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Environmental Effects on Germination and Seedling Emergence of Weedy Rice

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ABSTRACT

The objective of this study is to determine the germination and growth of weedy rice seedlings that were grown under four different environmental conditions simulated in either growth chamber or room conditions. Photoperiod (24 h light, 24 h dark, 12 h light-dark), drought (0, 5, 10, 20 %) and temperature (25, 30, 35, 40 °C) effects were tested in growth chamber while the flooding depth (0, 2.5, 5, 10 cm) effects was tested in room condition. Germination percentage was not significantly affected ($p > 0.05$) by either of the environmental factors except for flooding depth at 10 cm. Temperature treatment specifically at 30 °C showed a pronounced effect on hypocotyl length, radicle length and root-shoot ratio compared to other temperature range. Highest hypocotyl length and root-shoot ratio were recorded in 10 % drought treatment and longest radicle length obtained in control treatment. Meanwhile, a continuous dark condition in photoperiod treatment showed the highest hypocotyl length in comparison to 24 hours of light and alternate 12 hours of light and dark. Highest radicle length and root-shoot ratio were obtained in 24 hours of light and 12 hours alternate light and dark respectively. Growth of weedy rice exhibited a declining trend in increasing flooding depth. The result of this study suggests that germination was retarded by deep flooding. Temperature and flooding depth affects the vegetative growth of weedy rice the most and sustained growth was observed at higher temperature regime. The information gained from this study may be useful in assessing the growth performance of weedy rice in relation to cultivated rice, and further helps in developing effective Malaysian weedy rice control strategies.

Keywords: Drought, flooding depth, photoperiod, temperature, weedy rice

INTRODUCTION

The emergence of weedy rice becomes a concern globally and in Asia, sightings of weedy rice infestation were reported primarily in Malaysia during 1988, Phillippines in 1990 and Vietnam in 1994 (Wahab & Suhaimi, 1991; Mortimer et al., 2000). Weedy rice is a notorious weed in the rice field, as it possesses similar morphological and physiological traits to the cultivated rice, hence it cannot be distinguished during critical stages of weeding. It causes severe yield loss to rice producers, which easily makes it one of the most problematic weeds in the 21st century (Varshney & Tiwari, 2008). Counter measures are taken to alleviate the infestations and lowers the yield loss using different weed controls. Introduction of herbicide-resistant rice that is commercialized in 2002, Clearfield™ (CL) that are mutated without insertion of any foreign gene can tolerate imidazolinone herbicide (Croughan et al., 1996) brings the hope of a selective approach in controlling weedy rice. However, there are potential for outcrossing between CL cultivar and weedy rice. Scott and Burgos (2004) corroborated the information by confirming an outcrossing event of CL161 and weedy rice in a farmer's field in Arkansas. So, other alternatives need to be utilized, such as using various cultural weed management like proper water management to replace the dependency on the toxicity of herbicides that poses a residual hazard on environmental safety.

Another concern on rice production and weedy rice management is the global climate changes, which could cause implications in rice production by worsening weed management (Rodenburg et al., 2011). Global climate change can lead to modifications in land and water resources as well as lowering rice crop productivity all across the world. Weeds are also affected by global climate change by inducing an alteration in the composition of arable weed species (Peters et al., 2014). Evidence provided by Ziska et al. (2012) showed climate change fosters weed-crop gene flow and the adaptive fitness values of weedy strains are enhanced in addition to altered phenological traits. Furthermore, global climate change could inflict drought effect by changes in seasonal rainfall, and inconsistent pattern of precipitation that can lead to frequent drought and flood (Korres et al., 2017). Additionally, aside from cold tolerance, several weedy rice accessions had been identified with higher tolerance to drought and salinity stress (Chen & Suh, 2015). Thus, weedy rice imposes a threat to rice production by better growing adaptibility in a rice field in spite of water deficit.

Climate change causes the temperature to rise (Rosenzweig & Parry, 1994) which affects weed species like *Rottboellia* spp. and *Striga* spp. of their dispersal, growth and reproduction (Mohamed et al., 2006). Ziska et al. (2012) reported that in higher temperature, weedy rice displayed high biomass production and seed yield. This shows that weedy rice can potentially endure and outperform the growth performance of rice crops at elevated temperature in future. Photoperiod also affects plant responses as plants exhibit different physiological reaction based on the relative length of day and night which is known as photoperiodism. Other responses of plants commonly associated with photoperiod are flowering time, where 14 quantitative trait locus (QTL) controlling the flowering time of hybrid japonica and indica rice in response to photoperiod was found out in a study by Yano et al. (2001). However, less study is conducted on photoperiodism in weedy rice and responses to vegetative growth, which requires clarification on this matter. Thus, this study is conducted to determine the effect of different environmental conditions on the germination and growth of weedy rice under growth chamber or room conditions.

MATERIALS AND METHODS

Seed collection

Samples of weedy rice were taken from a paddy rice field in Pendang, Kedah prior to harvesting period at coordinate 5.985390 N, 100.452831 E. The weedy rice plants were randomly collected from three sampling areas on the field, with three weedy rice stalk for each area. Then, the stalks were put into different plastic bags to separate them before being taken back to Plant Physiology Lab of Universiti Sultan Zainal Abidin Besut Campus.

Seed storage

The weedy rice seeds were separated from the panicles by shaking the plastic bag. Afterwards, the separated seeds were put into three different plastic jars and let for air-dry to remove moisture under room temperature. Then, the jars were closed and put into chiller at 20 °C inside the Plant Physiology Lab before being used later.

Drought effect treatment

Effect of drought on weedy rice was tested by the induction of water stress effect through the inclusion of polyethylene glycol 8000 (PEG) to make different osmotic potential [0 (control), 5, 10 and 20 %] in 200 ml distilled water solution for each treatment (Ahmad et al., 2018). Seed preparation and viability test were conducted and the seeds were put into petri dishes for germination on top of two layers of filter paper added with 20 ml of the various concentration of polyethylene glycol solution. The Petri dishes were incubated at a temperature of 28 ± 2 °C with 12 hours of light and dark (12 L-12 D) inside the growth chamber for a duration of 14 days. If the need arised, the filter paper was replaced and polyethylene glycol was added if dried up. Each osmotic potential treatment had five seeds in Petri dishes with triplicates. Germination percentage, length of hypocotyl and radicle as well as the root-shoot ratio was recorded at 14 days after sowing (DAS).

Photoperiod effect treatment

Three different photoperiods were induced to discern the effect of day length on germination. The seeds were sown in Petri dishes with five seeds each in three replicates with photoperiod conditions of constant darkness (24 D), constant daylight (24 L) and alternating exposure to light and dark (12 L-12 D) (Humphries et al., 2018). Incubation was conducted inside a growth chamber with RH 60 % and temperature 28±2 °C for 14 days. For the 24 h replicates, aluminium foil was used to cover the Petri dishes to block out the light exposure. Germination percentage, length of hypocotyl and radicle as well as the root-shoot ratio were observed and recorded at 14 DAS.

Temperature effect treatment

Germination test for observing temperature effects was conducted in the growth chamber with different temperature levels. Preparation of seeds and seed viability test were conducted and temperature used were in the range of 25 °Cto 40 °C. This experiment had exposed the seed to the different temperature of 25, 30, 35, 40 °C following Puteh et al. (2010) inside the growth chamber. This experiment was performed in three replication and comprised of five seeds per replicate. The seeds were sown in petri dishes to germinate on double layer of filter paper moistened with distilled water. Germination percentage, length of hypocotyl, length of radicle and the root-shoot ratio were observed and recorded at 14 DAS.

Flooding depth effect treatment

Plastic pots were added with soil taken from paddy field in Kedah, and were sown with five seeds containing four different water level on the top surface of soil [0 (control), 2.5, 5.0, 10.0 cm] (Chauhan, 2012). The pots were placed in the outdoor condition with some shades and approximate outdoor temperature of 30 ± 2 °C. Germination under flooding condition was recorded at 14 DAS and germination percentage, hypocotyl length, radicle length and root-shoot ratio were recorded in the table. For each pot, there was three replication. Germination was considered when the emergence of radical reaches 5 mm or more.

Statistical analysis

Complete Randomized Design (CRD) was used to determine the effects of environmental factors on germination and seedling emergence of weedy rice in three replications. Statistical analysis of the data was performed using one-way Analysis of Variance (ANOVA) and the significance of variation between means were tested using Duncan's Multiple Range Test (DMRT) using IBM Statistical Package for Social Science (SPSS) version 14.0.

RESULTS AND DISCUSSION

Effects of environmental factors on germination percentage of weedy rice

There were no significant differences observed ($p > 0.05$) of photoperiod, drought and temperature treatments on germination of weedy rice (Fig. 1). However, there was a significant difference in flooding depth treatments ($p < 0.05$), which implies that flooding depth can inhibit germination of weedy rice.

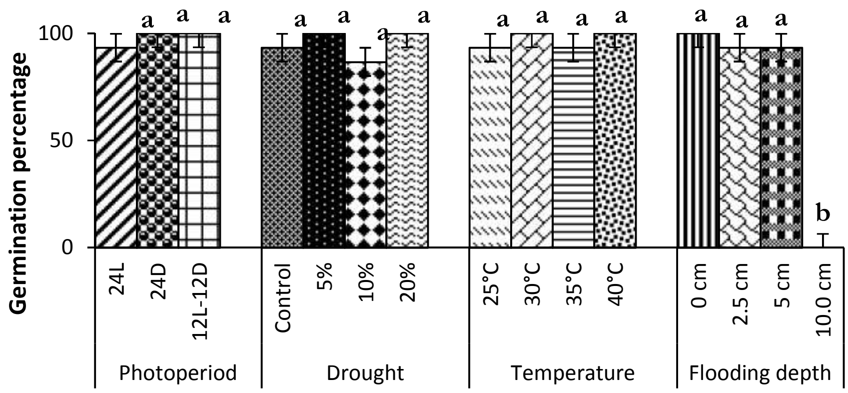

Fig. 1. Effects of photoperiod, drought, temperature and flooding depth on the germination percentage of weedy rice.

Weedy rice seed germination was insensitive to light or dark, hence this indicates the weedy rice species, in particular, was non-photoblastic and able to germinate in the presence or absence of light. A study by Rosli (2008) presented that the weedy rice seeds do not show sensitivity to light during germination. However, there were weedy rice species that were photoblastic, such as in the study by Lee et al. (2018) where the Korean weedy rice germination was inducible by light.

Weedy rice germination also was insensitive to drought treatment, and this may be attributed to the drought tolerance in weedy rice. Suh and Ha (1993) stated that many useful genes are present in weedy rice, one of it being drought tolerance. A study by Ding et al. (2013) also revealed no significant effect of drought stress on germination percentage using 29 accessions of weedy rice and nine varieties of cultivated rice. Additionally, insensitivity of weedy rice towards temperature was displayed in this study, with slightly higher germination at 30 °C regime but was not significant. This is in partial agreement with the study by Puteh et al. (2010) where an increase in temperature from 10 °C to 30 °C enhance germination percentage of both cultivated rice and weedy rice strain, while temperature > 30 °C exhibits a rapid decline in germination percentage of both cultivated rice and weedy rice strain.

Flooding depth can inhibit weedy rice emergence and is an important component in cultural weed management in rice. 10 cm water level was found to significantly inhibit the emergence of weedy rice totally, with lower water level than 10 cm unable to significantly inhibit germination of weedy rice. This is corroborated by a study by Chauhan (2012) where flooding depth of 0 – 8 cm had little to no effect on the emergence of four weedy rice accessions. On the other hand, Azmi and Karim (2008) suggested 5 – 10 cm flooding depth to inhibit weedy rice emergence in Malaysia.

Effects of environmental factors on hypocotyl length of weedy rice

There were significant differences observed ($p < 0.05$) for all environmental factors tested namely photoperiod, drought, temperature and flooding depth treatments on hypocotyl length of weedy rice (Fig. 2).

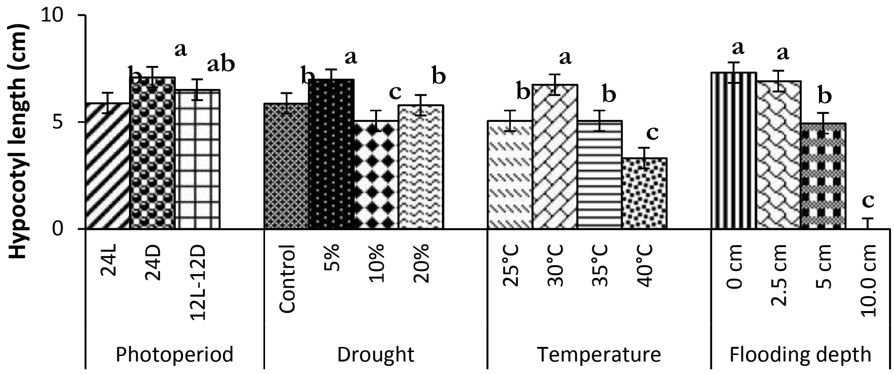

Fig. 2. Effects of photoperiod, drought, temperature and flooding depth on hypocotyl length of weedy rice.

Weedy rice in continuous dark condition had its hypocotyl elongated the most in comparison to other photoperiod conditions. Abnormal elongation of the hypocotyl in dark is not specific only to weedy rice species, but rather to all plants. A study by Woloszynska et al. (2018) presented that skotomorphogenesis or development of seedling in dark causes etiolation of seedlings where elongation of hypocotyl, closed apical hooks and folded cotyledons. The study using the model plant *Arabidopsis* also stated that hypocotyl elongation is controlled by elongator via phytochrome interacting factors (PIFs) and another pathway in darkness condition, while inhibiting hypocotyl growth under light conditions. This also might explain the short hypocotyl exhibited in continuous light conditions.

Drought treatment at low level which is 5 % managed to promote hypocotyl elongation of weedy rice. Manikavelu et al. (2006) disclosed that drought stress greatly impacts the reduction of plant growth and development during the vegetative stage of the rice plant, and this was displayed at 10 % treatment. The opposite occured at 5 % treatment which is a promotion, and this might be attributed to balance between plant responses and sensitivity that determines the positive effect (eustress) or negative effect (distress) of a stressor to plant metabolism and growth (Kaciene et al., 2015). Demkura and Ballare (2012) had mentioned that positive plant response that leads to growth and quality enhancement can be induced by several non-biological stressors from the chemical or physical origin. Additionally, hypocotyl length in 20 % drought treatment was higher than in 10 % treatment which contradicts the supposedly progressive decline in increasing level of drought stress. Chutia and Borah (2012) reported the gradual plant height decrement of 12 traditional rice in India when the intensity of artificial drought simulated by PEG 6000 was increased. Thus, there might be mechanical error involved in the process like accidental switching of PEG solution used.

Meanwhile, temperature treatment presented the longest hypocotyl length of weedy rice at 30 °C when compared to other temperature ranges. Weedy rice has similarities in terms of physiology, morphology and anatomy to cultivated rice (Olajumoke et al., 2016), thus both cultivated rice and weedy rice responses to environmental factors are quite similar in a sense. However, at > 35 °C treatment, the declining trend of hypocotyl length occured, as corroborated by Mispan (2008) where the seedling height of weedy rice at 20 DAS was tremendously reduced at a temperature more than 30 °C onwards.

Flooding depth showed a progressive decrement in hypocotyl length as the water level increased up to 10.0 cm. A study by Ismail et al. (2008) revealed that shoot length of both tolerant and intolerant genotypes of rice to flooding was reduced when submerged in 10.0 cm water level, with a greater decrease in intolerant rice. The effects of physical, chemical and biological properties of flooded soil may relate to the response of weedy rice to flooding (Chauhan, 2012**)**. Oxygen concentration reduced that leads to hypoxia, CO_2 and toxic gaseous accumulation derived from anaerobic decomposition added with the reduced form of chemical radicals plays a major role in flooding effects on weedy rice emergence and growth (Smith & Fox, 1973).

The use of flooding in cultural weed management is indispensable to reduce weedy rice infestation at an early stage (Chauhan & Johnson, 2011). The growth and abundance of weedy rice can be affected by the duration, flooding depth and optimal timing of flooding (Chauhan, 2012). Extended duration of flooding causes decay of many weed species (Abraham et al., 2012). The suggestion of keeping a water depth of 5 to 10 cm was proposed by Azmi and Karim (2008) to impede weedy rice emergence. This indicates good water management in conjunction with a period of flooding can reduce the infestation of weedy rice in the rice field.

Effects of environmental factors on radicle length of weedy rice

There was no significant differences ($p > 0.05$) observed for photoperiod effect on radicle length of weedy rice. However as observed in Fig. 3, there were significant differences ($p < 0.05$) for other environmental factors tested namely drought, temperature and flooding depth treatments on radicle length of weedy rice.

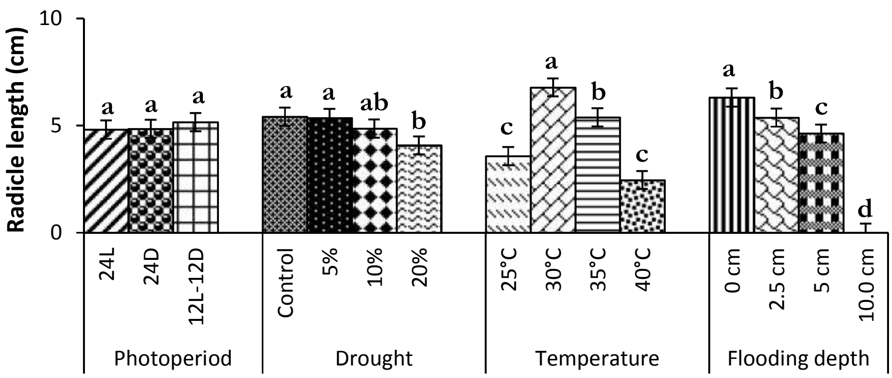

Fig.3. Effect of photoperiod, drought, temperature and flooding depth on radicle length of weedy rice.

Photoperiod of 12 h alternate light-dark had slightly longer radicle than other range of daytime and nighttime and was not significant. A study conducted by Iijima et al. (1998) found little fluctuation on root elongation rate for rice through the day/night period in constant temperature. The study also suggested for detailed mathematical analysis on root elongation rate under continuous dark or light condition to elucidate the circadian rhythm or endogenous rhythmicity in root elongation growth.

Radicle length of weedy rice had a serial decline when the concentration of PEG 8000 was increased. Water stress acts by decreasing the rate of germination and seedling growth as reported in corn (Farsiani and Ghobadi, 2009) and wheat (Gholamin and Khayatnezhad, 2010), which are monocots like weedy rice. Aside from that, a study by Midaoui et al. (2003) stated that the reduction of the shoot and root growth at higher osmotic stress affected root volume and root length. The study also reported reduced root volume under osmotic stress was triggered not only from growth inhibition but also from loss of turgidity.

Weedy rice had longer root development at 30 °C than other temperature range. A study by Sanchez et al. (2014) presented mean optimum temperature (T_{opt}) for rooting of Oryza *sativa* sp at 27.6 °C, which is closer to 30 °C and higher than 25 °C. The study also presented maximum temperature (T_{max}) for rooting of 35.9 °C, which indicates temperature above T_{max} such as 40 °C causes root development to be susceptible to heat damage. Flooding depth treatment reduced radicle length progressively with rising water levels. Likewise, a study by

Prakash et al. (2016) reported all rice cultivars to have diminishing root, shoot and total dry matter production in flooding treatments, with a pronounced reduction in higher than lower flooding levels (5 vs 1 cm).

Effects of environmental factors on root-shoot ratio of weedy rice

There were no significant differences ($p > 0.05$) of photoperiod and temperature on the root-shoot ratio of weedy rice (Fig. 4). However, there were significant differences ($p < 0.05$) for drought and flooding depth treatments on the root-shoot ratio of weedy rice.

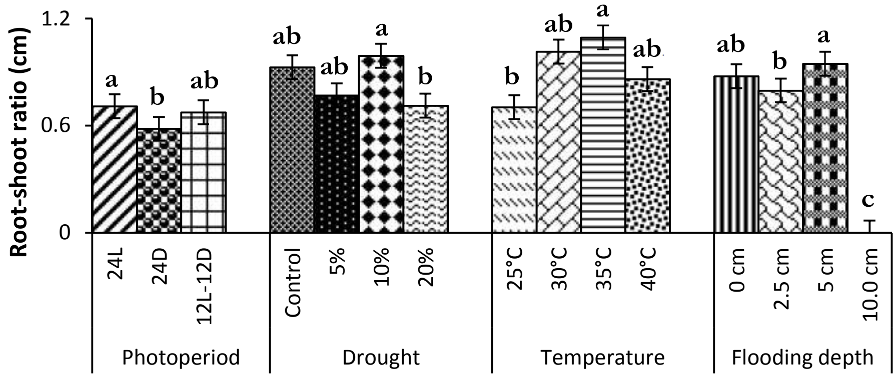

Fig. 4. Effect of photoperiod, drought, temperature and flooding depth on the root-shoot ratio of weedy rice.

Photoperiod treatment showed the increasing root-shoot ratio when the weedy rice had higher exposure to light, with the highest ratio at 24 h continuous light. Hilbert et al. (1991) reported analysis prediction of increased root-shoot ratio in conjunction with increased N at high light which is a general trend often observed empirically. According to Srivastava (2002), the generally high root-shoot ratio is obtained under low levels of nutrient supply, reduced water availability in soil and high intensity of light.

The root-shoot ratio of weedy rice in drought treatment was ambiguous as it showed increment in 10 % treatment, but reduction occurred at 5 % and 20 % treatment. Increase in the root-shoot ratio in low water content can be explained, as stated by Ho et al. (2005) where an inverse proportional relationship between the root-shoot ratio of crops to both nutrient and water availability, due to more allocation of photosynthates to plant roots which allows for more soil exploration in limiting conditions. On the other hand, the reduced root-shoot ratio had also been observed in the study by Asch et al. (2005) using three rice cultivars, which indicated more photoassimilates were invested in shoot growth rather than root growth and reduced root dry matter. Another factor to reduced shoot-ratio under drought stress can be the higher formation of aerenchyma as a stress response (Postma & Lynch, 2011).

Temperature ranges from 25 °C to 35 °C shows an elevated root-shoot ratio but declined at 40 °C. Hakeem (2015) stated temperate species have an inclination for the root-shoot ratio to increase at low temperature, however the minimum is 20 to 30 °C and tends to increase at high temperature. Reduction of root-shoot ratio at a higher temperature like 40 °C may be explained. According to Arai-Sanoh et al. (2010), the root-shoot ratio decreased in soil temperature at 37 °C in 2 different growing stages of *Oryza sativa* cv. Koshihikari. The reduction of root-shoot ratio in high soil temperature was caused by inhibition to formation and elongation of the main root (Sattlemacher et al., 1990) and less distribution of carbohydrate to root (Xu & Huang, 2000).

The root-shoot ratio of weedy rice in flooding treatment exhibited both high ratio in deeper depth and low ratio in shallow depth at 5.0 cm and 2.5 cm water level respectively. The high root-shoot ratio was found out in a study by Zhang et al. (2015) using juvenile or young plants instead of the mature plant with a substantial increase in deep flooding, which was probably resulted from leaf abscission under water that lowers the shoot biomass.

On the other hand, the reduced root-shoot ratio is common in response to the prolonged flood in microcosms (Topa & McLeod, 1986). There are two possible reasons for this phenomenon. First, adequate water and nutrients in root zone allow carbon allocation to shift from root to shoot (Tilman, 1988), Second, flooding causes extensive root mortality, inhibit translocation of carbon to roots or limits root cambial activity (Nadelhoffer et al., 1985).

CONCLUSION

It is concluded that the environmental factors like photoperiod, drought and temperature does not significantly affect the percentages germination of weedy rice. Flooding treatment preferably at 10.0 cm was the most important limiting factor to prevent germination of weedy rice seedlings in the fieldImplementation of flooding the rice field is definitely required in DSR field to reduce infestation and disrupts the growth of weedy rice. Flooding supressed both root and shoot growth at higher efficiency in increasing water level. This study suggests that weedy rice have a high tolerance to water stress and temperature, thus need to be controlled through good water management practices and suppression by the flood. Hence improvements to this study can be made by increasing the experiment duration until flowering or panicle initiation. The optimal temperature of weedy rice coincides with cultivated rice, which requires integrated weed management when rice is feasibly grown in a controlled environment in future.

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