Morpho-Physiological Dynamics of Weedy Rice Seeds Collected from Two Contrasting Agro-Ecological Zones in Sri Lanka

D. Ratnasekera[1*] and K.M.A.S.K. Rupasingha[1]

[1]*Department of Agricultural Biology, Faculty of Agriculture, University of Ruhuna, Sri Lanka*

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Abstract

Weedy rice (*Oryza sativa* L. *f. spontanea*), seeds remain a longer period in soils at different depths enriching soil seed bank and that contribute to the success of weedy rice as a "weed." Hence basic information on the level of longevity, dormancy and germination behaviour of weedy rice seeds with relation to its morphology is very important to implement efficient control measures. In this study, weedy rice seeds were collected from six infested locations in Ampara and Matara districts representing two different agro-ecological zones in Sri Lanka. Two widely grown improved varieties (At 362 and Bg 379-2) were assembled as check lines. Thirty panicles per population were randomly collected from each location to determine their morphological characteristics including awn lengths, seed shape, hull colour, pericarp colour along with physiological phenologies such as degree of dormancy, viability, longevity and rate of survival.

Significant variability of seed shape, awn length, hull colour and pericarp colour was observed. Germination rate and survival rates were highly variable and closely associated with awn characteristics. Our study clearly indicated that prolong longevity (more than 24 weeks) and viability of weedy rice seeds in field conditions implying their key role as a weed by enriching soil seed bank. Awned populations are dormant and influence weedy rice population dynamics leading to the competitiveness of this weed. Therefore, management practices have essentially to take them into account and be adapted accordingly. Further, this study inferred that the morpho-physiological variation of the weedy rice seed populations was not associated with the agro-ecological conditions; for example, the dry and the wet zone suggesting rapid seed mediated gene flow throughout the country.

Keywords: dormancy, germination, seed viability, seed morphology

1. Introduction

Weedy rice (*Oryza sativa* complex) has become the most dominant and competitive conspecific weed relative of cultivated rice (*Oryza sativa* L.) that occurs in rice fields worldwide (Michael et al., 2010). It is the main competitor with cultivated rice, affecting both growth and yield, especially in regard to space and nutrient availability (Zainudin et al., 2010). Weedy rice is vegetatively very similar to cultivated rice but has some key differences *viz.* shattering seed dispersal, red pericarp pigmentation,

[*]*Correspondence: disnaratnasekera@gmail.com*
Tel: +94 718232808
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and the ability of seeds to persist in the soil (Delouche et al., 2007). The management of weedy rice infestations is much more difficult than that of other rice weeds because of the high biological similarity with cultivated rice and the extended germination over a long period of rice growth (Fogliatto et al., 2010). Weedy rice has close affinity with cultivated rice, in terms of morphological and physiological characteristics (Fogliatto et al., 2011). This similarity has even led weedy rice to be classified as the same species as cultivated rice (Vaughan et al., 2001). Morphologically, weedy rice is highly variable in almost all the vegetative and reproductive characteristics with each other and appears to be an intermediate between wild and cultivated rice (Cao et al., 2006). In addition, it is considered as a useful germplasms, as it has successfully adapted to the natural growing conditions (Heu et al., 1990). It also has many useful genes for cold tolerance (Chang et al., 2004), grain quality and germination characteristics (Ma et al., 2008) and high salinity and drought tolerance (Jiang et al., 1985).

Weedy rice was first identified in mid 1990’s as a threat from Vavunia, Ampara, and Batticaloa districts (Marambe and Amarasinghe, 2000) and at present that is most common in all rice growing areas in Sri Lanka. The superior competitive ability of weedy rice over cultivated rice has contributed to its rapid spread in the country (Abeysekera et al., 2010). The seed-mediated gene flow of weedy rice along with the long-distance exchange of farmer-saved rice seeds between weedy-rice contaminated regions in Sri Lanka has significant influence in spreading weedy rice throughout the country (He et al., 2014). Lack of a selective herbicide for the control of weedy rice, or other effective measures, has made its control a subject of national significance in the country. Morphological traits such as seed colour and seed shapes and awn characteristic are highly variable in weedy rice while physiological traits such as degree of dormancy, germination ability, viability and longevity also showed high diversity. Higher levels of seed shattering and seed dormancy have enriched the soil seed bank of weedy rice in infested fields. Seeds in the soil seed bank may germinate as soon as conditions are favourable (e.g., soil moisture, oxygen, and temperature), while other seeds will germinate at later days or when other factors change. The variable and prolonged periods during which seeds remain dormant are major factors that contribute to the success of weedy rice as a “weed”. Strategies for the control of weedy rice are diverse and their implementation depends on the specific site conditions. However, any control measure should aim to reduce the weedy rice seed bank in soil in the medium or long term (Labrada, 2010). Weedy rice seed banks together with other seed characteristics therefore play an important role in determining the severity of infestation in rice fields (Abeysekera et al., 2010). It also highlighted the importance of testing germination behaviour of weedy rice that similar to field conditions to implement efficient control measures.

This study attempted to determine the basic seed characteristics and physiological phenologies *viz.* germination percentage, viability over time under field conditions and survival rate with relation to contrasting ecological conditions. The information gathered in this study will provide a useful guideline for designing effective strategies to control and manage weedy rice in Sri Lanka.

2. Methodology

2.1 Sample collection

An extensive field survey was carried out at Ampara (representing dry zone) and Matara (representing wet zone) districts in Sri Lanka and total of six weedy rice infested locations were selected intentionally as sampling sites. The selected sites were Akuressa, Thihagoda and Mulatiyana from Matara district and Akkareipattu, Ampara and Lahugala from Ampara district. Panicles from 30 individuals were collected from each location twice in Yala and Maha season in year 2012 and 2013 and tested separately.

2.2 Seed germination and morphology

Seed morphology was recorded by considering awn traits, seed shape, seed size and pericarp pigmentation. The collected mature seeds were dried to 13% of moisture and 10 weedy rice seeds were randomly collected from each panicle per individual and totally 300 seeds from each population, mixed well and randomly collected 100 seeds per one population were used for dormancy and germination test. Weedy rice seeds were soaked in water for 24 hours, covered for 24 hours by cloth bag (standard germination percentage testing method) and were kept on the humid filter paper in the petri dish with sufficient light. Every population was tested four times and germinated seeds were transferred into mud trays and healthy plants were counted after 21 days. Survival rate was assessed as the percentage of germinated seeds.

The rate of seed germination was calculated according to Maguire (1962) using $GR=\Sigma Dn/\Sigma n$ where, D is the number of days counted from the beginning of sprouting and n is the number of newly emerged seedlings on day D.

2.3 Seed viability

Germination and viability of samples were tested in all test lines before commencing the experiment. Seeds were soaked for 18 hours in water and select the 100 seeds and they were split in two halves trough embryo. Splited seeds were kept in 1% triphenyl-tetrazolium chloride (TTC) solution for two hours in dark for staining and reddish-pink stained seeds were counted as viable seeds.

2.4 Burial test

The burial test was conducted twice during 2013 in a rice field of the experimental garden at faculty of Agriculture, University of Ruhuna, Sri Lanka. Weedy rice seeds collected from different locations in each district were mixed well to get a representative sample, and they were put into separate nylon bags at the rate of 200 seeds per bag. Four bags representing each district, but collectively three locations from each district were prepared per each depth for the burial test. In addition to weedy rice two widely grown improved varieties (At 362 and Bg 379-2) were included in the study as check lines. Bags were buried 15 and 30 cm depths in paddy field respectively with four replicates. Over a period of 24 weeks, bags of the buried seeds were dug up at two-week intervals commencing from the four weeks after burying (WAB) for testing their viability and germination ability.

3. Results

3.1 Seed morphological diversity

Our results showed that there was a great diversity in seeds in terms of seed shape, awn length, hull colour and pericarp colour. However, those variations showed no association with agro-ecological conditions. Morphological and topographical characteristics of plant organs such as the shape and size of seeds and the structure of incidental features have been useful weapons in identifying and classifying the plant and weed species (Noda et al., 1985; Prathepha, 2009).

Weedy rice seeds collected from Matara and Ampara districts showed greater variation in awn length, which varied from 0 (awnless) to 10 cm (Figure 1a). A considerable diversity was observed in seed shape (Figure 1b) and hull colour, which varied from pale white to ashy-black (Figure 1c). Pericarp colour varied from white to brownish red (Figure 1d). The variability observed were comparatively distributed among populations as well as within the populations in same extend in both locations. The plants observed from the study showed grains with and without awns. The size and the colour of the awn were also highly variable.

a. Variation in awn length **b.** Variation in seed shapes

c. Variation in hull colour **d.** Variation in pericarp colour

Figure 1: Morphological variability of weedy rice seeds collected from Ampara and Matara districts.

Table 1: Percentages of seed morphological traits of weedy rice collected from Matara and Ampara districts in 2012-2013.

Population	Hull colour (%)			Pericarp colour (%)			Awn length (%)			Seed shape (%)		
	S	B	G	R	Br	W	L	M	A	L	I	Ro
Akuressa	60	10	30	88	8	4	25	35	40	30	45	25
Thihagoda	70	5	25	80	15	5	22	26	52	32	43	25
Mulatiyana	62	13	25	90	6	4	48	34	18	30	55	15
Ampara	50	22	28	81	15	4	30	25	45	45	43	12
Akkareipattu	56	15	29	83	11	6	35	25	40	48	38	14
Lahugala	63	16	21	81	12	7	50	32	18	55	35	10

A=awnless; B=black; Br=brown; G=gray; I=intermediate; L=long; M=medium; R=red; Ro=round; S=straw; W=white

Referring to all populations, straw hull colour seeds occur in a higher percentage (>50%) than black (<25%) and gray (<30%) hull coloured seeds. The pericarp colour red is more prominent (>80%) compare to white pericarp seeds (<7%) (Table 1).

3.2 Dormancy and Germination Rate

The degree of seed dormancy of weedy rice was not uniform. The Lahugala (LH) population of Ampara district representing dry zone showed strong dormancy during 5-50 DAH while the rest of the two populations from the same district displayed medium seed dormancy similar to check lines used. The Mulatiyana (ML) population of Matara district representing wet zone has relatively high degree of dormancy similar to Lahugala population inferring variation of the weedy rice populations was not associated with the agro-ecological conditions. In general, all test lines required nearly three months to be overcome dormancy (Table 2).

The majority of tested weedy rice populations had the medium seed dormancy as same with the cultivated rice Bg 379/2 and At 362. However, weedy rice populations do not had GP of seeds uniformly as compared to cultivated rice. This trait may be contributed to the reason why weedy rice is present in the field for the succeeding seasons when they meet favourable conditions in the long run.

Table 2: Dormancy and Germination behaviour of weedy rice populations collected from Ampara and Matara districts representing 03 locations from each district.

	5 DAH	20 DAH	50 DAH	80 DAH	100 DAH	120 DAH	140 DAH
AK	10.47^{d}	73.41^{d}	85.77^{b}	94.66^{a}	96.64^{b}	94.68^{b}	68.53^{b}
AM	25.42^{e}	73.66^{d}	87.77^{b}	98.00^{b}	98.34^{b}	90.66^{a}	66.66^{b}
LH	2.66^{a}	11.00^{a}	63.67^{a}	93.83^{a}	98.64^{b}	85.98^{a}	60.22^{ab}
TH	30.22^{e}	74.26^{d}	90.66^{b}	99.00^{b}	98.66^{b}	95.33^{b}	70.32^{b}
ML	9.76^{cd}	46.54^{b}	58.78^{a}	98.66^{b}	90.00^{a}	87.00^{a}	54.66^{a}
AR	27.54^{e}	60.75^{c}	90.00^{b}	98.72^{b}	98.00^{b}	90.00^{a}	61.21^{ab}
Bg379/2	5.51^{bc}	58.44^{c}	87.45^{b}	98.00^{b}	98.33^{b}	95.00^{b}	73.65^{c}
At362	3.87^{ab}	57.66^{c}	90.33^{b}	99.00^{b}	98.00^{b}	91.00^{a}	73.00^{c}

DAH=days after harvest; AK=Akkareipattu; AM=Ampara; LH=Lahugala; TH=Thihagoda; ML=Mulatiyana; AR=Akuressa

Data in a column followed by the same letter are not significantly different by DMRT.

All the test lines showed lower germination percentages (GP) when seeds germinated after at 5 days after harvesting (DAH), indicating considerable degree of dormancy.

3.3. Seeding servival rates

Survival percentage was highly variable in weedy rice population in Ampara and Matara districts. Ampara population showed highest survival percentage (90%) while Lahugala population showed lowest survival percentage (43.33%). Except Lahugala and Mulatiyana populations, other populations showed high survival rates (Figure 2).

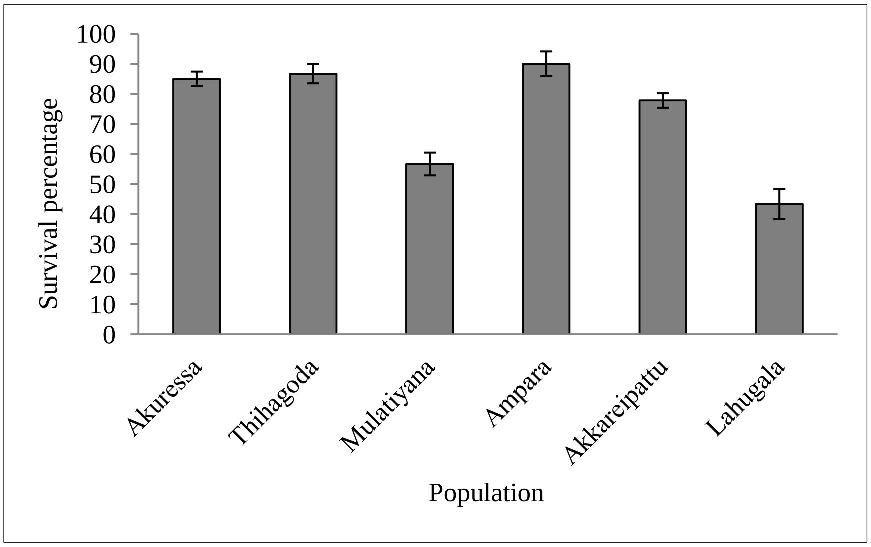


Figure 2: Survival percentage of weedy rice populations collected from Ampara and Matara districts. Vertical bars on the columns showed standed errors.

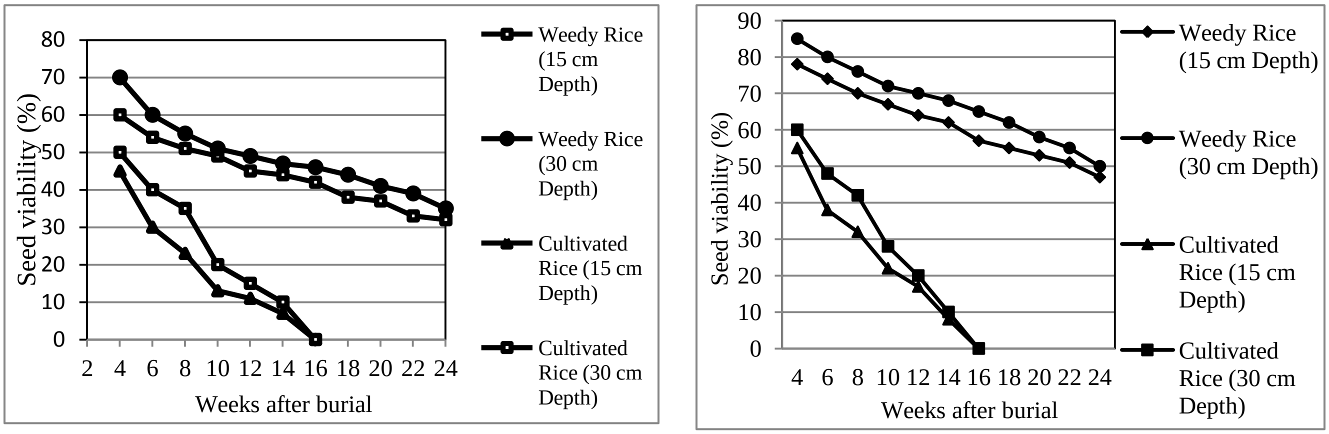
3.4. Burial test

Both weedy rice and improved rice varieties showed more than 85% viability and more than 80% germination before burying. The difference between viability and germination of weedy rice was low, inferring that the degree of seed dormancy of weedy rice was not considerable between two locations tested.

Germination percentage of improved rice buried at 15 cm and 30 cm depth was 45% and 50% respectively at 4WAB and it declined sharply to zero at 16WAB (Figure 3a). The same pattern of results followed a similar pattern for the two improved rice varieties.

Germination percentage of weedy rice buried at 15 cm and 30 cm depth was 60% and 70% respectively at 4WAB and it declined gradually to 32% and 35% respectively at 24WAB. Viability percentages also followed similar pattern (Figure 3b) for improved and weedy rice seeds buried at 15 cm and 30 cm depth.

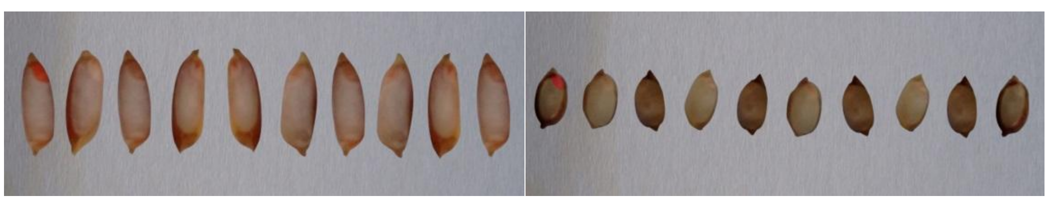
These experiments revealed that weedy rice seeds could remain viable under soil for more than 24 weeks compared with the improved rice varieties, which did not remain viable beyond 16 WAB. Viability and germination ability of seeds always higher in deep soil (30 cm) compared with surface soil (15 cm depth) for both weedy and improved rice (Figures 3 and 4). These results supported the observations of the persistent nature of weedy rice. Further, the findings highlight the importance of management measures to decrease the weedy rice soil seed bank of infested fields and of long-term strategies to minimize the soil seed bank of weedy rice.



(a) (b)

Figure 3: Germination and viability percentage of improved rice and weedy rice seeds from 4 to 24 weeks after burying in paddy soil.

Weedy rice seeds at 15 cm depth   Weedy rice seeds at 30 cm depth



Improved (At 362) seeds at 15 cm depth   Improved (Bg 359) seeds at 30 cm depth

Figure 4: Comparison of Seed viability in weedy and improved rice at two different depths (15 cm &30 cm) at 12 WAB by using TTC test.

4. Discussion

Constantin (1960) reported three types of red rice based on hull colour. Further, straw hull red rice is more common than black hull (Huey and Baldwin, 1978; Smith, 1981). Presence of awn in seeds was a characteristic feature to weedy rice, but occurrence of awn less seeds also in a considerable percentage (>35%) in the all populations was noticed. Similarly, Perera et al. (2010) reported that higher variation in weedy rice seeds among and within the locations of Amapara district, and reported 4 categories of awn types, which has been supported by the observation by Marambe and Amarasinghe, in 2000.

Our study clearly indicated that prolong longevity (more than 24 weeks) and viability of weedy rice seeds in field conditions implying their key role as a weed by enriching soil seed bank. One previous study found that certain weedy rice populations from temperate rice planting regions have either extremely weak or no seed dormancy (Delouche et al., 2007). Xia et al. (2011) confirmed that weedy rice in temperate rice planting regions evolved directly from domesticated rice and have weak or no dormancy. By contrast, the seed dormancy of tropical weedy rice may be attributable to a hybrid ancestry involving a wild species that donated genes for dormancy (Veasey et al., 2004).

In addition, morphological diversity, not only among the weedy rice populations but also within them, offers an array of traits that could be studied and incorporated to future rice-breeding programs (Griselda et al., 2004). Previous studies have reported that diversity of seed traits such as hull colour in weedy rice is greater than cultivated rice (Fogliatto et al., 2011). Our results showed that great diversity in weedy rice seeds and the favourable characteristics such as high germination percentage, high survival ability, awn less seeds, proper seed shape and pericarp colour can be incorporated into cultivated rice varieties in rice breeding programs. In particular, the awned populations showed the greater diversity in traits that can impact species' weediness (Fogliatto et al., 2011). We found large variability in germination pattern characteristics known to influence seed bank dynamics and infestation evolution. From an evolutionary point of view, awned populations would be favoured under different environmental and cropping systems, being able to adapt more easily. According to this study, awned populations are usually dormant and influence weedy rice population dynamics and eventually the competitiveness of this weed. Therefore, management practices have essentially to take them into account and be adapted accordingly. Our study also inferred that the morpho- physiological variation of the weedy rice seed populations was not associated with the agro-ecological conditions; for example, the dry and the wet zone. This is probably caused by seed-mediated gene flow via farmers' frequent exchange of rice cultivar seeds and through other media such as the machinery used for rice harvesting and water canals.

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