL2	10.1690±0.432 a	2.36400±0.169 c	4.3219±0.352 a
Weedy rice	V1	8.6080±0.587 b	2.52500±0.224 b	3.4339±0.382 b
	V2	7.7555±0.332 c	2.69400±0.271 a	2.9125±0.382 d
	V3	8.3975±0.783 b	2.5300±0.238 b	3.4367±0.434 b
	V4	8.6084±0.567 b	2.72211±0.225 a	3.1600±0.279 c
LSD		0.34	0.14	0.23

LSD= least significant difference; Within a column, means sharing same alphabets are not significantly different at P=0.05 probability level according to LSD test

Table 3: Plant height and tillering ability of Clearfield® rice and weedy rice variants at different growth stages

Varieties/Variant		Plant height (cm)				Tillering ability (no./hill)		
Clearfield® rice		7 DAT	28 DAT	70 DAT		7 DAT	28 DAT	70 DAT
CL1		18.89 b	64.90 b	94.50 b		5.35 a	19.10 cd	17.70 b
CL2		16.05 b	57.10 c	91.45 b		4.30 b	12.80 d	13.25 b
Weedy rice								
V1		29.05 a	80.16 a	142.68 a		3.40 bc	29.16 ab	34.74 a
V2		26.65 a	80.95 a	141.47 a		3.40 bc	32.70 a	33.63 a
V3		26.76 a	80.69 a	143.11 a		2.85 c	21.72 bc	31.22 a
V4		28.55 a	81.06 a	139.68 a		2.60 c	25.33 abc	32.63 a
LSD		2.85	6.61	5.36		0.93	7.63	6.29

LSD= least significant difference; Within a column, means sharing same alphabets are not significantly different at P=0.05 probability level according to LSD test

Table 4: Correlation between plant height and tillering ability of different Clearfield® rice and weed rice variants

Rice variants	Pearson Correlation Coefficient
CL1	0.51566
CL2	0.79193
V1	0.86072
V2	0.84174
V3	0.83995
V4	0.85729

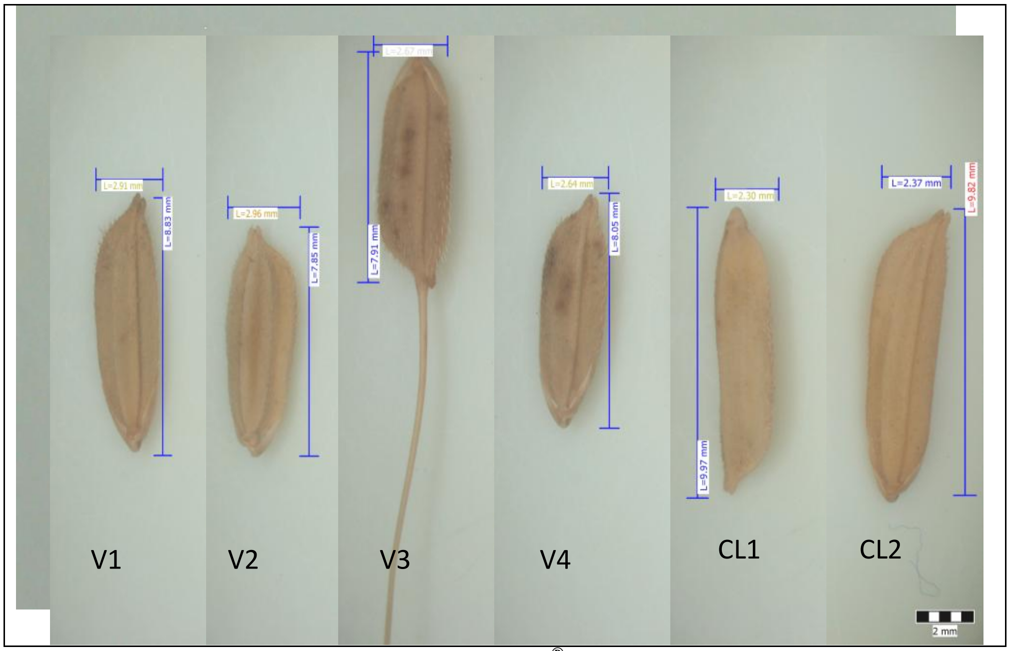

Figure 1: Seeds of different Clearfield® rice and weedy rice variants