

## Germination behaviour of Macedonian weedy rice

Ўртливоста кај Македонскиот див ориз

Trajche DIMITROVSKI<sup>1\*</sup>, Danica ANDREEVSKA<sup>1</sup>, Dobre ANDOV<sup>1</sup>

<sup>1</sup>*Institute of Agriculture in Skopje, Ss. Cyril and Methodius University, Republic of Macedonia*

### Abstract



The main aim of this study was to evaluate the germination behaviour of weedy rice, a problematic and persistent weed in Macedonian rice production. Seeds from six morphotypes and the cultivars San Andrea and Onice from which weedy rice was isolated were collected and stored in typical conditions (room temperature). The germination energy, total germination and germination speed were examined at 2 weeks, as well at 4, 8, 12 and 16 months of storage. The seedling vigor index I, seedling length (root, shoot and total length) and vigor index II were examined after the release from dormancy in weedy rice. Weedy rice exhibited varied degree of dormancy at the beginning of the study (total germination from 4% to 63%), while cultivated rice showed no dormancy. Weedy rice had a significantly higher vigor index I (from 2092.78 to 2366.77 across morphotypes), seedling length (21.46 cm to 24.96 cm) and root length (11.61 cm to 13.69 cm) compared to cultivated rice (seedling length: 19.64 cm in San Andrea and 20.18 cm in Onice; root length: 9.02 cm in San Andrea and 10.42 cm in Onice). Weedy rice exhibited significantly lower to non-significant vigor index II (from 1939.19 to 2110.04) compared to cultivated rice (2788.03 in San Andrea and 2112.26 in Onice). The vigor index II and dry seedling weight were in significant positive correlation with the 100 grains weight of paddy.

The germination energy, total germination and germination speed in weedy rice had increased values at 8 months of storage, that is the period when paddy fields are prepared for new sowing in the next vegetative season. This information is useful for the application of good agricultural practices for the control of weedy rice.

**Keywords:** germination energy, total germination, germination speed, seedling length, vigor index.

Во оваа студија беше испитана ѳртливоста на дивниот ориз во оризопроизводството во Република Македонија. Семенски материјал од шест дивни морфотипови ориз и култивираниите сорти од кои беа изолирани San Andrea и Onice беше колектиран и одржуван во типични услови на складирање (на собна температура). Енергијата на ѳртење, вкупната ѳртливост и брзината на ѳртење беа испитани за временски период од 2 недели, како и за 4, 8, 12 и 16 месеци од складирањето. Вигорот на семето (индекс I и индекс II), должината на младите растенија (корен, стебло и вкупна) беа испитани по прекилот на дормантноста кај дивниот ориз. Дивните форми на ориз покажаа различен степен на дормантност на почетокот на испитувањето (вкупна ѳртливост од 4% до 63%), додека пак, култивираниот ориз не покажа дормантност. Кај дивниот

Submitted: 18.04.2018

Accepted: 10.09.2018

ориз беа констатирани значајно зголемени вредности за вигор-индекс I (од 2092,78 до 2366,77 кај дивите форми), должина на растението (21,46 cm to 24,96 cm) и должина на коренот (11,61 cm to 13,69 cm) во споредба со култивираниот ориз (должина на растение: 19,64 cm кај San Andrea и 20,18 cm кај Onice; должина на коренот: 9,02 cm кај San Andrea и 10,42 cm кај Onice). Вигор-индексот II кај дивиот ориз (од 1939,19 до 2110,04) имаше значајно пониски до статистички незначајни разлики во споредба со култивираниите сорти (2788,03 кај San Andrea и 2112,26 кај Onice). Вигор индексот II и сувата маса кај испитуваните примероци покажаа значајна позитивна корелација со масата на 100 зрна арпа. Енергијата на `ртење, вкупната `ртливост и брзината на `ртење кај дивиот ориз имаа зголемени вредности на 8 месеци од складирањето, а тоа е периодот на припрема на оризовите полиња за нова сеидба во наредната вегетативна сезона. Оваа информација е корисна од аспект на примена на практики за контрола на дивиот ориз.

**Клучни зборови:** енергија на `ртење, вкупна `ртливост, брзина на `ртење, должина на растение, вигор.

## Introduction

Weedy rice is a common and widespread weed in the rice production region of the Republic of Macedonia. This term refers to populations belonging to the *Oryza sativa* L. species exhibiting weedy characteristics, such as seed shattering (Delouche et al. 2007) and dormancy (Vidotto and Ferrero 2000, Gu et al. 2003), that negatively affect rice production (Ottis et al. 2005, Xu et al. 2017). Due to easy seed shattering (Akasaka et al. 2009, Thurber et al. 2010), weedy rice forms seed-banks in the soil and infests the rice fields the next planting season, when they are prepared and sown with the cultivated rice (Zhang et al. 2014). The infestation of the rice fields in the Republic of Macedonia with weedy rice could be attributed to several factors. Rice is generally cultivated in monoculture. Due to the variable area under rice from year to year, on some parts rice is grown in crop rotation, yet this is done without professional analysis and plan. (Ilieva 2015). The rice is direct seeded in wet conditions- sowing is done manually by broadcasting seed material in flooded paddy fields (Andreevska et al. 2013). This conditions are beneficial for the widespread of weedy rice. While in the past, until the 1960's weeds were controlled by hand weeding combined with regulation of the water bed in the rice field, this practice nowadays is replaced with herbicides application (Ilieva 2015). Weedy rice is conspecific with cultivated rice (Ilieva et al. 1998, Xia et al. 2015). Therefore, the applied herbicides against the other weed species in the rice field are ineffective regarding weedy rice.

In order to determine important biological

features of the weedy rice, the current study was performed in the main rice production region. This is the first study regarding the germination characteristics of Macedonian weedy rice. The findings obtained in this study will be useful for better understanding of weedy rice persistence in this temperate rice producing region and developing a good agricultural practices and protocols for its management.

## Material and methods

The examined rice fields are located in the Sredorek area in Kochani and belong to the Rice experimental station of the Institute of Agriculture Skopje . The fields were sown with Italian cultivars Onice and San Andrea, which are common cultivars in the rice production of the Republic of Macedonia. The latter is the most prevalent variety for cultivation. Tested area is located within the main rice producing region representing the typical rice growing conditions in the country and it is classified as the temperate continental-sub-Mediterranean region (Filipovski et al. 1996). Six morphotypes were identified on the basis of the panicle and grain characteristics (Table 1). At time of maturity, seed material (paddy) was collected from the weedy types and the cultivars in which they were found.

The paddy rice was air dried at moisture content of around 14% and stored in typical rice storing conditions (warehouse). The following parameters were evaluated: germination energy (GE), total germination (TG), germination speed (GS), root length (RL), shoot length (SL) and total seedling length (root +

shoot) (TSL), dry seedling weight (DSW), seedling vigor index I (VI-I) and vigor index II (VI-II). The GE, TG and GS were examined at two weeks, as well at 4, 8, 12 and 16 months after collecting the material. The dormancy present at two weeks and 4 months of storage resulted in high number of non-germinated seeds in the weedy rice accessions. Therefore, the vigor traits- RL, SL, TSL, DSW, VI-I and VI-II were determined after dormancy in weedy rice was overcome (at 8, 12 and 16 months of storage).

From each weedy rice morphotype and cultivar, four replications of 25 seeds (total of 100) were used. The seed was presoaked in distilled water for 24 hours before testing. Each replication was germinated in Petri dish between filter paper wetted with distilled water. The Petri dishes with seed samples were incubated in a germination cabinet at 25 °C. The germinated seeds for each replication were daily calculated after incubation until the end of the trial (day 14). A seed was considered germinated when both the coleoptile and the radicle were emerged, and radicle was minimum 2 mm long. In order to calculate the VI- I and VI- II, seeds from each type and cultivar were germinated in open Petri dish in the germination cabinet along with the replications. The RL and SL of 40 random seedlings from each type and cultivar developed in dark conditions were measured at the end of the trial (day 14). After the RL and SL were measured, the SDW was determined by drying the plants from each type in air-oven at 100 °C to constant weight.

The GE was determined on the fifth day, while the TG on the fourteenth day after incubation. These parameters were calculated as % from the total number of inoculated seeds in the Petri dish (Official gazette of the Republic of Macedonia No 61/2007).

The GS was calculated according to Maguire (1962) and AOSA (1983), by calculating the germinated seeds every day from the first day to the last day (day 14) of trial:  $GS = \text{Sum} (N_i \text{ seeds} / N_i \text{ days})$ , where  $i = 1$  to 14;  $N_i$  seeds = number of seeds germinated on day  $N_i$ ;  $N_i$  days = number of days (1 to 14).

The seedling vigor was determined on the basis of the vigor indexes (Abdul-Baki and Anderson 1973):

Vigor index I: VI-I = Seedling length (cm) Germination (%)

Vigor index II: VI-II = Seedling Dry Weight (mg) Germination (%)

The results were statistically analyzed by the analysis of variance (ANOVA) followed by LSD test at 0.05 and 0.01 significance level. Correlation analysis was determined by calculating the Pearson's correlation coefficient.

## Results and discussion

### Germination energy

The results for GE of the weedy rice types and the rice cultivars are shown in Table 2. During the study, cultivated rice showed constantly high GE ranging from 89% to 98% in San Andrea and from 92% to 99% in Onice. This trait was not observed for the weedy rice types, which showed significantly lower GE at 2 weeks after collection (from 0% to 49%). At 4 months of storage, the values of GE in weedy rice were still significantly lower compared to the cultivars, ranging from 4% to 58%. The GE values increased in time with a peak at 12 months of storage (from 99% to 100%). The differences in GE between the weedy rice accessions and the cultivar San Andrea were non-significant. The weedy accessions WR1200234 and WR1321232 had

**Table 1** Morphological and grain characteristics of the examined weedy rice accessions\*.

Code	PT	MAA	AP	AC	LPC	CS	CC
WR1200234	1	2	0	0	2	3	4
WR3311234	3	3	1	1	2	3	4
WR1313632	1	3	1	3	6	3	2
WR1323232	1	3	2	3	2	3	2
WR1311234	1	3	1	1	2	3	4
WR1321232	1	3	2	1	2	3	2

\* PT - panicle type; MAA - main axis attitude; AP - awns presence; AC - awns color; LPC - lemma and palea color; CS - caryopsis shape; CC - caryopsis color. The numbers correspond to Descriptors for wild and cultivated rice (*Oryza* spp.) by Bioversity International, IRRI and WARDA (2007).

**Table 2** Germination energy (%) of the examined weedy accessions and rice cultivars\*.

	at 2 weeks	at 4 months	at 8 months	at 12 months	at 16 month
WR1200234	21.00 ± 3.83b	51.00 ± 10.00cd	92.00 ± 7.30bc	100.00 ± 0.00b	94 ± 4.00
WR3311234	49.00 ± 8.25c	48.00 ± 24.66cd	99.00 ± 2.00d	99.00 ± 2.00ab	96 ± 5.66
WR1313632	1.00 ± 2.00a	4.00 ± 5.66a	84.00 ± 3.27a	99.00 ± 2.00ab	93 ± 6.00
WR1323232	1.00 ± 2.00a	36.00 ± 9.80bc	87.00 ± 6.00ab	99.00 ± 2.00ab	95 ± 3.83
WR1311234	24.00 ± 7.30b	58.00 ± 12.44d	95.00 ± 2.00cd	99.00 ± 2.00ab	95 ± 5.03
WR1321232	0.00 ± 0.00a	25.00 ± 3.83b	98.00 ± 4.00d	100.00 ± 0.00b	98 ± 2.31
San Andrea	92.00 ± 3.27d	89.00 ± 8.87e	98.00 ± 2.31d	97.00 ± 2.00ab	96 ± 3.27
Onice	98.00 ± 2.31d	98.00 ± 2.31e	99.00 ± 2.00d	96.00 ± 3.27a	92 ± 3.27
LSD (0.05)	6.72	17.60	5.29	3.05	NS
LSD (0.01)	9.14	23.95	7.20	4.16	NS

\* Means are average values of 4 replications standard deviation. Analysis of variance (ANOVA) followed by LSD test at 0.05 and 0.01 levels of difference. The values in the column marked with different lower-cases denoted significant differences at  $p < 0.05$  between samples.

**Table 3** Total germination (%) of the examined weedy accessions and rice cultivars\*.

	at 2 weeks	at 4 months	at 8 months	at 12 months	at 16 month
WR1200234	40.00 ± 3.27c	61.00 ± 20.23bc	98.00 ± 4.00b	100.00 ± 0.00b	94.00 ± 4.00ab
WR3311234	63.00 ± 6.00d	60.00 ± 16.65bc	99.00 ± 2.00b	99.00 ± 2.00ab	97.00 ± 3.83ab
WR1313632	13.00 ± 3.83b	19.00 ± 11.49a	88.00 ± 5.66a	99.00 ± 2.00ab	95.00 ± 3.83ab
WR1323232	5.00 ± 2.00ab	50.00 ± 7.66b	97.00 ± 3.83b	99.00 ± 2.00ab	96.00 ± 3.27ab
WR1311234	36.00 ± 11.78c	77.00 ± 10.52c	100.00 ± 0.00b	99.00 ± 2.00ab	97.00 ± 6.00ab
WR1321232	4.00 ± 3.27a	72.00 ± 10.33c	98.00 ± 4.00b	100.00 ± 0.00b	98.00 ± 2.31b
San Andrea	99.00 ± 2.00e	97.00 ± 3.83d	98.00 ± 2.31b	97.00 ± 2.00ab	96.00 ± 3.27ab
Onice	98.00 ± 2.31e	99.00 ± 2.00d	99.00 ± 2.00b	96.00 ± 3.27a	92.00 ± 3.27a
LSD (0.05)	8.21	17.46	4.83	3.05	5.90
LSD (0.01)	11.17	23.76	6.57	4.16	8.04

\* Means are average values of 4 replications standard deviation. Analysis of variance (ANOVA) followed by LSD test at 0.05 and 0.01 levels of difference. The values in the column marked with different lower-cases denoted significant differences at  $p < 0.05$  between samples.

significantly higher values compared to Onice. At 16 months of storage, GE in weedy rice ranged (from 93% to 98%). In comparison to the cultivars, the differences were non-significant.

Among the weedy rice morphotypes, significant differences in GE were found at 2 weeks, as well at 4 and 8 months of storage. On the other hand significant differences in GE among the morphotypes were not observed at 12 and 16 months.

### Total germination

The TG in the weedy rice types and cultivated rice followed similar pattern as the GE. As presented in Table 3, the cultivars had constantly high and similar TG values during

the storage period of 16 months, ranging from 96% to 99% in San Andrea and from 92% to 99% in Onice indicating no dormancy occurred in cultivated rice. This pattern of germination was not observed by the weedy rice types, which showed varying degree of dormancy at the first and second trial (Fig. 1).

Weedy rice found in temperate rice growing regions exhibits diverse level of dormancy. While no or low dormancy is reported in some populations (Delouche et al. 2007, Xia et al. 2011), high primary dormancy is found in others (Fogliato et al. 2012). At the beginning of this study (2 weeks after collection) weedy rice showed significantly lower TG compared to both cultivars ranging from 4% to 63% across the morphotypes (Table 3). All tested weedy morphotypes have a pigmented

pericarp. According to Gu et al. (2011) the association between pericarp color and seed dormancy, arises from pleiotropy. The pleiotropic gene expressed in early developing seeds promoted expression of key genes for biosynthesis of abscisic acid (ABA), resulting in an increase in accumulation of the dormancy-inducing hormone; activated a conserved network of eight genes for flavonoid biosynthesis to produce the pigments in the lower epidermal cells of the pericarp tissue; and enhanced seed weight.

Significant differences among weedy types were also observed. Even the TG of weedy rice was increased at 4 months of storage (from 19% to 77%), it was still significantly lower compared to the cultivars. The weedy morphotype WR3311234 with the highest TG at the start (63%) showed similar value at 4 months (60.00%).

Depending on the morphotype, weedy rice reached the highest TG values from 8 to 12 months of storage (from 88% to 100% and from 99% to 100%, respectively). In comparison to the cultivars, most of the weedy morphotypes showed non-significant differences in TG. Significant differences were found for WR1313632 (lower TG compared to both cultivars) at 8 months, and WR1200234 and WR1321232 (higher TG compared to Onice) at 12 months of storage. At 8 months of storage, the dormancy was completely overcome in weedy morphotypes WR1321232, WR3311234, WR1323232, WR1311234 and WR1200234. Certain level of dormancy was still present in WR1313632. The TG of this morphotype (88.00%) was significantly lower compared to the rest of the weedy accessions and the cultivars. At 16 months of storage, the weedy types had similar TG compared to the cultivars ranging from (94% to 98%). Significant difference was found between WR1321232 with the highest TG (98%) and Onice which had the lowest TG value (92%).

The varying degree of dormancy resulted in significant differences in TG among weedy rice accessions. Once dormancy was completely overcome, the differences in TG between the different weedy accessions were non-significant.

The study by Vidotto and Ferrero (2000) showed that environmental conditions following the shattering affect the length of seed dormancy in red rice.

The results for GE and TG suggested that the examined weedy rice accessions are characterized by seed dormancy, which disables massive germination after maturation. This could explain the persistence of weedy rice populations in Macedonian rice fields, as dormancy secures for a large portion of the shattered seeds to remain dormant in autumn, when favorable environmental conditions for germination in the rice fields still exist. The rice producing region has mean monthly, mean monthly minimal and mean monthly maximal temperatures for October of 14.7 °C, 6.9 °C and 20.4°C, respectively (data obtained from the meteorological station at the Institute of Agriculture Skopje-Rice research station in Kochani for the period 1998- 2014). This also avoids subsequent killing of the seedlings due to upcoming later low temperatures and freezes. It should be noted that the trial at 8 months of storage when the weedy rice dormancy was naturally broken overlapped with the time when the rice fields are prepared for cultivation and sown with paddy rice (beginning of May).

### Germination speed

The results for the GS are presented in Table 4. The highest GS during the course of the study was determined in the cultivar Onice (from 8.08 to 20.10) and it was significantly higher compared to the weedy rice, as well as the cultivar San Andrea.



**Figure 1.** Varying degree of dormancy in weedy rice at 2 weeks of storage. Pictured at the end of the trial: A- WR3311234, B- WR1323232, C- WR1200234, D- San Andrea cultivar

**Table 4** Germination speed of the examined weedy accessions and rice cultivars\*.

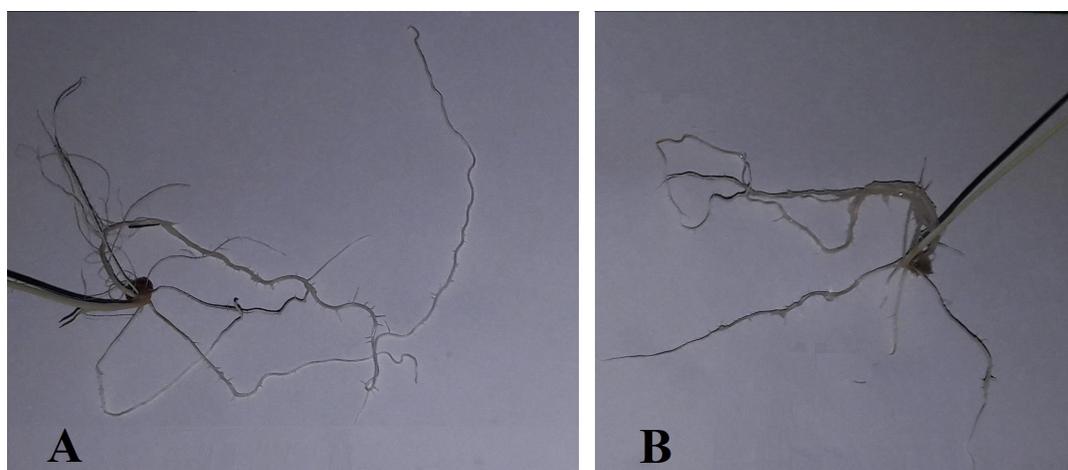
	at 2 weeks	at 4 months	at 8 months	at 12 months	at 16 months
WR1200234	1.90 ± 0.35b	3.38 ± 0.91bc	6.70 ± 0.63c	11.58 ± 0.17bc	10.08 ± 0.20abc
WR3311234	3.68 ± 0.33c	3.90 ± 1.65c	6.53 ± 0.28c	12.33 ± 0.97c	9.85 ± 0.84abc
WR1313632	0.41 ± 0.08a	0.74 ± 0.53a	5.13 ± 0.35a	9.81 ± 0.69a	9.15 ± 1.16ab
WR1323232	0.16 ± 0.08a	2.57 ± 0.54b	5.79 ± 0.45b	10.79 ± 0.37ab	8.67 ± 0.45a
WR1311234	1.92 ± 0.49b	4.11 ± 0.53c	6.54 ± 0.24c	11.78 ± 0.27bc	10.24 ± 0.79bc
WR1321232	0.14 ± 0.14a	3.65 ± 0.52bc	6.44 ± 0.48c	12.17 ± 1.24bc	10.21 ± 0.80bc
San Andrea	6.74 ± 0.51d	6.26 ± 0.32d	7.39 ± 0.20d	10.83 ± 0.41ab	10.81 ± 0.88c
Onice	8.08 ± 0.19e	8.11 ± 0.17e	10.80 ± 0.34e	20.10 ± 1.94d	17.40 ± 2.00d
LSD (0.05)	0.48	1.13	0.50	1.45	1.49
LSD (0.01)	0.65	1.54	0.69	1.97	2.03

\* Means are average values of 4 replications standard deviation. Analysis of variance (ANOVA) followed by LSD test at 0.05 and 0.01 levels of difference. The values in the column marked with different lower-cases denoted significant differences at  $p < 0.05$  between samples.

**Table 5** Vigor index I of the examined weedy accessions and rice cultivars.\*

	after 8 months	after 12 months	after 16 month	Average
WR1200234	2020.76 ± 82.48b	2418.00 ± 0.00c	1839.58 ± 78.28bc	2092.78
WR3311234	2202.75 ± 44.50cd	2585.88 ± 52.24d	2085.50 ± 82.34d	2291.38
WR1313632	2135.76 ± 137.29c	2885.85 ± 58.30f	2038.70 ± 82.19d	2353.44
WR1323232	2173.77 ± 85.82c	2908.62 ± 58.76f	2017.92 ± 68.65d	2366.77
WR1311234	2293.00 ± 0.00de	2805.66 ± 56.68e	1835.24 ± 113.52bc	2311.30
WR1321232	2349.06 ± 95.88e	2887.00 ± 0.00f	1851.22 ± 43.62c	2362.43
San Andrea	1757.14 ± 41.41a	2272.71 ± 46.86b	1686.72 ± 57.38a	1905.52
Onice	1931.49 ± 39.02b	2103.36 ± 71.56a	1759.04 ± 62.45ab	1931.30
LSD (0.05)	108.72	77.83	115.87	
LSD (0.01)	147.98	105.93	157.71	

\* Means are average values of 4 replications standard deviation. Analysis of variance (ANOVA) followed by LSD test at 0.05 and 0.01 levels of difference. The values in the column marked with different lower-cases denoted significant differences at  $p < 0.05$  between samples.

**Figure 2.** Seminal root system of seedlings. A: Weedy rice. B: cultivated rice

In comparison to San Andrea, the weedy types had significantly lower GS from the beginning of the study to 8 months of storage. Afterwards, most of the weedy types showed non-significant differences compared to San Andrea, except for WR3311234 at 12 months of storage (significantly higher GS) and WR1313632 and WR1323232 at 16 months of storage (significantly lower GS). The germination speed of both weedy and cultivated rice was lowest at the start of the study and increased over time with a peak at 12 months of storage.

According to Cho (2010), seed germination speed of Korean and Southeast Asian red rice is increased with increasing the temperature.

### Vigor index I and seedling length

After dormancy was overcome, the VI-I of weedy types ranged from 2135.76 to 2349.06 at 8 months, from 2418.00 to 2908.62 at 12 months and from 1835.24 to 2085.50 at 16 months of storage, as shown in Table 5. Generally, weedy rice showed significantly higher vigor index I compared to cultivated rice. The WR1200234 at 8 and 16 months and WR1311234 at 16 months showed non-significant differences compared to Onice.

The results for the total length of the seedlings incubated 14 days at 25 C in dark

conditions are given in Table 6. At 8 and 12 months, the weedy accessions generally developed longer seedlings compared to the cultivars, except for WR1200234, which showed similar results to Onice at 8 months and to San Andrea at 12 months. At 16 months, WR3311234, WR1313632 and WR1323232 had statistically higher values for seedlings length compared to both cultivars, while WR1200234 had significantly higher seedling length compared to Onice. The seedling length in weedy rice was positively correlated with the root length (Table 9).

The results for the root and shoot length are given in Table 7. The weedy rice had longer roots (from 11.61 cm to 13.69 cm among morphotypes at 8, 12 and 16 months) compared to cultivated rice (9.02 cm in San Andrea and 10.42 cm in Onice). The weedy rice seedlings had visibly longer and thinner (finer) roots compared to the cultivars, which had shorter and thicker roots (Fig. 2).

At 8 months of storage, WR1323232 and WR1321232 had significantly higher seedling shoot length compared to Onice. WR1313632 had significantly higher shoot length compared to both cultivars, while WR1311234 had significantly lower shoot length compared to San Andrea. At 12 months, in WR3311234, WR1313632 and WR1321232 significantly higher shoot length values were determined in

**Table 6** Total seedling (root + shoot) length (cm) of the examined weedy accessions and rice cultivars.\*

	at 8 months	at 12 months	at 16 month	Average
WR1200234	20.62b	24.19bc	19.58bcd	21.46
S	3.60	5.13	4.23	
WR3311234	22.25c	26.12c	21.50e	23.29
S	3.63	5.70	4.51	
WR1313632	24.27e	29.15d	21.46e	24.96
S	5.01	6.67	4.29	
WR1323232	22.67cd	29.39d	21.02de	24.36
S	3.70	5.21	3.65	
WR1311234	22.92cde	28.35d	18.92ab	23.40
S	3.18	3.94	3.59	
WR1321232	23.97de	28.87d	18.89ab	23.91
S	3.68	4.49	3.64	
San Andrea	17.93a	23.43ab	17.57a	19.64
S	1.70	4.30	2.34	
Onice	19.52b	21.91a	19.12abc	20.18
S	3.55	3.98	3.98	
LSD (0.05)	1.59	2.20	1.69	
LSD (0.01)	2.09	2.90	2.22	

\* Means are average of 40 samples per weedy rice morphotype/cultivar; S - standard deviation, CV - coefficient of variation. Analysis of variance (ANOVA) followed by LSD test at 0.05 and 0.01 levels of difference. The values in the column marked with different lower-cases denoted significant differences at  $p < 0.05$  between samples.

**Table 7** Root and shoot seedling length (cm) of the examined weedy accessions and rice cultivars.\*

	Seedling root length			Seedling shoot length		
	8 months	12 months	16 months	8 months	12 months	16 months
WR1200234	10.72bc	12.92b	11.19c	9.90ab	11.27a	8.39ab
S	3.03	3.80	3.41	1.68	2.47	1.35
Average	11.61			9.85		
WR3311234	12.13de	11.88ab	11.39c	10.12abc	14.24c	10.11d
S	2.81	4.11	3.81	1.47	3.78	1.38
Average	11.80			11.49		
WR1313632	12.63de	13.12b	10.47bc	11.64e	16.03d	10.99e
S	2.93	3.71	2.95	2.94	4.88	2.42
Average	12.07			12.89		
WR1323232	11.84cd	15.92cd	11.46c	10.83cde	13.47bc	9.56cd
S	3.09	3.68	3.54	1.92	2.94	1.83
Average	13.07			11.29		
WR1311234	13.27e	17.00d	10.79bc	9.65a	11.35a	8.13a
S	2.65	3.42	3.21	1.36	2.24	1.20
Average	13.69			9.71		
WR1321232	12.89de	15.00c	9.73ab	11.08de	13.87c	9.16c
S	3.24	4.23	3.01	1.60	2.86	1.43
Average	12.54			11.37		
San Andrea	7.23a	11.25a	8.59a	10.70bcd	12.18ab	8.98bc
S	1.35	3.86	1.62	1.00	2.25	1.40
Average	9.02			10.62		
Onice	9.69b	10.37a	11.22c	9.83a	11.54a	7.90a
S	2.70	3.19	3.24	1.77	2.19	1.40
Average	10.42			9.76		
LSD (0.05)	1.22	1.66	1.39	0.83	1.36	0.70
LSD (0.01)	1.61	2.18	1.83	1.10	1.79	0.92

\* Means are average of 40 samples per weedy rice morphotype/cultivar; S - standard deviation, CV - coefficient of variation. Analysis of variance (ANOVA) followed by LSD test at 0.05 and 0.01 levels of difference. The values in the column marked with different lower-cases denoted significant differences at  $p < 0.05$  between samples.

comparison to both cultivars. WR1323232 had significantly higher value compared to Onice. At 16 months, significantly higher values were determined in WR3311234 and WR1313632 compared to both cultivars and WR1323232 and WR1321232 compared to Onice. WR1311234 had significantly lower value compared to San Andrea. As shown in Table 9, the shoot length in weedy rice was positively correlated with the 100 grains weight of paddy. Significant positive correlation between these characteristics was also reported by Zhang et al. (2017).

### Vigor index II (VI-II)

As shown in Table 8, the cultivar San Andrea showed significantly higher vigor index II (VI-II) (2628.70 to 2933.14), compared to the weedy rice accessions (1980.00 to 2031.54 in WR1200234, 1945.35 to 2270.07 in WR3311234, 2012.10 to 2168.10 in

WR1313632, 1981.98 to 2264.95 in WR1323232, 1860.46 to 2088.00 in WR1311234, 1975.00 to 2345.14 in WR1321232) and the cultivar Onice (2024.00 to 2200.77). In comparison to Onice, WR1200234 and WR1313632 had significantly lower vigor index II, while WR1321232 had significantly higher index at 8 months of storage. At 12 month of storage, the weedy types had significantly lower index compared to Onice, except WR1313632. At 16 month of storage, the differences between the weedy accessions and Onice were non-significant, except WR1311234 with significantly lower index.

As shown in Table 9, both the vigor index II (VI-II) and the seedling dry weight were in significant positive correlation with the 100 grains weight of paddy. The results for the dry seedling weight are given in Fig. 3. Significant correlation between the 1000-seed weight and seedling dry weight has previously been reported by Zhang et al. (2017).

**Table 8** Vigor index II of the examined weedy accessions and rice cultivars.\*

	at 8 months	at 12 months	at 16 month	Average
WR1200234	2031.54 ± 82.92a	1980.00 ± 0.00b	1983.40 ± 84.40ab	1998.31
WR3311234	2270.07 ± 45.86cd	1945.35 ± 39.30b	2002.08 ± 79.05b	2072.50
WR1313632	2048.64 ± 131.69a	2168.10 ± 43.80c	2012.10 ± 81.11b	2076.28
WR1323232	2264.95 ± 89.42cd	1981.98 ± 40.04b	2083.20 ± 70.87b	2110.04
WR1311234	2088.00 ± 0.00ab	1869.12 ± 37.76a	1860.46 ± 115.08a	1939.19
WR1321232	2345.14 ± 95.72d	1975.00 ± 0.00b	1987.44 ± 46.83b	2102.53
San Andrea	2933.14 ± 69.12e	2628.70 ± 54.20d	2802.24 ± 95.33c	2788.03
Onice	2200.77 ± 44.46bc	2112.00 ± 71.85c	2024.00 ± 71.85b	2112.26
LSD (0.05)	114.62	66.70	126.93	
LSD (0.01)	156.00	90.78	172.76	

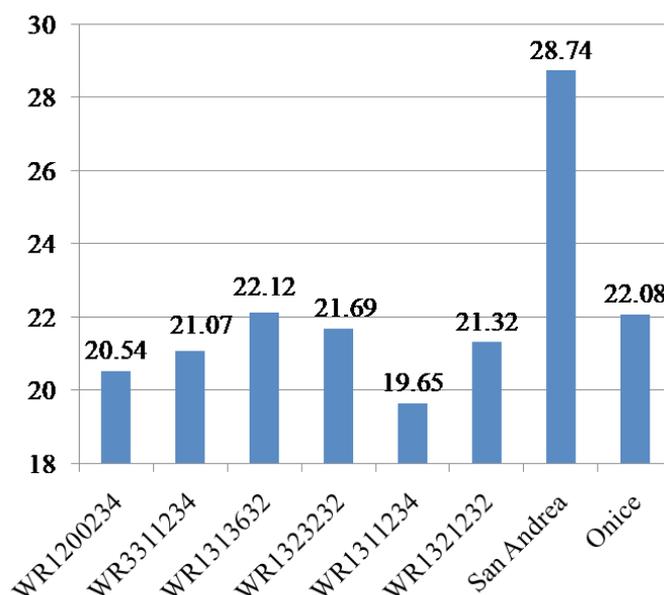
\* Means are average values of 4 replications standard deviation. Analysis of variance (ANOVA) followed by LSD test at 0.05 and 0.01 levels of difference. The values in the column marked with different lower-cases denoted significant differences at  $p < 0.05$  between samples.

**Table 9** Correlation analysis of the root length (RL), shoot length (SL), total seedling length (TSL), dry seedling weight (DSW), 100 grains weight of paddy (W100), seedling vigor index I (VI-I) and seedling vigor index II (VI-II) of the examined weedy accessions and rice cultivars.\*

	RL	SL	TSL	DSW	W100	VI-I	VI-II
RL	1						
SL	-0.310 ns	1					
TSL	0.362 ns	0.774 ns	1				
DSW	-0.370 ns	0.909 *	0.645 ns	1			
W100	-0.578 ns	0.917 **	0.515 ns	0.870 *	1		
VI-I	0.531 ns	0.593 ns	0.935 **	0.453 ns	0.382 ns	1	
VI-II	-0.283 ns	0.758 ns	0.555 ns	0.893 *	0.825*	0.514 ns	1

df = 4; Critical values: 0.811 ( $\alpha$  0.05); 0.917 ( $\alpha$  0.01)

\* df- degrees of freedom (n - 2);  $\alpha$  0.05,  $\alpha$  0.01- levels of probability. Significant correlation at 0.05 level (\*) and at 0.01 level of significance (\*\*).

**Figure 3.** Seedling dry weight (mg) - average of the results obtained at 8, 12 and 16 months of storage

## Conclusions

Macedonian weedy rice exhibits variable degree of seed dormancy, which allows the persistence of weedy populations in rice fields by inhibiting the germination process during favorable autumn conditions. After dormancy was overcome, the weedy rice had a significantly higher Vigor index - I, longer seedlings and significantly lower Vigor index - II. The germination energy, total germination and germination speed in weedy rice had increased values at 8 months of storage. This period overlaps with the time when the paddy fields were prepared for new sowing in the next vegetative season. This information is useful for the application of good agricultural practices for the control of weedy rice.

## References

- Abdul-Baki, A.A. & Anderson, J.D. (1973). Vigor determination in soybean seed by multiple criteria. *Crop Science*, **13**: 630-633. doi:10.2135/cropsci1973.0011183X001300060013x
- Andreevska, D., Menkovska, M., Andov, D. (2013). Overview of the current condition, in production consumption and the research potential of the rice crop in the Republic of Macedonia. *Macedonian Journal of Animal Science*, **3**: 219-228.
- Akasaka, M., Ushiki, J., Iwata, H., Ishikawa R., Ishii, T. (2009). Genetic relationships and diversity of weedy rice (*Oryza sativa* L.) and cultivated rice varieties in Okayama Prefecture, Japan. *Breeding Science* **59**: 401-409.
- Association of Official Seed Analysis AOSA (1983). *Seed Vigor Testing Handbook*. Contribution No. 32 to the handbook on Seed Testing. 88 pp. AOSA.
- Bioversity International, IRRI, WARDA (2007). *Descriptors for wild and cultivated rice (Oryza spp.)*. 63 pp. Bioversity International, Rome, Italy; International Rice Research Institute, Los Banos, Philippines; WARDA, Africa Rice Center, Cotonou, Benin.
- Cho, Y.S. (2010). Germination characteristics of Korean and Southeast Asian red rice (*Oryza sativa* L.) seeds as affected by temperature. *Asian Journal of Plant Sciences*, **9**: 104-107. DOI: 10.3923/ajps.2010.104.107
- Delouche, J.C., Burgos, N.R., Gealy, D.R., de San Martin, G.Z., Labrada, R., Larinde, M., Rosell, C. (2007). *Weedy rices: Origin, biology, ecology and control*. FAO plant production and protection paper 188. 144 pp. FAO, Rome, Italy.
- Filipovski, Gj., Rizovski, R., Ristevski, P. (1996). *Karakteristiki na klimatsko-vegetacisko-pochvenite zoni (regioni) vo R. Makedonija*. 177 pp. Macedonian Academy of Sciences and Arts, Skopje, Republic of Macedonia.
- Fogliatto, S., Vidotto, F., Ferrero, A. (2011). Morphological characterisation of Italian weedy rice (*Oryza sativa*) populations. *Weed Research*, **52**: 60-69.
- Gu, X., Chen, Z., Foley, M.E. (2003). Inheritance of seed dormancy in weedy rice. *Crop Science*, **43**: 835-843. doi:10.2135/cropsci2003.8350
- Gu, X.Y., Foley, M.E., Horvath, D.P. et al. (2011). Association between seed dormancy and pericarp color is controlled by a pleiotropic gene that regulates abscisic acid and flavonoid synthesis in weedy red rice. *GENETICS*, **189**: 1515-1524. <https://doi.org/10.1534/genetics.111.131169>
- Ilieva, V. (2015). Otstranuvanje na plevelite nekogash i sega. In: Dimitrovski, R. et al. (eds.) *Kochanskiot oriz*. Opshtina Kochani, Kochani, 65-73.
- Ilieva, V., Stojkovski, C., Maznevska, S. (1998). Inheritance of some productive traits in hybrids between cultivated white and red-grain rice genotypes. *Macedonian Agricultural Review*, **45**: 29- 37.
- Maguire, J.D. (1962). Speed of germination - aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, **2**: 176-177.
- Official gazette of the Republic of Macedonia No 61, 2007. Pravilnik za nachinot na rabota, prostornata i tehnickata opremenost na ovlastenite laboratorii i metodi za ispituvanje na kvalitetot na semenskiot materijal kaj zemjodelskite rastenija.
- Ottis, B.V., Smith, K.L., Scott, R.C., Talbert, R.E. (2005). Rice yield and quality as affected by cultivar and red rice (*Oryza sativa*) density. *Weed Science*, **53**: 499-504.
- Thurber, C.S., Reagon, M., Gross, B.L., Olsen, K.M., Jia, Y., Caicedo, A.L. (2010). Molecular evolution of shattering loci in U.S. weedy rice. *Molecular Ecology*, **19**: 3271-3284. <http://doi.org/10.1111/j.1365-294X.2010.04708.x>
- Vidotto, F. & Ferrero, A. (2000). Germination behaviour of red rice (*Oryza sativa* L.) seeds in field and laboratory conditions. *Agronomie*, **20**: 375-382. DOI: 10.1051/

- agro:2000134
- Xia, H.B., Wang, W., Xia, H., Zhao, W., Lu, B.R. (2011). Conspecific crop-weed introgression influences evolution of weedy rice (*Oryza sativa* f. *spontanea*) across a geographical range. *PLoS ONE* **6**: e16189. <https://doi.org/10.1371/journal.pone.0016189>
- Xu, X.M., Li, G., Su, Y., Wang, X.L. (2017). Effect of weedy rice at different densities on photosynthetic characteristics and yield of cultivated rice. *Photosynthetica*. DOI: <https://doi.org/10.1007/s11099-017-0707-2>
- Zhang, Z., Dai, W., Song, X., Qiang, S. (2014). A model of the relationship between weedy rice seed-bank dynamics and rice-crop infestation and damage in Jiangsu Province, China. *Pest Management Science*, **70**: 716-724. doi:[10.1002/ps.3649](https://doi.org/10.1002/ps.3649)
- Zhang, A., Liu, C., Chen, G. et al. (2017). Genetic analysis for rice seedling vigor and fine mapping of a major QTL qSSL1b for seedling shoot length. *Breeding Science*, **67**: 307- 315. doi:10.1270/jsbbs.16195
- Xia, H., Xia, H., Ellstrand, N.C., Yang, C., Lu, B. (2011). Rapid evolutionary divergence and ecotypic diversification of germination behavior in weedy rice populations. *New Phytologist*, **191**: 1119-1127. doi:[10.1111/j.1469-8137.2011.03766.x](https://doi.org/10.1111/j.1469-8137.2011.03766.x)