

Field Evaluation and Molecular Detection of Allelopathic Potential for Some Rice Genotypes to Improve Weed Management and Grain Yield of Broadcast-Seeded Rice

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ABSTRACT

A field study was carried out at the Experimental Farm of Rice Research Dept., Sakha Agricultural Research Station, ARC, Egypt through 2016 and 2017 seasons to study allelopathic activity of selected rice genotypes to integrate with herbicides use under field conditions and to assess genetic diversity using SSR markers linked to allelopathic activity. Six rice entries were used in field study included MBG-41, HHZ-12-SAL8-Y1-Y2, FFZ-1, Weed tolerant-1, Sakha 106 and non-allelopathic check Sakha 101. Pre-mixed herbicide Top Shot 6% OD (penoxsulam 1% + cyhalofop-butyl 5%) was applied at recommended and half doses as compared with untreated (weedy check) plots. Sakha 106 and Weed tolerant-1 performed the best in weed control, yield and yield attributes in both seasons, while Sakha 101 recorded the highest dry weights of studied weeds and lowest values of rice dry weight as well as grain yield and its attributes during both seasons. The recommended dose of Top Shot 6% OD was the best as compared to either half dose or untreated plots. Sakha 106 as allelopathic rice cultivar had same behavior under both recommended and half doses of herbicide in controlling grasses, sedges, broad leaves and total weeds, and produced the highest rice dry weight, grain yield and its attributes in both seasons. For molecular study, Rikuto Norin22 was used as identified allelopathic genotype. Four previously identified SSR markers linked to allelopathic activity in rice were used to study genetic diversity among studied rice genotypes. A total number of 13 alleles were generated and number of alleles per locus varied from 2 to 5. Heterozygosity (H_e) values ranged from 0.490 to 0.735 with an average of 0.607. Polymorphic Information content (PIC) values ranged from 0.37 to 0.685. Genotypes were clustered based on genetic background and allelopathic activity. The results demonstrated the power of SSR markers in detecting molecular diversity as they separated indica from japonica genotypes. SSR 227 was able to detect Weed tolerant-1 and Rikuto Norin22 and Sakha106. This marker could be a potential candidate for MAS-based allelopathic selection.

Keywords: Allelopathic activity, Molecular diversity, Microsatellites, Cluster analysis.

INTRODUCTION

Rice is considered as the main source of calories for more than half of the world's population (Carrizo *et al.*, 2017). The per capita consumption of rice is more than 50 kg per year globally (FAOSTAT, 2016). The total harvested area in the world was approximately 167.25 million hectares produced about 769.66 million tons of paddy rice by average of 4.602 tons ha^{-1} (FAOSTAT, 2017). In Egypt, about 6.38 million tons of paddy rice was produced from 685,908 hectares by average of 9.302 ($t ha^{-1}$) as the highest yield from unit area over in the world (FAOSTAT, 2017).

Weed control is considered the most serious challenge for rice cultivation especially direct seeded rice. Weeds can directly or indirectly negatively affect rice yield (Mahajan *et al.*, 2009). Weeds increase rice production cost because of herbicides use in addition to degrading rice grain quality consequently net income of rice production.

Weed flora in direct-seeded rice is a complex of grassy weeds (*Echinochloa crus-galli*, *E. colona* and *Dinebra retroflexa*), sedges (*Cyperus difformis*) and broad leaf weeds. Thus, individual herbicide application through rice growing season in broadcast-seeded rice is not effective. This is because of the ability of some weeds to still survive and/or had a quick recovery again after herbicide application in addition to the new generations of weeds that can grow during different times in the same season. Moreover, herbicide-resistance can occur because of frequent application for the same herbicide at the same area every year. This leads to usage huge amounts of herbicides (more than 40 registered herbicides) in rice cultivation in Egypt (Egyptian Agricultural Pesticide Committee, 2017) which causes environmental pollution.

Allelopathic activity is defined as the direct or indirect harmful or beneficial effects of one plant to another plant through release of biochemicals, known as allelochemicals into the environment (Rice, 1984). Thus, allelopathy is a phytotoxic interference in most circumstances (Romeo, 2000). Allelochemicals are present in all plant parts such as the root, stem, leaf, bud and flower

(Inderjit, 1996). Under certain conditions, allelochemicals are released into the environment as exudates from living plants as well as decomposition of plant residues in abundant quantities to inhibit germination and growth of adjacent and successive plants (Seigler, 1996 and Einhellig, 1999).

Selecting or breeding for allelopathic rice cultivar which has the ability to prevent weed seeds germination or suppress their growth is an effective method to reduce herbicide use and pollution. Hassan *et al.*, (1995) identified allelopathic rice cultivar that inhibited root development and emergence of the first or second leaf of *Echinochloa crus-galli*. The high allelopathic potential rice cultivar may be effective against one weed or many weeds, so that it must be tested against every weed species to determine allelopathic activity of certain rice genotype.

Herbicide rates used in rice can be minimized by exploiting weed suppressive allelopathic rice cultivars (Gealy *et al.*, 2003) which may not add any extra cost (Jabran *et al.*, 2015). Allelopathy could be employed by integration with other cultural practices such as planting spaces, planting method and reduced herbicide rates (Shebl *et al.*, 2007 and Abd El-Razek *et al.*, 2014).

Molecular markers improve selection efficiency for desired genotypes by using markers closely linked to the desired traits. Some studies have identified QTLs controlling allelopathic activity in rice. Jensen *et al.* (2001) identified four QTLs located on the three chromosomes, 2, 3 and 8, and collectively explained 35% of the total phenotypic variation. Ebana *et al.* (2001) and Okuno and Ebana (2003) identified seven QTLs associated with the allelopathic effect with LOD scores higher than 2.0 and explained 16.1, 15.1, 13.2, 12.5, 10.4, 9.6, and 9.4% of phenotypic variation. Zeng *et al.* (2003) detected four QTLs related to rice allelopathic activity on chromosomes 3, 9, 10 and 12 with additive effects of 1.65, -1.44, 1.43 and -1.58, respectively. Lee *et al.* (2005) identified nine QTLs controlling allelopathic effects of rice on *E. crus-galli* on chromosomes 1, 2, 3, 4, 5, 8, 9 and 12. Of these, QTLs on chromosomes 1 and 5 were the most allelopathic and explained 36.5% of total phenotypic variation. Jensen

et al. (2008) identified two QTLs located on chromosomes 4 and 7 and explaining 20% of the phenotypic variation. El-Denary et al. (2016) reported that among ten SSR markers studied, RM439 and RM164 were able to distinguish the rice genotypes with strong allelopathic activities.

The present study aimed to evaluate possible allelopathic potential of some rice genotypes against common weeds and the integration with herbicides use in the field and assess genetic diversity among studied rice genotypes using SSR markers previously identified for allelopathic activity in rice, as well as employ allelopathy phenomenon to reduce herbicide use in rice.

MATERIALS AND METHODS

A- Field study:

A field study was conducted at the Experimental Farm of Rice Research Dept., Sakha Agricultural Research Station, ARC, Egypt during 2016 and 2017 summer seasons to study the role of allelopathic activity in reducing herbicide use for rice weed control in Egypt. Pre-germinated seeds of studied rice genotypes were broadcast-seeded at seed rate of 120 kg ha⁻¹ at 17th and 23th of May in both seasons, respectively. A split-plot design arranged in randomized complete block with three replications was used in both seasons. Main plots were devoted to the six rice genotypes while, the sub-plots included weed control treatments in both seasons. Plot size was 15 m² (3 x 5 m) in both seasons. The agricultural practices were applied as recommended for broadcast-seeded rice according to Rice Research & Training Center (RRTC, 2016).

Studied factors:

Rice genotypes:

Six rice genotypes were given by breeding program at Rice Dept., Field Crops Research Institute (FCRI), ARC, Giza, Egypt. Data on pedigree, origin and duration of studied rice genotypes are presented in Table (1). Allelopathic rice genotypes MBG-41, HHZ-12-SAL8-Y1-Y2, FFZ-1 and weed tolerant-1 were selected from allelopathy screening field, Rice Res. Dept., from the previous screening seasons, Sakha 106 was selected as high allelopathic potential cultivar (Abd El-Naby, 2013), while Sakha 101 was non-allelopathic check according previous screening of RRTC.

Table 1. Pedigree, origin and duration of utilized rice genotypes.

Rice genotype	Pedigree	Origin	Duration (days)
MBG-41	-----	Egypt	130
HHZ-12-SAL8-Y1-Y2	HUANG-HUA-ZHAN/TE QING	IRRI	132
FFZ-1	-----	China	129
Weed tolerant-1	-----	China	137
Sakha 106	Giza177 x Hexi 30	Egypt	125
Sakha 101	Giza176 x Milyng 79	Egypt	145

Weed control treatments were as follow:

- 1- Weedy check (untreated).
- 2- Top Shot 6% OD at 500 ml fed⁻¹ as a half dose (penoxsulam 5 g ai ha⁻¹ + cyhalofop-butyl 25 g ai ha⁻¹) at 15 days after seeding (DAS).
- 3- Top Shot 6% OD at (1 L fed⁻¹) at recommended dose (penoxsulam 10 g ai ha⁻¹ + cyhalofop-butyl 50 g ai ha⁻¹) at 15 DAS.

Top Shot 6% OD (penoxsulam 1% + cyhalofop-butyl 5%) as pre-mix herbicide was sprayed at 15 DAS in 300 liter water per hectare on wet land using Knapsack sprayer, then the soil was flooded after 24 hours from

herbicide application and water was kept for three days after herbicide treatment.

Data collection:

I- Weed measurements:

At 60 DAS; weeds were sampled from 50 x 50 cm, replicated four times for each plot. Weeds were cleaned, classified into species, and air dried for two days, then dried in the oven at 70 °C up to constant weights, and the average weight was recorded in g m⁻².

II- Rice growth measurements:

1- Dry weight of rice plants: was recorded at 60 DAS from four random samples (50 x 50 cm) of each plot, which was cleaned and weighed as fresh weight, then samples were air dried for two days, then oven dried at 70 °C up to constant weight and the average weight was recorded as g m⁻².

III- Grain yield and its attributes:

At harvest, the following data on rice plants were recorded.

- 1- Number of panicles m⁻²:** The average number of random 1/4 m² replicated four times was counted in each sub-plot and panicles m⁻² was recorded.
- 2- Panicle weight (g):** It was estimated by weighing random ten panicles per plot and their average was estimated.
- 3- Number of filled grains per panicle:** Average number of filled grains of random ten matured panicles was recorded.
- 4- Thousand-grain weight (g):** It was recorded in random samples from 1000-grain weight (g) for each sup-plot.
- 5- Grain yield (t ha⁻¹):** A central area of 6 m² for each sup-plot were manually harvested, air dried and thrashed, rice grain yield of each plot was adjusted to 14% moisture content and converted into tons per hectare.

Statistical analysis:

The collected data were subjected to proper statistical analysis of variance according to Snedecor and Cochran (1971). Weed data were statistically analyzed by *MSTAT-C* Program after transformed according to square-root transformation ($\sqrt{x + 0.5}$), while rice collected data were directly analyzed by *MSTAT-C* software then the means of both weeds and rice characteristics were compared by using Duncan's Multiple Range Test (Duncan, 1955).

B- Molecular study:

Molecular analysis

Genomic DNA was isolated from fresh leaves of the studied rice genotypes seedlings at 21 days old using CTAB method described by (Murray and Thompson, 1988) in Rice Biotechnology Lab., RRTC. The extracted DNA was quantified on 0.8% agarose gel comparing to known concentrations of uncut λ genomic DNA. The DNA concentration was adjusted to approximately 50 ng/ μ l. Four SSR markers linked to allelopathic activity were used. The sequences of primer pairs are found on the Web database (<http://www.gramene.org>). Primers names, repeat motifs, chromosome number are found in Table (9). PCR reactions were done in 15 μ l reaction mixtures, containing 1.5 μ l of template DNA, 1 μ l of each forward and reverse primer (10 pmole/ μ l), 7.5 μ l of 2X PCR master mix (Promega, USA) and 4 μ l ddH₂O. PCR profile was as following: initial denaturation step at 94°C for 5 min, followed by 35 cycles of amplification (denaturation at 94°C for 1 min, primer annealing at 55°C for 30 seconds and extension at 72°C for 1 min) with a final extension at 72°C for 7 min followed by storage at 4°C. PCR was

carried out using thermocycler machine from Biometra, Germany. Ten µl of DNA amplified products were loaded into 3 % agarose gel. using 1X TAE as running buffer and 50 bp DNA ladder (0.5 µg / µl, Fermentas) to determine the molecular size of the amplified fragments. Electrophoresis was conducted at 70 Volts for 2 hours.

Gels were then photographed using Biometra gel documentation unit (BioDoc, Biometra, Germany). The amplified fragments were scored for each marker based on the presence or absence of amplified fragments, generating a binary data matrix of 1 and 0 for each marker. Heterozygosity (H_e) and Polymorphic information content (PIC) for each SSR marker was calculated using POWERMARKER Ver3.25 (Lui and Muse, 2005). The data matrix was then analyzed using PAST, ver. 1.90 (Hammer *et al.*, 2001) to calculate Jaccard's similarity coefficients, and construct the phylogenetic tree using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

RESULTS AND DISCUSSION

A- Field study

Weeds:

Effect of rice genotypes

Data in Table (2) showed significant differences among rice genotypes effects for dry weight of studied weeds during 2016 and 2017 seasons. Allelopathic rice genotypes significantly reduced weed biomass more than non-allelopathic one (Sakha 101) in both seasons of study. Sakha 106 rice cultivar recorded the lowest dry weight of

Echinochloa crus-galli, *Cyperus difformis*, *Ammannia baccifera* and total weeds with no significant differences with Weed tolerant-1 rice genotype in both seasons. The FFZ-1 rice entry significantly equaled Sakha 106 and Weed-tolerant-1 in dry weight of *C. difformis* in 2016 season and total weeds in the second season. While the highest dry weights of studied weeds as well as total weeds dry weight were observed with Sakha 101 the non-allelopathic rice cultivar in the two seasons. These findings reflect high allelopathic potential of Sakha 106 and Weed tolerant-1 in suppressing germination of weed seeds and reducing growth of certain weeds, consequently providing a high weed management. It might be due to the released allelochemicals by both rice genotypes which transferred to weeds (recipient plant) and inhibited or minimized weed germination or/and decreasing both of shoot or root growth of undesirable weeds. Hassan *et al.*, (1995) found high allelopathic effect of rice plants in inhibiting root development and emergence of the first or second leaf of barnyard grass. In Egypt, about 4000 rice entries were evaluated in field, greenhouse and laboratory from 1993 to 2001 for allelopathic activity, fifty three rice genotypes self-controlled *E. crus-galli* by 20-90% in broadcast-seeded rice, seven rice entries controlled *C. difformis* by 50-70% and three rice entries self-controlled *Dinebra retroflexa* by 40-60% (Rice in Egypt, 2002). Dass *et al.*, (2017) reported that Japonica rice showed greater allelopathic activity than Indica and Indica- Japonica hybrids.

Table 2. Dry weight (g m⁻²) of *Echinochloa crus-galli*, *Cyperus difformis*, *Ammannia baccifera* and total weeds as affected by rice genotypes in 2016 and 2017 seasons.

Genotype	Dry weight (g m ⁻²)							
	2016 season				2017 season			
	<i>Echinochloa crus-galli</i>	<i>Cyperus difformis</i>	<i>Ammannia baccifera</i>	Total weeds	<i>Echinochloa crus-galli</i>	<i>Cyperus difformis</i>	<i>Ammannia baccifera</i>	Total weeds
MBG-41	227.86 (13.67 b)	95.04 (9.0 b)	18.82 (4.28 b)	341.72 (17.07 b)	194.53 (12.68 b)	47.67 (6.44 a)	13.61 (3.63 b)	255.81 (16.68 b)
HHZ-12-SAL8-Y1-Y2	97.83 (8.90 c)	66.16 (6.9 c)	4.06 (2.07 d)	168.06 (11.49 c)	77.74 (8.03 c)	29.11 (4.92 b)	2.61 (1.74 d)	109.47 (9.58 c)
FFZ-1	87.67 (8.26 cd)	19.90 (3.90 d)	5.94 (2.49 c)	113.51 (9.43 d)	65.11 (7.32 cd)	14.44 (3.07 c)	3.62 (2.02 c)	82.38 (8.1 d)
Weed tolerant-1	85.46 (8.19 cd)	15.56 (3.30 d)	4.16 (2.06 d)	105.18 (9.01 de)	59.07 (6.93 d)	10.39 (2.69 cd)	3.41 (1.87 cd)	72.83 (7.6 de)
Sakha 106	73.84 (7.58 d)	13.79 (3.14 d)	3.81 (1.97 d)	91.44 (8.39 e)	55.14 (6.73 d)	9.04 (2.54 d)	3.13 (1.78 d)	67.32 (7.34 e)
Sakha 101 (Non-allelopathic)	316.63 (16.23 a)	143.29 (10.64 a)	22.83 (4.58 a)	482.76 (19.99 a)	276.72 (15.18 a)	49.28 (6.46 a)	17.49 (4.07 a)	343.49 (16.97 a)
F. test	**	**	**	**	**	**	**	**

** indicate P < 0.01. In a column, means of transformed data followed by the same alphabetical letter are not significantly different at 5% level, using Duncan's Multiple Range Test (DMRT).

Effect of weed control treatments

As shown from data in Table (3), the application of pre-mix herbicide Top shot 6% OD at recommended and half doses markedly reduced dry weight of *E. crus-galli*, *C. difformis*, *A. baccifera* and total weeds as compared with un-treated plots for the two seasons.

Top Shot 6% OD at recommended rate (penoxsulam 10 g ai ha⁻¹ + cyhalofop-butyl 50 g ai ha⁻¹) achieved the best weed control and produced the lowest dry weight of studied weed flora as well as total dry weight of total weeds during 2016 and 2017 seasons followed by the application of half dose of the same herbicide. On the

other hand, weedy check plots recorded the highest values of *E. crus-galli*, *C. difformis*, *A. baccifera* and total weeds in both seasons of study. These results might be due to the high efficiency of such pre-mixed herbicide in controlling different species of weeds in broadcast-seeded rice by inhibition of cell-division and elongation, weakness of weed root growth (Raj and Elizabeth, 2015), and negative effect of penoxsulam on protein structure for weed growth by inhibition of ALS synthase enzyme (Kogan *et al.*, 2011). Sen *et al.*, (2018) reported that penoxsulam + cyhalofop butyl as a new mix-herbicide was effective in reducing dry biomass of sedges, grasses and broad leaf weeds, as well as higher growth, yield and yield attributes.

Table 3. Dry weight (g m⁻²) of *Echinochloa crus-galli*, *Cyperus difformis*, *Ammannia baccifera* and total weeds as affected by weed control treatments in 2016 and 2017 seasons.

Weed control treatment	Dry weight (g m ⁻²)							
	2016 season				2017 season			
	<i>Echinochloa crus-galli</i>	<i>Cyperus difformis</i>	<i>Ammannia baccifera</i>	Total weeds	<i>Echinochloa crus-galli</i>	<i>Cyperus difformis</i>	<i>Ammannia baccifera</i>	Total weeds
Weedy check	305.59 (17.02 a)	124.84 (10.38 a)	17.05 (3.92 a)	447.48 (20.43 a)	247.12 (15.10 a)	56.80 (7.31 a)	12.32 (3.34 a)	316.23 (17.09 a)
Top Shot 6% at half dose	111.97 (9.24 b)	44.97 (5.52 b)	8.51 (2.74 b)	165.45 (11.12 b)	93.59 (8.50 b)	18.48 (3.67 b)	6.64 (2.43 b)	118.72 (9.59 b)
Top Shot 6% at full dose	27.09 (5.20 c)	7.06 (2.54 c)	4.26 (2.06 c)	38.41 (6.11 c)	23.45 (4.83 c)	4.68 (2.08 c)	2.98 (1.79 c)	30.71 (5.46 c)
F. test	**	**	**	**	**	**	**	**

** indicate P < 0.01. In a column, means of transformed data followed by the same alphabetical letter are not significantly different at 5% level, using Duncan's Multiple Range Test (DMRT).

Effect of interaction between rice genotypes and weed control treatments.

The interaction between rice genotypes and weed control treatments obviously influenced dry weight of total weeds during 2016 and 2017 growing seasons as presented in Table (4).

The results showed that rice genotypes which have allelopathic effect *i.e.* Sakha 106, Weed tolerant-1, FFZ-1 and HHZ-12-SAL8 treated with the recommended rate of mixed-herbicide Top shot 6% achieved the best weed control and recorded the lowest dry biomass of total weeds. No significant differences between Sakha 106 allelopathic rice cultivar treated with Top shot 6% OD at half dose in the two seasons, as well as Weed tolerant-1 in the second season. The maximum values of

total weeds dry weight were obtained from un-treated plots cultivated with non-allelopathic rice cultivar Sakha 101 during both seasons. The high allelopathic activity of Sakha 106 and Weed tolerant-1 achieved best weed control and reduced herbicide dosage by 50%, thus allelopathy is considered effective aspect in weed management, reducing production cost and increase net income of rice crop. Similar results were obtained by Kim (2001), Shebl *et al.*, (2008), Duke (2010) and Abd El-Razek *et al.*, (2014). Xu *et al.*, (2018) concluded that planting allelopathic rice cultivars significantly decreased herbicides use and protect environment and biodiversity.

Table 4. Dry weight (g m⁻²) of total weeds as affected by interaction between rice genotypes and weed control treatments in 2016 and 2017 seasons.

Genotype	2016 season			2017 season		
	Weedy check	Top shot 6% at half dose	Top shot 6% at full dose	Weedy check	Top shot 6% at half dose	Top shot 6% at full dose
MBG-41	636.6 (25.2 b)	331.1 (18.2 e)	57.5 (7.6 i)	489.6 (22.1 b)	233.6 (15.3 d)	44.2 (6.7 hi)
HHZ-12-SAL8-Y1-Y2	393.5 (19.9 d)	77.8 (8.8 h)	32.9 (5.8 jk)	235.9 (15.4 d)	64.7 (8.0 g)	27.8 (5.3 jk)
FFZ-1	272.3 (16.5 f)	36.9 (6.1 j)	31.3 (5.7 jk)	193.6 (13.9 e)	32.9 (5.8 ij)	20.6 (4.6 k)
Weed tolerant-1	254.5 (16.0 fg)	35.9 (6.0 j)	25.1 (5.1 jk)	172.5 (13.2 ef)	26.7 (5.2 jk)	19.3 (4.5 k)
Sakha 106	221.6 (14.9 g)	32.2 (5.7 jk)	20.6 (4.6 k)	157.7 (12.6 f)	25.9 (5.1 jk)	18.4 (4.3 k)
Sakha 101 (Non-allelopathic)	906.4 (30.1 a)	478.9 (21.9 c)	63.0 (8.0 hi)	648.1 (25.4 a)	328.4 (18.1 c)	53.9 (7.4 gh)

Within a season, means transformed data followed by a common letter are not significantly different at 5% level, using Duncan's Multiple Range Test (DMRT).

Rice characteristics:

Effect of rice genotypes

Data on rice dry weight, grain yield and its attributes as influenced by rice genotypes during 2016 and 2017 seasons are presented in Tables (5 and 6).

Allelopathic rice cultivar Sakha 106 achieved the highest dry weight, number of panicles m⁻², panicle weight per panicle, 1000-grain weight and rice grain yield during both seasons of study. Sakha 106 significantly equaled with Weed tolerant-1 for number of panicles per unit area in the first and second seasons, as well as rice dry weight and thousand grain weight in 2017 growing season.

For number of filled grains per panicle, Weed tolerant-1 surpassed all the tested rice entries in this trait

during both seasons. On the other hand, the lowest dry weight, rice grain yield and its studied attributes were recorded by Sakha 101 as non-allelopathic rice cultivar through 2016 and 2017 seasons. The superiority of Sakha 106 and Weed tolerant-1 allelopathic rice genotypes might be due to the ability of such entries on reducing weed population and species by decreasing shoot and root systems growth, which resulted in better rice growth, producing more dry matter, more effective tillers, panicle weight and grain yield as a final task of previous condition. Shebl *et al.*, (2008) who showed that all tested allelopathic rice entries significantly reduced dry weight of *E. crus-galli* and recorded the highest number of panicles m⁻² and higher grain yield as compared to non-allelopathic rice

cultivar Giza 176. Abd El-Naby (2013) found that allelopathic rice genotypes minimized weed distribution, reduced competition and produced desirable growth

characteristics and enhanced yield. The obtained results are also in harmony with those obtained by Dass *et al.*, (2017).

Table 5. Dry weight (g m⁻²), Number of panicles m⁻² and panicle weight (g) of rice as affected by rice genotypes in 2016 and 2017 seasons.

Genotype	2016 season			2017 season		
	Rice dry weight (g m ⁻²)	Number of panicles m ⁻²	Panicle weight (g)	Rice dry weight (g m ⁻²)	Number of panicles m ⁻²	Panicle weight (g)
MBG-41	667.36 d	337.0 d	1.92 c	715.79 c	366.3 e	2.24 d
HHZ-12-SAL8-Y1-Y2	742.24 c	448.0 c	1.93 c	800.27 b	504.9 c	2.14 e
FFZ-1	841.62 b	471.1 bc	2.05 c	939.86 a	529.8 bc	2.32 c
Weed tolerant-1	856.12 b	483.6 ab	2.20 b	945.59 a	535.1 ab	2.47 b
Sakha 106	926.00 a	508.4 a	2.56 a	976.00 a	561.3 a	2.62 a
Sakha 101 (Non-allelopathic)	592.86 e	360.9 d	2.00 c	650.24 d	401.0 d	2.07 f
F. test	**	**	**	**	**	**

** indicate P < 0.01. In a column, means followed by the same alphabetical letter are not significantly different at 5% level, using Duncan's Multiple Range Test (DMRT).

Table 6. Number of filled grains per panicle, 1000-grain weight (g) and grain yield (t ha⁻¹) of rice as affected by rice genotypes in 2016 and 2017 seasons.

Genotype	2016 season			2017 season		
	No of filled grain per panicle	1000-grain weight (g)	Grain yield (t ha ⁻¹)	No of filled grain per panicle	1000-grain weight (g)	Grain yield (t ha ⁻¹)
MBG-41	91.9 c	20.67 de	5.760 d	96.1 c	22.67 b	6.024 d
HHZ-12-SAL8-Y1-Y2	97.3 b	20.56 e	7.158 c	99.8 b	21.56 c	7.648 c
FFZ-1	95.3 b	22.00 c	7.417 bc	100.6 b	23.00 b	7.944 bc
Weed tolerant-1	104.6 a	23.00 b	7.676 ab	107.2 a	24.11 a	8.149 ab
Sakha 106	98.3 b	24.06 a	7.978 a	99.0 bc	24.83 a	8.406 a
Sakha 101 (Non-allelopathic)	81.6 d	21.44 cd	5.150 e	88.1 d	22.78 b	5.516 e
F. test	**	**	**	**	**	**

** indicate P < 0.01. In a column, means followed by the same alphabetical letter are not significantly different at 5% level, using Duncan's Multiple Range Test (DMRT).

Effect of weed control treatments

It is obvious from data in Tables (7 and 8) that, weed control treatments significantly affected all studied traits of rice during 2016 and 2017 seasons.

The recommended rate of Top shot 6% achieved the highest values of rice dry weight, panicles m⁻², panicle weight, number of filled grains per panicle as well as 1000-grain weight and grain yield followed by the reduced rate of the herbicide in 2016 and 2017 seasons. Meanwhile untreated plots (weedy check) produced the lowest dry biomass, yield and yield attributes of rice through the first

and second seasons. These results indicates the high efficiency of such herbicide penoxsulam + cyhalofop butyl when applied at recommended dose in killing grasses, sedges and broad leaf weeds in early stage, decreasing weed competitiveness ability against rice plants on growth resources (nutrients, water, light and space) leading to better growth, yield and yield components of rice. These findings are in harmony with those reported by Hassan *et al.*, (2008), Kogan *et al.*, (2011) and Sen *et al.*, (2018).

Table 7. Dry weight (g m⁻²), Number of panicles m⁻² and panicle weight (g) of rice as affected by weed control treatments in 2016 and 2017 seasons.

Weed control treatment	2016 season			2017 season		
	Rice dry weight (g m ⁻²)	Number of panicles m ⁻²	Panicle weight (g)	Rice dry weight (g m ⁻²)	Number of panicles m ⁻²	Panicle weight (g)
Weedy check	481.03 c	279.2 c	1.70 c	527.40 c	364.7 c	1.89 c
Top Shot 6% at half dose	755.49 b	484.7 b	2.08 b	836.00 b	515.6 b	2.34 b
Top Shot 6% at full dose	1076.57 a	540.6 a	2.54 a	1150.48 a	568.9 a	2.69 a
F. test	**	**	**	**	**	**

** indicate P < 0.01. In a column, means followed by the same alphabetical letter are not significantly different at 5% level, using Duncan's Multiple Range Test (DMRT).

Table 8. Number of filled grains per panicle, 1000-grain weight (g) and grain yield (t ha⁻¹) of rice as affected by weed control treatments in 2016 and 2017 seasons.

Weed control treatment	2016 season			2017 season		
	Filled grain panicle ⁻¹	1000-grain weight (g)	Grain yield (t ha ⁻¹)	Filled grain panicle ⁻¹	1000-grain weight (g)	Grain yield (t ha ⁻¹)
Weedy check	82.2 c	19.86 c	3.149 c	85.8 c	20.89 c	3.904 c
Top Shot 6% at half dose	95.9 b	21.81 b	7.912 b	99.4 b	23.53 b	8.220 b
Top Shot 6% at full dose	106.4 a	24.19 a	9.508 a	110.2 a	25.06 a	9.719 a
F. test	**	**	**	**	**	**

** indicate P < 0.01. In a column, means followed by the same alphabetical letter are not significantly different at 5% level, using Duncan's Multiple Range Test (DMRT).

Effect of interaction between rice genotypes and weed control treatments.

As observed from data in Figures (1 and 2), significant interactions between rice genotypes and weed control treatments were observed for number of panicles m^{-2} and grain yield of rice during 2016 and 2017 seasons.

The application of mixed-herbicide penoxsulam + cyhalofop butyl at full dose with Sakha 106, Weed tolerant-1, FFZ-1 and Sakha 101 produced the largest number of panicles m^{-2} and grain yield with no significant differences with the combination of allelopathic cultivar Sakha 106 treated with Top shot 6% OD at half dose for both traits through 2016 and 2017 seasons. No significant differences were observed with Weed tolerant-1 rice entry under the application of Top shot 6% OD at half dose for

number of panicles m^{-2} in the first season. On the other side, the lowest number of panicles per unit area and grain yield of rice was achieved by un-treated plots cultivated with Sakha 101 and MBG-41 rice entries for grain yield in 2016 and 2017 growing seasons. These results reflect the importance of allelopathic rice genotypes to reduce herbicide use for weed management, moreover allelopathic rice decrease production cost and save environment by reducing herbicide pollution, in addition it increase net return from rice production. These findings are in agreement with those observed by Shebl *et al.*, (2008), Abd El-Naby (2013), Abd El-Razek *et al.*, (2014) and Dass *et al.*, (2017).

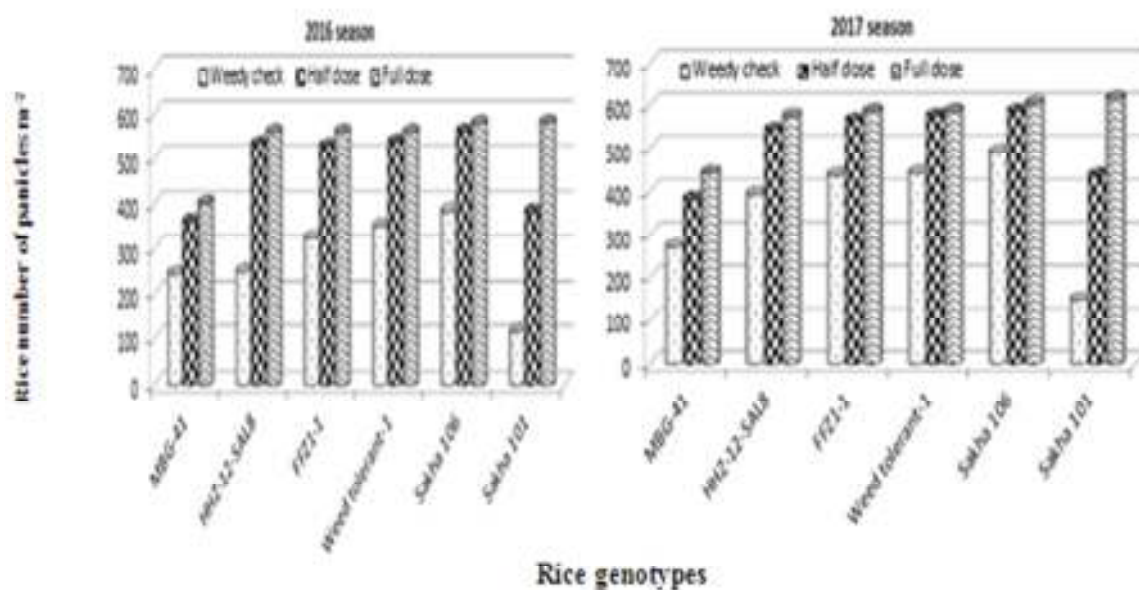


Figure 1. Effect of the interaction between rice genotypes and weed control treatments on rice number of panicles m^{-2} in 2016 and 2017 seasons.

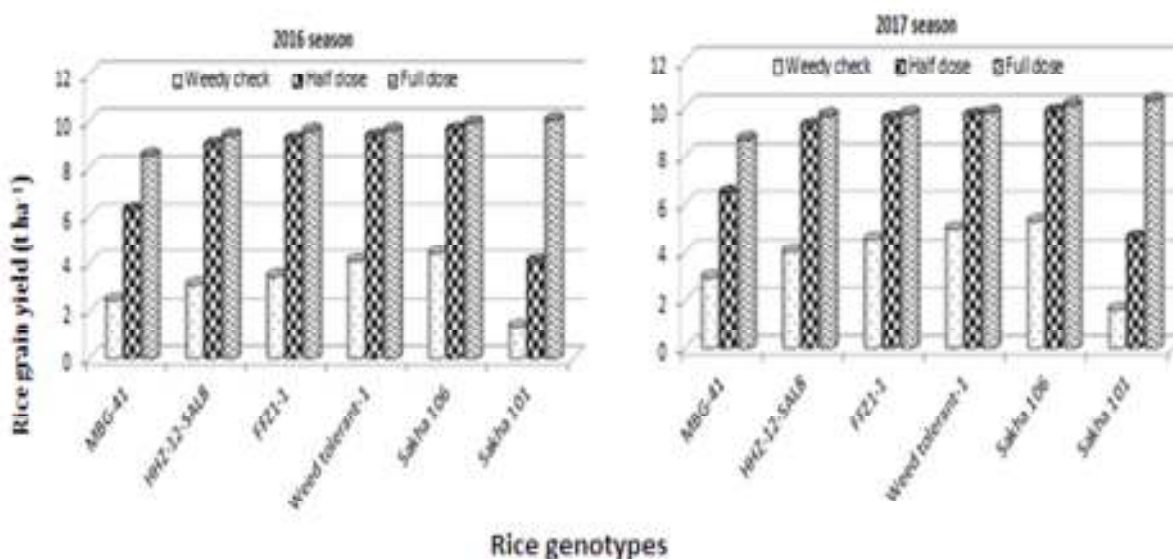


Figure 2. Effect of the interaction between rice genotypes and weed control treatments on rice grain yield ($t ha^{-1}$) in 2016 and 2017 seasons.

B- Molecular analysis

Results of SSR analysis are presented in Table (9). A total number of 13 alleles were generated by SSR markers across the studied rice genotypes. The number of alleles per locus for each marker varied from 2 to 5 with an average of 3.25 alleles per locus. RM164 generated the highest number of alleles (5), while both RM335 and RM511 generated the lowest number (2).

Heterozygosity (H_e) or gene diversity of a specific locus is defined as the probability that an individual is heterozygous for this locus in the studied population (Liu, 1998). The higher the H_e value, the more informative the studied locus. As observed in Table (9), H_e values ranged from 0.490 to 0.735 with an average of 0.607. The highest values were observed for RM227 (0.735) followed by RM164 (0.622). Similar findings for high H_e values were obtained by Ramadan *et al.* (2015). PIC value estimates the discriminating power of the DNA marker (Nagy *et al.*, 2012). As shown in Table (9), the highest PIC value of 0.685 was observed for RM227. Significant correlation coefficient ($r = 0.977^*$) was estimated between PIC values

for SSR markers and number of alleles for each marker. This result is in agreement with Lapitan *et al.* (2007). Ramadan *et al.* (2015) reported wide range of PIC values varied from 0.21 to 0.79 with an average of 0.46. Figure (3), indicated the amplified polymorphic alleles among the studied rice genotypes using four SSR markers. Among SSR used in the current study, RM227 was able to distinguish the varieties with allelopathic properties from the other genotypes. Both Japonica rice varieties Rikuto Norin22 and Sakha106 had specific allele with molecular size of 113 bp. In addition, another allele with molecular size of 135 bp was found in the indica rice variety Weed Tolerant-1. The other rice genotypes had different alleles with different molecular sizes. This result is in agreement with Jensen *et al.* (2001) who identified four QTLs controlling allelopathic potential in recombinant inbred lines (RILs) mapping population derived from a cross between the *japonica* cultivar with strong allelopathic activity IAC 165 and the indica rice variety with weakly allelopathic activity. CO 39. One of these identified QTLs was flanked by both SSR markers RM16 and RM227.

Table 9. List and results summary of SSR markers used in current study.

SSR Marker	Ch. No.	Repeat motif	Primer sequences (3' → 5')	Band size (bp)	No of alleles	H_e	PIC
RM335	4	(CTT)25	(F) GTACACACCCACATCGAGAAG (R) GCTCTATGCGAGTATCCATGG	194-200	2	0.490	0.370
RM511	12	(GAC)7	(F) CTTCGATCCGGTGACGAC (R) AACGAAAGCGAAGCTGTCTC	122-125	2	0.490	0.370
RM227	3	(CT)10	(F) ACCTTTCGTCATAAAGACGAG (R) GATTGGAGAGAAAAGAAGCC	113-150	4	0.735	0.685
RM164	5	(GT)16TT (GT)4	(F) TCTTGCCCGTCACTGCAGATATCC (R) GCAGCCCTAATGCTACAATTCTTC	262-327	5	0.714	0.671

Table (10) indicated the Jaccard's similarity coefficients among all studied rice genotypes. It was observed that the similarity values ranged between zero and 0.8 with an average value of 0.213. This result indicated the presence of high level of diversity among the studied genotypes. The highest similarity coefficient value was obtained between both *indica* genotypes HHZ12-SAL8-Y1-Y2 and FFZ-1. While, the lowest similarity coefficient value was found between both of *japonica* genotypes Rikuto Norin22 and Sakha101 from one side and the *indica* genotypes Weed Tolerant-1, HHZ12-SAL8-Y1-Y2 and FFZ-1 from the other side. Similar results were reported by Chakravarthi and Naravaneni (2006), Ramadan *et al.* (2015), Salgotra *et al.* (2015) and Ammar (2017) who observed wide range of genetic similarity values among rice genotypes

The genetic relationships among the studied rice genotypes were constructed using UPGMA method (Fig. 4). All studied genotypes grouped into two major groups at similarity coefficient of 4% based on Jaccard's similarity index. The first major cluster A consisted of the 3 *indica*

rice genotypes, while the second major cluster B formed from the 4 *japonica* rice genotypes. This result indicated the potential of SSR markers to distinguish *indica* from *japonica* rice genotypes. The main cluster A in the dendrogram was subdivided into two subclusters A1 and A2 at similarity value of 27%. The subcluster A1 included the allelopathic variety Weed tolerant-1, meanwhile the subcluster A2 included both genotypes HHZ 12-SAL8-Y1-Y2 and FFZ-1. Similarly, the main cluster B was also grouped into two subclusters, B1, B2, at about similarity value of 34%. The subcluster B1 included both japonica rice genotypes Sakha101 and MBG-41, while the subcluster B2 included both allelopathic varieties Sakha106 and Rikuto Norin22. These results indicated the power of used SSR markers to distinguish indica and japonica genotypes. The clustering of genotypes was largely based on genetic constitution and allelopathic activity. Similar results were obtained by Zeng *et al.* (2004), El-Malky *et al.* (2007), Zhu *et al.* (2012) and Ramadan *et al.* (2015) who reported the ability of SSR markers to identify the indica rice genotypes from the japonica ones.

Table 10. Jaccard's similarity coefficients among studied rice genotypes based on SSR markers

	Rikuto Norin22	Sakha 106	Sakha 101	MBG-41	Weed tolerant-1	HHZ12-SAL8-Y1-Y2
Sakha106	0.600					
Sakha101	0.600	0.333				
MBG-41	0.333	0.143	0.600			
Weed Tolerant-1	0.000	0.125	0.000	0.125		
HHZ12-SAL8-Y1-Y2	0.000	0.125	0.000	0.125	0.250	
FFZ-1	0.000	0.143	0.000	0.143	0.286	0.800

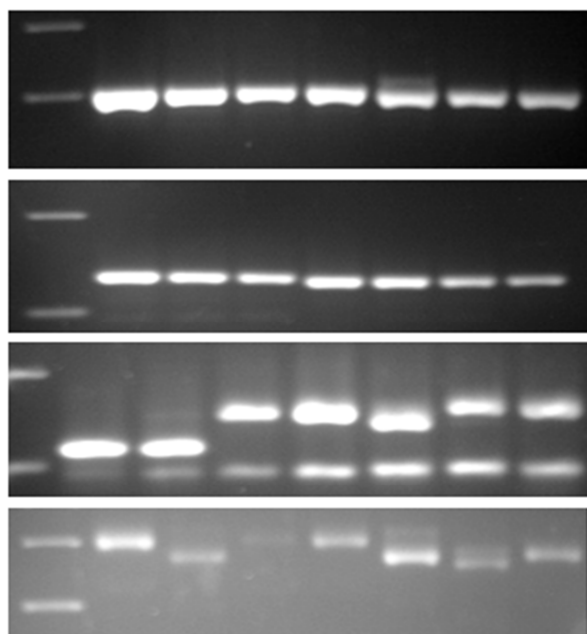


Figure 3. Agarose gel electrophoresis of PCR amplified fragments generated by four SSR markers i.e. RM335, RM511, RM227 and RM164. M is 100 bp DNA ladder; 1, Rikuto Norin22; 2, Sakha106; 3, Sakha101; 4, MBG-41; 5, Weed Tolerant-1; 6, HHZ12-SAL8-Y1-Y2 and 7, FFZ-1.

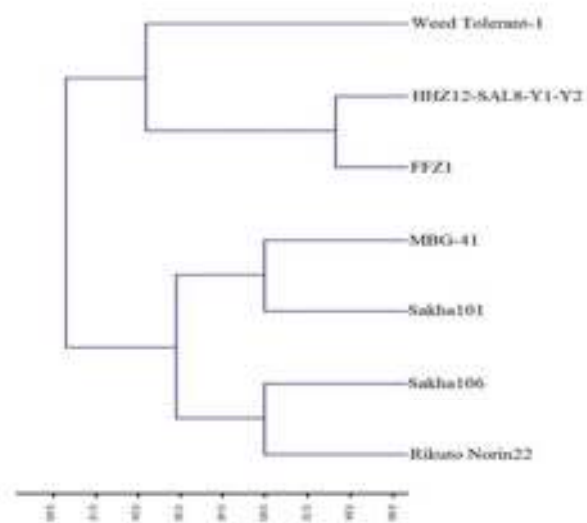


Fig. 4. Dendrogram derived from UPGMA cluster analysis for studied rice genotypes based on Jaccard's coefficient using 4 SSR markers.

CONCLUSION

Based on the obtained results it could be concluded that Sakha 106 rice cultivar was the best in allelopathic activity against *E. crus-galli*, *C. difformis*, *A. baccifera* and total weeds and producing the highest rice dry weight, grain yield and its attributes when treated by Top shot 6% herbicide at recommended or half rates. SSR 227 marker detected Weed tolerant-1, Rikuto Norin22 and Sakha 106, so this marker could be a potential candidate for MAS-based allelopathic selection.

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التقييم الحقل والجزيني لصفة تثبيط نمو الحشائش ذاتياً (الأليلوباثي) في بعض التراكيب وراثية من الأرز لرفع كفاءة مكافحة الحشائش ومحصول الأرز البدار

صبري صبحي محمد عبد النبي ، أحمد مصطفى أحمد القندور، إبراهيم عبدالسلام رمضان و سماح منير عبدالخالق
قسم بحوث الأرز- معهد بحوث المحاصيل الحقلية- مركز البحوث الزراعية - مصر

أجريت دراسة حقلية في المزرعة البحثية لقسم بحوث الأرز - محطة البحوث الزراعية بسخا- مركز البحوث الزراعية - جمهورية مصر العربية خلال موسمي 2016 و 2017. وكان الهدف من هذه الدراسة هو دراسة القدرة الذاتية لبعض التراكيب الوراثية من الأرز على تثبيط نمو الحشائش (أليلوباثي) ومقارنتها بالصنف سخا 101 الذي لا تتوافر فيه هذه الصفة تحت ظروف الحقل بالإضافة لدراسة التنوع الوراثي باستخدام أربعة من الدلائل SSR التي تم تحديدها سابقاً والمرتبطة بمقاومة الحشائش في الأرز، وكذلك معرفة إمكانية الاعتماد على أصناف الأرز ذات القدرة الذاتية على تثبيط نمو الحشائش في تقليل معدلات مبيد الحشائش المركب توب شوت 6% (بينوكلولام + سسيهالوفوب بيبوتيل) المستخدم في مكافحة حشائش الأرز البدار. استخدمت ستة تراكيب وراثية من الأرز في الدراسة الحقلية وهي Weed tolerant-1, FFZ-1, MBG-41, HHZ-12-SAL8-Y1-Y2, سخا 106 مقارنة بالصنف سخا 101 الذي لا تتوافر فيه صفة الأليلوباثي، كما أضيف مبيد توب شوت 6% بالمعدل الموصى به ونصف الجرعة مقارنة بالقطع التجريبي غير المعاملة (كنترول). أظهرت النتائج وجود فروق معنوية بين التراكيب الوراثية في الصفات المدروسة لكل من الحشائش والأرز، حيث حقق الصنفان سخا 106 و Weed tolerant-1 أفضل مكافحة للحشائش وأعلى وزن جاف للأرز و محصول الحبوب ومكوناته في كلا الموسمين، في حين سجلت أعلى قيم للوزن الجاف للذنبية، العجيرة و رجل الحمامة بالإضافة للحشائش الكلية بواسطة الصنف سخا 101 مصحوبة بأقل وزن جاف للأرز و محصول حبوب ومكوناته خلال موسمي الدراسة. كما تفوق المعدل الموصى به من المبيد على كل من نصف الجرعة والقطع غير المعاملة بالمبيد (الكنترول) في مكافحة الحشائش. أعطى الصنف سخا 106 نفس النتائج عند المعاملة بالمعدل الموصى به أو نصف المعدل من مبيد الحشائش حيث حقق أقل وزن جاف للذنبية، العجيرة، رجل الحمامة والحشائش الكلية كما سجل أعلى وزن جاف للأرز و محصول حبوب ومكوناته في الموسمين. كما أظهرت الدراسات الوراثية إنتاج عدد إجمالي من 13 أليل وتراوحت أعداد الأليلات لكل بادئ من 2 إلى 5 بمتوسط 3.25 أليل لكل موقع وراثي. تراوحت قيم معامل الخلط الوراثي (He) من 0.490 إلى 0.735 بمتوسط 0.607. كما تراوحت قيم محتوى المعلومات متعددة الأشكال للدلالة المستخدمة (PIC) من 0.37 إلى 0.685. تم تجميع التراكيب الوراثية في شجرة القرابة الوراثية على أساس الخلفية الوراثية والنشاط الأليلوباثي وأظهرت النتائج قدرة دلائل SSR في الكشف عن التنوع الجزيني وفصل التراكيب الهندية عن اليابانية. ولم يوجد دليل واحد قادر على التمييز بين جميع التراكيب ذات النشاط الأليلوباثي بسبب الطبيعة الكمية للصفة و تحكم عدد كبير من العوامل الوراثية بها. ومع ذلك، كان البادي SSR 227 قادراً على اكتشاف الأصناف Weed tolerant-1 و Rikuto Norin22 و Sakha106 وبالتالي يمكن اعتبار هذا البادئ من البوادئ الواحدة المرشحة كدليل مساعد للانتخاب لصفة النشاط الأليلوباثي في الأرز .