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CROP PRODUCTION

Environmental factors on seasonal germination of different weedy rice (*Oryza sativa* L.) biotypes

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ABSTRACT: *Weedy biotypes of Oryza sativa L., (weedy rice) are a serious threat to rice production because of their flexibility in seed germination timing, variable growth forms, and high genetic diversity. Experiments were designed to determine the effects of storage conditions, osmotic stress, pH, salt stress, and burial depth on germination of weedy rice seeds in flooded or non-flooded conditions. Mature weedy rice seeds were gathered from rice fields in the different regions of Turkey. Three biotypes were selected and classified according to awn length; long awn, short awn and awnless. Seasonal germination patterns of weedy rice seeds in the laboratory after retrieval from various depths and timing were affected by burial depth, water regime, and exhumation timing across the treatments for all biotypes. The long and short awn biotypes had higher germination rates than the awnless biotype and did not have a seasonal germination pattern. Seed germination was initiated in the spring, peaked in summer and declined in the fall in flooded and non-flooded treatments for the awnless biotype. The most distinct differences in seedling emergence patterns were observed in awnless and long awn biotypes, and they emerged from 10 cm depth. Our results suggested that flooded conditions reduced the germination of weedy rice biotypes. Flooding rice paddies for a period of time after harvest may improve weedy rice control and decrease the weed population. In addition to deeper cultivation would be more effective in controlling all weedy biotypes since the majority of weed seedling emergence was from shallow depths.*

Key words: *dormancy, seedling emergence, flooded and non-flooded germination, burial depth, integrated weed management.*

Efeitos de fatores ambientais na germinação sazonal de diferentes biótipos de arroz vermelho (*Oryza sativa* L.)

RESUMO: *Biótipos de Oryza sativa L., arroz vermelho, são uma séria ameaça à produção de arroz devido à sua flexibilidade no tempo de germinação das sementes, formas de crescimento variáveis e alta diversidade genética. Os experimentos foram projetados para entender os efeitos das condições de armazenamento, estresse osmótico, pH, estresse salino e profundidade de semeadura na germinação de sementes de arroz vermelho em condições de inundação ou não. Sementes maduras de arroz vermelho foram colhidas em campos de arroz em diferentes regiões da Turquia. Três biótipos foram selecionados e classificados de acordo com o comprimento da arista longa, curta e sem arista. Padrões sazonais de germinação de sementes de arroz vermelho no laboratório após a recuperação em várias profundidades e tempos foram afetados pela profundidade de semeadura, regime de água e tempo de permanência no solo entre os tratamentos para todos os biótipos. Os biótipos de arista longa e curta tiveram taxas de germinação mais altas do que o biótipo sem arista e não apresentaram um padrão de germinação sazonal. A germinação das sementes foi iniciada na primavera, atingiu o pico no verão e diminuiu no outono em tratamentos com alagamento e sem alagamento para o biótipo sem armação. As diferenças mais nítidas nos padrões de emergência de plântulas foram observadas em biótipos sem e com arista longa, e emergiram a partir de 10 cm de profundidade. Nossos resultados sugerem que as condições de inundação reduziram a germinação de biótipos de arroz vermelho Parece que inundar os arrozais por um período de tempo após a colheita pode melhorar o controle do arroz vermelho e diminuir a população de arroz vermelho. Além disso, o cultivo mais profundo seria mais eficaz no controle de todos os biótipos de arroz vermelho, uma vez que a maioria da emergência de plântulas de arroz vermelho ocorreu em profundidades rasas.*

Palavras-chave: *dormência, emergência de plântulas, germinação inundada e não inundada, profundidade de semeadura, manejo integrado de arroz vermelho.*

INTRODUCTION

The genus *Oryza* includes edible rice as well as several weedy species and biotypes occurring in rice throughout the world (MAITI, 2020). The species in this genus have the potential to hybridize with other *Oryza* species (NOLDIN et al., 2002; LU & SNOW 2005) and this gene interaction may lead to regulating phenotypic and genotypic variation for many traits in inherent biotypes. Weedy rice is a troublesome weed in rice production owing to its variability in the timing of seed germination, variable growth forms, multiple annual lifecycles, cold tolerance, high dispersal ability, and high genetic

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diversity (XIA et al., 2011; CHAUHAN 2013; ZISKA & MCCONNELL 2015).. The highest weedy rice infestation (80%) has been reported in Cuba (SALES et al., 2011; BAEK & CHUNG, 2012) followed by 40% to 75% in Europe (FERRERO, 2003). Weedy rice has become a very significant weed species in rice production in Turkey even though it was only recently introduced to the country (KAYA ALTOP et al., 2015).

Seed germination is an important event in determining the success of a weed in an agricultural ecosystem and may be regulated by various factors such as temperature, light, soil salinity, moisture, and pH. Temperature plays an important role in determining the seed germination periodicity and distribution of species (GUAN, 2009; SURIYASAK et al., 2020). Light is an important ecological marker for germination; The absence of light serves as an indicator of soil depth, which prevents the germination of many seeds (CRISRAUDO et al., 2013). Similarly, the ability to germinate under moisture stress or high salinity conditions may enable a weed to benefit from conditions that limit the growth of other species. Fracture of weedy rice dormancy often results in a decrease in pH in the embryo tissues (FOOTITT & COHN, 1992). Comprehensive knowledge of weedy rice seed dormancy is important for management in rice production (OARD et al., 2000; GIANINETTI & COHN 2008). GU et al., (2005) indicated that weedy rice seed dormancy was correlated with the presence of awns, pericarp/testa colours. In other studies, it was confirmed that weedy rice populations that exhibited different morphological characteristics have different dormancy levels at harvest (NOLDIN, 1995). Nevertheless, DELOUCHE et al., (2007) and XIA et al., (2011) reported that seeds from some weedy rice populations were not dormant. Besides those features, many environmental conditions such as seed position during the development stage, photoperiod, temperature, nutrition, light intensity, and quality can affect dormancy (BEWLEY & BLACK 1982; BENVENUTI et al., 2001). It is that about 60% of plant species are non-dormant and their seed germination is regulated by other mechanisms (BASKIN & BASKIN, 1998). Previous research has shown that optimal seed germination is closely associated with habitat temperatures in non-dormant species. Changes in the level of dormancy after the burial of seeds have been reported for summer and winter annual species. TEEKACHUNHATEAN (1985) reported that annually there are two seasonal germination periods of weedy rice in temperate areas. The first germination period is in early spring, and the second begins in autumn and ends in early winter. After an initial field survey of weedy rice in different regions of Turkey, three different biotypes were classified as long awn, short awn, and awnless, and some of the biotypes had low or no seed dormancy (MENNAN et al., 2018). However, it is not known whether biotypes with these morphological differences exhibit differential germination and dormancy patterns. Understanding differences in dormancy may help us design more effective control strategies.

This study determined seasonal germination and emergence patterns of different biotypes of weedy rice in flooded or non-flooded burial conditions and to demonstrate the effects of storage conditions, salt stress, Ph, and osmotic stress on seed germination.

MATERIALS AND METHODS

Seed collection

Seeds of 76 weedy rice biotypes were collected from natural populations in rice fields in the main rice-growing regions of Turkey: Trachea, South Marmara, and the Black Sea in early September 2014 (Figure 1a). Seeds were classified according to awn length as long awn, short awn and awnless (Figure 1b). The harvested seeds were stored in the laboratory for 15-20 days at 24 °C, until germination experiments were initiated.

Seed mass

Seed mass was determined by weighing 10 samples of 1000 seeds from each biotype in accordance with the International Seed Testing Association rules (ISTA, 2009). At the same time, the awn length of short and long biotypes was measured on 1000 seeds of each biotype to quantify length differences.

Seed germination test

Before germination tests, seeds collected from 76 fields were sterilized using 0.25% NaOCl solution for 2 min then rinsed with deionized water. Fifty solid seeds from each biotype were selected and germinated in 9 cm diameter Petri dishes on two layers of filter paper wetted with 5 Ml of deionized water. In order to avoid contamination and drying, Petri dishes were covered with parafilm. The seeds were germinated in growth chambers at 10, 15, 20, and 30 °C with a 12/12 h light/dark alternating period. Germination was evaluated for 14 days in accordance with the International Rules for Seed Testing (ISTA, 2009) for rice.

One seed source from each biotype was selected according to awn length for further

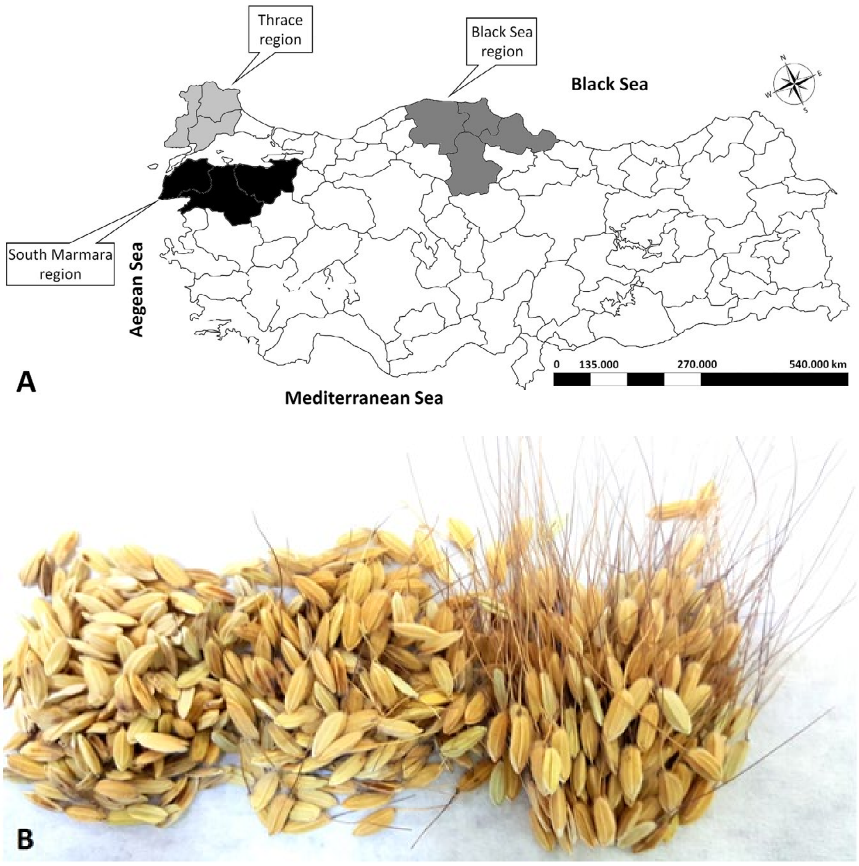

Figure 1 - (A) Gray area indicates rice crop-production regions of Turkey and sample localities which were used in the experiment; (B) Classification of seeds according to awn length; from left to right, awnless, short awn and long awn.

germination and burial experiments. Randomized designs with four replications were used in the experiment. All experiments were repeated twice. Non-germinated seeds in all experiments listed below were tested with triphenyl tetrazolium chloride (TTC) in order to enhance the viability of seeds before and after the experiments.

Burial experiment

For each seed biotype, 250 seeds were wrapped in nylon mesh and buried at 5 or 10 cm in pots (20 cm width and 25 cm length) or located on the soil surface at the end of September 2014. Seeds were placed in nylon mesh to simulate natural soil conditions such as air and water movement, microorganism diffusion, and temperature in the pots. The soil was obtained from a depth of 50 cm in a rice field to prevent contamination from other wild rice weed seeds. The soil was not sterilized to preserve natural soil microorganisms. The soil type in the field at 0–50-cm depth was silty loam with 35% silt, 49% clay, and 12% sand, Ph 8.12, and 1.21% organic matter. A randomized design with four replications was used in the experiments either flooded or non-flooded. A total of 576 pots were used for both experiments. Pots were maintained in a screen house and exposed to natural weather conditions. The long-term average precipitation and temperatures are given in figure 2 for the experimental area. For flooded pots, water levels were kept above 5 cm from May 1 to September 30, which included both germination periods. Four pots were exhumed from each burial depth every month for 24 months starting at the end of October 2014. Exhumed seeds were cleaned and

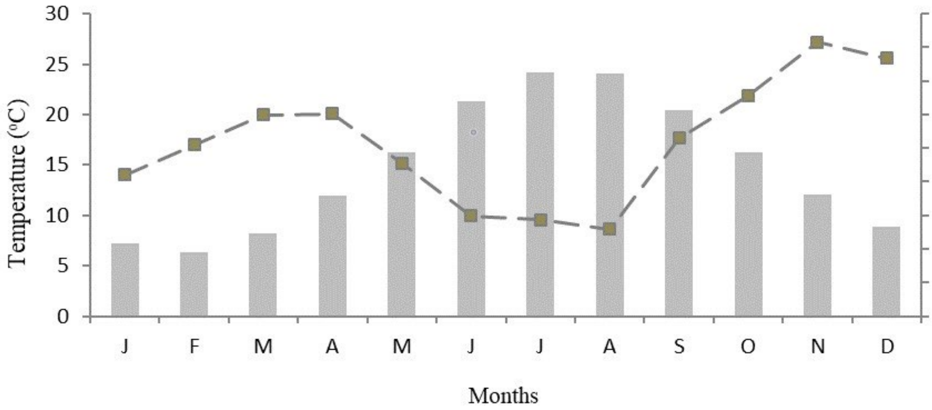

Figure 2 - Average temperatures and precipitation in Samsun, Turkey for the year of experiments. The bars show the average monthly temperatures and the line shows the average monthly precipitation.

twenty-five seeds were selected from each replication and depth. Seed germination tests were conducted as described above at 25/15 °C with a 12/12 h night/day-light regime.

Effects of storage conditions

For this experiment, 48 subsamples of 50 g of seeds from each biotype were exposed to each of the following treatments: (i) kept in 400 ml distilled water at 4 °C in 1500 ml covered plastic containers, and (ii) dry storage in a paper bag at 24 °C. Seeds were kept in a growth chamber to ensure consistent storage temperatures. Four bags from each storage condition were taken from storage monthly starting in October 2014 for 12 months. On each sampling date, twenty-five seeds from each storage condition were tested for germination.

Effects of Ph, osmotic pressure and salt stress

Seven different Ph solutions including 4, 5, 6, 7, 8, 9 and 10 were created as described by CHACHALIS & REDDY (2000). In order to acquire a 0, −0.2, −0.4, −0.6, −0.8 and −1.0 Mpa osmotic potential, polyethylene glycol was dissolved in distilled water (MICHAEL & KAUFMAN, 1973). To test the effects of salt stress on seed germination, ten Ml of NaCl solution from 0, 25, 50, 100, 200, 400, 800 and 1000 mole was added to Petri dishes. All germination experiments were designed as described above. Ten month old seeds were subjected to each treatment. A logistic model was applied to germination (%) at different osmotic potentials and salinity levels (CHAUHAN et al., 2006a):

$$G(\%)=G_{max}/[1+(x/x_{50})G_{rate}] \quad (1)$$

In this equation, where G is the total germination (%) at salinity level (NaCl concentration) x or osmotic potential x, G_{max} is the maximum germination (%), x_{50} is the salinity level or osmotic potential for 50 % inhibition of the maximum germination, and G_{rate} represents the slope.

Seedling emergence in the field

One hundred seeds of each biotype were sown in plastic pots (20 cm width; 25 cm height) at the end of September 2014. The same seeds from each biotype and similar soil structure were used as described above. A randomized complete block design with four replications was used for pots buried in an experimental field. Seeding depths for different biotypes in these pots were 0, 2, 5, 10, and 15 cm in flooded conditions. Total seedling emergence was counted weekly and seeds were considered emerged once the cotyledons appeared.

MET (Mean emergence time) was estimated as:

$$MET \quad \sum = (n \times d)/N \quad (2)$$

where n is the number of seedlings emerging per day, d is the number of days needed for emergence, and N is the total number of emerged seeds.

Statistical analyses

In order to enhance the homogeneity of variance, data were arcsine-transformed as necessary. To estimate the differences between treatments, seedling emergence and germination data were analyzed using ANOVA, followed by Tukey Multiple Range test. Different modeling approaches were used to determine the best model for seed germination in the buried experiment. The best-fit model was selected based on the homogeneity of variances, *P*-values, and residual normality. Statistical analyses were conducted in SPSS (version 13.0; IBM SPSS Statistics, Chicago, IL, USA).

RESULTS AND DISCUSSION

Seed mass

The long awn was the most common biotype in rice fields. Of the 76 populations, 56, 16, and 4 were classified as long awn, short awn, and awnless, respectively (Table 1). Statistical analyses indicated remarkable variation in awn length of biotypes between short and long awn biotypes. There was a significant difference among biotypes for fresh seed mass (Table 1). Seeds of short awn have less weight than those of awnless and long awn. Mean seed mass for awnless and long awn was around 30 mg per seed. In the field, the flowering period of weedy rice biotypes overlap. However, the short awn type shatters seeds one week earlier than the long and awnless type. Evidently, environmental conditions that cause early seed ripening may result in reduced seed mass and embryo nutrition throughout seed development.

Seed germination

After one week of storage, the germination rate increased gradually with increasing temperature in all three biotypes at alternating dark-light conditions. Germination of long awn, short awn, and awnless seeds occurred at a broad range of temperatures, and optimum temperature for germination was 25 °C for all biotypes. The mean germination was low for the awnless biotype, with rates ranging from 0% to 38% just after harvest (Table 2). The long awn biotype had the highest germination in all germination temperature profile parameters. Mean germination for the short and long awn populations were 82 and 98%, respectively, roughly double the mean for the awnless biotype at 25 °C. At this temperature, the viability of awnless, short and long awn biotypes seeds after germination experiment was 94, 96 and 100%, respectively. Freshly matured seeds exhibit primary dormancy, which prevents germination until environmental conditions are favourable. The establishment of dormancy occurs during seed development and involves both genetic and environmental factors. We concludedwith these data, that the harvested awnless seeds of three different biotypes had a high degree of primary dormancy. In addition, the short awn seeds had an intermediate degree of primary dormancy while stored in dry conditions at 24 °C and germinated with a 12 h photoperiod. Long awn seeds did not have primary dormancy, and all populations of these biotypes germinated over 90%. Morphological traits may affect the level of dormancy in diverse biotypes. GU et al., (2005) defined a high level of dormancy in a weedy rice biotype having awns and black hulls. FOGLIATTO et al., (2011) observed the varied response to germination within the examined awned populations. They indicated that the population referred to as P1 was the most dormant, whereas P50 showed the lowest dormancy level in buried experiments. Nevertheless, strong seed dormancy has been considered the most distinct feature for this species (OARD et al., 2000; GIANINETTI & COHN 2008), with limited exceptions (DELOUCHE et al., 2007; SCHWANKE et al., 2008). A recent report indicated that a low dormant or non-dormant weedy rice population has been introduced in temperate regions of China (XIA et al., 2011). Those outcomes imply that, in addition to a suitable germination environment, dormancy can be affected by biological and genetic factors (BASKIN & BASKIN, 1998; GUTTERMAN, 2000; MILBERG et al., 2000; MENNAN 2003; MENNAN & NGOUAJIO 2006).

Burial experiment

The seasonal germination pattern of weedy rice seeds in the growth chamber after burial

Table 1 - Fresh seed mass (mean ± SE) of weedy rice biotypes and average awn length.

	Number of biotypes	Seed mass (mg seed^{-1})	Awn Length (cm)
Long awn	56	31 ± 0.41	2.90 ± 0.39
Short awn	16	25 ± 0.36	0.91 ± 0.11
Awnless	4	30 ± 0.85	-

[a] Seeds were collected in early September 2014, (±SE) standard error.

Table 2 - Average germination of different biotypes incubated at 5, 10, 15, 20, 25 and 30 °C temperatures at 12 h (light/dark) conditions soon after collection in mid of September 2014.

	----------Temperature (°C)----------					
	5	10	15	20	25	30
Long awn	2 ± 0.14	8 ± 1.27	28 ± 2.41	88 ± 3.53	98 ± 6.84	92 ± 4.63
Short awn	0 ± 0.0	8 ± 0.95	16 ± 1.58	56 ± 2.96	72 ± 5.99	56 ± 4.82
Awnless	0 ± 0.0	4 ± 0.57	14 ± 1.24	22 ± 1.85	38 ± 3.46	32 ± 2.31

[a]Seeds were collected in early September 2014, (±SE) standard error.

at various depths and time was affected by burial depth, water regime and exhumation timing across awn biotypes (P < 0.001) (Table 3). In general, the long awn biotype had high seed germination, 90-96%, regardless of burial depth or flooding treatment (Figures 3 and 4), indicating that this biotype doesn't have primary seed dormancy. Germination rate was 88% and 96% depending on the depth of burial in non-flooded conditions after retrieving seeds in the first month. Similar results were obtained in flooded conditions from the first exhumation. In the following months, the percent germination gradually decreased in both conditions until the end of the experiment. Seeds of the long awn biotype showed a non-cyclic germination mechanism after exhumation from different depths throughout the experiment.

In contrast to the long awn biotype, the short awn seed did not exceed 54% germination after the first month of exhumation (Figures 3 and 4). Seeds of this biotype have a moderate level of primary dormancy. In this biotype, seed germination was strongly affected by flooding conditions and depths. Starting in October 2014, percent germination increased in non-flooded conditions until November 2015 and peaked at 66% when placed on the soil surface. In the following months, germination decreased through January 2016 and approached 50% in September. At other depths, short awn seeds under non-flooded conditions did not attain 70% germination under any test condition. In flooded conditions, peak germination occurred at 10 cm depth and began to decline after 4 months. Germination for all burial depths peaked in October 2014 with 44%, 52%, and 46% at 0, 5, and 10 cm, respectively. As in the long awn biotype, a non-cyclic germination mechanism was observed in short awn biotypes retrieved from different depths throughout the experiment.

The germination of awnless seeds in the burial trials varied significantly among depths (P < 0.001) during the season. At the beginning of the experiment from October to March, the

Table 3 - ANOVA results for the effects of seed burial depth, exhumation date and water regime on weedy rice germination.

Source of variation	DF[a]	Percentage germination[b]
Biotypes (B)	2	***
Water regime (WR)	1	**
Depth (D)	2	**
Month (M)	23	*
B x WR	2	*
B x D	4	*
B x M	46	*
WR x D	2	NS
WR x M	23	*
D x M	46	NS
B x WR x D x M	92	***

[a]Degree of freedom. [b]Significance: P < 0.05 (*), P< 0.01 (**), P< 0.001 (***), and NS (non significant).

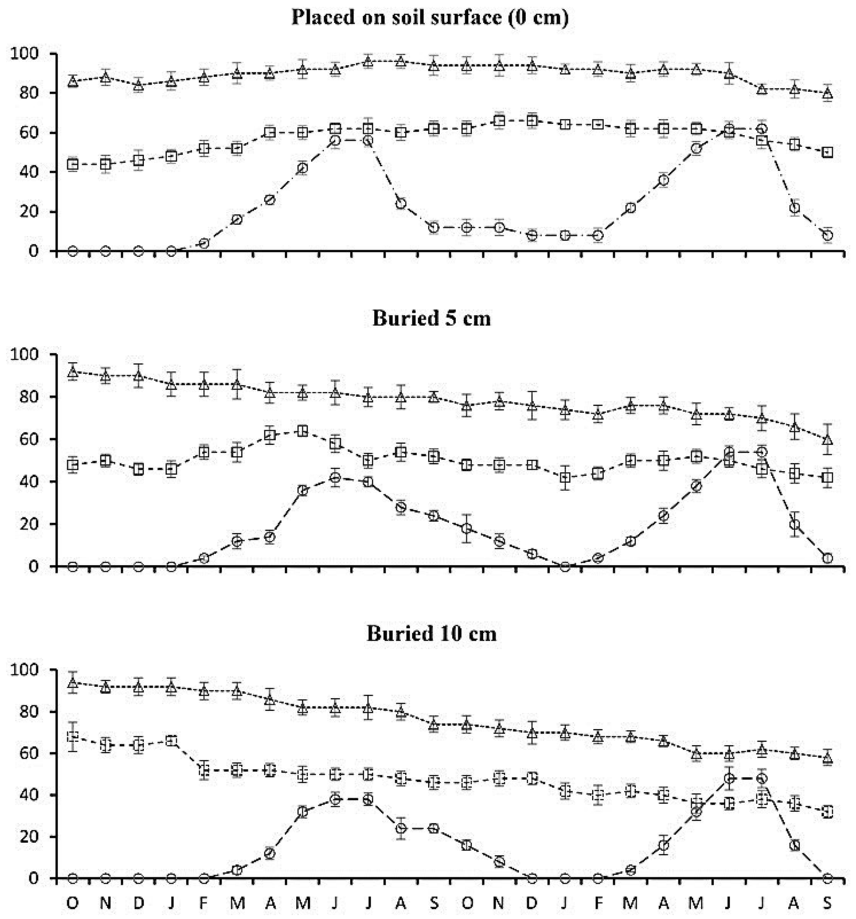

Figure 3 - Different weedy rice population seed germination in a growth chamber at 25/15 °C with a 12/12 h night/day regime after burial at a 0, 5 or 10 cm depth in non-flooded conditions. The bars indicate standard errors of the mean. (○), Awnless biotype; (□), Short awn; (Δ), Long awn.

seeds of awnless biotype buried in the field were dormant in non-flooded conditions (Figures 3 and 4). Germination rate was 0 and 4% in March according to a depth of burial and then started to increase until July 2015. Thereafter, the rate of germination declined and approached zero in September 2015. The seeds remained dormant until March of 2016. Similar results were reported for flooded conditions with lower germination in both depths and months. This biotype germinated in the spring became dormant in fall and germinated again in spring under both conditions. The seed of this biotype showed strongly cyclic behavior, and characteristic seasonal cyclic changes in germination recurred with moderate alteration in the following months.

Different biotypes of seeds tested by using a TTC assay after the germination test showed that the non-germinated seeds retained a high level of viability (data not shown). The non-germinated seeds from both the flooded and the non-flooded conditions and in all depths were 92–98% viable after the first exhumation and germination test. This rate steadily declined over the course of the experiment.

Burial depth and flooded conditions affected the seasonal change in germination of different weedy rice biotype seeds in the field. Long and short awn biotypes germinated all year without seasonal differences. We confirmed that there is no or low-level primary dormancy in those biotypes. The high germination capability of the awned

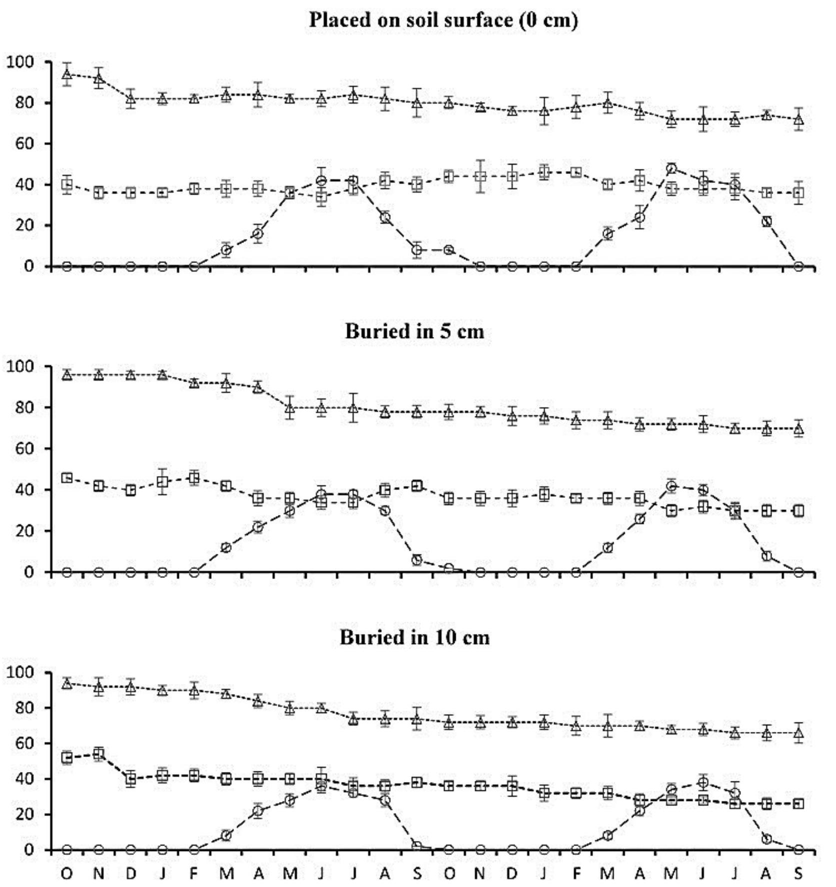

Figure 4 - Different weedy rice seed germination in a growth chamber at 25/15 °C with a 12/12 h night/day regime after burial at a 0, 5 or 10 cm depth in flooded conditions. The bars indicate standard errors of the mean. (○), Awnless biotype; (□), Short awn; (Δ), Long awn.

seeds after harvest has been recorded previously in various tested conditions (DELOUCHE et al., 2007; SCHWANKE et al., 2008), and weedy rice biotypes from Asian rice fields have either exceedingly low or no seed dormancy (XIA et al., 2011). The seeds of the awnless biotype had an extreme degree of primary dormancy initially then germination increased with time. The seeds apparently require a ripening period after harvest to overcome dormancy. This biotype exhibited a typical annual seed dormancy/non-dormancy cycle. This type of dormancy pattern is not common for weedy rice but it was demonstrated in some populations (TEEKACHUNHATEAN, 1985). The continuous loss of dormancy in certain weed species is generally connected with summer annuals, and the differences in dormancy among populations may be due to many environmental factors (BASKIN & BASKIN, 1998). Similar to other weed species, secondary dormancy induction has been seen in weedy rice under suboptimal conditions (GIANINETTI & COHN, 2008). Our findings demonstrated that there are large differences among weedy rice biotypes in primary dormancy levels, and the ripening time not only influences germination of weedy rice but also its dormancy status.

Seeds of all biotypes placed on pot surfaces had higher germination rates than those buried 5 or 10 cm in either non-flooded or flooded condition. Comparing the effects of burial on dormancy and germination rate, it is clear that the germination cycle throughout the experiment was affected by burial depth. Therefore, the seeds placed on the soil surface lost primary dormancy quicker than the seeds buried at 5 or 10 cm. Changes in temperatures in the field helped break dormancy and encouraged germination of non-dormant seeds. These outcomes concur with previous research (MENNAN, 2003; MENNAN & ZANDSTRA, 2005).

Effect of storage condition

As indicated previously, freshly harvested weedy rice biotypes had different levels of primary dormancy when stored either in distilled water at 4°C or in dry conditions at 24 °C (Figure 5). In dry storage conditions, the long awn biotype germinated (100%) until July 2015 and then declined to 88% (Figure 5). Short awn seeds germinated 68% after the first month of dry storage. The rate of germination increased with time and peaked at 84% in April and May 2015, then declined to 76% in the last month of the experiment. Germination of awnless seeds was not over 34% at the beginning of the experiment, then germination increased monthly and reached 66% in August 2015, and subsequently decreased to 60% at the end of the experiment. The germination rate of all biotypes stored wet at 4 °C was lower than similar seeds stored dry (Figure 5). Germination increased slowly for 10 months, until August, and then declined. Our germination experiments on seeds stored at ambient conditions (24 °C) or stored at 4 °C demonstrated that no biotype had induced dormancy in any tested conditions. It is clear that weedy rice biotypes can germinate when they have suitable germination conditions.

Effect of osmotic pressure, pH and salt stress

Cumulative seed germination decreased as the level of water stress (osmotic pressure) increased in all biotypes (Figure 6). Starting at −0.4 Mpa, the proportion of germinating seeds of long and short awn biotypes decreased gradually and reached their minimum at −1 Mpa. The awnless biotype was more sensitive to water stress; the level of germination decreased after −0.2 Mpa and it reached 0% at -0.8 Mpa. Long and short awn biotypes showed different germination patterns than awnless, and they germinated until −0.8 Mpa. Weedy rice populations can germinate with high water stress, and similar

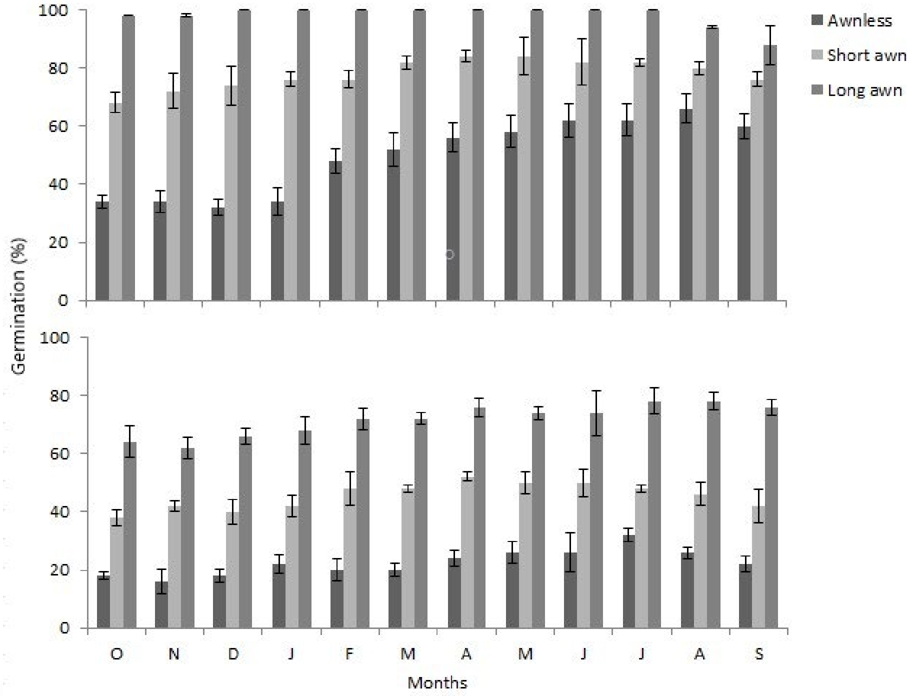

Figure 5 - Different weedy rice population seed germination in a growth chamber at 25/15 °C with a 12/12 h night/day regime for 14 d after stored dry at 24 °C (above) or stored in distillated water at 4 °C. The bars indicate standard errors of the mean.

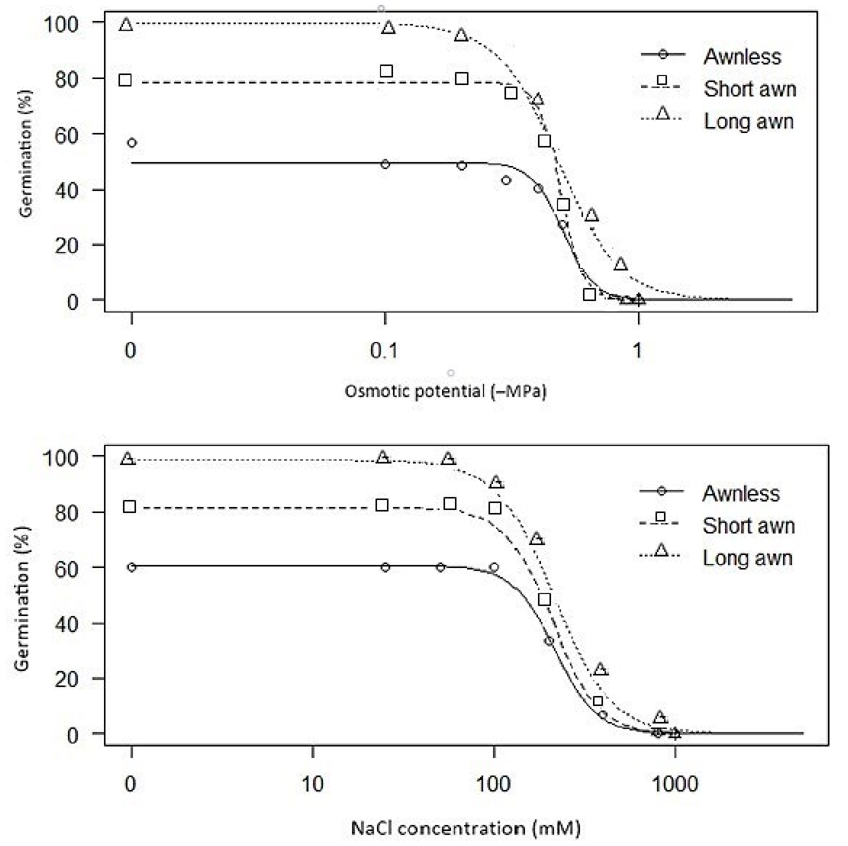

Figure 6 - Effect of osmotic potentials (-MPa) on germination percentage of different weedy rice populations. LSD= 6.87; Long awn population G (%) = 54.20/[1 + (X/0.23)4.01], r2 = 0.81; Short awn: G (%) = 41.08/[1 + (X/0.29)5.17], r2 = 0.83; Awnless: G (%) = 48.19/[1 + (X/0.64)7.31], r2 = 0.79 (above); Effect of NaCl concentration (mM) on germination percentage of different weedy rice populations. LSD= 6.87; Long awn population G (%) = 102.61/[1 + (X/0.87)7.19], r2 = 0.89; Short awn: G (%) = 121.95/[1 + (X/0.62)9.11], r2 = 0.80; Awnless: G (%) = 109.71/[1 + (X/0.83)9.12], r2 = 0.86. (below).

results have been reported (PUTEH et al., 2013). It may be concluded from our and other research that weedy rice seeds are highly adapted to the high water pressure as well as the submerged conditions in rice fields. But, initial germination in all biotypes was delayed as water stress increased. Results from our study suggested that the long awn biotype had greater tolerance to drought stress compared with short awn and awnless, and may be adapted very well to the dry seed rice cropping system which is becoming more common in countries with water shortages.

The interaction between weedy rice biotypes and salt concentration was significant; salinity reduced weedy rice seed germination of all biotypes. Similarly to water stress, increasing NaCl concentration from 0 to 1000 mole decreased the germination rate of weedy rice biotypes (Figure 6). In the long awn biotype, germination was > 95% at a salinity of 800 mM NaCl but decreased to <45% at 200 mM and then to < 20% at 100 mM NaCl. Seeds failed to germinate at 800 mM NaCl or greater. Similar results were obtained from the short awn biotype, but with an overall lower germination rate. Awnless seeds had a lower germination rate than long and short awn biotypes, and germination ceased at 400 mM NaCl. According to our results, long and short awn biotype seeds could tolerate salinity stress better than the awnless population. Awnless biotype seeds were very sensitive to salinity. HAKIM et al., (2011) & IRAKOZE, et al., (2020) reported that weedy rice populations were moderately tolerant to salinity. Soil salinity is affected by cropping, irrigation, land use, and application of fertilizer, manure, and compost, so the response of biotypes may be diverse.

Germination was affected by pH, and the interaction between biotype and pH was significant (Figure 7). Germination of the long awn biotype occurred at all pH ranges tested, with the highest germination (100%) at a pH of 7. The lowest germination rate (44%) occurred at a pH of 10. The short awn biotype responded similarly to the long awn biotype. In the awnless biotype, germination was 58% at a pH of 7, but decreased to 8% at pH 10. Germination occurred across a wide pH range. This has been confirmed in many weed species such as *Eleusine indica* (L.) Gaertn. and *Lolium rigidum* Gaud. (CHAUHAN et al., 2006b; CHAUHAN & JOHNSON 2008). In Turkey, the soil pH was between 5.8-9.3 in rice-growing areas, so it may be concluded that all weedy biotypes can survive at various pH levels.

Seedling emergence in the field

Seasonal emergence in the field burial experiment differed significantly between biotypes and depths ($P < 0.05$) (Table 4). Seedling emergence decreased with increasing burial depth (Figure 8). Seedlings did not emerge during the first 5 months of the experiment from any depth. Fewer than 10% of seedlings emerged from the 5 and 10 cm depths in April. In subsequent months, percent emergence increased moderately until August. Maximum emergence from all depths occurred in May. The awnless biotype seedlings emerged from all depths, with the highest germination (48%) from the 5 cm burial depth. Short awn seedling emergence was 42, 65 and 78% from 0, 2 and 5 cm respectively. Long awn seedlings emerged from all depths except 15 cm, and maximum germination was observed from 5 cm (88%). There were large differences in the mean seedling emergence times between weedy rice populations (Table 4). Differences in the mean emergence time ranged from 7.3 to 10.9 months when seeds were germinated on the surface. The most distinct differences in mean seedling emergence time were observed between long awn and awnless; long awn seedlings emerged earlier and reached their maximum germination after 7 months from 5 cm whereas the awnless biotype took more than 11.5 months to reach its maximum germination (48%). The mean emergence time increased with increasing depth for all biotypes. Low levels of seed dormancy and rapid emergence from the surface or shallow depths are typical germination behavior of weedy rice reported in many countries. Over half of weedy rice biotype seedlings from Arkansas, Louisiana, and Mississippi in the USA emerged from a depth of 7.5 cm in recent studies (GEALY et al., 2000). Contrary to this, CHAUHAN (2012) reported that Asian

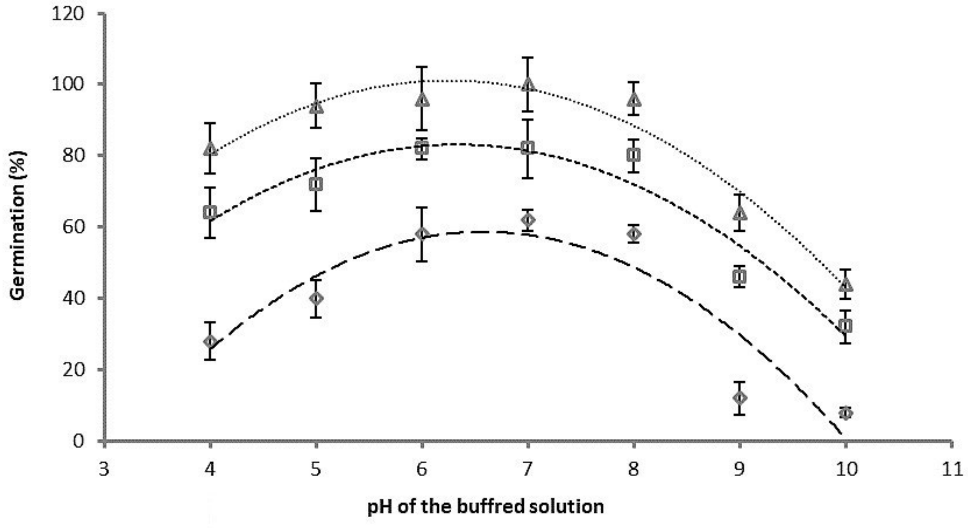

Figure 7 - Effect of pH on different weedy rice seed germination in a growth chamber at 25/15 °C with a 12/12 h night/day regime. The bars indicate standard errors of the mean.
(○), Awnless biotype; (□), Short awn; (Δ), Long awn.

Table 4 - Effect of seedling depth on the mean emergence time (mean ± standard error) of different weedy rice biotypes in flooded conditions.

	---Mean emergence time (month)---				
Biotypes	---Seedling depth (cm)---				
	0	2	5	10	15
Long awn	7.3 ± 0.8*	6.1 ± 0.7*	5.6 ± 0.4*	19.2 ± 0.4	-
Short awn	8.1 ± 1.1	5.9 ± 1.3	6.0 ± 1.1	21.9 ± 0.9	-
Awnless	10.9 ± 0.9	9.1 ± 1.2	11.5 ± 1.3	18.9 ± 0.9	-

*Significant within the column: $P < 0.05$, (±SE) standard error.
(-) The model did not calculate because of lack of emergence.

weedy rice biotypes were not able to emerge from 8 cm. Our result for the short awn biotype is similar to previous research, but awnless and long awn biotypes had greater mass and higher seedling emergence than short awn. Seed mass is clearly related to the depth of emergence and germination. This has been shown in other species (PAOLINI et al., 2001). However, the reasons for decreasing seedling emergence of weedy rice with increasing burial depth may be hypoxia and low rates of gaseous dissemination. The concentration of CO_2 produced by soil microbial activity is very high at deeper levels. Consequently O_2 level is low at these depths (CHAUHAN & JOHNSON 2010). This may cause hypoxia which can minimize seed germination and, ultimately, seedling emergence. Comparison of results reported here with previous research on weedy rice ecology clearly indicated a major change in seed dormancy within biotypes. Differences in seed dormancy, seasonal germination, germination rate, seedling emergence and the high adaptive ability of weedy rice to different environmental conditions among long, short and awnless biotypes may relate to their different evolutionary origins. Dormancy of weeds that may cross with each other and with cultivated rice promotes survival of weed seeds in disturbed environments. Genes interact with each other and with environments to regulate phenotypic variation for many adaptive traits in natural populations. Seed dormancy is a complicated trait controlled by multiple

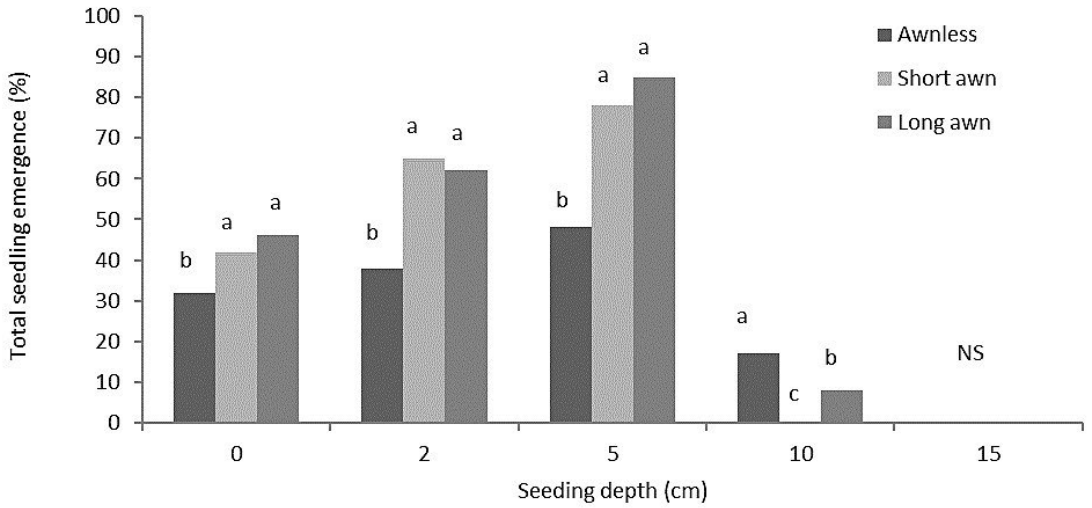

Figure 8 - Total seedling emergence of different weedy rice population seeds from the soil surface or different burial depths within 1 year. The bars with the same letters are not statistically different ($P < 0.05$).

genes and a variety of environmental conditions (GU et al., 2004; YE et al., 2010). Due to strong interaction with environmental factors, it is not fully understood at the molecular level. The genetic diversity in seed dormancy between weedy and cultivated populations have been used to investigate with quantitative trait locus (QTL) analysis (YE et al., 2010). The Sdr4 locus on chromosome 7 can explain the functional difference between accessions. The Sdr4 is positively regulated by OsVP1 alleles which are the genetic regulator of seed dormancy. In addition, Vp1 encodes a seed-specific regulatory transcription factor and acts in the genetic regulation of seed maturation. In previous studies, the seed dormancy of weedy rice populations, which differed in characteristics such as awn length and pericarp colour, was also reported to be different (JING et al., 2008; Chena et al., 2021).

CONCLUSION

Weedy rice will be one of the most serious weeds in rice production in Turkey, despite recent efforts to minimize it. Economic returns and field characteristics are major determinants for growers to continue rice monoculture. Especially rice fields are levelled with laser, and because ploughing the soil will destroy the laser layer, it is not preferred by the producers in weed control. Field storage conditions, either flooded or non-flooded during the season, also affected the germination behaviour of weedy rice biotypes. Flooded conditions partly inhibited the germination of all weedy rice biotypes. Weed management strategies should include flooding for some period following rice harvest to reduce weed populations. Our results suggested that deeper cultivation would be more effective in controlling all weedy biotypes since the majority of weed seedling emergence was from shallow depths. Deep tillage below 15 cm depth could be applied as a tool in an integrated weed management program. If farmers prefer no or reduced tillage, weedy rice populations will likely expand.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

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