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Prof. Dr. Amir Hamzah Soemintapoera, Ir.
Dr. Agung Karuniawan

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BIOLOGY AND ECOLOGY OF WEED

ALLELOPATHY IN RICE : SEARCHING FOR DNA OF RICE GENOTYPES TOLERANT TO BARNYARD GRASS

Irawati Chaniago* and Jamsari

Department of Agroecotechnology, Agricultural Faculty, Andalas University, Padang 25163

* Corresponding author : e-mail : ichaniago@faperta.unand.ac.id

ABSTRACT

Barnyard grass (*Echinochloa crus-galli* L. (Beauv.)) is one of important weeds associated with rice and causes the reduction growth and yield of rice. Preliminary studies have been carried out in searching for rice genotype potential for controlling the early growth of the weed through allelopathic mechanisms. The studies were aimed at isolating and characterizing DNA of rice genotypes with allelopathic potentials. Three series of experiments were set. The first was bioassay of rice and barnyard grass using modified relay seeding technique. One hundred genotypes of rice seeds were germinated three days prior to weed seeds in 10-cm diameter Petri dishes. Data were collected at fourteen days after the seeding of the weed. The rice genotypes resulted in $\geq 50\%$ suppression of weed growth were used at the second stage of the research. At this stage, the rice genotypes were germinated together with the weed seeds for measuring the activity of peroxides enzyme. Finally, the rice genotypes 'tolerant' and the ones 'not-tolerant' to the weed would have been subjected to the isolation and characterization of DNA. Results indicate that two genotypes (Cempo lutut and Badik/Gadiah kabalai) were 'most tolerant' to barnyard grass. In contrast, the genotypes of Pulu palappa and Harapan were found to be most intolerant to the weed. There is no difference in the activity of peroxides enzyme between the four genotypes. DNA characterizations demonstrate differences between 'tolerant' rice genotypes and 'intolerant' ones.

Key words : allelopathy, rice, barnyard grass, DNA, peroxides enzyme

INTRODUCTION

Pressures on agriculture, caused by the reduced amounts of arable land, and the impact of pests, diseases and weeds have prompted people to find appropriate methods to maximize agricultural production. Sustainable agriculture aims for long-term maintenance of natural resources and agricultural productivity with minimal adverse impact on the environment (Narwal, 1999). This will help in achieving the long term goals of increasing and sustaining agricultural yield while causing minimum environmental damages.

Weeds have always been recognized as one of the major problem in agricultural practices. The problems of weeds has been as old as the history of agricultural practice itself and will always be as long as crops being cultivated to feed the world population. Weed

definition varies among weed scientist, the simplest of which is 'a plant growing out of place'. A more precise definition might reflect the economic losses resulting from weed problems in agriculture. In this paper, we refer to weeds as plants that unusually persistent and pernicious, that significantly interfere with the growth of crops, and are optimally adapted to agro ecosystems (Ross and Lembi, 1999). Therefore, weed management is considered to be a normal and necessary work for farmers.

Weed control, mostly by herbicide application, is one of the key management factors of most agricultural systems. However, this practice has resulted in increasing herbicide resistance in weeds (Foes et al., 1998; Tranel et al., 2004) and widespread concern about the environmental side effects of herbicides. Herbicides have resulted in a range of effects on fauna and flora living on or close to farmland (Cooke and Burn, 1995). Increasing herbicide resistance in certain weeds, rising costs, and widespread concerns about the environmental effects have resulted in great pressure to reduce the use of commercial herbicides (Swanton and Murphy, 1996). Future weed control practices must therefore eliminate or at least minimize the herbicide use and if possible, utilize other practices for weed management

In rice cultivation, resource competition may occur between rice and weeds which reduces yield up to 82% (Solfiyeni and Setiawati, 2003). The competition between weeds and rice may result from similarities in growth habit, similarities in resource requirements, and similarities in their niches (Soerjani *et al.*, 1987). In addition to resource competition, weeds may release secondary metabolites (allelochemicals) to the environment and may influence the growth and development of agricultural and biological systems (Narwal, 1999).

Barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) is an important weed in rice cultivation and has been one of the worst weeds in the world. When allowed growing in association with rice, this weed may result in reduction of yield for as much as 90% (Kwesi et al., 1991). Barnyard grass produces abundant seeds per plant. The seeds are major mean of dispersal of this species and therefore have been important to maintain its existence (Kim and Park, 1996). The seeds may persist in the soil for three years.

Weed control, mostly by herbicide application, is one of the key management factors of most agricultural systems. However, this practice has resulted in increasing herbicide resistance in weeds (Foes *et al.*, 1998; Tranel *et al.*, 2004) and widespread concern about the environmental side effects of herbicides. Herbicides have resulted in a range of effects on fauna and flora living on or close to farmland (Cooke and Burn, 1995). Therefore, alternatives to chemical herbicides have been sought.

Allelochemicals may provide an opportunity to address such problems owing to their potential as natural herbicides. Some allelochemicals have shown great potential for use as natural herbicides. These include artimisinin (Lydon *et al.*, 1997), phenolic and hydroxamic acids (Reigosa *et al.*, 2001), allelochemicals from rye mulch (Nagabhushana *et al.*, 2001), and microbial-derived allelochemicals. Extensive studies have resulted in the isolation and identification of several hundred allelopathic compounds, e.g. phenolic acids (Wu *et al.*, 2001), sesquiterpene lactones (Macias *et al.*, 1999) and cyan amide (Kamo *et al.*, 2003). These compounds arise from many chemical classes and exhibit a large array of structural complexities (Einhellig, 1995). Therefore, it is unrealistic to expect that they may have a common mode of action

Einhellig and Leather (1988) proposed that strategies capitalizing on allelopathy might help in efforts towards this ideal of a more sustainable agriculture. Researchers in the South Korea have tried to find rice genotypes that were able to suppress the growth of barnyard grass. They tested 100 rice genotypes and reported that rice husk were more potential in suppressing barnyard grass growth compared to the terrestrial parts of the rice plants. Rice straw has also reduced the growth of barnyard grass (Ahn dan Chung, 2000; Chung *et al.*, 2003). However, similar work using rice genotype originated from Indonesia has not been widely reported. Research on rice allelopathy has been commenced in 1980s in the USA. Rice plants produced and released secondary metabolites (allelochemicals) able to reduce growth of barnyard grass (Chung *et al.*, 2001; Rimando *et al.*, 2001) and some other species.

In this article, we report on our efforts in searching for rice genotype having potentials to suppress the early growth of barnyard grass. The so-called 'tolerant' rice genotype was subjected to DNA isolation and characterization to further explain the phenomenon of growth suppression.

MATERIALS AND METHODS

Genotype screening through bioassay

The experiments were carried out at the Laboratory of Plant Physiology and Laboratory of Plant Biotechnology & Genetic Molecular of Agricultural Faculty, Andalas University, Padang in 2008. One hundred rice genotypes were obtained from Balai Besar Penelitian dan Pengembangan Bioteknologi & Sumberdaya Genetik Pertanian (Balitbiogen) Bogor. Rice seeds were surface sterilized by immersion in 70% aqueous ethanol solution for 1 minute

followed by washing in 1% (w/v) of sodium hypochlorite (NaOCl) solution for 15 minutes. The source of the NaOCl was commercial bleach (Bayclean™). Sterilized seeds were rinsed with sterile deionizer water 4 times prior to germination. Twenty rice seeds were germinated on 1 layer of Whatman No. 1 filter paper in a 9 cm diameter Petri dish, and supplemented with 10 mL of distilled water. The dishes were placed in an incubation room with 12 hour light and temperature of 25°C. Three days later, ten rice seedlings were left at the dish and 10 seeds of barnyard grass were placed side by side with the rice. To keep the seedling grow upright, 30 g of sterile sand was added to the dish. Five mL of distilled water was added to each dish every other day to keep the moisture. Two weeks after the placement of the weed seeds, the plants were harvested for data collection including shoot and root length, shoot and root fresh and dry weight). Rice genotypes showing $\geq 50\%$ of weed growth suppression was then subjected the next step of screening for the activity of peroxides enzyme.

Determination of peroxides enzyme - POD

Rice genotypes showing $\geq 50\%$ of weed growth suppression were germinated following similar procedures described above and were subjected to extraction and analysis of the activity of peroxides enzyme. The extraction and analysis of POD enzyme was conducted following the method of Dodd (1996) and Ng *et al.* (2003).

DNA Isolation and Characterization

Two genotype of rice most 'tolerant' (suppressed weed growth) and most 'intolerant' (least suppression to the weed growth) were subjected to DNA isolation and characterization. Rice seeds were surface sterilized as mentioned above prior to germination in Petri dish for 14 days. Isolation and characterization of DNA were carried out following 'modified' CTAB extraction protocol (Porebski *et al.*, 1997). Selection of suitable primer was conducted as two consecutive steps. The first selection was aimed at finding primer produce more than 5 DNA fragment. The second stage of primer selection was to select individual through DNA pool 'tolerant' and 'intolerant' to the barnyard grass. At this stage, we may determine whether polymorphism occurred or not.

RESULTS AND DISCUSSION

Different responses of barnyard grass were observed at different rice genotype. Rice genotypes of Cempo lutut, Badik/Gadiah kabalai, Komas B, dan Soegon resulted in the suppression of root length of barnyard grass as much as 60.35; 58.09; 52.96; and 50.91%,

consecutively. However, shoot growth and dry weight of the weed were not significantly affected by the rice tested. None of the rice genotype reduced shoot growth and dry weight accumulation for $\geq 50\%$. These indicated that the weed growth suppression happened for the roots but not the shoots. In this experiment, the roots of rice and weeds grew together at the same media simultaneously; therefore, root exudates responsible for allelopathic interference affected roots via direct contact in the growth medium. This phenomenon could be explained through analogy of the activity of phytohormones through which allelochemicals, secondary metabolites, may either promote or reduce the growth of target species depending on various factors such as concentration (Koitabashi, *et al.*, 1997).

Root growth reduction for $\geq 50\%$ observed in barnyard grass demonstrated that root growth was determined more by cell elongation following cell division. This can further explained why root dry weight of the weed was not reduced for more than 50%. It is assumed that secondary metabolites from rice suppressed lateral growth of weed roots. Chaniago (2006) reported that rice cv. Cisokan reduced root cell mitotic index of barnyard grass for 54.88%. This shows that the rice genotype may reduce weed growth through the release of allelochemicals.

Extraction and analysis of POD enzyme from barnyard grass grown together with rice genotypes of Cempo lutut, Badik/Gaduh kabalai, Komas B, dan Soegon has been conducted to further explain the mechanism of growth suppression of weeds by rice allelopathy. Data demonstrated that the activity of POD enzyme was not affected (**Table 1**). As the activity of POD enzyme was not affected by rice, it is assumed that the weed responded to rice allelopathy through other physiological mechanisms. Peroxides enzyme is known for its roles in response to environmental stress (Sanchez *et al.*, 1996) as well as catalyzing the oxidation of some phenolic compounds. However, this experiment did not reveal such mechanisms in all treatments. This result is in accordance with another work with canola (*Brassica napus* L.). ferulic acid did not affected POD enzyme in cotyledons of canola. In contrast, cinnamic and benzoic acids at 1.0 mM increased the activity of POD enzyme in cotyledon as well as reduced the germination of canola (Ng *et al.*, 2003). The activity of POD enzyme was also reported to be increased in soybean germination following an increase of aqueous extract of Powell's amaranth and nut grass from 10 to 40% (Chaniago, 2004).

Isolation and characterization of DNA has been conducted from two rice genotypes 'tolerant' (Cempo lutut and Badik/Gaduh kabalai) and two 'intolerant' (Pulu palappa and Harapan). Results demonstrated different pattern of distribution of DNA in each genotypes. We found two primers showing polymorphic strands of DNA. We found 5 primers causing

polymorphic fragments of DNA pool of 'tolerant' rice genotypes (**Table 2**). Subsequent analysis shows that primer OPN-16 only resulted in specific DNA fragment from rice genotypes Cempo lutut and Badik/Gaduh kabalai (the 'tolerant' genotypes). The size of the fragments was 800 bp obtained by comparing them to the marker of 1 kb ladder from Promega (**Figure 1**). This finding is promising and in accordance with future direction of allelopathic studies using molecular biology to explain the phenomenon (Dayan *et al.*, 2000). Researchers have been focusing on bioassay aspects of allelopathic studies (Bruckner, 1998) and need to redirect their orientation to biotechnology. Dendogram showing genetic relatedness between barnyard grass and the four rice genotypes is presented in **Figure 2**.

Results of this study demonstrate differences in DNA strands in rice genotypes adding to the explanation on how the rice suppressed the early growth of barnyard grass. However, it is too early to jump to such conclusion. Further works is needed on more DNA characterization, sequencing of specific nitrogenous base, and finding appropriate DNA responsible for reducing the growth of barnyard grass which in turn can be inserted to rice genomes by plant breeders to create rice genotype having ability to suppress barnyard grass growth in rice field.

Improving plants' ability to produce allelochemicals could be achieved via the approaches of manipulating genomic and proteonomic (Einhellig, 1995). Reports on such areas are currently limited but having wide arrays of achieving sustainable and environmentally-friendly agricultural practices. Identification of gene(s) responsible for allelopathy in rice is not impossible to be realized through DNA fingerprinting and sequencing or other genomic-base technology (Weller *et al.*, 2001).

CONCLUSION

1. Rice genotypes of Cempo lutut, Badik/Gaduh kabalai, Komas B, and Soegon resulted in suppression of root growth of barnyard grass as much as 60.35; 58.09; 52.96; and 50.01%.
2. DNA characterization analysis shows different types of distribution of DNA strands between 'tolerant' and 'intolerant' rice genotypes.
3. Rice genotype Cempo lutut has specific fragments of DNA having same position with specific fragments of DNA of barnyard grass leading to the presumption that those DNA have gene(s) responsible for allelopathy in rice.

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Table 1. The activity of peroxidase (POD = 470 nm absorbance/minute/g fresh weight) of barnyard grass roots 14 days after germination. Weeds and rice were co-cultured in Petri dish.

| Treatments | POD activity*) | Suppression (% control) |
|------------|---------------------------------|-------------------------|
| 32,8684 | 0 , 0 0 0 0 | |
| 32,4020 | 1 , 4 1 9 1 | |
| 33,4076 | - 1 , 6 4 0 5 | |
| 34,8337 | - 5 , 9 7 9 4 | |
| 34,2124 | - 4 , 0 9 4 4 | |

*) according to analysis of variance, $P > 0.05$

Table 2. Selected primers showing polymorphic fragments from DNA pool of 'tolerant' rice genotypes

| No | Primers | Size (base pairs) |
|----|---------|-------------------|
| 1 | OPA-09 | 1300 |
| 2 | OPE-18 | 900 |
| 3 | OPK-15 | 500 |
| 4 | OPN-16 | 1300 |
| 5 | OPY-20 | 200 |

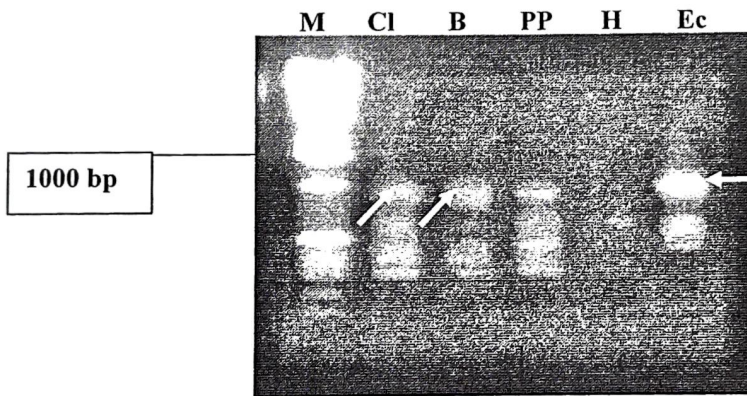


Figure 1. Visualisation of individual selection using OPN-16 primer following 1% agarose electrophoresis at 100 Volt after 60 minutes running. M = marker, Cl = Cempo lutut, B = Badik/Gadiah kabalai, PP = Pulu palappa, H = Harapan, Ec = barnyard grass. Specific fragments shown by arrows

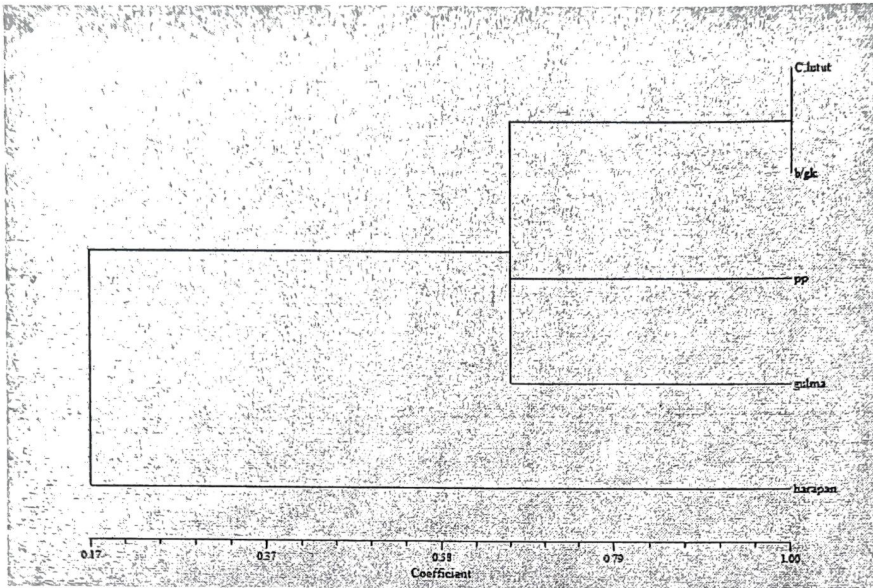


Figure 2. Dendrogram of genetic relatedness between rice and barnyard grass.
 C.lutut = rice genotype Cempo lutut ('tolerant'), bgk = rice genotype Badik/Gadiah kabalai ('tolerant'), pp = rice Pulu palappa ('intolerant'), harapan = rice harapan ('intolerant'), and weed = barnyard grass