Towards the production of allelopathic cultivars for low input rice farming systems

Sophea Pheng[1,2], Steve Adkins[1], Maria Olofsdotter[2,3], Harry Nesbit[2] and Gary Jahn[2]
[1] Tropical and Subtropical Weeds Research Unit, University of Queensland, St. Lucia, Qld 4072, Australia
[2] Cambodia-IRRI-Australia Project, PO Box 1, Phnom Penh, Cambodia
[3] Weed Science, Department of Agricultural Science, KLV, Agrovej, 2630 Denmark

Summary The allelopathic effects of 357 accessions or cultivars of rice (*Oryza sativa* L.) on awnless barnyard grass (*Echinochloa colona* (L.) Link.) were evaluated using a Petri dish test, then with small umbrella sedge (*Cyperus difformis* L.) and barnyard grass (*Echinochloa crus-galli* (L.) P.Beauv.) in a field trial. These experiments were conducted as part of a broader attempt to evaluate the suitability of ancient accessions of rice as donors of allelopathic traits to modern rice to produce new rice cultivars with weed suppressing ability. Such traits would be highly desirable in Southeast and Southern Asia where herbicide cost restricts their use. The Petri dish test used a 'relay seeding technique', in which awnless barnyard grass seeds were planted next to 7-day old rice seedlings and the two grown together for 10 further days. There were 14 rice accessions or cultivars (3%) that significantly reduced the root and in most cases, the shoot growth of the weed and hence exhibited allelopathic effects. A field experiment conducted in Cambodia during 1999/2000 wet season was undertaken to determine the weed-suppressing ability of six of these 14 rice accessions or cultivars against awnless barnyard grass and small umbrella sedge. All showed significant reductions in shoot biomass. The effect of the soil-incorporated residues from these rice accessions or cultivars was also studied on the establishment of awnless barnyard grass and rice plants in the following season. The incorporation of residues was found to be suppressive towards the early growth and establishment of awnless barnyard grass.

Keywords Allelopathy, rice, weed control.

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important crop in Cambodia. It dominates in terms of its cultivated area, the volume of its harvested product, and the number of people involved in its production. However, the national average yield of about 1.45 t ha^{-1} is still one of the lowest in Asia (Javier 1997). This low productivity results from a number of unhelpful abiotic factors, including poor soil fertility and unpredictable rainfall. In addition, biotic constraints such as weed and pest out breaks are always a major threat to production in any tropical country. Of the land devoted to rice production in Cambodia, 86% is planted to rain fed lowland rice (Javier 1997) where water management and other cultural practices are not fully effective in controlling weeds. The resulting hand weeding approach is time-consuming and impractical. Herbicides can be used to control these in-crop weeds and is a labour-saving option, but most are too expensive for Cambodian farmers to use. Recent studies on allelopathy (*viz*. the production of chemicals by one plant that can influence the growth of another plant, Rice 1984) have revealed that this phenomenon may have a significant role in many agricultural systems (Olofsdotter 1998). As with some other crops, the existence of allelopathic activity in rice has been investigated for its potential role in weed management (Dilday *et al.* 1998, Olofsdotter *et al.* 1995). The planting of allelopathic rice cultivars could significantly reduce the use of synthetic herbicides in rice production. This would create a weed management approach that is 'built' into the crop and provides the farmer with an affordable alternative to hand weeding.

The aims of the present study were to (1) determine the ability of 357 rice cultivars or accessions (hereafter called cultivars) to suppress the growth of weed seedlings growing in a laboratory bioassay and to (2) determine the growth suppressing ability of six of these cultivars on two annual weeds growing in the field in Cambodia. A second aspect of this study was to assess the effect of post-harvest crop residuals on weed growth.

MATERIALS AND METHODS

Materials A total of 357 rice accessions or cultivars (311 from Cambodia, seven from the International Rice Research Institute, and 48 from the Australian Rice Germplasm Collection) were used in this study. The weeds used were awnless barnyard grass (*Echinochloa colona*, ABG) obtained from the Darling Downs Region of South East Queensland, and small umbrella sedge (*Cyperus difformis*, SUS) and barnyard grass (*E. crus-galli*, BG) from Cambodia.

The laboratory screen Twenty washed, highly viable rice seeds of uniform size were imbibed in a 9 cm Petri dish on a single Whatman No. 1 filter paper moistened with 15 mL distilled water. Dishes for all 357 accessions were placed in germination incubators (photoperiod 12 hours day, 12 hours night, temperature 25°C day, 20°C night) for three days. Ten rice seedlings of uniform root and shoot length were then selected from the 20 and used in a screening procedure (the relay seeding technique) adapted from Navarez and Olofsdotter (1996). Seven days after the sowing of the rice seeds, 20 washed ABG seeds were placed next to the 10 rice seedlings (two per seedling). All seedlings were then covered with 4.0 g of a washed, granular perlite and an additional 25 mL of water added. Four days later, the ABG seedlings were thinned to 10 uniform seedlings per Petri dish. For a control, 40 ABG seeds were sown in a Petri dish and incubated for a further 10 days under the same conditions as described above. Seventeen days after the initial sowing of rice seeds, the ABG seedlings were gently removed, washed, and their shoot and root lengths measured. The data was then entered into a spreadsheet program and the average root and shoot length calculated. Analysis of variance (ANOVA) and mean comparison (DUNNETT's test) were performed with SigmaStat® (Jandel Corporation).

The field experiment The experiment was conducted at the Cambodian Agricultural Research and Development Institute, Phnom Penh, Cambodia. The soil at the experimental site was a sandy, light textured soil overlaying a loamy- or a clay-textured subsoil. The six month-long experiment was started in the wet season but concluded two months into the dry season. Annual rainfall and other climatic variables were similar to the mean values previously reported for this region of Cambodia (Nesbitt 1997). Six of the eight rice cultivars (*viz*. CAR-3, CAR-4, CAR-8, Neang Kong, Neang Khat, and Pa Thaut) used in this trial were selected based on their inhibitory effects in the Petri dish and an unreported pot test procedure. The other two rice cultivars, Taichung Native 1 (TN-1) and ST-3, were used as they represented allelopathic and non-allelopathic rice controls, respectively (Navarez and Olofsdotter 1996). As controls, weed monocultures involving the two weed species; BG or SUS was also prepared. The experiment employed a randomised complete block design with four replications. The plot size for each treatment was 2.0 × 5.2 m forming a 10.4 m^2 plot area. Each plot received fertilizer at a rate of 50 kg ha^{-1} of nitrogen, 23 kg ha^{-1} of phosphorous (P), and 30 kg ha^{-1} potassium (K). P and K were applied before planting (basal application). Nitrogen was split into two applications, half applied before planting and half applied 45 days after seeding the rice. The trial was undertaken in two phases. In the first phase, the rice and the weeds were sown and grown together in mixed-culture and the effects of the growing rice plants tested on the growth of the two weed species. To achieve this seeds of each rice cultivar were direct-seeded onto the assigned plot at a rate of 100 kg seed ha^{-1}. Twenty-five days after rice seeding (DAS), BG and SUS were direct-seeded into half (2.5 × 2.0 m) of each plot at a rate of 7.5 kg ha^{-1} and 3.75 kg ha^{-1}, respectively. Non-target weeds emerging in the trial were removed from each plot regularly by hand. Shoot biomass of weeds was assessed at harvest. In the second phase, the unharvested rice stubble was chopped into pieces of *ca*. 5 cm length, and then spread evenly back into the plot and incorporated manually into the soil. The effect of this incorporation was then tested on the early establishment of BG and rice (ST-3). Weed plots were also set up on non-stubble incorporated sites. Seven days after rice straw incorporation, seeds of BG were direct-seeded at the rate of 7.5 kg ha^{-1}. By using the same data collection technique in Phase-1, shoot biomass of these newly established seedlings was assessed 30 DAS.

RESULTS

The laboratory screen There were 14 cultivars whose effects on the growth of ABG significantly reduced root and/or shoot lengths (Figure 1). In most cases, root lengths were affected much more than shoot lengths. The active cultivars reduced root and shoot length of weed seedlings by as much as 78% for roots and 58% for shoots.

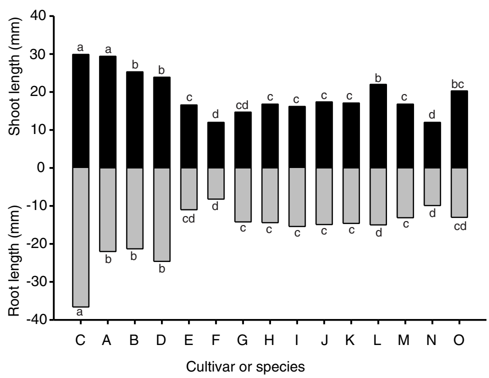

Figure 1. The growth parameters of *Echinochloa colona* seedlings when grown together with one of 14 cultivars of rice (A–O) or on its own (C). In a column, means followed by a common letter are not significantly different. C control, A CAR 3, B CAR 4, D CAR 8, E Neang Chhmar, F Neang Chhol, G Neang Khat 3034, H Neang Khat 3409, I Neang Knog 3029, J Neang On, K Pa Thaut 3051, L Saloio, M Srau Chrey, N Srau Kheng, O Srau Neang Lay.

The field experiment The shoot biomass of BG was significantly reduced (Figure 2) by the six test rice cultivars and this suppression was significantly greater than that seen for the allelopathic rice control, TN-1. The ABG biomass in the ST-3 (non-allelopathic) plot was greater than that seen in the TN-1 (allelopathic) plot as expected and all biomasses were much lower than that seen in the weed monoculture plot. All six rice cultivars also reduced the shoot biomass of SUS (Figure 2).

In the second phase of the experiment, BG and ST-3 seed sown onto land after the incorporation of rice straw residues produced lower shoot biomass than seedlings in the residue-free controls (Figure 3). For BG, the dry matter production in the residue-free plots was significantly greater than all residue treated plots. Among the plots treated with the residues of tested rice cultivars, the order of growth inhibition was Neang Khat >CAR-3>CAR-4>CAR-8>Pa Thaut. There was no significant difference in shoot biomass between the plots treated with ST-3 and TN-1 residues, however, ABG shoot biomass in these plots were about four times smaller than the weed monoculture plots. In terms of biomass production, ST-3 in the residue-free plots produced dry matter 3.0 to 6.7 times greater than those in the residues treated plots.

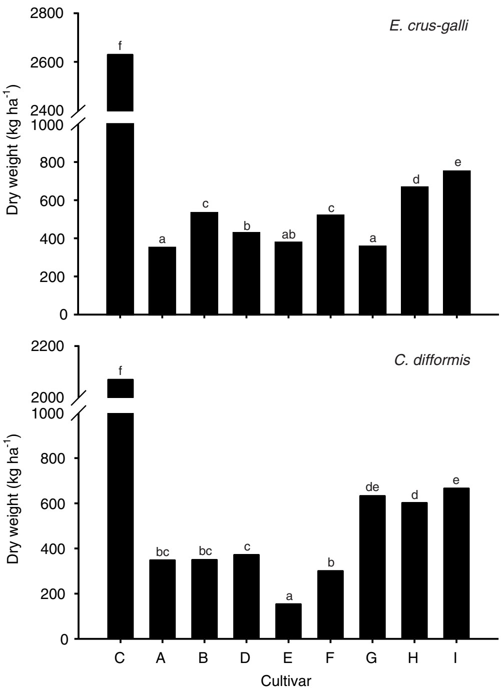

Figure 2. The growth parameters of *Echinochloa crus-galli* (L.) P. Beauv. and *Cyperus difformis* L. when grown together with one of the eight cultivars of rice (A–I) or on its own (C). Across treatments, means followed by a common letter are not significantly different. C control, A CAR 3, B CAR 4, D CAR 8, E Neang Kong 3029, F Neang Khat 3034, C Pa Thaut 3051, H TN-1 (allelopathic), I ST-3 (non-allelopathic).

DISCUSSION

The laboratory screen The relay seeding technique is believed to eliminate competition for water, nutrients and light (Navarez and Olofsdotter 1996). Using this approach 14 rice accessions significantly inhibited root and shoot elongation of ABG (Figure 1). In most cases the reductions in shoot length were less than those for root. This uneven response is believed to indicate an allelopathic interaction, rather than one due to competition, which would have resulted in an even reduction in both the root and shoot growth (Navarez and Olofsdotter 1996). Only 3% (14) of the screened cultivars (357) exhibited allelopathic effects on ABG. This is in line with other studies (Dilday *et al.* 1998), which have shown *ca.* 3% of screened rice germplasm to exhibit allelopathy against a selected weed species. Of the 14 accessions identified, all were screened in a glasshouse pot test (not reported in this paper) and from these the six best were used in the field trial.

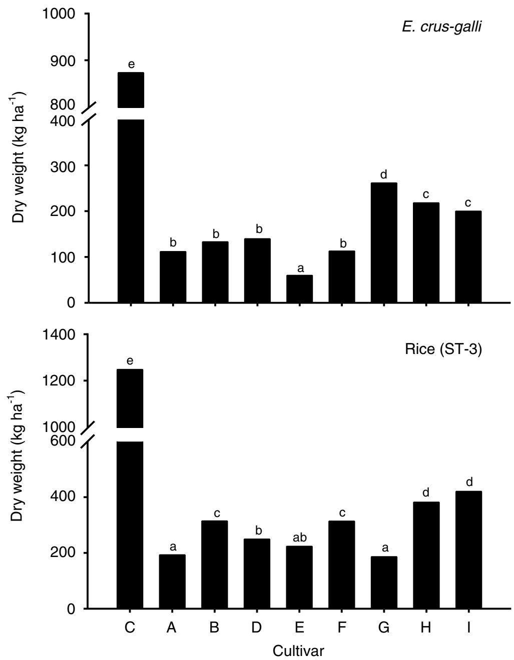

Figure 3. Performance of *Echinochloa crus-galli* (L.) P.Beauv. and rice (ST-3) grown after rice straw incorporation. In a column, means followed by a common letter are not significantly different. Cultivar code is as Figure 2.

The field experiments A general perception of using laboratory bioassays in the science of allelopathy is that they allow researchers to work with large amounts of test plant material in a short time, to eliminate certain interfering climatic factors, and to study only those effects due to allelopathy (Inderjit and Olofsdotter 1998). However, even when this is done successfully those laboratory experiments must always be validated in the field. The reductions in weed shoot biomass attained (Figure 2) suggest that there were growth suppressive actions from all of the rice cultivars studied and upon both weed species tested. For the test cultivars coming out of the laboratory test, their suppressive ability was as great or greater than those seen for an already characterized allelopathic rice cultivar.

The residue effect During plant tissue decomposition, chemicals are released directly from the residues, or indirectly through the action of microbes, and these compounds can be stimulatory, have no effects, or be inhibitory on the growth of other plants depending on their nature and concentrations in the soil (Fischer 1986). The degree of microbial activity is generally dependent on the soil environments such as water content or nutrient concentration (Inderjit and Foy 1999), thus the microbial activity in the residue-treated plots in this study should be equal as all plots received the same management practices. For that reason, it is possible to conclude that the residues of all tested rice cultivars had an inhibitory effect on the growth of ABG. As chemical release is absent in the residue-free plots, weeds performed better on these plots. The data indicates that all rice residues, regardless of whether they came from known allelopathic or non-allelopathic rice cultivars, have the ability to suppress weed growth.

Summary Modern agriculture is being challenged to reduce environmental damage and to prevent health hazards from chemical inputs. This, at the same time as maintaining crop production (Einhellig 1995). Thus, crop protection strategies based on allelopathy would be one way of helping achieving this ideal (Einhellig and Leather 1988). The concept of using allelopathic rice for potential weed control was first developed 14 years ago (Olofsdotter 1998). In countries where herbicides are too costly to use and crop water management is not an option, as in the rain fed regions of Cambodia, an economically sound solution to weed control might be to develop allelopathic rice cultivars. A weed management method that is already built into the seed would provide farmers with a control method, which is cost effective, labour saving, and environmentally safe.

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