

Allelopathic Effect of West Java Local Black Rice Varieties on Barnyard Grass (*Echinochloa crus-galli* (L.) Beauv.) at Germination Stage

Muhamad Kadapi*, Denny Sobardini, Elissa Helena, Haritsa Hanindianingrum,
Fachryansah Noor, Nolahdi Wicaksana and Sumadi

Faculty of Agriculture Universitas Padjadjaran, Jatinangor, Indonesia

Received: 20 July 2020, Revised: 4 March 2021, Accepted: 23 March 2021

Abstract

Some colored rice varieties are able to release root exudates that contained allelochemicals which can inhibit the growth of other plant species including weeds. This capability is required if rice is to compete with weeds from the germination stage onwards. One of the dominant weeds in rice fields is barnyard grass, which can have negative effects on rice growth at every stage, particularly at the germination stage. Therefore, the objective of this study was to evaluate the response of local black rice varieties originated from West Java, Indonesia against barnyard grass at the germination stage. In this study, four local black rice varieties that originated in three West Java districts, two from Subang (SB and SB II), one from Sukabumi (SMI) and one from Tasikmalaya (TSK), were sowed together with *Echinochloa crus-galli* in petri dishes with six replications. The experiment was conducted in a plant breeding and seed technology laboratory in order to observe T_{50} , germination percentage, vigour index, shoot and root length at 7 and 14 days after sowing (DAS). The results showed that the four local black rice cultivars had allelopathic potential, as indicated by vigour traits at germination stages of barnyard grass. Besides, the prepresence of barnyard grass showed an inhibitory or stimulatory effect on black rice growth in its early germination stage (7 DAS). However, the local black rice that originated from Subang (SB II) showed growth recovery and inhibitory effect towards barnyard grass at the second germination stage (14 DAS), as measured by vigour index, shoot length and inhibitory percentage. This study suggested that the ability to recover at the second stages of germination is a useful trait to be considered when conducting breeding program for the efficiency of weed management.

Keywords: allelochemical; tolerance; variation; black rice; weed
DOI 10.14456/cast.2021.54

1. Introduction

Rice is one of the important crops for more than half of human consumption in the world. The average of world rice consumption has been increasing every year [1]. Among the countries of Asia, Indonesia is the third country in rice consumption after India and China [2]. More than 40,000

*Corresponding author: Tel.: (+62) 8812279027
E-mail: kadapi@unpad.ac.id

varieties of rice have been registered around the world which can be differentiated according to shape and color [3]. Colored rice, such as black rice is mainly cultivated in Asia and the biggest country that produces this kind of rice is China, followed by Sri Lanka, Indonesia, India and Philippines [3]. The color of black rice is commonly found in the aleurone coat that contains anthocyanin [4].

Breeding program for black rice has been paid much attention on anthocyanin content due to its benefits to human health [5]. However, another potential of black rice has been reported by Kato-Noguchi *et al.* [6] that Japanese black rice has allelopathic potential that can inhibit hypocotyls and root growth of lettuce and white clover. This potential is important for rice during the growth and developmental stage to compete with weeds [7]. The control of weeds in rice fields over several periods involved the use of synthetic chemicals as herbicides that caused the ecological problems and finally led to the emergence of rice weeds resistant to herbicides [8-10]. Allelopathy is well known as a phenomenon that occurs due to interactions among plants and causes allelochemicals release into the plant rhizosphere. Allelochemicals can be released by several mechanisms such as root exudation, degradation of plant residues, volatilization and leaching from leaves. Allelochemicals can influence the neighboring plants causing inhibitory and stimulatory effects on plant growth [11]. Therefore, the development and cultivation of allelopathic rice that can release allelochemicals causing damage to weeds or having inhibitory effect on weeds, has become an important step in the reduction of the negative impact of herbicides [12, 13]. At the preliminary stage, the revealing of the allelopathic potential of various rice strains can provide important information needed to improve the new rice varieties in breeding program [14, 15]. It has been reported that the allelopathic rice varieties depend on genetic factors [16-18].

Variations of allelopathic rice potential have been reported to inhibit the growth of several weed species when planted together in field conditions or in the laboratory [19, 20]. One of the dominant and most harmful weeds in rice cultivation field is barnyard grass (*Echinochloa crus-galli* (L.) Beauv.). This weed can reduce the yield from 35% to 95% [21-23]. Therefore, the current study of allelopathic rice on the barnyard grass growth has become more intensive [7, 24]. In the quest to evaluate and discover the allelopathic potential of rice varieties, several screening approaches have been established [19, 25, 26]. One of the useful methods is laboratory screening and the results showed a significant correlation between laboratory and field performance of rice seed vigour traits [25]. However, environmental conditions affect the correlation between field and laboratory investigations [27]. In addition, Berendji *et al.* [28] reported that barnyard grass seedling growth traits were inhibited by Iranian rice cultivars. The inhibition caused by rice was due to allelochemicals released by rice root exudates [20]. Furthermore, seed vigour is a physiological trait that is required to ensure the uniform emergence of plants in the field under sub-optimal conditions [29]. This trait is useful for the breeding program, even though this trait is often absent from breeding programs, which are frequently directed toward yield traits [30, 31]. Therefore, the aims of this study were to evaluate the allelopathic potential of local black rice against barnyard grass and to reveal the particular vigour traits that might be useful for the breeding program in order to develop new allelopathic rice as one of practices in environmentally friendly of weed management by farmers.

2. Materials and Methods

Four out of seven West Java local rice (*Oryza sativa* L.) seeds from a plant breeding and seed technology laboratory were selected according to their allelopathic potential against lettuce, color and viability of seed [32]. The screening method used was in accordance with the following method with brief modification [33] in which 20 seeds of rice and 50 seeds of check plant were planted

together. The local black varieties were collected from local farmers in three districts of West Java, Indonesia (Subang, Sukabumi, and Tasikmalaya) (Figure 1) while barnyard grass (*Echinochola crus-galli* (L.) Beauv.) seeds were purchased from (Prairie Moon Nursery) Minnesota, USA.

The experiment was conducted in a plant breeding and seed technology laboratory at the Universitas Padjadjaran under room conditions, with 26/24°C day/night temperature, 60-70% relative humidity (RH), and 12 h daily light. The germination test used was the top of paper method [7]. In this study, we put 20 rice seeds of each variety into 90 mm petri dishes containing a filter paper (Whatman™ no. 42) and 10 ml distilled water. The first day count (FDC) of germination percentage (%) was calculated at 7 days after sowing (DAS) and 14 DAS as last day count (LDC) using ISTA formula [34]:

$$GP (\%) = \frac{\text{Total seeds germinated}}{\text{Number of initial seed used}} \times 100\% \quad (1)$$

The germination test for barnyard grass seed used 100 seeds with the same method. The potential allelopathy of rice was tested using the method that was adapted [18, 19, 35] with a slight modification. Fifteen seeds of each variety of rice and 30 seeds of barnyard grass were planted together in 90 mm petri dishes that contains filter paper (Whatman No. 42) and 10 ml distilled water. All of the petri dishes were placed at room condition. The germination rate (%) of weeds and black rice was recorded every day until 14 days after sowing as LDC of rice seed using the same formula as mentioned above and vigour parameter such as T_{50} using the methods described by Coolbear *et al.* [36] and Farooq *et al.* [37], vigour index, and inhibition percentage were calculated by the following equation [38].

$$\text{Vigour Index} = \frac{N}{\text{Total number of seeds}} \times \text{Total length of seedling} \quad (2)$$

N: total of normal seeds germinated at 14 days after sowing

$$T_{50} = ti + [(N / 2 - ni) (ti - tj)] / ni - nj \quad (3)$$

N: the final number of germination and ni, nj cumulative and number of seeds germinated by adjacent counts at times ti and tj

$$\text{Inhibition Percentage} = \frac{\text{control} - \text{treatments}}{\text{control}} \times 100\% \quad (4)$$

In this study, we also measured the shoot length (cm) and root length (cm) of weeds at 7 and 14 days after sowing of both species. The inhibition percentages of the treatments were to identify the interaction between two species, and the negative values of this trait indicated a stimulatory effect whereas positive values indicated inhibitory effect [12]. The treatments were conducted with six replications, and in order to reveal the differences between treatments and control, t-test at α 0.05 levels was performed. A statistical analysis was calculated using software SPSS v. 2

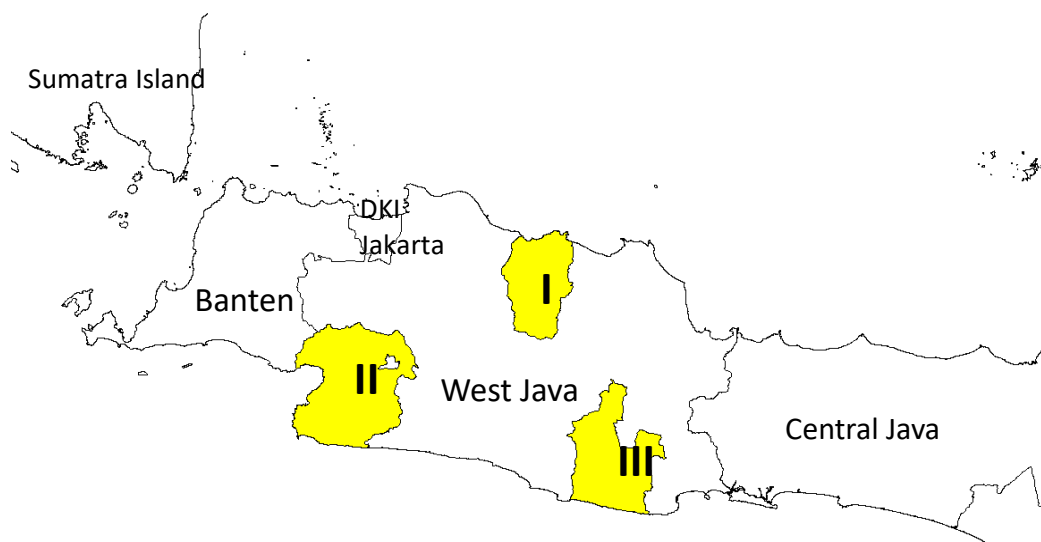


Figure 1. The location of local black rice varieties in West Java Province, Indonesia, I is Subang District, II is Sukabumi District, and III is Tasikmalaya District. The map was created with DIVA-GIS at scale 1: 2000000.

3. Results and Discussion

This study observed the allelopathic potential of local black rice root exudates that originated from West Java, Indonesia. The selected local black rice varieties that originated from different districts of West Java were used against barnyard grass, which was the dominant weed in rice fields. Two local varieties were derived from Subang (SB and SB II), one variety from Sukabumi (SMI), and one variety from Tasikmalaya (TSK). Various tests at germination stage to examine the potential of local black rice against barnyard grass were used in this study including time of 50% germination (T_{50}), germination percentage (GP), vigour index (VI), shoot and root length (SL and RL), and inhibitory percentage (IP), and the results are presented in Table 1 and Figure 2.

The results showed that the various different responses of local black rice against barnyard grass were revealed using these assessments. However, the GP at 7 and 14 days showed no significant difference to control. The speed of germination was revealed by the time of 50% germination (days), and the results showed that three out of four varieties (SB, SB II, and TSK) were significantly different when compared to control. The SB and TSK varieties germinated at 3.37 and 2.38 days, which were faster than the controls at 4.2 and 3.4 days, respectively. However, the SB II variety germinated at 3.65 days, which was slower than the control at 2.53 days, as displayed in Table 1. Interestingly, Interestingly, the germination percentage at 7 and 14 days after sowing showed that there were no significant different compared to control, where the range of GP was 87.78% to 95.56% at 7 days after sowing and 91.11% to 97.78% at 14 days after sowing. However, the SB II local black rice varieties showed negative inhibitory percentage (IP) at 7 and 14 days after sowing, while the other varieties were positive (-0.58% and -1.15%) as presented in Figure 2A. The vigour index of SB (7.83) and SMI (8.91) varieties were higher than the controls, which

Table 1. The response of West Java local black rice against barnyard grass at germination stages

Traits	Treatments	Rice Varieties (Mean \pm Std Err)			
		SB	SB II	SMI	TSK
T ₅₀ (days)	Control	4.16 \pm 0.10	2.53 \pm 0.26	3.40 \pm 0.25	3.44 \pm 0.30
	+BG	3.37 \pm 0.22	3.65 \pm 0.06	3.29 \pm 0.26	2.38 \pm 0.38
	t test	*	*	ns	*
GP Rice 7 (%)	Control	91.75% \pm 0.02	95.00% \pm 0.02	91.67% \pm 0.03	93.33% \pm 0.03
	+BG	90.00% \pm 0.04	95.56% \pm 0.01	87.78% \pm 0.05	90.00% \pm 0.02
	t test	ns	ns	ns	ns
VI Rice 7	Control	4.37 \pm 0.28	8.29 \pm 0.37	5.21 \pm 0.20	9.31 \pm 0.77
	+BG	7.83 \pm 0.57	7.38 \pm 0.26	8.91 \pm 0.51	7.80 \pm 0.40
	t test	*	ns	*	ns
GP Rice 14 (%)	Control	92.96% \pm 0.02	96.67% \pm 0.02	96.67% \pm 0.02	93.15% \pm 0.03
	+BG	91.11% \pm 0.04	97.78% \pm 0.01	93.33% \pm 0.05	94.44% \pm 0.01
	t test	ns	ns	ns	ns
VI Rice 14	Control	11.14 \pm 0.47	14.70 \pm 1.02	20.19 \pm 0.66	20.20 \pm 0.95
	+BG	14.76 \pm 1.01	17.38 \pm 0.63	18.44 \pm 1.05	18.78 \pm 0.73
	t test	*	*	ns	ns
SL 7 (cm)	Control	2.87 \pm 0.13	4.10 \pm 0.10	2.76 \pm 0.14	4.87 \pm 0.48
	+BG	4.71 \pm 0.18	3.81 \pm 0.10	6.21 \pm 0.15	4.71 \pm 0.17
	t test	*	ns	*	ns
SL 14 (cm)	Control	8.49 \pm 0.24	7.30 \pm 0.98	11.13 \pm 0.50	10.76 \pm 0.90
	+BG	11.72 \pm 0.66	12.34 \pm 0.53	14.53 \pm 0.43	15.20 \pm 0.53
	t test	*	*	*	*
SL Δ 714 (cm)	Control	5.62 \pm 0.11	4.75 \pm 1.03	8.37 \pm 0.63	5.88 \pm 0.69
	+BG	7.00 \pm 0.61	8.53 \pm 0.47	8.32 \pm 0.36	10.90 \pm 0.41
	t test	ns	*	ns	*
RL 7 (cm)	Control	1.87 \pm 0.09	4.61 \pm 0.13	2.92 \pm 0.08	5.12 \pm 0.45
	+BG	3.95 \pm 0.26	3.91 \pm 0.19	3.95 \pm 0.20	3.95 \pm 0.26
	t test	*	*	*	*
RL 14 (cm)	Control	3.47 \pm 0.06	8.00 \pm 0.38	9.82 \pm 0.46	10.93 \pm 0.55
	+BG	4.47 \pm 0.24	5.42 \pm 0.33	5.27 \pm 0.23	4.67 \pm 0.34
	t test	*	*	*	*
RL Δ 714 (cm)	Control	1.60 \pm 0.04	3.98 \pm 0.77	6.90 \pm 0.40	5.81 \pm 0.87
	+BG	0.52 \pm 0.24	1.51 \pm 0.35	1.32 \pm 0.36	0.87 \pm 0.40
	t test	*	*	*	*

Note: * indicates significance at 0.05 by t test. SB is Subang, SB II is Subang II, SMI is Sukabumi, and TSK is Tasikmalaya, +BG is the rice placed together with barnyard grass, T₅₀ is time to reach 50% germination, GP is germination of percentage, VI is vigour index, SL is shoot length, RL is root length, and Δ 714 is the deviation of the traits between two times (7 and 14 days after sowing).

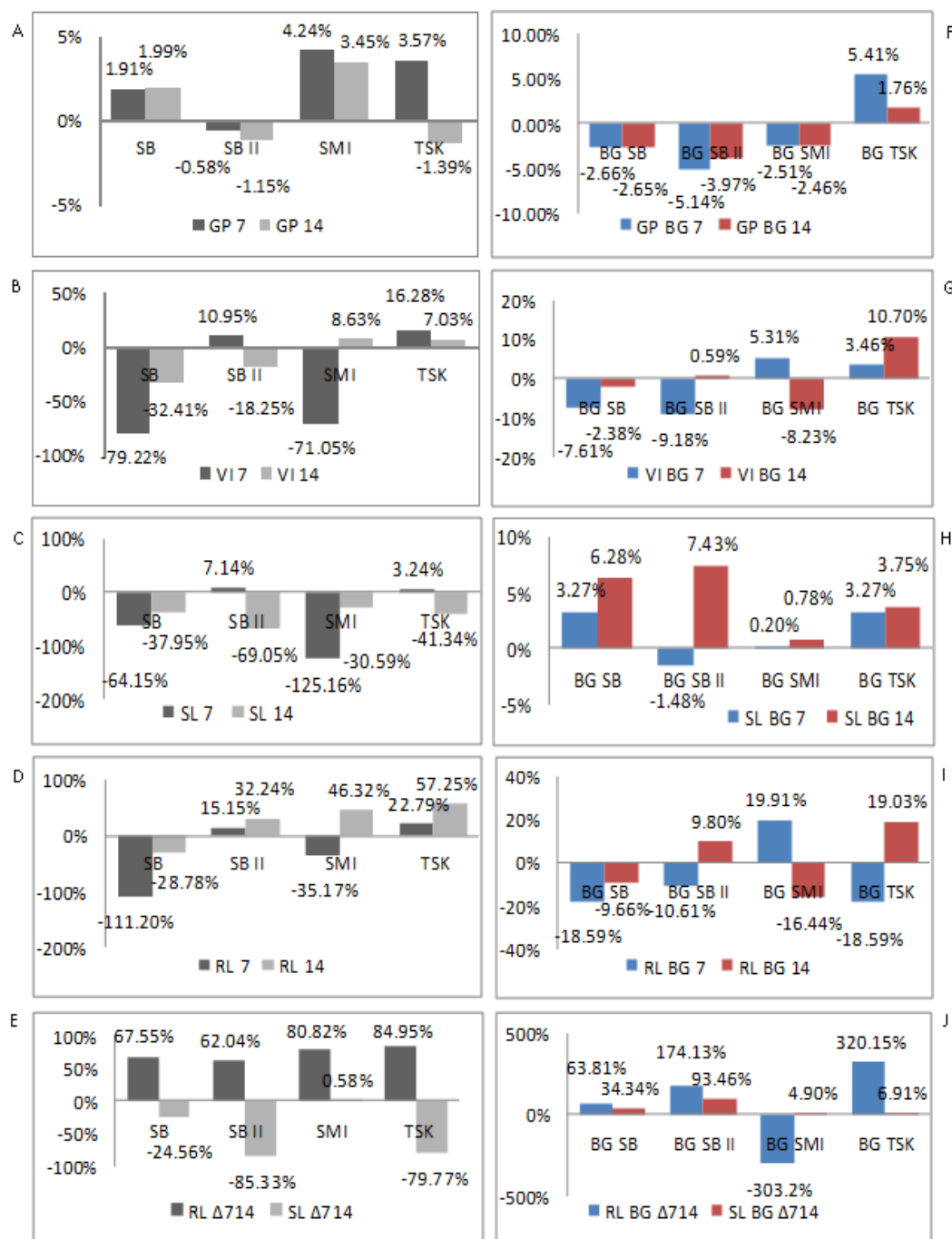


Figure 2. The inhibitory percentage of rice against barnyard grass (A-E) and barnyard grass against rice (F-J) in vigour traits. BG is Barnyard grass, SB is Subang, SB II is Subang II, SMI is Sukabumi, and TSK is Tasikmalaya. The positive values indicated inhibitory effect and negative values indicated stimulatory effect.

were 4.37 and 5.21, respectively, after sowing for 7 days. Moreover, the vigour index of SB (14.76) and SB II (17.38) varieties had a higher value than the controls at 11.14 and 14.70, respectively, after sowing for 14 days as could be seen in Table 1. In Figure 2B, only the SB local black variety showed negative IP at 7 and 14 days after sowing (-79.22% and -32.41%).

Table 1 shows the growth seedling traits, shoot and root length (SL and RL) at 7 to 14 days after sowing. Furthermore, the inhibitory percentages are shown in Figures 2C and 2D. Table 1 shows that based on two observations, the local varieties, SB and SMI had significantly higher SL traits at 4.71 and 6.21 cm length compared to the controls, which were 2.87 and 2.76 cm, respectively. In addition, at day 7 after sowing, the SL traits of these two varieties were 11.72 and 14.53 cm, compared to the controls, which were 8.49 and 11.13 cm respectively at 14 days. The other two local varieties, SB II and TSK showed higher significance in SL traits at 12.34 and 15.20 cm compared to those of the controls, which were 7.30 and 10.76 cm, respectively, at 14 days after sowing. Also, the RL trait of the SB local black rice variety was significantly higher at 3.95 cm than was the control which was 1.87 cm, at the 7 day. While at 14 days, the RL trait of this variety and the control were 4.47 and 3.47 cm, respectively. Thus, the value of inhibitory percentage of the SB variety showed negative value at two observation times, -111.20% and -28.78% respectively. The deviation between two times of SL and RL measurement (SL Δ 714 and RL Δ 714) showed the increase in the growth values. In SL Δ 714, all local black rice varieties showed values higher than control. However, the SB II and TSK varieties showed significantly higher values compared to those of control in SL Δ 714 traits. The SL Δ 714 traits of the SB II and TSK varieties were 8.53 and 10.90 cm, respectively. These values were higher than the controls of these varieties, which were 4.75 and 5.88 cm, respectively. Besides, these two local black rice varieties had negative values of more than 50%, at -85.3% and -79.8%, respectively. Furthermore, the controls showed significantly higher than all local varieties for the RL Δ 714 trait, as the values of the trait were 0.52 cm for SB, 1.51 cm for SB II, 1.32 cm for SMI and 0.87 cm for TSK. Meanwhile, for the controls, they were 1.60, 3.98, 6.90, and 5.81 cm, respectively. Thus, there were no negative values in the inhibitory percentage (IP) for all varieties (Figure 2E).

The local black rice varieties provided different allelopathic potential on barnyard grass at germination stage. A comparison of the responses of barnyard grass between the control (without rice) and barnyard grass sowed with rice showed inhibitory and/or stimulatory effects on barnyard grass at germination stage (Table 2 and Figures 2F to 2J). The local black rice originated from Subang (SB II) showed significant stimulatory effects on barnyard grass T_{50} . The barnyard grass reached 50% at 1.04 days after sowing compared to control at 1.38 days, while the other local black varieties had no stimulatory and/or inhibitory effects on T_{50} of barnyard grass. The germination percentage (GP) of barnyard grass was not affected by the presence of local black rice (Table 2). However, inhibitory percentage (IP) of GP at 7 and 14 days after sowing showed that the barnyard grass was inhibited by the presence of the local black rice from Tasikmalaya (5.41% and 1.76%), while the other varieties caused stimulatory effects on GP of barnyard grass at 7 and 14 days after sowing (Figure 2F). Furthermore, the vigour index (VI) of TSK variety during observation at 7 and 14 days had values of 8.75 and 9.08 (Table 2). It also significantly inhibited barnyard grass VI (9.06 and 10.17) at 7 and 14 days after sowing, respectively. The IP of the TSK variety at 7 and 14 days after sowing showed that the TSK variety could inhibit barnyard grass VI (3.46% and 10.7%) as presented in Figure 2G. Furthermore, the shoot and root length (SL and RL) were used to analyze the seedling growth traits of barnyard grass at 7 and 14 days after sowing, as shown in Table 2. The result showed that the growth of barnyard grass SL (6.07 cm) was inhibited in the presence of the SB and TSK varieties at 7 days after sowing, while, the control was 6.28 cm. In addition, the SL values of barnyard grass were lower than control when grown together with the SB, SB II, and TSK varieties, being 6.49 cm, 6.41 cm, and 6.67 cm, respectively, while the control was 6.93 cm. Furthermore, the inhibitory percentages of SB variety on the SL of barnyard grass at 7 and 14 days after sowing were 3.27% and 6.28%, while the SL of the TSK variety were 3.27% and 3.75%, as

shown in Figure 2H. For the root length trait, the SMI variety could inhibit the RL of barnyard grass at 7 days after sowing. This could be seen from results which showed that the RL of the grass was 3.0 cm when grown together with the SMI variety, while the control was 3.74 cm (Table 2). However, the other varieties stimulated the RL of the grass.

The SB II and TSK varieties of the local black rice showed inhibition effect on the RL trait of barnyard grass, compared to control after 14 days of sowing. This is shown in Table 2 and Figure 2I, where the RL traits of the varieties were 3.79 and 3.40 cm, while that of the control was 4.20 cm. These results were also shown by the IP, where the SB II and TSK local black rice varieties showed inhibitory effects of 9.80 and 19.03% on the RL of barnyard grass at 14 days after sowing. Furthermore, the deviation of the SL from the two times measurements (SL Δ 714) of barnyard grass that was planted with local black rice of SB II (0.04 cm) was the lowest among the effect of other varieties on this trait, compared to the control (0.64 cm) and the IP of SB II local black rice variety on this trait was 93.46%. The TSK local black rice variety gave the lowest value on RL Δ 714 of barnyard grass (-1.03 cm) and the highest positive value of inhibitory percentage (320.15%) which indicated higher stimulatory effect as displayed in Table 2 and Figure 2J.

According to the results, the exploration of local rice potential in Indonesia is limited in breeding programs particularly for improving local black rice. Local rice has been reported to have genetic diversity and adaptability to various environmental conditions [39]. Several researchers reported that black rice varieties have the potential to release allelopathic compounds that can inhibit the growth of other plants including weeds and increase the competitiveness of rice plants [7, 12]. This study reveals that the local black rice varieties originated in West Java have allelopathic potential which is characterized by inhibitory effects on the germination and growth of barnyard grass at germination stage. By using dendrogram obtained from Ward's method based on inhibitory percentage, it showed that the presence of barnyard grass stimulated the growth of the local black rice variety that originated from Subang (SB II) and at the same time this variety inhibited the growth of barnyard grass as revealed by the inhibitory percentage of vigour traits at germination stage (dendrogram not shown).

Various biotic or abiotic factors may play roles in the stimulation of releasing allelochemicals in rice. Barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) is one of the dominant weeds in Indonesia rice field that interrupts every stage of growth and development in rice [40]. Barnyard grass has previously been shown to reveal the allelopathic potential of rice [13]. The advantages of the use allelopathic varieties are to avoid the negative effects of weeds in field and to use as breeding material for breeders [40-42]. Various inhibitory effects of rice varieties on barnyard grass at germination stage has been presented in this study that may reflect the allelopathic potential of the rice varieties and the results are in agreement with numerous studies [5, 6, 43, 44]. However, most of the previous studies examined the allelopathic effect of rice on barnyard grass or the allelopathic effect of barnyard grass on rice, and there were limited studies that observed this relationship at both sides. Thus, this study examined the effect of rice on barnyard grass and barnyard grass on rice in order to determine which varieties are useful for breeding materials and also to provide information on various responses of each rice variety against barnyard grass at germination stage. In this study, the presence of barnyard grass stimulated one TSK local black rice variety in T50, while the other varieties were inhibited by the presence of barnyard grass. However, the TSK local black rice variety did not inhibit the T50 of barnyard grass and the SMI local black rice variety stimulated the T50 of barnyard grass. This indicated that there was a different plant defense to the presence of allelochemicals that were released by barnyard grass in the early stage of germination. Also, barnyard grass responded to the presence of rice in this trait by accelerating germination. This result was in agreement with Zhang *et al.* [44] which reported that barnyard grass could recognize the presence of rice. This phenomenon occurred at every stage of germination, and was more pronounced in the more specific vigour traits such as in shoot and root length, at two times of observation.

Table 2. The response of barnyard grass against West Java local black rice at germination stages

Traits	Control	Barnyard grass + Rice Varieties (Mean \pm Std Err)			
		BG SB	BG SB II	BG SMI	BG TSK
T ₅₀ (days)	1.38 \pm 0.03 a	1.20 \pm 0.04 ab	1.38 \pm 0.02 a	1.04 \pm 0.12 b	1.29 \pm 0.05 a
GP BG 7 (%)	90.0 \pm 0.02 a	93.0 \pm 0.02 a	95.0 \pm 0.02 a	93.0 \pm 0.03 a	85.0 \pm 0.03 a
VI BG 7	9.06 \pm 0.31 a	9.75 \pm 0.23 a	9.89 \pm 0.24 a	8.58 \pm 0.28 a	8.75 \pm 0.14 a
GP BG 14 (%)	91.0 \pm 0.03 a	94.0 \pm 0.01 a	95.0 \pm 0.01 a	94.0 \pm 0.02 a	90.0 \pm 0.01 a
VI BG 14	10.17 \pm 0.27 b	10.41 \pm 0.21 ab	10.11 \pm 0.18 b	11.01 \pm 0.32 a	9.08 \pm 0.21 c
SL 7 (cm)	6.28 \pm 0.11 ab	6.07 \pm 0.10 b	6.37 \pm 0.06 a	6.27 \pm 0.09 ab	6.07 \pm 0.10 b
SL 14 (cm)	6.93 \pm 0.10 a	6.49 \pm 0.13 b	6.41 \pm 0.07 b	6.88 \pm 0.14 a	6.67 \pm 0.10 ab
SL Δ 714 (cm)	0.64 \pm 0.09 a	0.42 \pm 0.12 ab	0.04 \pm 0.09 b	0.61 \pm 0.16 a	0.60 \pm 0.18 a
RL 7 (cm)	3.74 \pm 0.10 b	4.44 \pm 0.12 a	4.14 \pm 0.10 a	3.00 \pm 0.11 c	4.44 \pm 0.12 a
RL 14 (cm)	4.20 \pm 0.09 bc	4.61 \pm 0.09 ab	3.79 \pm 0.25 cd	4.89 \pm 0.15 a	3.40 \pm 0.23 d
RL Δ 714 (cm)	0.47 \pm 0.13 b	0.17 \pm 0.14 bc	-0.35 \pm 0.26 c	1.90 \pm 0.22 a	-1.03 \pm 0.24 d

Note: Means followed by a different letter are indicated significant at 0.05 by t test. SB is Subang, SB II is Subang II, SMI is Sukabumi, and TSK is Tasikmalaya, BG initial rice is the barnyard grass placed together with rice, T₅₀ is time to reach 50% germination, GP is germination of percentage, VI is vigour index, SL is shoot length, RL is root length, and Δ 714 is the deviation of the traits between two times (7 and 14 days after sowing)

The rice and barnyard grass reached the maximum germination percentage (GP) as shown by value of GP above 80%. However, the vigour index (VI), shoot and root length (SL and RL) revealed that there were different responses of rice and barnyard grass when they were placed together. These indicated that need for more specific traits to discover the allelopathy of both plants. Xuan *et al.* [7] reported that the root exudates released by barnyard grass possessed phytotoxic effect on root and shoot length of rice. However, this study revealed that the presence of barnyard grass may stimulate the shoot and root growth of rice during germination stage. Similar results have been reported by Khoo *et al.* [5] that the reactions of allelopathic rice in the presence of barnyard grass increased shoot and root length. As presented in this study, the local black rice variety originated from Subang (SB II) underwent recovery during 7 to 14 days after sowing. The germination percentage (GP), vigour index (VI), and shoot length (SL) of SB II variety were stimulated by the presence of barnyard grass after sowing for 14 days. Additionally, during the recovery of SB II

growth, this variety showed an inhibitory effect on the growth of barnyard grass as displayed by positive values of inhibitory percentage in the VI, SL, and RL traits of barnyard grass.

These results were consistent with a recent study which showed that allelopathic rice influenced the plant architecture of barnyard grass [44]. This indicated that this rice had a different defense mechanism from the other varieties; one in which the variety struck back at the competitor, probably by releasing an allelochemical. This phenomenon is called “counter-offensive defense mechanism”. This mechanism probably occurs by two factors. The first factor is that the level of allelopathic effect of barnyard grass on this variety did not interfere in the beginning stage. The second factor is that allelochemical compounds arose more than released by this rice variety at later germination stage. However, the actual mechanisms in terms of the metabolism of the allelopathic interactions between these two species remain unclear and further research is needed. According to these results, the selection of rice varieties with allelopathic effects gave promising results in early stages of plant growth (germination stage), and this was indicated by the morphological traits observed during the seedling stage, through the vigour index, shoot and root length, and inhibitory percentage. These traits were useful in rice breeding programs, particularly in the effort to release new allelopathic rice varieties. As suggested by Kim and Shin [45], the identification of allelopathy effects should precede efforts to reveal allelopathy genes, knowledge of which can be used to improve cultivars by conventional or modern techniques of breeding.

4. Conclusions

The allelopathic potential of local black rice originated from West Java, Indonesia against barnyard grass varies as indicated by vigour traits such as T_{50} , germination percentage, vigour index, shoot and root length and inhibitory percentage. In the early (7 DAS) and the second germination stages (14 DAS), it could be seen that several varieties were inhibited and stimulated by barnyard grass presence. Furthermore, the inhibitory percentage showed that barnyard grass was inhibited by rice presence. The Subang (SB II) variety displayed recovery mechanism that occurred in the second stage of germination, whilst it had shown the sign of suppressed in the early germination stage as revealed by inhibitory percentage in several vigour traits. These results unveiled that rice allelopathy may increase as part of the recovery mechanism. Besides, the vigour index and shoot length that also reflected by the inhibitory percentage in these two traits were indicated as useful traits for allelopathic rice breeding program. These findings suggest that an allelopathic rice variety that contains a richness of allelopathic compounds and can inhibit the growth of barnyard grass will be, in the future, one part of an environmentally-sound weed management program used by farmers. Furthermore, further study is needed to identify the physiological mechanisms of the release of allelochemicals from root exudates in rice and barnyard grass and also to obtain the right concentration of allelochemicals that could stimulate and inhibit the interactions of these species at several growth stages.

5. Acknowledgements

The authors are grateful to Universitas Padjadjaran for funding this work through HIU (internal grant of UNPAD).

References

- [1] FAO, 2018. *Supply and Demand World Rice*. [online] Available at: <https://app.amis-outlook.org/#/market-database/supply-and-demand-overview>
- [2] Sawe, B.E., 2019. *World Atlas Top 10 Rice Consuming Countries 2019* [online] Available at: <https://www.worldatlas.com/articles/top-10-rice-consuming-countries.html>
- [3] Kushwaha, U.K.S., 2016. *Black Rice: Research, History and Development*. Geneva: Springer.
- [4] Hao, J., Zhu, H., Zhang, Z., Yang, S. and Li, H., 2015. Identification of anthocyanin in black rice (*Oryza sativa* L.) by UPLC/Q-TOF-MS and their *in vitro* and *in vivo* antioxidant activities. *Journal Cereal Science*, 64, 92-99.
- [5] Khoo, H.E., Azlan, A., Tang, S.T., and Lim, S.M., 2017. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research*, 61(1), 1361779, <https://doi.org/10.1080/16546628.2017.1361779>
- [6] Kato-Noguchi, H., Nitta, K. and Itani, T., 2013. Allelopathic potential of white, red and black rice cultivars. *Plant Production Science*, 16, 305-308.
- [7] Xuan, T.D., Chung, I.M., Khanh, T.D. and Tawata, S., 2006. Identification of phytotoxic substances from early growth of barnyard grass (*Echinochloa crus-galli*) root exudates. *Journal of Chemical Ecology*, 32, 895-906.
- [8] Kropff, M.J. and Walter, H., 2000. EWRS and the challenges for weed research at the start of a new millennium. *Weed Research*, 40, 7-10.
- [9] Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P. and Hens, L., 2016. Chemical pesticides and human health: The urgent need for a new concept in agriculture. *Frontiers in public health*, 4, 148, <https://doi.org/10.3389/fpubh.2016.00148>
- [10] Lushchak, V.I., Matviishyn, T.M., Husak, V.V., Storey, J.M. and Storey, K.B., 2018. Pesticide toxicity. A mechanistic approach. *Experimental and Clinical Science Journal*, 17, 1101-1136.
- [11] Chung, I.M., Kim, J.T. and Kim, S.H., 2006. Evaluation of allelopathic potential and quantification of momilactone A, B from rice hull extracts and assessment of inhibitory bioactivity on paddy field weeds. *Journal of Agricultural and Food Chemistry*, 54, 2527-2536.
- [12] Kong, C.H., Xuan, T.D., Khanh, T.D., Tran, H.D., and Trung, N.T., 2019. Allelochemicals and signaling chemicals in plants. *Molecules*, 24, 2737, <https://doi.org/10.3390/molecules24152737>
- [13] Khanh, T.D., Cong, L.C., Chung, I.M., Xuan, T.D. and Tawata, S., 2008. Variation of weed-suppressing potential of Vietnamese rice cultivars against barnyard grass (*Echinochloa crus-galli*) in laboratory, greenhouse and field screenings. *Journal of Plant Interactions*, 4, 209-218.
- [14] Li, Y., Xu, L., Letuma, P. and Lin, W., 2020. Metabolite profiling of rhizosphere soil of different allelopathic potential rice accessions. *BMC Plant Biology*, 20, 265, <https://doi.org/10.1186/s12870-020-02465-6>.
- [15] Olofsdotter, M., Jensen, L.B. and Courtois, B., 2002. Improving crop competitive ability using allelopathy -An example from rice. *Plant Breeding*, 121, 1-9.
- [16] Duke, S.O., Scheffler, B.E., Dayan, F.E., Weston, L.A. and Ota, E., 2001. Strategies for using transgenes to produce allelopathic crops. *Weed Technology*, 15, 826-834.
- [17] Gealy, D.R., Estorninos, L.E., Gbur, E.E. and Chavez, C.R.S., 2005. Interference interactions of two rice cultivars and their F3 cross with barnyard grass (*Echinochloa crus-galli*) in a replacement series study. *Weed Science*, 53, 323-330.
- [18] Olofsdotter, M., 2001. Getting closer to breeding for competitive ability and the role of allelopathy—an example from rice (*Oryza sativa*). *Weed Technology*, 15, 798-806.

- [19] Khang, D.T., Anh, L.H., Thu, H.P.T., Tuyen, P.T., Quan, N.V., Minh, L.T., Quan, N.T., Minh, T.N., Xuan, T.D., Khanh, T.D and Trung, K.H., 2016. Allelopathic activity of dehulled rice and its allelochemicals on weed germination. *International Letters of Natural Sciences*, 58, 1-10.
- [20] Kato-Noguchi, H., 2011. Barnyard grass-induced rice allelopathy and momilactone B. *Journal of Plant Physiology*, 168, 1016-1020.
- [21] Kim, S.Y., Madrid, A.V., Park, S.T., Yang, S.J. and Olofsdotter, M., 2005 Evaluation of rice allelopathy in hydroponics. *Weed Research*, 45, 74-79.
- [22] Rao, A.N., Johnson, D.E., Sivaprasad, B., Ladha, J.K. and Mortimer, A.M. 2007. Weed management in direct-seeded rice. *Advances in Agronomy*, 93, 153-255.
- [23] Heidarzade, A., Esmaili, M. and Pirdashti, H., 2012. Common allelochemicals in root exudates of Barnyard grass (*Echinochloa crusgalli* L.) and inhibitory potential against rice (*Oryza sativa*) cultivars. *International Research Journal of Applied and Basic Science*, 3, 11-17.
- [24] Gealy, D.R., Rohila, J.S. and Boykin, D.L., 2019. Genetic potential of rice under alternate-wetting-and-drying irrigation management for barnyard grass (*Echinochloa crus-galli*) suppression and grain yield production. *Weed Science*, 67, 453-462.
- [25] Seal, A.N., Pratley, J.E. and Haig, T.J., 2005. Evaluation of rice varieties for allelopathic effects on Australian rice weeds-linking laboratory to field. *Proceedings of 4th World Congress on Allelopathy*, Wagga Wagga, New South Wales, Australia, 21-26 August 2005, 164-167.
- [26] Wu, H., Pratley, J. and Lemerle, D., 2001. Screening methods for the evaluation of crop allelopathic potential. *The Botanical Review*, 67, 403-415.
- [27] Olofsdotter, M., Navarez, D., Rebulanan, M. and Streibig, J.C., 1999. Weed-suppressing rice cultivars - Does allelopathy play a role? *Weed Research*, 39, 441-474.
- [28] Berendji, S., Asghari, J. and Matin, A.A., 2008. Allelopathic potential of rice (*Oryza sativa*) varieties on seedling growth of barnyard grass (*Echinochloa crus-galli*). *Journal of Plant Interactions*, 3, 175-180.
- [29] Ventura, L., Dona, M., Macovei, A., Carbonera, D., Buttafava, A. and Mondoni, A., 2012. Understanding the molecular pathways associated with seed vigour. *Plant Physiology and Biochemistry*, 60, 196-206.
- [30] Munamava, M.R., Goggi, A.S. and Pollak, L., 2004. Seed quality of maize inbred lines with different composition and genetic backgrounds. *Crop Science*, 44, 542-548.
- [31] Goggi, A.S., Caragea, P., Pollak, L., McAndrews, G., DeVries, M. and Montgomery, K., 2008. Seed quality assurance in maize breeding programs: Tests to explain variations in maize inbreds and populations. *Agronomy Journal*, 100(2), 337-343.
- [32] Helena, E., 2019. *Evaluasi Vigor Benih Padi Hitam (Oryza sativa L.) Lokal Jawa Barat Pada Beberapa Taraf Konsentrasi Eksudat Akar Gulma Echinochloa crus-galli (L.) Beauv.* BSc. Universitas Padjadjaran. (Indonesian)
- [33] Ma, Y., Zhang, M., Li, Y., Shui, J. and Zhou, Y., 2014. Allelopathy of rice (*Oryza sativa* L.) roots exudates and its relations with *Orobanche cumana* Wallr. and *Orobanche minor* Sm. germination, *Journal of Plant Interactions*, 9, 722-730
- [34] International Seed Testing Association, 1999. International rules for seed testing. Rules 1999. *Seed Science and Technology*, 27 (Suppl.), 1-288.
- [35] Khanh, T.D., Cong, L.C., Chung, I.M., Xuan, T.D. and Tawata, S., 2009. Variation of weed-suppressing potential of Vietnamese rice cultivars against barnyard grass (*Echinochloa crus-galli*) in laboratory, greenhouse and field screenings. *Journal of Plant Interactions*, 4, 209-218.
- [36] Coolbear, P., Francis, A. and Grierson, D., 1984. The effect of low temperature pre-sowing treatment under the germination performance and membrane integrity of artificially aged tomato seeds. *Journal of Experimental Botany*, 35, 1609-1617.

- [37] Farooq, M., Basra, S.M., Ahmad, N. and Hafeez, K., 2005. Thermal hardening: A new seed vigour enhancement tool in rice. *Journal of Integrative Plant Biology*, 47, 187-193.
- [38] Abdul-Baki, A.A. and Anderson, J.D., 1973. Vigour determination in soybean seed by multiple criteria. *Crop Science*, 13, 630-633.
- [39] Muhamad, K., Ebana, K., Fukuoka, S. and Okuno, K., 2015. Diversity and differentiation of *Oryza sativa* and *O. rufipogon* in Indonesia. *Genetic Resources and Crop Evolution*, 64, 41-54.
- [40] Usman, P., B.S., Syukur, M. and Guntoro, D., 2016. Toleransi Galur Harapan Padi Sawah (*Oryza sativa* L.) pada Persaingan dengan Gulma *Echinochloa crus-galli*. *Jurnal Agronomi Indonesia*, 44, 111-118. (in Indonesian)
- [41] Khanh, T.D., Xuan, T.D. and Chung, I.M., 2007. Rice allelopathy and the possibility for weed management, *Annals of Applied Biology*, 151, 325-339.
- [42] Kong, C.H., Chen, X.H., Hu, F. and Zhang, S.Z., 2011. Breeding of commercially acceptable allelopathic rice cultivars in China. *Pest Management Science*, 67(9), 1100-1106.
- [43] Ahn, J.K. and Chung, I.M., 2000. Allelopathic potential of rice hulls on germination and seedling growth of barnyardgrass. *Agronomy Journal*, 92(6), 1162-1167.
- [44] Zhang, T., Fan, B. and Wang, P., 2018. Barnyardgrass root recognition behaviour for rice allelopathy. *Agronomy*, 8(4), 39, <https://doi.org/10.3390/agronomy8040039>
- [45] Kim, K.-U. and Shin, D.-H., 2008. Progress and prospect of rice allelopathy research. In: R.S. Zeng, A.U. Mallik and S.M. Luo, eds. *Allelopathy in Sustainable Agriculture and Forestry*. New York: Springer, pp. 189-213.