CHAPTER 6

GENERAL DISCUSSION

6.1 General - "They Stand Among Equals".

The present series of studies on new biotypes of weedy rice (NBWR) were conducted to answer several pertinent ecological questions arising from the prevalence of NBWR in the Selangor North-West Project (PBLS) granary, Malaysia. Previously, weedy rices in Malaysian rice granaries displayed dominantly taller biotypes. The advent of new biotypes with similar morphological traits, principally with almost similar plant height *vis-à-vis* the commercial rices warrant detailed studies on the ecology of the former. Primarily, the study focuses on the extent of prevalence of NBWR in the granary, and the morphological variations, if any, among the new biotypes.

This strong display of crop mimicry by NBWR to any cultivated rice in Malaysia such as MR84, MR219, MR220 or MR235 has led Baki, B.B. (*pers comms.*) to label NBWRs as **"They stand among equals"**. If former biotypes of weedy rice is taller than cultivated rice and very easy to spot and identify but it is almost practically impossible to differentiate NBWR and any other cultivated rice. It needs details and a lot of scrupulous to be able to differentiate NBWR and their commercial counterparts in the field.

The intrigues on how these NBWR accessions spread and dispersed in the granary, and the patterns of spatio-temporal distribution can be explained via dispersion analyses of the collated data generated through seasonal surveys. Spatial attributes such as Lloyd's mean crowding, Lloyd's mean index of patchiness, variance-to-mean ratio, patchiness value index and quantitative indices of different accessions helps to denote the locality- and time-mediated differences on patterns of distribution in the granary. From this information, some theoretical considerations could be used to elucidate the distribution patterns of different accessions found in the granary. Characterization of the biotypes based on descriptive analyses of the NBWR accessions helps to delineate the major accessions prevailing in the granary.

In order to assess the competitive ability of dominant NBWR accessions *vis-à-vis* their commercial counterparts, growth patterns of both MR220 and an accession of NBWR were studied. Statistical treatments such as path analysis helped to delineate the principal and important yield components and clonal parameters and attributes that explain direct and indirect effects of competition when MR220 and an accession of NBWR, either in monoculture or mixtures.

6.2 Weedy and Wild Rices

Baker (1965) described a number of general characteristics that can increase the propensity of a plant to become a weed. It does include adaptation to different habitats, out-crossing versus selfing ability, dormancy and persistent seed bank, efficient seed dispersal and vegetative regeneration. This description fits well with those traits in weedy rice. Weedy rice (*Oryza sativa* L.) is one of serious weed in rice agriculture. The term weedy rice generally includes all the species of genus *Oryza* which behaves and mimics like the rice crop but have the ability to disseminate their grains before rice harvesting which can reduce the production of rice in the paddy field. Weedy rice populations have been reported in many paddy areas in the world where the crop is directly seeded (Parker & Dean 1976).

Weedy rice (*Oryza sativa* complex) in Malaysia was first observed at Sekinchan, Selangor in 1987 (Azmi *et al.* 2005a, Wahab & Suhaimi 1991). It is one of the most serious threats to the rice industry and weedy rice infestations in paddy fields are deleterious to rice production in Malaysia. Nowadays it has spread throughout Peninsular Malaysia in majority paddy field regions (Azmi *et al.* 2005a; Baki 2008). The loss caused by weedy rice in Malaysia was estimated *ca.* RM137, 876,375 per year just by 5% field infestation of weedy rice (Baki 2005). Such extent of infestations lead to an economic impact in yield lost of *ca.* 64,880 tons of rice per year.

Weedy rice tends to mimic the growth attributes of the cultivars with which it grows, germinating simultaneously and growing at a similar rate, although earlier maturity is not uncommon. This mimicry is likely to be due to their origins in hybridization between those cultivars and the wild species (Abdullah, M.Z, *pers. Comms.*). The populations of weedy rice tend to be genetically diverse and highly heterogeneous (Chang 2003). They have a high seed shedding rate and buried seeds will germinate with the crop the following season. In addition, their seeds can contaminate farmers' seed stocks and be sown with the cultivar each season (Anon 2005).

In this study, new biotypes of weedy rice (NBWRs) were found in the Selangor North-West Project (PBLS) granary, Malaysia. These NBWRs displayed similar morphological characteristics as the cultivated rice especially in plant height. Morphologically it mimics with cultivated rice, namely, MR84, MR219, MR220 and MR235 as this weedy rice grows as tall as cultivated rice, thus becoming a new threat for the rice production in Malaysia. It has similar a common characteristic of easy shattering with existing weedy rice accessions, thus both has become a perennial and constant threat in rice granaries. Some of these new accessions have a red pericarp, while others display white or colourless pericarp similar to the cultivated rice.

6.3 Spatio-Temporal Distribution Pattern of New Biotypes of Weedy Rice.

The extent of infestation based on distribution studies of NBWRs seems to be in an early stage as discussed in Chapter 2. However, the trend cannot be underestimated. Taller biotype of weedy rice also was first found at Sekinchan, one of the rice farm blocks in PBLS in 1987 (Wahab and Suhaimi, 1991). After a few years, the infestation went northward to other rice granaries all over Peninsular Malaysia and becoming the worst ever weed to be controlled (Azmi & Baki 2003; Baki 2006, 2008). Such patterns of infestation and extent of spread may also occur for NBWR, if there is no serious action to prevent this ensuing infestation.

Rice farms practicing good weed management programmes such as in Sekinchan have the least infestation of NBWR. On the contrary, rice farms in Sawah Sempadan were the worst. Through random surveys with farmers, there were some farmers who practised 3T (*Tabur, Tinggal, Tuai* or literally translated to mean Sow, Leave, Harvest, *sensu* Baki, B.B. *pers. comms*). Such ill-management practices can allow any weed, including weedy rices to easily spread and infest the farms.

Basically, weediness traits in *O. sativa* have a tendency to manifest itself (or becoming weedy) in areas where wild and cultivated rice plants grow sympatrically. In these areas, wild and cultivated rice plants can hybridize, producing plants that compete with the rice cultivars, and by producing inferior seeds, thus decreasing the yield from the rice crop (Anon 2005). However, weedy rice can also develop in areas without native wild rice populations (Bres-Patry *et al.* 2001). The origins of weedy rice under these conditions are currently under investigation, but they are believed to be derived from hybridization between different cultivars, selection of weedy traits present in cultivars, relics of abandoned cultivars, or to have been brought into the growing region through contaminated seed stocks (Vaughan & Morishima 2003).

Arguably, hybridization can be the main reason for NBWR existence in Malaysian rice granaries. Hybridization between crops and their wild relatives sometimes brings genes into wild populations, occasionally resulting in the evolution of aggressive weeds and/or endangering the rare species. According to Roberts *et al.* (1961), cultivated rice, *O. sativa,* is an autogamous plant, with a low out-crossing rate of 0-1%. These are exemplified by cases where wild relatives originated in Central and South America were found to hybridizing with the rice crop, including *O. rufipogon* and *O. glumaepatula* (Oka & Chang 1961; Vaughan & Tomooka, 1999). Genes from rice varieties may transfer quickly into red rice (at 1% to 52% hybridization rate) (Langevin *et al.* 1990). However, most of the hybridization rate estimates have been done under temperate conditions.

There is a probability for hybridization between rice cultivars occurs in PBLS. Active introduction in fertilizer and herbicide in this particular area may cause the existence weedy rice re-emerge and adapted as new biotypes to survive. Manual weeding by roughing the panicle of the taller biotype of weedy rice actually was the best solution to control the weedy rice when herbicide or other chemical control method failed to control the weed. However, such actions were seen as an opportunity for weedy rice to evolve to shorter biotypes. Therefore, a further study needs to be done especially in predicting the distribution of this NBWR using climatic measurement and the possibility to assess genetic resemblance in this NBWR to find the origin and genetic characters which allow the NBWR to evolve based on the environment change and gene introgressions.

Most accessions of NBWR exist in clump or cluster in rice farms especially those accessions which have high population i.e. Acc8, Acc12, Acc3, Acc4 and Acc7. This type of distribution may occur because the control of taller biotype with certain areas may be overlooked by the farmers and leave the seed bank of hybridized weedy rice. Another argument on the prevalence of NBWR is that such accessions have existed alongside the taller biotypes, but the former were left hitherto unnoticed (Baki, B.B. *pers. comms.).*

The main sources of NBWR are, rice seeds contaminated with NBWR seeds, and NBWR seed bank in soil. Therefore, any control measure should be aimed at the reduction of infestation from these sources. As direct seeded was implemented in almost all rice farms, it may be possible for NBWR seeds to be in certain areas randomly and in cluster or single. Unnoticed NBWR plants as it as tall as any commercial rice varieties make it easy to grow and distribute in the farms. If there is no control from the beginning, the distribution pattern may be uniform and cluster in massive account throughout the farms as what had happened by former taller biotypes.

With the concern on the active hybridization of these weedy rices, it is equally important to study the population genomics and the phylogenetics of this weed, and I believed that these areas serve as a new research frontier in the future, not only in Malaysia but also in Malesia and other countries in South-East Asia. Reports of wide distribution of weedy rices in South-East Asia may lead to question whether the distribution of weedy rices may have the relationship genetically, and such intricacy remains.

Climate is an important determinant of pest species distribution and abundance and this relationship has been used by the computer simulation program, CLIMEX^{TM} (Sutherst *et al.* 1999) to estimate potential distribution and abundance of pest species. The simulation program is based on climate and uses an inferential approach to forecasting potential pest distribution and abundance. CLIMEX^{TM} has been applied to different biological entities including several insect pest species in Canada (Sutherst *et al.* 1999).

Plant species distribution basically influences by climatic factors mainly temperature and rainfall. However, in some cases, the distribution also related to edaphic factors (Gates *et al.* 1956).

It is a new area of research on weedy rices to forecast the potential distribution and abundance (severity) of weedy rice using under both current long-term climatic conditions and potential climate change scenarios in predicting plant species distribution and the possibility to predict weedy rice invasion in South-East Asia particularly based on the CLIMEX^{TM} computer program.

Studies on genetic diversity of weedy rice have been widely reported. Federici *et al.* (2001) investigated Uruguayan weedy rice using AFLP markers and found that weedy rice adapts either to the natural environment or to cultivation. Several studies, based on morphological and physiological traits, isozymes, RFLP, and RAPD markers, indicate that weedy rice strains appear to be differentiated into indica and japonica types (Suh *et al.* 1997). A recent study using SSRs show that some weedy rice is closely related to *Oryza sativa* while others are related to *Oryza rufipogon* (Vaughan *et al.* 2001). Thus, as pointed out by Watanabe *et al.* (2000), different rice-growing locations often show different patterns of genetic diversity, depending on the specific combination of germplasm from which weedy rice emerges**.**

Various molecular methods have been used to clarify phylogenetic relationships within the *Oryza* genus, mainly within the weedy rices, but information and data concerning genome molecular organization in the weedy rices especially in South-East Asia, is still limited.

Reports suggest that weedy rice may include other *Oryza* species including *O. barthii*, *O. glaberrima*, *O. longistaminata*, *O. nivara*, *O. punctata*, *O. sativa*, and *O. latifolia* (Holm *et al.* 1997). In addition to seed shattering, weedy rice seeds may possess secondary dormancy and some types are morphologically indistinguishable from rice varieties yet still shatter seed (Lentini & Espinoza 2005). Natural gene flow estimates in the field from herbicide-resistant rice into weedy rice under temperate conditions indicate hybridization rates of under one percent (Chen *et al.* 2004), as confirmed by genetic analysis (Estorninos *et al.* 2002).

Weedy rice also occurred by gene transfer. Gene transfer can occur within a species (between cultivars and/or weedy varieties of the same species) or between different species of the same or other genera. These are referred to as intraspecific and interspecific gene transfer respectively. Successful gene transfer requires that three criteria are satisfied. The plant populations must overlap spatially, overlap temporally including flowering duration within a year and flowering time within a day and be sufficiently close biologically that the resulting hybrids are able to reproduce normally (Anon 2005).

As a diploid crop with a relatively small genome, rice holds a great potential for understanding the genetic mechanisms of crop domestication and improvement. Beside all efforts to understand the genetic basis of weediness in weedy rices, the study will also provide a lot of useful outcomes. The completion of rice genome sequencing can accelerate the study of the history and process of rice domestication. The phylogenetic of weedy rices may lead to the understanding of the origin and the parenting of hybridize weedy rices.

Only recently we have phylogenetic studies with increased taxonomic sampling and genomic markers come together with population and molecular genetics analyses to reveal the dynamic process of rice domestication. Therefore, there is a need to review recent advances in the genetics and phylogenetic of rice domestication and discuss major challenges for sorting out the complex evolutionary history of cultivated rice.

According to Mortimer *et al.* (2000), three factors that determine the population of weedy rices are seed remaining dormant in the soil over crop seasons, dissemination through crop seed contamination and seeds returning from plants in the previous crop. No single control measure will effectively control weedy rice. An integrated approach involving cultural, physical, and chemical interventions is expected to be effective in managing the weedy rice problem in a sustainable manner. Unless the problem is addressed, weedy rice in many areas poses a major threat to sustainable direct-seeded rice production. (Azmi *et al* 2004)**.**

6.4 Descriptive analyses of NBWR

Chapter 3 discussed the descriptive analyses of NBWRs in Selangor North-West Project (PBLS). Sixteen accessions were assessed with various characteristic which deferred from each other. However, all of them sharing one particular character, i.e. relatively similar plant height with recent cultivated rice varieties. They also share the capability to shatter grain easily during maturity except for Acc 9 and Acc 11. However, the grains of these two accessions have close resemblance with taller biotype of weedy rice with red pericarp. This gives an indication that there is also a biotype which has a hard shattering but closely related to weedy rice. The existence of these Acc 9 and Acc 11 in rice granaries if massive can contaminate the rice yield with red grains.

In general, it was hard to identify and characterize these NBWRs either it is more related to former weedy rice or cultivated rice varieties because the traits and the characters of these NBWRs were mixed between both two cultivars (MR219 and MR220).

Multivariate analysis was also performed to assess the relationship between traits and the relationship between accessions. Basically, multivariate analysis is the simultaneous statistical consideration of relationships among many measured properties of a given system (Gould 1996). Four analyses in multivariate analysis have been performed to study the relationship. The analyses were factor analysis (FA), principal component analysis (PCA), cluster analysis and canonical analysis.

This multivariate analysis gave us an idea that most NBWR accessions were different morphologically. There is still no evidence that can support this result. The difference between accessions will remain uncertain if further study especially in genetic aspect not be carried out.

PCA and FA had identified some similar characters as the most important for classifying the variations among the accessions. The most important characters or traits were pericarp colour and panicle type. In general, most accessions will show either dark red or white pericarp and either opened or closed panicle. However, the PCA also identified seedling height and time of booting as additional characters which can be identification keys to differentiate NBWR accessions. Nevertheless, these two characters were difficult to be used in the field, but can only be employed in the greenhouse. On the other hand, pericarp colour and panicle type can be a critical measurement to differentiate NBWR.

The canonical analysis gave another different summary of the variability within the characters and traits. Mostly, this analysis has given quantitative measurement rather than qualitative measurement as the most dominant characters to differentiate between accessions. Grain length, time of booting and time of mature were the main characteristics given by canonical analysis. This result was expected as in canonical analysis they calculate the characters to correlation with canonical variables.

The clustering of NBWR accessions had shown that some accessions were closely related. The accessions, Acc 13 and Acc 15, and Acc 10 and 14 for example have a similarity up to 98%. The most distinct accession based on characters and traits using cluster analysis was Acc 7.

The close similarity of NBWR and any cultivated rices (MR220, MR219 *etc.*) morphologically can lead to the using of genes identification. The similar characteristics of NBWR with cultivated accessions of rice may be differentiated genetically. These differences are very useful especially when we are trying to find the best herbicides or creating the best cultivated rice varieties to control the weedy rice from the serious infestations to rice granaries.

We need to determine the genetic diversity represented by accessions of NBWR and to identify DNA markers that might be useful in identifying hybrids between weedy rice and cultivated rice. This hopefully can identify the origin and population structure of NBWR, weedy and red rices using both DNA sequence data from a neutral nuclear locus as well as microsatellite genotype data.

The genetic diversity from this region will be analyzed to enable the genetic structure of these gene pool complexes to be better understood. The completion of this study may afford an opportunity to widen our understanding of the genomes of weedy rices especially NBWR and help to answer questions related to domestication, speciation, polyploidy and ecological adaptation that cannot be answered by studying weedy rices alone.

6.5 Growth pattern and seed germination of NBWR

Seed germination and early growth pattern were also studied (Chapter 4). This study will be the basis for further study on seed dormancy of NBWR in the future. The study generally test the capability of NBWR seeds to germinate and survive to various environmental effects such as temperature, light, soil depth and soil-moisture content and depth of inundation.

The NBWRs were found to be affected by different temperature regimes, soil depth and water inundation. NBWR was not affected by light regimes. NBWR seeds can germinate with or without light. Even though NBWR was affected by temperature, the temperature margin for NBWR to germinate was still high (around 20^oC). Rice granaries in Malaysia only have alternate temperature ranging from 20^oC to 38^oC.

In soil depth test, NBWR seeds cannot germinate after being buried below 5cm depth. NBWR seeds also cannot germinate after being soaked below 7cm water level. However, the dormancy of NBWR seeds still not proved yet. We still not have any information on how long the NBWR seeds can survive under such environment. The study on dormancy of NBWR will be ahead light on the possible causes or seed dormancy under different environmental regimes.

The dormancy of weedy rices for instance is one of the major threats in rice cultivation. Generally, seed dormancy in weedy rice is stronger than in cultivated rice (Oka, 1988; Cho *et al.,* 1995; Suh *et al.,* 1997; Tang & Morishima, 1997). Gu *et al.* (2006) reported that dormancy genes have been observed in weedy rices. The genes were identified to have selective advantages in adapting to environments. The weedy rices seem to have a genetic mechanism to alleviate the dormancy of weedy rices especially the adaptation to different seasons and temperatures. It is also suggested that it will be useful to further investigate the underlying bases for seed covering-imposed dormancy using comparative genetic mapping of genotypes with hull- and pericarp-imposed dormancy (Gu *et al.* 2003).

All data from this chapter are taken from control condition experiment. Temperature, humidity, water, sunlight and fertilizer were assumed to be same for all experimental replicate. This study can be expand and continued by testing all variables in real condition or precisely the test can be done in the rice farm itself. The results can be compared with data from a control condition.

6.6 Differential competitive ability of NBWR and cultivated rice *var.* MR220

Chapter 5 has discussed on the competition between NBWR and cultivated rice *var.* MR220. This study generally discussed the growth and differential competitive ability of NBWR and cultivated rice *var.* MR220 and also the relationship between grain yield and some yield components based on the quantitative growth indices and path analysis.

Surprisingly, NBWR did not affect growth and yields of the cultivated rice as *var.* MR220 in mixtures. In all tests, NBWRs were less competitive *vis-à-vis* cultivated rice in mixtures. On the other hand, NBWR seemed to be affected more.

Both MR220 and NBWR experiencing a reduction of plant height and tillers number albeit inconsistent as the density increased in monoculture. This indicated that both of these plants had an intra-specific competition among themselves (Fig 5.3.1 to Fig 5.3.4). However, the pattern changed in mixture regimes. Apparently MR220 did not to have any problems in mixture when there was no significance different in plant height in any mixture with NBWR (Table 5.3.1 and 5.3.2). We can conclude that vegetative growth was not affected for both MR220 and NBWR when grown sympatrically.

In yield production, NBWR was badly affected by increasing number of MR220 in mixture. The filled grain of NBWR dropped up to 45.73% of total filled grain in single plant monoculture, but MR220 filled grain reduced only by 20.5%. No significance difference in grain weight for both plants in any density regimes was registered. Relative yield was affected for both MR220 and NBWR when their proportions of plants changed. As taller biotypes always become a threat to reduce production yield of cultivated rice, NBWR not a very good competitor compared with taller biotypes of weedy rice to cultivated rice *var.* MR220. This is maybe because NBWR was not an aggressive plant in competition with MR220 as shown in aggresivity index.

The quantitative growth indices (reproductive effort, vegetative effort and harvest index) showed some significant results in the relationship between these two sympatric plants. MR220 produced more yield than NBWR in any mixture regimes. However, the yield reduced as the rice density reduced. This indicated that NBWR in rice cultivation will not have so much effect as their existences were not reducing the yield. However, this situation cannot be underestimated because it is generally believe that NBWR may evolve to become better plant to compete with any cultivated rice varieties.

The path analysis model had illustrated the direct and indirect effect of some yield components on plant densities and fecundity. The direct effect of rice and weedy rice densities on yield components was always negative. This indicated that density will affect all yield components for both MR220 and NBWR. The direct effect of yield components on rice fecundities was almost all were positive except for the yield per plant and grain per plant of rice have a negative path coefficient value of panicle per plant and also path coefficient of FP on GPP in MR220 and NBWR were the indication of the direct effect of FP to GPP in both rice and NBWR.

The indirect effect of all yield components for MR220 and NBWR were always positive. The result gives an indication that the yield components had variable influence on each other. TP of rice for example had a very strong influence on PP for rice and *vice-versa* but TP of NBWR had only a minor influence to PP. Conversely, TP of rice and NBWR not have a strong influence on GP and FP except for a slight effect on GP and FP for NBWR.

Differences in the path coefficient values between densities of MR220 and NBWR to yield components of rice and weed species perhaps explained the inherent differences in response of rice and NBWR to density subjected to. These differences will reflect the differential competitive ability of this NBWR to the rice crop.

6.7 Epilogue

NBWR is a new threat in Malaysian rice granaries especially in Selangor North West Project. Even as a taller biotype, there is no single weed control method can effectively manage weedy rice problem because the plants have similar growth characteristics with any cultivated rice varieties. Adaptation to the new biotype which stands as tall as commercial rice makes the control even worst.

As Wahab and Suhaimi (1991) founding of the first weedy rice in Sekinchan in 1987, the distribution of this taller biotype of weedy rice throughout Peninsular Malaysia was fast and uncontrolled. Only after a few years, the infestation went northward to other rice granaries all over Peninsular Malaysia and becoming the worst ever weed to be controlled (Azmi & Baki 2003; Baki 2006, 2008). It is worried if this NBWR will follow the same distribution pattern and it will be very hard to control as it very difficult to identify because of the similar plant height.

Even though Selangor North West Project rice granaries use a very systematic and incorporated system to control weeds and to increase production, weedy rices in this area seem to be evolved and as the result, NBWR has taken place. NBWR existence is very unpredictable because most farmers in this area used high quality, clean and certified seeds provided by various company and even from the government through Agricultural and Agro-Industry Department. An integrated approach to control former weedy rice biotypes also have been adopted in Selangor North West Project based on ecological methods and involving indirect and direct control methods especially at pre-planting stage but still weedy rice is exist and now in a new form.

Still we have no record of any NBWR existence in other rice granaries in Peninsular Malaysia but if there is no serious action to prevent this ensuing infestation, such patterns of infestation and extent of spread as in taller biotype of weedy rice may occur for NBWR.

However as we can see the distribution of NBWR in the third season (2007/2008 season) which has been discussed in Chapter 2, the number of NBWR seems to be reduced. This is because during this season, most of rice farms especially in Sekinchan, Pasir Panjang and Sungai Leman have been using machine to transplant paddy. Farmers were first sowing their seeds in other place before transplant it in the flooded farms. This can prevent the seed bank to germinate and compete with the cultivated rice. Sungai Burung and some rice farms in Bagan Terap also have started to use this technique in 2008 season.

All possible control measures in an integrated way should be adopted to check the spread, and to reduce the NBWR population in rice fields. Preventing possible hybridization between weedy and cultivated rice, where applicable, should be done. Farmers should be made aware on the weedy rice problems, and adoption of effective control measures could made through active participation of extension agents and farmers via appropriate training programs and demonstration.

Last but not least, within the context of integrated management, it is necessary to conduct regular studies of the behaviour of available rice cultivars and also former and new biotype of weedy rices in terms of their ability to compete with each other, life-cycle, morphology, genetics, phyto-chemistry and tolerance to submersion during flooding.