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GENETIC BEHAVIOR FOR MOMILACTONE B IN SOME LINES AND THEIR HYBRIDS OF RICE

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ABSTRACT

The allelopathy-competition separation (ACS) based approach was used to explore the biointerferance relationship between rice accessions and barnyardgrass exposed to different nitrogen (N) supplies in hydroponics. Some crosses had higher percentage for allelopathic activity as follow; IR69625A × Rikuto Norin 22, IR69625A × IR65617-52-2-3-3-2-3, IR69625A × Vener1A and IR69625A × Giza182 and their values were 87, 85, 85 and 85%, respectively. The varieties Rikuto Norin22, IR67413-71-4-2-2, Giza182, IR65617-52-2-3-3-2-3 and Vener1A exhibited the highest activity against E. crus-galli during 2009 season. These results referred to highly genetic effect which controlled these traits since the environmental effect is not significant. The additive and dominance genetic variances might be important in the inheritance of weed control, although the dominance genetic variance played more important role in this case. Rice accession Rikuto Norin22 exhibited high allelopathic potential to suppress the growth of accompanying weeds, especially when the culture solution had low N content. Although rice accession Giza182 has restoring fertility but it also exhibited high allelopathic activity under field conditions. Allelopathic activity of rice seedlings (Rikuto Norin22) exhibited 5.3–6.3-fold increase when rice and barnyardgrass seedlings were grown together, where there may be the competitive interference between rice and barnyardgrass for nutrients. Barnyardgrass is one of the most noxious weeds in rice cultivation. The momilactone B concentration in rice seedlings incubated with barnyardgrass seedlings was 6.9-fold greater than that in rice seedlings incubated independently. Low nutrient growth conditions also increased allelopathic activity and momilactone B concentrations in rice seedlings. However, the increase in the low nutrient-induced allelopathic activity and momilactone B concentration were much lower than those in barnyardgrass-induced allelopathic activity and momilactone B concentration. Root exudates of barnyardgrass seedlings increased allelopathic activity and momilactone B concentration in rice seedlings at concentrations greater than 30 mg/L of the root exudates, and increasing the exudate concentration increased the activity and momilactone B concentration. Therefore, barnyardgrass-induced allelopathic activity of rice seedlings may be caused not only by nutrient competition between two species, but also by components in barnyardgrass root exudates. As momilactone B shows strong allelopathic activities, barnyardgrass-induced allelopathic activity of rice may be due to the increased concentration of momilactone B in rice seedlings.

INTRODUCTION

Allelopathy of plants is strongly coupled with external biotic and abiotic stress factors. Abiotic stress factors such as heavy metal, weak solar radiation, higher temperature, and nutrient starvation increase allelopathic activity due to increased production of allelochemicals (*Einhellig, 1999; Wu et al., 1999; Belz, 2007 and Kato-Noguchi, 2009*). Many plants are able to induce defense mechanism against biotic stress factors such as pathogen and insect attacks (*Pena-Cortes et al., 2005 and Okada et al., 2007*). Allelopathy of rice has been shown to be induced by jasmonic and salicylic acids, which are important signaling molecules regulating inducible defense genes against the pathogen infection (*Bi et al., 2007 and Kato-Noguchi, 2009*). The production of sorgoleone, sorghum allelochemical was also induced by velvetleaf root extracts (*Dayan, 2006*), although it is unclear how active components in the extracts reach sorghum plants in natural ecosystems.

Phenolic acids are often mentioned as putative allelochemicals, and are the most commonly investigated compounds among potential allelochemicals since they have been found in a wide range of plants (*Inderjit, 1996 and Dalton, 1999*). However, phenolic acids are unlikely to be responsible for rice allelopathy because their concentrations in rice root exudates never reach phytotoxic levels (*Olofsdotter et al., 2002 and Seal et al., 2004*). Another potential allelochemical isolated from root exudates of the rice cultivar Koshihikari is momilactone B (*Kato-Noguchi et al., 2002*). Momilactone B inhibits the growth of typical rice weeds like barnyardgrass and *Echinochloa colonum* at concentrations greater than 1µmol/L. Rice plants secrete momilactone B from the roots into the rhizosphere over their entire life cycle (*Kato-Noguchi et al., 2008*). The secretion of momilactone B was also confirmed for other rice cultivars (*Kong et al., 2004*). These observations suggest that rice allelopathy may be primarily dependant on the secretion of momilactone B (*Kato-Noguchi et al., 2008, and 2010*).

Despite the influence of biotechnology and genetic engineering in changing future production methods for breeding new crop varieties, traditional plant breeding research and breeding methodology will play a significant role in future breeding programmes. Further refinement of these methods and better knowledge of classical knowledge are a prerequisite for the rational use of new tools such as molecular markers. The objectives of this study were; production of hybrid rice have heterosis in allelopathic potential and yield components by traditional plant breeding methods and Investigation of possible involvement of chemical-mediated rice/barnyardgrass interaction in rice allelopathy. Thus, effects of barnyardgrass, nutrient-limited conditions and root exudates of barnyardgrass on alleloapthic activity and momilactone B concentration in rice seedlings were examined in a series of experiments.

MATERIALS AND METHODS

Plant materials and growth conditions

Fourty three genotypes using a line x tester model, which including; three CMS lines; ten testers and thirty crosses were used to study the allelopathic activity and yield components. Observations were taken on five random plants from each plot. The crop was raised following the package of recommendation practices for summer season at the research farm of RRTC.

Seeds of rice (*Rikuto Norin22*) were surface sterilized in 70% (v/v) aqueous ethanol and grown on a sheet of moist filter paper (No.1; DBNRR.

Arkansas, USA) at 25°C with a 12-h photoperiod for 10 days in a growth chamber as described by (*Kato-Noguchi et al., 2002*). Seeds of barnyardgrass (*Echinochloa crus-galli* (L.) Beauv) were germinated and grown for 10 days as described above.

Uniform rice and barnyardgrass seedlings (fifty of each) were then transferred onto a holed plate of polystyrene foam that was floated on the medium of 1L Hoagland nutrient solution in plastic container (15cm×10cm×10 (height) cm; (*Kim et al., 2005 and Song et al., 2008*). Only roots of both plants were immersed in the medium through the hole of the plate and all manipulations were carried out under sterile conditions. The pH value of the medium was maintained at 6.0 throughout the experiments by using 0.5mol/L NaOH or H_2SO4 (*Kim et al., 2005 and Song et al., 2008*). After 10 days incubation at 25°C with a 12-h photoperiod, rice seedlings were harvested for bioassay and momilactone B determination.

Nutrient stress treatment

Four concentrations of Hoagland solution, 1N (normal strength), 0.3N (0.3-fold strength), 0.1N (0.1- fold strength) and 0.03N (0.03-fold strength) were prepared for the medium of rice incubation. After germination, uniform 50 rice seedlings were transferred onto a holed plate of polystyrene foam that was floated on 1L of these medium, and grown for 10 days as described above.

Barnyardgrass bioassay

Rice seedlings (10 g fresh weight) were homogenized in 100mL 50% (v/v) cold aqueous methanol and the homogenate was filtered through filter paper (No.2; DBNRR. Arkansas, USA). The residue was homogenized again with 100mL of methanol and filtered and the two filtrates were combined. An aliquot of the extract was evaporated to dryness, dissolved in 0.2mL of methanol and added to a sheet of filter paper (No.2) in a 3-cm Petri dish. Methanol was evaporated in a draft chamber. Then, the filter paper in the Petri dishes was moistened with 0.8mL of a 0.05% (v/v) aqueous solution of Tween 20. The final assay concentration was one rice plant equivalent extract per mL. After germination in the darkness at 25°C for 120 h, 10 uniform barnyardgrass seedlings were placed into the Petri dishes. The length of their shoots and roots was measured after 48h of incubation in the darkness at 25°C. Inhibitory activity (%) was determined by the formula: *[(control plant length−plant length treated with rice extract)/control plant length]×100*. Control bioassays did not contain rice extracts. The bioassay was repeated three times using a completely randomized design with 10 plants for each determination. Significant differences were evaluated by Tukey's HSD test.

Determination of momilactone B concentration in rice seedlings

Rice seedlings were homogenized and filtered as mentioned above. Then, the aqueous residue was loaded onto a column of synthetic polystyrene adsorbent and purified, and momilactone B was quantified as described by (*Kato-Noguchi et al., 2002*). The experiment was repeated three times with three assays for each determination. Significant differences were evaluated by Tukey’s HSD test.

Statistical analysis

All collected data were subjected to statistical analysis using ANOVA as described by *Gomez and Gomez (1984).* The treatment means were compared using Duncan's multiple range test Duncan, (1955).All statistical analyses were performed using analysis of variance technique by means of "MSTAT" computer soft ware package.

RESULTS AND DISCUSSION

Allelopathic characters

Distinct differences in allelopathic potential were observed among the tested rice genotypes, ranging between 25-87% as shown in Table 1, and six out of 13 rice germplasms showed allelopathic activity from 60-87% on *Echinochloa crus-galli* during 2009 season. The parents were crossed with their CMS lines and produced 30 crosses with weed control ranging between 30-87% as shown in Table 2, and 22 out of 30 crosses showed allelopathic activity from 60-87% on *E. crus-galli* during 2010 season. The following varieties; Rikuto Norin22, Giza182, IR67413-71-4-2-2, IR65617-52-2-3-3-2-3 and Vener1A originated from different countries and showed higher allelopathic activity during 2009 season, their values were 87, 80, 85 and 83 %, respectively as shown in Table 1. *Dilday et al. (1994*) reported that the varieties Shmokita and Rikuto Norin22 provided at least 80 and 70% weed control against duck salad in field experiment at Stuttgart, Arkansas, USA.

On the other hand, among the crosses, there were some crosses had higher percentage for allelopathic activity as follow; IR69625A × Rikuto Norin 22, IR69625A × IR65617-52-2-3-3-2-3, IR69625A × Vener1A and IR69625A × Giza182 and their value were 87, 85, 85 and 85%, respectively.

Table 1: Mean performance of weed control and radial area for the parental lines

No.	Parents	Radial area (cm)	Weed control %
1	**IR 65617-52-2-3-3-2-3**	11.37	83
2	**IR 671017-124-2-4**	10.33	80
3	**IR 67413-71-4-2-2**	10.33	85
4	**IR 67418-238-6-2-3-2**	5.17	37
5	**Vener 1 A**	11.83	83
6	**Upl-Ri-S**	2.17	27
7	**Giza 182**	11.33	80
8	**Giza 178**	9.33	44
9	**Rikuto Norin 22**	11.33	87
10	**Sakha 103**	2.30	25
11	**IR 69625 A**	5.33	39
12	**IR 70368 A**	6.17	46
13	**IR 58025 A**	7.33	50

For radial area character, we found that the parents IR65617-52-2-3-3-2-3, Rikuto Norin22 and Giza182 had higher values among all parents used during 2009 season as shown in Table 1. These parents were used to be crossed with their CMS lines and produced crosses with values ranging between 4.83–12.33cm, and the high desirable values which were expressed in; IR69625A × Rikuto Norin22, IR70368A × IR65617-52-2-3-3-2-3 and IR58025A × Giza182 during 2010 season as shown in Table 2.

From the obtained results, the varieties Rikuto Norin22, IR67413-71-4-2-2, IR65617-52-2-3-3-2-3 and Vener1A exhibited the highest activity against E. crus-galli during 2009 season. These results referred to highly genetic effect which controlled these traits since the environmental effect is not significant. In a similar study, (*Park and Lee, 1996*) found that the cultivars; Tang Gan, Koukets Umuchi and PSBRC 10 provided at least 70% weed control in transplanted rice against *E. crus-galli* under Korean conditions.

Table 2: Mean performance of weed control and radial area for the obtained crosses

No.	Crosses	Radial area (cm)	Weed control %
1	**IR69625A × IR 65617-52-2-3-3-2-3**	11.33	85
2	**IR69625A × IR 671017-124-2-4**	11.33	84
3	**IR69625A × IR 67413-71-4-2-2**	11.33	83
4	**IR69625A × IR 67418-238-6-2-3-2**	8.33	65
5	**IR69625A × Vener 1 A**	11.33	85
6	**IR69625A × Upl-Ri-S**	5.17	37
7	**IR69625A × Giza 182**	11.66	85
8	**IR69625A × Giza 178**	8.17	65
9	**IR69625A × Rikuto Norin 22**	12.33	87
10	**IR69625A × Sakha 103**	6.33	30
11	**IR70368A × IR 65617-52-2-3-3-2-3**	11.83	83
12	**IR70368A × IR 671017-124-2-4**	9.00	65
13	**IR70368A × IR 67413-71-4-2-2**	8.33	35
14	**IR70368A × IR 67418-238-6-2-3-2**	6.33	42
15	**IR70368A × Vener 1 A**	9.67	68
16	**IR70368A × Upl-Ri-S**	4.83	37
17	**IR70368A × Giza 182**	11.17	84
18	**IR70368A × Giza 178**	6.33	65
19	**IR70368A × Rikuto Norin 22**	10.17	81
20	**IR70368A × Sakha 103**	7.00	30
21	**IR58025A × IR 65617-52-2-3-3-2-3**	11.17	83
22	**IR58025A × IR 671017-124-2-4**	8.33	65
23	**IR58025A × IR 67413-71-4-2-2**	6.67	65
24	**IR58025A × IR 67418-238-6-2-3-2**	10.00	62
25	**IR58025A × Vener 1 A**	11.67	81
26	**IR58025A × Upl-Ri-S**	6.67	37
27	**IR58025A × Giza 182**	11.83	83
28	**IR58025A × Giza 178**	8.83	60
29	**IR58025A × Rikuto Norin 22**	11.00	85
30	**IR58025A × Sakha 103**	5.33	30

Analysis of variance in the two seasons showed highly significant differences in allelopathic potential against *E. crus-galli* among the rice genotypes as shown in Table 3. In order to emphasis these results, heritability in broad sense was estimated in weed control and radial area characters and found to be 75.67% and 98.93%, respectively during 2010 season. These characters could be transferred in the genetic background of the varieties by involving such donor variation in the breeding programs. According to the behavior of the screened material, the Five varieties; Rikuto Norin22, IR67413-71-4-2-2, Giza182, IR65617-52-2-3-3-2-3 and Vener1A were used to generate the genetic materials for this study.

Table 3: Mean square estimates of the ordinary analysis for weed control and radial area

S.O.V	df	Radial area	Weed control
Rep	2	0.04 $^{n.s}$	0.001 $^{n.s}$
Treat	42	46.66 **	0.17 **
Crosses	29	4.87 **	0.02 **
Parents	12	56.49 **	0.19 **
P.vs cross	1	1131.13 **	4.02 **
Lines	2	20.53 **	0.04 **
Testers	9	70.75 **	0.25 **
L*T	18	-29.81 **	-0.10 **
Error	84	0.11	0.01
h^2.b.s %		98.93	75.67

n.s: not significant **: high significant

Jensen et al. (2008) showed that allelopathy in rice is a typical quantitative trait involving several loci and possibly some degrees of epistasis, as well as, the cultivar IAC 165 showed strong and consistent allelopathic activity against barnyardgrass, whereas Co 39 showed weakly allelopathy. The estimate of the broad-sense heritability for allelopathic activity was reasonably high with a value of 0.68.

Table 4: Mean square estimates of the ordinary analysis for yield and its component characters

Source of variance	d.f.	No. of panicles/ plant	Panicle length	Panicle Weight	Grain yield/ plant	No. of grains/ panicle	1000-grain weight	Seed set
Reps	2	1.03 $^{n.s}$	0.93 $^{n.s}$	0.21 $^{n.s}$	0.82 $^{n.s}$	0.75 $^{n.s}$	0.60 $^{n.s}$	0.67 $^{n.s}$
Entries	42	91.67 **	18.73 **	3.05 **	799.03 **	4946.35 **	91.29 **	828.47 **
Parents	12	11.17 **	2.08 **	0.29 **	80.33 **	574.83 **	11.58 **	40.98 **
Crosses	29	29.76 **	13.39 **	3.73 **	447.50 **	3710.58 **	63.18 **	1758.50 **
P* V.C	1	2939.68 **	524.85 **	72.77 **	23561.6 **	133991.9**	2701.75**	12398.08**
Lines	2	7.00 **	5.34 **	0.52 **	37.00 **	1533.00 **	0.78 **	133.00 **
Testers	9	38.12 **	16.66 **	4.86 **	588.44 **	4606.77 **	84.07 **	2315.11 **
L*T	18	1.84 **	5.57 **	2.02 **	168.91 **	1547.61 **	23.46 **	1106.30 **
Error	84	2.71	0.46	0.02	27.34	149.48	0.44	1.26

The results summarized in Table 4 revealed that highly significant differences were recorded among treatments, parents, crosses, parent vs. crosses, lines, testers and line x testers for most of yield and its components characters i.e., panicle length, panicle weight, No. of panicles per plant, number of grains per panicle, 1000-grain weight, grain yield per plant and seed set %. On the contrary, insignificant differences among reps for all yield and its component characters were recorded.

From the above results which are summarized in Table 4, it could be conducted that the pedigree method can be used to improve all traits under study and develop the new hybrid rice verities. While, the mean square values of all characters were highly significant for, treatments, parents, crosses, parent vs. crosses, lines, testers and lines x testers. Similar results were obtained by (*El-Mowafi et al., 2005*). He estimated heterosis and combining ability of ten cytoplasmic genetic male sterile lines and five Egyptian testers (restorers) by using line x tester analysis for some agronomic characters and yield and its components to obtain useful information for hybrid rice program in Egypt. Among the ten cytoplasmic male sterile lines (CMS), IR58025A, IR69625A, IR68899A, and IR69625A were the best general combiners for grain yield. But, the restorers, Giza 178 and Giza182 were the best general combiners among the testers for grain yield and most studied characters.

In addition, the highly significant mean squares of lines x testers for all traits indicated that they interacted and produced more kindly differences in combining ability effects, indicating the presented wide genetic diversity among the lines and testers.

The interactions of parental lines and the resultant crosses were significant for all traits under investigation, indicating that the average heterosis overall crosses of these traits was inconsistent. It could therefore be concluded that the test of potential parents for the expression of heterosis would be necessarily conducting over a number of environmental conditions. Also, genetic diversity alone would not guarantee the expression of heterosis but the suitability of the environmental conditions would be required.

Estimation of the genetic components and heritability in broad and narrow senses

The genetic variance components i.e. additive genetic variance and dominance genetic variance were estimated for all traits under study. Also, these components were used to compute the heritability estimates in broad and narrow senses. The results presented in Tables 5 and 6 show the estimated values of genetic components and heritability in broad and narrow senses for allelopathic characters and yield and its component characters, respectively.

Allelopathic characters

As shown in Table 5, the partitioning of genetic variance for allelopathic characters, recorded high estimates of dominance component of variance in comparison with the additive genetic variance and ranged between 0.030 to 9.900 for weed control and radial area, respectively.

As well as, for weed control as an example for allelopathic characters, the data in Table 5 showed that the dominance genetic variance as a portion of the total genetic variance was larger than the additive genetic variance. Their respective values were 0.030 and 0.001. These results indicated that, the two genetic variance components might be important in the inheritance of weed control, whereas the dominance genetic variance played the more important role in this case.

Table 5: Estimates of genetic parameters and heritability for the studied allelopathic characters

Source	Radial area	Weed control
Additive gene	0.317	0.001
Dominance gene	9.900	0.030
Environment effect	0.110	0.010
Heritability broad %	98.93	75.67
Heritability narrow %	3.10	2.68

Regarding heritability estimates, Table 5 illustrates that high value 75.67% was recorded in broad sense. On the other hand, very low narrow sense heritability value of 2.68% was estimated. These findings were in agreement with those obtained earlier from the partitioning of genetic variance in this study. These results indicated that this character was influenced by environmental effect. This means that selection for allelopathic characters might be practiced successfully in late generations.

Yield and its component characters

As shown in Table 6, the partitioning of genetic variance for all yield characters, recorded high estimates of dominance component of variance in comparison with the additive genetic variance and ranged between 0.043 to 466.040 for number of panicles per plant and number of grains per panicle, respectively.

Table 6: Estimates of genetic parameters and heritability for yield and its components

Source	No. of panicles / plant	Panicle length	Panicle weight	Grain yield / plant	No. of grains / panicle	1000-grain weight	Seed set
Additive gene	0.364	0.096	0.020	3.464	26.630	0.494	5.410
Dominance gene	0.043	1.703	0.670	47.190	466.040	7.670	368.350
Environment effect	1.710	0.460	0.02	27.340	149.480	0.440	1.260
Heritability broad %	19.23	79.64	97.18	64.95	76.72	94.89	99.66
Heritability narrow %	17.19	4.25	2.82	4.44	4.15	5.74	1.44

In general, panicle length as an example for yield and its component characters, the data in Table 6 showed that, the dominance genetic variance as a portion of the total genetic variance was larger than the additive genetic variance. Their respective values were 1.703 and 0.096. These results indicated that the two genetic variance components might be important in the inheritance of panicle length, whereas the dominance genetic variance played the more important role in this case.

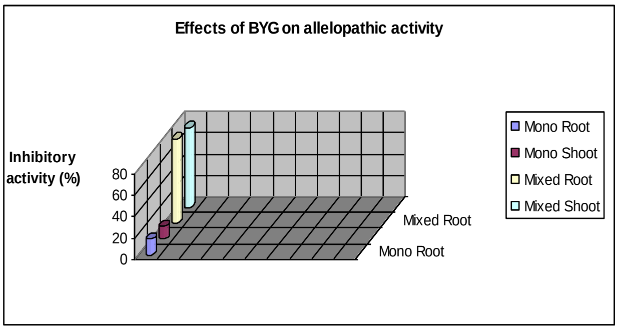
Regarding heritability estimates, Table 6 illustrates that high value of 79.64% was determined in broad sense. On the other hand, very low narrow sense heritability value of 4.25% was estimated. These findings were in agreement with those obtained earlier from the partitioning of genetic variance in this study. These results indicated that, this character was influenced by environmental effect. This means that, selection for panicle length might be practiced successfully in late generations.

Effects of barnyardgrass on the allelopathic activity and momilactone B concentration in rice

Rice seedlings of Rikuto Norin22 variety were incubated with (mix-incubation) or without (mono-incubation) barnyardgrass seedlings for 10 days, and allelopathic activity of rice extracts was determined by barnyardgrass bioassay (Fig.1A). The extracts of mono-incubated rice inhibited the growth of barnyardgrass roots and shoots by 15 and 12%, respectively, and the extracts of mixed-incubated rice inhibited the growth of barnyardgrass roots and shoots by 79 and 75%, respectively. Thus, the mixed-incubation induced 5.3– 6.3-fold increases in allelopathic activity in the rice extracts against barnyardgrass roots and shoots, respectively.

The concentration of momilactone B in mono-incubated rice seedlings was 2.7nmol/seedling and that in mixed-incubated rice seedlings was 18.6nmol/seedling. Thus, the concentration was 6.9-fold greater in mixed-incubated rice seedlings than in mono-incubated rice seedlings (Fig.1B). This result suggests that the production of momilactone B in rice seedlings may be increased by the presence of barnyardgrass seedlings. Momilactone B inhibited the growth of roots and shoots of barnyardgrass at concentrations greater than 1µmol/L. The effectiveness of momilactone B on the growth inhibition of rice seedlings themselves was less than 1% of that on the growth inhibition of barnyardgrass (*Kato-Noguchi et al., 2008*). As described in "Introduction," it was reported that momilactone B among rice allelochemicals may play a critical role in rice allelopathy (*Kato-Noguchi et al., 2002, 2010 and Kato Noguchi, 2004*). Therefore, the increased concentration of momilactone B in rice seedlings under mixed-incubation with barnyardgrass (Fig.1B) may be associate with increased allelopathic activity of rice seedling (Fig.1A)

A



(A) and momilactone B concentration in rice (B).

B

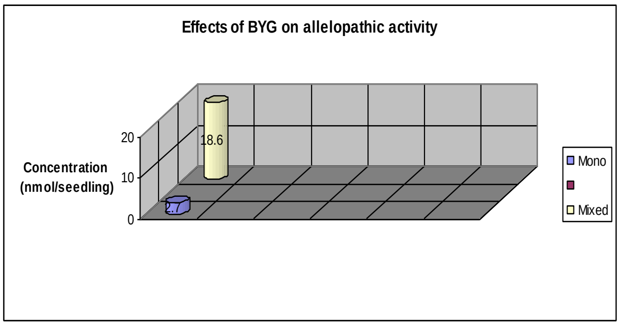


Fig.1. Effects of barnyardgrass on allelopathic activity

Effects of nutrient concentration in rice medium on the allelopathic activity and momilactone B concentration in rice

Rice seedlings were incubated without barnyardgrass seedlings in the media containing different nutrient concentrations for 10 days, and allelopathic activity of the rice extracts was determined by barnyardgrass bioassay (Fig.2). The allelopathic activity of rice extracts was increased significantly at nutrient concentrations lower than 0.1N. The extracts of rice seedlings incubated in 1N (normal strength) medium inhibited the growth of barnyardgrass roots and shoots by 16 and 14%, respectively, whereas the extracts of rice seedlings incubated in 0.03N medium inhibited the growth of barnyardgrass roots and shoots by 44 and 46 %, respectively.

Thus, allelopathic activities of rice seedlings incubated with 0.1N medium against barnyardgrass root and shoot growth were 2.6 and 3.1 fold greater than those of rice seedlings incubated with 1N medium, respectively. These results indicate that the growth conditions under nutrient starvation may increase allelopathic activity of rice seedlings. It was also reported that phosphate or nitrogen limited growth conditions increased allelopathic activity of rice (*Shen and Lin, 2007 and Song et al., 2008*). When rice and barnyardgrass are grown together, there may be greater competition for nutrients between rice and barnyardgrass. This competition may cause nutrient deficiency stress and thus increase the allelopathic activity of rice seedlings.

Concentrations of momilactone B in rice seedlings were increased significantly at nutrient concentrations lower than 0.1N (Fig.3). The momilactone B concentration in the rice seedlings incubated in 0.1N medium was 2.6-fold greater than that of rice seedlings incubated in 1N (normal strength) medium, which suggests that the growth conditions under low nutrient concentration may increase the momilactone B production in the rice seedlings. The elevated rice allelopathic activity by low nutrient conditions (Fig.2) may be caused by the accumulation of momilatone B in rice seedlings (Fig.3) because of the strong allelopathic activity of momilactone B against barnyardgrass (*Kato-Noguchi et al., 2008, 2010*).

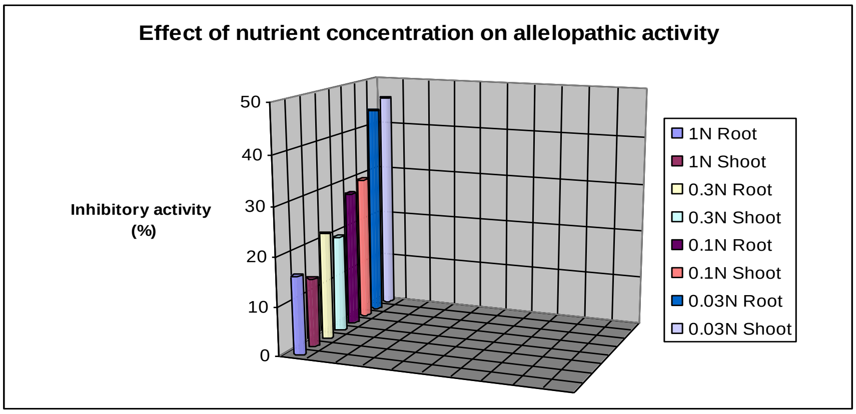


Fig.2. Effect of nutrient concentration in medium on allelopathic activity of rice.

However, inhibitory activities of rice seedlings incubated in 0.01N medium against barnyardgrass root and shoot growth, respectively, were still 1.9 and 1.7 fold less than those of rice seedlings incubated with barnyardgrass (Fig.1A). The momilactone B concentration in rice seedlings incubated with 0.01N medium was 2.7-fold less than in rice seedlings incubated with barnyardgrass (Fig.1B). Therefore, there may be some other mechanisms by which allelopathic activity and momilactone B concentration are increased in rice seedlings by mixed-incubation of rice with barnyardgrass, other than potential nutrient competition between two species.

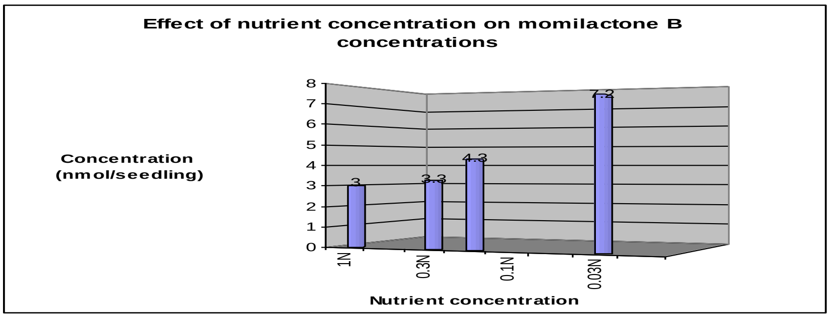


Fig.3. Effect of nutrient concentration in medium on momilactone B concentrations in rice.

Effects of barnyardgrass root exudates on the allelopathic activity and momilactone B in rice

Rice seedlings were incubated with barnyardgrass root exudates for 10 days, and allelopathic activity of rice extracts was determined by barnyardgrass bioassay. The presence of root exudates increased the allelopathic activity of the rice extracts at concentrations greater than 30mg/L of root exudates, and increasing the concentration increased the activity

(Fig.4). At concentration of 200 mg/L of root exudates, which was equivalent to that obtained from the incubation of 50 barnyardgrass, the inhibitory activity of rice extracts against barnyardgrass root and shoot growth was 76 and 74%, respectively. The root exudates of barnyardgrass also increased momilactone B at concentrations greater than 30mg/L of root exudates, and increasing the root exudate concentration increased the momilactone B concentration in rice seedlings (Fig.5).

At concentration of 200 mg/L of root exudates, momilactone B concentration in rice was 6.5-fold greater than that in the control (zero mg of root exudate). There was no significant difference in the osmotic potential between the medium containing barnyardgrass root exudates and control medium (all about 10 mmol/kg), and pH value of the medium was maintained at 6.0 throughout the experiments as described in the section of “Materials and methods”. These results suggest that chemical constituents in barnyardgrass root exudates may induce the allelopathic activity and momilactone B production in rice seedlings. Thus, the chemical constituents may affect gene expressions involved in momilactone B biosynthesis.

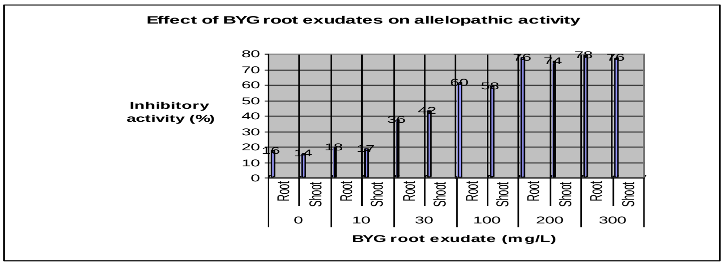


Fig.4. Effect of barnyardgrass root exudates on the allelopathic activity of rice.

Many plants are able to accumulate phytoalexins around infection sites of pathogens soon after sensing elicitors of pathogen origin. This accumulation of phytoalexins can protect the plant from further pathogen infection (*Pena-Cortés et al., 2005 and Okada et al., 2007*). Plants are also able to activate defense mechanisms against attacking herbivores by sensing volatile compounds, such as methacrolein and methyl jasmonate, released by herbivore attacked plant cells. Transcripton and signal cascade analyses of the volatile-exposed plants suggest that plants elicit various defenses such as increasing production of phenolics, alkaloids, terpenes, and defense proteins, which reduce further herbivory attacks (*Baldwin et al., 2006 and Ton et al., 2007*). Therefore, plants are able to elevate the defense mechanisms against several biotic stress conditions by detection of various compounds. Interestingly, momilactone B in rice was also increased by jasmonic acid treatment (*Kato-Noguchi, 2009*).

The chemical cross talk between host and symbiont plants is an essential process for the development of physical connections in symbiosis and parasitism (*Callaway, 2002; Palmer et al., 2004 and Bais et al., 2006*). Barnyardgrass is one of the most common and noxious weeds in rice paddy fields (*Xuan et al., 2006*). Although barnyardgrass is adapted rice production system due to its similarity in growth habit, the reason why barnyardgrass so often invades into the rice paddy fields is unknown. There might be some special interactions between both plant species. The present research suggests that rice may be aware of the presence of neighboring barnyardgrass by detection of certain key in barnyardgrass root exudates, and this sensorial function may trigger a signal cascade resulting in increasing rice allelopathy through increasing production of momilactone B. Therefore, rice allelopathy may potentially be an inducible defense mechanism by chemical-mediated plant interactions between rice and barnyardgrass.

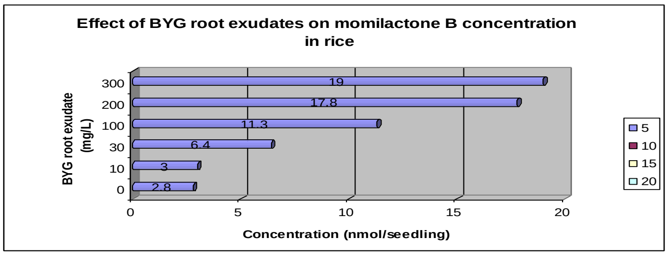


Fig.5. Effect of barnyardgrass root exudates on momilactone B concentration in rice.

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السلوك الوراثى للـ Momilactone B فى بعض سلالات الأرز الهجين

مدحت عراقى الدينارى[1] ، سعيد عبدالسلام درة[2] ، محمود إبراهيم أبويوسف[3] و عصام عادل الشامى[3]

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أجريت هذة التجربة لدراسة السلوك الوراثى للـ momilactone B فى بعض سلالات الأرز الهجين. و يتضح أن طريقة المقاومة الذاتية للحشائش تعتمد على النهج القائم على المنافسة بين الأرز والحشائش بعد تعرضة لتركيزات مختلفة من النيتروجين فى البيئة المائية. من خلال التجربة هناك بعض الهجن لديها قدرة عالية على المقاومة الذاتية للحشائش كما يلى IR69625AxRikutoNorin22, IR69625AxVener1A, IR69625AxIR65617-52-2-3-3-2-3 و IR69625AxGiza 182 وكانت القيم كما يلى بالتتابع 87% ، 85% ، 85% و 85% بالتتابع. وكانت أصناف الأرز المنزرعة IR65617-52-2-3-3-2-3, Giza182, IR67413-71-4-2-2, Rikuto Norin22, و Vener1A أظهرت مقاومة عالية ضد حشيشة الدنيبة خلال موسم 2009. وأوضحت النتائج أن التأثير الوراثى كبير فى حين أن التأثير البيئى غير معنوى. وأوضح الصنف Rikuto Norin22 قدرة عالية على المقاومة الذاتية للحشائش فى البيئة المائية المنزرع فيها خاصة عندما كان التركيز النيتروجينى أقل ما يمكن. وأيضا الصنف Giza 182 الذى لدية القدرة على أعادة الخصوبة أظهر قدرة فائقة على مقاومة الحشائش تحت ظروف الحقل.وقد أظهر الصنف Rikuto Norin22 قدرة عالية على مقاومة الحشائش بمعدل 5.3 – 6.6 مرة ضعف عندما تم زراعتة مع الحشائش فى بيئة واحدة. ومن خلال ما سبق وجد أيضا أن التركيز الكيماوى لمادة momilactone B 6.9 مرة ضعف عندما تم تنمية الصنف المنزرع مع الحشائش فى بيئة واحدة ، وأيضا الزراعة فى تركيز نيتروجينى منخفض يحفز المقاومة الذاتية للحشائش و يزيد التركيز الكيماوى للـ momilactone B فى بذور الارز أكثر من 30ملجم/لتر.

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