

SPATIAL DISTRIBUTION OF WEED SEEDBANK IN RICE
ECOSYSTEM AND ABANDONED LAND

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RICE ECOSYSTEM AND ABANDONED LAND**

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SPATIAL DISTRIBUTION OF WEED SEEDBANK IN RICE ECOSYSTEM AND ABANDONED LAND

ABSTRACT

Seed longevity and durability in the seedbank are important traits for weed survival especially for the escape and successive mechanism in the nature. This study was developed to investigate the spatial dynamics of seedbank in rice agroecosystem and in diverse abandoned agricultural land. The objectives of the study were: (i) to characterize vertical and horizontal seedbank weedy rice distribution pattern in rice agroecosystem; (ii) to determine seedbank distribution pattern at different types of abandoned agricultural lands in Jelevu, Negeri Sembilan; and (iii) to evaluate weed infestation potential from seedbank. The weedy rice seedbank survey was conducted in IADA Barat Laut Selangor (IBLS) rice granary at two locations, Sawah Sempadan and Sungai Burung, of different land preparation practices. A total of fifty soil cores at 20 cm depth were extracted from each location and weedy rice seeds were counted from every 5 cm soil core intervals to characterize the vertical weedy rice seedbank dynamic. The horizontal seeds distribution survey was conducted by randomly placed one hundred 10 cm² quadrat on the rice fields to count weedy rice shattered seeds on the ground. All collected seeds (vertical and horizontal) was germinated using standard germination test to evaluate the viability of the seeds. The density of weedy rice seeds at both locations has no significant difference vertically and horizontally. However, Sawah Sempadan displayed significantly higher germination rate for all soil depths indicating poor land preparation contributes to higher potential of weedy rice escape for successive season. Determination of seedbank distribution pattern in abandoned agricultural lands was conducted in Glami Lemi Biotechnology Research Center (GLBRC), University Malaya, Jelevu, Negeri Sembilan at 10 sites with three ecological types. A total of five soil samples were randomly

collected at each site from 5 cm deep top soil in a 10 cm² quadrat. The samples were put in a separate container, wetted with 10 ml distilled water and left in a room temperature with light. Germinated seeds were counted and determined for weed types (broadleaf and grass) at 3, 6 and 9 days after soaking. Another set of soil from the similar site for germination test was collected, placed in a 9 mm petri dish and air-dried for 3 days in the greenhouse. The presence of seed in the soil was counted under light microscope. The non-active sites showed the highest density of seedbank by direct seed count while the active sites with on-going agriculture activities displayed significantly higher germinated seedlings than other sites. Broadleaf weeds have higher germination rate as compared to grasses. Active land activities such as agriculture promoted weed emergence probably due to enhancement of soil suitability for seedbank germination. Abandoned lands especially from agriculture land will accumulate various weeds through seedbank. Reintroduction of these lands might provide suitable conditions for these weeds to re-emerge. The spatial distribution pattern of the weed seedbank from two agriculture landscapes of this study indicates; (i) proper land preparation will reduce weed infestation, (ii) soil depth might provide extra advantage for weedy rice seed to survive in the seedbank, and (iii) an abandoned land can be a pool for diverse weeds through seedbank. Therefore, weeds seed longevity and durability study in the seedbank need to be emphasized and strategized in the future to reduce weed infestation.

Keywords: land management; spatial distribution; weeds; weed management; weedy rice

TABURAN SPASIAL BAGI BANK BIJI BENIH DALAM AGROEKOSISTEM PADI DAN TANAH PERTANIAN TERBIAR

ABSTRAK

Kelangsungan dan ketahanan biji benih dalam bank biji benih adalah satu ciri-ciri penting bagi kemandirian rumpai terutama mekanisme lepas dan berturutan dalam alam semulajadi. Kajian ini dijalankan untuk mengkaji ruang dinamik bagi bank biji benih dalam agroekosistem dan pelbagai tanah pertanian terbiar. Objektif kajian ini adalah: (i) untuk mencirikan bentuk taburan benih secara menegak dan mendatar bagi agroekosistem; (ii) untuk menentukan taburan bank biji benih pada tanah pertanian terbiar yang berbeza di Jelevu, Negeri Sembilan; (iii) untuk menilai kebolehan infestasi rumpai dari bank biji benih. Tinjauan untuk bank biji benih bagi padi angin dijalankan di jelapang padi IADA Barat Laut Selangor (IBLS) pada 2 lokasi, Sawah Sempadan dan Sungai Burung, yang berbeza amalan penyediaan tanah. Sejumlah 50 teras tanah pada kedalaman 20 cm telah diambil dari setiap lokasi, kemudian biji benih padi angin direkodkan bagi setiap 5 cm jarak teras tanah untuk mencirikan dinamik bank biji benih secara menegak. Bagi tinjauan pengedaran biji benih secara mendatar, ia dijalankan secara rawak dengan meletakkan secara rawak 100 kali 10 cm quadran di atas sawah padi untuk mengira jumlah biji benih padi angin yang gugur di atas tanah. Kesemua biji benih (menegak dan mendatar) dicambahkan menggunakan ujian percambahan untuk menilai daya tahan benih. Ketumpatan biji benih rumpai untuk kedua-dua lokasi tidak mempunyai perbezaan untuk menegak dan mendatar, walau bagaimanapun Sawah Sempadan menunjukkan kadar percambahan signifikan yang tinggi bagi kesemua kedalaman tanah menunjukkan amalan penyediaan tanah yang teruk membantu meningkatkan potensi pelarian biji benih padi angin untuk musim yang berikut. Kajian pola taburan bank biji benih bagi tanah pertanian terbiar dijalankan di Pusat Penyelidikan Bioteknologi Glami Lemi (PPBGL), Universiti Malaya, Jelevu, Negeri Sembilan pada 10 tapak dibahagikan kepada 3 berbeza

ekologi. Sebanyak 5 sampel tanah diambil secara rawak pada setiap tapak dengan kedalaman 5 cm x 10 cm² quadrat. Sampel diletakkan di dalam bekas berasingan, dibasahkan dengan 10 ml air suling dan ditinggalkan di dalam suhu bilik dan bercahaya. Benih yang bercambah dikira dan ditentukan mengikut jenis (daun lebar and rumput) pada 3, 6 dan 9 hari selepas perendaman. Sejumlah sampel tanah yang lain dari tapak yang sama diambil untuk ujian percambahan, diletakkan di dalam 9 mm piring petri dan dikeringkan udara selama 3 hari di dalam rumah hijau. Kehadiran biji benih di dalam tanah di kira menggunakan mikroskop cahaya. Kawasan tidak aktif menunjukkan ketumpatan bank biji benih yang paling tinggi berdasarkan pengiraan terus biji benih, manakala kawasan aktif yang masih berlaku aktiviti pertanian pula mencatatkan percambahan yang tinggi berbanding tapak yang berbeza ekologi. Rumpai daun lebar mempunyai kadar percambahan yang tinggi berbanding rumput. Tanah aktif seperti tanah pertanian menggalakkan pertumbuhan rumpai berkemungkinan disebabkan penambahan kesesuaian tanah bagi percambahan bank biji benih. Tanah terbiar terutama tanah pertanian akan mengumpulkan pelbagai rumpai melalui bank biji benih. Pengenal semula tanah ini kemungkinan akan membekalkan kondisi yang sesuai untuk rumpai tumbuh semula. Pola sebaran spasial bank biji benih bagi kedua-dua tapak pertanian ini menunjukkan (i) penyediaan tanah yang bagus membantu dalam mengurangkan infestasi rumpai; (ii) kedalaman memberikan kelebihan ekstra untuk benih padi angin untuk mandiri di dalam bank biji benih; dan (iii) tanah terbiar boleh menjadi takungan untuk pelbagai rumpai melalui bank biji benih. Oleh itu, kelanjutan usiada ketahanan benih rumpai perlu diberi penekanan dan strategi di masa depan untuk mengurangkan infestasi rumpai.

Kata Kunci: pengurusan tanah; ruang dinamik; rumpai; pengurusan rumpai; padi angin

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CHAPTER 1: INTRODUCTION

1.1 History of Agriculture in Malaysia

Agriculture is one of the major key sectors in Malaysian economy that contributes about 8.3% of Gross Domestic Product (GDP) (Department of Statistics Malaysia, 2017). Agricultural sector was broken down into several subsectors: industrial crops, food crop, and other miscellaneous crops that continue to supply variety of commodity for both domestic consumption and export production. The importance of agricultural sector in Malaysia can be traced back during the early decade after gaining sovereignty as a primary driver for Malaysian economy due to better net returns for export commodity on industrial crops such as rubber and palm oil. However due to the shifting of policy, Malaysia as a developing country started to venture and invest more heavily into industrial sector such as steel production, automobile, electrical and electronics. Industrial sectors were envisioned by Mahathir Muhammad, the fourth prime minister of Malaysia as a way forward for this budding nation to continue to prosper and propels itself towards modernization.

Despite the enthusiasm, Malaysia financial crisis in 1997 coupled with global economic depression dealt a heavy blow toward industrial sector and economy indirectly. During this hard time, agriculture sector had contributed RM 16.9 billion in 1995 up to RM 18.3 billion in 2000 (Eighth Malaysia Plan) shielding the economy from worsen, which later reintroduce government interest to highlight the importance of this sector (Department of Statistics Malaysia, 2017).

1.2 Food Security in Malaysia

As Malaysia continued their effort to empower agriculture sector, there were enormous increase in the land use for industrial crop plantation for export profits. However, there is

minimal growth in land use for food crop such as paddy fields. Upon deeper inspections, land competition between lucrative cash crop such as palm oil and rubber causing minimal increase in land used for paddy plantation area from 644,873 hectares in 1987 to 684,111 hectares in 2012 due to high revenue returns for the economy (Othman & Jafari, 2014). This can be observed in Sarawak where the palm oil plantation grew 23-fold for planted area in comparison to paddy plantation that dwindled about 24 percent of planted area during the same period (Norhidayu *et al.*, 2017). Although these problems were eased by the increase in paddy yield for all three locations (Peninsular Malaysia – 3.192MT/ha, Sabah – 2.084 MT/ha and Sarawak – 1.702 MT/ha) (Department of Statistics Malaysia, 2017), this advantage were soon starting to diminish as the nation population started to grow bigger resulting in higher consumption rate. It was reported that Malaysian population grew about 73% from 15.67 million populations in 1985 to 32.02 million in 2018 (Department of Statistics Malaysia, 2017). These situations caused Malaysia to end up relying to imports to satisfy and fill the gap between production and domestic consumption of rice.

Above scenario worsen when food crisis hit Malaysia in 2008. The cheap imported rice that were brought in for the past decades come to a halt as the price started to rise in 2005 and continue to soar in 2007 and 2008. Control were announced and causing increase in domestic price mainly by exporting nations such as Vietnam and India introduced export reductions followed by China and Cambodia during the same period of early 2008 (Tey, 2010). Thailand were struck by flooding disaster causing rice price in the nation to increase up to a maximum 30% due to the significant decrease in rice production (Fahmi *et al.*, 2013). Rice riot in Vietnam also contributes to the control restriction (Wong *et al.*, 2009). Furthermore, major rice importing nations were deeply stricken by lack of supply such as Philippines ended up forced to purchase from other importing nation at a higher price to compensate. This alerting other importing nation and

battled to secure enough supply for domestic consumption and stockpile for their respective nations. Malaysia, for example, announced plans in mid-January 2008 to increase their stockpile levels as a precaution steps from 92,000 tons to 550,000 tons (Dawe, 2012).

Learning from above experience, Malaysian government set up an objective to secure a safe self-sufficiency level (SSL) as an index to food security especially rice as it is the nation main staple food. SSL was targeted around 65 percent in 3rd Agricultural Plan (1998-2010) and increase to 70% under the new Agro-Food Policy (2011–2020). To ensure that staple food supply especially rice achieve its target for the nation, various plan have been concocted and ongoing: various form of subsidies, such as price subsidy, fertilizer subsidy and cash aid for rice farmers; price control for stability directly under government; development of important infrastructure for farming such as irrigation system; as well as proper farm mechanization projects have been introduced. Malaysia government also attempt to increase rice production is by the adoption of more scientific method throughout the production line from seeds to post harvest. The research not only centered on development of high yield and quality rice, but also emphasizes on optimal utilization of resources by developing cost efficient, sustainable and easy system for farmers to employ. Other than that, the research includes key pest and disease management, post-harvest technology and proper product development.

1.3 Rice Industry in Malaysia

One of the major problems encountered by Malaysian government that lead to substantial loss of yield of rice quality and quantity were caused by the infestation of weeds primarily weedy rice. Poor land preparation, inappropriate herbicide timing, application of herbicides, and late flooding after sown are some of the issue that could lead to increase on rice weed infestation problem. Weedy rice can compete with cultivated

rice for nutrients, space, light, and water, thus high risk on rice growth and production. Furthermore, certain weed species possess allelopathy mechanism that could hamper the surrounding species growth that associated with them, bringing yield down to 96% (Mesquita, 2017).

Lack or unsuitable implementation of weed control program can caused serious infestation issue for farmers. When weed seeds were shattered from their mother plant, the seeds fall on the top soil thus capable to enter the existing seedbank through several entryway. Dispersal agent such as wind, water, animal and human activities; like tillage and harvesting could facilitate weed seed recruitment into the field. For example, dispersal of weed seed can occurs horizontally through animals (Zhou *et al.*, 2018)

Weed seedbank establishment were known to safeguard weed seed from many detrimental elements towards the seeds such as harsh environmental conditions, sudden changes in weather (Rao *et al.*, 2017), and control methods (herbicide, burning) thus allowing the seed to remain viable for longer period. This can be advantageous for the weed plant population survival to diversify its genetics to evade selection pressure and resulting in various combination of genetic makeup when it starting to emerge making the management of weeds harder than before. (Rao *et al.*, 2017). Plenty of weed seedbank studies focused more on seed longevity in the seedbank and density of different species in the seedbank throughout the world and Malaysia especially where there are insufficient studies on topic of seedbank itself. There is also lack of information on the issue of the aboveground vegetation and how does it reflect on the composition and distribution of weed seed in the soil seedbank.

1.4 Problem Statement

Weedy rice (*Oryza sativa* L.) is a problematic weed in Malaysian rice agro-ecosystems which seed longevity and durability in soil seed bank is one of the key traits for its

survival. Integrated weedy rice management strategies should include effective approaches to minimize the weed soil seed bank size, buildup and longevity. Weed seed aging/longevity has been widely studied but there is lack of information not only between the natural variation in weed seed bank longevity and the distribution pattern and spatial dynamics of weed seed in the seedbank but also its relationship with the aboveground weed community.

Although contaminated crop seeds used by farmers for crop cultivation are one of the main issues that could trigger the start of weed infestation, shattered seeds from the mother plant that exist on the field are more likely the cause of infestation due to high concentration of weed seedbank and continuous buildup from earlier shattered seeds (Zhang *et al.*, 2014). Some of newly shattered weed seeds can remain viable and dormant in the seedbank for more than 2 years, while Zhang *et al.* (2014) also reported up to nine years or even more. This can be problematic as many farmers does not possess or ignorant of the importance of managing the seedbank. A constant addition of shattered weed seed into the seedbank resulting in increase the size, ultimately causing the population of weeds to increase while growing alongside cultivated crop, thus hinder the process of weed control management (Zhang *et al.*, 2014). Understanding the dynamics of weed seedbanks is an important initial step to take for better a framework in devising weed management plans (Nichols *et al.*, 2015). By understanding the general essential overview and weed seed persistency in the seedbank and how these knowledges of seedbank can be related to the aboveground vegetation community, an agriculturist could create most suitable weed management programs. Furthermore, predictive overview of the future can be made for preemptive weed control for infestation problems. These connections make understanding the weed seedbank considerably more important for expanding the proficiency of weed management. Therefore, this project is developed to

study the seedbank dynamics of weedy rice in rice agro-ecosystems and weed in abandoned agriculture land.

1.5 Objectives

This study was developed to investigate the spatial dynamics of seedbank in rice agro-ecosystem and in a diverse abandoned agricultural land. The objectives of the study were: (i) to characterize weedy rice vertical and horizontal seedbank distribution pattern in rice agro-ecosystem; (ii) to determine seedbank distribution pattern at different types of abandoned agricultural lands in Jelebu, Negeri Sembilan; and (iii) to evaluate weed infestation potential from seedbank.

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CHAPTER 2: LITERATURE REVIEW

2.1 Agriculture in Malaysia

Malaysia is a country situated in Southeast Asia with a total land area of 329,758 square kilometers and it is divided into 2 regions with almost similar in size which is peninsular Malaysia, a region connected to the mainland Asia continent neighboring Thailand in the northern part of peninsular, Singapore to the south across the Johor straits and Sumatra (Indonesia) to the west across Malaccan straits while east Malaysia (Borneo) region borders with Brunei and Kalimantan (Indonesia). overall, Malaysia is divided into 13 states, 11 located on the peninsular parts and another 2 on the island of Borneo. Malaysia boast around 28 million population and the labor force makes up about sixty eight percent of the total population in Malaysia (Department of Statistics Malaysia, 2017). Out of the total, agriculture sector takes up about 11 percent of the labor force that considered the third important key divers in Malaysian economy which contributes 8.3% of Gross Domestic Product (GDP) (Department of Statistics Malaysia, 2017).

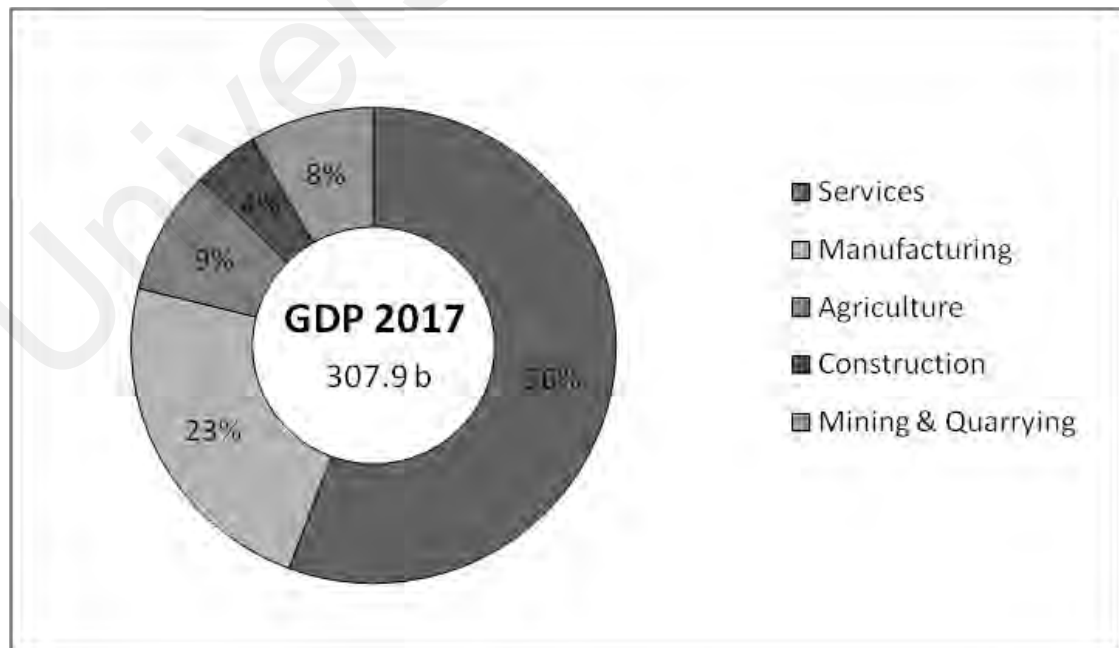


Figure 2.1 Percentage of Malaysia GDP on 2010
*Source: Department of Statistics Malaysia, 2017

Malaysian economy can be subdivided into 3 key sectors. In 2017, service sector compromise about 56% of total GDP followed by manufacturing at 23% and agricultural sector at 9% (Department of Statistics Malaysia, 2017; Figure 2.1). Agriculture is a significant sector in Malaysia. Since independence, this sector has been the foundation of Malaysian economy not only due to its contribution towards growth and prosperity but also by providing important staple food for the nation population.

Malaysian agriculture can be subdivided into 3 subsectors: industrial crops, food crop, and other miscellaneous crops. Industrial crop mostly defined as plantation-based crop with great focused given on crops that generate income, specifically oil palm, cocoa, tobacco and rubber which then served as export market. In addition, sizeable area is also devoted for such food crops like rice, fruit orchards (banana, coconut, mango), livestock and vegetables. lastly, other miscellaneous crops such as orchids, ornamental plants and other herbal plant that caters both export and domestic markets. Several key factors that will continue to shape the future of Agriculture sector in Malaysia include a) availability of natural resources and inputs, b) competition for land and land degradation, c) advances in science and technology, d) urbanization, e) trade liberalization and f) commercialization of agriculture and international agreements (PEMANDU, 2016).

2.2 Rice Industry in The World and Malaysia

Three leading food crops which are rice, wheat and maize supply the world with approximately provide more than 2891 kcal per capita per day ($\text{kcal cap}^{-1}\text{d}^{-1}$) for human population (Meyfroidt, 2018). In 2015, human population consumed 78% of total rice produce around the world, followed closely by wheat which reported at 64% and lastly maize at 14% of world total production (FAOSTAT, 2018). Statistics above shows that rice is the most important food source for human population around the world especially towards people in third world countries such as Vietnam and Thailand. However, given

that rice consumption is spread among third world countries, lowest income countries more likely to consumed relatively little wheat.

Rice is the commonplace staple food for half of the world population especially in Asia region, which is around 3.5 billion people depend on rice as primary food consumption around 100 kg of rice. (Friedrich *et al.*, 2017). Asia region recorded the highest percentage of consumption of rice which at 90% and the total number of demands are expected to continue grow. However, other regions other than Asia such as Africa, rice is the fastest growing demand and one of the fastest growths in Central and Latin America.

Rice consumption will continue to remain strong, caused by ever increase population growth and economic expansion especially in many developing Asian and African region. On average, 155.5 million hectares were used as rice field around the world with an average growth around 0.39% a year based on the last trends in rice industry over 30 years (Friedrich *et al.*, 2017). In many developing Asian and African nation, global rice consumption continues to rise, strongly backup by economic growth and population boom. On average, the world use around 155.6 million ha with an average growth rate of 0.4% a year, in the last 30 years for rice production (Friedrich *et al.*, 2017).

The biggest producers of rice are China with a production of 144,560 million tons of goods produce while the second spot belong to India which has produce about 104,800 million tons of rice. China and India together produce around 250,000 million tons of rice produce which are the 50% of world rice production (Figure 2.2). Although China dedicated most of their resource in total grain output toward rice production, India could keep their production on high level by large agricultural land used by rice production and cheap manual labor for work. Indonesia, Bangladesh and Vietnam also considered as one of the biggest rice producers in the world while Japan produce only 7842 million tons. Most rice producing nation are in Asia region and only Brazil, south American nation produce rice around 8,465 million tons for consumption.

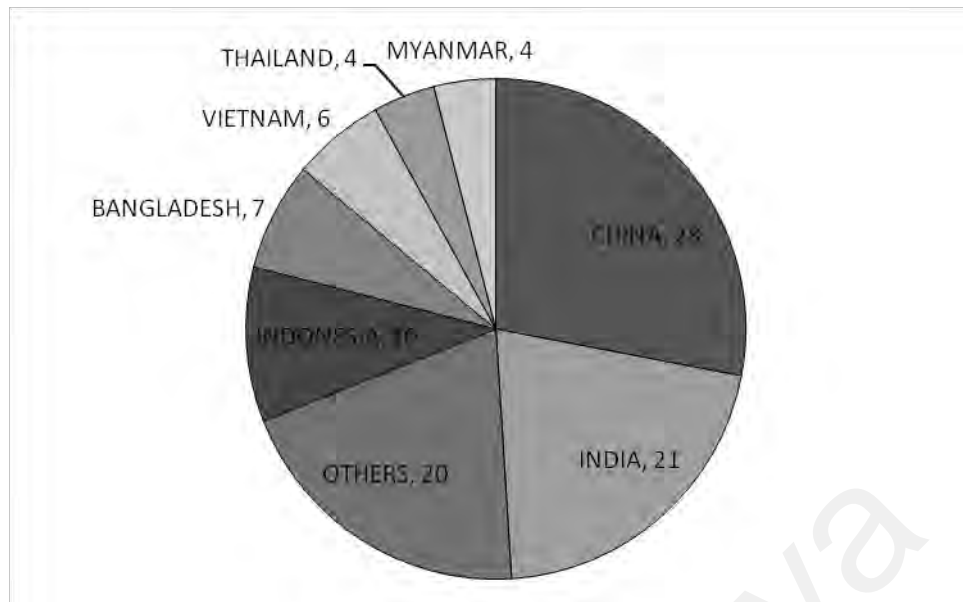


Figure 2.2: World Main Paddy Producers, 2014

*Generate from World Paddy Producer of Foreign Agricultural Services of United States Department of Agriculture (USDA) in www.fas.usda.gov/psdonline on January 2017

For Malaysia, 514,381 hectares of land use in Peninsular and Borneo Island registered about 164,858 hectares primarily just for rice farming (Table 2.1). On average, Malaysia population consumed about 82.3 kilograms of rice each year. As for rice production, the average only recorded at 3.0 tons per hectares (Department of Agriculture Malaysia, 2017). Thus, the agriculture sector could only satisfy the local consumption demand around 60-65% of total required causing a shortage in the rice market. So, this put Malaysia as a net importer nation as it still needs import rice from other countries, like Vietnam, Thailand, Pakistan and China. Malaysia has several granaries around the country such as Seberang Perai in Penang (1 300 ha), Seberang Perak in Perak (9 510 ha), Kerian in Perak (24 010 ha), Kemubu in Kelantan (32 400 ha), Besut in Terengganu (5 100 ha), Sungai Manik in Perak (6 510 ha), Projek Barat Laut Selangor (PBLs) (19 920 ha) and the largest one which is located in Muda, Kedah (98 860 ha).

Table 2.1: Hectarage of overall planted paddy area by state, Malaysia, 2005-2014

States	Year	
	2005	2014
Johor	2,885	2,976
Kedah	210,036	212,401
Kelantan	66,411	69,412
Melaka	1,095	2,608
N. Sembilan	1,684	2,070
Pahang	6,561	11,872
Perak	82,085	81,503
Perlis	49,203	52,088
P. Pinang	25,344	25,564
Selangor	37,180	37,842
Terengganu	17,004	16,045
Pen. Malaysia	499,488	514,381
Sabah	40,117	41,387
Sarawak	127,218	123,471
Malaysia	666,823	679,239

*Generate from hectarage of paddy area of Foreign Agricultural Services of United States Department of Agriculture (USDA) in www.fas.usda.gov/psdonline on January 2017

Rice provide 3 million Malaysian population two third of their caloric intake daily making it an important staple food crop for large chunk of the population. Different from the cash crop industry such as rubber and palm oil, rice industry in Malaysia are protected due to food security arguments. Coupled with rapid rises in demand and continuous growth of population, the future looks bleak due to decrease in supply of food. This situation can be problematic to this country as it leads to needs to import rice from other countries to satisfy the ever-growing market demand for population consumption. This also caused Malaysia to be vulnerable to food crisis caused by sudden changes such as disease outbreak, unpredictable weather and many more.

Rice is a major staple food for majority of household in Malaysia. Considering its importance in economic and wellbeing of the society and in a bigger sense country, it is certain that the government has been playing and monitoring this sector more than others. throughout the years, the government has listed 3 main objectives of different rice policies that need to be fulfilled to safeguard our household staple food. the first one is to safeguard food security followed by increase in food crop productivities and lastly to

establish a reasonable price for all food supply. Malaysian government concerned at the already poor farmers would suffer if weedy rice infestation problem continues to persist resulting in low production yield. Land preparation, water depth, lack of weed control program, improper timing of seeding and herbicide application are some of the factor that can encourage weedy rice infestation. Rice crop low productivity can be attributed to several factor, lack of usage of newer technology, erratic weather, quality of seeds and soil, low manpower available for management and lack of maintenance of agricultural infrastructure (Baki, 2004; Fisher & Connor, 2017). There are also other contributing factors such as insect pest, animal and disease outbreaks that were cause by lack of biosecurity measure.

To ensure that staple food supply especially rice achieve its target demand for the nation consumption, various policies have been concocted and already ongoing: various form of subsidies for struggling farmers, such as price subsidy, fertilizer subsidy and cash aid for rice farmers; price control for stability directly under government; development of important infrastructure for farming such as irrigation system; as well as proper farm mechanization and technological projects have been introduced. Malaysian government introduces price subsidy as a strategy to help already poor farmer by providing subsidy for fertilizer and seeds for their farming use.

Malaysia government also attempt to increase rice production is by the adoption of more scientific method throughout the production line from seeds to post harvest. The research not only centered on development of high yield and quality rice, but also emphasizes on optimal utilization of resources by developing cost efficient, sustainable and easy system for farmers to employ. Other than that, the research includes key pest and disease management, post-harvest technology and proper product development at MARDI (Table 2.3).

Table 2.2: List of cultivars in Malaysia

Cultivated Rice	Year Released	Cultivated Rice	Year Released
Malinja	1964	MR 81	1988
Mahsuri	1965	Pulut Hitam 9 PH9	1990
Ria (IR8)	1966	MR 103	1990
Bahagia	1968	MR 106	1990
Murni	1972	MR 123	1991
Masria pulut	1972	MR 127	1991
Jaya (C4-63)	1973	MR 159	1995
Sri Malaysia I	1974	MR 167	1995
Pulut Malaysia I	1974	MR 185	1997
Sri Malaysia II	1974	MRQ 50	1999
Setanjung	1979	MR 219	2001
Sekembang	1979	MR 220	2003
Kadaria	1981	Maswangi MRQ 74	2005
Pulut Siding	1981	MR 232	2006
Seberang	1984	MRM 16	2010
Manik	1984	MR 220 CL1	2010
Muda	1984	MR 220 CL2	2010
Makmur	1985	MR 253	2010
MR 84	1986	MR 263	2010

*Note: this table was constructed based on MARDI 2011 Yearly Report accessed from www.moa.com.my on January 2017.

Currently, Malaysia only able to satisfy up to 72% of its population demand for rice crop production (Ismail & Ngadiman, 2017). However, Malaysia still need to rely on rice imports from other rice exporting countries even after steps that had been taken to boost its rice industry (Table 2.4). This predicament worsens when there is a rice crisis where many rice exporting nations such as Vietnam and India introduced export restriction followed by China and Cambodia at same period of early 2008 (Tey, 2010).

Table 2.3: Total imported rice on 2017 by top rice importer countries in the world

Importer	2017 Rice Imports	% world Total
China	US\$1.8 billion	8.5%
Benin	\$1.1 billion	5%
Saudi Arabia	\$1 billion	4.8%
Iran	\$853.9 million	4%
Bangladesh	\$734.3 million	3.4%
United States	\$727.7 million	3.4%
UAE	\$593.5 million	2.8%
United Kingdom	\$546.6 million	2.5%
South Africa	\$513.3 million	2.4%
Iraq	\$512.2 million	2.4%

Table 2.3, continued.

Importer	2017 Rice Imports	% world Total
France	\$474 million	2.2%
Ivory Coast	\$423.5 million	2%
Mexico	\$408.1 million	1.9%
Senegal	\$382.6 million	1.8%
Japan	\$358.1 million	1.7%
Germany	\$352.7 million	1.6%
Malaysia	\$343.6 million	1.6%

*Generate from Imported Rice of Foreign Agricultural Services of United States Department of Agriculture (USDA) in www.fas.usda.gov/psdonline on January 2017

2.3 Weeds in Malaysia

In general, weeds are plant whose undesirable traits outweigh its benefits in designated area. For poet Ralph Waldo Emerson in one of his literary works *Fortune of the republics*, states that a weed was merely a “plant whose virtues have not yet been discovered”. Weeds also describe as a competitive, persistence, and interfere negatively with human activity such as agriculture crops (Peterson *et al.*, 2016). Thus, weed is a pest because it competes with crops for nutrients such as sunlight, water, air and space ultimately causing negative effect towards the crop (Mortensen *et al.*, 2012). For farms and plantations, weed management control is an important step to minimize commercial loss caused by reduced crop production. Many weed species colonized roadsides and have been kept in check by regular mowing (Kiew & Tan, 2014).

Although no single unarguable statement can be used to clearly define “weed”, most weed scientists agree that weeds are not desirable. This array of definitions gives a signal to weed scientists and vegetation workers to have a good consideration in equating which plants to be controlled in human-related environments (Neher, 2018). Most weeds species develop a special mechanism for swift dispersal of seeds. Certain weed species have developed a special structure such as exploding seed pod to send it further away from the mother plant (Ogudchen *et al.*, 2018). There is also other structure that give advantage against other crop such as ability to cling, fly, or float. Animals such as birds and small rodents can spread weed seeds when eating fruit produced and deposited the seed in their

manures (Ogudchen *et al.*, 2018). Contaminated weed seed, straw, and grain can also become a dispersal agent for weed seeds.

Weeds generally characterized by their abilities that allow the survival of their own species. Most weeds possessed the ability to produce an abundant amount of seed, rapid population establishment compared to cultivated crops, high seed dormancy and survival of seed in the soil (longevity) for long term strategies, adaptation for far and wide spreading, abilities to reproduce through vegetative parts and lastly ability to occupy (infestation) sites (Mispan *et al.*, 2019). The most accepted impact of weed infestation is by decrease in production yields by clashing with cultivar for necessities (water, soil nutrients, light and space). Other than that, weeds also are said to reduce crop quality by contamination the commodity (weedy rice), interfere with harvesting programs, can act as a host for many kinds of crop disease by providing host for pathogens and also provides shelter for insect (pest), and weeds capable to produce chemical that which are toxic to animals, humans and crops alike.

Despite the negative connotation surrounding weeds, certain weeds do bless the environment in certain ways. Some weeds can turn into habitat for important microbe as well as food source for animal to feed such as pollen supply for bees. It also promotes soil stabilization and an active source of organic matters for the soil as they continue to grow and eventually die, and some weed can be used for human consumption. Moreover, weed also act as a cover for land and this can act as a protection from wind and water erosion. Last but not least, weed can be our natural diagnostic tool to inform us of the condition and nutritional balance of the soil. Lastly, some weed can break hard soil deadpan and control erosion. In conclusion, weeds have a controversial nature as it has many sides to look at. But in this context, the negative impacts of weeds indirectly affect all living beings.

Today, the Malaysian agro-ecosystems are homes of more than 500 invasive weed species including nine of the world's worst weed species (Heap, 2014). Several of these weeds are categorized as scheduled pests under the Malaysia Plant Quarantine Act 1976 and Plant Quarantine Regulation 1981. These naturalized plants have decorated our roadsides, backyards and public area (Table 2.4). The notorious one turns into nuisance as it primarily clogging our drains, polluting our fields, reducing our agriculture produce, interfere existing ecosystem and ultimately able to change the local landscape forever.

Table 2.4 Some invasive species in Malaysian agro-ecosystems (Citation report table is derived from Baki, 2006, Universiti Malaya Press, Copyright University of Malaya Press.)

Invasive species	Code ^A	Mode of spread ^B	Origin
<i>Acacia mangium</i> Willd.		S	Australia
<i>Ageratum conyzoides</i> L.	AGECO	S	Tropical America
<i>Alternanthera philoxioides</i> (Mart.) Griseb ^{# a}	ALRPH	C/S	Tropical America
<i>Amaranthus lividus</i> L.	AMALI	S	East Asia
<i>A. spinosus</i> L.	AMASP	S	Unknown
<i>Azolla pinnata</i> R. Br	AZOPI	C/S	Tropical Asia
<i>Asystasia intrusa</i> Blume	ASYIN	C/S	India
<i>Borreria alata</i> (Aubl.) DC	-	S	Tropical America
<i>Brachiaria mutica</i> (Forsk.) Stapf.	-	C/S	Tropical Africa
<i>Chromolaena odorata</i> (L.) King and Robins	EUPOD	C/S	Africa
<i>Cleome rutidesperma</i> DC	CLERT	S	West Tropical Africa
<i>Clidemia hirta</i> (L.) D.Don.	CXAH	S	Tropical America
<i>Cordia curassavica</i> Jacq.) Roem. And Schult	CRHCY	S	Tropical America
<i>Crassocephalum crepidioides</i> (Benth.) S.Moore***	CRSCR	S	Tropical Africa
<i>Croton hirtus</i> L'Herit	CVNHI	S	Tropical America
<i>Cuscuta australis</i> R. Br.	CUCAV	C/S	?
<i>Cynodon dactylon</i> (L.) Pers.	CYNDA	C/S	Asia/Africa
<i>Cyperus digitatus</i> Roxb.	CYPDG	S	Tropical Asia?
<i>C. difformis</i> L.	CYPDI	S	Old World Tropics
<i>C. esculentus</i> L.	CYPES	C/S	India?
<i>C. iria</i> L.	CYPIR	C/S	Asia
<i>C. kyllingia</i> Endl.	CYPKY	S	Asia
<i>C. malaccensis</i> LAM	CYPMA	C/S	South East Asia
<i>C. pilosus</i> Vahl.	CYPPI	C/S	Asia
<i>C. rotundus</i> L.	CYPRO	C/S	India
<i>Digitaria ciliaris</i> (Retz.) Koel.	-	C/S	Taiwan
<i>D. setigera</i> R. and S.	-	C/S	Tropical Asia
<i>D. ternata</i> (A.Rich.) Stapf.	DIGTE	C/S	Tropical Asia

Table 2.4, continued.

Invasive species	Code ^A	Mode of spread ^B	Origin
<i>E. crus-galli</i> ssp. <i>formosensis</i> P. Beauv. Ohwi	ECHCS	S	Asia, Taiwan
<i>E. glabrescens</i> Munro ex. Hook.f./Kossenko	ECHGL	S	Old Tropics, India?
<i>E. oryzicola</i> Vasing	ECHCR	S	Old Tropics
<i>E. stagnina</i> (Retz.) P. Beauv.	ECHST	C	Africa
<i>Eclipta Prostrata</i> L.	ECLAL	S	Unknown?
<i>Eichhornia crassipes</i> (Mart.) Solms.	EICCR	C/S	South America
<i>Eleocharis acicularis</i> (L.) Roem. And Schult ^{#a}	ELEOC	S	Northern Hemisphere
<i>Eleusine indica</i> (L.) Gaertn.**	ELEIN	S	South America
<i>Eragrostis pilosa</i> (L.) P. Beauv.	ERAPI	S	Old World Tropics
<i>Erechtites Valerianaefolia</i> DC.	EREVA	C/S	Tropical America
<i>Erigeron sumatrensis</i> (Retz.) Walker	-	S	Tropical America
<i>Eriochloa polystachya</i> H.B.K.	-	C/S	Tropical America
<i>Euphorbia heterophylla</i> L.	EPHHL	S	Tropical America
<i>Fimbristylis dichotoma</i> (L.) Vahl.	FIMDI	C/S	Southeast Asia
<i>F. globulosa</i> (Retz.) Kunth.	FIMGL	C/S	Southeast Asia
<i>F. miliacea</i> (L.) Vahl*	FIMMI	C/S	South America
<i>Fuirena umbellate</i> syn. <i>F. quinquangularis</i> Roxb.	FUICI	S	Unknown
<i>Gleichenia linearis</i> syn. <i>G. dichotoma</i> Hook.	GLCDI	C/S	Tropical Asia
<i>Hydrilla verticillata</i> , (L.F.) Casp./Royle	HYLLI	C/S	Asia
<i>Hymenachne acutigluma</i> , (Stued.) Gilliland	-	C/S	India
<i>Hyptis capitata</i> Jacq.	HYPCA	S	Tropical America
<i>Imperata cylindrica</i> (L.) P. Beauv./Raeusch	IMPCY	C/S	Tropical Asia
<i>Ipomoea aquatica</i> Forssk.	IPOAQ	C/S	Southeastern Asia
<i>Isachne globosa</i>	-	C/S	Tropical Asia
<i>Ischaemum rugosum</i> Salisb.	ISCRU	C/S	South East Asia
<i>Lantana camara</i> L.	LANCA	S	Tropical America
<i>Leersia hexandra</i> Sw.	-	C/S	Tropical America
<i>Lemna purpusilla</i> syn. <i>L. minor</i>	LEMMI	C	Unknown
<i>Leptochloa chinensis</i> (L.) Nees.	LEFCH	S	Tropical Asia
<i>Limnocharis flava</i> (L.) Buchenau*	-	C/S	Tropical America
<i>Lindernia crustacea</i> (L.) F. Muell	LICR	C/S	Tropical Asia
<i>Ludwigia adscendens</i> (L) Hara	LUDAC	C/S	Tropical Asia
<i>L. hyssopifolia</i> syn. <i>L. linifolia</i>	LUDLI	S	Tropical America
<i>Lygodium flxuosum</i> (L.) Sw.	LYFFL	S	Old World Tropics
<i>Marsilea minuta</i> L.	MASMI	C/S	Unknown
<i>M. crenata</i> Presl.	MASCR	C/S	Unknown
<i>Melastoma malabathricum</i> L.	MESMA	S	Asia
<i>Melochia corchorifolia</i> L.	MEOCO	S	Malesia
<i>Mikania micrantha</i> H.B.K.	MIKMI	C/S	South America

Table 2.4, continued.

Invasive species	Code ^A	Mode of spread ^B	Origin
<i>M. pudica</i> L.	MIMPU	S	Tropical America
<i>Murdannia nudiflora</i> (L.) Drennan.	MUDNU	C/S	Unknown
<i>Myriophyllum aquaticum</i> (Vell.) Verc. ^{# a}	MYPBR	C/S	Tropical America
<i>Nephrolepis biserrata</i> (Sw.) Scott.	NEHBI	C/S	Old World Tropics
<i>Nymphoides indica</i> (L.) O.K.	NYPIN	C/S	South America
<i>Oldenlandia corymbosa</i> L.	OLDCO	S	Unknown
<i>Oryza sativa</i> L. (weedy rice)	ORYSA	S	Southeast Asia
<i>Ottochla nodosa</i> (Kunth) Dandy	-	C/S	Southeast Asia
<i>Panicum repens</i> L.	PANRE	C/S	Asia
<i>Paspalum conjugatum</i> Berg.	PASCO	C/S	Tropical America
<i>P. distichum</i> L.	PASDS	C/S	Unknown
<i>P. vaginatum</i> syn. <i>P. virginatum</i> L.	PANVI	C/S	Unknown
<i>Pennisetum polystachion</i> (L.) Schult. [#]	PESPO	C/S	Tropical Africa
<i>P. setosum</i> (Sw.) L. Rich. [#]	PESSE	C/S	Tropical Africa
<i>Pistia stratiotes</i> L.	PIIST	C/S	Unknown
<i>Rhynchospora corymbosa</i> L. Britt.	RHCAUS C/S	C/S	Unknown
<i>Rottboellia cochinchinensis</i> (Lour.) W.D. Clayton [#]	-	C/S	India Tropical
<i>Sagittaria guyanensis</i> H.B.K.	SAGGU	C/S	Africa/Southeast Asia
<i>Salvinia cucullata</i> Aubl.	SAVMO	C	South America
<i>S. molesta</i> D.S. Mitchell	-	C	South America
<i>S. natans</i> (L.) All. ^{# a}	SAVNA	C	Old World
<i>Scirpus grossus</i> L.	SCPGR	C/S	South East Asia
<i>S. juncooides</i> Roxb.	SCPJU	C/S	Asia
<i>S. mucronatus</i> L.	SCPMU	C/S	Asia
<i>Scleria sumatrensis</i> Retz.	SCLSU	C/S	South East Asia
<i>Sphenoclea zeylanica</i> Gaertn. [*]	SPDZE	S	Tropical Africa
<i>Stenochlaena palustris</i> Bedd.	-	C/S	Asia
<i>Utricularia speciosa</i> Vahl.	-	C/S	Asia

* Resistant to 2,4-D; ** Resistant to glyphosate; *** Resistant to paraquat; A Bayer code; ^B C – Clonal growth; S-seeds/spores; # Scheduled pests under Plant Quarantine Act 1976 and Plant Quarantine Regulation 1981; ^a Detected but not attained invasive status.

2.4 Weedy rice biology and features

Weedy rice which is also known as red rice in some parts of the world due to its red pericarp belongs to the same genus (*Oryza*) as cultivated rice. It is one of the weeds alongside *Echinochloa* spp., *Leptochloa chinensis* (L.) Nees, *Ischaemum rugosum* Salisb,

Paspalum spp, *Digitaria ciliaris* (Retz.) Koeler, and *Cynodon dactylon* (L.) Pers that seriously infest rice field reducing the productivity and quality of cultivated rice. Weedy rice is known to exhibit high phenotypic and genetic diversity even among themselves (Tseng *et al.*, 2018). Habitat location and ecotype are able to effect weedy rice diversity which can be seen in Asia (Thailand, Sri Lanka, Malaysia, Vietnam and Philippines) where each weedy rice exhibit variability in seed characteristic and growth response towards cultivated rice from different region (Chauhan & Johnson, 2010). For example, weedy rice in Malaysia has a high morphological variation that can be categorised into four different group. In Vietnam, weedy rice was observed to have higher potential growth while in Philippines, yield produce by weedy rice were found out to be the highest and also weedy rice in Thailand was the shortest (Perera *et al.*, 2012).

Natural crossing between cultivated rice and wild rice can generated many different types of weedy rice in the region they grew sympatrically (Saha *et al.*, 2014). Wild species, such as *O. rufipogon*, *O. nivara* and *O. longistaminata* and cultivated species such as Asian rice (*O. sativa*) and African rice (*O. glaberrima*) share genome 'AA' thus can be easily crossed with other species of cultivated *O. glaberrima* and *O. sativa* species (Olajumoke *et al.*, 2016).

Weedy rice was discovered and recorded for the first time in United States of America (USA) in 1846 (Londo & Schaal, 2007). There is also another study that states weedy rice first brought into USA as a contaminant from imported rice seed at much earlier date (Delouche *et al.*, 2007). For South East Asia, weedy rice infestation only reported around three decades ago where the first report in Malaysia, Philippines and Vietnam was in 1988, 1990 and 1994, respectively (Mortimer *et al.*, 2000). Weedy rice is one of threat to rice production as it can competes with cultivated rice for every important nutrients and necessities (light, space) causing the cultivated rice unable to grow as intended, diverse and very difficult to control because of very similar in physiology and morphology, which

eventually leads to yield loss (Sales *et al.*, 2011, Gealy, 2015). Under normal circumstances, weedy rice can be easily drawing a distinction with cultivated types only during the tillering and post tillering stages when typical traits of weedy rice are more evident, such as taller plant stature, weaker culms, able to emerge from greater depths, awn with variable length, rapid seedling growth when compared with cultivated rice, easily shattered seeds, the color of the pericarp, high number and slenderer tiller and bristle on both side the leaves (Mispan *et al.*, 2019). These characteristics can be used as a tool to differentiate both weedy rice and cultivars. For example, red pericarp was favored by natural selection due to its link with seed shattering and dormancy which translated to persistence of the seeds in the seedbank.

The success of a weedy rice can be attributed to two main factors which is high dormancy and easy seed shattering. Shattering trait are the earliest and important trait for grain domestication (Fuller & Allaby, 2018). The ability to shatter seeds before crop harvesting gave weedy rice an advantage over cultivated rice to maintain and evade various weed management practices (Zhao *et al.*, 2010). Unlike weedy rice, cultivated rice often have undergone selection to reduce its shattering potential during domestication, so the amount harvestable by human can increase thus minimizing the needs of manual labour (Dilipkumar *et al.*, 2017). There are four gene linked in weedy rice shattering potential which is sh1, sh2, Sh3 and sh4 (Subudhi *et al.*, 2012). A single dominant gene sh1 mutated can resulted in reduce seed shattering due to the effect towards abscission zone development (Jiang *et al.*, 2019).

Seed dormancy is an innate barrier that temporary block any viable weed seed from germination even if the environmental cue permits it (Debeaujon *et al.*, 2018). Baskin & Baskin (2004) suggested that seed dormancy was the inability of a seed to germinate at a given period when the physical environment is fit for germination. Finch-Savage & leubner (2006) suggested that dormancy should be define by the seed characteristic for

germination rather than absence of germination. He argues that by changing the environment cue needed for germination, is by definition changing dormancy. Most cultivated rice crop show a lower dormancy level in contrast with its wild relative (Debeaujon *et al.*, 2018). This is intended effect for cultivated rice crop to have high emergence rate as well as to ensure simultaneous germination achieved (Choi & Purugganan, 2018).

Uneven dormancy level can cause uneven germination of newly seeds resulting in early germination and this will cause a substantial yield loss for cultivated crop (Simsek *et al.*, 2014). However, weedy rice seed show a much higher level of dormancy level when compared with cultivated crop. Seed dormancy for weed seed is an advantageous feature as it allows weed seed to escape weed control that usually kill them in seedling stages. Seed dormancy is determined by the combination of plant hormone such as abscisic acid (ABA) and gibberellic acid (GA) coupled with its genetic that influence by environmental cue surrounding it (Finch-Savage & Leubner, 2006).

2.5 Weedy Rice in Malaysia

Begum *et al.* (2005) shows that weedy rice is still a dominant grass species in Malaysia, infesting almost 100% of rice fields with high level of occurrence and economic loss in the area was high. In Malaysia, weedy rice was first observed in Tanjung Karang rice fields in 1988 and then found randomly in the one part of Muda rice granary area in 1990 (Mispan *et al.*, 2019). Now, weedy rice infestation has started to cover large area such as MADA Kedah; Ketara, Terengganu; Sungai Manik, Perak; and Seberang Perak (Mispan *et al.*, 2019). Furthermore, 74% of rice yield loss were reported by Azmi *et al.* (2008) in Malaysia due to direct seeding methods (Table 2.5). Ziska *et al.* (2015) observed that reduction of rice yield can rise to 60% for tall cultivars and 90% reduction for short variety

when densities hit 35 to 40 weedy rice plants m⁻² in which overshadow the yield losses by other grass weed.

Table 2.5: Weedy rice infestation across the world

Country	Infestation (%)	References
Europe	40-75	Ferrero, 2003
Italy	70	Vidotto and Ferrero, 2009
Malaysia	74	Chauhan, 2013
USA	60	Sales et al., 2011; Baek and Chung, 2012
Brazil	40	Fogliatto et al., 2011; Baek and Chung, 2012
Senegal	55	Fogliatto et al., 2011; Baek and Chung, 2012
Cuba	80	Fogliatto et al., 2011; Baek and Chung, 2012
Costa Rica	60	Fogliatto et al., 2011; Baek and Chung, 2012

*Generate from Weedy rice infestation of Foreign Agricultural Services of United States Department of Agriculture (USDA) in www.fas.usda.gov/psdonline on January 2017

In Malaysia, some farmers took almost zero preventative action to remove or inhibit weedy rice infestation in their respective field and largely disregard their associated yield and income losses in the beginning as something trivial (Azmi *et al.*, 2012). Thus, they continue with their current and not very effective conventional cropping practices. In the end, because of lackluster interest in managing weedy rice and continually increase in seedbank size and density, disastrous infestation starting to take roots in their rice fields (Zhang *et al.*, 2014). Close morphological and biology between rice variety and weedy rice has most likely render herbicide application useless (Song *et al.*, 2017; Sudianto *et al.*, 2016), causing weedy rice management almost impossible task to achieve chemically (Mispan *et al.*, 2019).

Because of lack of weed management at the time of crop emergence in direct seeding, weedy rice and cultivated rice emerge simultaneously and the two will compete for vital necessities. This method was viable when Malaysian farmers practicing hand weeding in their direct seeded rice field to remove weedy rice (Azmi *et al.*, 2012). One of many factors which causes the weedy rice infestation are due to the changes of rice planting technique practiced by the local farm from the method of transplanting rice seedlings from seedling nursery to the rice fields, to practice of direct seeding. However, due to

high amount of labour needed to carry out the task, many farmers abandoned their rice field when weedy rice infestation was too high to manage (Mispan *et al.*, 2019). Moreover, when cultivated rice seedlings are less competitive when compared to its weedy counterpart (Rao *et al.*, 2017) and new biotype of weedy rice started to emerge that can mimic cultivated rice variety making it hard to distinguish between these two (Baki & Shakirin, 2010). Weeds are also known not only able to absorb nutrients more efficiently than crops but also competing for light source, space and moisture throughout the growing season (Hayat, 2004; Nichols *et al.*, 2015). One of many factors which causes the weedy rice infestation are due to the changes of rice planting technique practiced by the local farm from the method of transplanting rice seedlings from seedling nursery to the rice fields, to practice of direct seeding. The usage of technique like direct seeding also provides an additional advantage for weedy rice to emerge because it is more capable to be well adapted to different environments and is more stress-tolerant than cultivated rice. Thus, causes the problem to be more severe (Azmi *et al.*, 2012). Therefore, one of the major threats of current direct seeded rice production is weedy rice.

Other than that, while rice technology has undergone massive advancement in recent years especially with the addition of automation such as rice seed planter, tillage tractors, thresher and combine harvesters, these machines were often shared between farmers in the same region because of its high price and maintenance issue. So, there was a risk of weedy rice seed being transported from one field to another and thus it became one of many carriers of weedy rice seeds. The seeds were attached to on the body of automation. This was reported by Baki & Shakirin (2010) who revealed that the transfer of weedy rice from one field to another is probably due to the movement of farm automation such as harvester resulting in onset of weedy rice infestation problems.

2.6 Weedy Rice Seedbank in Agriculture

Weed seeds are a major ingredient in its life cycle as they carry the torch for the weed species as an agent for propagation for future populations, especially for annually and simple perennial species such as hibiscus (*Hibiscus rosa-sinensis*) which only reproduce by seed only. For weed seeds to become effective agent for propagation, it needs to be able to disperse away to establish new colony away from the mother plant or enforcing and maintaining weed species in an area thus resulting in expansion of population size. Shattering of seed from the mother plant allow it to disperse both directions (horizontally and vertically) in through the soil (Ball, 1992). Dispersal of weed seed mainly occurs horizontally through animals (Van der Putten & Wim, 2012), wind-blown (Katul *et al.*, 2005; Bohrer *et al.*, 2008), water (Benvenuti, 2007). Weedy rice is known to be able to stay dormant better and longer compared to cultivated rice (Chang, 1991; Moldenhauer & Gibbons, 2003; Ye *et al.*, 2015), higher viability in soil and also longer seed longevity (Noldin *et al.*, 2006).

As weedy rice seed either shattered around the mother plant or transported through other medium, they fall down onto the soil surface of a new area. However, these newly weedy rice seed on the soil surface are very vulnerable to many kinds of dangers such as predation (Navntoft *et al.*, 2009), decay (Gomez *et al.*, 2014) and prolonged exposure to sunlight (Botto *et al.*, 1998), thus show that mortality of newly produced weedy rice seeds is one of the most influential parameters in the life-cycle of a weed. Common ground beetles, cricket can reduce up to 15% weedy rice seed emergence and large animal such as birds or mice also known to consume large amount of weedy rice seeds when it remains on the surface without enough cover (Menalled *et al.*, 2018). So, to prevent these seeds from being at risk, they are able to enter the soil through several methods and thus resulting in an establishment of a seedbank for weed seed to remain safe until germination.

Weedy rice ability to maintain viability over an extended period of time proved to be a useful feature as it allows weedy rice to survive in extreme conditions such as heat and high humidity to escape seed deterioration especially in tropical regions (Baek & Chung, 2012). We can maintain weedy rice seed dormancy or trigger its second dormancy by relocating it deeper into the soil from the soil surface, giving them enhanced seed longevity. Other than that, moving the buried viable seeds towards the soil surface can enhance dormancy release and result in germination (Fogliatto *et al.*, 2011; Roham *et al.*, 2014; Bhullar & Chauhan, 2015).

2.7 Factors Contribute to Seedbank Dynamics

2.7.1 Predation Effects on Weed Seedbank Dynamics

Seed predation is one of the major causes for weed seed loss in the field, effectively reducing the density of weed seeds in the soil seedbank for future years (Quinn *et al.*, 2016). Survivability of weed seeds is one of the key players in determining density and composition of weed seeds in the existing soil seedbank (Quinn *et al.*, 2016). Seed predation effect and amount of its influence depends greatly on many deciding natural elements such as seed position in the field (Quinn *et al.*, 2016), agricultural residue and tillage practices (Blubaugh *et al.*, 2015) and canopy cover (Qian *et al.*, 2016). Despite all these deciding factors, the studies on weed seed predation still weigh heavily on weed seed mortality in the region.

Several management practices such as tillage, herbicide application and mulching could make an impact on seed predation effectiveness. This study from Blubaugh *et al.* (2015) indicates that different tillage types could give out different results on seed predation. The experiment was done in South Ontario and found that no-till fields produce the best result in seed removal, followed by fields that use moldboard and lastly, chisel-plowed which has the lowest intended output. However, he suggested that certain factors such as crop

residue in no tilled field can influence the rate of predation. For Qian *et al.* (2016), his studies show that highly disturbed area had the lower predation rate when compared to heavily vegetated region.

Most seed predation occur mainly on the aboveground surface of seedbank (Bertiller & Carrera, 2015). Time span between the seed start to be dispersed from mother plant and seed burial determine the vulnerability of weed seed to predation. This is conceptual model made by (Pannwitt *et al.*, 2017) that combine the seed burial, dispersal, and demand to roughly estimate the weed seed losses toward predation. Weed seed that are left exposed on the top soil for a long period of time has higher chance for it to be eaten by predator. So, a valid strategy can be concocted using this knowledge by the farmers to take advantage of by delaying any practice that cause seed burial thus maximizing seed predation rate in the field. Delaying harvest date can also create the same opportunity for predators to feed on to reduce seed rain (Pannwitt *et al.*, 2017).

2.7.2 Seed Decay Effects on Weed Seedbank Dynamics.

When weed seeds fall on the soil surface, they may experience different fates. Overtime, these weed seeds that inhabit the soil may undergo decomposition by microbes or other factors which plays an important role in managing the weed seedbank (Müller-Stöver *et al.*, 2016). Seed decay is loss of quality, viability and vigor of a seeds because of environmental changes that determine the ability of the seeds to survive (Müller-Stöver *et al.*, 2016). However, to determine the effect of weed seed decay for different weed species is a daunting task as there exist several significant factors that can influence the weed seed decay process making it inconsistent such as time, location and species.

Several studies had been reviewed that give a very contrasting observation regarding weed seed decay process. Davis *et al.* (2006) shows that conventionally managed system exhibits a higher rate of weed seed decay in the soil when compared to low external input

system for species *S. faberi* and *A. theophrasti*, while for Ullrich *et al.* (2011) found that there is no consistent correlation between type of cropping systems (organic, conventional). Other than that, Davis (2007) also shows that high nitrate levels in the soil influenced seed decay; high fertility causing negative impact on longevity as nitrate able to encourage germination in many weed species. Kremer & Li (2003) also notes that high level of nitrates can be associated with high level of weed inhibiting microbes that may increase weed seed mortality. Bacteria and fungi are one of the agents that can promote weed seed decay which infestation mainly dependent on moisture content of seeds, temperature and seed resource storage. They induce infestation by producing chemicals that can damage the outer shell of seeds thus allowing for quick and easy access for microflora to enter and use the seeds resource (Shelar, 2008).

2.7.3 Fatal Germination Effects on Weed Seedbank Dynamics.

One of the alternatives that farmers had employed for control strategy against weeds is by creating an environment that can force the weed seeds to germinate and develop into a fatal outcome (Davis, 2007). This situation can be achieved by exposing the weed seed to germination triggers such as light signal and temperature timely agricultural practice such as tillage to incite germination followed by death of the seedlings. Light has been shown as one of major stimuli for weed emergence on the field from the seedbank (Ballare & Casal, 2000). A momentary exposure to daylight were enough to trigger germination from the seedbank especially during tillage operation as observed in *Datura stramonium* L. (Ballare & Casal, 2000). Many studies also indicate that burial depth can also effect weed seeds germination potential; the deeper the seeds burial, the lower the germination (Burmeier *et al.*, 2010).

This technique is called fatal germination which means that seedlings dies out before reaching the ground surface caused by exhaustion of resource due to unsuitable

germination time and environment (Limon & Peco, 2016). This event can also cause by seeds germinating too deep in the soil as it were buried by tillage practices. Small seeded species were shown to have larger percentage of surviving during burial when compared to large seeded species (Burmeier *et al.*, 2010). She observed that while small seeded species had low survival rate on the surface possibly due to selection pressure, these species develop depth sensing mechanism that cause depth-mediated inhibition preventing it from fatally germinate during unfavorable depth to emerge (Rydberg & Milberg, 2000). However, large seeded species capable to germinate and emerged from greater depth due to larger resource thus shielding it from fatal germination situation (Burmeier *et al.*, 2010).

2.8 Importance of Seedbank in Agriculture

The dynamics of weed seedbank determine by its inputs and outputs. Seed rain from the mother plants into the seedbank makes up for the input while any event that deplete or reduce the seeds in the seedbank such as predation, decay and germination fall into outputs categories (Chauhan *et al.*, 2013; Mohler *et al.*, 2012). Seed banks also provides us historical evidence of the past and future of a species and plant populations, as well as one of the factors that govern the local species composition and its dynamics in each habitat (Thompson & Grime, 1979). Most agronomic research area prioritize on the attributes of weed species and weed management practice but lacking on related knowledge and research that is focused on its seedbank dynamics. Weed seedbank characteristic not only was influenced primarily by weed population in the region but also by the success of the management system (Bhullar & Chauhan, 2015).

Other than that, seed banks allow species such as annual weeds to persist through the unforgiving environment and sudden changes in weather of winter (Gulden & Shirliffe, 2009). Seedbank in the soil able to provide and improve the capabilities of these seeds to

survive by providing buffer against highly effective control methods (herbicide, burning) as well as harsh environmental changes and condition such as acidic or flooding and allowing them to germinate over a period of many years. These advantages allow these seeds to slow the genetic shift of a weed population causing many seedlings that emergence in one year are not of the same genetic background due to intense selection pressure making the management of weeds harder than before. (Gulden & Shirliffe, 2009).

Furthermore, some weeds can turn into habitat for important microbe as well as food source for animal to feed such as pollen supply for bees. It also promotes soil stabilization and an active source of organic matters for the soil as they continue to grow and eventually die, and some weed can be used for human consumption. Moreover, weed also act as a cover for land and this can act as a protection from wind and water erosion. Last but not least, weed can be our natural diagnostic tool to inform us of the condition and nutritional balance of the soil. Lastly, some weed can break hard soil deadpan and control erosion. In conclusion, weeds have a controversial nature as it has many sides to look at. But in this context, the negative impacts of weeds indirectly affect all living beings.

2.9 Conclusion

As it stands, the study of weedy rice seedbank is a relatively new endeavor for Malaysia region. It is reported that more than 50 percent of the rice granary in Malaysia suffer from the infestation of weedy rice which is largely due to the weedy rice seedbank buildup in the soil (Mispan *et al.*, 2019). Dependency of direct seeded method to grow cultivated rice by the farmer for more several decades has caused the establishment of robust and efficient weedy rice soil seedbank that continue to become refuge for weedy rice seeds. Seed bank enables weedy rice to evade a wide range of weed management practices such as pre-emergence herbicide (Shivrain *et al.*, 2009), tilling (Chauhan, 2013),

hand weeding (Delouche & Labrada, 2007), and mulching (Kanapeckas, 2016). Other than that, A constant addition of shattered weed seed into the seedbank resulting in increase the size, ultimately causing the population of weeds to increase while growing alongside cultivated crop, thus hinder the process of weed control management (Zhang *et al.*, 2014).

Integrated weedy rice management strategies should include effective approaches to minimize the weed soil seed bank size, buildup and longevity. Weed seed aging/longevity has been widely studied but there is lack of information not only between the natural variation in weed seed bank longevity and the distribution pattern and spatial dynamics of weed seed in the seedbank but also its relationship with the aboveground weed community. By understanding the general essential overview and weed seed persistency in the seedbank and how these knowledges of seedbank can be related to the aboveground vegetation community, an agriculturist could create most suitable weed management programs. Furthermore, predictive overview of the future can be made for preemptive weed control for infestation problems. These connections make understanding the weed seedbank considerably more important for expanding the proficiency of weed management.

CHAPTER 3: METHODOLOGY

3.0 MATERIALS AND METHODS

Two experimental design were used to address the objective of this study. This study was developed to investigate the spatial dynamics of seedbank in rice agro-ecosystem and in a diverse abandoned agricultural land. The first experiment was done in Tanjung Karang, Selangor between two rice field. It was carried out to satisfied the first objective of this study which is to characterize weedy rice vertical and horizontal seedbank distribution pattern in rice agro-ecosystem. The second experiment were carried out at GBRLC Negeri Sembilan which to tackle the next objective which is to determine seedbank distribution pattern at different types of abandoned agricultural lands in Jelebu, Negeri Sembilan.

3.1 Weedy Rice Seedbank in Two Rice Field

3.1.1 Study Sites

A field experiment was conducted from May to July 2016 to investigate the spatial distribution of weedy rice (*Oryza sativa* L.) soil seedbank at two farms with different tillage practices in Sawah Sempadan (3° 26' 55.7232" N; 101° 11' 24.2808" E) and Sungai Burung (3° 27' 16.7508" N; 101° 9' 23.4288" E) at IADA Barat Laut Selangor, Malaysia. Both farms are about 10 kilometers apart (Figure 3.1). Sawah Sempadan is an individual, locally owned farm while Sungai Burung farm is a research field station managed by the Malaysian Agriculture Research and Development Institute (MARDI). During the survey season, Sungai Burung farm followed a recommended 3-till round tillage while Sawah Sempadan only tilled twice during land preparation before planting.

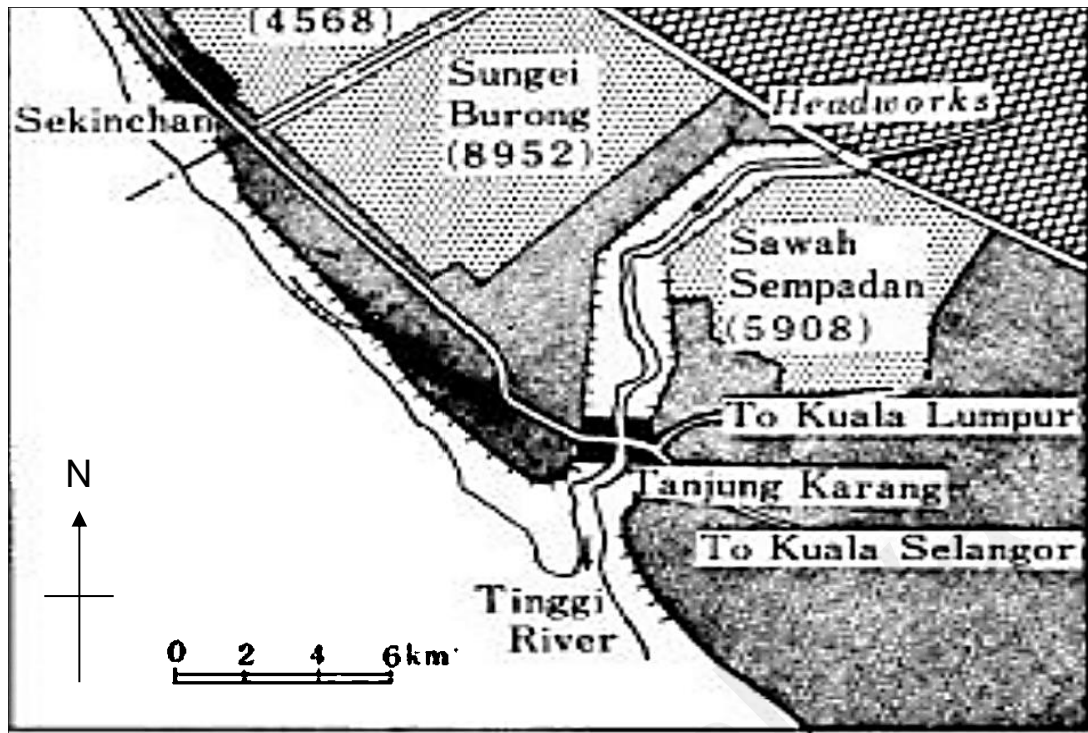


Figure 3.1: Map of Sawah Sempadan and Mardi Sungai Burung location

3.1.2 Horizontal Distribution of Weedy Rice Seedbank

W-shaped transect were laid on the farm to examine the weedy rice soil seedbank horizontal distribution (Figure 3.2), presumably covering or representing the whole area of the farm (Mansur *et al.*, 2012). The survey was conducted immediately after the rice was harvested. A 10cm² (0.01m²) quadrat was placed at every 10-meter interval following the W-shaped transect for a total of 100 quadrats (Appendix 1 & 2). The weedy rice seeds within the quadrat were counted but damaged seeds were excluded from the count. All seeds were collected and placed in a plastic bag. The seeds were air-dried for 3 days before they were stored in a -20°C freezer for further analysis.

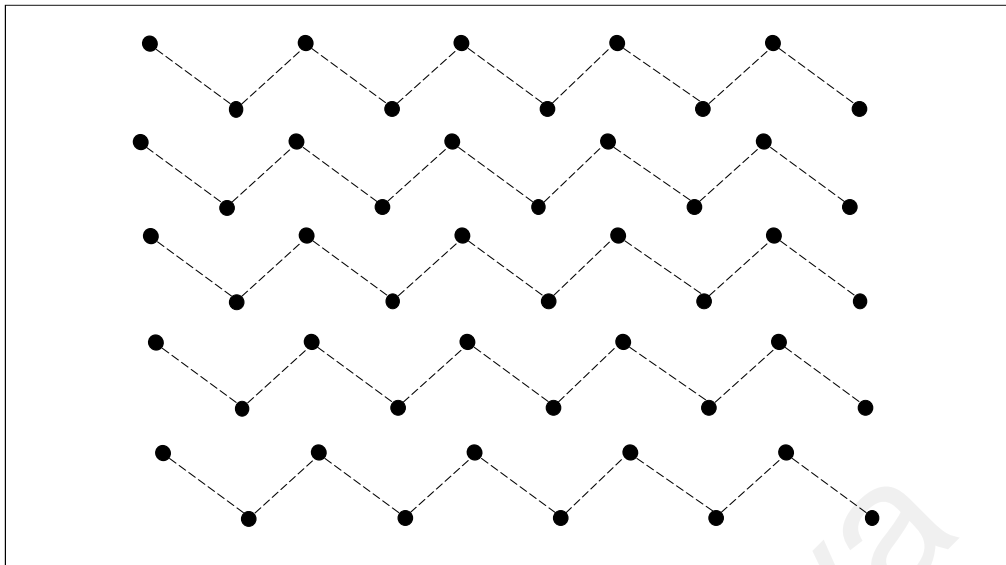


Figure 3.2: Visual representation of horizontal collection of samples in both 2ha rice plots. Each dot represents the approximate location sampling took place

3.1.3 Vertical distribution of weedy rice seedbank

A 5 cm diameter soil core was extracted from the seedbank at 20cm deep for the vertical seedbank distribution evaluation, using soil recovery probe at every quadrat on the W-shaped transect as described (Figure 3.3). The soil cores were divided into four different depths (0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm), then wrapped with aluminum foil and were immediately stored in a -4°C freezer to prevent seedbank germination during storage (Appendix 3). Prior to seed count, the soil cores were air-dried for 3 days. The cores were carefully broken using a hammer and a chisel to avoid unnecessary damage on the seeds and to remove large debris. The cores were broken into fine soil so all other impurities such as roots and rocks were removed. Weedy rice seeds were then separated from the soil and counted; damaged seeds were excluded. The seeds were then dry-cleaned from intact soils and kept in a -20°C freezer for further analysis.

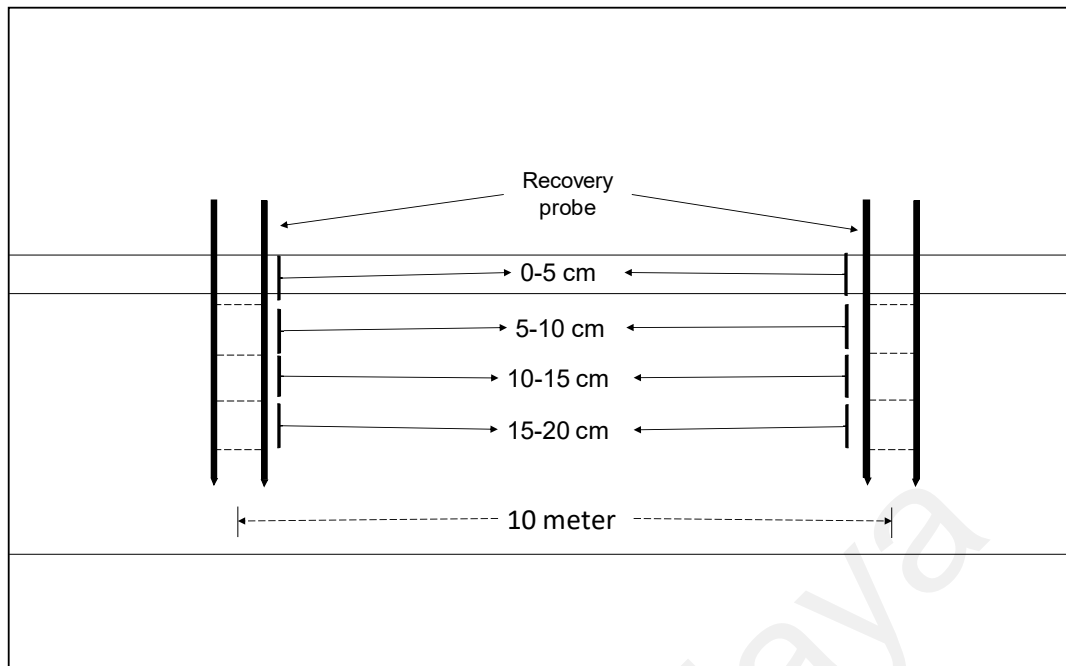


Figure 3.3: Vertical samples collection on both rice field using soil recovery probe

3.1.4 Soil seedbank viability

The viability of collected seeds was determined using a standard germination test (Mispan *et al.*, 2013). The seeds from the surface (horizontal) and at each different depth (vertical) were pooled for every ten quadrats based on their respective depth. This was because the number of seeds for each quadrat was too low for germination test. It was assumed that the newly pooled seeds were from similar conditions/environment since the samples were extracted from the same farm (Appendix 4).

Prior to germination test, the seeds were washed using distilled water to remove soil remnants on the seeds. The seeds were set down on a petri dish and was lined using a filter paper and was wetted with 5ml distilled water. Samples were placed in an incubator set at 30°C and 100% relative humidity in dark. Germinated seeds were determined by the emergence of radical or coleoptiles. The germination rate was counted up to 21 days after imbibition at 3-day interval.

3.1.5 Statistical analysis

Summary statistics (mean, standard deviation, variance, Anova and Duncan grouping) were calculated using SAS software (University Edition).

3.2 Weed Seedbank in an Abandoned Land

3.2.1 Study sites

Weed composition of the above-ground communities was assessed in May 2016 at Glami Lemi Biotechnology Research Center (GLBRC), located in district of Jekebu, Negeri Sembilan, Malaysia (3.053361, 102.063997). A total of 10 random sampling areas were selected in this 120-acre land as described in Figure 4.1.

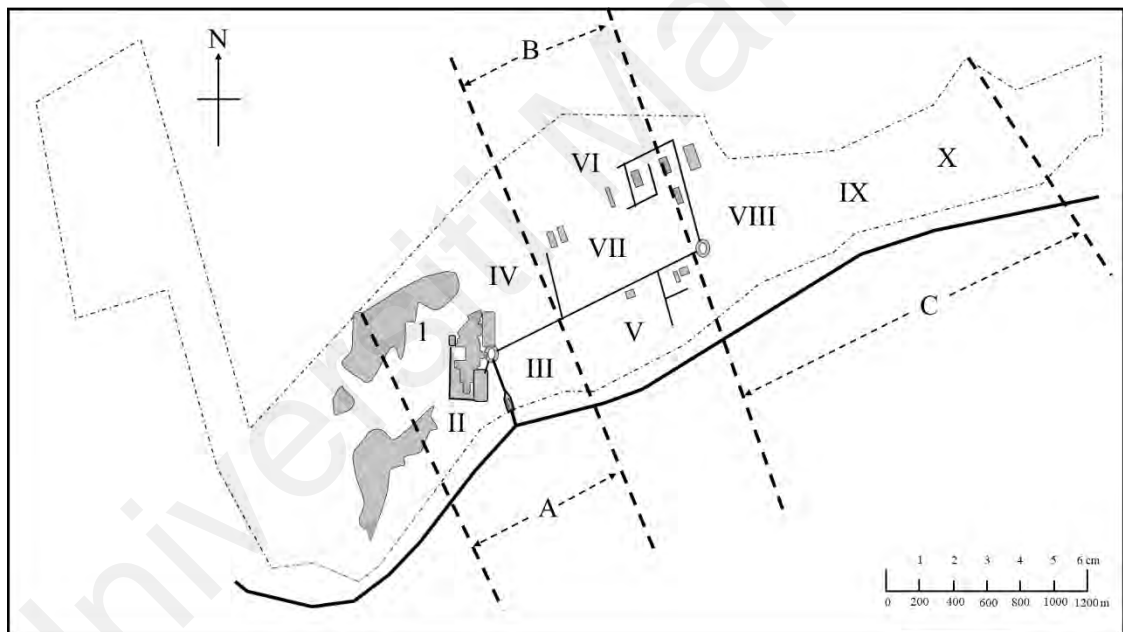


Figure 3.4: Map of GLBRC with approximate 10 sampling areas shown

For each site, it can be loosely grouped into 3 categories, active sites, non-active sites, and lastly abandoned agricultural sites (Table 4.1). For active sites (V – VII), it was located near active agriculture sites with various type of vegetation being planted such as corn, napier, pumpkin. Most of it were grown for research project or commodity. Next, non-active site (I – IV), was located near the administration and hostel building area. There were sparsely infested with weeds that were mostly primary succession type of

vegetation. These sites were mostly foreign soil brought in for development and construction purpose. Soils consist mainly of sand type. Other than that, site that were located near the active sites (VIII–X) that were abandoned were heavily invested with weeds. There were no active agricultural practices done and no crops found whether for commercial use or research purpose. These sites were on standby until future need for usage.

Table 3.1: Description of survey sites in Glami Lemi Biotechnology Research Center, Univeresity Malaya, Jelevu, Negeri Sembilan

Survey locations	Survey Sites	Description
A	Site I - IV	<ul style="list-style-type: none"> • Near building areas. No vegetation. Mainly primary succession. • Mostly foreign soil brought in for development purpose. • Sandy
B	Site V – VII	<ul style="list-style-type: none"> • Active agriculture sites. • Various type of vegetation being planted: corn, napier, pumpkin. • Vegetation grown as research project or commercialization
C	Site VII – X	<ul style="list-style-type: none"> • Near active site. • No active agricultural practices or vegetation grown. • Primarily infested with weed.

3.2.2 Vegetative record and seedbank sampling

The vegetation mainly weed species for GLBRC were identified and recorded. The species were characterized as grasses, broadleaves, sedges and ferns. Some species were identified directly by observing their morphology, but some were sampled and brought back to lab for further identification. Distribution of seedbank in GLBRC was access by two methods, namely germination test and direct seedbank counting.

For every sampling site, 5 random soil samples were taken for sail seedbank assessment (50 samples). Each sample were about 10cm³ soil surface (5cm deep) each

site. The samples were immediately put in a plastic bag and placed in a greenhouse to be air-dried for three (3) days.

To estimate composition of the seedbank, each soil sample was sieved to eliminate any rocks or roots and evenly spread in a plastic container (16 cm x 11 cm x 4 cm) lined with paper towels and placed inside a greenhouse. The containers were maintained at ambient temperatures and the soil samples were soaked with about 10ml distilled water as requirement to keep the soil moistened to field capacity. Most of seedling emergence from the seedbank emerged during the first week of experiment. Germinated seeds were identified as grass seedling or broadleaf seedling, counted and removed for every 3 days interval. The germination was observed for 6 weeks.

Another set of soil from the similar site for germination test was collected, placed in a 9mm petri dish and air-dried for 3 days in the greenhouse. Then, big debris like rocks and roots were removed from the soil samples. The presence of seed in the soil was counted under light microscope. Data was analyze using Microsoft excel to produce means, variance and standard deviation. Lloyd's patchiness index (lp) was used to assess the distribution pattern of soil seedbank on all sites from the relationship between mean crowding (m^*) and mean density (m) (lp) (Baki & Shakirin, 2010). The distribution pattern can be determined using Iwao line from m^*/m plotting.

CHAPTER 4: RESULTS

4.1 Weedy Rice Seedbank Density

The density of weedy rice seeds for Sawah Sempadan was significantly the highest ($p < 0.001$) at 0 – 5 cm soil depth than those at 0 (surface), 5 - 10 and 10 - 15 cm (Table 4.1). The same pattern also prevailed at Sungai Burung rice farms. Only 23.8% and 25.8% of weedy rice seeds were recorded above the soil surface from the total of weedy rice seedbank densities for Sawah Sempadan and Sungai Burung, respectively. The density of weedy rice seedbank for Sawah Sempadan and Sungai Burung rice farms increased about 16% and 12% from the surface to 5 cm depth, it then decreased by 17% and 14% at 10 cm depth, and eventually 11% and 10% at 15 cm (Table 4.1).

Table 4.1: Mean density (10cm^{-2}) of weedy rice seeds at various depths in the rice seedbank

Depth	Locations [§]		<i>F.value</i>	<i>P.value</i> [†]
	Sungai Burung	Sawah Sempadan		
0 cm (surface)	22.33 ^b + 8.05	22.18 ^b + 7.86	0.02	0.900
0-5 cm	33.16 ^a + 2.38	38.57 ^a + 2.63	6.53	0.012
5-10 cm	20.30 ^b + 2.52	21.94 ^b + 2.20	0.87	0.354
10-15 cm	10.71 ^c + 1.40	10.61 ^a + 1.14	0.06	0.938
<i>F.value</i>	2.641	2.641		
<i>P-value</i> [‡]	<0.0001	<0.0001		

[†] *P-value* to indicate the difference between depths for both locations.

[‡] *P-value* to indicate the difference between two locations for depths.

[§] LSD groups for Sungai Burung and Sawah Sempadan are denominated by small letters, respectively.

Based on two factorial experiment (Appendix 5 & 6), there is no interaction effect ($p = 0.09$) between two location when compared based on seed density for both location and all depths. Other than that, p value of = 0.19 shows that different location (Sungai Burung and Sawah sempadan) are not associated with density of weedy rice seeds. For depth level, it shows p value < 0.0001 means there is a very significant level of depth associated with density. Since depth has a significant value ($p < 0.5$), Table 4.1 shows the mean

density assessment were there are no significant differences of seed density between both locations at various depths individually, except at 5 cm depth where Sawah Sempadan recorded a significantly higher density ($p = 0.012$) than Sungai Burung.

4.2 Spatial Distribution Pattern

Weedy rice seeds at various seedbank depths for both locations showed that the frequency was equal to one, indicating that the seeds can be found at every plot taken. Table 4.2 displayed spatial distribution variations of weedy rice seeds at different soil depths for both locations. The variance-to-mean ratio (VMR) values indicate the distribution patterns of weedy rice seeds in the seedbanks, based on Poisson probability distribution. If the distribution is random, the values of variance and means can be modelled by the Poisson: $VMR = 1$, while a clumped or a clustered distribution pattern is indicated by $VMR > 1$; a $VMR < 1$ corresponds to a uniform distribution pattern (Baki & Shakirin, 2010). Based on VMR values, both locations show cluster distribution pattern of weedy rice seedbank for various depths. Sungai Burung displayed a higher VMR than Sawah Sempadan respective to their depths, indicating that the clustering at Sungai Burung was more endearing.

Table 4.2: Variance-to-mean ratio (VMR), mean crowding (m^*) and patchiness index (lp) of weedy rice seeds at various depths in the rice seedbank

Locations	Depth	VMR	m^*	lp
Sungai Burung	0 cm (surface)	2.871	24.201	1.084
	0-5 cm	4.089	36.253	1.093
	5-10 cm	8.588	27.894	1.374
	10-15 cm	4.689	14.403	1.344
Sawah Sempadan	0 cm (surface)	2.754	23.934	1.079
	0-5 cm	4.404	41.980	1.088
	5-10 cm	5.612	26.551	1.210
	10-15 cm	3.124	12.736	1.200

Distribution pattern of the weedy rice seedbank can also be determined from the correlation between mean density (m) and mean crowding (m^*) based on the Lloyd's patchiness index (lp) (Lloyd, 1967; Baki & Shakirin, 2010). Table 4.2 showed the lp values for weedy rice seedbank at both locations. The distribution pattern can be determined using the Iwao line from the m^*/m plotting. Values located on the line indicate a random distribution. Values plotted above and below the line correspond to clustered and uniform distribution, respectively (Iwao, 1968). All depths that display- lp values > 1 indicates a cluster distribution pattern (Table 4.2; Figure 4.1). However, both locations showed lp values close to one for seedbank at soil surface and 5cm deep, indicating that the distribution pattern was almost random. The clumping of the seedbank distribution became eminent as the seeds got deeper.

4.3 Weedy Rice Seedbank Viability

Weedy rice seeds extracted from the seedbank were tested for germinability to determine their viability. Respective to the various depths (Table 4.3), Sawah Sempadan seeds have a significantly higher germination percentage (43% - 72%) as compared to Sungai Burung's (10% - 31%) rice field.

Table 4.3: Germination rate (%) of weedy rice seeds extracted from various locations and depths in the seedbank

Depth	Locations [†]		F.value	P.value [†]
	Sungai Burung	Sawah Sempadan		
0 cm (surface)	15.66 ^{ab} + 12.02	43.58 ^b + 22.33	12.12	0.003
0-5 cm	31.41 ^a + 19.63	61.37 ^{ab} + 10.11	9.20	0.016
5-10 cm	14.20 ^b + 3.76	75.30 ^a + 9.76	170.76	<0.001
10-15 cm	10.35 ^b + 17.30	72.19 ^a + 20.87	26.03	0.001
F.value	2.241	4.591		
P.value[§]	0.113	0.0127		

[†] P-value to indicate the difference between depths for both locations.

[‡]P-value to indicate the difference between two locations for depths.

[§] LSD groups for Sungai Burung and Sawah Sempadan are denominated by small letters, respectively.

The germination rate for Sawah Sempadan weedy rice seedbank has significantly increased by depth up to 10 cm deep. Germination rate has increased by 20% and 14% from surface to 5 cm, and from 5cm to 10cm deep, respectively. The germination rate was significantly different from surface to 10 cm depth indicating that different depths preserve the weedy rice seedbank differently, and it then became constant beyond the 10 cm deep line. On the other hand, Table 4.3 also displayed that there is no significant variation of seedbank germination between depths at Sungai Burung rice field. The highest germination rate at Sungai Burung rice field was at 5 cm depth (31.41%) while the seedbank at surface and 10 cm lower have germination rate of less than 16%. The surface seedbank has only 15.66% seeds germinated before it doubled (31.41%) at 5 cm deep, decreased about half at 10cm, and has continued to be reduced by 27% at 15 cm deep.

Based on Two-way Anova data (Appendix 5 & 6), p -value for depth level is 0.063, which indicates that the levels of depths are not associated with different germination rate. Other than that, p -value for location are <0.0001 shows that location is associated with germination rate. Furthermore, the interaction effect between depth level and location is statistically significant, indicates that we cannot interpret the factors without considering the interaction effect. Table 4.4 shows that there is significant difference for both location (Sungai Burung and Sawah Sempadan) when compared by depth individually. However, interaction effect indicates that the relationship between location and germination rate still depends on depth level. Table 4.3 shows that Sawah Sempadan has the highest germination rate at 75.30% on 5 – 10 cm depth level, while Sungai Burung had the highest germination rate at 31.41% on 0 – 5 cm depth level.

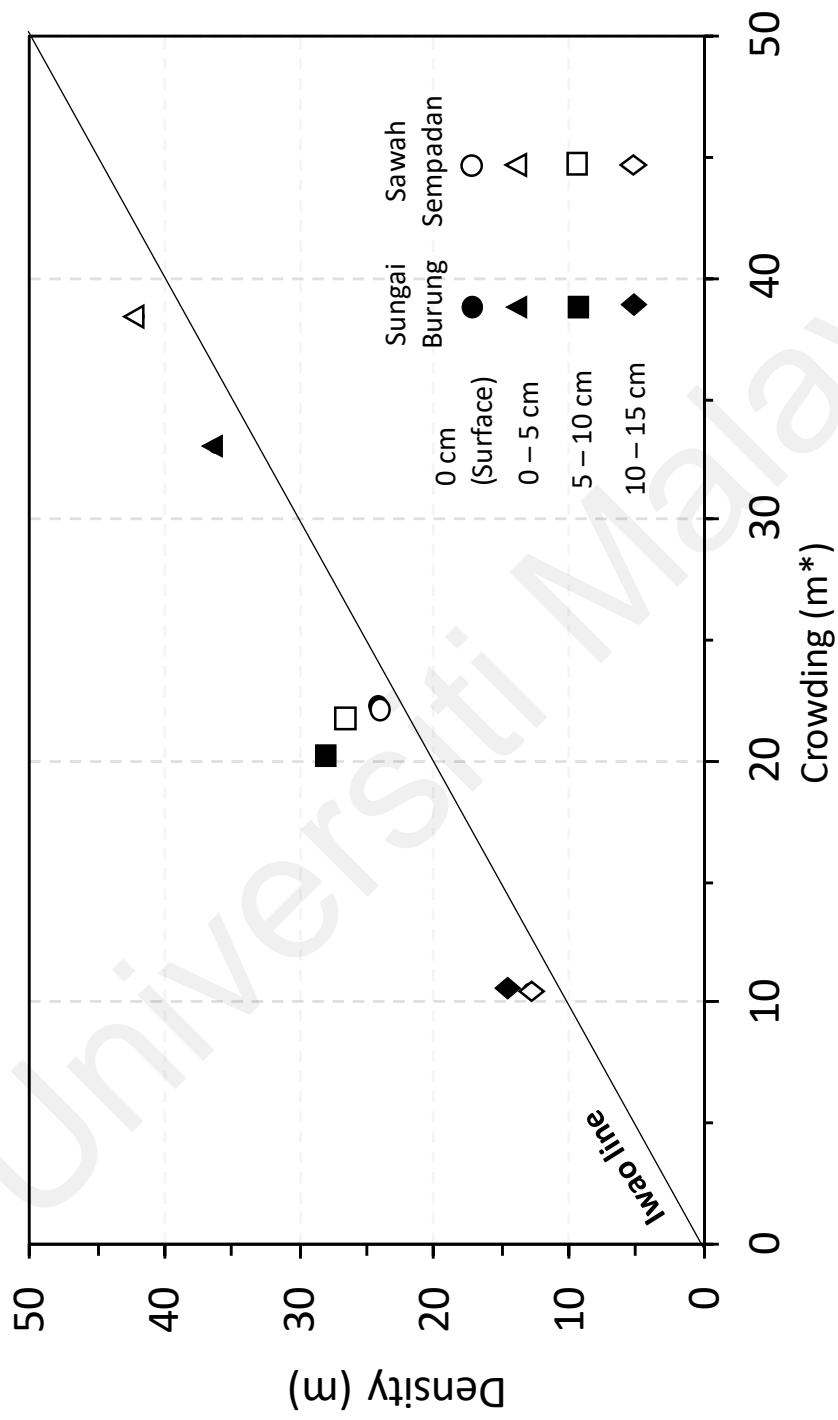


Figure 4.1: Scatter plot Lloyd patchiness index (I_p) of weedy rice soil seedbank for Sungai Burung (black dots) and Sawah Sempadan (white dots). Distributions at 0 cm (surface), 5 cm, 10 cm and 15 cm deep are represented by circle-, triangle-, square-, and diamond-shaped dots, respectively. Each dot (black and white) represent total mean average of 50 samples. Points above, on, and below Iwao line ($m^*/m = 1$) indicate the cluster, random and uniform distribution patterns, respectively.

4.4 Aboveground Weed Composition

A total of 53 species has been identified and recorded in Glami Lemi Biotechnology Research Centre (GLBRC). There are 17 families of weed consist of four different types of weeds such as broadleaf, grass, sedge and fern (Figure 4.2). Each of the type of weed recorded have the number of species of 36, 14, 2 and 1 respectively. Finding shows that broadleaved weed has the highest number of species found with the total of 36 species. It comes from various weed families such as Asteraceae, Fabaceae, Euphorbiaceae etc. Whilst Fabaceae has the highest number of weed species found within the broadleaved weeds, Poaceae from grass weed has been the highest number of species among all of other type of weeds found there. It shows that broadleaf has the highest number while fern has the least number of species found.

Broadleaf weeds consist from family of Acanthaceae, Asteraceae also known as Compositae, Capparidaceae, Convolvulaceae, Cucurbitaceae, Dilleniaceae, Euphorbiaceae, Leguminosae also called Fabaceae, Loganiaceae, Malvaceae, Melastomaceae, Rubiaceae and Verbenaceae. Poaceae and Cyperaceae are the only family found grass and sedge while fern recorded family of Blechnaceae.

4.5 Seedling Emergence from Seedbank/ Seedbank Composition

Seedlings started to emerge at 3d after soaking (Table 4.4) and reached full germination at 9 d. No new seedlings emerged after 9 d of imbibition. For each site, 10 replicates of samples taken from their respective sites were pooled and number of average seedlings germinated are taken. Sites II, VI and VII showed fast seedling emergence with more than 90% of total seedlings emerged at 3d after germination while Site IV emerged at slow rate with only 29.6% at 3d after imbibition.

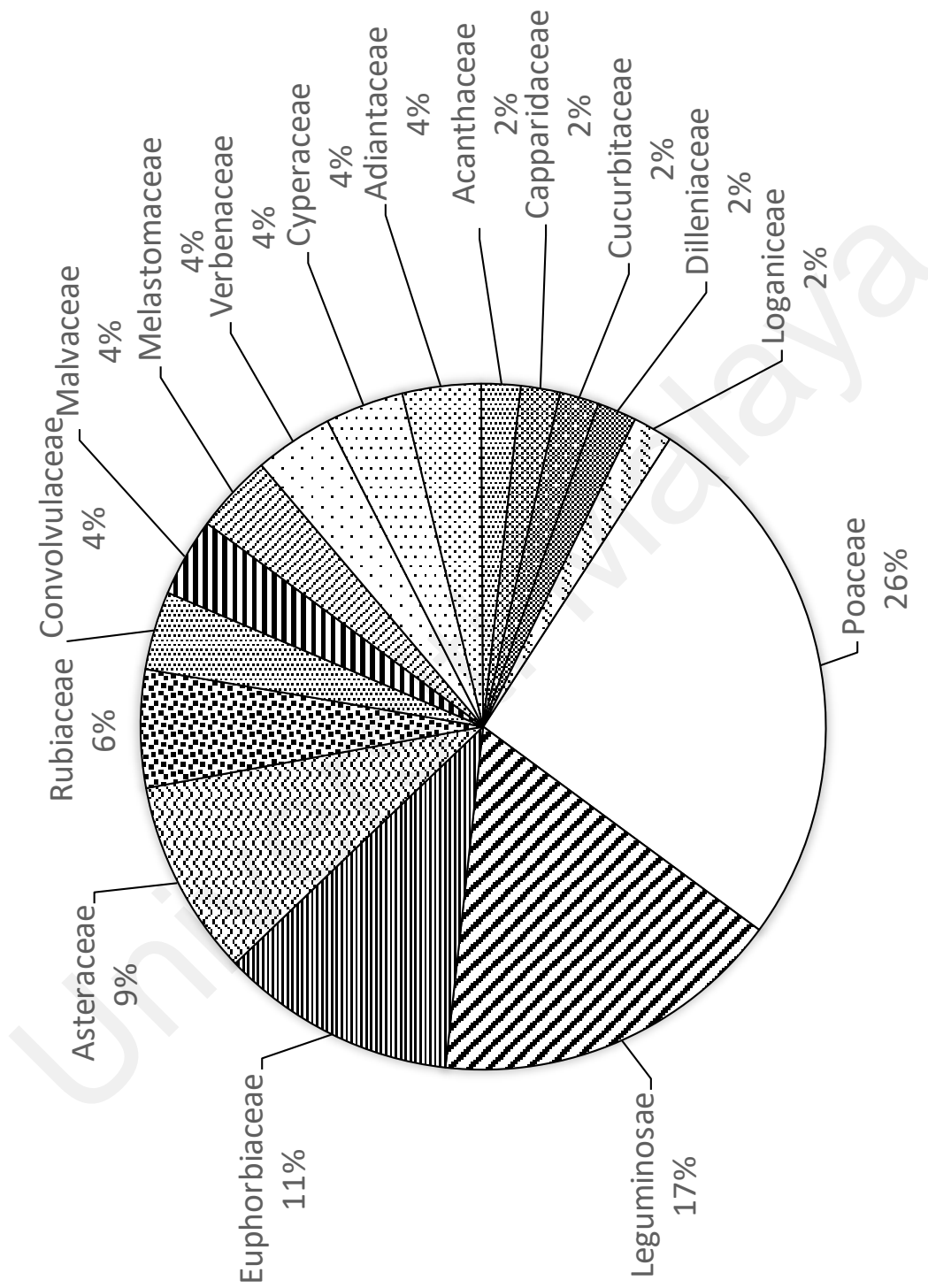


Figure 4.2: Type of weed species found in GLBRC

Table 4.4: Number of seedlings germinated from collected soil surface at three interval periods

Survey sites	3 d	6 d	9 d
Site I	8.2 (75.9)	9.8 (90.7)	10.8
Site II	17 (92.4)	17.2 (93.5)	18.4
Site III	7.2 (56.3)	10 (78.1)	12.8
Site IV	5.8 (29.6)	18 (91.9)	19.6
Site V	13.4 (60.9)	15.6 (70.9)	22.0
Site VI	197 (92.9)	210.6 (99.3)	212.0
Site VII	131.8 (91.2)	142 (98.6)	144.0
Site VIII	43.8 (83.0)	51 (96.6)	52.8
Site IX	26.6 (60.7)	42.6 (97.3)	43.8
Site X	18.6 (63.7)	25 (85.6)	29.2

Note: The number inside the bracket are the percentage (%) of germinated weed seeds for survey site on different interval period respectively.

Number of seedlings emerged from the seedbank varies between sites (Figure 4.3). Sites VI and VII have the highest number of seedlings recorded with average of 55.5 and 60.3 seedlings, respectively. Sites V, VIII, IX and X have a moderate seedlings number ranged from 20 to 60 seedlings, and Sites I to IV recorded less than 20 seedlings. This indicates that every site has different potential of seedbank germinability. Emerged seedlings were differentiated as grasses and broadleaves, based on their seedlings leaf characteristics (Figure 4.3). Sites VI and VII significantly recorded the highest number of grass and broadleaf seedlings, respectively. Majority of the sites showed higher density for broadleaves than grasses except at Sites II and VI. The biggest difference was shown within location B where agriculture activities were located.

Seeds from the seedbank were hand sorted and directly counted (Figure 4.3). Across all sites, locations B and C displayed higher density of seedling emergence compared to direct counting, while location A displayed the opposite. Soil composition in location A was primarily foreign sand that were brought in for development purpose as compared to other locations which has diverse compositions of higher silt and clay. Location A also was highly maintained from weeds. The difference ratio between emerged seedlings and counted seeds can be used as indicator to the potential of seedbank to germinate once the

environment favors, depending on dormancy status and/or the viability of the seedbanks.

4.6 Seedbank Spatial Distribution

When comparing patchiness index (Figure 4.4, 4.5 & 4.6) graph for both grasses and broadleaves, both weeds exhibit an aggregated distribution pattern at GLBRC ($LI > 1$). For grass species, only one point located on the straight line indicating a random distribution while for broadleaf, 3-point falls on the straight line ($LI = 1$). Total emergence spatial distribution also exhibits an aggregated distribution ($LI > 1$). Values located close to the line indicate a random distribution. Values plotted above and below the line correspond to clustered and uniform distribution, respectively.

None of the weed seedbanks for both broadleaf and grass types form uniform distribution at all sites. Majority of the sites displayed a cluster distribution of seedbank for both weed types. Broadleaves showed random distribution at sites I, IV and VII, while grasses only displayed random distribution at site II.

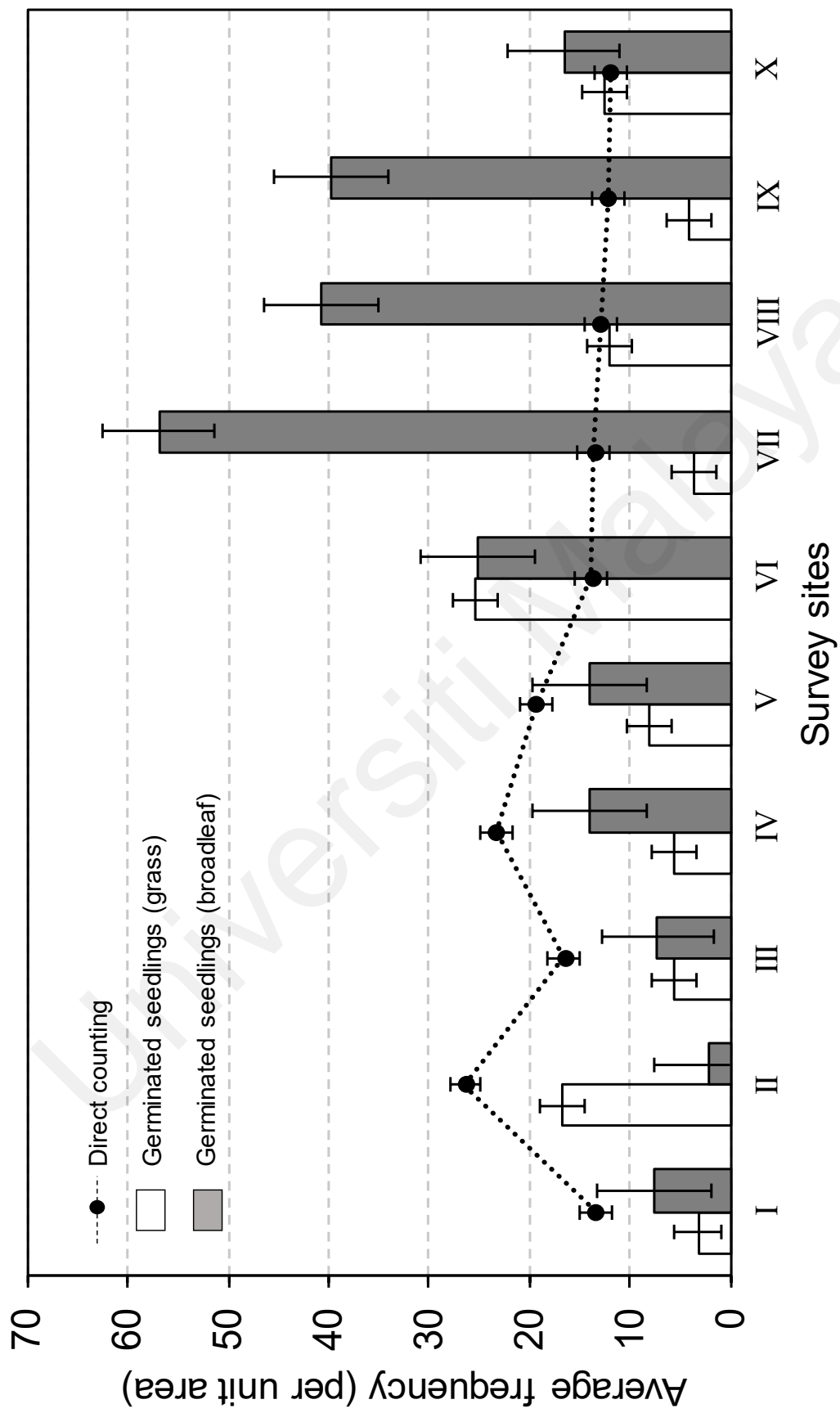


Figure 4.3: Seedling emergence for direct counting and germination test.

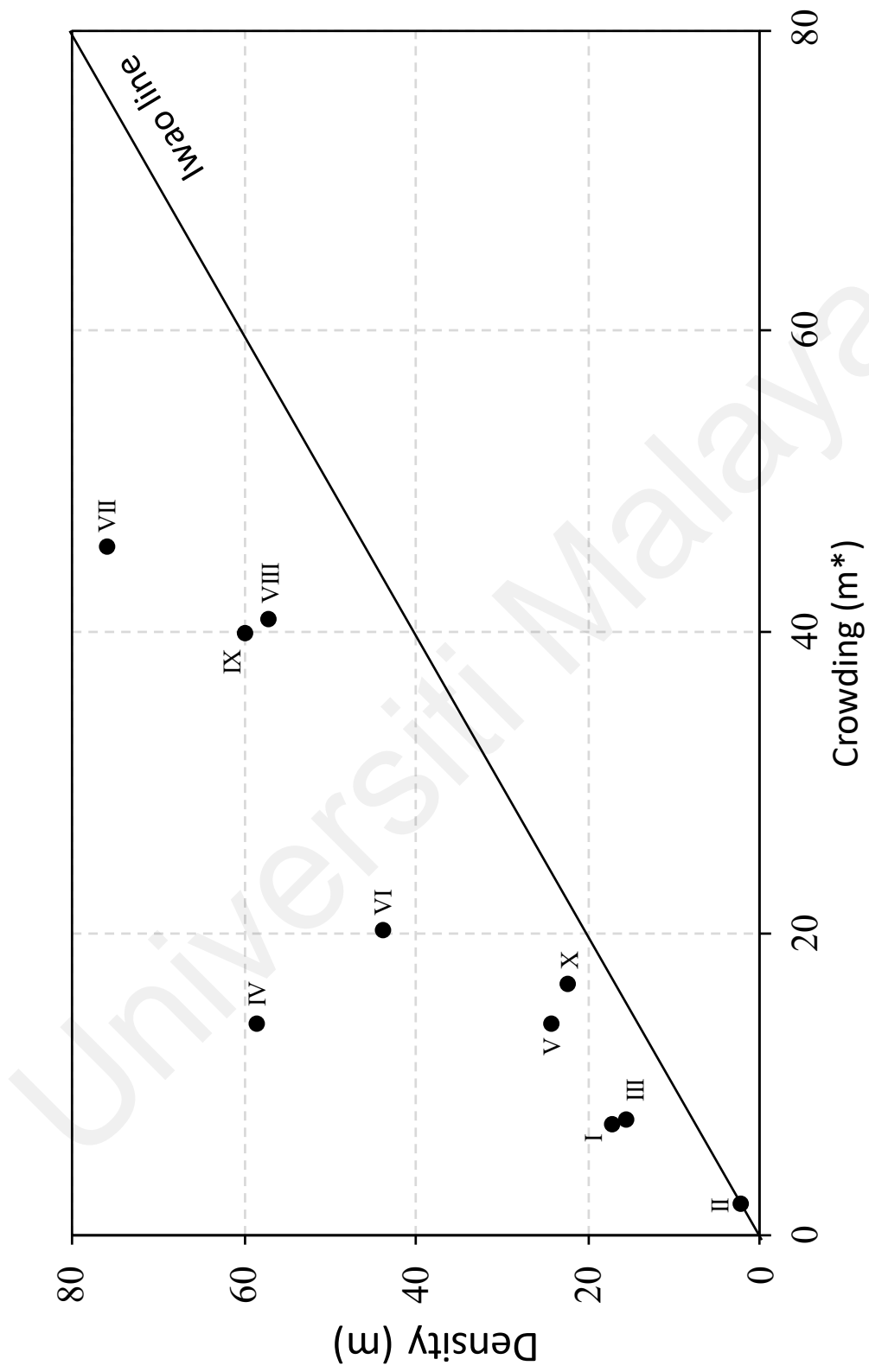


Figure 4.4: Scatter plot of Lloyd patchiness index (*lp*) of grass weed species soil seedbank for all sites. Each dot represents 50 samples for respective site.

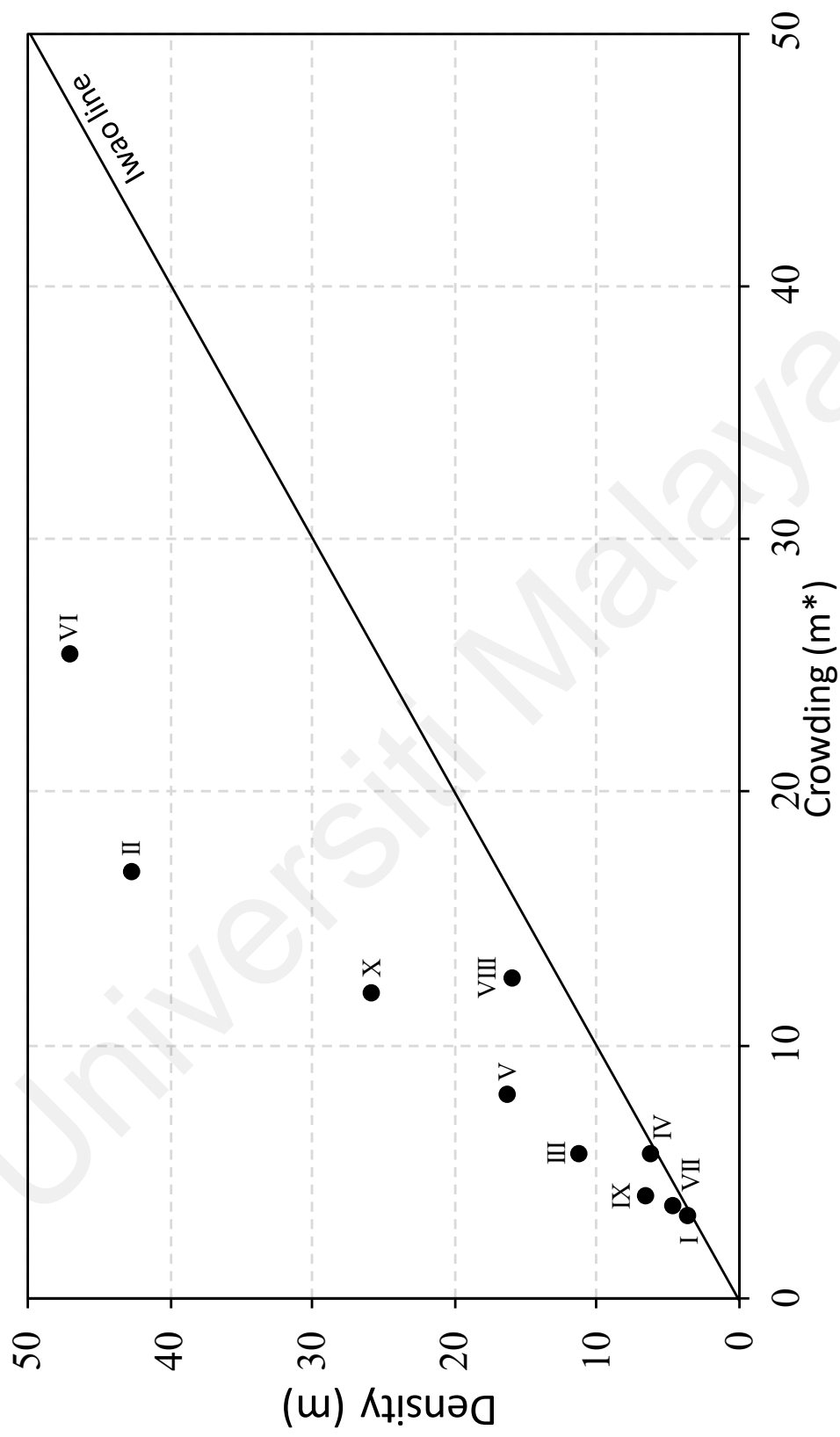


Figure 4.5: Scatter plot of Lloyd patchiness index (p) of broadleaf weed species soil seedbank for all sites. Each dot represents 50 samples for respective site.

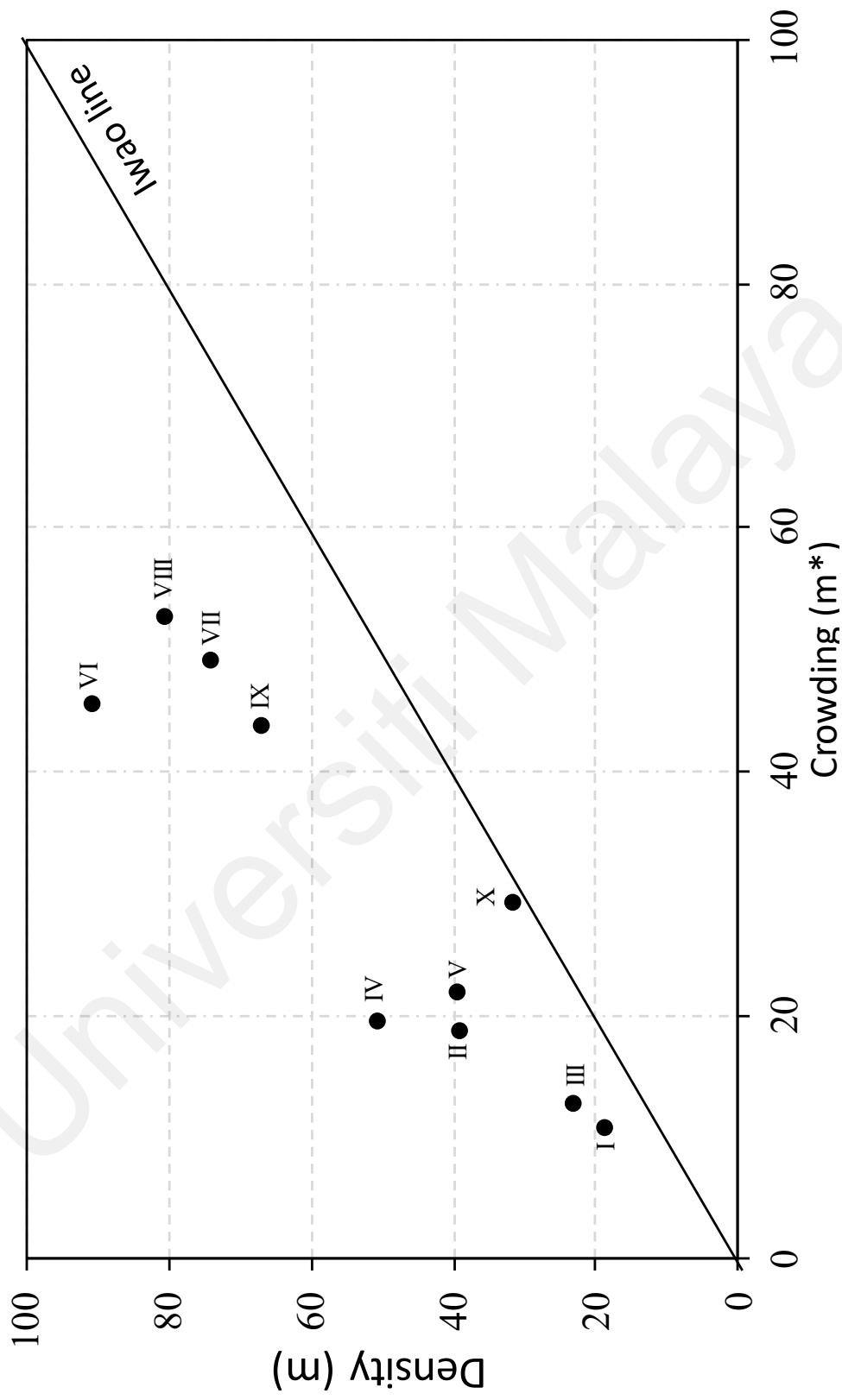


Figure 4.6: Scatter plot of Lloyd patchiness index (*lp*) of total weed species soil seedbank for all sites. Each dot represents 50 samples for respective site.

CHAPTER 5: DISCUSSION

Weedy Rice Seedbank Density

The availability of weedy rice seed in all survey plots at every depth shows that the seeds were spread across the fields even with different land preparation practices. Both farms were direct seeded fields and had applied dry rototilling or shallow tillage and wet rototilling (land levelling) at 30d and 2d before sowing. The only difference was Sawah Sempadan farm skipped wet rototilling at 15d before sowing.

Based on Two-way Anova results (Appendix 5 & 6), there is no interaction effect of weed seed density on depth levels and locations were found. However, depth level has a significant effect on the weed seed density ($p < 0.0001$). The result indicated that both locations (Sungai Burung and Sawah Sempadan) had no significant difference on the density of weedy rice seeds for majority of the depth level except for 0 -5 cm depth level (Table 4.1). These might due to Sawah Sempadan skipped the wet rototilling at 15 days before rototilling causing weedy rice seeds in the 0 – 5 cm soil depth from previous seasons to maintain its location underneath the soil and not brought out to the surface.

Identical pattern of weedy rice seeds for both locations showed increasing density from surface towards 0 – 5 cm and continue to decrease towards 10 – 15 cm indicates that weed management practices that rely on tillage would produce a pattern that accumulate buried weedy rice seeds at 0 – 5 cm depth level (Table 4.1). More than 70% of weedy rice seeds are found below the soil surface indicating that the seedbank was developed from previous seasons and through a similar mode (tillage) (Table 4.1). This is in line with previous findings by Konstantinovic *et al.* (2011) and Roham *et al.* (2014) that the majority of seeds will end up in the lower depths of the soil. These can cause a problematic weed problem as it allows the weedy rice seed to maintain its viability when buried, ready to emerge when next tillage brought then out towards the surface. Weedy rice needs to

deposit as many seeds as possible into the seedbank to ensure the successful emergence of seedlings. Weedy rice as a notorious weed might use this seed dispersal mechanism to co-exist with cultivated rice as a trade-off between colonization ability and competitive exclusion, when the seed fails to enter or persists in the seedbank. The spread of weedy rice in rice fields is most likely due to poor land preparation which can increase the survivability of weedy rice seeds in the seedbank (Chauhan, 2013)

Spatial Distribution Pattern

Despite the weedy rice seeds spreading throughout rice fields, the distribution of the seedbank tends to cluster or clump (Figure 4.1). The clumping distribution also indicates robust seedbank of the area which can provide suitable environments for further germination (Mohammad *et al.*, 2007). Furthermore, it may show that the weedy rice plants only cluster at certain specific areas since weed seeds are generally dispersed in less than two meters away from the parent plant (Roham *et al.*, 2014). However, based on simulation on tree population, the seeds degree of clumping decline with increasing tree density (Bleher *et al.*, 2002). It can be assumed that the seedbank is most likely to form cluster in a low-density weedy rice population. Therefore, the above ground weedy rice infestation in Sungai Burung farm was milder than Sawah Sempadan farm since clustering was more prominent in Sungai Burung, based on the VMR values (Table 3.3). The patchiness of the weedy rice seeds has not been mapped in the field. Therefore, there is no information where the clumping is located.

The movement of the seeds to “weed free area” became possible due to interference of human activities especially during tillage and harvesting. This may cause concern of future infestation of weedy rice and how it should be dealt with for its management. In general, weed vegetations always clusters at various densities with various patchiness sizes (Roham *et al.*, 2014). Seeds of the weed can be easily dispersed by multiple agents

according to the size and shape of reproductive organs, environmental conditions (e.g. wind, water, animals), and anthropological activities including planting patterns, tillage system or management and harvesting practices (McConkey *et al.*, 2012). As the distribution of weed seeds are dynamically changing due to various dispersion factors mentioned, some seedbank of the abandoned fields may contain seeds below the economic thresholds while the other parts may have higher abundance of seedbank (López-Toledo & Martínez-Ramos 2011) which are often overlooked in agricultural practices (Mispan *et al.*, 2015; 2019). Weed management especially by herbicide application is typically applied uniformly throughout the field. It was always assumed that weeds are distributed homogeneously (Roham *et al.*, 2014). Conversely in this study, it has been shown that the seedbanks were heterogeneously distributed. The weed seedbank management could be effectively improved if the control methods (e.g. herbicide application) has been based on the clumping locations (Loghavi & Mackvandi, 2008) and precise prediction on seedbank density.

Weedy Rice Seedbank Viability

High weedy rice seeds germination rate in Sawah Sempadan indicates that weedy rice seedbank has a high viability and can pose as a major threat for future infestation of the area (Table 4.3). Wet rototilling (2nd tillage) during land preparation in Sungai Burung farm has significantly reduced germinability of the seedbank up to seven times respective to the different depths (Table 4.3). The second tillage has rotovated the soil up to 7.5 cm with the presence of shallow water to remove perennial weeds and to encourage weedy rice seeds to emerge (Azmi & Muhammad, 2003; Azmi & Karim, 2008). Dry period between the first and third tillage in Sawah Sempadan might provide ample condition for the weedy rice seeds to persist in the seedbank (Chauhan, 2013). This study shows that the failure of the farmers to follow the proposed standard operating procedures for land

preparations (in this case, skipped only one recommended tillage) has increased weedy rice infestation potential from 2- to 7-fold. Seedbank size of the weedy rice can be managed by land preparation which direct or indirectly minimizes the severity of weedy rice infestation. A series of tillage operations during land preparation and pre-planting management practices have been reported to reduce weedy rice seedbanks in majority of direct-seeded

Based on the Two-way Anova data (Appendix 5 & 6), the interaction effect for germination rate are significant for location and burial depth ($p = 0.013$). This means that we can't interpret factors such as burial depth without taking account of location effect on germination rate. Table 4.3 indicate that each burial depth has significant different when we compared both location (Sungai Burung and Sawah Sempadan). However, both locations have different highest germination rate based on burial depth, 75.30% on 5 – 10 cm burial depth for Sawah Sempadan and 31.14% on 0 – 5 cm burial depth for Sungai Burung (Table 4.3). farms in Malaysia (Azmi *et al.*, 2000; Chauhan, 2013).

The burial depth is also an important characteristic for regulations of germination and seedling emergence of weeds to the soil seedbank, thus it affects the ability of the seeds to remain viable for extended time (Ren *et al.*, 2002). This characteristic provides adaptability advantages for the weedy rice seeds to survive from heat and high humidity and to escape seed deterioration especially in tropical areas (Baek & Chung, 2012). Based on the result (Table 4.3), Sawah Sempadan showed a very high germination potential for weedy rice seeds increased by 20% and 14% from surface to 5cm, and from 5cm to 10cm deep, respectively. These showed that different burial depth has different degree preserving the weedy rice seed to maintain its viability throughout many seasons. For Sungai Burung, the overall germination potential on all depth are lower than Sawah Sempadan. However, Sungai Burung exhibit higher germination potential on 0 -5 cm depth level at 31% while Sawah Sempadan is at 75% germination potential at 5 -10 cm

as the highest value (Table 4.3). These differences can be attributed to failure of the farmer to follow the weed management properly causing viable weedy rice seed to stay deeper in the soil and maintain its dormancy.

Aboveground Weed Composition

This study shows that the aboveground weed vegetation can be reflected to the seedbank composition of grass and broadleaf weeds. Broadleaf weeds show higher density of germinated seedling compared to grasses (Figure 4.3) which tallied with our aboveground weed composition findings in Figure 4.2. Takim *et al.* (2013) reported that broadleaf weed seeds were easy to germinate in the agricultural fields. Although the result of this study could give a fair estimation of future weed emergence in the field, the result is only representation of a small and variable fraction of weed seed in the soil seedbank. Continuous cultivated maize farm has significantly higher density of broadleaf weed seedlings when compared with cultivated cowpea farm and closely followed by natural fallow fields that show to be the least significant in term of density of broadleaf weed (Takim *et al.*, 2013). In contrast, Tozer *et al.* (2010) reported that aboveground weed composition did not reflect the seedbank germinated seedling in a pasture. Weed assessment in a pasture recorded domination of grass weeds in all regions, but only one grass weed species germinated from the seedbank. Weedy herb (broadleaf) species were the most frequent seedlings emerging from the seedbank but not the most frequent species found aboveground in all surveyed regions.

Seedling Emergence from Seedbank/ Seedbank Composition

Based on the graph (Figure 4.3), overall total seedling yields more results compared direct counting. Site I - V (Location A) has a very low result for both total seedling and direct count. displayed significantly the lowest density for germinated seedlings

compared to other locations. Active weed control of the areas might have resulted in low seedbank number. Application of herbicide for instant can suppress germination or might push toward death of the weed seed itself. Kumar *et al.* (2013) states that herbicide use produced a shift in the weed seedbank in favor of germination of weed species that are less susceptible compared with other species to applied herbicides. However, Smith *et al.* (2015) showed that pesticide seed treatment can reduce the abundance of the seed natural enemies such as pathogens (bacteria, fungi) and soil dwelling predator that able to damage or destroy seeds in the soil seedbank thus treated plot had much higher but less diverse weed seedbank compared to untreated plot. Herbicides significantly able to suppress seedling emergence from the seedbank and can harm both non-native and native plants at some growth stages such as seed stage (Wagner & Nelson, 2014). These sites at Location A also has soil composition that was primarily foreign sand that were brought in for development purpose. These sites devoid of vegetation and small soil organism to support higher species of plant. As most of the soil composition were sand particle, it was easier to hand sorted as the difference in size and shape between weed seed and soil were very apparent, thus resulting in high number of observations.

Sites at locations B and C showed higher density of germinated seedlings (sites VI to X) displayed different pattern of the non-active sites where the number of germinations is higher than hand sorted count indicating the environmental conditions of these areas was favorable for weed seed germination. These sites were mostly fertile soil that was once used for agriculture. So, it was easier for the buried seed to emerge because of favorable environment for germination. The soil samples collected here were finer soil than the previous sites causing it harder to hand-sorted the seed from the soil. Other than that, we could also say that these fertile lands had more active soil organism. Earthworm, or soil microorganism had shown to feed on these seeds (Navntoft *et al.*, 2009).

This study also reveals that broadleaf and followed by grasses made up of the large quantity of seeds in the germinable seedbank at the target sites and are consistent with findings in several studies (Gaisberger *et al.*, 2017) that there are high proportion of viable seeds among grasses, and broadleaves species when compared with tree species. This is due to the fact that grasses and broadleaves seeds ability to produce numerous, small sized, and persistent seeds that allow these seeds to enter or establish seedbank easily. Large sized seed could be problematic as they cannot be easily incorporated into the soil seed bank and thus remain on the surface. Thus, causing the seed to be heavily predated and decomposed readily.

Location B and C also has higher germination rate when compared to direct counting from separation method (Figure 4.4). At the same time, average frequency of direct counting for all locations was not significantly different despite variation in germination data. This indicates that determination of composition of seeds in the soil cannot be highly dependent on physically count the seeds. Determination of seedbank composition in the soil by separation method is a tedious process with some inadequacies. Problems of this method are the residue still contains soil material, and hand-sorting under a binocular microscope to collect the seeds is needed which was very time consuming and only suitable for finding large-seeded species but ineffective for small-seeded species, especially in large-scale studies (Price *et al.*, 2010). At the same time, seeds in the soil has diverse morphological characteristics with huge magnitude of sizes (Leslie *et al.*, 2017; Price *et al.*, 2010).

Seedbank Spatial Distribution

Majority of the sites displayed cluster or clump distribution pattern for both grass and broadleaf weeds (Figure 4) based on Lloyd's patchiness index. This distribution pattern suggests a robust seedbank of the area which can provide suitable environments for

further germination (Mohammad *et al.*, 2007). Moreover, it may indicate that the grass and broadleaf weeds only cluster at certain specific areas since weed seeds are generally dispersed in less than two meters away from the parent plant (Roham *et al.*, 2014). However, for seeds to travel further from mother plant, they will require dispersal agent to help facilitate their dispersal (McConkey *et al.*, 2012). It can be assumed that the seedbank is most likely to form a cluster in a low-density weed population. This is possible since the seedbank samples of this study were taken only at the accessible areas where the vegetations were not dense due to safety issue.

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CHAPTER 6: CONCLUSION

This study was done to further the general knowledge and understanding of the weed seedbank dynamics and its affect to agricultural lands especially in Malaysian scenario where there is insufficient study regarding this topic that could facilitate effort to improve the current weed management strategy. For the first objective, the experiment observed the vertical and horizontal spatial distribution of weedy rice seeds by comparing density and germination of both locations from the first experiment. Based on the result, there are no significant difference on the density of weedy rice seeds for majority of the depth level except for 0 -5 cm depth level. Identical pattern of weedy rice seeds for both locations showed increasing density from surface towards 0 – 5 cm and continue to decrease towards 10 – 15 cm with 70 % of the seed accumulated on the surface (Horizontal).

However, germination test showed that rice fields with poor land preparation procedure (Farm Sawah Sempadan) has higher potential of emerging seedbank once the condition permits. Sawah Sempadan seeds have a significantly higher germination percentage (43% - 72%) as compared to Sungai Burung's (10% - 31%) rice field. The highest germination rate at Sungai Burung rice field was at 5 cm depth (31.41%) while the seedbank at surface and 10 cm lower have germination rate of less than 16%. It can be concluded that management practice had an impact on the spatial distribution horizontally and vertically and thus effecting the weed seedbank buildup and weed seed preservation.

For second objective, the second experiment were devised to determine seedbank distribution pattern at different types of abandoned agricultural lands which was in Jelebu, Negeri Sembilan. As a result, different types of land have different seedbank distribution patterns. Experiment shown that different type of land (composition, function) can somehow affect the seeds to maintain its persistency (viability, dormancy) when buried inside the soil seedbank. Weed management program can somehow suppress the

germination or outright kill the seeds as shown in non-active sites (I-V). Fertile land type found in active site (VI-X) could provide enough and better environment for the seeds to form seedbank and continue to remain viable.

Based on the result of both experiments, the third objective in evaluating the infestation potential from the seedbank can be concluded that high density of seedbank in agriculture land may lead to future weed infestation problem. Result showed us that seedbank able to house newly shattered seeds into the seedbank via tillage and continue to remain viable until next season. Higher density of seed inside the seedbank mean higher the amount of seeds that can emerge from the seedbank. Reduction of seeds can be carried out either not allowing the newly shattered seeds to enter the seedbank or induce germination for weedy rice seeds before the seeding period and applying herbicide to kill it. So, human activities and interference can determine the weed seedbank patterns and longevity. Proper land management such as herbicide application and no-till can affect the buildup of seedbank making it a good choice of practices to follow.

Based on the experiments, it shows that the dynamics of seedbank such as density, germination, aboveground vegetation, spatial distribution can be a crucial aspect that can affect positively or negatively for weeds to infest target region. This variety of approach allowing the weed to maintain its species diversity and act as an archive for all the information regarding their dynamics and structure. Studies of the dynamics of seedbank and its succession triggers present us possibility to predict the composition of future vegetation on the field thus, give an opportunity to make a preemptive management plan to counteract its recruitment. Recommendations for future experimentation is the implementation of seed viability testing. It can be done after the seed extraction and hand sorting steps for experiment 2 as an extra procedure to determine whether extracted seeds were viable for germination.

Seed metabolomic research is a new breakthrough in science by providing a more accurate profiling of seeds through metabolites studies. Metabolomics studies has the ability to detect a mass number of metabolites from a one extract, thus allowing precise dan quick analysis of metabolites. These allow opportunities to expand the understanding on dynamics of weed seedbank on more complex and deeper level than what has been done in this experiment. Thus, better weed management program can be developed.

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