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Genetic Studies on the Relationship between Isozymes and Allelopathic Activity in Hybrid Rice

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Abstract

Genetic parameters play an important role in rice breeding for heterosis. Twenty three rice genotypes in line × tester model, comprising, three CMS lines; five testers and fifteen crosses were used to study the genetic of allelopathic activity, yield and related characters, all recommended agricultural practices were applied as usual for the ordinary rice field during seasons 2014 and 2015. Some crosses had high percentage of allelopathic activity as follow; IR69625A × Rikuto norin 22 and IR69625A × Giza 179, with values of 87 and 85 %, respectively. The varieties Rikuto norin 22 and Giza 179 exhibited the highest activity against *E. crus-galli*. The obtained results referred to high genetic effect controlling studied traits. The dominance genetic variances playing the major effect in the inheritance of weed control more than additive variance. The activities of POD from the samples of rice leaves were higher than those from rice roots. A potential allelopathic Rikuto norin-22 showed 0.20 $OD_{470} \cdot g^{-1}$ $FW \cdot min^{-1}$ activity of POD in the leaf parts, but significantly reduced 0.12 OD470/g FW/min in the roots. Among the five testers of rice, the potential allelopathic rice cultivars; Rikuto norine-22, HR 195 and Giza 179 showed stronger activities of POD than non-allelopathic varieties, Sakha 103 and Giza 178, in their leaves. The bands of EST isozyme were varied among the five varieties of rice, allelopathic rice variety, Rikuto norin-22, had six bands (as numbers) of A, B, C, D, E and F. Allelopathic rice variety Giza 179 had five bands of A, B, C, D and F. The EST of C was the characteristic brand of both allelopathic rice cultivars. However, the non-allelopathic rice cultivars had much less concentration of EST isozyme, it was absent in Sakha 103, the non-allelopathic rice cultivar. So, Potential allelopathic rice had greater activity of peroxidase isozyme and more spectrum bands of esterase isozyme than non-allelopathic rice varieties.

Keywords: rice, allelopathy, peroxidase, esterase, additive, dominance, heritability

1. Introduction

In Egypt, the average national yield level of rice has to be increased by 25 – 30 % to meet the demands of the increasing population. This seems difficult considering the narrow gap between potential yield and actual yield (12 t/ha) in 2015. However, among available technologies to increase yield above the present ceiling, the exploitation of heterosis in hybrid rice appears to be practical approach for Egypt. The cytoplasmic male sterile (CMS) lines are utilized for developing the popular "three line system" hybrids .The majority of the rice hybrids that are currently under commercial cultivation in the world derive their cytoplasm from the Wild Abortive (WA) source.

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Allelopathy in crops is one of the hot topics in agricultural ecology and chemo ecology. In recent years, rice allelopathy has been extensively studied as it is considered as one of the environment-friendly alternative approaches in weed control. Evidences in earlier studies have indicated that rice allelopathy is a quantitative trait, which is mediated by both genetic effects and environmental conditions [1]. In rice germplasm there were only a very few cultivars with allelopathic potential, and the difference in allelopathic potentials among cultivars is determined by the genotype. Breeding of rice cultivars for higher allelopathic potential might provide natural herbicides for growers [2]. In this study we collected samples from roots and leaves, from roots because the secretion of allelochemicals through it and from leaves because the activities of enzymes were higher as possible.

Isozymes technique has been an effective tool to study the life phenomena at the molecular level, and is generally applied in the study of genetics, heterosis, and physiology [3]. However, the reports about the study of rice allelopathy with this method were very few. The objective of this study was to study the relationship of the allelopathy to the isozymes by analyzing the isozymes of peroxidase and esterase of allelopathic rice cultivars and the non-allelopathic rice cultivars that were determined by bioassay and its relation to some yield characters in some hybrids using Line x Tester mating. Hence, application of plant activators to induce resistance or increase allelopathic potential has become a new strategy for weed control and been expected to be an alternative for effective and sustainable management.

2. Materials and Methods

2.1 Plant materials

This experiment was conducted at experimental farm of Rice Research and Training Center (RRTC), Sakha, Kafr El Shekh, and Pathology lab, Faculty of Agriculture, Kafrelsheikh University, Egypt, during 2014 and 2015 seasons. Twenty three genotypes using a line x tester model, which including three CMS lines, five testers and 15 crosses were used to study the allelopathic activity and yield components. The experimental design was RCBD in three replicates. 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.

Rice leaves at the stage of three to four leaves were collected from the following varieties; Rikuto norin-22, HR 195 and Giza 179 (have allelopathic activity) and from non-allelopathic rice varieties (Sakha 103 and Giza 178). The homogenates of the rice leaves were prepared in a mortar with the sample of 2 g and 2 ml of extracting reagent under ice-bath condition. Samples of supernatant were prepared by extracting and centrifuging with an equal quantity of 40% sucrose solution. Electrophoresis of peroxidase (POD) isozyme and esterase (EST) isozyme were conducted to show the relationship between the activities of isozymes and rice allelopathy.

2.2 Growth conditions

The rice seeds were sterilized with 1% NaCl (w/v) solution for 30 min, and then rinsed 3 to 4 times with sterilized water, soaked in water for 48h, then incubated at 28°. The pre-germinated seeds were cultivated in the greenhouse, and the seeds of barnyardgrass were inoculated in the rice pot. The rice leaves were picked at the stage of tillering for analyzing peroxidase and esterase.

The activity of peroxidase (POD) in the rice leaves was determined using a modification of the method of Zhang [4]. The 0.5g fresh rice leaves and roots were ground into homogenate with 50 ml phosphate buffer (pH 7.0) under ice-bath conditions. Crude peroxidase solution samples were obtained after centrifuge (10,000 rpm; at 4°) for 15 min with the 10 ml homogenates. The reaction included 1.9 ml acetate buffer (pH 5.0), 1 ml 0.1% guaicol, 0.01 ml crude peroxidase solution and 1 ml 0.08% H_2O_2. The OD_{470} values were investigated after 2 min reaction. Distilled water was used to substitute 0.08% H_2O_2 to rectify the spectrophotometer. The activities of POD were measured in the form of $OD_{470}\cdot g^{-1}FW\cdot min^{-1}$.

2.3 Analysis of peroxidase isozyme and esterase

The activity of the esterase (EST) in rice leaves was investigated using a modification of the method of Asperen [5] using α-naphthyl acetate esterase substrate with Fast Blue as staining solution. Solutions of α-naphthyl acetate esterase 200 mg solute in 10 ml 50% acetone, and blue RR salt 200mg solute in 90 ml 0.2 mol/L phosphate buffer (pH 7.0), were mixed together and filtered for evaluating the esterase activities. 1 ml esterase crude solution mixed with 5 ml staining solution for 1 h at 37°. The activities of esterase were measured in the form of $OD_{600}\cdot g^{-1}FW\cdot min^{-1}$.

2.4 Statistic analysis

All collected data were subjected to statistical analysis using ANOVA as described by Gomez and Gomez [6]. All statistical analyses were performed using analysis of variance technique by means of "MSTAT" computer soft ware package and “Quantitative genetic analysis”.

3. Results and Discussion

3.1 Allelopathic characters

Distinct differences in allelopathic potential were observed among the tested rice genotypes, ranging between 25-87% as shown in Table 1, and eight out of 15 rice germplasms showed allelopathic activity from 81-87% on *Echinochloa crus-galli* during 2014 and 2015 season. The parents were crossed with their CMS lines and produced 15 crosses with weed control ranging between 30-87% as shown in Table 2, the following varieties; Rikuto Norin 22, Giza 179, and HR 195 showed higher allelopathic activity during 2014 and 2015 season, their values were 87, 85, and 55 %, respectively as shown in Table 1. Dilday *et al*. [7] reported that the varieties Shmokita and Rikuto Norin22 provided at least 85 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 × Giza 179, and IR58025A × Rikuto Norin 22 and their values were 87, 85, and 85 %, respectively.

For radial area character, it has been found that the parents; Rikuto Norin-22 and Giza 179 had higher values among all parents used during 2014 and 2015 seasons as shown in Table 1. These parents were used to be crossed with their CMS lines and produced crosses with values ranging between 5.33–12.33cm^2, and the high desirable values which were expressed in; IR69625A × Rikuto Norin22 (12.33 cm^2), IR69625A × Giza 179 (11.33 cm^2) and IR70368A × Giza 179 (11.33 cm^2) during 2014 and 2015 seasons as shown in Table 2.

These results referred to highly genetic effect which controlled these traits since the environmental effect had no significance. In a similar study, El Shamey [8], found that the cultivars; Rikuto norine 22, IR 65617-52-2-3-3-2-3 and Vener 1A provided at least 70% weed control in transplanted rice against *E. crus-galli* under Egypt conditions.

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

No.	Parents	Radial area (cm^2)	Weed control %
1	Giza 179	11.37	85
2	HR 195	9.83	55
3	Giza 178	9.33	44
4	Rikuto norin 22	11.33	87
5	Sakha 103	2.30	25
6	IR 69625 A	5.33	39
7	IR 70368 A	6.17	46
8	IR 58025 A	7.33	50

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

No.	Crosses	Radial area (cm^2)	Weed control %
1	IR69625A × Giza 179	11.83	85
2	IR69625A × HR 195	10.33	57
3	IR69625A × Giza 178	8.17	65
4	IR69625A × Rikuto norin 22	12.33	87
5	IR69625A × Sakha 103	6.33	30
6	IR70368A × Giza 179	11.33	83
7	IR70368A × HR 195	9.67	51
8	IR70368A × Giza 178	6.33	65
9	IR70368A × Rikuto norin 22	10.17	81
10	IR70368A × Sakha 103	7.00	30
11	IR58025A × Giza 179	11.17	83
12	IR58025A × HR 195	10.67	53
13	IR58025A × Giza 178	8.83	60
14	IR58025A × Rikuto norin 22	11.00	85
15	IR58025A × Sakha 103	5.33	30

Analysis of variance of 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 71.34% and 97.83%, respectively during two seasons. These characters could be transferred into the genetic background of the varieties by breeding programs. According to the behavior of the screened material, three varieties; Giza 179, HR 195 and Rikuto norin-22 were used to generate the genetic materials for this study Jensen *et al.* [9] 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.

The results summarized in Table 3 revealed that highly significant differences were recorded among treatments, parents, crosses, parent vs. crosses, lines, testers and line x testers for all studied traits. Similar results were obtained by Abo-Youssef *et al.* [10]. In addition, the highly significant mean squares of lines x testers for all traits indicated that they interacted and produced high differences in combining ability effects, indicating the presented wide genetic diversity among the lines and testers.

The interactions of parental lines and the 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.

Table 3. Mean square estimates of the ordinary analysis for yield and its component characters and allelopathic 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	Radial area	Weed control
Reps	2	1.01 n.s	0.82 n.s	0.11 n.s	0.75 n.s	0.67 n.s	0.58 n.s	0.59 n.s	0.04 n.s	0.001 n.s
Entries	22	90.46 **	16.56 **	3.03 **	675.01 **	4759.4 **	89.21 **	765.42 **	42.66 **	0.16 **
Parents (p)	7	10.09 **	2.04 **	0.23 **	78.23 **	568.32 **	10.34 **	34.56 **	4.17 **	0.02 **
Crosses (C)	14	27.54 **	11.32 **	2.99 **	357.42 **	3542.3 **	58.23 **	1678.1 **	49.49 **	0.16 **
P. V.C	1	2786.3 **	489.7 **	69.45 **	22431 **	12125**	2609.8**	11890**	10.11 **	3.91 **
Lines (L)	2	6.78 **	4.23 **	0.49 **	36.65 **	1489.3 **	0.67 **	123.00 **	18.33 **	0.03 **
Testers (T)	4	37.23 **	15.23 **	3.99 **	564.22 **	4321.3 **	78.13 **	2134.1 **	59.45 **	0.28 **
L*T	8	1.81 **	4.51**	2.12 **	158.78 **	1479.4 **	22.65 **	1098.2 **	-21.31 **	-0.13 **
Error	44	2.71	0.46	0.02	27.34	149.48	0.44	1.26	0.11	0.01

Notes: **= highly significant value and n.s = non significant value.

3.2 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. The resultes presented in Table 4 showed that the dominance genetic variance was more important than additive genetic variance for all studied traits. Also, these components were used to compute the heritability estimates in broad and narrow senses. The results presented in Table 4 show the heritability in broad and narrow senses for allelopathic characters and yield and its component characters.

3.3 Yield and its component characters

As shown in Table 4, 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.

In general, panicle length was an example for yield and its component characters, the data in Table 4 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 for the inheritance of panicle length, whereas the dominance genetic variance played the more important role in this case.

Table 4. Estimates of genetic parameters and heritability for yield and its components and Allelopathic characters

Source	No. of panicles/ plant	Panicle length	Panicle weight	Grain yield/ plant	No. of grains/ panicle	1000-grain weight	Seed Set%	Radial area	Weed control
Additive gene	0.364	0.096	0.020	3.464	26.630	0.494	5.410	0.299	0.007
Dominance gene	0.043	1.703	0.670	47.190	466.040	7.670	368.35	10.140	1.730
Environment effect	2.71	0.46	0.02	27.34	149.48	0.44	1.26	0.110	0.010
Heritability broad %	19.23	79.64	97.18	64.95	76.72	94.89	99.66	97.83	71.34
Heritability narrow %	17.19	4.25	2.82	4.44	4.15	5.74	1.44	2.83	1.75

Regarding heritability estimates, Table 4 illustrates that high value of 99.66% was determined in broad sense. On the other hand, very low narrow sense heritability value of 1.44% was estimated for seed set percentage. These findings were in agreement with those obtained earlier from the partitioning of genetic variance in this study. These results indicated that, this character (number of panicles / plant) was influenced by environmental effect. This means that, selection for panicle length might be practiced successfully in late generations and the F1-hybrid should be provied.

3.4 Allelopathic characters

As shown in Table 4, the partitioning of genetic variance for allelopathic characters, recorded high estimates of dominance component of variance in comparison with the additive genetic variance with values 1.730 and 10.140 for weed control and radial area, respectively.

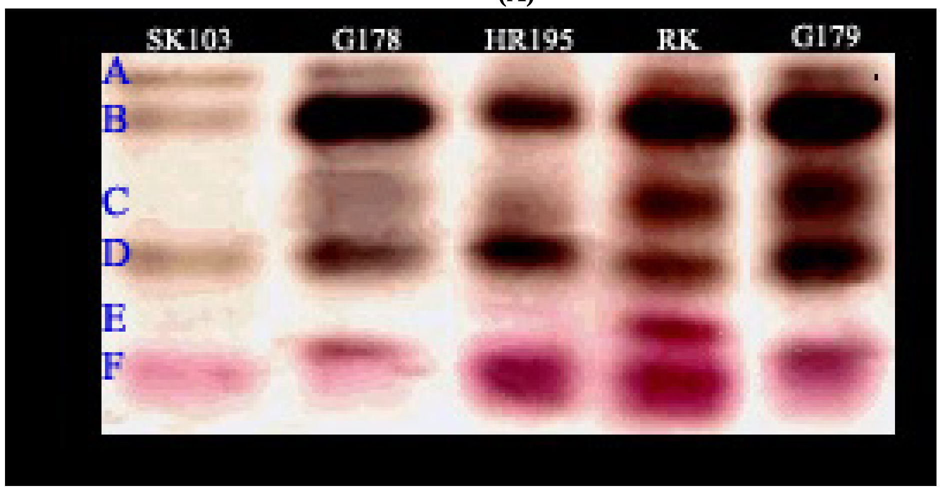
Also, for weed control as an example for allelopathic characters, the data in Table 4 showed that the dominance genetic variance as a portion of the total genetic variance was larger than the additive genetic variance. Their respective values were 1.730 and 0.007. These results indicated that the two genetic variance components might be important for the inheritance of weed control, whereas the dominance genetic variance played the more important role in this case.

Regarding heritability estimates, Table 4 illustrates that high value (71.34%) was recorded in broad sense. On the other hand, very low narrow sense heritability value of 1.75% 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 during pedigree method.

3.5 Analysis of the esterase activity (EST) in rice leaves

The activities of EST was the highest in variety Rikuto norin 22, and Giza 179. The activities of EST of these two allelopathic rice were higher if we compare with non-allelopathic rice cultivars Sakha 103, Giza 178. The allelopathic rice cultivar Rikuto norin 22 had six bands (A,B,C,D,E,F) after electrophoresis, and the allelopathic rice cultivar Giza 179 had five bands (A,B,C,D,F). Compared to the non-allelopathic rice cultuvars, Sakha 103 and Giza 178, the EST of C was the characteristic band of the allelopathic rice cultivars, which absent in the non-allelopathic rice, also, E band present in Rikuto norin 22 only as strong allelopathic cultuvar as shown in Figure 1. The same results obtained from Figure 2, the ideogram showed the estearase activity levels for studied varieties. The two varieties Rikuto norin 22 and Giza 179 scored the heist levels of esterase respectively, while the variety Sakha 103 scored the lowest level of esterase.

(A)



(B)

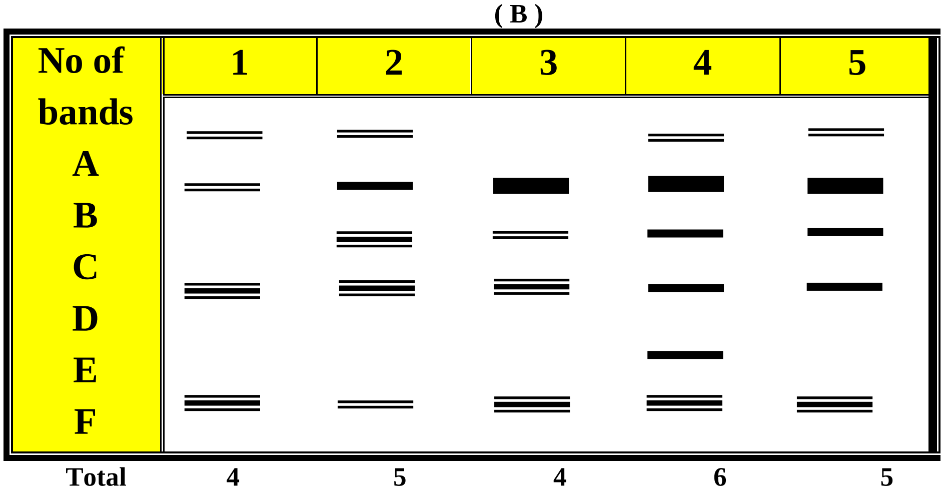


Figure 1. Isozyme zymogram (A) and diagram (B) of esterase to comparise among allelopathic and non-allelopathic rice varieties
1) SK.103, 2) G.178, 3)HR 195, 4) Rik and 5) G.179

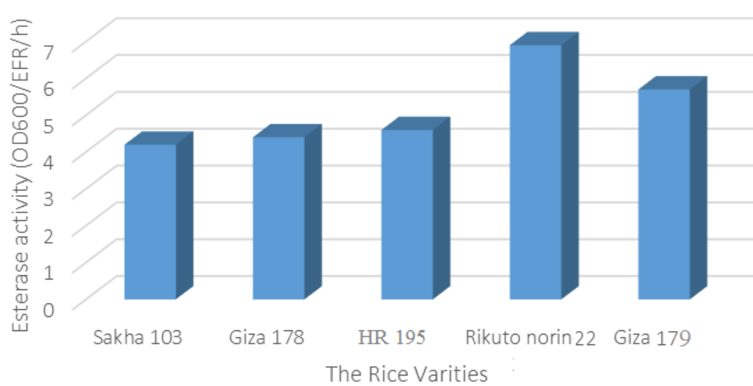
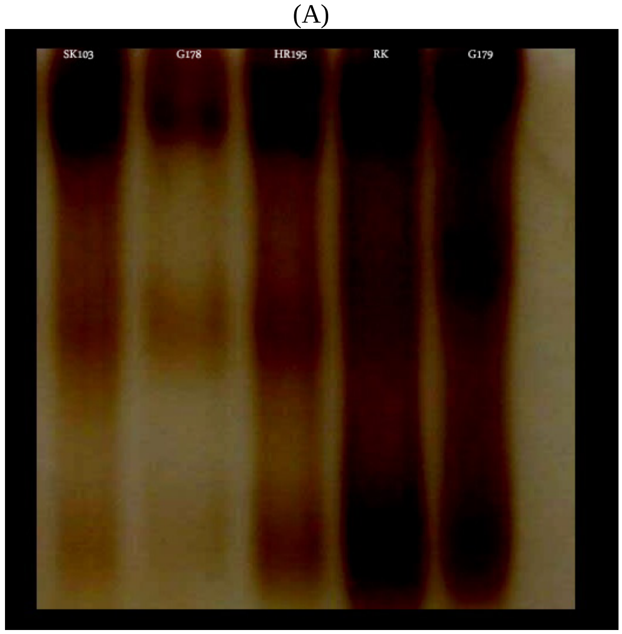


Figure 2. Esterase activity in rice among different varieties

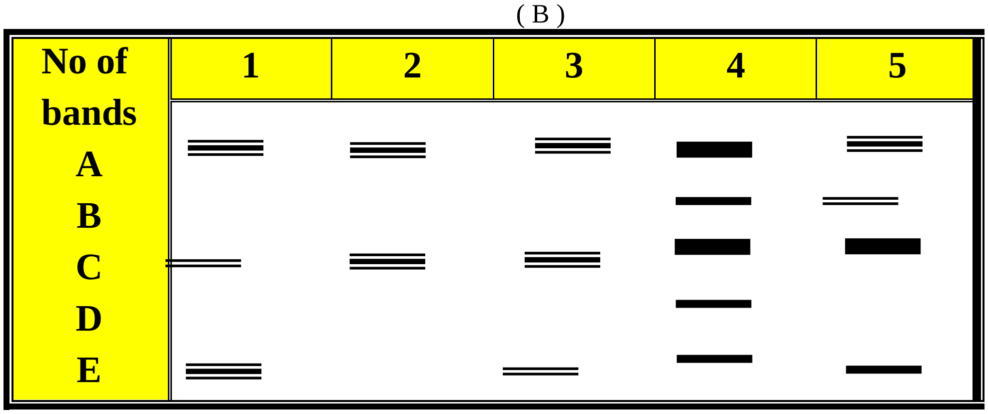
3.6 Analysis of the peroxidase activity (POD) in the rice leaves

The activities of the POD in the different rice cultivars were significantly different, the activities of Rikuto norin 22 was the highest, and that of Giza 179 was secondary, as shown in Figure 1, Numbers of bands of the POD in the rice from different cultivars on gel after electrophoresis were different, the allelopathic variety Rikuto norin 22 had five bands (A,B,C,D,E), and the Egyptian allelopathic rice variety Giza 179 had four bands (A,B,C,E). Compared to the non-allelopathic cultivars Sakha 103 and Giza 178, band B may be refere to allelopathic rice which both of the Rikuto norin-22 and Giza 179 had as shown in Figure 3. Figure 4 shows the Peroxidase activity levels for studied varieties, the results showed The two varieties Rikuto norin 22 and Giza 179 recorded the heist levels of Peroxidase respectively, while the varieties Giza 178 and Sakha 103 recorded the lowest levels of Peroxidase activity respectively.

(A)



(B)



Total 3 2 3 5 4

Figure 3. Isozyme zymogram (A) and diagram (B) of Peroxidase in the rice leaves to comparise among allelopathic and non-allelopathic rice varieties 1) SK.103, 2) G.178, 3) HR 195, 4) Rik and 5) G.179

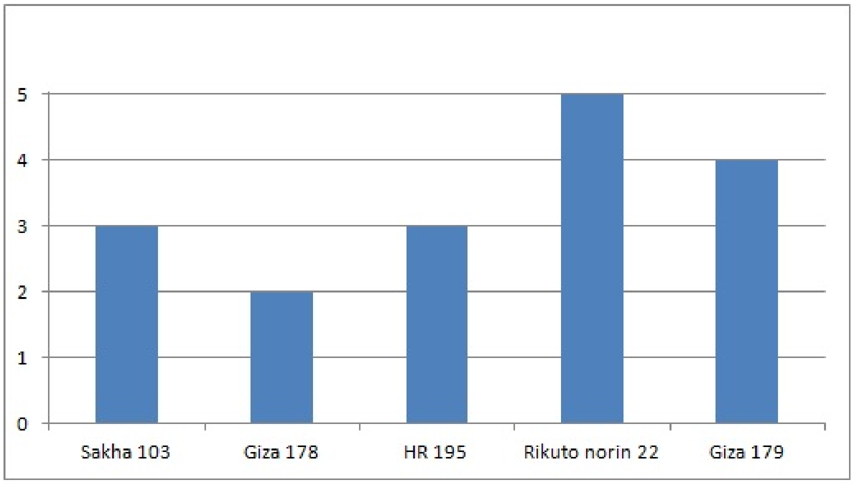


Figure 4. Peroxidase activity in rice among different varieties

Li *et al.* [11] evaluated the allelopathy of over 470 rice cultivars from different rice germplasm with the method of the root-length of barnyardgrass and the root-length of lettuce, and concluded that Japanese variety Rikuto norin 22, was a rice variety with allelopathic potential. Zhang *et al.* [12] identified Rikuto norin 22 as a rice variety with allelopathic potential using the method of allelopathic index (AI) with the specific secondary metabolites as HELP markers.

The allelopathic rice Giza 179 and Rikuto norin 22 had higher activities of POD and EST, both of them had the POD A and EST C bands, that did not exist in the non-allelopathic rice cultivars, indicating that the POD A and EST C bands represented the characteristics of the allelopathic potential rice cultivars and could be utilized in large scale evaluation of allelopathic activity in new developed rice genotypes.

4. Conclusions

In general, the dominance gene action playing the major role in inheritance of all yield and yield components traits under study, the studied allelopathic traits showed highly genetic variance effect. The varieties Rikuto norine-22 and Giza 179 and their crosses showed highly potential of allelopathy. The esterase and peroxidase analysis showed positive relation to emphases the importance of Rikuto norine-22 and Giza 179 in breeding for allelopathic ability.

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