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Sakha108 Egyptian Rice Variety Japonica Type High Yielding and Resistant to Blast

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Cross Mark

ABSTRACT

Sakha108 was released in 2018 as a high yielding cultivar amended for enhancing rice production in Egypt. Sakha108 was developed from backcrossing between Sakha101 and HR5824-B-3-2-3 to retrieve blast resistance that was broken in wide spread variety Sakha101. The cross between Sakha101 and HR5824-B-3-2-3 was initiated in 2003, F_1 then was backcrossed with Sakha101 as to produce F_1BC_1. This backcross was advanced up to 2008 to enrich the genetic background of the recurrent parent Sakha101 along with blast resistance and reach homozygosity up to F_1BC_6. Five selected promising sister lines were evaluated in preliminary yield trial (GZ8564-SP70-20-6-1-1, GZ8564-SP70-20-6-1-2, GZ8564-SP70-2-6-1-3, GZ8564-SP70-20-6-1-4 and GZ8564-SP70-20-6-1-5). The promising line GZ8564-SP70-20-6-1-1 surpassed all selected lines and was evaluated from 2010 to 2015 in multi-location yield trials, regional, final and verification yield trials. The results indicated that GZ8564-SP70-2-6-1-1 exceeded yield potential of recurrent parent Sakha101, yet with shorter growth duration and blast resistance. The selected line grain yield recorded 10.75 and 11.00 t/ha during 2015 and 2016 seasons with total duration 137 day, while Sakha101 recorded 10.32 and 10.50 t/ha, respectively with a total growth duration of 145 day. GZ8564-SP70-2-6-1-1 showed high level of blast resistance in the field and artificial inoculation under greenhouse conditions compared with Sakha101 that showed susceptible reaction to rice blast under both field and greenhouse conditions. The application of 165 kg N/ha^{-1} and wider space (25x20 cm) significantly increased growth, yield attributes and grain yield of the new cultivar Sakha108. The new cultivar exhibited multiple resistance to other important biotic stresses.

Keywords: Rice, Varieties, Grain yield, Blast resistance, Nitrogen, Plant spacing

INTRODUCTION

Rice *(Oryza sativa* L.) is the staple food for more than half of the world's population. Maintaining stable rice production is extremely important to feed the constantly growing human population. In Egypt, rice is one of the major strategic cereal crops, with annual cultivated area of about 600.000 ha^{-1}, producing 6.0 million tons of paddy rice. The 9.88 t ha^{-1} average yield, is considered one of the highest values worldwide (RRTC, 2018). Rice blast disease is caused by the Fungus *Pyricularia grisea*, is one of the most widespread and destructive crop diseases globally. Use of host resistance is the most effective and economic way to control plant diseases (Hulbert *et al.*, 2001., Chuanxu *et al* 2020). Utilization of hybridization and backcross has been approved as efficient method in rice breeding to improve the genetic background or introduce different genes related to high yield potential, disease resistance and improved

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specific traits (El-Namaky 2018 and and Dubina 2020). Plant breeding based on classical methods hybridization and selection, molecular marking methods are now widely used that allow identifying target genes and selecting the desired genotypes (Kostylev *et al*. 2018). Therefore, breeding efforts have aimed to introduce blast resistance genes into desirable genetic background. Blast disease is considered as a major constraint that minimizes yield because of its high level of variability and quick spread. Blast resistance has consistently been one of the most important objectives of Egyptian rice breeders to maintain widespread of disease resistant cultivars as effective and reliable method of disease management. In some instances, rice blast and many other important field crop diseases, the resistance of some cultivars could be broken few years after release (Marchetti and Bomman 1989, and Veillet *et al*., 1996 and Kumari *et al*., 2018). The rice blast fungus has a wide range of variability (Ou and Ayad, 1968, Kalboush 2019 and Agbowuro *et al* 2020. Sakha101, the leading rice cultivar that is widely accepted for its yield potential and grain quality was resistant from 1994 up to 2002. In 2003, the resistance was broken in some locations, and the "mega variety Sakha101" became susceptible due to appearance of specific virulent races (EL-Shafy 2002. and Sehly *et al.* 2008). Sedeek and El-Wahsh (2015)), indicated that the new rice line GZ8564-SP70-20-6-1-1 derived from Sakha101 was high yielding, resistant to rice blast under artificial inoculation in greenhouse and under normal conditions in blast nursery in multi-location rice research stations. Japonica rice variety Sakha101 one of the mega rice varieties in Egypt released in 1994, grain yield ranged between 10.0 -11.0/ha. Grain quality was preferred by consumer in Egypt because its short grain, high milling (70%) low amylose content (19) and excellent cooking properties (RRTC 2018). This investigation describe the procedures carried out to develop the new high yielding variety Sakha108, along with optimum culture practice to obtain maximum yield potential for the new cultivar.

MAERTIALS AND METHODS

Breeding Methodology: High yielding Sakha101was crossed with HR5824-B-3-2-3 in 2003 to develop new breeding population. Saka101 were crossed with the F_1 as recurrent parent to produce BC_1F_1. Figure 1, shows the procedures to advance BC_1F_1, BC_1F_2, BC_1F_3, BC_1F_4, BC_1F_5 and BC_1F_6 and yield trials (primary, regional, final and verification trials) evaluation of promising lines (GZ8564-SP70-2-6-1-1) at the distinction, uniformity and stability (DUS) and value of cultivation and use (VCU) tests as variety registration requirements. Grain yield, duration, plant height**,** phenotypic acceptability and resistance to biotic stresses (blast resistant in particular), were the main criteria to advance selected plants/lines the different generations. Grain quality, hulling%, milling%, head rice%, amylose content and cooking of promising variety Sakha108 and three check cultivars (Sakha101, Sakha102 and Sakha104) were evaluated according to Juliano (1971) and standard evaluation system (SES) (IRRI 2006) .

Agronomic evaluation: To identify the optimum nitrogen levels and plant spacing of Sakha108 and some promising lines, field experiments were conducted at the farm of Sakha Research Station, Kafrelsheikh, Egypt in 2016 and 2017 rice growing seasons (Elhabet *et al* 2018). Rice genotypes i.e. GZ8564-SP70-2-6-1-1 (Sakha108), GZ9461, GZ7112 and GZ9057 (Giza179) were evaluated under three spacing namely 15x20, 20x20 and 25x20 cm with three nitrogen levels i.e. T_1: control (without N-application), T_2: 110 and T_3: 165 Kg N/ha^{-1}. The experiment was laid out in split-split plot design.

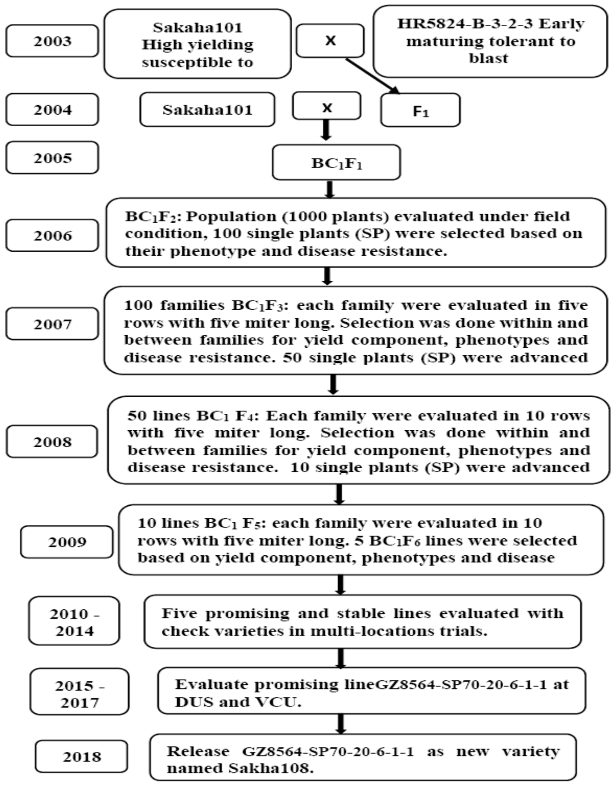

Figure 1. Diagram for development, registration and release of high yielding variety Sakha108.

Rice genotypes were arranged in the main plots while sub plots were devoted for plant spacing, and sub-sub were allocated for nitrogen levels. Bed nursery and permanent field were established and prepared as recommended according to Rice Research and Training Center (RRTC). Phosphorus fertilizer in form of mono super phosphate (15%) at the rate of 36 kg P_2O_5/ha and potassium in form of potassium sulphate (48% K_2O) at the rate of 57 kg K_2O/ha were applied during land preparation. Nitrogen levels (T_2 and T_3) in the form of urea (46.5% N) was applied to sub-plots in two splits, i.e, two thirds as basal application incorporated into the soil immediately before flooding, and on third after 30 days from the first dose. Seedling were transferred from nursery to the permanent field after 25 days after sowing and transplanted in sub-sub plots at different spacing treatments (15 x 20, 20 x 20 and 25 x 20 cm) between hills and rows, respectively. The grain yield and agronomic traits were estimated according to IRRI standard evaluation system (SES) (IRRI, 2006).

Diseases methodology: Behavior of backcross and advanced selected promising lines were evaluated for major diseases infection under field and artificial inoculations under greenhouse condition (Hammoud and Gabr 2014)

Blast reaction under field conditions: Rice genotypes were tested to blast disease at seedling stage under blast nursery (natural infection) at three locations i.e. Sakha (Kafr

El-Sheikh), Gemmiza (Gharbia) and Zarzora (Beheira) Governorates, with three replications for each genotype. Giza 159 was used as a susceptible spreader as a natural source of blast inoculum, while Giza 177 was used as resistant check. The sowing date was in the first week of July in both 2012 to 2013 rice growing seasons. Natural infection was developed and assessed 30-45 days after sowing, using the (0-9) scale according to SES of IRRI (IRRI, 2006). The severity of neck infection symptom was calculated using the formula adopted by Townsend and Huberger (1943).

Blast reaction under greenhouse: Rice entries including GZ8564-SP70-20-6-1-1 (Sakha108), were inoculated with blast isolates in greenhouse conditions to evaluate their blast resistance levels and identify virulent races (if any). Plastic trays (30 x 20 x 12 cm) were partially filled with fine soil, 5 g ammonium sulphate were added to each tray. Each tray was planted with 16 entries, and two rows in the ends of each tray as the susceptible check variety Giza 159. Plants were inoculated at 3-4 weeks after sowing. Single isolates were purified and grown on growth medium containing 200 g banana, 15 g glucose, 20 g agar /l medium for spore production. Two hundred ml of spore suspension was prepared from each isolate and adjusted to $5x10^4$ spores/ml. Each season twenty five isolates were used for inoculation using electrical spray gun. Plants were left in cages for 24 hours with 100% RH, then moved to the incubation room supplied with automatic system for temperature adjustment, between 25-30°C; and relative humidity was maintained at 100% by fine sprinklers. Seven days after inoculation, typical blast lesions appeared and scored using the 0-9 scale SES (IRRI, 2006).

Brown spot evaluation: Samples of rice leaves were collected at maximum tillering stage from the tested genotypes. Total number of brown spot lesion was counted on one hundred leaves, which were randomly collected from each plot to calculate severity of infection. After harvest, samples of one hundred grains were taken for estimating the discolored grain percentage according to SES (IRRI, 2006).

Bakanae disease evaluation: Seven genotypes i.e Sakha101, GZ8564-SP70-2-6-1-1, IRAT 112, IR65610, Sakha104, HR4856 and GZ7769 were treated with spore suspension of *Fusarium moniliforme* fungus. Symptoms of bakanae disease became visible about 15 to 20 days after planting. Samples and disease reactions were taken at the nursery (25-30 days after sowing) to ensure the full appearance of bakanae rice disease symptoms. Plant growth parameters i.e., the infected plants (%), dead plants % due to infection, were recorded. Furthermore, tested genotypes were tested under greenhouse with the most aggressive *Fusarium moniliforme* identified isolates. One hundred grains from each rice genotype was soaked in *Fusarium moniliforme* spore suspension (4 x 10^5 spores/ml) for 48 h. and incubated for 48 h. at 35ºC. Other one hundred grains from each rice entry was soaked in distilled water for 48 h. and incubated for 48 h. at (35ºC) as cheek. The treated rice genotypes were cultivated in 15x15 cm diameter pots with three replications and kept in greenhouse at 30-35ºC. The fertilization with urea 46.5% N at 3g/pot was applied. The number of infected plants was recorded 30 days after sowing.

White-tip nematode evaluation: Rice samples of hundred flag leaves were randomly collected from each plot before heading to determine severity of infection according to leaf area damage according to SES (IRRI, 2006).

Insect methodology: Parents, and advanced promising lines including GZ8564-SP70-2-6-1-1 were evaluated for stem borer infestation as the main insect pest for rice in Egypt. The reaction of evaluated genotypes was classified into five categories according to SES (IRRI, 2006).

Molecular marker analysis: Assessment and evaluation of genetic diversity of some lines developed from Sakha101 Cv. of rice by using SSR markers (Hammoud *et al.* 2013). Primer sequences of six SSR markers used are present in Table 1.

Table 1. Primer sequences of SSR used markers.

No.	Marker	Primers
1	SSR206	Forward CCCATGCGTTTAACTATTCT Reverse CGTTCCATCGATCCGTATGG
2	SSR216	Forward GCATGGCCGATGGTAAAG Reverse TGTATAAAACCACACGGCCA
3	SSR302	Forward TCATGTCATCTACCATCACAC Reverse ATGGAGAAGATGGAATACTTGC
4	SSR209	Forward ATATGAGTTGCTGTCGTGCG Reverse CAACTTGCATCCTCCCCTCC
5	SSR262	Forward CATTCCGTCTCGGCTCAACT Reverse CAGAGCAAGGTGGCTTGC
6	SSR244	Forward CCGACTGTTCGTCCTTATCA Reverse CTGCTCTCGGGTGAACGT

RESULTS AND DISCUSSION

Progeny of the backcross BC_1F_1 of (Sakha101/ HR5824-B-3-2-3// Sakha101) were selfed to produce the BC_1F_2 generation. Figure (1), shows the procedures to select and advance early segregated populations and advanced breeding lines. Out of 1000 plant of BC_1F_2, 100 single plants, were selected based on agronomic performance and disease resistance. About 50 lines were selected Out of the 100 lines BC_1F_3, and advanced to BC_1F_4. Using yield performance and other selection criteria, ten promising lines were selected and advanced to BC_1F_5. Finally, five selected lines (BC_1F_6) has been selected as high yielding potential candidates and promoted to yield trials for further evaluation.

Mean performance: Plant height, duration and flag leaf area of five promising lines; GZ8564-SP70-20-6-1-1 (Sakha108) GZ8564-SP70-20-6-1-2; GZ8564-SP70-20-6-1-3; GZ8564-SP70-20-6-1-4 and GZ8564-SP70-20-6-1-5, and their parents Sakha101 and HR4856 during the two rice growing seasons 2012 and 2013, as well as their combined data are presented in Table (2).

The results showed a wide range of variability for all studied traits. Rice variety HR4856-1-1-1-1, was the shortest plant height in 2012 and 2013 with average 74.75 cm, the same variety had shortest duration in 2012 and 2013 with average 106.33 days. The promising line GZ8564-SP70-20-6-1-1 showed the highest flag leaf area in 2012 and 2013 with average (31.73 cm). Results of panicle traits and grain yield are presented in Table (3). Sakha101 showed the highest No. of panicles/ plant, GZ8564-SP70-20-6-1-3 give the highest values of 1000/grain weight for both seasons. The promising lines GZ8564-SP70-20-6-1-2 and GZ8564-SP70-20-6-1-4 revealed the highest No. of grains/ panicle, in the same time the two promising lines of GZ8564-SP70-20-6-1-1 and

GZ8564-SP70-20-6-1-4 gave the highest grain yield of (11.41 and 11.24 t/ha, respectively) compared with (10.89 and 6.57 t/ha) for their parents; Sakha101 and HR 4856, respectively, the lines were superior compared with their parents in all yield and yield components traits.

Table 2. Plant height, duration and flag leaf area of five promising lines and their parents in the preliminary yield trial in two growing seasons and their combined data.

Genotype	Plant height (cm)			Duration (day)			Flag leaf area (cm^2)		
	2012	2013	Comb.	2012	2013	Comb.	2012	2013	Comb.
Sakha101	87.39	88.18	87.78	145.00	145.33	145.17	29.13	28.31	28.72
GZ8564-SP70-20-6-1-1	91.78	92.84	92.31	135.67	136.00	135.83	32.44	31.01	31.73
GZ8564-SP70-20-6-1-1	93.72	93.85	93.79	136.33	135.00	135.67	31.14	29.78	30.46
GZ8564-SP70-20-6-1-3	94.18	92.91	93.54	134.00	134.67	134.33	31.44	30.78	31.11
GZ8564-SP70-20-6-1-4	90.80	92.64	91.72	136.67	135.33	136.00	30.15	29.05	29.60
GZ8564-SP70-20-6-1-5	99.34	95.98	97.66	130.00	129.33	129.67	29.27	31.61	30.44
HR4856-1-1-1-1	73.02	76.48	74.75	105.00	107.67	106.33	18.85	20.29	19.57
L.S.D $_{0.05}$	2.43	2.77	0.96	2.01	2.33	2.06	2.39	1.31	1.82
L.S.D $_{0.01}$	3.41	3.82	1.31	2.82	3.27	2.80	3.35	1.83	2.47

Results on grain quality for tested genotypes are presented in Table (4). GZ8564-SP70-20-6-1-5 gave the highest values for the hulling%, milling% and head rice% of five promising lines and their parents in the preliminary yield trial in two growing season. In general most of the five promising lines exhibited desirable values for hulling %, milling% and head rice% in the two growing seasons compared with parental genotypes Sakha101 and HR4856-1-1-1-1.

Table 3. Grain yield and its components of five promising lines and their parents in the preliminary yield trials across 2012 and 2013 growing seasons and their average.

Genotypes	No. of panicles/ plant			1000-grain weight (g)			No. of grains/ panicle			Grain yield (t/ha)		
	2012	2013	Comb.	2012	2013	Comb.	2012	2013	Comb.	2012	2013	Comb.
Sakha101	23.15	24.75	23.95	27.97	28.11	28.04	146	157	151.5	10.99	10.79	10.89
GZ8564-SP70-20-6-1-1	22.85	23.16	23	30.4	30.77	30.58	153.3	147.7	150.5	11.32	11.50	11.41
GZ8564-SP70-20-6-1-2	22.93	22.89	22.91	29.9	30.33	30.12	156.7	157.1	156.8	11.14	10.98	11.06
GZ8564-SP70-20-6-1-3	21.76	21.57	21.67	32.2	31.67	31.94	163.7	138.7	151.2	10.95	11.01	10.97
GZ8564-SP70-20-6-1-4	21.9	22.05	21.98	30.83	30.1	30.47	164.7	147.7	156.2	11.30	11.17	11.24
GZ8564-SP70-20-6-1-5	18.45	19.02	18.73	30.87	31.86	31.36	145.7	152.3	149	10.53	10.64	10.59
HR4856-1-1	13.27	9.97	11.62	25.47	24.06	24.76	76.3	80.7	78.5	6.59	6.54	6.57
L.S.D $_{0.05}$	2.61	1.13	1.91	1.54	0.94	1.23	10.48	13.21	21.84	0.82	0.88	0.63
L.S.D $_{0.01}$	3.66	1.58	2.58	2.16	1.37	1.66	14.69	18.52	29.59	0.9	0.97	0.85

Table 4. Grain quality measures of five promising lines and their parents in the preliminary yield trial in two growing seasons 2012 and 2013.

Genotype	Hulling %			Milling %			Head rice %		
	2012	2013	Comb	2012	2013	Comb.	2012	2013	Comb.
Sakha101	81.75	82.39	82.07	71.83	70.57	71.2	64.52	63.45	63.98
GZ8564-SP70-20-6-1-1	82.10	82.79	82.45	72.03	72.05	72.04	65.64	63.14	64.39
GZ8564-SP70-20-6-1-2	82.43	82.76	82.6	73.64	72.44	73.04	64.35	63.17	63.76
GZ8564-SP70-20-6-1-3	84.7	84.91	84.8	75.57	74.24	74.9	66.48	65.37	65.92
GZ8564-SP70-20-6-1-4	78.68	80.28	79.48	70.54	70.36	70.45	66.19	60.58	63.38
GZ8564-SP70-20-6-1-5	84.5	86.2	85.35	77.33	76.08	76.45	68.33	66.63	67.48
HR4856-1-1-1-1	72.69	70.81	71.75	57.81	57.81	57.81	48.17	45.5	46.83
LSD at 0.05%	1.91	2.04	2.03	1.72	1.69	1.72	2.6	2.09	2.19
0.01%	2.67	2.76	2.84	2.41	2.37	2.41	3.65	2.93	2.87

Results of grain yield and growth duration of the new promising line compared with Sakha101 Sakha104 as medium duration checks for five constitutive seasons 2011-2015. The results of this study are shown in Table (5). The obtained results indicated that the new variety Sakha108 (GZ8564-SP70-20-6-1-1) exhibited the highest values of grain yield t ha1 under normal conditions compared with checks cultivars Sakha101 and Sakha104. The new variety also exhibited early growth duration compared with studied checks. Sakha108 was earlier than Sakha101 by about 7 days without any significant difference with Sakha10

Table 5. Duration and grain yield of Sakha108 and two check cultivars Sakha101 and Sakha104 during five growing season (2011 -2015) under normal soil at Sakha.

Genotype	Grain yield (t/ha)						Duration (day)					
	2011	2012	2013	2014	2015	Mean	2011	2012	2013	2014	2015	Mean
Sakha101	10.11	10.25	10.56	10.34	10.41	10.334	145	145	144	145	144	144.6
Sakha108	10.45	10.77	10.68	11.00	10.95	10.77	137	138	137	138	138	137.6
Sakha104	9.80	9.59	9.60	9.43	9.83	9.65	135	134	135	135	135	134.8
LSD .05%	0.65	0.76	0.64	.91	0.77	0.746	2..7	3.1	2.1	2.4	2.6	2.58

Performance of Sakha108 and the two checks (Skha101 and Skha104) were superior under the three normal locations and one saline location as presented in Table (6). Sakha108 exhibited the highest grain yield in the three normal locations (Sakha, Gemmiza and Zarzora) compared with the two checks. Also the duration ranged from 137 to 138 days without any significant difference from the earliest check Sakha104. However Sakha104 showed the highest grain yield and shortest duration under the saline stress conditions at El-Sirw location.

Table 6. Duration and grain yield of Sakha108 and two check varieties Sakha101 and Sakha104 under four locations Sakha, Gemmiza, Zarzora and El-Sirw during 2015-2016 growing seasons.

location	Grain yield (t/ha)						Duration (day)					
	Sakha101		Sakha108		Sakha104		Sakha101		Sakha108		Sakha104	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Sakha	10.56	10.41	11.0	10.95	9.43	9.83	145	145	138	137	135	134
Gemmiza	10.89	11.0	11.2	11.48	10.15	9.92	145	146	137	138	135	136
Zarzora	9.95	10.30	10.84	10.50	9.59	10.1	144	145	137	137	134	135
El-Sirw	4.35	4.5	4.20	4.68	5.50	5.30	140	141	134	133	130	131
Mean	10.47	10.57	11.01	10.98	9.72	9.95	145	145	137	137	135	135
LSD 0.05%	0.94	1.1	1.3	0.90	0.89	0.79	2.1	3.4	1.7	2.3	2.7	1.9

Performance of Sakha108 under distinction, uniformity and stability (DUS) test: Performance of promising line GZ8564-SP -20-1-1 (Sakha108) with three cultivated varieties as the checks in DUS trials are presented in Table (7). In general terms, the grain yield average of Sakha108 under one experiment of primary yield trial, three experiments of regional yield trails and 21 experiments of the final trials was 10.89 t/ha compared with 10.42, 9.60 and 9.25 t/ha for check varieties Sakha104, Sakha101 and Sakha102, respectively. The yield advantage of Sakha108 over the three check varieties were 0.43, 1.29 and 1.64 t/ha, respectively. Sakha108 had almost the same growth duration of Sakha104 (135 days), while Sakha102 was (125 days). Sakha101 was the shortest stature (91 cm), while plant height of Sakh108 was (100 cm). Water consumption under normal condition ranged from 5400 – to 5840 m3, Sakha102 was the lowest consumption of 5400 m3 because the short growth duration (only 125 days). Sakha108 and Sakha102 were resistance to blast while Sakha101 and Sakha104 were susceptible. Sakha108 and all check varieties were resistance to stem borer and leaf minor infestations. Highest milling% (72%) was recorded for promising line GZ8564-SP70-20-6-1-1 (Sakha108), compared with other check varieties.

Table 7. Performance of (GZ8564-SP70-20-6-1-1) Sakha108 and check varieties at Distinction, Uniformity and Stability (DUS) test.

Traits	Sakha108	Sakha101 (check)	Sakha104 (check)	Sakha102 (check)
Grain yield (t/ha)				
Primary yield trial (1Exp)	10.75	10.32	9.50	9.10
Regional yield trails (3Exp)	10.82	10.43	9.60	9.30
Final yield trials (21Exp)	11.10	10.50	9.70	9.35
Average of (25 Exp)	10.89	10.42	9.60	9.25
Yield advantage (t/ha) compared with checks		0.43	1.29	1.64
% Yield advantage over checks		4.3%	11.855	15.01%
Duration (day)	135	145	135	125
Plant height (cm)	100	91	107	110
Water consumption (m^3)	5840	6350	5800	5400
Blast resistance	R	S	S	R
Insect resistance				
Stem borer	R	R	R	R
Leaf minor	R	R	R	R
Grain quality				
Milling%	72	70	70	70
Amylose content	19	18	18	18
Cooking	Excellent	Excellent	Excellent	Excellent

Expansion during cooking due to water absorption and hence increase in cocked rice volume is directly affected by amylose content. Low amylose content rice varieties (17-22%) are Egyptian consumer`s preference. Sakha108 and check varieties exhibited excellent cooking quality with amylose content ranged between 18 to 19%.

Agronomy evaluation: The nitrogen requirements, seed rate and plant spacing differed from variety to variety. Investigation on the effect of different nitrogen levels and plant spacing on the productivity of new released varieties is very important (Elhabet *et al* 2018).

The number of filled grains/panicle, 1000-grain weight, grain and straw yield as affected by space of transplanting and nitrogen level are presented in Table (8).

Table 8. Number of filled grains /panicle, thousand grain weight (g), grain yield t/ha and straw yield t/ha as affected by plant spacing (cm) and nitrogen levels (kg N/ha) of some different rice genotypes during 2016 and 2017 seasons.

Genotype	Number of filled grains /panicle		1000- weight (g)		Grain yield (t/ha)		Straw yield (t/ha)	
	2016	2017	2016	2017	2016	2017	2016	2017
Sakha108	113.95b	115.75b	26.881a	27.147a	9.671a	9.898a	11.074b	11.302b
GZ9461	107.51c	109.31c	25.159b	25.415b	9.750a	9.977a	11.243b	11.471b
GZ7112	117.41b	119.21b	25.190b	25.456b	8.662c	8.890c	11.297b	11.524b
GZ9057 (Giza179)	122.85a	124.66a	25.237b	25.503b	9.261b	9.489b	11.613a	11.840a
F-Test	**	**	**	**	**	**	**	**

The rice line GZ9057 (Giza179) gave the highest number of filled grains/panicle followed by GZ7112 and GZ8564-SP70-20-6-1-1 (Sakha108) rice genotypes while, GZ9461 gave the lowest value of filled grains/panicle.

For 1000-grain weight, (Sakha108) exhibited the heaviest 1000-grain weight, followed by GZ9461, GZ7112 and GZ 9057 (Giza179) which gave nearly the same values (25.159- 25.503 g) without any significant differences over the two studied seasons. Regarding to grain yield, both rice genotypes GZ9461 and Sakha108 produced the highest grain yield followed by GZ9057 (Giza179) rice genotype, while GZ7112 gave the lowest value in the both studied seasons. The rice variety Giza179 recorded the highest straw yield followed by the other tested rice genotypes which gave nearly the same straw yield without any significant differences among them.

To determine the best plant density, various spacing treatments were applied. Results showed that each genotype produced its best yield under wide spacing of 20 x25 cm (Table 9). In both seasons, Sakha108, gave highest yield potential under the wide spacing, this was the same with other three tested genotypes. Whereas, GZ9461 and GZ9057 (Giza179) obtained statistically the same yield under wide and moderate spacing. Meanwhile the lowest yield was obtained by GZ7112 under moderate and narrow spacing in both seasons.

Table 9. Grain yield (t/ha) as affected by the interaction between some different rice genotypes and plant spaces (cm) in 2016 and 2017 seasons.

Plant space (cm)	Rice genotype							
	2016				2017			
	Sakha108	GZ9461	GZ7112	GZ9057	Sakha108	GZ9461	GZ7112	GZ9057
15x20	9.186bc	9.255bc	8.293d	8.670cd	9.413bc	9.482bc	8.520d	8.897cd
20x20	9.461b	9.810ab	8.519d	9.324b	9.688b	10.122ab	8.777d	9.551b
25x20	10.365a	10.183a	9.174bc	9.790ab	10.592a	10.326a	9.402bc	10.018ab

The increases in grain yield of GZ8564-SP70-2-6-1-1 (Sakha108) and GZ9461 rice lines when cultivated under wider or medium spacing might be due to the vigorous growth in both shoots (canopy) and roots, so the wider or medium spaces are suitable for minimizing the competition among both shoots and roots which led to increase both nutrient uptake and light penetration through the leaves of their canopy specially flag leaf plus second and third leaves which are representative for about 75% from total photosynthesis consequently increase the photosynthesis process and its products (assimilates) that translocate to the panicle and efficiently fill most of the spikelet's resulted in increase the number of filled grains consequently grain yield. These results coincidence with that recorded by Abd Alla (1996), Abd EL-Hamed (2002); Koutroubas and Ntanos (2003) and Sorour, *et al*., (2016).

Grain yield as influenced by the interaction between tested rice genotypes and nitrogen levels is presented in Table 10. Data showed Sakha108, GZ7112 and GZ9057 (Giza179) produced highest grain yield at 165kg N/ha, while GZ9461 obtained statistically the same grain yield when it was fertilized by 110 or 165kg N/ha. This increase could be attributed to the increase in nitrogen uptake which make continuous supply of nitrogen requirements to rice plants. Also, one of the most important role of nitrogen is the significant increase in both enzymatic activities and chlorophyll content. This increase viability of flag leaf, and cause delay flag leaf senescence that leads to more efficient photosynthetic processes and enhance metabolites stream that immediately translocate to the panicles and fill most of the spikelets leading to higher grain yield. These results obtained were in accordance with that reported by Abd Alla (1996), Abd EL-Hamed (2002); Koutroubas and Ntanos (2003) and Sorour, *et al*., (2016).

Table 10. Grain yield (t/ha) as affected by the interaction between some different rice genotypes and nitrogen levels (kg N/ha) in 2016 and 2017 seasons.

Nitrogen Levels (Kg N/ha)	Rice genotype							
	2016				2017			
	Sakha108	GZ9461	GZ7112	GZ9057	Sakah108	GZ9461	GZ7112	GZ9057
Control	7.17e	7.75e	6.20f	6.47f	7.75e	7.89e	6.34f	6.60f
110 kg N	10.17c	10.44bc	8.92d	9.78c	10.36c	10.55bc	9.12d	9.98c
165 kg N	11.23a	11.05ab	10.87ab	11.54a	11.58a	11.40ab	11.21ab	11.88a

Diseases resistance evaluation

Blast Resistance: Eight rice genotypes including GZ8564-SP70-2-6-1-1 were tested under greenhouse and field conditions to access their blast resistance levels. The reaction of tested rice genotyppes under 25 isolates of rice blast are presented in Table 11. The results show that the 25 isolates were represented 8 races as follow; the IG-1 race (6 isolates), IC-13 race (5 isolates), IC-8 (4 isolate), both ID-5 and ID-7 races (3 isolates), IH race (2 isolates), both IB-45 and IF-4 races (one isolates). The results indicated that rice varieties IRAT 112, IR 65610-105-2-5, and Giza177 were resistant to all rice blast races under greenhouse and field conditions. The two commercial cultivars Sakh101 and Sakha104 were susceptible under both greenhouse and field conditions along with susceptible check Giza159. Gabr (2004), El-Wahsh and Hammoud (2007) found that commercial cultivars Sakha101 and Sakha104 showed susceptible reaction to 20 blast pathogen isolates in a greenhouse test. Concerning natural infection under field conditions, Sakha101 was susceptible in the first two seasons (2011 and 2012), and it became highly susceptible from 2013 -2015. On the other hand the promising line GZ8564-SP70-2-6-1-1 together with IRAT 112, IR 65610-105-2-5, and Giza177 were resistant to all rice blast races under field conditions. Sedeek and El-Wahsh (2015) reported that Sakha108 (GZ8564-SP70-2-6-1-1) was resistant to rice blast under natural infication in blast nursury at Sakha, Gemmiza and Zarzoura and artificial inculation in the greenhouse compared with Sakha101 was secebtable by blast under the same conditions.

Table 11. Reaction of leaf blast disease for eight tested genotypes under greenhouse and field conditions during 2011-2015 growing seasons.

Blast races	Greenhouse								Field reaction				
	IG-1	IB-45	IC-8	IC-13	ID-5	ID-7	IF-11	IH	2011	2012	2013	2014	2015
No of isolate	6	1	4	5	3	3	1	2					
Genotype													
Sakha101	S	R	HS	S	S	S	HS	HS	S	S	HS	HS	HS
GZ8564-SP70-2-6-1-1	R	R	R	R	R	R	R	R	R	R	R	R	R
IRAT 112	R	R	R	R	R	R	R	R	R	R	R	R	R
IR 65610-105-2-5	R	R	R	R	R	R	R	R	R	R	R	R	R
Sakha104	R	S	R	R	R	R	R	R	S	S	S	S	S
HR4856-1-1-1-1-2	R	R	S	R	R	R	S	R	R	R	R	R	R
Giza177 (R check)	R	R	R	R	R	R	R	R	R	R	R	R	R
Giza159 (S check)	S	S	S	S	S	S	S	S	S	S	S	S	S

R = (Resistant) MR = (Moderately Resistant) S = (Susceptible) HS = (Highly Susceptible)

Reactions to brown spot and white tip nematode infestations: Brown spot %, Brown spot severity % and White tip nematode % of some rice genotypes are presented in Table (12). The results indicated that, promising line GZ8564-SP70-2-6-1-1, gave the lowest brown spot infection, severity and White tip nematode %. On the other hand, the parent HR4856-1-1-1, gave the highest infection percent of brown spot and white tip nematode . White tip disease of rice leaves caused by the rice leaf nematode, it has a widespread and found nearly in all different world rice ecosystems, Ou (1985). Giudici *et al*. (2003) reported that *Aphelenchoides besseyi* is a seed transmitted plant parasitic nematode that can dramatically affect rice growth and yield. Abdel Hadi *et al*. (2005) investigated *A. besseyi* damage, infection, dynamic symptoms and epidemic features in different locations. They found that the disease is widely spread all over Egyptian governorates, causing remarkable yield reduction in the susceptible old rice cultivars Giza171 and Reiho as well as some new rice cultivars (Giza177, Sakha102 and Sakha103) with different levels of infection and yield losses reaching 47% in old cultivars, as recorded by El-Shafey (2007), who reported the positive effects of seed priming with NaCl solutions and sulfur control the disease.

Table 12. Mean performance of some rice genotypes as affected by some rice diseases at the two seasons 2012 and 2013 and their combined data.

No.	Genotype	Brown spot %			Brown spot severity			White tip nematode %		
		2012	213	Comb	2012	213	Comb	2012	213	Comb
1	Sakha101	44.67	45.5	45.08	215.5	220	217.8	50.84	52.5	51.67
2	GZ8564-SP70-2-6-1-	7.84	8.17	8	36.75	38.5	37.62	0.15	0.2	0.17
3	IRAT 112	62	63.15	62.57	295.5	298.8	297.1	33.25	34.5	33.87
4	IR 65610-105-2-5	45.23	46.5	45.86	210	215.8	212.9	27.56	28.5	28.03
5	Sakha104	67.53	66.5	67.01	305.8	308.5	307.1	56.48	60.15	58.31
6	HR4856	71.52	70.66	71.09	320.8	326	323.4	67.55	65.5	66.52
	L.S.D 05 %	3.69	4.25	3.97	10.37	9.29	9.83	4.93	4.13	4.53

Reaction to bakanae disease: Bakanae is a seed and soil-borne disease. When seeds of rice plants are infected by the fungus, the typical characteristic symptoms of the disease is the appearance of tall thin plants, markedly over-growing than uninfected plants. The active metabolic product of the pathogen is gibberellins which was isolated and proved to play an important role in the pathogenicity of this organism by many scientist Kumar (1984), Ou (1985), Li and Luo (1997), Jeff (2001), Rood (2004) and Li *et al*. (2005) Hammoud and Gaber (2014). All entries gave the lowest infection of Bakanae rice disease under natural infection as illustrated in Table (13). Concerning artificial inoculation under lab conditions, Sakha101 gave the highest infection under non-inoculated and inoculated conditions (1.667 and 89.33 % respectively) while, Sakha104 gave the lowest infection under both non-inoculated and inoculated (0.767 and 25.67 % respectively). GZ8564-SP70-2-6-1-1, IRAT112 and IR657 showed the lowest infection percentage of 0.133% by bakanae under non-inoculated conditions, while Sakha104 followed by IRAT112 then GZ8564-SP70-2-6-1-1, showed percentage infection of 25.67, 52.33 and 62.67 respectively for the same genotypes under artificial inoculation conditions.

Table 13. Reaction of rice genotypes to bakanae disease (*Fusarium moniliforme*) under lab conditions during 2012 season.

NO	Genotype	Bakanae %		Bakanae % under inoculated	
		Non-inoculated	inoculated	Treated with Vitavax 2g/l	Treated with sodium chloride 50/l
1	Sakha101	1.667	89.33	4.67	29.33
2	GZ8564-SP70-2-6-1-1	0.133	62.67	2.33	16.76
3	IRAT 112	0.133	52.33	1.67	14.33
4	IR 65610-105-2-5	0.133	64.33	2.33	26.76
5	Sakha104	0.767	25.67	1.00	13.33
6	HR 4856	0.133	79.33	3.33	27.33
	L.S.D. 5 %	1.0070	10.964	2.018	4.785

Molecular marker analysis: Rice is a self-pollinated diploid crop, microsatellite markers (SSR) usually reveal single-copy, homozygous loci and allelic heterogeneity is rare in pure line varieties. These facts simplified the work of microsatellites for the analysis of genetic diversity of rice cultivars. In this study, the level of polymorphism among genotypes was evaluated by six SSR markers (Table 1). The results in Fig.(2) showed that the primers SSR206, SSR209 and SSR216 showed existence of two alleles. While, the primers SSR302 and SSR262 gave three and four alleles, respectively. The primer SSR206, produced two different alleles , the first allele (550bp) was found in all genotypes except GZ8564-SP70-2-6-3 line (lane # 4). While, the second allele (300bp) was found in all genotypes except the same line (lane 4). For RM209, Figure 2 shows that two alleles were found; the first one (300Kb) was found in all genotypes except GZ8564-SP70-2-6-1-3 (lane2), whereas the second one (170Kb) was found in Sakha101; GZ8564-SP70-2-6-1-2; GZ8564-SP70-2-6-1-3 and GZ8564-SP70-2-6-1-4 (lanes1, 3, 4, and 5). The second allele (170Kb) gave the same trend. For RM216, (Figure 2) shows that two alleles were present; the first one (850Kb) was found in genotypes GZ8564-SP70-20-6-1-1, GZ8564-SP70-20-6-1-2, GZ8564-SP70-20-6-1-3, GZ8564-SP70-20-6-1-4, GZ8564-SP70-20-6-1-5 (lanes3, 4, 5, and 6). While, the second one (570Kb) was found in all genotypes except GZ8564-SP70-20-6-1-4; and GZ8564-SP70-20-6-1-5 (lanes; 5 and 6). The marker SSR244 gave two alleles; the first one (300Kb) was found in all genotypes, whereas the second one (250Kb) was found in the lines; GZ8564-SP70-2-6-1-4 and GZ8564-SP70-2-6-1-5 (lanes5 and 6) (Hammoud *et al.* 2013)

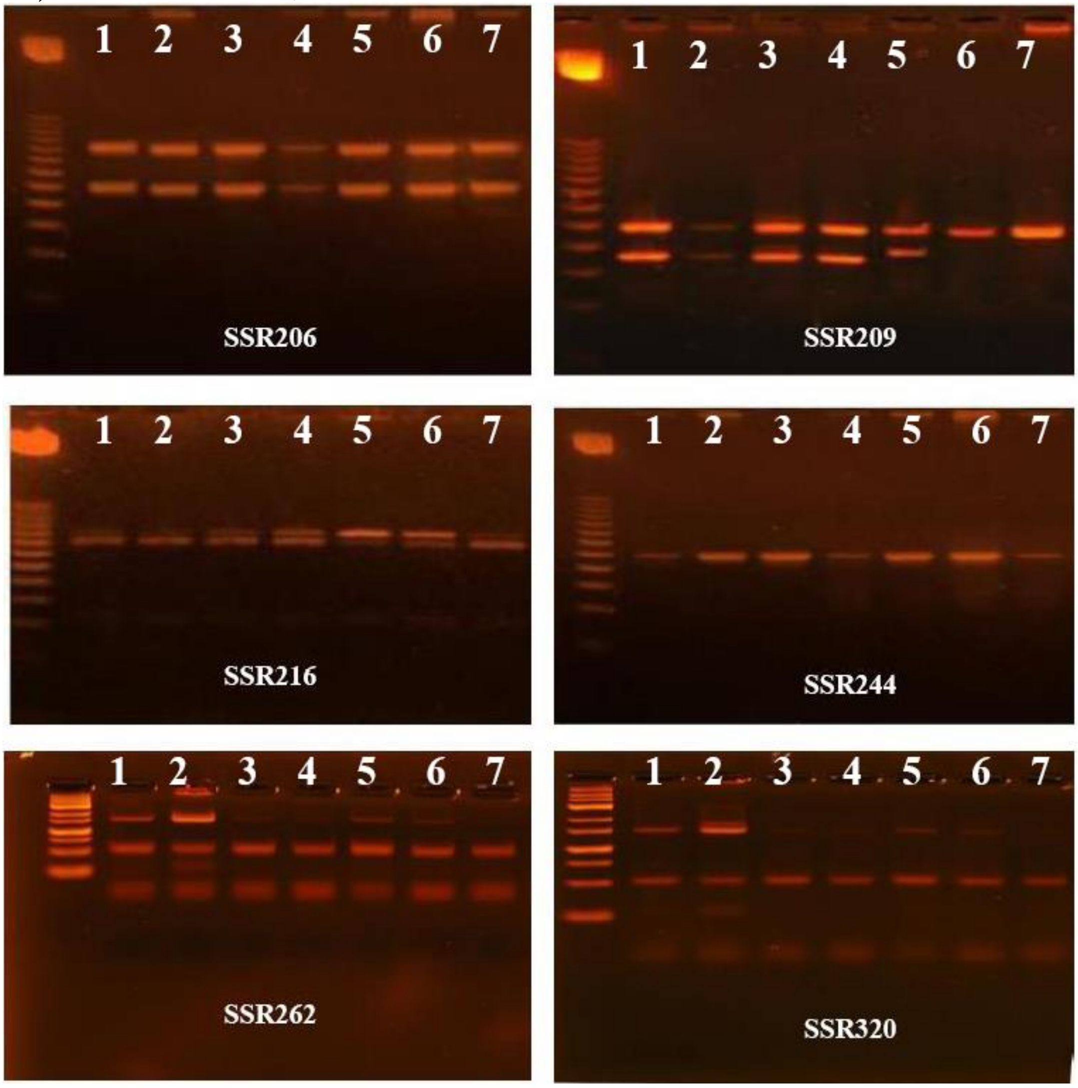

Figure 2. The electrophotogram of DNA amplified fragments using SSR262 for selected genotypes. First lane, 100bp DNA ladder; 1= Sakha101, 2= SP70-101-1, 3= SP70-101-2, 4= SP70-101-3, 5= SP70-101-4, 6= SP70-101-5 7= HR4856-1-1-1-1-2.

CONCLUSION

In general the new rice cultivar Sakha108 exhibited significant advantages over the widespread Sakha101 variety. The new cultivar has higher yield potential compared with Sakha101 with excellent grain quality, yet with shorter growth duration. Furthermore , the new variety is blast resistant and hence, it could be ideal candidate to replace Sakha101 gradualy. This will significantly help to improve /maintain varietal policy with new high yielding and biotic stress resistant varieties to replace old/succiptble varieties. This is also a model of continuous flow of new rice varieties to cope with highly variable biotic stress agent such as blast fungus. The results of the new cultivar demonstrate the power of backcross breeding in developing blast resistant rice genotypes using succeptible parent and suitable blast resistance donor. Sakha108 is now taking place on rice belt as a leading rice variety. This will indeed enhance productivity per unit area and minimize the negative impact of blast disease on national rice production. Attaining such policy could greatly help in achiving food security of such strategic crop, enhance farmer`s net return and improve the welfare of such rural areas .

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سخا 108 صنف أرز مصرى يابانى الطراز عالى المحصول مقاوم لمرض اللفحة

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سخا108 صنف أرز مصرى يابانى الطراز قصير الحبة عالى المحصول مقاوم لمرض اللفحة تم تسجيلة ونشرة عام 2018 . تم استنباط سخا 108 من خلال التهجين الرجعى بين الصنف سخا101 والصنف HR5824-B-3-2-3 حيث تم أجراء التهجين عام 2003 وفى عام 2004 تم زراعة الجيل الاول F_1 الذى تم تهجينة رجعيا بالصنف سخا101 للحصول على الجيل الاول الرجعى BC_1F1. ثم زراعة الجيل الاول الرجعى للحصول على الجيل الثانى BC_1F_2. تم الانتخاب فى الاجيال من الثانى الى السادسر للمحصول العالى والتبكير والمقامة للامراض والشكل المظهرى. أظهرت خمس سلات مبشرة وهى GZ8564-SP70-20-6-1-1, GZ8564-SP70-20-6-1-2, GZ8564-SP70-20-6-1-3, GZ8564-SP70-20-6-1-4, GZ8564-SP70-20-6-1-5, تفوقا فى المحصول ومقاومة لمرض اللفحة. تم تقييم السلالة GZ8564-SP70-20-6-1-1 - فى ثلاثة مواقع تحت الظروف العادية فى سخا الجميزة والزرزورة بالاضافة الى موقع عالى الملوحة بالسرو. أظهرت النتائج فى التجارب التأكيدية ان الصنف الجديد سخا108 (GZ8564-SP70-20-6-1-1) كان افضل فى المحصول (t/ha 11.0-10.75) والعمر 137 يوم مقارنة بالصنف سخا101 (10.32-10.50 طن للهكتار) والعمر 145 يوم. ايضا أظهر الصنف سخا108 مقاومة لمرض اللفحة تحت ظروف الحقل وتحت ظروف العدوى الصناعية مقارنة بالصنف سخا101 الذى أظهر قابلية للأصابة تحث ظروف الحقل والعدوى الصناعية. كما اظهرت النتائج أن إضافة 165 كجم نيتروجين / هكتار سجلت زيادة فى صفات النمو ومكونات المحصول ومحصول القش والحبوب. مسافات الزراعة الواسعة (20x 20) , (25 x 20) أعطت محصول أعلى من الزراعة على مسافات ضيقة (15x 15) , (15 x20).