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Allelopathic Potential Evaluation of Rice Varieties on *Echinochloa crus-galli*

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돌피(*Echinochloa crus-galli*)에 대한 벼품종의 알레로파시 잠재성 평가

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ABSTRACT

Rice (*Oryza sativa* L.) contains water-soluble substances that inhibit the germination of *Echinochloa crus-galli*. This study investigated the allelopathic potential of rice in which rice had been grown with *E. crus-galli* in the field. Extracts of forty-seven rice varieties were screened for allelopathic potential in the laboratory. Double distilled water was used as a control. Based on the germination percentage, the varieties may be classified in the following order of decreasing inhibition : Nam-weon(36%) and Gyehwa(38%) hulls extracts and Sambaeg(43%) and Seoan(46%) leaves extracts. In the comparison of allelopathic activity test between leaves and hulls extracts, hulls extracts contain more allelopathic toxic substances. In the effect of concentration treatment on the six levels of extract solutions(3, 6, 12, 25, 50, 100 %, v/v) germination percentage was significantly inhibited as the extract concentration increased. The highest concentration(100 %) caused the greatest reduction in *E. crus-galli*. Allelopathic potential effects were significantly different among the various varieties and between '94 and '95 year extracts. The results indicate that there are genetic differences among varieties for allelopathic potentiality on *E. crus-galli*. Allelopathic potential in the ecological aspects can be decided through variety selection.

Key Words : Allelopathic Potential, Rice Extracts, *Echinochloa crus-galli* Germination Percentage

INTRODUCTION

Effective weed control programs for rice include preventive, cultural, mechanical, chemical, and ecological practices. The most recent and perhaps least exploited control practice is the ecological method. This method may be of importance in minimum tillage and zero tillage farming, where crop residues are left on the field soil surface after harvest as well as intensive cropping systems like intercropping and crop sequence.

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In recent years, one ecological strategy of weed control, allelopathy, has received increased attention. A number of weed scientists have attempted to exploit allelopathy directly as a weed management strategy through screening for allelopathic types in germplasm collections of crops. This characteristic appears to be incorporated into a cultivar by conventional plant breeding or other genetic recombination strategies. Superior weed-suppressing types have been reported in cucumber (*Cucumis sativus* L.)[9,11], oat (*Avena fatua*)[5], sunflower (*Helianthus annuus* L.)[8], soybean (*Glycine max* [L.] Merr.)[10,13], and sorghum (*Sorghum bicolor* L.)[1]. However, in some instances, the accessions showing good results in the laboratory or greenhouse were either less effective or not thoroughly tested in the field.

Putnam and Duke[11] evaluated the world collection of 500 cucumber accessions for growth suppression of proso millet (*Panicum miliaceum* L.) and white mustard (*Brassica hirta* Moench.). Of the plant introductions (PIs) tested in a controlled environment, one accession inhibited indicator plant growth 87 % and 25 others inhibited growth by 50% or more. Leachate transferred from several toxic PIs to proso millet grown in separate containers inhibited emergence and plant growth. It was demonstrated that within the world collection of cucumber there are accessions capable of biochemically inhibiting the growth of certain species in a relatively sterile media.

Lockerman and Putnam[9] discovered two cucumber accessions to be very effective in suppressing wild proso millet, barnyard grass (*E. crusagalli*[L.] P. Beauv.) and redroot pigweed (*Amaranthus retroflexus* L.) under field condition. Emergence of the weed species at 10 days after planting and weed fresh weight at harvest were significantly reduced by cucumber accessions that had demonstrable allelopathic properties in earlier leachate studies. And, the total overseed and volunteer weed population was reduced 54% when grown in association whit PI 169391. PI 169391 was approximately twice as effective as a standard cultivar in suppressing proso millet growth, but weed species were not inhibited consistently in the field. The allelopathic effect of cucumber was suppressed during periods of increased rainfall. Steinsiek et al.[15] reported that aqueous extracts of wheat inhibited the germination and seedling growth of six species of weeds. The degree of inhibition varied with the method of extraction of the wheat straw, the weed species tested, and the temperature at which seed germination and seedling root growth was observed. Of the species tested, ivyleaf morningglory (*Ipomoea hederacea*[L.] Jacq.) and velvetleaf (*Abutilon theophrasti* Medic.) were the most inhibited. These tests demonstrated that certain allelopathic plants appear to contain considerable selectivity against different species, but the results were not considered consistent enough to warrant a plant breeding effort.

At this time, there are no published references on improving allelopathic potential through genetic manipulation of crops. The purpose of this study was to determine which rice parts contain more allelopathic toxic substances, to evaluate the effects of aqueous extract concentration of hull extracts on barnyard grass germination, to screen varieties differences in allelopathic potential of rice and to compare the allelopathic yearly allelopathic potential variation. This study may provide plant breeders with information for selecting rice varieties that contain high concentrations of allelopathic compounds.

MATERIALS AND METHODS

Plant Sampling and Preparation of Extracts

Forty-seven entire mature rice varieties which were grown at the Department of Crop Science Farm, Kon-Kuk University were harvested in

October, 1994 and 1995. All rice varieties were separated into leaves and seed. The materials were dried at room temperature(24℃), and leaves and hulled were ground in a Wiley mill through a 40 mesh screen after seeds were hulled by milling machine and then stored at 5 ℃ until needed. Aqueous extracts(w/v) were prepared by extracting leaves and hulled ground materials(5g) with 100 ml distilled water in a water shaker for 1 hours. To compare with '94 and '95 year harvested hull extracts on the allelopathic toxicity were also extracted at the same concentration. Thus, the obtained extract gave a concentration of 5 % on a tissue dry weight basis.

Also, the bioassay concentrations of 3, 6, 12, 25, 50, 100 %(V/V) using hull extracts were made by appropriate dilutions of the 10 %(V/W) solution. These solutions were filtered through four layers of cheese cloth to remove the fiber and centrifuged at a low speed (3000 rpm) for 4 h and then, the supernatant was filtered through filter paper (Whatman No. 42). These solutions were filtered again by using a 0.45 μM Nalgene filterware unit (Becton Dickinson Labware, Lincoln Park, NJ) to obtain microorganism free extracts. pH (Beckman pH meter) on the all hull and leaves extracts was measured to give an indirect explanation on the two extract difference.

Seed Germination Test

Germination tests using extracts from forty seven varieties were performed in the following manner. Five grams of barnyard grass (*E. crus-galli*) which was harvested from rice field seeds were sterilized at the ratio of 1 : 10(Clorox : water) and were broken dormancy at 10 % (H_2SO_4) for 5 minutes and then rinsed several times with distilled water and were imbibed by soaking in distilled water for 12 h at room temperature(24 ℃). One hundred seeds were placed in sterilized 9 cm petri dishes with moistened filter paper (Whatman No. 1). Ten ml aqueous solution from each variety extract was added to petri dishes and covered. The petri dishes were then placed in a lighted growth chamber at 24 ℃. After 96 h, the percentage of germination was determined. If the root radicle protruded totally from the seed coat as observed with the naked eye, the seed was counted as a germinating seed. The control was treated with distilled water instead of an aqueous extract. Fungal contamination was not found during this experiment. Treatments were arranged in a completely randomized design with four replications. All method above mentioned was applied by some modification Chung and Miller's method[7].

Statistical Analysis

All of the above mentioned experiments were repeated twice. Analysis of variance for all data was accomplished using the general linear model procedure of the statistical analysis system program[14]. The pooled mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level. Since there was no significant difference between the all types of controls, these results were combined and the average used as a control for comparison.

RESULTS

Germination Test

The comparison between leaves and hull aqueous extracts on *E. crus-galli* seed germination are presented in Table 1. The germination percentage

Table 1. The comparison of *Echinochloa crus-galli* germination percentage between hulls and leaves extracts.

Extracts	Germination percentage (%)
Hulls	45.8
Leaves	71.3
LSD(0.05)	1.7

Table 2. Effect several concentration treatments on the *Echinochloa crus-galli* germination by days using rice hulls extract.

Concentration(%)	Day1	Day2	Day3	Day4	TG
			germination (%)		
3	33.3	13.5	1.2	0.7	97.4
6	29.3	13.5	2.6	0.7	92.2
12	25.3	19.3	2.7	0.8	96.3
25	16.1	22.2	5.4	1.5	90.3
50	10.3	21.2	7.5	2.2	82.4
100	3.6	11.6	10.1	5.8	62.1
Control	40.0	9.0	0.0	0.0	98.0
LSD(0.05)	2.8	2.5	1.5	0.8	6.08

by leaves and hull extracts was 71.3 % and 45.8 % respectively at the same(5 %) extract concentration. The degree of germination inhibition was statistically larger in the hull than in the leaves extracts. The effect of various concentration treatment by hulls extracts showed the maximum inhibition occurred at 100 % concentration treatments(Table 2). As the extract concentration progressively increased from 25 % to 100 % degree of reduction on total germination percentage increased significantly 90 %, 82 % and 62 %, and others extracts concentrations showed no significant effect on germination as compared to the control (Table 2). As indicated by the data in Table 3, all varieties hulls aqueous extracts significantly reduced the *E. crus-galli* seed germination as compared to the control. The lowest germination percentage, 36 % and 38 % was occurred Gyehwa and Namweon varieties hull extracts. The leaves extracts caused maximum 43 % and 46 % inhibition at the Sambaeg and Seoan extracts, repetitively. However, Donghae, Cheongmyeong and Hawyeong leaves extracts significantly stimulated *E. crus-galli* seed germination as compared to the control(Table 3). In the comparison test of '94 and '95 year extracts, '95 hull extracts was more effective to the *E. crus-galli* seed germination inhibition(Table 4).

DISCUSSION

In this study an attempt was made to evaluate the inhibitory qualities of rice varieties that are frequently grown in association with the *E. crus-galli* and to screen the inhibitory characteristics of extracts from separated rice leaves and hulls. Extracts from hull extracts solutions showed more significantly inhibitory effects than those of leaves on seed germination (Table 1). These results are similar to those of Ballester et al.[2] who reported that the most inhibitory effect of Erica plants was produced by reproductive tissue extracts.

The degree of germination inhibition increased with the extract concentration (Table 2). These inhibition responses were dependent upon the extract concentration and the source of the extract (Tables 1 and 2). These results were supported by the comparison of pH values from the leaves and hulled extracts implies a difference in chemical components between the extracts and the correlation relationship between hull and leaves extracts pH value on *E. crus-galli* germination (Tables 5 and 6). These data provides an indirect explanation for the different germination response to two extracts. The most extensive inhibition observed in this study occurred in treatments using a 100 % extract concentration. When using these bioassays

Table 3. Effect on *Echinochloa crus-galli* germination using hulls and leaves extracts.

Varieties	Hulls	Leaves
	%	%
Nagdong	49.0	70.0
Jinbuchal	53.3	80.3
Chucheong	41.8	82.8
Yeongdeog	43.3	72.8
Palgong	43.3	68.0
Daecheong	53.8	75.3
Dongjin	49.8	65.3
Hwaseong	48.8	79.3
Yeongsan	45.3	65.8
Hwajin	44.3	74.3
Seohae	49.8	78.3
Hwacheong	42.8	67.8
Hwaseonchal	50.3	63.0
Gancheok	49.3	78.8
Seoan	46.0	46.3
Tamjin	45.3	50.3
Donghae	45.8	83.5
Joryeong	46.3	74.8
Cheongmyeong	43.0	83.0
Sinkeumo	46.0	70.5
Hwajung	39.3	77.0
Sambaeg	42.3	42.5
Sangsan	44.0	67.3
Hawnam	43.3	65.0
Bonggwang	43.0	68.8
Yeomyung	41.8	74.0
Shinseonchal	49.8	73.0
Daegwan	41.8	52.3
Daeseong	40.0	77.5
Sinunbong	51.0	71.0
Namweon	36.3	73.3
Sangju	46.3	82.5
Obong	43.0	67.5
Hawyeong	42.8	85.3
Keumo	51.3	82.8
Unbong	45.3	80.5
Mankeum	45.8	73.3
Odae	48.8	80.3
Gyehwa	38.0	68.0
Sobaeg	47.3	73.8
Jinbu	47.0	80.0
Ilpum	41.0	71.8
Jinbuolbyeo	48.3	69.3
Dunnae	48.0	69.8
Chilseong	40.0	58.3
Yongmoon	43.0	64.0
Samgang	41.0	69.5
Control	74.8	74.8
CV(%)	5.3	7.9
LSD(0.05)	3.4	7.9

to screen allelopathic crops for weeds, it is advisable to insure that the concentration of the extracts is similar to the concentration in the natural ecosystem. Without authentic allelochemical separation and identification, only various concentration estimates can be suggested. The concentration of inhibitory substances of the extracts employed in this study is greater than what would be encountered under natural conditions because concentrations of as low as 25μg of phenolic compounds are inhibitory to seedling growth[3].

Variation of allelopathic activity exists among varieties. The activity of allelopathic potential varied among the rice varieties studied (Table 3). Among the different rice varieties extracted, Namweon and Gyehwa in the hull extracts and Sambaeg and Seoan in the leaves extracts were found to have the most inhibitory effect on germination. These variations could be attributed to the genetic differences among varieties, since all extracts used the same concentrations in this experiment. These high allelopathic potential varities might be used for the selection of cultivars with strong weed germination inhibitory ability in the crop-weed competition. It is possible that cultivars having good early seedling vigor may ultimately cause less input to control weeds in the rice field.

Environmental factors affect the amount of toxic substances produced in plants. This result is supported by germination test using the '94 and '95 hulls extracts (Table 4). Plants vary in their

Table 4. The effect of *Echinochloa crus-galli* germination percentage by two years rice hulls extracts.

Extracts	Germination percentage (%)
94' Hull extracts	68.5
95' Hull extracts	89.2
CV(%)	8.5
LSD(0.05)	2.1

Table 5. The comparison of pH value on hull and leaves extracts.

Varieties	Hulls	Leaves
Nagdong	6.20	6.79
Jinbuchal	5.77	7.19
Chucheong	6.16	6.75
Yeongdeog	6.12	6.94
Palgong	6.19	6.54
Daecheong	6.02	6.02
Dongjin	5.98	7.26
Hwaseong	5.96	6.72
Yeongsan	6.07	6.95
Hwajin	6.05	6.73
Seohae	5.90	7.21
Hwacheong	6.60	7.37
Hwaseonchal	5.91	6.64
Gancheok	6.11	7.34
Seoan	5.91	7.19
Tamjin	5.80	7.57
Donghae	6.00	7.35
Joryeong	5.78	6.64
Cheongmyeong	5.86	6.85
Sinkeumo	5.91	6.79
Hwajung	6.09	7.14
Sambaeg	6.03	6.94
Sangsan	5.88	7.36
Hawnam	5.85	6.99
Bonggwang	6.07	6.93
Yeomyung	5.94	6.91
Shinseonchal	6.13	7.01
Daegwan	5.77	7.20
Daeseong	5.75	7.10
Sinunbong	5.73	6.98
Namweon	5.47	6.98
Sangju	5.73	6.81
Obong	5.72	6.86
Hawyeong	6.01	6.92
Keumo	5.88	6.78
Unbong	5.63	6.86
Mankeum	5.92	6.75
Odae	5.68	6.88
Gyehwa	5.99	6.95
Sobaeg	5.63	6.71
Jinbu	5.90	6.45
Ilpum	6.14	7.01
Jinbuolbyeo	5.73	5.98
Dunnae	5.99	6.45
Chilseong	5.86	6.74
Yongmoon	5.92	6.94
Samgang	5.56	6.61
Control	6.76	6.64
CV(%)	0.47	0.96
LSD(0.05)	0.03	0.09

Table 6. The correlation relationship between hulls and leaves extracts pH value on *Echinochloa crus-galli* germination.

Extracts pH	Germination percentage (%)
Hulls extracts	0.396***
Leaves extracts	0.164**

production of allelopathic chemicals depending on the environment in which their are grown and stresses that they encounter as Rice[12] mentioned.

Purification and identification of suspected growth-inhibitory compounds from such extracts may be a better way of duplicating the conditions that occur in a natural ecosystem than testing individual extracts. Previous researchers have conducted chemical analysis of individual extracts and have identified several phenolic compounds[4,6,7]

In conclusion, this study indicates that variations of inhibitory effects exist among rice varieties and it is an inherited characteristic. Further investigation is needed to select variety showing high herbicidal activity under field conditions and identify the substances involved in allelopathy which would facilitate selection and breeding of varieties with such tolerance or resistance.

적 요

본 실험은 allelopathic물질을 함유한 것으로 알려진 국내 재배벼(Nagdong 외 46종)의 왕겨와 잎의 조 추출액을 이용하여 제초활성 벼품종 선발과 천연식물성 제초제를 개발하기 위한 기초 연구로서 피(*E. crus-galli*)의 발아에 대한 영향을 조사하기 위하였다.

피 종자에 대한 발아율은 왕겨 추출액의 경우 남원벼가 36%, 계화벼가 38%의 발아율을 보였으며, 잎 추출액은 삼백벼 43%과 서안벼 46%로서 가장 큰 억제 작용을 보였다.

왕겨 추출액과 잎 추출액의 피에 대한 발아 억제 정도 비교 실험에서는 왕겨 추출액이 46%로서 잎 추출액 71% 보다 더 효과적으로 작용하였다. 추출액의 농도처리 효과에서는 농도

가 증가됨에 따라서 발아율이 억제되었고 왕겨 추출액의 발아억제정도는 연차간의 차이를 보였다.

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