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Full Length Research Paper

Identification of new fungi isolated from *Echinochloa* spp., as potential biological control agents in paddy fields in Iran

Mohammad Reza Safari Motlagh

Department of Plant Pathology, Faculty of Agriculture, Islamic Azad University, Rasht Branch, Rasht, Iran.
E-mail: ssafarimotlagh@yahoo.com.

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***Echinochloa* spp. are the most important weeds of rice. Fungal pathogens can be exploited as biological agents for the management of agricultural weeds. Two pathogenic fungal species were isolated from naturally infected *Echinochloa* species and identified. In order to isolate the fungus from disease tissues, the obtained samples were cultured on potato dextrose agar medium. Isolates were cultured due to sporulation on water agar medium. Morphological characters of isolates were studied in order to identify the taxonomy. According to the results, isolates belonged to *Bipolaris maydis* (Nisikado and Miyake) Shoem., and *Bipolaris australiensis* (Tsuda and Ueyama) Alcorn. Pathogenicity test of isolates in species was done in desiccators, and revealed the pathogenicity of the species and their ability to cause leaf blight on *Echinochloa* spp.. Inoculation was done using a spore suspension consisting of 10^5 spore/ml distilled water and 1%Tween-20 at the 2 to 3 leaf stage. Results indicated that the disease rating caused by *B. maydis* and *B. australiensis* in *Echinochloa* spp. was higher than that observed in the studied rice cultivar. Hence *B. maydis* and *B. australiensis* can be considered as a probable mycoherbicide for controlling *Echinochloa* species.**

Key words: *Echinochloa* spp., fungi, biological control, *Bipolaris* spp.

INTRODUCTION

Rice (*Oryza sativa*) is one of the most important crops in the world, being the staple food for millions of people in Asia (Deluna et al., 2002). Weeds are considered a major constraint to world rice production (Moody, 1995). The *Echinochloa* spp. are major weeds wherever rice is grown (Holm et al., 1977). Their distribution is truly global from temperate to tropics in a wide variety of crops (Gressel, 2002). *Echinochloa* spp., severely reduce both yield and quality of rice (Holm et al., 1977). Current weed management practices of mechanical, cultural, and chemical methods need to be reassessed in the wake of increasing concerns about economical and environmental sustainability of intensive rice production (Deluna et al., 2002). Fungal pathogens can be exploited as biological agents for the management of agricultural pests and diseases (Evans, 1999). *Bipolaris hawaiiensis* had been reported on bermudagrass (*Cynodon dactylon*) and other *Cynodon* spp. from subtropical areas around the world (Pratt, 2001). Virulence of *Bipolaris hawaiiensis* on bermudagrass, compared with *B. cynodontis* and *B. spicifera.* All three pathogens induced symptoms of chlorosis and necrotic lesions (Pratt, 2001). Six pathogenic fungal species were isolated from naturally infected *Echinochloa* species in rice (Zhang et al., 1996). *Bipolaris sacchari, Curvularia geniculata, Dactylaria dimorphospora*, and *Exserohilum monoceras* were pathogenic only to *Echinochloa* species (Zhang et al., 1996). Two fungal pathogens, *B. sacchari* and *Drechslera gigantea* were identified as promising biological control agents for cogongrass (*Imperata cylindrica*) (Yandoc et al., 2005). *B. sacchari* was capable of causing foliar blight on cogongrass (Yandoc et al., 2005). In Japan, a fungal pathogen, identified as *Drechslera monoceras* is being evaluated as a bioherbicide for control of *Echinochloa* species in paddy fields (Gohbara et al., 1996; Goto, 1992). In Malaysia and Indonesia, ten barnyardgrass (*Echinochloa crus-galli* var. *crus-galli*) ecotypes were tested for variation in their susceptibility to the leaf blight

pathogen (*Exserohilum longirostratum*) (Jurami et al., 2006). *Alternaria alternata* and *Fusarium equiseti* were reported as eventual biological agents for the management of *Echinochloa* spp. (Safari Motlagh, 2010). The principle goal of this research was to identify new genus and species of fungi isolated from *Echinochloa* spp., as eventual agents biological control of this weed in Guilan province of Iran.

MATERIALS AND METHODS

Collection and culture of fungal isolates

Diseased leaves of *Echinochloa* spp. were sampled from five locations in each field of Guilan province in Iran. Each sampled location was approximately 5 × 8 m and locations were approximately 35 m apart together (Xia et al., 1993). Leaves were transferred to the laboratory and the fungi was then isolated from disease samples. Leaf pieces with lesions were surface sterilized with 0.5% sodium hypochlorite solution, washed by sterile distilled water and placed on potato dextrose agar in Petri dishes at 27 to 30ºC for 2 to 3 days. Related to fungus, potato dextrose agar or water agar was used for sporulation. Then Petri dishes containing media were incubated at 27ºC in the dark or artificial light supplied by fluorescent light on a 12 h light/dark photoperiod for 5 to 30 days (Montazeri et al., 2006).

To avoid bacterial contamination, sulfate streptomycin antibiotic was used (Safari, 2008). Conidia were single- sporulated. Monoconidial isolates of the recovered fungi were maintained on half- strength potato dextrose agar slants in test tubes as stock cultures or colonial of fungal placed onto sterilized filter paper, then cuts of these filters were incubated in sterilized vials at freezer on -20ºC (Safari, 2008).

Study and identification of fungi

Morphological studies were carried out on potato dextrose agar and water agar media. Cuts of colonies or each of filter papers were placed onto potato dextrose agar medium for 2 to 3 days. Then, section of colonies was transferred to water agar medium for 7 to 30 days in incubator at 27ºC and 12 h photoperiod. Afterward, morphological observations were taken based on colony, conidium and conidiophore morphology and other morphological characters (Ellis, 1971; Sivanesan, 1987).

Pathogenicity tests

Pathogenicity tests of weed were carried out in desiccators. In each of two desiccators (one desiccator as control) two Petri dishes were placed each containing 10 germinated seeds of *Echinochloa* spp. At first, seeds of *Echinochloa* spp. were placed on moistened filter paper in Petri dishes and incubated at 28ºC for 24 h in a germinator with 12 h light/ dark photoperiod. Then, seeds were surface sterilized with 0.2% sodium hypochlorite solution for 2 min. After washing with distilled water, ten germinated seeds (coleoptile and radical just emerged) were planted per 10- cm- Petri dishes filled with saturated soil (Zhang et al., 1996), and were incubated at room temperature. Distilled water was added to Petri dishes. Seedlings at the 2 to 3 leaf stage were inoculated with 10^5 conidia per ml. To increase the surface adsorption, 1% tween-20 was applied. Evaluation of symptoms was performed 7 days after inoculation. Therefore, standard evaluation system and Horsfall- Barratt system were applied for *Echinochloa* spp. (Zhang et al., 1996; Bertrand and Gottwald, 1997).

$$\text{Disease rating} = \frac{(N_1 \times 1) + (N_2 \times 2) + ... + (Nt \times t)}{(N_1 + N_2 + ... + N_t)}$$

where N is number of leaves in each of rate and t is number of treatments.

Pathogenicity tests of rice were carried out in desiccator. To do so, in each of two desiccator (one desiccator as control) were placed two Petri dishes and in each Petri dish was placed 10 seeds of rice, Khazar cultivar. Then, seeds were sterilized in water bath at 52 to 57°C and cultivated in saturated soil and incubated at 25°C. Distilled water was added to Perti dishes. After 16 to 18 days, 2 to 3 foliages seedlings were inoculated by suspension of spores (Safari Motlagh and Kaviani, 2008). Other conditions including concentration of conidia and evaluation systems were similar.

Data analysis

Data analysis was done using NTSYS software (Sneath and Sokal, 1973).

RESULTS

The fungi isolates belonged to *Bipolaris* spp. These isolates were divided into 2 groups based on morphological characters, as follows:

Characteristics of first group

Colonies fast-growing, fluffy, with concentric rings is given in Figure 1. Conidiophores single or often in groups from flat, dark brown to black stromata, straight to flexuous, septate, smooth, geniculate, mid to dark brown, paler towards the apex, up to 700 µm long, 5 to 10 µm thick (Figure 2). Conidiogenous nodes verruculose. Conidia distinctly curve, fusoid, pale to mid dark golden brown, smooth, 5-11-distoseptate, 70 to 160 × 15 to 20 µm, hilum 3 to 4.5 µm wide (Figure 3). The characteristics of this group corresponded with *B. maydis* (Nisikado and Miyake) Shoem (Sivanesan, 1987; Ellis, 1971).

Characteristics of second group

Conidial colonies effuse, grey to blackish brown, velvety (Figure 4). Hyphae pale to dark brown, smooth, septate. Stromata erect, straight, cylindrical, black, formed in culture on rice grains. Conidiophores single, flexuous, geniculate, septate, smooth, cylindrical, reddish brown, up to 150 µm long, 3 to 7 µm thick (Figure 5). Conidiogenous nodes verruculose. Conidia straight, ellipsoidal or oblong, rounded at the ends, pale brown to mid reddish brown, usually 3, rarely 4-5-distoseptate, 14 to 40 × 6 to 11 µm (Figure 6). The characteristics of this group corresponded with *Bipolaris australiensis* (Tsuda

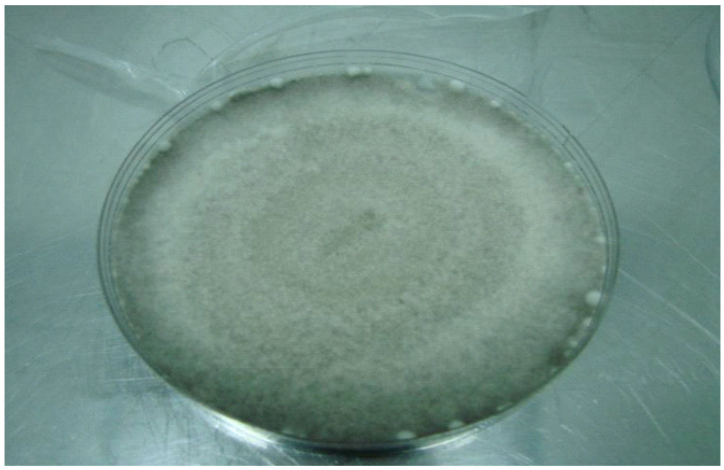

Figure 1. Colony of *B. maydis* on PDA.

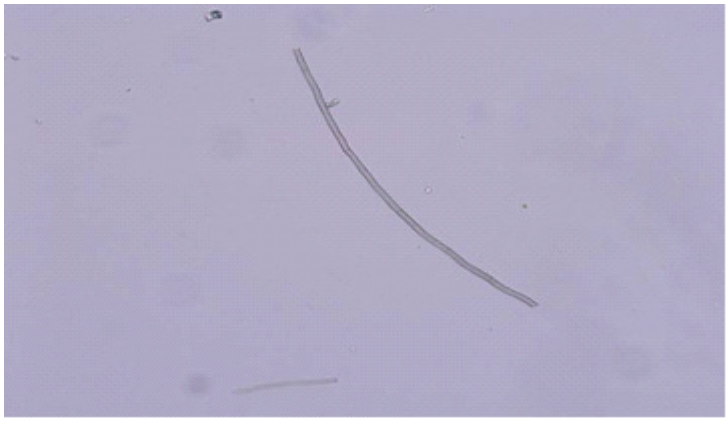

Figure 2. Conidiophore of *B. maydis* (×460).

Figure 3. Conidium of *B. maydis* (×1200).

and Ueyama) Alcorn (Sivanesan, 1987; Ellis, 1971).

The first symptoms of *B. maydis* appeared 24 h after inoculation on barnyardgrass and 36 h after inoculation on rice. Symptoms on *Echinochloa* spp. were chlorotic spots that expanded and produced necrotic spots. On rice appeared chlorotic and necrotic spots.

Figure 4. Colony of *B. australiensis* on PDA.

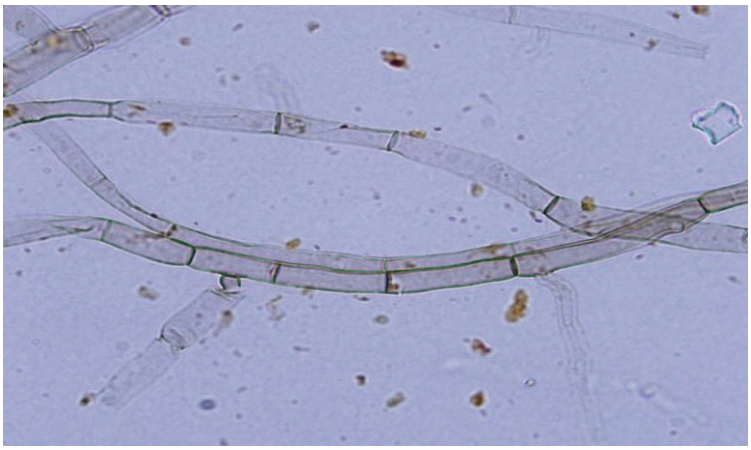

Figure 5. Hyphae and conidiophore of *B. australiensis* (×1200).

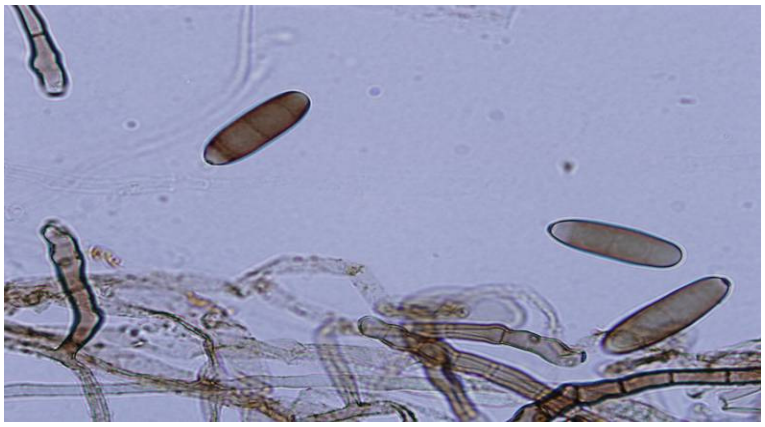

Figure 6. Conidia of *B. australiensis* (×1200).

The first symptoms of *B. australiensis* appeared 4 days after inoculation on barnyardgrass and 2 days after inoculation on rice. Symptoms on *Echinochloa* spp. were chlorotic and necrotic spots that expanded and produced

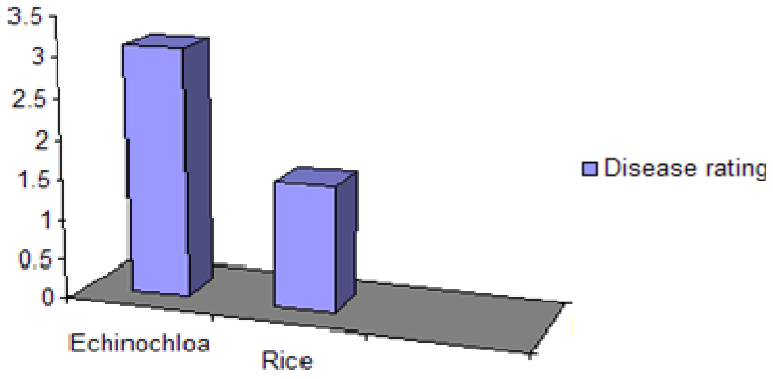

Figure 7. Diagram of the comparison of *B. maydis* mean disease rating in rice and *Echinochloa*.

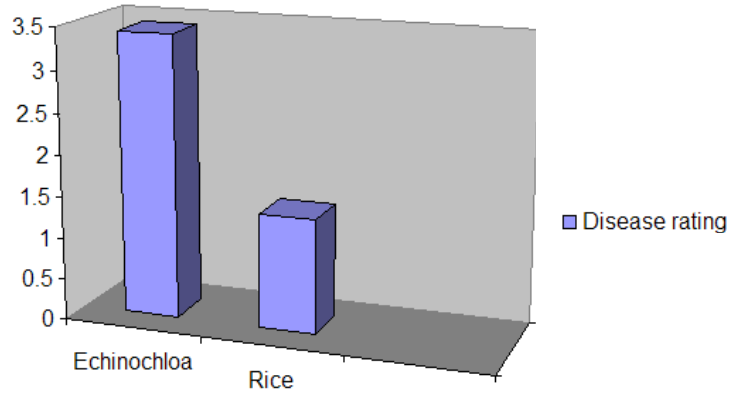

Figure 8. Diagram of the comparison of *B. australiensis* mean disease rating in rice and *Echinochloa*.

fluffy colonies. On rice appeared pinhead specks and on top of leaves was created blight.

Based on the sizes and types of the spots that appeared on the rice and Horsfall-Barratt system, this cultivar was less affected by the *B. maydis* compared with *Echinochloa* spp., and its disease rating was lower and showed much tolerance (Figure 7).

Based on dendrogram from cluster analysis, *B. maydis* isolates in disease rating index on barnyardgrass divided into 2 groups. The first group consisted of 2 isolates. The second group consisted of 1 isolate. Also, in first group, 2 isolates had similarity coefficient of 0.95 (Figure 9).

Based on dendrogram from cluster analysis, *B. maydis* isolates in disease rating index on rice is divided into 2 groups. The first group consisted of 1 isolate. The second group consisted of 2 isolates. Also, in second group, 2 isolates had similarity coefficient of 0.95 (Figure 10).

Moreover, the rice cultivar was less affected by the *B. australiensis* compared with *Echinochloa* spp. and its disease rating was lower and showed much tolerance (Figure 8).

Disease rating index of *B. maydis* compared with it in *B. australiensis* on rice and *Echinochloa* spp. did not show any significant difference (Figures 7 and 8). Because the number of *B. australiensis* was limited (one isolate), cluster analysis was done. But, on the base of dendrogram, cluster analysis of *B. maydis* and *B. australiensis* together in disease rating index on barnyardgrass, isolates divided into 2 groups. In first group, 2 isolates of *B. maydis* and 1 isolates of *B. australiensis* were placed. In second group, 1 isolate of *B. maydis* was placed (Figure 11).

On the base of dendrogram cluster analysis of *B. maydis* and *B. australiensis* together in disease rating index on rice, isolates were divided into 2 groups. In the first group, there was 1 isolate of *B. maydis* and in the second group, there were 1 isolate of *B. maydis* and 1 isolate of *B. australiensis* (Figure 12).

DISCUSSION

In the study of the reaction of *Echinochloa* spp. to *B. maydis* and *B. australiensis,* it was found that the disease rating caused by these fungi on the said weed was higher than what was observed in the studied rice cultivar.

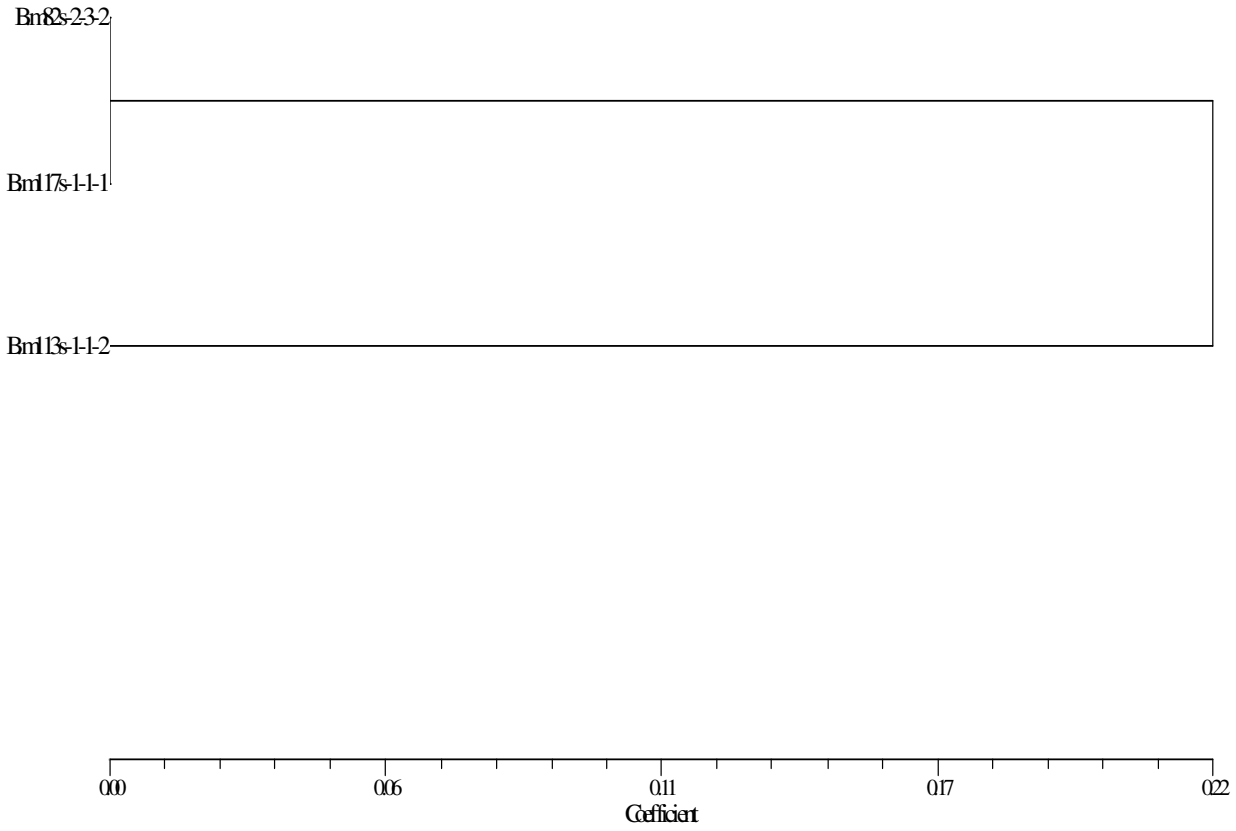

Figure 9. UPGMA-dendrogram for *B. maydis* isolates on *Echinochloa*.

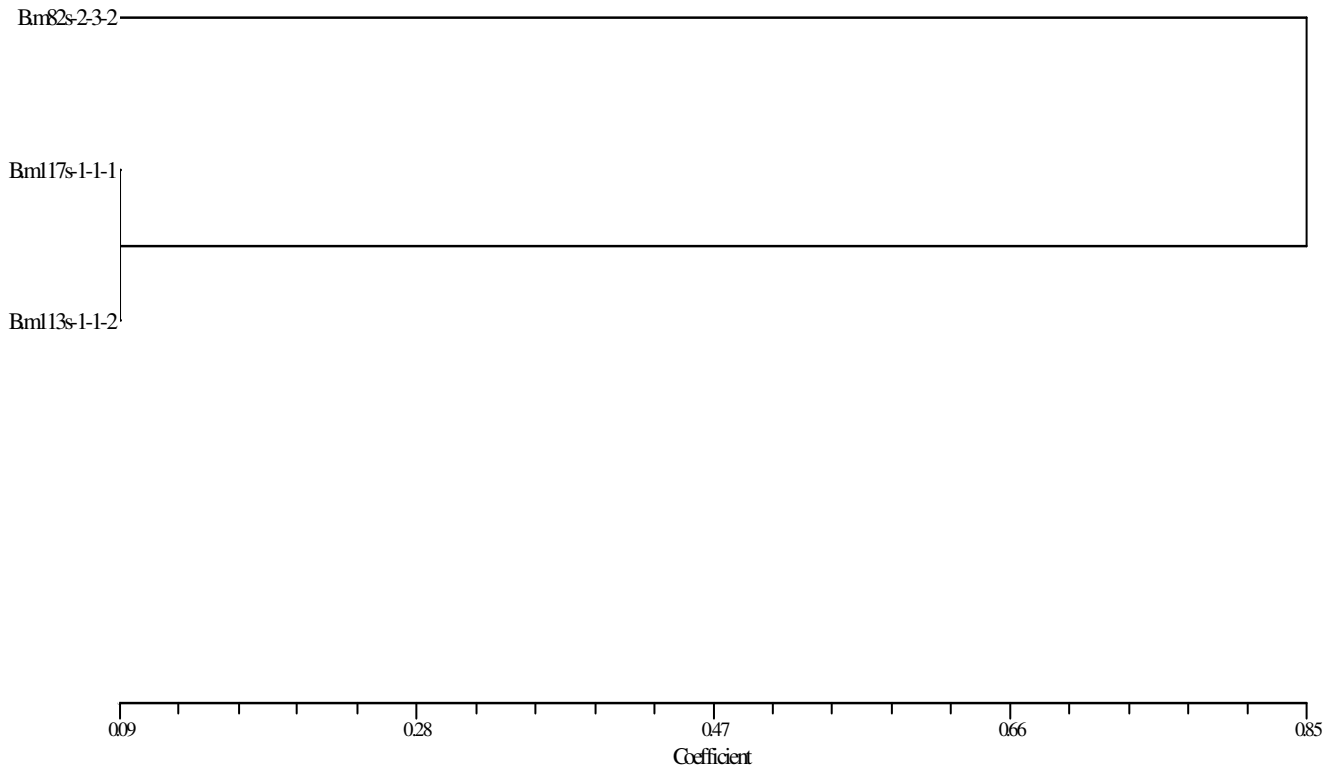

Figure 10. UPGMA-dendrogram for *B. maydis* isolates on rice.

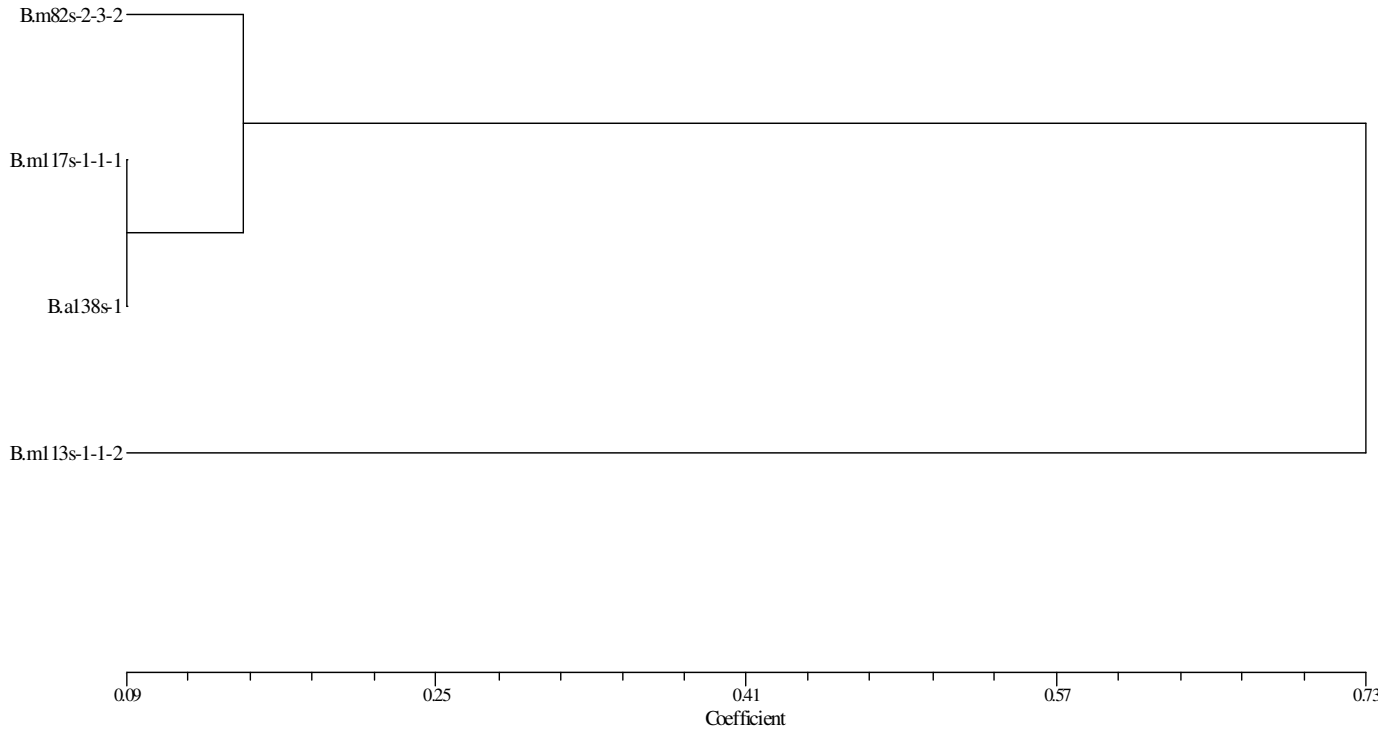

Figure 11. UPGMA-dendrogram for *B. maydis* and *B. australiensis* isolates on *Echinochloa*.

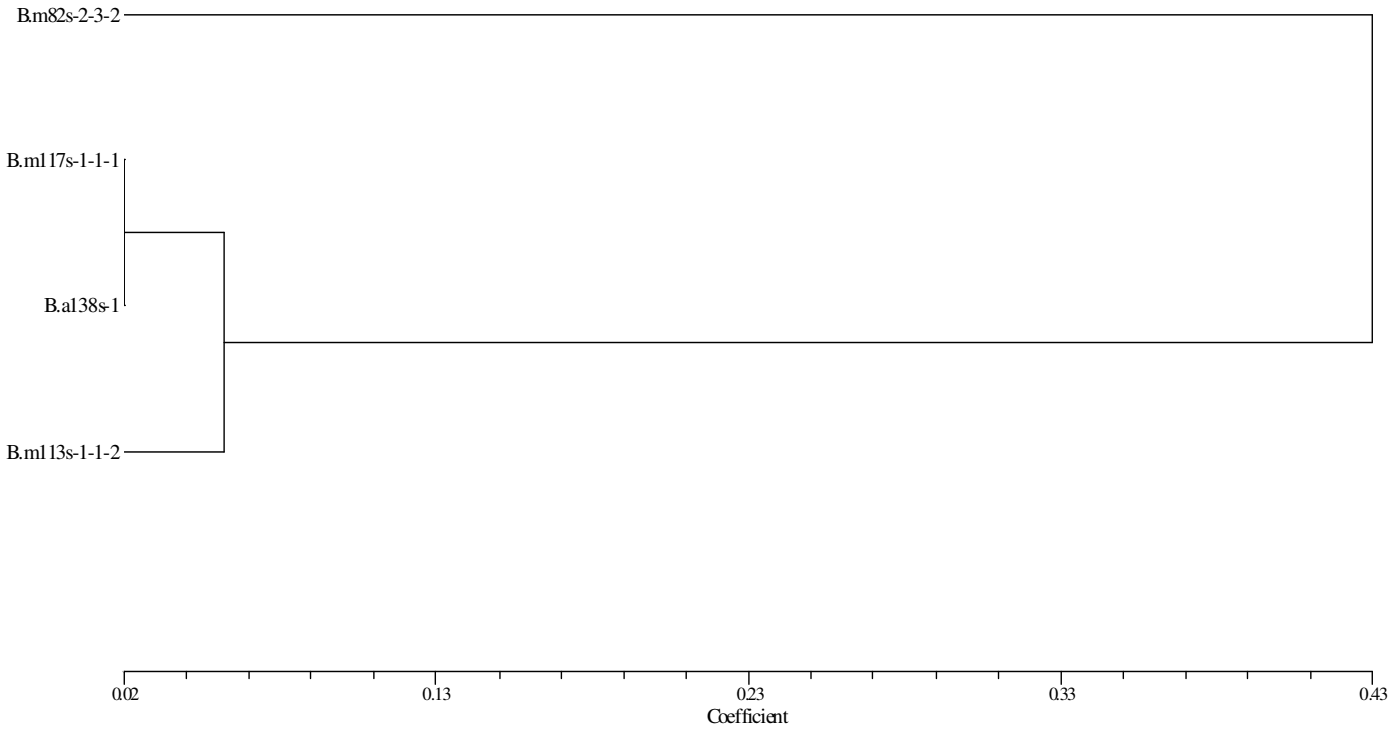

Figure 12. UPGMA-dendrogram for *B. maydis* and *B. australiensis* isolates on rice.

The results indicated that not only the symptoms but also the virulence in these two fungi was similar (Figures 7 and 8).

In this research based on cluster analysis, isolates of *Bipolaris* spp. indicated similar reactions. But this similarity in isolates of same species was more than other species of this genus. This subject can be related to more genetic diversity in different species (Oliveira et al., 2002).

The virulence of *B. hawaiiensis* on bermudagrass is compared with *B. cynodontis* and *B. spicifera.* All three pathogens induced symptoms of chlorosis and necrotic lesions (Pratt, 2001).

In other study, sporulation by nine species of *Bipolaris, Curvularia, Drechslera* and *Exserohilum* was observed on symptomatic leaves of ryegrass. The most isolates of *B. cynodontis* were virulent and caused necrosis (Pratt, 2006).

Studies indicated that *Bipolaris sacchari* was pathogenic only to *Echinochloa* species and was not pathogenic to rice. When provided a 24-h dew period, *B.* sacchari resulted in 100% mortality of seedlings of *Echinochloa* spp. (Zhang et al., 1996). Therefore, *B. sacchari* have potential to control *Echinochloa* species (Zhang et al., 1996). Also was indicated that *B. sacchari* was capable of causing foliar blight on cogongrass (Yandoc et al., 2005).

Phytotoxins have been reported to be produced by *B. sacchari* (Steiner and Stroble, 1971) that are biologically active on *Echinochloa* spp. (Zhang et al., 1996). It is often assumed that a virulent, highly aggressive pathogen (that is, one that causes a high level of mortality) is a preferred bioherbicide candidate (Zhang et al., 1996). Therefore, identification of fungi associated with weeds, helps us in finding biological control agents of weeds.

In conclusion, *B. maydis* and *B. australiensis* can be exploited as eventual biological agents for the management of *Echinochloa* spp. in paddy fields.

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