NATURAL DISTRIBUTION OF WEED SEEDBANK IN DIFFERENT LAND ACTIVITIES DUE TO ABANDONED LAND RECLAMATION FOR AGRICULTURE

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Abstract. Most studies on weed infestation mainly focused on the aboveground infestation despite the fact that the importance of the seedbank dynamics in influencing weed abundance is well acknowledged. Effective weed management for development or rejuvenation of an abandoned land should consider the potential of weed seed emergence from the seedbank. Soil samples from three different locations were collected from abandoned agriculture lands in Glami Lemi Biotechnology Research Center, Negeri Sembilan (GLBRC), Malaysia to determine the density and distribution pattern of the weed seedbank using seed separation and seedling emergence methods. A total of 53 weed species, mainly broadleaves, were identified in the area. Broadleaf weeds showed a higher number of emerged seedlings compared to grasses which reflected the aboveground weed vegetation composition. Seedling emergence method provided better representation of weed seedbank composition in this study compared to separation method. Lloyd's patchiness index (*lp*) determined that the majority of survey sites displayed cluster distribution pattern for the seedbank of both broadleaf and grass weeds indicating the robust weed seedbank composition of the area. The weed seedbank management could be effectively employed for the development of abandoned lands based on the clustering areas and precise prediction of seedbank density.
Keywords: *distribution pattern, land management, soil seedbank, tillage, weed management*

Introduction

Periodical weed field emergence strongly depends on the amount, botanical composition and vertical arrangement of the seedbank (Benvenuti and Perdossi, 2017). The vertical arrangement is key for weed survival via seedbank where light-dependent small seeds are capable of germination and seedling emergence at the shallowest seed bank possible (Batlla and Benech-Arnold, 2014). In addition, high seed longevity is typical for many weeds (Gu et al., 2006) which facilitates their long-term persistence in the soil (Mispan et al., 2019).

The number of viable seeds in the soil can be affected by ecological conditions and agronomic practices ranges from about 2300 seeds per m^2 in the conventional cropping systems (Sosnoskie et al., 2006), to almost 10,000–50,000 seeds per m^2 in the organic management systems (Albrecht, 2005; Graziani et al., 2012). In Malaysian rice agro-ecosystem for instance, weedy rice seedbank plays a crucial role in weedy rice infestation for future successive seasons (Mispan et al., 2019).

This seedbank accumulation occurs since most annually produced weed seeds undergo dormancy in the soil due to genetic, species, and/or environmental soil-depth mediated mechanisms (Gu et al., 2006; Benvenuti and Mazzoncini, 2019). Consequently, knowing the quantity, botanical composition and vertical distribution of buried seeds can play a crucial role in predicting the likeliness of the dynamics of weed emergence. As seedbank

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plays an important role for plant species survivability and viability, this phenomenon also brings several impacts towards many human activities such in the agriculture practices (Hossain et al., 2014), and forestry (Meers et al., 2012). This information facilitates planning on weed control strategies before weed emergence. In other words, a seedbank evaluation acts as a non-chemical weed management method, thus making it a useful agronomic tool.

Malaysia is a tropical country with warm climate and adequate rainfall. These conditions permit the luxuriant growth of various weed species almost all year round. Today, the Malaysian agro-ecosystems are homes of more than 500 weed species including nine of the world's worst weed species (Heap, 2014; Ruzmi et al., 2017). Some of these species are classified as scheduled pests under the Malaysia Plant Quarantine Act 1976 and Plant Quarantine Regulation 1981. Farmers in Malaysia normally employ a battery of control methods to achieve satisfactory results in managing weeds. These include, principally, the agro-technical and preventive methods comprising land preparation and tillage, water management, crop manipulation through competitive cultivars, chemical control, and in certain cases, biological control using bio-control agents and bio-herbicides is instituted (Mispan et al., 2015). The aim of these weed management strategies was not only eliminating the weeds that emerge but also include limiting the seed production and dispersal (Nichols et al., 2015) to minimize the input of weed seeds in the soil – the seedbank (Mispan et al., 2019).

Abandoned land was known to be infested with weed flora and volunteer crop species when not more in use for any anthropogenic activities. The abilities of these weeds and vegetation to continue to infest the land are supported by the dynamics of their respective seedbank to provide adequate seedling to continue its survival even after abandonment (Valko et al., 2016). Therefore, reclamation of the land for agriculture activities will need to consider the potential of weed infestation from the existing seedbank. Thus, the seedbank dynamics play an important role as a developmental potential for future weeds infestation and vegetation species in the region (Bajwa et al., 2016).

The objective of this study was to observe and determine the ecology of seedbank via seed distribution patterns at different types of soils in an abandoned agricultural land. Such information will be useful for decision making for future weed management based on potential risks and incurred costs for further development of the area. The results can be used for future land-use weed control program for a given types of abandoned land.

Materials and Methods

Study sites

This study was conducted in June 2018 at the Glemi Lemi Biotechnology Research Center (GLBRC), Universiti Malaya (3.053361N, 102.063997E) which is in the district of Jelebu, Negeri Sembilan, Malaysia. This 120-acre facility was built on an abandoned farming land and an ex-tin mining site with the purpose to be a field-research hub for agricultural biotechnology. A total of 10 sampling sites were randomly selected across the land (*Fig. 1*) and was grouped into three categories based on the current land activities (*Table 1*).

Sites I to IV were located within approximately 30 m from the facility's buildings parameter. These areas were regularly maintained from weed infestation mainly by mowing and scheduled herbicide (mainly glyphosate) application. Soil for these areas were sandy with mostly from foreign soil brought in for development and construction

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purpose. Sites V to VII were located near field research experiment sites. During this study, these areas were growing Napier grass and pineapple. Weed management of these sites followed standard weed management practices. Sites VIII to X were located on undeveloped areas where there were no active agricultural practices on the land since the opening of the facility in 2012.

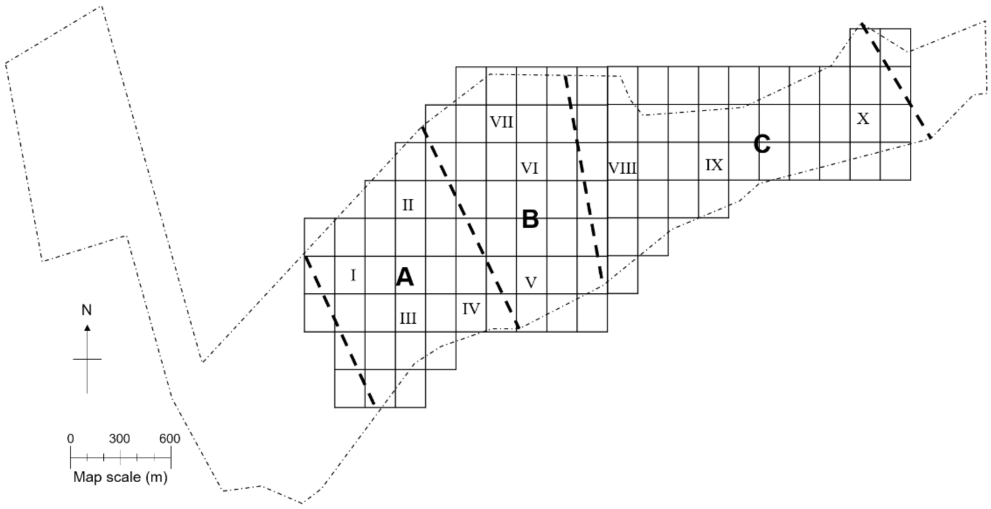

Figure 1. *Locations (A-C) of the seedbank collection at ten sampling sites (I-X). The sampling sites were randomly determined from the aerial grid*

Table 1. *Description of seedbank survey locations in Glami Lemi Biotechnology Research Center, Universiti Malaya, Jelebu, Negeri Sembilan*

Locations	Sites	Description
A	Site I - IV	• Near building areas. No vegetation. Mainly primary succession. • Mostly foreign soil brought in for development purpose.
B	Site V – VII	• Active research sites. • Various type of vegetation being planted: corn, napier, pumpkin. • Vegetation grown for research project.
C	Site VII – X	• Near active location (*B*). • No active agricultural practices or crop grown. • Primarily covered with shrubs and weeds.

Vegetative recording

The weed species of GLBRC were identified and recorded. The species were characterized as grasses, broadleaves, sedges and ferns. Some species were identified directly by observing their taxonomical characteristics, but some were sampled and brought back to lab for further identification.

Seedbank sampling

Distribution of seedbank in GLBRC was accessed by two methods, namely germination test and direct seed counting. A total of five (5) spots were randomly plotted at every sampling site for soil collection. Distance for each spot was more than 20 m apart. Soil was extracted in a 10 cm x 10 cm square and 1 cm deep at each spot. The

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

To estimate composition of the seedbank by germination test, each soil sample was concentrated by washing the soil with water through a course (4.0 mm mesh width) and a fine sieve (0.212 mm mesh width), to remove both coarse and fine soil material, roots and vegetative parts. The concentrated samples were mixed with water until they became fluid then thinly and evenly (~ 5 mm thick) poured in a plastic container (15 cm x 15 cm x 10 cm) filled with 1 cm thick steam-sterilized potting soil and 5 mm thick layer of sterile sand preventing contact between sample and the potting soil. The sand and potting soil were separated with a fine nylon mesh net. The samples were placed in the greenhouse at natural temperatures and humidity. Germinated seedlings were counted at every 3-day interval for six weeks. The containers were kept moist for the entire experiment. Germinated seeds were identified as grass or broadleaf weed based on seedling leaf morphological characteristic.

Additional soil samples from the similar plots for germination test were used for direct seed counting. A 1 cm thick layer of soil samples were collected and placed in a 9 mm petri dish. The samples were air-dried for 3 days in the greenhouse. The presence of seed in the soil was directly counted under light microscope.

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) (Baki and Shakirin, 2010). The distribution pattern can be determined using the Iwao line from the m*/m plotting. Values located close to the line indicate a random distribution. Values plotted above and below the line correspond to clustered and uniform distribution, respectively.

Results

Aboveground weed composition and seedbank composition

Survey of the aboveground vegetation identified and recorded a total of 53 weed species within the facility. There were 17 families of weed characterized as broadleaf, grass, sedge and fern with 36, 14, 2 and 1 number of species, respectively (*Fig. 2*). This information will be the base to corroborate with the seedbank composition.

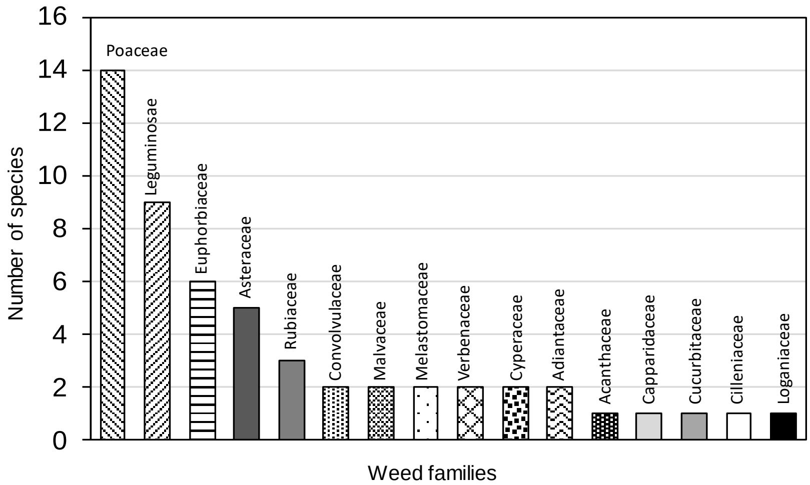

Figure 2. *Number of weed species according to respective family identified in survey sites*

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Germination test on the soil samples showed that the seedlings started to emerge from the soil at 3 d after soaking and reached full germination at 9 d (*Fig. 3*). No new seedlings emerged after 9 d of imbibition. Sites VI and VII displayed the highest number of germinated seedlings with an average of 212.0 (±352.26) and 144.0 (±352.26) seedlings, respectively especially in Plots 6.1 and 7.1.

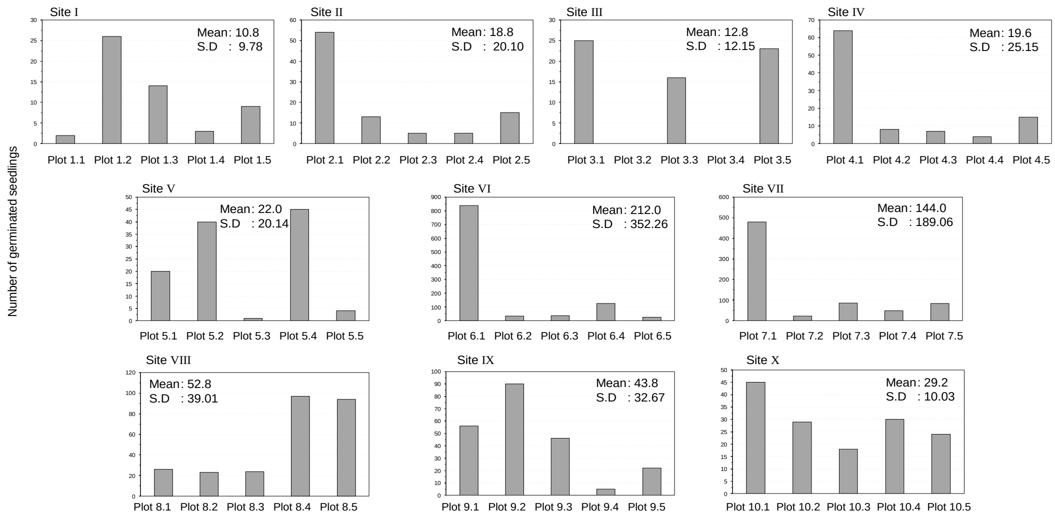

Figure 3. *Number of emerged seedlings recorded at every site (I-X). Mean and standard deviation (S.D.) were calculated based on five plots in each site*

Due to high standard deviation of number of seedlings in the majority of the sites, numbers for all plots at each site were combined to represent the site. Emerged seedlings were differentiated as grasses and broadleaves, based on their seedlings leaf characteristics (*Fig. 4*). Site VI recorded the highest density of both grass and broadleaf seedlings, while Sites I and II displayed the lowest density for broadleaf and grass seedlings, respectively. Majority of the sites showed higher density for broadleaves than grasses except at Sites II. The biggest difference was shown within location B where agriculture activities were located.

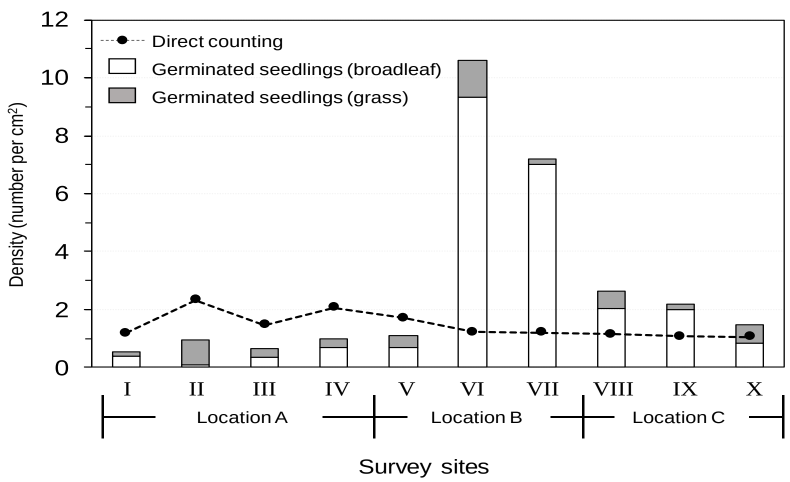

Figure 4. *Density (number per cm^2) of germinated seedlings for grass and broadleaf weeds (bar) and number of seeds from direct counting (dotted line) from the sampling sites (I-X)*

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Seeds from the seedbank were hand sorted and directly counted (*Fig. 4*). Across all sites, locations B (except Site V) 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

Seedling spatial distribution

None of the weed seedbanks for both broadleaf and grass types form uniform distribution at all sites based on Lloyd's patchiness index (*Fig. 5*). Majority of the sites displayed cluster or clump distribution pattern for both grass and broadleaf weeds. Broadleaves showed random distribution at sites I, IV and VII, while grasses only displayed random distribution at site II.

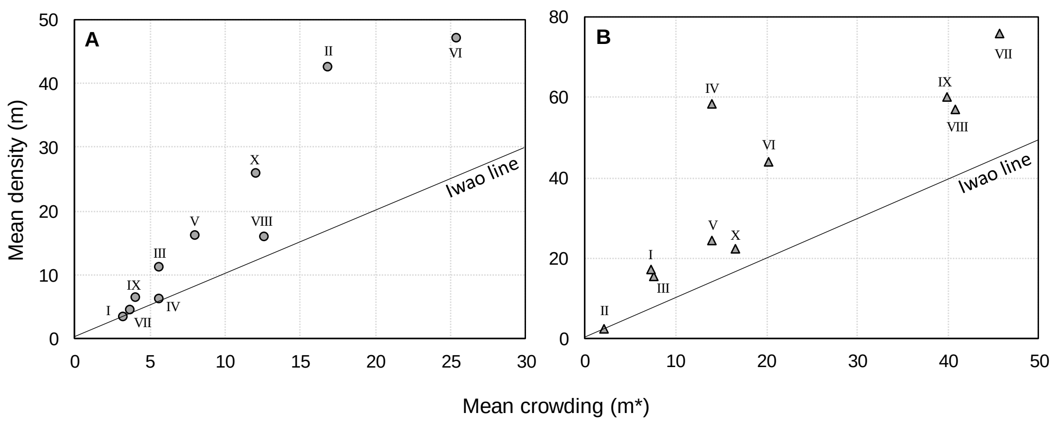

Figure 5. *Distribution pattern of broadleaf (A) and grass (B) weeds based on mean density (m) and mean crowding (m*) for every survey site (I-X). Sites located on, above, and below Iwao line indicate random, clustered and uniform distribution pattern, respectively*

Discussion

An effective management plan for invasive weed infestation must consider the potential for the emergence of weeds from the soil seedbank (Head et al., 2015). Weeds can persist in an agricultural land through their seed's survivability in the seedbank. Maintaining viability over longer periods in the seedbank might provide several adaptive advantages for weed seeds to survive heat and high humidity and escape seed deterioration especially in tropical areas. Therefore, knowing the composition of weed in the seedbank is crucial to allow effective management strategies to be designed to reduce or ultimately eliminate the possible seed escape (Mispan et al., 2019).

This study showed that the aboveground weed vegetation can be reflected to the seedbank composition of grass and broadleaf weeds. Broadleaf weeds displayed higher density of germinated seedling compared to grasses (*Fig. 4*) which tallied with our aboveground weed composition findings in *Fig. 2*. Takim et al. (2013) reported that

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broadleaf weed seeds were easy to germinate in the agricultural fields. 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.

Sites at locations B and C showed higher density of germinated seedlings as compared to direct counting from separation method (*Fig. 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 (Ter Heerdt et al., 1996). 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 (Price et al., 2010; Leslie et al., 2017).

While germination data provides better representation of seeds information in the seedbank, many other factors can contribute towards seeds germination ability which the extent of variation is still unknown (Price et al., 2010; Leslie et al., 2017). Seedlings emergence from the seedbank can be affected by field management practices (Chauhan et al., 2012), weed seedbank diversity (Mesquita et al., 2013), seed dormancy and viability (Kleemann and Gill, 2013), and seed placement (Mohler et al., 2006). Chauhan et al. (2006) reported that seed decay in no-tillage systems (48 to 60%) was much higher than under minimum tillage (12 to 39%). As these factors and their impacts are considered, the efficiency and efficacy of weed seedbank management can be increased.

It was also clear that sites at Location A displayed significantly the lowest density for emerged seedlings compared to other locations. Active weed control of the areas might have resulted in the low seedbank number. Application of herbicide for instance can suppress germination or might push toward death of the weed seed itself. Kumar et al. (2013) reported that herbicide usage produced a shift in the weed seedbank in favor of germination of weed species that are less susceptible compared with other species. However, Smith et al. (2016) 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 and Nelson, 2014).

High standard deviation at each site (*Fig. 2*) indicates the variation of seedbank distribution in the field. Analysis of spatial distribution of the seedbank might provide new insight on how the seeds of the weeds dispersed into the seedbank. The cluster distribution pattern at the majority of the sites suggests a robust seedbank in the area

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which can provide suitable environments for further germination (Mohammadvand 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 dispersion (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, despite being random sampling, were taken only at the accessible areas where the vegetations were not dense due to safety issue.

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 and 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 and Mackvandi, 2008) and precise prediction on seedbank density.

Although this study could give a fair estimation of future weed emergence in the field, the result is only a representation of a small and variable fraction of weed seed in the soil seedbank. Therefore, it is proposed, the assessment on seed content in the soil especially in a large-scale study needs a reliable, quick and space-savvy method. A new approach can be developed to determine seedbank composition in the soil using molecular methods. Molecular techniques have been developed to detect and quantify a range of organisms in soil (Ophel-Keller et al., 2008) but very limited studies are available on seeds in the soil. Riley et al. (2010) developed quantitative DNA assays for *Lolium perenne* (ryegrass) and *Trifolium subterraneum* (subterranean clover) to include seeds determination in the soil in mixed plant populations. DNA extraction from seeds in general is feasible (Barrett et al., 2005) indicating the potential of using molecular techniques on seeds. Osterbauer and Rehms (2002) used Polymerase Chain Reaction (PCR) techniques to detect single seed of broomrape (*Orobanche minor* Smith.), which are extremely small, averaging 200 to 300 μm in size. This method was expanded by Rolland et al. (2016) by using High Resolution Melting assay where they successfully and accurately determined the targeted broomrape species from the soil. Accurate seedbank information will assist in decision support system in the future for weed management via seedbank.

Conclusion

Effective weed management for development or rejuvenation of an abandoned land should consider the potential of weed seed emergence from the soil seedbank. This study

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shows that broadleaf weeds were highly likely to emerge first under favorable condition. Any potential weed management including application of pre-emergence herbicide should consider this possibility. Different activities also influenced soil seedbank distribution. Active weed management will reduce weed seedbank capability to emerge as the density was significantly reduced. On the other hand, abandonment of the land produced patchiness or clustering of weed seeds in the seedbank which contributes to uncertainties of future weed infestation. Molecular techniques are proposed as a way forward to determine weed seedbank composition in agricultural soils to overcome the limitations in the seedling emergence and seed separation methods. Understanding the nature on how seed of weeds dispersed and settled in the soil as seedbank is significant to provide better strategy(s) to manage/control weeds.

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