UNIVERSITI PUTRA MALAYSIA

HERBICIDE TOLERANCE IN SELECTED LOCAL RICE CULTIVARS

HUSSAIN TAHIR

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By

HUSSAIN BIN TAHIR

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To,

My beloved parents Mr. Tahir , Mrs. Rashida
who taught me that the best kind of knowledge to have is that which is learned for its own sake…..

And my brothers and sister,
Fatimah Zahara, Ibrahim, Abdul Rahman and Abdul Rahim
…….Thank you for your tender care, love and support

ABSTRACT

This study was carried out to evaluate tolerance in selected local rice cultivars (*Oryza sativa* L.) to different concentrations of the herbicide metolachlor. The study was conducted in a netted greenhouse at University Putra Malaysia Bintulu Campus, Sarawak using 44 x 32 x 12 cm high trays filled with a sandy clay loam soil. A total of 13 rice cultivars were screened for tolerance to metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide]. The cultivars were MR 220, MR 219, MR 185, MR 84, Serendah Kuning, Padi Satu, Acheh 62, Buntar B, Sampangan B, Lasak, Serasan Puteh, Rotan and Bario. The treatment consisted of three levels of metalachlor at 0.5, 0.25, and 0.125 kg ai/ha and an untreated control in 8 replications. The cultivar tolerance responses to metolachlor were evaluated with respect to seedling emergence, height and weight. The result of the study showed that, Serendah Kuning, Acheh 62, and Buntar B appear to have some degree of tolerance to metolachor with tolerance indices for seedling growth ranging between 1.056 and 1.602 at the highest herbicide rate tested.

ABSTRAK

Kajian ini dijalankan untuk menilai toleransi dalam kultivar padi tempatan terpilih (*Oryza sativa* L.) atas kepekatan berbeza racun rumpai metolachlor. Kajian ini dikendalikan di dalam rumah kalis serangga di Universiti Putra Malaysia Kampus Bintulu, Sarawak mengunakan bekas 44 x 32 x 12 cm tinggi yang diisi dengan tanah lom lempung berpasir. Sejumlah 13 kultivar dinilai toleransi terhadap metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide]. Kultivar yang digunakan ialah MR 220, MR 219, MR 185, MR 84, Serendah Kuning, Padi Satu, Acheh 62, Buntar B, Sampangan B, Lasak, Serasan Puteh, Rotan dan Bario. Rawatan mengandungi 3 kadar metolachlor iaitu 0.5, 0.25, 0.125 kg ai/ha dan kawalan tanpa rawatan dalam 8 replikasi. Respon toleransi kultivar terhadap metolachlor dinilai dengan kemunculan anak benih, ketinggian dan berat. Daripada kajian ini, Serendah Kuning, Acheh 62, dan Buntar B kelihatan mempunyai toleransi kepada metolachlor dengan toleransi menunjukkan julat antara 1.056 dan 1.602 pada kadar racun yang tertinggi yang dikaji pada pertumbuhan anak benih padi.

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I certify that this research project report entitled "Herbicide tolerance in Selected Local Rice Cultivars" has been examined and approved as a partial fulfilment of the requirement for the degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus.

Assoc. Prof. Dr. A. Rajan
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia Bintulu Campu
(Supervisor)

Prof. Dato' Dr. Nik Muhamad Bin Ab. Majid
Dean
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia Bintulu Campus

Date:

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LIST OF ABBREVIATIONS

1. **ai**-active ingredient
2. **ha**-hactare
3. **ANOVA**-Analysis of Variance
4. **MARDI**-Malaysia Agriculture Research and Development Institute.
5. **kg**-kilogram
6. **cm**-centimetre
7. **DOA**-Department of Agriculture

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is a very important food crop. It has been the primary staple food for millions of people for centuries. Rice is also the main livelihood of rural populations in many Asian, African and Latin American countries (Labrada, 1998). Half the world's population depends on a diet based on rice (*Oryza sativa* L.). Increasing populations in Asia is predicted to raise rice demand by 30% in 2020 despite possible changes in the preferred diet from rice to other cereals as a result of economic growth (Anon., 1998).

Rice is generally planted by transplanting and direct seeding methods. However, transplanting involves costly labour, compaction of soil structure due to puddling and sometimes failure in the nursery due to factors such as unfavourable weather conditions, nutrient deficiencies and toxicities (Sohail, 1999). Moreover, the nursery in transplanted rice engages the field for nearly two or three weeks prior to transplanting which requires additional expenditure and intensive care unlike direct seeding. Due to these reasons, most farmers are more in favour of raising rice crops through direct seeded culture. Research has proved that accurate seed rate, timely seeding, efficient weed control and water management under direct seeded condition give yields as high as transplanted rice with comparatively lower production cost (Awan, 1989; Baloch, 1994) while in some experiments yields were higher than transplanted rice (Shad, 1983).

Weed control is one of the major inputs in rice production (Seaman and De-Datta, 1968). Various methods are used for weed control such as cultural, biological, mechanical and chemical control. The later method of control is the most popular method because it is the most efficient means of reducing weed competition with minimum labour cost (Seaman and De-Datta, 1968; Mian and Mamun, 1969; Baloch, 1994). However, removal of weeds at the critical period by traditional means may not be possible at peek periods of labour demand. (Rao and Pillai, 1974; De Datta, 1980). Labour for transplanting and hand weeding is increasingly scarce, forcing farmers to switch to direct seeding thereby losing the early season advantage the crop has under a flooded condition to suppress initial weed growth, especially of grasses.

Rapid industrialization and urbanization also compete with agriculture for limited resources, especially water. Additionally, poor maintenance of the irrigation infrastructure further reduces water availability for rice production. Measures to conserve water in rice production, such as intermittent flooding and shallow water depths are generally less efficient with respect to weed control. Labour and water shortages limit weed control alternatives thus increasing reliance on herbicides. Generally, herbicides are more effective, easy to use and less expensive than hand labour.

The shift to direct seeding accompanied by widespread, often exclusive use of herbicides for weed control in rice promotes changes in the weed flora from relatively easily controlled broad-leaved weeds to more aggressive grass weeds, especially weedy *Oryza* species (Moody, 1992; Mortimer & Hill, 1999). Worldwide, continuous use of herbicides has also led to evolution of herbicide-resistant weeds.

Some herbicides are promising alternatives in controlling weeds and are reported to not only control weeds, but also increase the rice yield (Chowdhury, 1995). Not all herbicides are selective and may cause injury to rice plants. The development of rice cultivars which are tolerant to an herbicide that controls wild rice would help control wild rice in commercial rice. Intraspecific variation in tolerance to herbicides has occurred in crops such as barley (*Hordeum vulgare* L.) (Derscheid *et al.*, 1952); oats (*Avena sativa* L.) (Smith *et al.,* 1964; Williams 1953); corn (*Zea mays* L.) (Williams, 1953; Wright *et al.,* 1973); sorghum [*Sorghum bicolor* (L.) Monech] (Scifres *et al.*, 1970); sugarcane (*Saccharum officinarum* L.) (Matherne and Millhollon, 1973); soybeans [*Glycine max* (L.) Merr.] (Barrentine *et al.*, 1982, Hays and Wax, 1975, Wax *et al.*, 1974, Williams, 1953); potato (*Solanum tuberosum* L.) (Graf and Ogg, 1976); and tomato (*Lycopersicon esculentum* Mill.) (Stephenson *et al.*, 1976). Rice cultivars and advanced lines that were tolerant to molinate, an herbicide which controls barnyard grass, have been reported (Richard and Baker, 1979; Smith, 1970).

Resistant biotypes that are now common in rice often do not lack vigour compared to susceptible ones, and some are hard to control with alternative herbicides. There are weed populations that have evolved resistance to several herbicide groups. Currently more than 200 cases of resistance to at least 16 major herbicide groups have been documented in almost 150 weed species worldwide in several crops and non-agricultural lands and the area infested with herbicide resistant weeds is increasing (Heap, 2000). Adverse effects of increased herbicide use on health and on the environment are also of major concern.

During the past decade however there has been keen interest to develop herbicide resistant crops. Justification for this development is the potential improvement in weed control and environmental benefits brought about by being able to use more efficient and environmentally benign herbicides in place of older-generation chemicals. Also developing new herbicides with new modes of action is becoming increasingly difficult and expensive, making it profitable to develop new niches for existing products.

Clearly, there is a need for further research in determining which varieties are more tolerant to the herbicide applied. Most literature, reported use of herbicides to kill weeds; where the rice is tolerant and gives higher yields. Many herbicides have been developed over the years and these herbicides have been widely used in most parts of the world. However, changes in weed populations to grasses have been frequently reported. These include *Oryza spp.* which mostly respond in the same way as cultivated rice. Therefore, use of herbicides which are more selective against grasses will be more effective in controlling these grass weeds including wild rice. However, rice cultivars that are tolerant to these herbicides are needed. Hence, the objective of the present study is to investigate on tolerance of selected rice cultivars to the grass herbicide metolachlor.

CHAPTER 2

LITERATURE REVIEW

2.1 Cultivated rice and other *Oryza* species

Oryza sativa L. and *O. glaberrima* Steud. are cultivated species of rice. The first one, known as Asian rice, is produced worldwide; the second species (African rice) is produced in areas of West Africa. Cultivated *O. sativa* is divided into two main groups, *indica* and *japonica*; the latter is further divided in to tropical (= *javanica*) and temperate Japonicas (Khush, 1997). According to their genetic relationships, species are grouped in four complexes (sativa, officinalis, ridleyi and meyeriana), except *O. brachyantha*, *O. schlechteri* and *O. neocaledonica*, which do not belong to any of these complexes (Aggarwal *et al*., 1999)

The cultivated rice (*O. sativa*) and its closely related wild species, such as *O. rufipogon, O. nivara* and *O. longistaminata* share the AA genome. These wild species can be crossed with *O. sativa* and desirable genes from them can be transferred to cultivated rice by conventional crossing and back crossing procedures. However, wild species with genomes other than AA are difficult to cross with *O. sativa* (Brar & Khush, 1997). As cultivated rice is predominantly selfing, most gene flow occurred from the cultivated plants to the wild species, which has a greater outcrossing rate (Akimoto *et al.,* 1999). This emphasises the relevance of risk evaluation for movement of herbicide resistance genes from the crop to related weedy species.

2.2 Weed Problems in Rice Fields

Weed control is one of the major inputs in rice production (Seaman and De-Datta, 1968). Various methods are used for weed control such as cultural, biological, mechanical and chemical control. The later method of control is the most popular method because it is the most efficient means of reducing weed competition with minimum labour cost (Seaman and De-Datta, 1968; Mian and Mamun, 1969; Baloch, 1994). However, removal of weeds at their critical period by traditional means may not be possible at peek period of labour demand. (Rao and Pilla, 1974; De Datta, 1980). Some herbicides are promising alternatives in controlling weeds and are reported to have not control weeds, and increase rice yield (Chowdhury, 1995). Not all herbicides are selective and may cause injury to rice plants.

2.3 'Red rice' or 'Weedy rice'

Common names to describe weedy *Oryza* species vary among regions in the world, so the term "weedy rice" is used in its broad sense for weedy *Oryza* species, including red rice. Some weedy rice can be considered as feral forms of cultivated rice (*Oryza sativa* L.). In the USA and Latin America these weedy species are known as red rice although in Central America they are also termed 'contaminant' rice; in Asia the term weedy rice is more widely used. As weedy species, these feral *O. sativa* forms are undesirable, early-shattering off-type rice with seed dormancy mechanisms.

Weedy rice is an increasing problem in all rice growing areas in the world. Weedy rice can be morphologically very similar to, and naturally cross with, cultivated rice. In the USA, weedy rice problems mostly occur in fields under continuous rice cropping. In Asia, however, continuous rice cropping has been practiced for thousands of years without development of serious weedy rice problems. Thus, continuous rice cropping is hardly the sole explanation for the increased importance of weedy rice. Direct seeding, saving and planting contaminated seed are major contributing factors. Many weedy rice populations in the USA have a red pericarp (thereby the name 'red rice') and are therefore relatively easy to distinguish from cultivated rice.

In the USA, red rice has been known as a weed since 1846 and was probably introduced from India (Craigmiles, 1978). Red rice was described as having red pericarp, pubescent light-green leaves and pubescent seeds (Diarra, 1985). Recent studies have added more information on morphological traits. Weedy rice generally has more tillers and panicles per plant and produces less seed of comparable size per panicle than cultivated rice. Weedy rice grows taller than the cultivated varieties it associates with and shatters seeds that are predominantly (about 80%) dormant (Noldin *et al.,* 1999). Oka (1991) characterised weedy rice as intermediate between wild and domesticated forms occurring in direct-seeded rice fields, irrigation ditches, and dikes, which could not survive in natural habitats.

Weedy rice retains wild-plant characters such as seed shedding and dormancy, and awns. Similarity between weedy rice and cultivated rice in the vegetative stage makes it difficult to remove by hand. Weedy rice tolerates adverse conditions and has a higher outcrossing rate than domesticated cultivars. They usually mature before the cultivars they associate with. Asian weedy rice is phenotypically variable, the white pericarp being more common than the red pericarp. Suh *et al.* (1997) classified 152 accessions of weedy rice, originating from 10 countries, in to four groups based on morphological and physiological characteristics, isozyme patterns and DNA markers.

The first group (Group I) was composed of 54 accessions from temperate countries (mostly from Japan, Bhutan, USA, Nepal and Brazil) and was identified as '*indica*-type, similar to cultivars.' A few of the accessions in this group, however, exhibited characteristics associated with *japonica*, indicating the possibility of gene flow between *indica* and *japonica*. Group II (24 accessions from more tropical climates including India, Bangladesh and Thailand) was designated as '*indica*- type, similar to wild rice'. Group III, '*japonica*-type similar to cultivars', included 63 accessions originating from Korea and Bhutan, which were assumed to be old rice cultivars that reverted to a weedy form. The remaining 11 accessions from China and Korea comprised Group IV ('*japonica* – similar to wild rice') which is likely to have evolved from gene flow between *japonica* cultivars and wild rice from China. Similarly, Oka (1991) suggested two categories of weedy rice: those occurring together with wild rice (most frequent in India and Thailand) and those distributed in regions where no wild rice is found.

2.4 Climate, Soil texture and pH

Rainfed lowland rice (*Oryza sativa* L.) is grown in bunded fields by some of the poorest subsistence farmers in the world. Crop performance is variable, due to different seasonal conditions and spatial heterogeneity over soil types and topographic positions. Consequently, agro hydrology may vary from field to field (Wade *et al.*, 1999). Drought stress is commonly considered the most severe limitation to productivity, but ponding or even complete submergence may occur during the cropping season. Drought is complex in rainfed lowland, as it may occur early in the growing season or from flowering to grain filling, and may follow a period in which soils were flooded and anaerobic (Wade *et al.,* 1999).

The grain yield in the dry season is generally higher than in the wet season. The dry season crop is dependent on irrigation water while the wet season crop is dependent on rainfall. Soil organic carbon (OC), clay content, water content, and pH often influence the bioactivity of soil-applied herbicides, and these soil properties can vary greatly within fields. Soil-applied herbicides are used widely in agriculture production in the United States, and several factors influence their efficacy. Those factors include pesticide properties, formulation, application technique, agricultural management, characteristics of crops and weeds, climatic conditions, and soil properties. Failure to recognize such factors in practice can result in unsatisfactory herbicide effectiveness and non target effects such as crop injury.

2.5 Herbicide resistant rice and weed management

Major research efforts have been directed towards the development of herbicide-resistant field crops (Dekker *et al*., 1995). The primary focus has been on rice cultivars resistant to either glufosinate (Datta *et al*., 1992), sulfonylureas, imidazolinones or glyphosate, have already been developed and were being field-tested, mostly in the USA but also in South America and Japan (Anon., 1998, 1999; Sissell, 1999). The main reason for developing herbicide resistant rice is to attain better control of weedy *Oryza* species that cannot be controlled selectively in rice (Gealy and Dilday, 1997).

Additionally, two major benefits of herbicide resistant rice are generally stated. First, the introduction of herbicide resistant rice would improve current cropping systems, with more efficient weed control measures, and could reduce the amount of land required to satisfy the global rice needs. This would preserve marginal land from exploitation resulting in conservation of ecosystems and habitats and thereby genetic resources. The increased efficiency in cropping systems would lead to the second benefit: reduction in the fossil fuel input into agricultural production (Mannion, 1995). If attainable for rice, these proposed benefits would enhance the probability of meeting the increasing demand for rice in the coming years. Specifically, herbicide resistant rice provides the farmer with new efficient chemical options for weed control. Thus, herbicide resistant rice could result in adequate control of hard-to-kill weeds in addition to weedy rice.

Weed control strategies with the herbicide to which a crop has been bred or transformed for resistance must be developed in relation to time of application, dose, crop phytotoxicity and integration with other herbicides or agronomic practices. Almost complete control of weedy rice and other grasses, including *Echinochloa crus-galli* (L.) Beauv. was achieved in glufosinate-resistant rice in Arkansas (USA) by sequential applications of glufosinate (0.42 kg ha-1) in early pre-emergence and at flooding. Two of the transformed cultivars (Bengal and Cypress) were not injured by the herbicide. The cultivar Gulfmont, however, was severely affected by glufosinate (Wheeler *et al.*, 1998). Undoubtedly, the introduction of herbicide resistant rice will provide farmers with additional tools for controlling weedy rice and other weeds that either escape or are resistant to currently available herbicides. Herbicide resistant rice would also allow production in fields which are heavily infested with weedy rice and, in areas where growers are forced to rotate rice with less profitable soybeans to control this weed, to extend the period between rotations (Croughan, 1998).

2.6 Varieties of rice

Although there are many rice varieties in the world, no one variety can be found everywhere. The most suitable variety in one place is selected on the basis of its ability to give a high and constant yield under the circumstances of the climate and the soil condition of that district.

Studies have shown that rice cultivar performance differs among the environments of the region, with the variance due to cultivar by environment interaction being 3-5 times greater than that due to cultivar alone (Cooper and Somrith, 1997; Fukai and Cooper, 1999). Traditional, subsistence farmers of the rainfed lowlands, however, favour taller cultivars because of their tolerance to flooding and their competitiveness against weeds.

MR 220 variety is a sister line to variety MR 219. It was developed from a cross between the two potential lines, MR 151 and MR 137. This variety is high yielding, capable of producing yields around 10 tonnes per hectare if grown under conducive environments and precise management practices. It requires slightly high fertilizer inputs than usual in order to produce high yields. This variety is also very suitable for direct seeding culture. The plant type is similar to MR 219 which is erect and slightly tall. The flag leaves are also erect with green to dark green colour. MR 220 is classified as a short maturation variety. The grains belong to long grade rice with good eating quality. The variety is also resistant to most major pests and diseases of rice. (MARDI, 2005)

MR 219 is a common cultivar used by Malaysian rice planters. MR 219 mature at around 105-111 day with direct seeding. This cultivar has 150 spikelets per panicle with 63.4% fertile spikelets. The weight of 1000 grains is about 27.11 g and the yield is between 5.9-10.7 tonnes per hectare. This cultivar is resistant to rice blast and sheath blight. This cultivar has moderate resistant to brown plant hopper attack, tungro and green plant hopper attact.

MR 185 is another cultivar developed by Malaysia Agriculture Research and Development Institute. This cultivar has a maturity of around 112-115 days with direct seeding method. This cultivar produces 13-16 panicles per plant and 140 spikelets per panicle with 90% fertile spikelets. The 1000 grain weight is about 25.3 g and yield is around 5-7 tonnes per hectare. This cultivar is resistant to rice blast and moderate resistant to tungro, green and brown hopper attack.

MR 84 has a maturity period of around 115-125 days, with 13-17 panicles per plant, has 130 spikelets per panicle with 84% fertile spikelets. The 1000 grain weight is around 26 grams and produce around 4.0 to 6.2 tonne yield per hectare. This cultivar is resistant to rice blast. (MARDI, 2003)

Serendah Kuning is a short term rice variety suitable for double cropping in Sarawak. This variety is the outcome of extensive selection and evaluation of material. The plant is of medium height with stem (culms) measuring about 105-110 cm. The stems are strong and do not lodge easily. This variety is suitable for transplanting and direct seeding. Serendah Kuning grows well in both irrigated and rainfed areas. During the main season, this variety can be harvested 118 days from the date of sowing, while during the off season 116 days. Compared to other varieties in Sarawak, Serendah Kuning matures at about 3-4 weeks earlier than MR 30, 4-6 weeks earlier than traditional varieties such as Biris, Rotan and Bario. In addition, Serendah Kuning is also a good yielder capable of producing 4.5-6.0 t/ha compared to 3.0-4.9 t/ha for MR 30 and 2.8-3.5 t/ha for traditional varieties in an area with good in water and crop management. Serendah Kuning is highly resistant to blast. (Sarawak, DOA, 2005)

2.7 Methods of cultivation

Rice is generally planted by transplanting and direct seeding methods. However, transplanting involves costly labour, compaction of soil structure due to puddling and sometimes failure in the nursery due to factors such as unfavourable weather conditions, nutrient deficiencies and toxicities (Sohail, 1999). Transplanting rice requires additional expenditure and intensive care unlike direct seeding. Hence, most farmers are in favour of raising rice crop through direct seeded culture and direct seeded flooded rice is gradually replacing transplanted rice in Peninsular Malaysia. Some estimates indicate that the extent of direct seeding area in Muda, Seberang Perai, Seberang Perak, Barat Laut Selangor and Besut is in the order of 70 %, > 60 %,> 70 %,> 50 % and 90 %, respectively (Supaad *et al.*, 1990). Farmers use the current high-yielding varieties bred for transplanted culture.

2.8 Tolerance to herbicide.

Six experiments involving two concentrations of alachlor and 4,567 accessions from the USDA/ARS small grains collection were conducted from 1983 to 1985 (Dilday, 1988). Plants were drill seeded (3 g of seed/m) in single row plots 1.23 m long with a 19 cm row spacing in two replications. Prior to seeding, 4.58 and 6.88 kg ai/ha of alachlor [2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl) acetamide] was incorporated to a soil depth of 5 to 8 cm. Plant height and grain type were determined at maturity. Two replicated tests were conducted in 1986. Each test included 2 row plots, 1.23 m long with each row being 19 cm apart and the tests were treated with 4.58 and 6.88 kg ai/ha of alachlor.

The 4,567 rice accessions that were evaluated for tolerance to alachlor originated from 34 countries. Sixteen accessions that had a higher level of tolerance to alachlor than the others are originated from Pakistan, India, Sierra Leone, Madagascar, Sri Lanka and Philippines. The accessions included short, medium, and long grain types that produced plant heights ranging from 90 to 157 cm. Seedling emergence was significantly greater for two accessions, 'IR 1317-152' and ' Jhona White Pak 83'.

In 1985 five seeds per accession were germinated in disposable petri dishes which on filter paper, moistened with 3 ml of 2 and 3 ppm of alachlor. The mesocotyl and coleoptile were measured after following germination chamber for seven days at 28°C. Seedlings which produced leaves were washed in water and transplanted in herbicide-free soil in pots in the greenhouse for 3 weeks to observe their growth and development. In 1986, 16 of the most tolerant accessions were further evaluated. After 7 days the mesocotyl coleoptile, primary leaf, secondary roots, and radicle of each seedling were measured. Alachlor is absorbed primarily through the mesocotyl and coleoptile tissue of the seedling and ultimately disrupts the development of the radicle, secondary roots, and primary leaves (Pillai *et al.*, 1979). Furthermore, the elongation potential of the mesocotyl and coleoptile is heritable and varies according to the genetic constitution of the germplasm (Dilday *et al.,* 1988). Inge and Loomis (1937) demonstrated that mesocotyl elongation is inhibited when the tip of the coleoptile is exposed to light. Also, the elongation of the mesocotyl and coleoptile is influenced by the depth of seeding.

Results from the above experiments demonstrated that the mesocotyl or coleoptile elongation in tolerant accessions was reduced by about 50% at 0.1 ppm to 1 0.0 ppm. Furthermore, secondary root development in some tolerant accessions occurred at even the highest concentration of 10.0 ppm and primary leaf development continued to occur at the highest concentration (10.0 ppm). Two tolerant germplasm accessions responded differently to alachlor which suggests that the tolerance is due to separate genetic mechanisms. It was suggested that pyramiding of tolerant genes from different germplasm could increase the overall level of germplasm tolerance to alachlor (Dilday, 1988).

Rice growers throughout the world could benefit from the introduction of herbicide-resistant rice cultivars that would allow in-crop, selective control of weedy *Oryza* species. Other perceived benefits are the possibility to control 'hard-to-kill' weed species and weed populations that have already evolved resistance to herbicides currently used in rice production, especially those of the *Echinochloa* species complex. Introduction of herbicide resistant rice could also bring areas heavily infested with weedy rice that have been abandoned back into rice production, allow longer term crop rotations, reduce consumption of fossil fuels, promote the replacement of traditional chemicals by more environmentally benign products, and provide more rice grain without adding new land to production.

Many herbicides have been developed over the years and these herbicides have been widely used in most parts of the world. However, changes in weed populations to "grasses" and related species with similar characteristic as rice plants have become more serious problems. Therefore, use of herbicides which are more selective against grasses will be more effective in controlling these grass weeds including wild rice. However, rice cultivars that are tolerant to these herbicides are needed. Hence, the present study is a preliminary investigation on tolerance of selected rice cultivars to the grass herbicide metolachlor.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

The study was conducted in trays placed in a netted greenhouse at University Putra Malaysia Bintulu Campus, Sarawak.

3.2 Experimental design and treatments

The experiment was laid out in a split-plot arrangement with four herbicide levels as the main plot treatments within each of which were sown 13 rice cultivars. There were a total of 4 x 13 treatment combinations. Herbicide treatment were 0, 0.125, 0.25 and 0.5 kg ai/ha metolachlor

3.3 Soil Preparation, Treatment Application and Establishment of Test Plant

3.3.1 Soil Preparation

Trays of dimension 44 x 32 x 12 cm high were filled with a sandy clay loam soil with 48% sand, 25% silt and 27% clay (with pH 3.60 in KCl and 4.1 in H_2O). The soils in the trays were maintained water saturated at all time.

3.3.2 Source of rice cultivars

There were 13 cultivars used for the study. There were 9 cultivars acquired from Sarawak Department of Agriculture (DOA) and 4 cultivars from Malaysian Agriculture Research and Development Institute (MARDI).

3.3.3 Treatment Application

The metolachlor treatments were sprayed onto the soil using a RB 15 knapsack sprayer. The herbicide was applied at 0.125, 0.25 or 0.5 kg ai/ha in eight replicates.

3.3.4 Seed Preparation and Sowing

The rice seeds were soaked in the water for 5 hours prior to sowing in the trays. The seeds were partially buried on the soil surface. Ten seed were sown at each of the herbicide treatments in all replicates.

3.4 Crop Maintenance

Carbofuran was applied to control stem borers. Malathion was sprayed at 0.5 kg active ingredient per hectare every 3 days to prevent damage from insects and other pests. All trays were irrigated and soils maintained at saturated condition until harvest.

3.5 Parameters measured

At one week after sowing, the number of seedlings surviving were recorded. Seedlings were removed after 2 weeks and shoot length were recorded. Plants were then oven dried at 80°C for 24 hours and dry weights were determined.

3.6 Soil analysis

Soil samples were taken randomly and air dried for 3 days, sieved using a 2 mm sieve, and analyzed for texture and pH.

3.6.1 Determination of Soil pH

A) pH in water

The following method was used to determine the pH. A 10 g sample of air-dried soil was weighed into plastic vials, 25 mL distilled water was added at 1:2.5 ratio, and shaken at 180 rpm for 15 minutes. The vials were left to stand overnight for 24 hours. The pH was then determined using a pH meter.

B) pH in KCl

To a 10 g of air-dried soil was added 25 mL 1 M KCl (1:2.5) in plastic vials and shaken at 180 rpm for 15 minutes. The pH was then determined using a pH meter. The result of the pH determinations are reported in Appendix 4.

3.6.2 Determination of soil texture

Soil samples were air dried and sieved using a 2 mm sieve. Sample of 50 g of soil sample were placed in the blender cup, water was added to within 10 cm of the top (rim), and 10 mL of 2.0M sodium hydroxide was added as dispersing agent. Samples were blended for 15 minutes, transferred into a measuring cylinder, and the volume made up to 1130 mL. The samples were then thoroughly mixed and the hydrometer readings were recorded after 40 seconds and again after 2 hours. The percentage of silt, clay and sand were then derived from the data obtained. The soil texture was derived from a standard chart (USDA textural triangle). The soil texture analysis is presented in Appendix 4.

3.7 Data analysis

The data collected were subjected to analysis of variance using the NCSS program. Where appropriate data were transformed using either the Logarithmic or Square-root transformation.

CHAPTER 4

RESULTS

4.1 Seedling Emergence

The treatments and interaction effect on seedling emergence were significant (Appendix 1). Comparison between treatments showed that treatment with 0.5 kg ai/ha metolachlor had significantly lower emergence than treatments with 0.25 kg ai/ha metolachlor and the untreated control. Treatments with 0.125 kg ai/ha metolachlor were not different from 0.25 kg ai/ha of metolachlor. The untreated control had the highest emergence.

There were no significant differences in seedling emergence amongst cultivars within 0.5 kg ai/ha metolachlor treatments (Table 1). All cultivars gave the same response within this treatment. However when compared to the untreated control Serendah Kuning, Buntar B, Acheh 62 and Rotan showed reduced seedling emergence in this treatment compared to other cultivars.

There were also no significant differences in seedling emergence amongst cultivars within 0.25 kg ai/h metolachlor treatments. Most cultivars showed suppressed emergence. However when compared to the untreated control Sarawak cultivars Buntar B, Serendah Kuning, Acheh 62, Lasak, Serasan Puteh, Padi Satu, Rotan and Sampangan B showed greater reduction in emergence in this treatment compared to other cultivars.

Table 1: Effect of metolachlor treatments on seedling emergence of 13 rice cultivars (nos/ plot)

Cultivars	Metolachlor levels (kg ai/ha)			
	0 kg ai/ha	0.125 kg ai/ha	0.25 kg ai/ha	0.5 kg ai/ha
MR 220	1.6004 efgh	1.4142 fgh	1.4142 fgh	1.4142 fgh
MR 219	1.5334 efgh	1.4539 fgh	1.4539 fgh	1.4142 fgh
MR 185	1.5272 efgh	1.4142 fgh	1.4142 fgh	1.4142 fgh
MR 84	1.4142 fgh	1.4142 fgh	1.4142 fgh	1.4142 fgh
Serendah Kuning	3.0224 b	1.5436 fgh	1.9399 dfgh	1.5902 efgh
Padi Satu	2.9001 b	1.7133 efgh	1.5436 efgh	1.4142 fgh
Acheh 62	3.3886 a	1.8325 dfh	1.8843 dfgh	1.4937 fgh
Buntar B	2.9877 b	1.6361 efgh	1.9542 dfgh	1.5607 fgh
Sampangan B	1.8853 defgh	1.5436 efgh	1.4937 fgh	1.4142 fgh
Lasak	2.9443 b	1.4874 fgh	1.7724 dfgh	1.4142 fgh
Serasan Puteh	2.1998 def	1.4937 fgh	1.6004 efgh	1.4142 fgh
Rotan	2.5975 c	1.4142 fgh	1.4937 fgh	1.4539 fgh
Bario	2.0405 defg	1.4142 fgh	1.4142 fgh	1.4142 fgh

Data were transformed to square-root (X + 2) prior to statistical analysis.
Means with the same alphabet are not significantly different at $p \leq 0.05$ (Duncan's Multiple Range Test)

Table 2: Tolerance index based on seedling emergence for cultivars receiving the metolachlor treatments.

Cultivars	Metolachlor levels (kg ai/ha)		
	0.125 kg ai/ha	0.25 kg ai/ha	0.5 kg ai/ha
MR 220	1.000	1.000	1.000
MR 219	1.028	1.028	1.000
MR 185	1.000	1.000	1.000
MR 84	1.000	1.000	1.000
Serendah Kuning	1.092	1.371	1.130
Padi Satu	1.211	1.092	1.000
Acheh 62	1.295	1.332	1.056
Buntar B	1.157	1.382	1.104
Sampangan B	1.092	1.056	1.000
Lasak	1.052	1.253	1.000
Serasan Puteh	1.056	1.132	1.000
Rotan	1.000	1.056	1.028
Bario	1.000	1.000	1.000

Tolerance Index = Relative survival compared to treatments with zero tolerance.

There were also no significant difference in seedling emergence among cultivars within 0.125 kg ai/ha metolachlor treatments. Within the treatment, all cultivar gave a similar response. When compared to the untreated control Acheh 62, Padi satu, Buntar B, Serendah Kuning, Sampangan B, Serasan Puteh and Lasak showed significant decline in emergence compared to the other cultivars.

There were also significant differences in seedling emergence among cultivars in the untreated control. Acheh 62 recorded the highest seedling emergence while Serendah Kuning, Lasak, Buntar B and Padi satu, Bario, and Rotan were comparable. The MR cultivars had very low seedling emergence even in the untreated controls.

However, values for tolerance index with seedling emergence data, indicate that cultivars Serendah Kuning, Acheh 62 and Buntar B may have mechanisms for higher tolerance to the herbicide with tolerance indices greater than 1.050 to the highest herbicide rate tested (Table 2).

4.2 Seedling Height

All treatments and interaction effects on seedling height response were significant (Appendix 2). There were significant differences between the untreated control and herbicide treatments. Treatments with 0.50 and 0.25 kg ai/ha metolachlor were not different from treatments with 0.125 kg ai/ha metolaclor (Table 3).

There were no significant differences in seedling height among cultivars in treatment with 0.5 kg ai/ha metolachlor. Most cultivar were stunted in this treatment.

However, when compared to the control Acheh 62, Serendah Kuning, Buntar B, and Rotan showed reduction in height due to treatments compared to other cultivars.

There were also no significant differences in seedling height among cultivars within treatments with 0.25 kg ai/ha metolachlor. However when compared to the untreated control Lasak, Serendah Kuning, Buntar B, Acheh 62 and Serasan Puteh showed reduction in height compared to the other cultivars.

There were no significant differences in seedling height among cultivars within treatments with 0.125 kg ai/ha metolachlor. All cultivar gave the same response within this treatment. However, when compared to the untreated control, Acheh 62, Buntar B, Serasan Puteh, Lasak, Sampangan B, Padi Satu and Serendah Kuning showed reduction in height compared to the other cultivars. There were also significant differences in seedling height among cultivars in the untreated control.

However, values for tolerance index with seedling height data indicate that Serendah Kuning, Acheh 62, and Buntar B may have mechanisms for higher tolerance to the herbicide with tolerance indices ranging from 1.565 to 1.602 (Table 4).

Table 3: Effect of metolachlor treatments on Seedling height of 13 rice cultivars (cm)

Cultivars	Metolachlor levels (kg ai/ha)			
	0 (Control)	0.125 kg ai/ha	0.25 kg ai/ha	0.5 kg ai/ha
MR 220	0.5909 defg	0.3010 defg	0.3010 defg	0.3010 defg
MR 219	0.6972 defg	0.3955 defg	0.3607 defg	0.3010 defg
MR 185	0.5121 defg	0.3010 defg	0.3010 efg	0.3010 defg
MR 84	0.3010 defg	0.3010 defg	0.3010 defg	0.3010 defg
Serendah Kuning	1.1941 abc	0.3625 defg	0.6367 defg	0.4711 defg
Padi Satu	1.2796 abc	0.7158 def	0.3952 defg	0.3010 defg
Acheh 62	1.2096 abc	0.6463 defg	0.6021 defg	0.4821 defg
Buntar B	1.2171 abc	0.5416 defg	0.6352 defg	0.4754 defg
Sampangan B	1.1117 abc	0.3698 defg	0.4138 defg	0.3010 defg
Lasak	1.2328 abc	0.4136 defg	0.6917 defg	0.3010 defg
Serasan Puteh	0.8773 bcde	0.4642 defg	0.5692 defg	0.3010 defg
Rotan	0.9037 bd	0.3010 defg	0.4298 defg	0.3474 defg
Bario	0.9010 bcd	0.3010 efg	0.3010 efg	0.3010 defg

Data were transformed to Log (X + 2) prior to statistical analysis.
Means with the same alphabet are not significantly different at $p \leq 0.05$ (Duncan's Multiple Range Test)

Table 4: Tolerance index based on seedling height for cultivars receiving the metolachlor treatments

Cultivars	Metolachlor levels (kg ai/ha)		
	0.125 kg ai/ha	0.25 kg ai/ha	0.5 kg ai/ha
MR 220	1.000	1.000	1.000
MR 219	1.314	1.295	1.000
MR 185	1.000	1.000	1.000
MR 84	1.000	1.000	1.000
Serendah Kuning	**1.204**	**2.115**	**1.565**
Padi Satu	2.378	1.313	1.000
Acheh 62	**2.147**	**2.000**	**1.602**
Buntar B	**1.799**	**2.110**	**1.579**
Sampangan B	1.212	1.375	1.000
Lasak	1.347	2.298	1.000
Serasan Puteh	1.542	1.891	1.000
Rotan	1.000	1.428	1.154
Bario	1.000	1.000	1.000

Tolerance Index = Relative seedling height compared to treatments with zero tolerance.

4.3 Seedling Dry Weight

All treatments and interaction effects on seedling weight were also significant (Appendix 3). Comparison between treatments showed that treatments with 0.5 kg ai/ha metolachlor had significantly lower seedling weight then treatments with 0.25 kg ai/ha metolachlor, treatment with 0.125 kg ai/ha metolachlor and the untreated control. Treatments with 0.25 kg ai/ha metolachlor were also not significantly different from treatments with 0.125 kg ai/ha metolachlor.

There were no significant differences in seedling weight amongst cultivars in treatments with 0.5 kg ai/ha metolachlor. However, when compared to the untreated control, Sarawak cultivars Serendah Kuning, Acheh 62, Buntar B and Rotan showed reduction in seedling weight when compared to the other cultivars.

There were also no significant differences in seedling weight among cultivars within treatments with 0.25 kg ai/ha metolachlor. However, when compared to the untreated control Sarawak cultivars Serendah Kuning, Padi Satu, Acheh 62, Sampangan B, Lasak, Rotan and Buntar B showed reduction in seedling weight compared to other cultivars.

There were also no significant differences in seedling weight among cultivars in treatments with 0.125 kg ai/ha metolachlor. However, when compared to the untreated control Padi Satu, Serasan Puteh, and Acheh 62 showed reduction in seedling weight compared to other cultivars.

Table 5: Effect of metolachlor treatments on Seedling dry weight of 13 rice cultivars (g)

Cultivars	Metolachlor Levels (kg ai / ha)			
	0 (Control)	0.125 kg ai/ha	0.25 kg ai/ha	0.5 kg ai/ha
MR 220	0.3021 def	0.3010 f	0.3010 ef	0.3010 ef
MR 219	0.3037 bcdef	0.3016 def	0.3013 def	0.3010 ef
MR 185	0.3020 def	0.3010 f	0.3010 ef	0.3010 ef
MR 84	0.3010 ef	0.3010 ef	0.3010 ef	0.3010 ef
Serendah Kuning	0.3054 abcde	0.3014 def	0.3038 abcdef	0.3021 def
Padi Satu	0.3063 abcde	0.3032 bcdef	0.3018 def	0.3010 ef
Acheh 62	0.3056 abcde	0.3033 bcdef	0.3034 bcdef	0.3021 def
Buntar B	0.3052 abcde	0.3031 bcdef	0.3029 cdef	0.3019 def
Sampangan B	0.3041 abcde	0.3014 def	0.3020 def	0.3010 ef
Lasak	0.3057 abcde	0.3017 def	0.3040 abcdef	0.3010 ef
Serasan Puteh	0.3035 bdef	0.3022 def	0.3029 bcdef	0.3010 ef
Rotan	0.3042 abcde	0.3010 ef	0.3023 def	0.3015 def
Bario	0.3034 bdef	0.30.10 f	0.3010 f	0.3010 ef

Data were transformed to Log (weight + 2) prior to statistical analysis.
Means with the same alphabet are not significantly different at $p \leq 0.05$ (Duncan's Multiple Range Test)

There were also no significant differences in seedling weight among cultivars in the untreated control. However, the value for tolerance index for seedling weight indicates that cultivars Serendah Kuning, Acheh 62, and Buntar B may have some mechanism for higher tolerance to the metolachlor herbicide compared to other cultivars in this study (Table 6), but the tolerance indices were less pronounced.

Table 6: Tolerance index based on seedling dry weight for cultivars receiving the metolachlor treatments.

Cultivars	Metolachlor levels (kg ai/ha)		
	0.125 kg ai/ha	0.25 kg ai/ha	0.5 kg ai/ha
MR 220	1.000	1.000	1.000
MR 219	1.001	1.001	1.000
MR 185	1.000	1.000	1.000
MR 84	1.000	1.000	1.000
Serendah Kuning	**1.001**	**1.009**	**1.004**
Padi Satu	1.007	1.003	1.000
Acheh 62	**1.008**	**1.008**	**1.004**
Buntar B	**1.001**	**1.006**	**1.003**
Sampangan B	1.001	1.003	1.000
Lasak	1.002	1.010	1.000
Serasan Puteh	1.004	1.006	1.000
Rotan	1.000	1.004	1.002
Bario	1.000	1.000	1.000

Tolerance Index = Relative dry weight compared to treatments with zero tolerance

CHAPTER 5

DISCUSSION

There were very few emerged seedlings in 0.5 kg ai/ha metolachlor treatments due to most rice cultivars had little or no tolerance to this treatment. The susceptible rice cultivars died right after emergence, as was also observed by Dilday (1988). However, when compared to untreated controls Sarawak cultivars Serendah Kuning, Buntar B, and Acheh 62 showed greater reduction in seedling emergence compared to other cultivars. The inhibitory effect of the herbicide relative to the untreated control was not detectable with the other cultivars (Bario, Rotan and MR cultivars), probably due to the very low or poor emergence of these cultivar even in the untreated controls.

There were more plants emerging in treatment with 0.25 kg ai/ha compared to 0.5 kg active ingredient per hectare treatment. There were significant difference between treatment 0.5, 0.25 and 0.125 kg ai/ha metolachlor and the controls for cultivars Serendah Kuning, Acheh 62, Buntar B, and Rotan due to the greater reduction in seedling emergence. Wei Zhang *et al.* (2000) also reported greater reduction in *Oryza sativa* emergence and plant dry weight with metolachlor applications.

There were significant differences in seedling height between treatment with 0.5, 0.25 and 0.125 kg ai/ha metolachlor and the untreateds Serendah Kuning, Acheh 62, and Buntar B These differences maybe due to genotype effects as elongation potential of the mesocotyl and coleoptile and tolerance is heritable and varies according to the genetic constitution of the germplasm (Dilday *et al.*, 1988).

Seedling weights in response to metolachlor were not significantly different among cultivars in all treatments. Differences in dry weights were not detectable compared to seedling height differences. This may probably be due to the small sample size, where dry weight differences were not clearly detectable.

In general the results of this study suggest that cultivars Serendah Kuning, Acheh 62, and Buntar B have some degree of tolerance to metolachlor.

CHAPTER 6

CONCLUSION

This preliminary study showed that of the 13 rice cultivars tested, Serendah Kuning, Acheh 62, and Buntar B have some degree of tolerance to metolachlor. Further research is needed to screen the vast collections of rice germplasm before any of these cultivars can be selected for transgenic studies.

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Appendix 1. ANOVA of data on seedling emergence of 13 rice cultivars.

Source	dF	SS	MS	F	Prob level
A: Rep	7	3.6417	0.5203	6.03	0.001**
B: Treat	3	49.6178	16.5393	191.75	0.001**
C: Cultivar	12	25.1654	2.0971	24.31	0.001**
BC	36	27.1820	0.7553	8.76	0.001**
Error	357	30.7925	0.0863		
Total (adjusted)	415	136.4073			

Data were transformed to Square root (X+2)

** Significant at alpha = 0.01

Appendix 2.ANOVA of data on seedling height of 13 rice cultivars.

Source	dF	SS	MS	F	Prob level
A: Rep	7	1.6220	0.2317	4.01	0.001**
B: Treat	3	21.6184	7.2061	124.77	0.001**
C: Cultivar	12	8.3083	0.6924	11.99	0.001**
BC	36	6.1784	0.1716	2.97	0.001**
Error	357	20.6187	0.0578		
Total (adjusted)	415	58.3458			

Data were transformed to Log (X+2)

** Significant at alpha = 0.01

Appendix 3. ANOVA of data on seedling dry weight of 13 rice cultivars

Source	dF	Sum of Squares	Mean Square	F	Prob level
Replicate	7	0.000056	0.000008	3.14	0.003**
Treatment	3	0.000442	0.000147	57.25	0.001**
Cultivar	12	0.000287	0.000024	9.28	0.001**
BC	36	0.000175	0.000004	2.89	0.002**
Errof	357	0.000918	0.000003		
Total (adjusted)	415				

Data were transformed to Log (X+2)

** Significant at alpha = 0.01

Appendix 4. Determinations of soil pH and soil texture

Soil pH:

pH in H_2O	pH in KCl
4.10	3.60

Soil texture:

Clay	27 %
Silt	25 %
Sand	48 %

From USDA texture triangle the soil texture for 48 % sand, 25 % silt and 27 % clay is sandy clay loam soil.

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Herbicide Tolerance in Selected Local Rice Cultivars" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus and published in the form approved by the Faculty.

Hussain Bin Tahir
Date: 30th April 2007