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A COMPARATIVE STUDY OF VEGETATIVE AND REPRODUCTIVE GROWTH OF LOCAL WEEDY AND CLEARFIELD® RICE VARIETIES IN MALAYSIA

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ABSTRACT

The movement from transplanting to direct seeding has brought weedy rice problems in Malaysia's rice granary areas. Weedy rice (*Oryza* spp.) is very difficult to control compared to other weeds due its close genetic relationship to the cultivated rice (*Oryza sativa* L.), therefore it cannot be controlled with conventional rice herbicides. Recently, a new technology for controlling weedy rice in rice fields which is known as Clearfield® Production System has been introduced by discovering the combination of herbicide imidazolinone and resistant trait containing variety. Two cultivars have been released by Malaysia known as MR 220CL1 and MR 220CL2 which were derived from crosses between CL1770 from Louisiana State University with a Malaysian local rice variety, MR 220. The objective of the study is to understand the growth patterns (vegetative and reproductive) of four different weedy rice morphotypes and two variants of Clearfield® rice in Malaysia. Weedy morphotypes were observed being significantly taller in all growth stages compared to Clearfield®. Tillering abilities of weedy morphotypes were not different from Clearfield® variants except for WR4 at 60 days after seeding (DAS). Flowering and maturity periods observed in weedy morphotypes were ranged widely where all weedy morphotypes flowered 10 to 20 days later than the Clearfield® rice varieties. Understanding all these morphological and physiological characteristics of weedy rice is useful to improve the weedy rice management and good agricultural practices for better control of escaped weedy rice in the Clearfield® planting areas.

Key words: *Oryza sativa* L., *Oryza* spp., Imidazolinone, MR 220CL1, MR 220CL2

INTRODUCTION

Rice is one of the major food source for more than half of the population in the world (Gealy et al., 2003) and it is the third most important crop in Malaysia (Karim et al., 2004) covering an area of about 402,800 ha. in Peninsular Malaysia while in Sabah and Sarawak is about 200,000 ha. (Ahmed et al., 2012). Currently, the national rice self sufficiency level (SSL) in Malaysia is only at about 70% (Sharif, 2013) which is not parallel with the increasing of country's population. The country requires about 1.85 million t/ha (tons per hectares) of rice a year in order to satisfy domestic requests but our country can only produce rice yield about 1.2 million tons (Hamid, 2008). Due to this, increasing rice production will keep on being a main focus in agriculture sector for Malaysia.

In rice cultures, many constraints are experienced by the farmers in obtaining high yields. Weeds have become the major yield - limiting constraint in rice production after the shift in methods of rice culture from transplanted rice to direct seeded rice (Olofsdotter et al., 2000). In Malaysia, weedy rice (*Oryza* spp.) had been appearing as a serious weed in recent years resulting in harvesting problem, loss of yield and quality by competing with the crop for water, light, nutrients, carbon dioxide and space (Hamid, 2008). Weedy rice seeds also can contaminate the harvested grain. Karim et al. (2004), reported about 10 – 35 % reduction in grain yield is caused by the competition of weeds with rice. In Peninsular Malaysia, loss of yield due to weedy rice infestation is estimated about RM 90 million per season in 2004 (Azmi, 2013).

Weedy rice generally includes species of genus *Oryza* which grows naturally and vigorously in and around rice fields (Suh et al., 1997). Therefore, it is very hard to control weedy rice in cultivated rice area since it is classified in the same genus and species as cultivated rice (Choudhary et al., 2011) and shares a lot of similarities in morphological, biochemical and physiological characteristics (Shivrain et al., 2007). However, there are several morphological and physiological differences between weedy rice and cultivated rice such as different in sizes, heights, leaf color, tillering capacity, panicle appearance, red pericarp, flowering, seed dormancy, longevity and shattering capacity (Kanwar et al., 2013; del Mar CatalaForner, 1995)

The introduction of herbicide-resistant rice offers farmers a good opportunity to manage weedy rice and other weeds and it is one of the latest technology used to control weedy rice problem in Malaysia (Azmi et al., 2012a). 'Liberty Link' rice was the first transgenic rice developed. However, due to concerns in the international market on genetically modified rice, it was not commercialized. Clearfield® rice, which was intentionally mutated to tolerate imidazolinone (IMI) herbicides without the insertion of any foreign gene was developed by Louisiana State University (LSU) Agricultural Center breeders and it was commercialized in 2002 (Shivrain et al., 2007). Imidazolinone herbicides control weeds by inhibiting the plant specific enzyme acetohydroxyacid synthase (AHAS). Enzyme AHAS involves in the biosynthesis pathway of the branched – chain amino acids; valine, leucine and isoleucine. This inhibition causes a disruption of protein synthesis, which constraints synthesis of DNA and growth of cell (Chin et al., 2007).

In Malaysia, development of local rice varieties tolerant to IMI started in 2003. Malaysian local rice variety MR 220 was backcrossed with donor Clearfield Rice Line IMI-TR No. 1770 introduced from LSU to get two inherited potential lines (B55 and B64A) that were tolerant to IMI. The new tolerant lines were released as MR 220CL1 and MR 220CL2 have been launched in 2010 at FELCRA Seberang Perak (Azmi et al., 2012b). Analysis of DNA between MR 220 with MR 220CL1 and MR 220CL2 showed 98.5% and 92.5% of genetic similarity respectively (Sudianto et al., 2013). The combination of certified IMI tolerant seeds (Clearfield®) with IMI herbicide (OnDuty®) and stewardship guidelines are known as Clearfield® Production System (CPS) (BASF, 2010). CPS can control the weedy rice with a single imizapic / imizapyr application at 214 g ha^{-1} applied at 0-7 days after sowing (Azmi et al., 2010). Clearfield® cultivars provide effective control of weedy rice and other noxious paddy weeds. The cultivars will help to reduce the cost of weed management in rice cultivation, yield a higher quality of rice. In addition, the use of the cultivars can reduce the amount of herbicides released into the environment and the ecosystem since imidazolinone herbicides are applied in much lesser volumes (Azmi, 2013). Satisfying outcome from fields planted with Clearfield® rice has been reported by BASF Malaysia which is double from 3.5 to 7 metric t/ha (Sudianto et al., 2013).

However, there are also concerns about the impact of releasing herbicide resistant rice in field for a long period of time. One of the concern is the possibility of transferring the resistant gene to compatible weedy *Oryza* species (Olofsdotter et al., 2000) which is likely to take place as the incidence of natural hybridization. This could produce herbicide resistant weedy rice and leads to the problem of controlling the weedy rice using herbicide. The risk of outcrossing between Clearfield®

and weedy rice in Asia is expected to be multiple times higher since rice monoculture is practiced broadly with 2-3 rice plantings per year compared to North America or other regions which apply diversified cropping systems (Sudianto et al., 2013). Therefore, it is very difficult and challenging to maintain the higher yields of Clearfield® rice without considering the evolution possibility of resistant weedy rice in the Clearfield® rice fields. Results from the recent study by Busconi et al. (2012) clearly show that Clearfield® herbicide resistant weedy rice plants were present in the field after 5 years of Clearfield® rice cultivation in Italy. Same findings on the occurrence of IMI – resistant weedy rice outcrosses were also reported on previous study by Shivrain et al. (2009) in Arkansas, U.S.A., Villa et al. (2006) in Brazil and Zhang et al. (2006) in Louisiana, U.S.A. The average of natural outcrossing rate in Brazil rice fields is 0.065% while the rate in the U.S.A. rice fields ranges from 0% to 1.26% (Sudianto et al., 2013).

The purpose of this study was to increase understanding on growth characteristics of different morphotypes of local weedy rice in comparison to Clearfield® rice varieties. It is very important to understand the vegetative and reproductive growth trend of weedy rice thus the escaped weedy rice can be controlled effectively as they are difficult to recognize in the Clearfield® rice field. Most importantly is to identify if there is any potential of hybridization between Clearfield® rice varieties with local weedy rice in the future.

MATERIALS AND METHODS

The growth development of four local weedy morphotypes and two varieties of Clearfield® rice were conducted in the glass house. Weedy rice morphotype samples were collected by random sampling from rice field area of Tanjung Karang and Kuala Rompin, Malaysia with no known history of planting Clearfield® rice and were classified by plant morphological characteristics (Table 1) according to the Standard Evaluation System for Rice (IRRI, 2002).

Seeds of Clearfield® rice varieties, MR 220CL1 and MR 220CL2 were obtained from the Malaysian Agricultural Research and Development Institute (MARDI) Seberang Perai. Each weedy morphotype was termed as WR for easy reference in the future while Clearfield® varieties were termed as CL.

Table 1: Description of weedy rice morphotypes found in Selangor and Pahang rice granaries and Clearfield® rice varieties. Numbers in parenthesis is corresponding to IRRI (2002) morphological rice descriptors.

Morphotype / Variety	Grain shape (length-width ratio)	Plant height	Panicle type	Awn	Lemma and palea colour
CL1	Slender (1)	Semidwarf	Intermediate	Awnless (0)	Straw (0)
CL2	Slender (1)	Semidwarf	Intermediate	Awnless (0)	Straw (0)
WR01	Slender (1)	Tall (9)	Compact (1)	Straw (1)	Straw (0)
WR02	Slender (1)	Tall (9)	Compact (1)	Awnless (0)	Straw (0)
WR03	Slender (1)	Tall (9)	Open (9)	Awnless (0)	Brown tawny (4)
WR04	Medium (3)	Tall (9)	Compact (1)	Awnless (0)	Brown tawny (4)

In the laboratory, the seeds of CL variants and WR morphotypes were soaked in water for 24 hours in order to produce pre-germinated seeds. The seeds then were sown in seedling trays with a one to one ratio of rice husk and top soil as a media and allowed to grow for 14 days. Germination rate was calculated for each morphotypes of weedy rice and Clearfield® varieties. Plastic pots (0.30 m deep, 0.27 m diameter, without bottom holes) were prepared by pouring 8.0 kg well – puddle clay soil of Lating series per pot. The soil was obtained from Kuala Rompin rice field. Three seedlings from rice's WR morphotype and CL rice variants were transplanted in the prepared pots and were arranged in randomized complete block design (RCBD) with three replications. Each replication has six treatments consisting of 2 CL rice variants and 4 morphotypes of WR and each treatment has three subsamples. NPK green (15:15:15) fertilizer was applied at 14 days after seeding (DAS), 35 DAS and 65 DAS at a rate of 200 kg ha^{-1} to supply elements required for plant growth and productiveness. Each individual plant of WR and CL rice were kept flooded at about 3 cm of water depth daily. Crop management was done according to the standard cultivation guidelines.

Growth and development of WR and CL rice plants were observed and examined closely from the stage of seedling to maturity. Two vegetative descriptors (plant height and numbers of tiller) were evaluated and recorded at 20, 30, 40, 50, 60 and 70 days after seeding (DAS). Plant height was measured based on the length from the soil surface to the top of the upper most leaf. In addition, five reproductive descriptors (booting stage, beginning of anthesis, 50% anthesis, maturity stage and anthesis duration) for *Oryza sativa* were also evaluated and recorded following the Standard Evaluation System for Rice (IRRI, 2002). The seeds then were harvested at maturity. Twenty seeds were randomly selected from each treatment of each replication and were digitally photographed. The grain length and width were measured in millimeters using the program QuickPHOTO MICRO 2.3 and the data were recorded. Grain shape was also determined from dividing the average length of the grain by the average width.

Data were subjected to Analysis of variance (ANOVA) and mean comparison between each morphotypic group was conducted by Least Significant Difference (LSD) test using computer program SAS version 9.2 (SAS, 2001). The significance differences were considered when $p \leq 0.05$.

RESULTS AND DISCUSSION

Vegetative Phase

The results obtained in this study showed a great diversification of growth and development between weedy morphotypes and Clearfield® varieties in both vegetative and reproductive phase. Regarding plant height, all WR morphotypes were significantly taller ($P \leq 0.05$) than CL varieties for the entire vegetative phase (Fig. 1). All WR morphotypes were 17-26%, 16-20%, 16-23%, 13-23%, 21-26% and 35-40% taller than the CL varieties at 20, 30, 40, 50, 60 and 70 DAS respectively. WR morphotypes showed a higher plant heights ranging from 134.2 ± 2.5 cm to 138.7 ± 8.3 cm at 70 DAS while plant heights for CL varieties were 98.2 ± 2.1 cm and 103.6 ± 1.3 cm at 70 DAS.

Similar results were reported by Noldin et al. (1999), ElenaR et al. (2007), Choudhary et al. (2011) and Ahmed et al. (2012) where all WR morphotypes were significantly taller than commercial rice varieties. At 70 DAS, CL2 variant was significantly the shortest plant whereas WR1 was significantly the tallest plant (35.21% taller than CL2). All WR morphotypes WR1, WR2, WR3 and WR4 showed no significant differences in height among them as well as CL varieties, CL1 and CL2 also showed no significant differences in height among the varieties at 70 DAS.

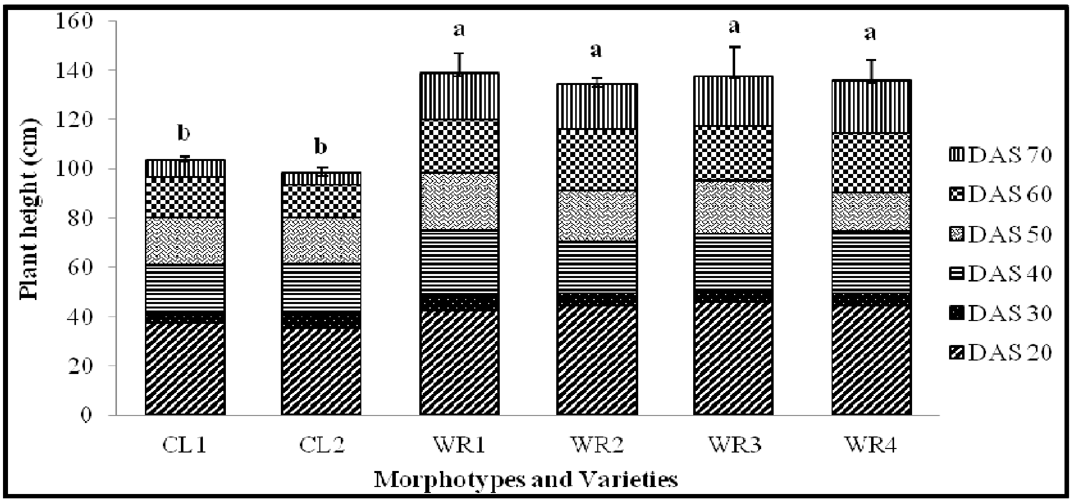

Fig. 1. Cumulative plant height of weedy rice morphotypes and Clearfield® rice varieties at 20, 30, 40, 50, 60 and 70 days after seeding (DAS). Bars showed standard deviation at 70 DAS. Means with the same letter represent values that do not differ significantly at the 5% level of probability using LSD test.

Tall characteristic of WR makes them compete more efficiently for light, nutrients and space in the rice fields compared to commercial rice varieties (Kwon et al., 1992). In this case, taller WR plants are capable of getting more sunlight and shade their adjacent rice plants and therefore it is known to reduce total rice yield more than the shorter weed plants (Chauhan and Johnson, 2010; IRRI et al., 2010). However, taller WR plants can be effectively identified and controlled during the post emergence period due to height differences between WR and commercial cultivated rice varieties (Fogliatto et al., 2011; Ahmed et al., 2012). Therefore, it is suggested to control the WR early before it becomes taller than cultivated rice in order to prevent high losses in grain yields. According to Ahmed et al. (2012), WR that has grown taller than cultivated rice can be controlled by applying methods such as manual roguing or treated with foliar systemic herbicides. Additionally, the height differences between WR and commercial cultivated rice varieties are probably because of heterosis pressure which exists in some of the WR morphotypes (Zainuddin et al., 2010).

There was a higher variation of plant height in WR compared to CL rice. The standard deviation values of plant height indicated that a high variation existed within WR morphotypes (2.5 to 11.9 at 70 DAS) those values for CL varieties were more consistent (1.3 and 2.1 at 70 DAS). This heterogeneous characteristic of weedy rice makes them difficult to be fully identified in the rice fields as some of these WR morphotypes might possess the same plant height of cultivated varieties.

Tillering is a very important agronomic trait that contributed in improving of yields significantly**.** The number of tillers produced is depend on both genetic and environmental factors. Chauhan and Johnson (2010) reported that cultivated rice with high tillering capacity could compete with WR effectively even though they are semidwarf in stature. In this study, the result showed there was no significant difference in number of tillers between both CL and WR varieties (WR1,WR2 and WR3 except for WR4) at 60 DAS ($p \leq 0.05$) (Fig. 2). Number of tillers for WR morphotypes and CL varieties used in this study reached their maximum at 60 DAS and then started to decrease at 70 DAS. Decreases in number of tillers were probably due to death of nonbearing tillers because of intraspecific competition among themselves in getting nutrients and sunlight (Kwon et al., 1992).

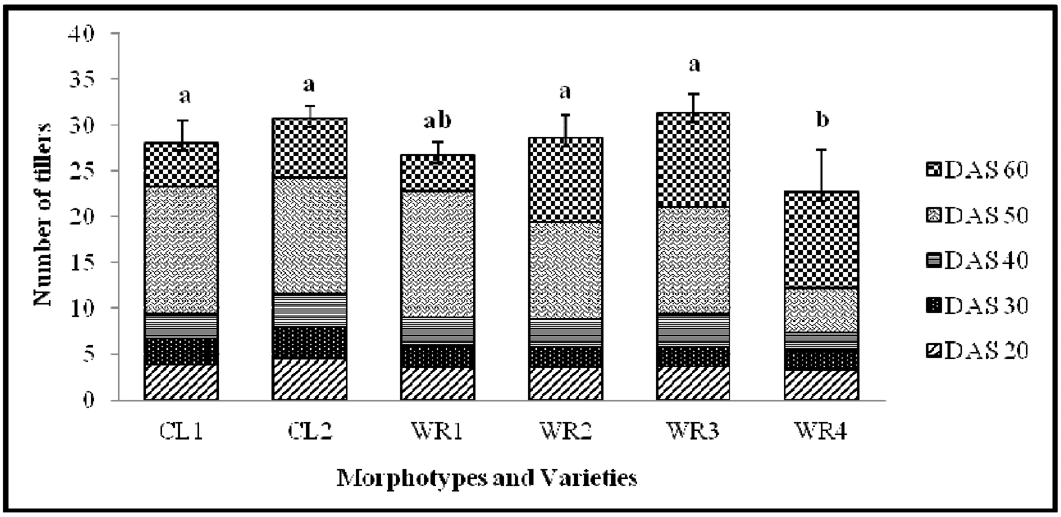

Fig. 2. Cumulative number of tillers produced by weedy rice morphotypes and Clearfield® rice varieties at 20, 30, 40, 50 and 60 days after seeding (DAS). Bars showed standard deviation at 60 DAS. Means with the same letter represent values that do not differ significantly at the 5% level of probability using LSD test.

There was no significant difference in number of tillers between CL1 and CL2 varieties were observed at all growth stages ($p \leq 0.05$). Among WR morphotypes, no significant difference in number of tillers were observed at initial vegetative stage (20 to 40 DAS). However, at later stage of 50 DAS and 60 DAS, there was a significant difference in number of tillers were observed among WR morphotypes. WR4 significantly produced minimum number of tiller (22.67 ± 4.6) whereas WR3 significantly produced highest number of tiller (31.33 ± 2.0). This proves that weedy morphotypes tend to have a vigorous growth only at initial growth stage but fails to produce more productive tillers at later growth stage.

Reproductive Phase

The reproductive phases of WR morphotypes and CL varieties were separated into booting, anthesis and maturity stage. WR morphotypes showed a wide range of flowering period where all flowered about 10 to 20 days later than CL rice varieties. This could reduce the opportunity for gene flow to occur to become low since the flowering periods are not synchronized. WR morphotypes reaching booting stage between 86.0 ± 2.4 and 95.4 ± 5.3 DAS whereas CL rice varieties reached this stage at 76.2 ± 2.7 and 78.7 ± 2.8 DAS (Table 2). Anthesis started within 3-4 days in CL rice varieties whereas that was started from 5 up to 7 days in WR morphotypes. CL varieties reached maturity at 101.4 ± 3.1 and 106.3 ± 3.2 DAS in contrast with WR morphotypes that reached this stage later, which are between 116.3 ± 2.9 and 126.0 ± 5.4 DAS. Early maturity of CL rice varieties will act as identification method during harvesting period.

All WR morphotypes required more time to complete their anthesis period (23.2 to 26.4 days) compared to CL rice varieties (21.4 and 22.9 days). High standard deviation values recorded in WR morphotypes at each reproductive growth stage presented more heterogeneous flowering and maturation than among CL rice varieties.

The uneven flowering and maturation observed in all weedy morphotypes act as a competitive strategy to overcome unfavorable weather circumstances. Similar findings were also observed by Ahmed et al., (2012). This characteristic ensures the progeny to have a better performance in the rice fields and can continuously produce seeds from different morphotypes (ElenaR et al., 2007).

Table 2. Days after seeding (DAS) for different growth stages in Clearfield® rice varieties and local weedy rice morphotypes (Means ± standard deviations are shown)

Morphotypes/ Varieties	Booting stage	Beginning of anthesis	50% anthesis	Maturity stage	Anthesis duration
CL1	78.7 ± 2.8	83.4 ± 2.5	89.5 ± 2.0	106.3 ± 3.2	22.9
CL2	76.2 ± 2.7	80.0 ± 2.3	84.6 ± 1.2	101.4 ± 3.1	21.4
WR01	86.0 ± 2.4	93.1 ± 2.3	98.2 ± 2.3	116.3 ± 2.9	23.2
WR02	90.3 ± 4.7	95.7 ± 3.4	103.3 ± 4.2	121.5 ± 4.2	25.8
WR03	95.4 ± 5.3	100.9 ± 5.1	106.8 ± 2.3	126.0 ± 5.4	25.1
WR04	91.6 ± 3.1	97.1 ± 3.4	104.1 ± 3.4	123.5 ± 3.1	26.4

Grain length, width and shape

The results obtained in this study showed significant difference of grain length between CL varieties and WR morphotypes at $p \leq 0.05$ (Fig. 3). No significant differences of grain length among CL varieties were observed but there was a significant difference among WR morphotypes ($p \leq 0.05$). CL1 was significantly the longest grain (10.34 ± 0.1 mm) and WR4 was significantly the shortest one (7.75 ± 0.2 mm).

In the case of grain width, there was no significant difference observed between CL varieties with WR1 and WR2 but there was a significant difference of the parameter between CL varieties with WR3 and WR4 ($p \leq 0.05$) (Fig. 4). WR1 was significantly the narrowest grain (2.36 ± 0.03 mm) and WR4 was significantly the widest one (2.78 ± 0.06 mm). For grain shape (length/width ratio) (Fig. 5), both CL1 and CL2 have a ratio of 4.35 and 4.25 respectively while WR morphotypes have a ratio from 2.79 to 3.36.

The length and width of a rice grain are important morphology that determines the class of the rice. The ratio of the length and the width is used internationally to define the shape of the variety. According to IRRI (2002), there are four classes of the rice based on grain length: short (5.5 mm or less), medium (5.51 to 6.6 mm), long (6.6 to 7.5 mm) and extra long (more than 7.5 mm). In this study, all CL varieties and WR morphotypes were classified as extra long grain.

For grain shape, there are four shapes of the rice based on IRRI (2002): slender (over 3.0 mm), medium (2.1 to 3.0 mm), bold (1.1 to 2.0 mm) and round (less than 1.1 mm). In this study, all CL varieties and WR morphotypes except for WR4 were observed as having a slender in shape while WR4 was observed as having a medium in shape.

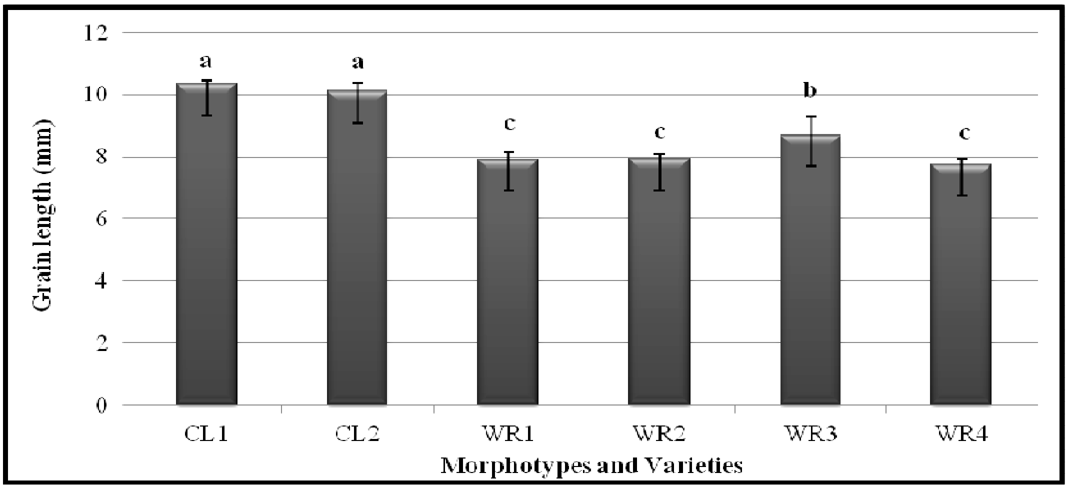

Fig. 3. Means of grain length of weedy rice morphotypes and Clearfield® rice varieties. Means with the same letter represent values that do not differ significantly at the 5% level of probability using LSD test

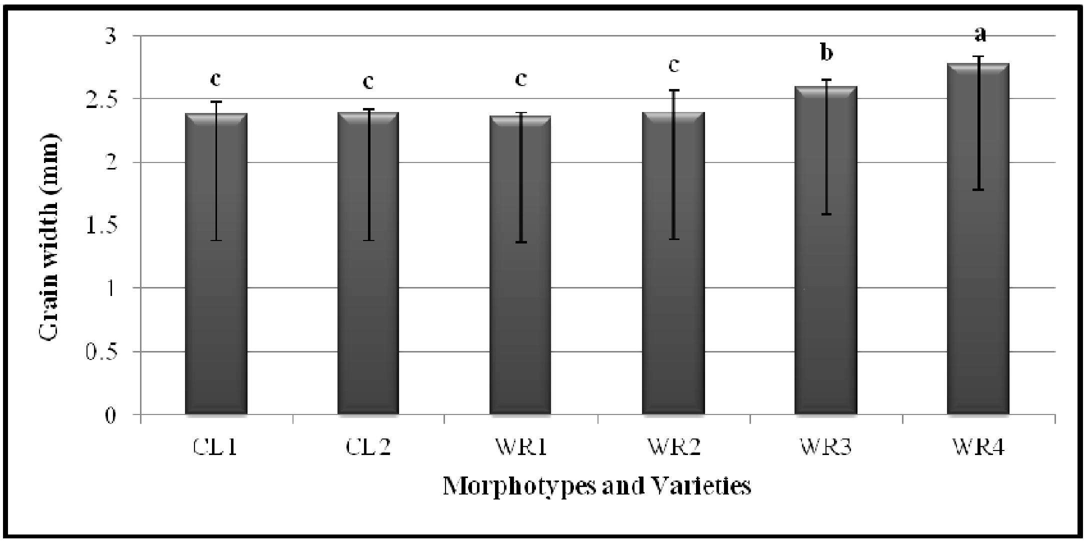

Fig. 4. Means of grain width of weedy rice morphotypes and Clearfield® rice varieties. Means with the same letter represent values that do not differ significantly at the 5% level of probability using LSD test

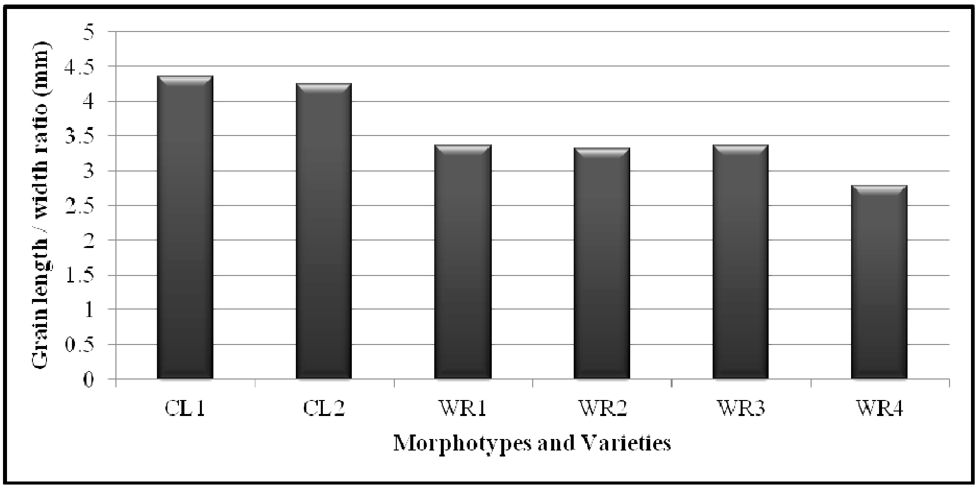

Fig. 5. Grain length / width ratio of weedy rice morphotypes and Clearfield® rice varieties.

CONCLUSION

From this study, all weedy morphotypes were observed being significantly taller than Clearfield® varieties in all growth stages. However, no significant differences in number of tillers were observed between Clearfield® variety and all weedy morphotypes except for WR4 at 60 DAS. All weedy morphotypes flowered 10 to 20 days later than the Clearfield® varieties. The results obtained from this study provide useful information to develop suitable weed management practices to control escaped weedy rice in the Clearfield® rice fields. Besides that, the information obtained on flowering periods will be useful in designing the gene flow studies among them in the future since the possibilities of outcrossing between Clearfield® rice and weedy rice in Asia are expected to be multiple times higher than other regions. More studies on vegetative and reproductive growth development urgently need to be carried out on other morphotypes of weedy rice to clearly understand and make a good comparison among all the weedy morphotypes existing in rice fields.

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