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Genomic Characterization and Identification of Effective Blast Resistant Genes for Sakha 101 and Sakha 108 as High Yielding Egyptian Rice Cultivars

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Abstract: Sakha 101 is one such short grained common Egyptian rice cultivar and known for its exquisite quality, however, is highly susceptible to blast disease that has led to considerable decline in its area. Sakha 101 was crossed to a blast gene donor line, HR5824-B-3-2-3 and followed through backcross-breeding method that helped to incorporate blast resistance genes and finally Sakha 108 was released as improved version of the most widespread Egyptian commercial rice Sakha 101. The study was to evaluate Sakha 101 and Sakha 108 for high yielding, blast resistance, and effective resistance genes to *Pyricularia oryzae* as well as assessment of genetic divergence based on genomic in these cultivars. There is a slight increase in the values of Sakha 108 than Sakha 101 in the most of studied traits. Also under this study, seventy isolates were identified as eight main groups *i.e.,* IA, IB, IC, ID, IF, IG, IH and II, but ID group was considered the most common races. On the other hand, *Pi-Z* and *Pii* - *Pi-k*^{s} resistance genes were the most effective genes to blast fungus. Sakha 108 proved resistance for all tested isolates under greenhouse condition compared with Sakha 101 which exhibited susceptible to 70% of tested isolates. On genomic level, out of 242 markers across the 7 chromosomes; only 6 markers (RM8236, RM13611, RM3839, RM17377, RM160 and RM27154) produced clear fragments and polymorphism between cultivars (Sakha 101 and Sakha 108) and were used to construct a genetic linkage map. A total of 39 candidate genes were identified around their regions on each chromosome. These results enrich our understanding of the differences between Sakha 101 and Sakha 108 and also provide a foundation for selecting candidate for marker-assisted selection breeding in rice.

Keywords: Rice, Yield, Blast Disease, Resistance Genes, Genomic Regions

1. Introduction

Rice is the world's most significant staple food crop, and is cultivated in a variety of environments based on topography, soil type, water regime, and climatic considerations. To meet rising demand, rice production will have to increase even more. A variety of obstacles will have to be solved in order to achieve sustainable production, including the loss of arable land, global water scarcity, and global climate change [36]. The majority of Egyptian rice cultivars are medium grain (Giza 177 and Sakha 101), with short grain variants making up the minority (Giza 178). Between May and September, the intense solar radiation, long days, and mild nights are ideal for a large rice output. That is why, with a yield of 10 tonnes per hectare, Egypt has one of the highest rice yields in the world [37]. Egypt's government has attempted to limit rice cultivation area due to limited water resources. The rice crop is subjected to a variety of biotic and abiotic challenges, the most serious of which is blast disease, which poses the greatest threat to high rice yield [24, 27]. In Egypt, because of its extensive distribution and strong destructive potential under favorable conditions, blast disease is considered the most serious threat to rice production. In normal or mildly infected seasons, rice diseases, particularly rice blast, can

reduce yearly rice yield by roughly 5%. During epidemic seasons, crop losses might range from 30 to 50% [42]. Blast disease is caused by *Pyricularia oryzae* (Cooke) Sacc. teleomorph, *Magnaporthe oryzae* (Hebert) Barr. It is a widespread and damaging disease in the most rice growing areas of the world [33, 47]. Rice blast fungus produces many physiological races which cause breakdown for some new rice cultivars, few years after released [41]. One of the most effective strategies to protect crops from the disease is to plant resistant types. Aside from the vast amount of data acquired over the course of a lengthy history of genetic studies on rice blast resistance, current advances in rice genomics have enabled us to employ DNA markers for breeding resistant varieties through marker assisted selection (MAS). This knowledge will aid rice breeders in improving resistance to rice blast caused by MAS, as well as expand our understanding of the molecular mechanisms governing race specificity [4]. Rice blast disease has been combated using a variety of ways, including the use of resistant cultivars [8]. Due to the recent breakdown of the blast resistance for the leading rice variety Sakha 101, Rice Research and Training Center (RRTC) has given up on the seed production of this variety in preparation for the abolition [41]. However, most committed farmers continued saving seeds for planting in the next seasons. Rice farmers in Egypt have expressed satisfaction with this variety despite the breakdown of the blast resistance and decided to use the pesticides to overcome the disease due to the high yield of this variety compared to other varieties. The rice breeders at RRTC decided to solve this problem through backcross-breeding and transfer of blast resistance genes to Sakha 101, and finally released a new high-yielding variety to farmers in the country under the name of Sakha 108 [14]. The statistics showed that a large segment of rice farmers accepted the cultivation of the new variety (Sakha 108), as it complies with their desires, in addition to its resistance to blast disease. In spite of this, there is another broad section of the farmers still grow the rice variety Sakha 101 [38]. Therefore, there was a great similarity between the two cultivars due to the convergence in the genetic background. The aim of this investigation was to study the differentiation between the two cultivars Sakha 101 and Sakha 108 in terms of morphological, pathological and genomic characteristics.

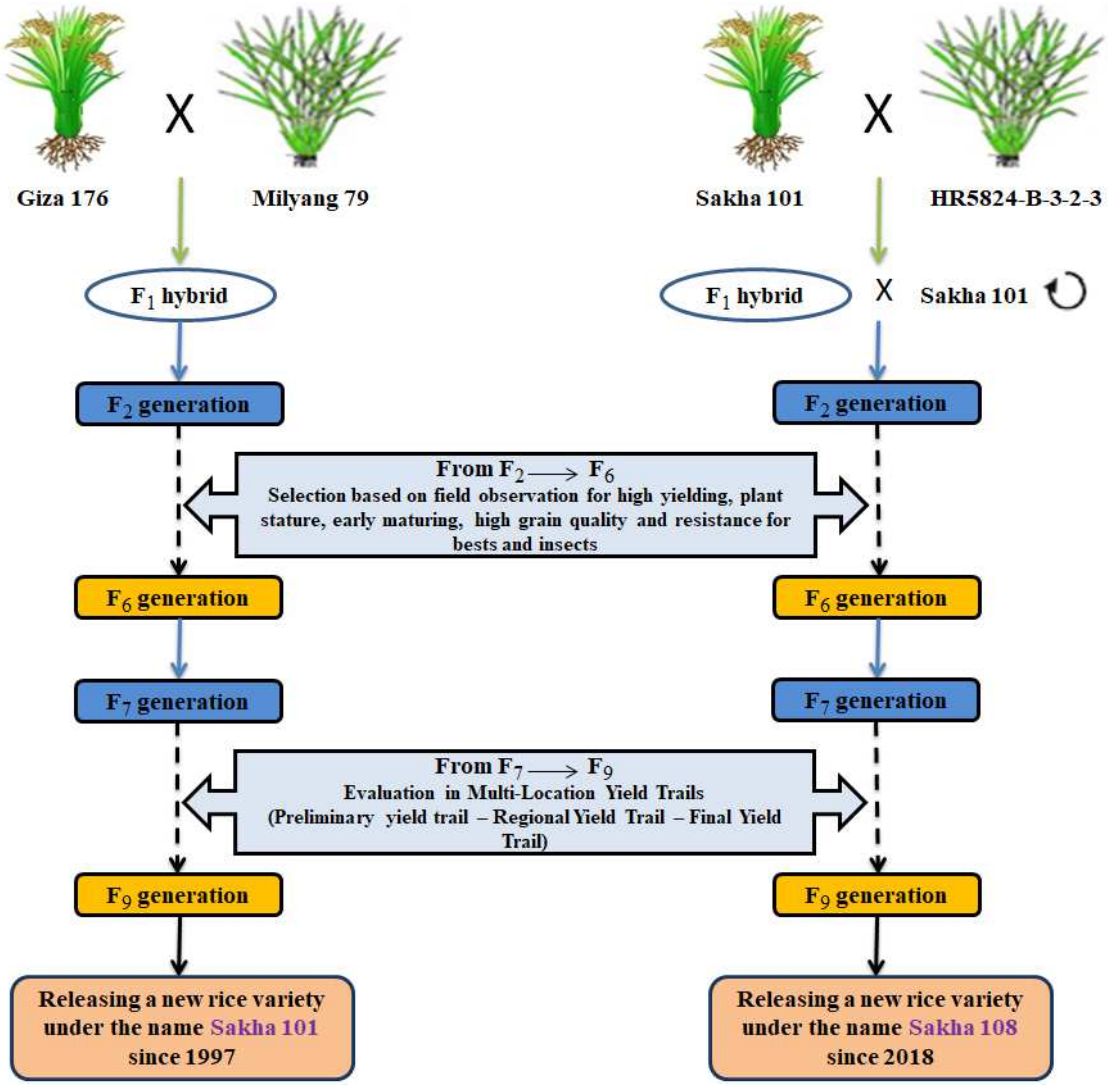

Figure 1. *Scheme for development and release of high yielding varieties Sakha 101 and Sakha108.*

2. Materials and Methods

2.1. Plant Materials and Field Experiments

The two common rice cultivars, Sakha 101 and Sakha 108, which were developed by Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt, were used in this study. These genotypes display a close range of their characters due to their same genetic background (Figure 1). The seeds of cultivars were sown under three different conditions, in Sakha, Gemmiza and Zarzora in May during two successful rice growing seasons; 2020 and 2021. The seeds were sown in nursery, and transplanted, a Randomized Complete Block Design (RCBD) with three replications 30 days later. Seedlings were transplanted at the experimental field in seven rows, each 5m long and 20 x 20 cm spaced between plants and rows. The standard package of recommendation practices were adopted for a good crop growth.

2.2. Measurements

Five plants from each replicate were chosen at random to measure and analyse basic agronomic characteristics, i.e. duration (day), plant height (cm), flag leaf area (cm^2), number of panicles/plant, panicle length (cm), panicle weight (g), 1000-grain weight (g), fertility percentage, and grain yield (t/h).

On the other hand, twenty rough grains were randomly selected from each cultivar and their dimensions were measured in order to determine the average grain length width, and thickness.

According to methodology of Adair [1], 150 g cleaned rough rice samples with a moisture content of 12-14% were used to determine percentage of hulling, milling and head rice percent. The amylose concentration and elongation ratio of rice were used to determine grain physicochemical qualities according to Nazmy, N. B. et al. [31].

2.3. Blast Study

During the rice growing seasons of 2020 and 2021, the research was conducted at the rice pathology laboratory, greenhouse, and farm, Sakha, Kafr Elsheikh, Egypt.

Isolation of rice blast fungus: from six governorates; Kafrelsheikh, Gharbia, Sharkia, Dakahlia, Damietta, and Beheira, rice blast samples were collected during 2020 growing season. According to Shabana, Y. M. et al. [43], typical blast lesions on leaves and panicles were isolated. For spore formation, the isolates were grown and multiplied on banana medium under fluorescent light for 10 days at 28°C. The spores were collected at a density of at least 25 spores per microscopic field, which was then inspected with a 10x objective.

2.4. Pathogenicity Test and Identification of Blast Physiological Races

The Two rice cultivars; SK 101 and SK 108 as well as eight international differential varieties (I.D.V) namely: Raminad Str.3, Zenith, NP-125, Usen, Dular, Kanto, CI 8970S and Caloro [7] were used to identify blast physiological races. In addition, ten international Japanese differential varieties (JDVs) *i.e*., Shin 2 (*Pik-*^{s}), Toride 1 (*Piz-*^{t}), Tusyake (*Pik-*^{m}), Kanto 51 (*Pik*), Fukunishiki (*Piz*), Ishikarishikie (*Pii-Pik-*^{s}), BL-1 (*Pib*), Yashiro-Mochi (*Pita*), Pi No. 4 (*Pita-*^{2}), Aichi Asahi (*Pia*) were used to determine effective resistant blast genes [41]. All tested entries were inoculated with seventy isolates under greenhouse conditions. The tested entries were seeded in plastic trays (30 cm). Each tray comprised 10 rows, with outer two rows [41]. The trays were kept at 28°C in the greenhouse and supplemented with urea (46.5 percent N; 5 g/tray). Seedlings were inoculated with spore suspension using an electrical spray pistol at the 3-4-leaf stage (approximately 3-4 weeks after sowing). The inoculated seedlings were kept in a damp chamber with a humidity of over 90% and a temperature of 28°C for 24 hours before being relocated to a greenhouse with comparable conditions.

2.5. Light Microscopy

Blast fungus-inoculated leaves were chopped into 0.5 cm-long pieces, vacuum-infiltrated in water, and dyed with a lactophenol–trypan blue solution containing 10 ml 90% lactic acid, 10 ml glycerol, 10 g phenol, and 10 mg trypan blue diluted in 10 ml distilled water [22]. Leaf segments were decolorized in a chloral hydrate solution containing 2.5 g of chloral hydrate diluted in 1ml of distilled water for at least 24 h after boiling for 3 minutes in the staining solution. For microscopy, stained leaf segments were placed in a chloral hydrate solution.

2.6. Leaf Anatomical

For the Sakha 101 and Sakha 108 rice cultivars, anatomical samples were taken after five days of inoculating leaves with blast pathogen. The leaves were washed and chopped into 1 cm^2 pieces. The samples were collected and placed in a flask containing 70% alcohol. The freehand section approach was used for the production and inspection of leaf anatomical samples. Sliced fresh leaves were fixed in a glycerin alcohol solution (30% glycerin and 70% alcohol). The staining techniques were carried out using safranin in 70% alcohol, and leaf cross sections were examined using a binocular light microscope.

2.7. Field Evaluation at Blast Nursery

Sakha 101 and Sakha 108 rice cultivars were investigated for blast resistance at seedling stage for key genes resistance under natural infection at blast nursery at two locations; Sakha and Gemmiza, in 2020 and 2021. Seedlings were ready by the first week of July, as a result of nitrogen fertilizer.

2.8. Disease Assessment

Under greenhouse conditions seven days after inoculation, blast reactions as typical blast lesions and field condition were scored on a 0-9 scale according to the standard evaluation system [17].

2.9. SSR Molecular Markers, PCR, and DNA Extraction

Ten seeds from each variety (Sakha 101 and Sakha 108) were soaked, germinated and placed in an incubator at 28°C for 20 days at China National Rice Research Institute (CNRRI). The DNA was isolated from fresh plants using the cetyl trimethyl ammonium bromide (CTAB) method described by Luo, Z. Y. et al. [28]. PCR protocol was performed by Creste, S. et al. [11]. For detection of polymorphism between Sakha 101 and Sakha 108, their DNAs were supposed to the PCR-amplification by 242 SSR markers on seven chromosomes 1, 2, 4, 9, 10, 11 and 12. Out of 242 SSR molecular markers, 6 markers gave polymorphism between the two cultivars. To identify the candidate gene around the polymorphic markers on each chromosome using Rice Genome Annotation Project, https://rapdb.dna.afrc.go.jp/index.html, we tried to take at least 5kb nucleotide sequences on both sides (before and after) of each marker.

3. Results and Discussion

3.1. Morphological Characterization

Morphological and agronomical characterizations are important and basic methodology to differentiate among the genotypes. In the present investigation, rice genotypes under study were characterized at different locations in terms of nine qualitative and quantitative traits (Table 1). Both Sakha 101 and Sakha 108 have a close range of agro-morphological traits mat be to the similarity in genetic background. With respect to days to maturing, the obtained data revealed early maturity for Sakha 108 compared to Sakha 101 under considered locations and years. This early flowering may be due to the transfer of some earliness genes from the extra-early maturing entry, HR5824-B-3-2-3 as one of the parents of the commercial Sakha 108 variety. On the other hand, the two cultivars; Sakha 101 and Sakha 108 are ideal and desirable for their stature under locations and years with values ranging from 92 to 98 cm. The rice cultivar Sakha 108 was a little bit taller than Sakha 101 during 2021 rice growing season. Several authors reported that transplantation time, water and soil condition, planting and sowing method affect rice plant height [16, 52, 19]. There is a significant difference between Sakha 101 and Sakha 108 for flag leaf area during 2020, and SK 108 recorded the highest values under locations. However, there were no significant differences between both cultivars in 2021. Flag leaf is the most essential organ for photosynthesis in rice and its size plays an important role in rice breeding for ideal plant-type and grain yield [55]. Yield component traits such as number of panicles/plant, panicle length, panicle weight, 1000-grain weight, fertility% and grain yield were measured for the studied genotypes. Generally, there are significant differences between the rice cultivars Sakha 101 and Sakha 108 for most of yield and its component traits except panicle length (2020 season), panicle weight (2021 season) and fertility% (2020 season). We may notice that there is a slight increase in the values of Sakha 108 compared to Sakha 101 in most of studied traits, which made the farmers accept the cultivation of Sakha 108 after breakdown Sakha 101 with blast disease. In this part, the two cultivars are studied to clarify the extent of the genetic relationship between them on the basis of morphology. This was also in confirmation with the findings of [15, 14].

Table 1. *Performance of agro-morphological traits for rice cultivars; Sakha 101 and Sakha 108 different locations.*

Genotype	Location	Duration (day)		Plant height (cm)		Flag leaf area (cm^2)	
		2020	2021	2020	2021	2020	2021
Sakha 101	Sakha	142^{a}	143^{b}	93	95^{b}	32.10^{f}	33.5
	Gemmiza	141^{a}	142^{ab}	92	93^{c}	33.55^{f}	34.11
	Zarzora	140^{a}	144^{a}	94	92^{d}	32.14^{e}	34.35
Sakha 108	Sakha	137^{b}	136^{c}	97	98^{a}	34.65^{c}	36.97
	Gemmiza	138^{b}	137^{c}	98	97^{a}	35.15^{b}	37.15
	Zarzora	137^{b}	137^{c}	98	98^{a}	36.17^{a}	37.01

Genotype	Location	No of panicles/plant		Panicle length (cm)		Panicle weight (g)	
		2020	2021	2020	2021	2020	2021
Sakha 101	Sakha	22^{ab}	21^{c}	22.2	22.11^{ab}	3.50^{ab}	3.15
	Gemmiza	23^{ab}	22^{bc}	21.3	20.90^{b}	3.41^{ab}	3.17
	Zarzora	21^{b}	21^{c}	22.0	21.25^{ab}	3.14^{b}	3.25
Sakha 108	Sakha	22^{ab}	23.6^{a}	21.83	23.0^{a}	3.68^{ab}	3.56
	Gemmiza	24^{a}	23^{ab}	22.01	22.11^{ab}	4.00^{a}	3.97
	Zarzora	23^{ab}	22.3^{ab}	22.65	22.15^{ab}	4.11^{a}	3.87

Genotype	Location	1000-grain weight (g)		Fertility%		Grain yield (t/h)	
		2020	2021	2020	2021	2020	2021
Sakha 101	Sakha	28.0^{b}	28.1^{c}	96.2	97.5^{a}	10.02^{e}	10.22^{e}
	Gemmiza	27.9^{b}	28.2^{bc}	95.7	95.6^{b}	9.90^{f}	10.09^{f}
	Zarzora	27.8^{b}	28.2^{bc}	96.0	95.3^{bc}	10.14^{b}	10.23^{d}
Sakha 108	Sakha	28.9^{a}	29.1^{ab}	92.8	91.9^{d}	10.62^{a}	10.35^{c}
	Gemmiza	28.7^{a}	28.9^{abc}	93.4	93.7^{cd}	10.12^{c}	10.56^{b}
	Zarzora	29.0^{a}	29.3^{a}	94.7	92.8^{d}	10.10^{d}	10.66^{a}

3.2. Physical and Chemical Properties

The physical dimensions Sakha101 and Sakha108 rice grains were measured and their results are shown in Figure 2. The grain length was 8.67 mm and 8.53 mm for Sakha101 and Sakha108, respectively. No significant difference (*P>0.05*) was observed between the two varieties, but Sakha101 was slightly longer than Sakha108 (Figure 3). The grain width was 3.37 mm for Sakha 101 and 3.53 mm for Sakha 108 with a highly significant difference (*P>0.05*) between the two varieties. On the other hand, grain thickness was 2.37 mm (Sakha 101) and 2.54 mm (Sakha 108). The results showed a significant difference (*P > 0.05*) between both cultivars as shown in Figure 3. Grain measurements are important features that influence the of rice quality. Also, physical grain quality is determined by hulling%, milling% and head rice%, as the higher values are more desirable. hulling percentage was higher in Sakha 101 than Sakha 108 with values of 81.2% and 79.78%, respectively. Milling percentage showed an opposite pattern to that of hulling percentage, and a mean value of 71.3% was recorded for Sakha 101 and 73.0% for Sakha 108 with a significant difference. A remarkable decrease was observed for head rice% for Sakha 108 with a value of 58.66% while, Sakha 101 showed a higher value.

The palatability of rice grains is one of the most important factors that rice breeder works on by studying some especial characteristics. Assessment of cooked rice quality can be determined by a combined evaluation of physical and physiochemical properties. Amylose content is used to predict the texture 4of cooked rice. Amylose contents were 18.4% and 19.5% for Sakha 101 and Sakha 108, respectively. The rice cultivar Sakha 108 was higher than Sakha 101 with elongation values of 42.5% and 39.5%, respectively. Results of [15, 14] on both cultivars are similar to the current results.

Table 2. *Number of rice blast samples collected from different cultivars and governorates during 2020 season.*

Cultivars	No. of isolates	No. of isolates/ Governorate					
		Kafrelsheikh	Gharbia	Dakahlia	Damietta	Sharkia	Beheira
Sakha 101	41	8	10	11	4	4	4
Sakha 104	18	4	2	4	5	2	1
Giza 171	7	1	3	1	0	1	1
Giza 159	2	0	0	0	0	1	1
PiNO.4	1	1	0	0	0	0	0
Reiho	1	0	0	0	0	0	1
Total	70	14	15	16	9	8	8

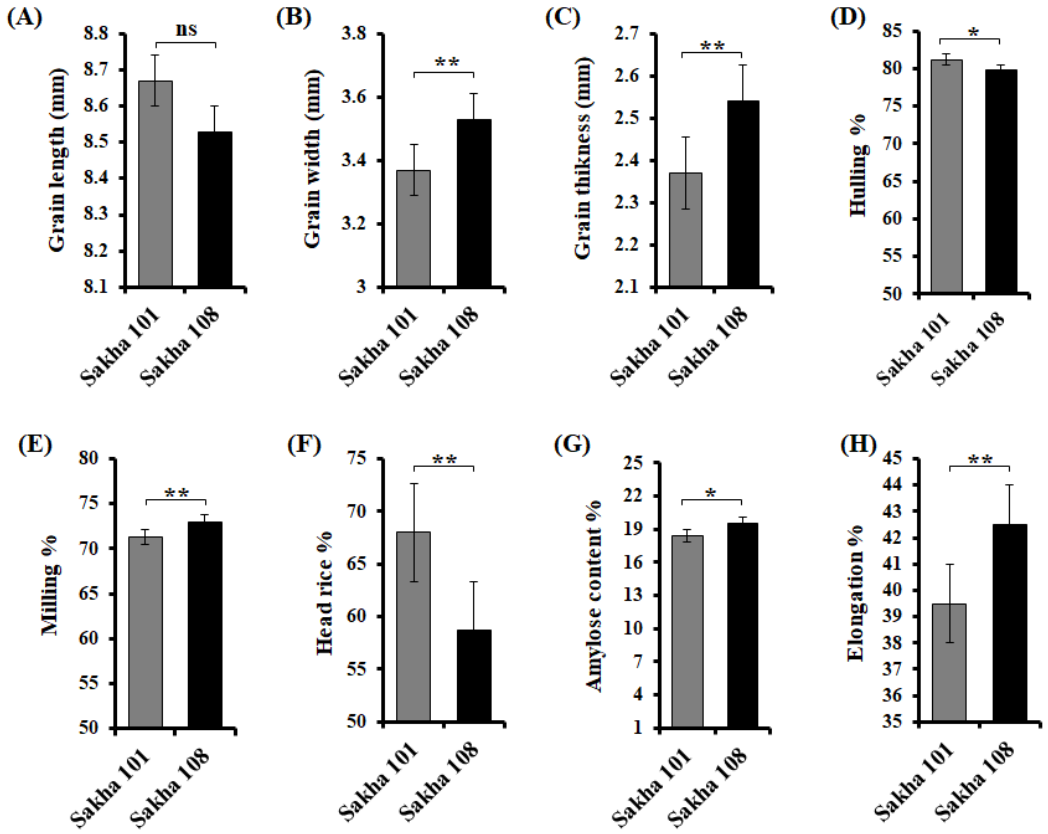

Figure 2. *Dimensions and shapes of Sakha 101 and Sakha 108 rice grains.*

Figure 3. *Phenotypic view of panicle length and some dimensions for Sakha 101 and Sakha 108 rice grains.*

3.3. Isolation of the Causal Organism of Blast Disease

Rice blast infected samples collected from different rice cultivars and locations during 2020 seasons are shown in Table 2. The rice cultivar Sakha 101 had the highest number of isolates (41) followed by Sakha 104 (18) and Giza 171 (7). The remaining cultivars had either one or two isolates. Higher numbers of isolates were collected from Dakahlia, Gharbia and kaferelshiekh governorates compared to Damietta, Sharkia and Beheira.

Seventy *P. oryzae* isolates were successfully isolated and purificated by the single spore technique. These isolates were identified as 22 races using the IDV under greenhouse conditions data in Table 3. Blast fungus spores of isolates no 9 as race group (ID-3) which isolated from Sakha 101 (Figure 4A) and isolates no 4 as race group (ID-15) which isolated from Sakha 101 (Figure 4E) and spore germination for two isolates (Figure 4B and F). Inoculated leaves after 5 days of inoculation for Sakha 108 and Sakha 101 were examined by light microscope (Figure 4C and G); C, show the Sakha 108 leaf surface free from conidia and hyphae; G, hyphae and conidia were appeared in inoculated Sakha 101 leaves. Lesion types appeared on Sakha 108 on (D) and Sakha 101 on (I). E, show the section of Sakha 108 leaf-anatomical and free from any hyphae and conidia of blast pathogen. While the section of Sakha 101 leaf-anatomical shows hyphae between the cells of leaf and covering the vessel (J). Rice blast fungus, *P. oryzae* is known to be highly variable [41]. Many investigators studied the physiological races of the fungus at different rice-growing areas [43, 20] who recorded many physiological races of *P. grisea* at different growing area of rice.

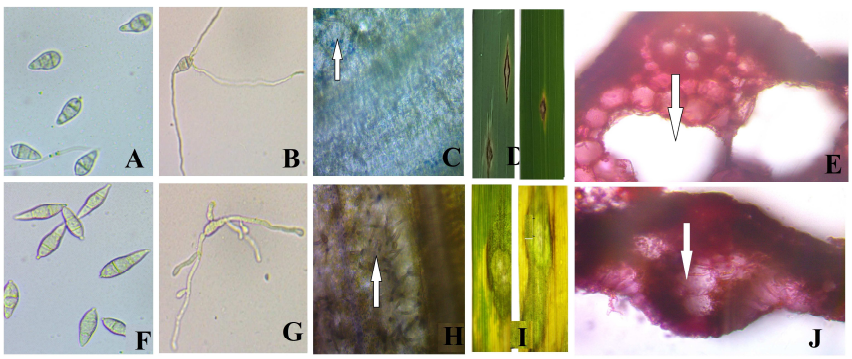

Figure 4. *Phenotypes of blast fungus-inoculated leaves of seedling rice leaves (A, B, C, D and E) Sakha 108 and (F, G, H and J) Sakha 101. (A) Blast fungus spores of isolates no 9 as race group (ID-3); (B) spore germination; C, S. 108 leaf surface were stained blue with lactophenol– trypan blue without mycelium; D, lesion on SK 108 with moderately resistance; E, leaf-anatomical of SK 108; F, Blast fungus spores of isolates no 4 as race group (ID-15); G, spore germination; H, Hyphae and conidia in inoculated leaves at 5 dpi were stained blue with lactophenol– trypan blue; H, lesion on SK 101 with highly susceptible; J, leaf-anatomical of SK 101.*

3.4. Pathogenicity Test and Race Identification

Seventy isolates were identified as eight groups using the IDV in (Table 3) and (Figure 5). The most occurring groups was ID (58.57%) followed by IC (17.2%). While, only one isolate was identified as IA (1.43%). Also, six isolates were identified as group IB race (8.57%). On the other hand, three isolates were identified as IH and II group races (4.3%), and two isolates were identified as IF and IG group (2.85). These results agree with the findings of Kalboush, A. Zeinab [20] that showed the distribution of races at different governorates. Physiological races play an important role for breakdown of the promising lines and new released cultivars; especially in case of expansion the growing areas of one or two cultivars. According to RRTC [38] cultivated areas with Sakha 101 were 21.26 and 19.25%, and that Sakha 108 was 19.44 and 16.40% out of total area in 2019 and 2020 rice seasons, respectively.

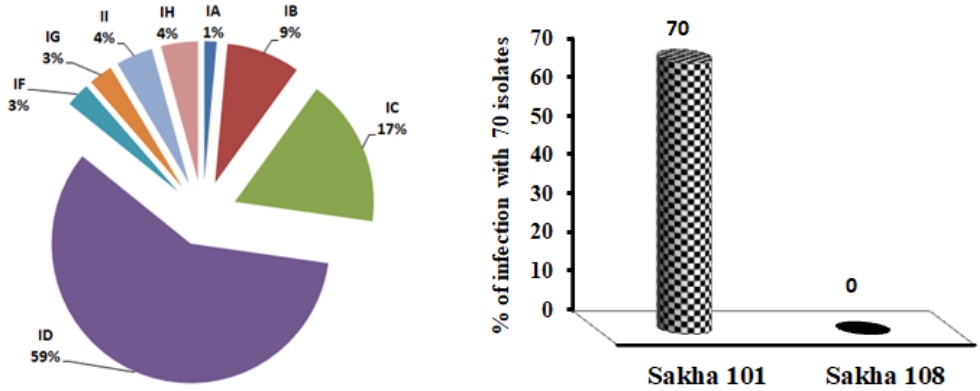

Figure 5. *Distribution of different blast race groups on the IDV and% of infection with 70% of isolates on SK 101 and SK 108 under greenhouse test during 2021.*

Many investigators studied the physiological races of the fungus at different rice-growing areas and discussed the role of physiological races in breakdown of the new promising lines. Also, data in Table 3 showed that isolate no. 9 collected from Sakha 101 was identified as ID-3 race. These results agree with those of Kalboush, A. Zeinab and Anis, et al. [20, 4] who showed the distribution of races with different rice entries and locations and this new physiological race was associated with breakdown of some new rice genotypes. Also, Anis, G. B. et al. [4] recorded race of ID-3 isolated from Giza 171 rice cultivar of Egypt. Data in (Table 4) showed that the forty nine isolates were infected Sakha 101 rice cv. While, only one isolate (9) as group ID-3 and gave reaction on Sakha 108 as moderately resistance (MR). Sehly [41] recorded that Sakha 101 rice cultivar was released in year 1997 and breakdown in year 2003 by *P. oryzae* fungus the cultivar growing 7 year before breakdown. While, Sakha 108 was released in year 2018 and until now still resistance for blast pathogen [14].

Table 3. *Race identification of Pyricularia oryzae isolates collected from different rice genotypes and governorates under greenhouse condition.*

Isolate number/Race/ Governorate /*										
Governorate	**Kafrelsheikh (SK 101)**									**PiNO.4**
District	**Desouq**	**Foaa**	**Sakha**	**Kallien**	**Metbol**	**Difria**	**Mesiar**	**Sidisalem**	**Sakha**	**Kafrelsheikh**
Isolate no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Race	ID-15	ID-15	ID-15	ID-15	IH-1	II-1	ID-7	IC-13	ID-3	ID-15

Isolate number/Reaction/ Governorate /*										
Governorate	**Kafrelsheikh (SK 104)**					**Gharbia (SK 101)**				
District	**Desouq**	**Kallien**	**Elhamol**	**Difria**	**Elsgaiea**	**Elmahala**	**Elmahala**	**Nemraelbassal**	**Gemmiza**	**Aga**
Isolate no.	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Race	IC-3	IB-47	ID-11	IC-11	ID-15	ID-15	ID-15	ID-11	ID-15	ID-15

Isolate number/Reaction/ Governorate /*										
Governorate	**Gharbia (SK 101)**					**Gharbia (Giza 171)**			**Gharbia (SK 104)**	
District	**Elmahala**	**Samanoud**	**Qotour**	**Qotour**	**Segeenelkom**	**Eldawakhlia**	**Qotour**	**Gemmiza**	**Qotour**	**Elmahala**
Isolate no.	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Race	ID-15	ID-11	ID-15	ID-15	ID-15	ID-15	ID-15	ID-11	ID-15	IB-11

Isolate number/Reaction/ Governorate /*										
Governorate	**Dakahlia (SK 101)**									
District	**Dekerns**	**Talkha**	**Mansoura**	**Mansoura**	**Mansoura**	**Talkha**	**Dekerns**	**Mansoura**	**Talkha**	**Talkha**
Isolate no.	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
Race	II-1	II-1	ID-16	ID-15	ID-11	IB-47	IC-11	IC-9	IB-3	ID-11

Isolate number/Race / Governorate /*										
Governorate	**Dakahlia (SK 101)**	**Dakahlia (SK 104)**				**Giza 171**	**Damietta (SK 101)**			
District	**Met sweed**	**Mansoura**	**Mansoura**	**Talkha**	**Dekerns**	**Mansoura**	**Zarka**	**Zarka**	**Kafr Saker**	**Kafr Saker**
Isolate no.	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**
Race	ID-15	ID-11	IA-111	ID-15	ID-9	IC-11	IF-3	ID-14	ID-11	ID-11

Isolate number/Reaction/ Governorate /*										
Governorate	**Damietta (SK 104)**					**Sharkia (SK 101)**			**Sharkia (SK 104)**	
District	**Kafr Saker**	**Kafr Saker**	**Kafr Saker**	**Kafr Saker**	**Zarka**	**Kafr Saker**	**Kafr Saker**	**Zagazig**	**Kafr Saker**	**Zagazig**
Isolate no.	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**
Race	IC-11	IH-1	IG-1	IB-4	IC-11	ID-15	ID-11	IC-15	IC-11	IC-11

Isolate number/Reaction/ Governorate /*										
Governorate	**Sharkia (Giza 159)**	**Sharkia (Giza 171)**	**Beheira (SK 101)**				**Beheira (SK 104)**	**Beheira (G.171)**	**Beheira (G.159)**	**Beheira (Reiho)**
District	**Kafr Saker**	**Kafr Saker**	**El-amriea**	**Mahmoudia**	**Itai-El-Barood**	**Mahmoudia**	**Mahmoudia**	**Itai-El-Barood**	**Itai-El-Barood**	**Itai-El-Barood**
Isolate no.	**61**	**62**	**63**	**64**	**65**	**66**	**67**	**68**	**69**	**70**
Race	ID-11	IH-1	ID-16	IF-3	ID-13	IG-1	IC-11	IB-15	ID-16	ID-9

These results should be considered in developing resistant rice cultivars, as the most important method for control of rice blast disease. Also, the highest infection was found in Sakha 101 (70%) while Sakha 108 was free from infection (Figure 5). Ten promising lines and their parents were evaluated against rice blast pathogen. Under artificial inoculation with blast pathogen the breeding lines were resistant in both greenhouse and field condition, but Sakha 101 was infected with the most isolates [40]. Also, Kalboush, A. Zeinab [20] found that Sakha 101 was the most susceptible cultivar when evaluated 21 rice genotypes with 20 isolates of blast pathogen and Sakha 101 was infected by 17 isolates of *pryicularia oryzae* under greenhouse condition.

Table 4. *Blast reactions of two rice genotypes inoculated with 70 Pyricularia oryzae isolates under greenhouse conditions.*

Genotype	*Isolate number/Reaction**																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Sakha 101	S	S	S	HS	S	S	S	S	R	S	R	R	R	R	S	S	S	S	S	S
Sakha 108	R	R	R	R	R	R	R	R	MR	R	R	R	R	R	R	R	R	R	R	R
	Race/Isolate number/Reaction																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Sakha 101	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Sakha 108	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	Race/Isolate number/Reaction																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Sakha 101	S	R	R	R	R	R	S	S	S	S	R	R	R	R	R	S	S	S	R	R
Sakha 108	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	Race/Isolate number/Reaction										*% of infecting SK 101 and SK 108 rice cultivars with 70 isolates*									
	61	62	63	64	65	66	67	68	69	70	SK 101					SK 108				
Sakha 101	S	S	S	S	S	S	R	R	S	S	70%					0.0				
Sakha 108	R	R	R	R	R	R	R	R	R	R										

* Reactions: R = resistant (1-2), MR = moderately resistant (3), S = susceptible (4-6), HS = highly susceptible (7-9) IRRI, 2013.

3.5. R-genes Effecency of Blast Resistance

Blast R-genes in improvement programs of rice are very important tools. Data presented in Figure 6 show the number of infected isolates on the JDVs to rice blast fungus isolates and effectiveness of blast resistant genes. The frequency of R- gene reaction of JDV to the 70 isolates ranged from 31.43 to 97.14%, depending on the effectiveness of the present R- gene. *Pi-Z* and *Pii* - *Pi-k*^{s} R-genes were the highest R- gene effective against tested blast isolates (97.14%), followed by *Pi-b*, *Pi-k*^{s} and *Pi-t*^{a} R-gene with efficiency of 94.28, 92.86 and 91.43%, respectively. Also, R-gene *Pi-z*^{t} and *Pi-ta*^{2} were moderately resistant (82.85%) followed by *Pik* and *Pi-k*^{m} (75.71 and 70%, respectively). R-gene *Pia* was the least which exhibited 31.43% resistance. The results are in agreement with [4] who found that *Pia R-* gene was the least effective gene under artificial infection with 26 isolates of *P. grisea* on JDVs. These genes are recommended to be used by rice breeders as donors for blast resistance under Egyptian conditions [41] or under China conditions [51]. Shabana, et al. [43] recorded the reaction of monogenic lines to 132 isolates of rice blast, and found that the reaction ranged between zero and 97.76%, depending on the effectiveness of the present resistance gene. *Piz-*^{5} gene was the most effective against blast isolates, followed by *pita-*^{2}, *Pi5 (t)* and then *Piz*, *Pii*, *Pi9*, *Pita-*^{2}, and *Pit*. Thus, these genes are recommended to be used by rice breeders as donors for blast resistance under Egyptian condition.

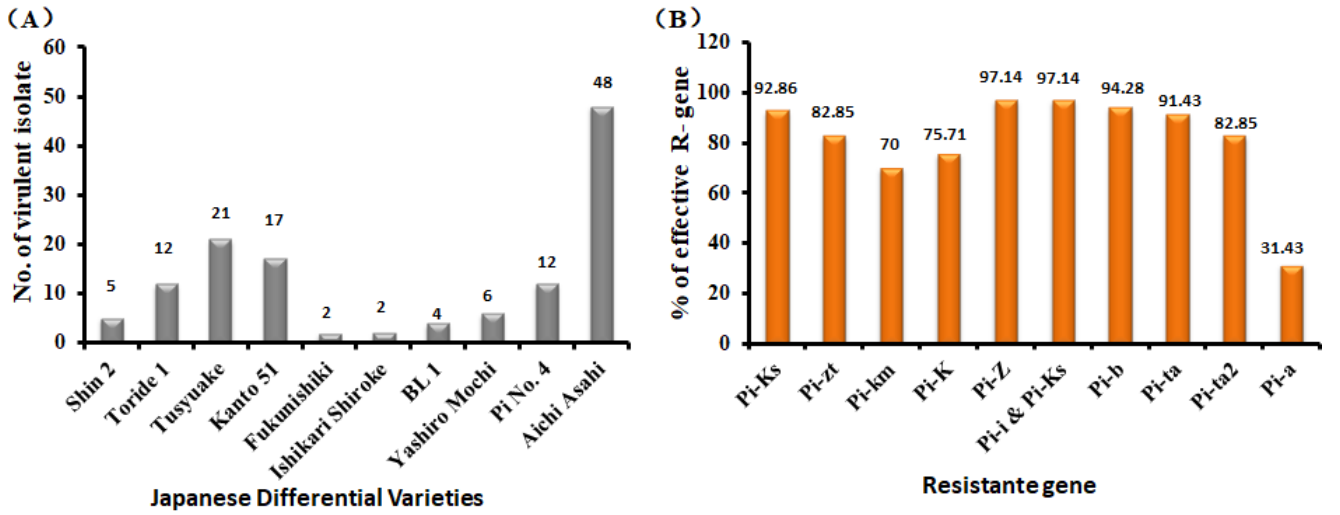

Figure 6. *Number of virulent isolates and effectiveness of blast resistant genes% against 70 blast isolates on JDV under greenhouse conditions during 2021.*

3.6. Under Field Condition

Sakha 108 at two locations was resistant while Sakha 101 was susceptible to blast disease at both seasons (Table 5). The results are in agreement with those of Hammoud S. A. et al. [14] who released Sakha 108 as a resistant rice cultivar and Sakha 101 as a check susceptible rice cultivar.

Table 5. *Reaction of Sakha 101 and 108 to blast disease under different locations and seasons.*

Cultivars	Sakha 101		Sakha 108	
	2020	2021	2020	2021
Sakha	HS	HS	R	R
Gemmiza	S	S	R	R
Zarzora	S	S	R	R

3.7. Polymorphisms Detected by SSR Markers Between Sakha 101 and Sakha 108

The distribution of SSR and linkage map information for Sakha 101 and Sakha 108 are shown in Table 6. The full genome was 213.55 cM in length and included 242 markers across 7 chromosomes (no. 1, 2, 4, 9, 10, 11 and 12). These markers were used in this study to survey the polymorphisms between Sakha 101 and Sakha 108. Among the screened primers, only 6 primers produced clear fragments and polymorphism, which were used to construct a genetic linkage map and find the candidate genes around their regions on each chromosome (Table 7 and Figure 8). The average distance between adjacent markers was 0.91 cM. The most saturated chromosome was chromosome 12, which had an average marker distance of 0.73 cM, while chromosome 11 had the largest average marker distance of 1.33 cM. The average chromosome length was 30.50 cM, with the longest being chromosome 1 (43.32 cM) and the shortest being chromosome 9 (22.56 cM). A total of 242 SSR markers used Sakha 101 and Sakha 108 and, only six markers exhibited polymorphism between the two cultivars, one marker on chromosomes 1, 2, 9 and 11 as well as two markers on chromosome 4 (Figure 7). One the other hand, no markers exhibited polymorphisms between the two varieties on chromosomes 10 and 12 (Table 7). The obtained data and less number of polymorphic markers support the conclusion that the Sakha 108 genetic background is somehow similar to Sakha 101 but the Sakha 108 has displayed very good yield potential, resistance to blast disease and its superiority over Sakha 101 in some other traits.

Table 6. *Distribution of polymorphic markers on the seven chromosomes.*

Chromosome	Chromosome length (cM)	No. of markers	Average distance between 2 markers (cM)	No. of polymorphic markers
Chr. 1	43.32	45	0.96	1
Chr. 2	35.30	46	0.76	1
Chr. 4	35.01	42	0.83	2
Chr. 9	22.56	30	0.75	1
Chr. 10	22.74	22	1.03	---
Chr. 11	28.03	21	1.33	1
Chr. 12	26.59	36	0.73	---
Total	213.55	242	0.91	6

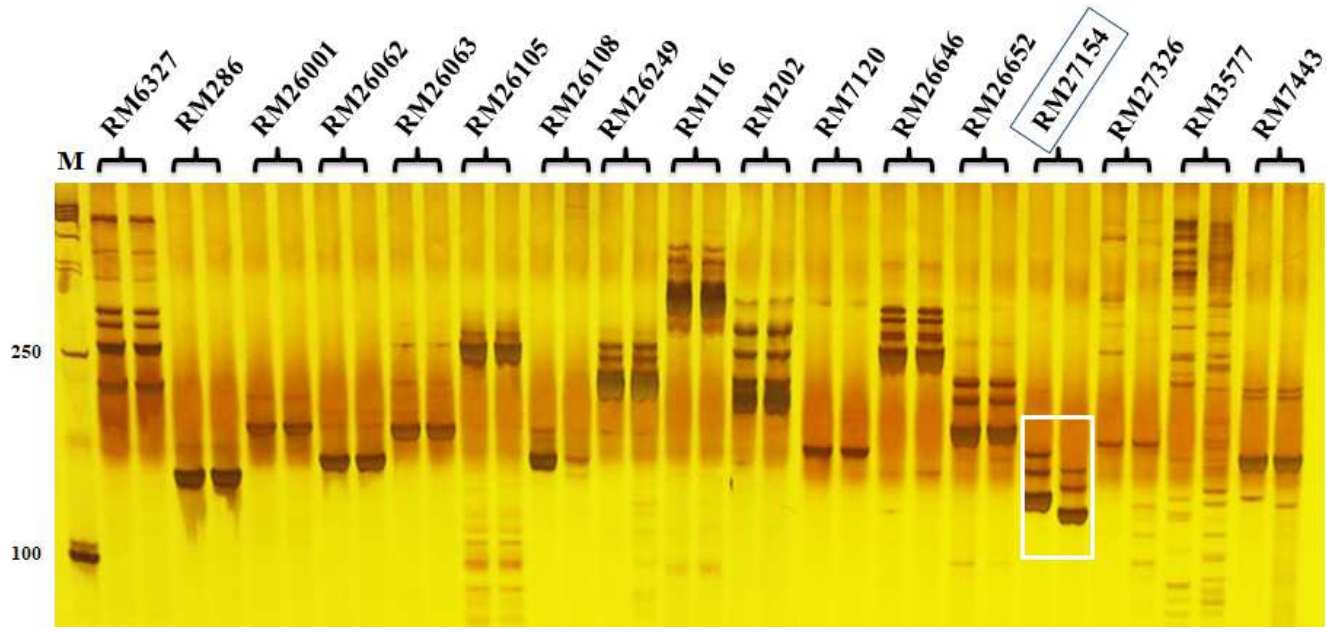

Figure 7. *SSR profiles of SK 101 and SK 108 amplified by 17 primers distributed on chromosome 11. The shaded primer from outside RM27154 the only marker gave polymorphic on chromosome 11.*

The six polymorphic markers are distributed over five chromosomes as follows; RM8236 on chromosome 1 and might be related to heading date and 1000-grain weight according to the finding of Zhou, J. A. et al. [58], RM13611 on chromosome 2 associated with grain size [34], RM3839 and RM17377 on chromosome 4 were associated with shoot dry weight and grain quality and this is in agreement with Cheng, X. X. et al. [10, 50], respectively. In the same time, RM160 on chromosome 9 and RM27154 on chromosome 11 may be associated with *R- genes Pi54*, *Pi1* and *Pita* and *Pi-K*^{m}, respectively (Table 7). Identification of the SSR primers polymorphism between the two cultivars will be useful in the rice breeding program to differentiate between them. Similar results were observed in some other studies [15, 14].

Table 7. *Marker information used for genotyping in the study and association between SSR markers and agronomic traits.*

Marker	Chr.	Primer	Start	Stop	Size bp	Related traits	Reference
RM8236	1	F- GGGATTATTTGAAATCTTTGC R- ATATAGCATTGCCAGTTTGC	40469905	40470075	170	Heading date and 1000-grain weight.	Zhou, *et al*. [58]
RM13611	2	F- CTCTTGAACGGCTGCACGAAAGG R- CGCGAGAATGGTAGGTGGATCG	24773556	24773698	142	Grain size	*Pao, et al.* [34]
RM3839	4	F- AATGGGACCAGAAAGCACAC R- AAAAAGAGCATGGGGGCTAC	23870630	23870962	179	Shoot dry weight	Cheng, *et al*. [10]
RM17377	4	F- ATATTACTTCGACGCTGGATCAGG R- GTCAGTTCGTCAGGCACAACG	28931144	28931312	168	Grain quality	Tahmina, *et al.* [50]
RM160	9	F- AGCTAGCAGCTATAGCTTAGCTGGAGATCG R- TCTCATCGCCATGCGAGGCCTC	19788247	19788377	130	*R- genes Pi54*, *Pi1* and *Pita*	Khan, *et al*., [21]
RM27154	11	F- TAGTCGGGCATCTCCTCTTCC R- GTTACCTCCGATGAAGGCAAGG	24729313	24729449	136	R- genes *Pi-K*^{m}	Shi, *et al*., [46]

3.8. Candidate Genes at the Target Regions on Five Chromosomes

On the basis of the Rice Genome Annotation Project Database (http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/) and within the targeted genomic regions of polymorphic markers on 5 chromosomes, there are predicted genes in each region (Table 8 and Figure 8). Forty -five SSR markers on chromosome 1 were tested between Sakha 101 and Sakha 108 to find the polymorphic markers and only RM8236 marker gave the target. On the target region on chromosome 1 and around marker RM8236 within the 40,464,905 - 40,475,075 kb, we found five predicted genes, including *LOC_Os01g69950.1* (Ribosomal protein L27, putative, expressed), *LOC_Os01g69960.1* (Expressed protein), *LOC_Os01g69970.1* (WD domain, G-beta repeat domain containing protein, expressed), *LOC_Os01g69980.1* (TCP family transcription factor, putative, expressed) and *LOC_Os01g69990.1* (GYF domain containing protein, putative, expressed). Several researchers used the marker RM8236 in their studies to identify different QTLs especially for disease resistance in rice. Orjuela [32] studied the resistance to rice yellow mottle virus (*RYMV2*) using the whole intraspecific mapping population and the results mapped *RYMV2* between RM12055 and RM8236 markers. In the same context, Sun, Z. et al. [48] studied the identification of quantitative trait loci for

small brown plant hopper in rice. Four QTLs, *viz. qSBPH1, qSBPH5, qSBPH8,* and *qSBPH9* were detected and one of them *qSBPH1* was localized between the RM3738 and RM8236 markers suggesting that this marker is close to a series resistance genes and associated with resistance in rice and we can speculate that one of the studied varieties includes this gene. Also, using 152 microsatellite markers across the rice whole genome, Zhou [58] studied the natural population comprising 128 japonica rice varieties to identify marker-trait associations, and the results revealed that RM8236 is associated to heading dates and 1000-grain weight traits. So, this marker might give polymorphism pattern between Sakha 101 and Sakha 108 based on the significant differences between them. One the other hand, the target region on chromosome 2 and within 24,768,556 - 24,778,698 kb around the marker RM13611 involved six candidate genes. These genes were; *LOC_Os02g40850.1* (Retrotransposon protein, putative, unclassified), *LOC_Os02g40860.1* (CK1_CaseinKinase_1.5 - CK1 includes the casein kinase 1 kinases, expressed), *LOC_Os02g40870.1* (Phosphatidylinositol N-acetylglucosaminyltransferase subunit C, putative, expressed), *LOC_Os02g40880.1* (Ribosomal protein L14, putative, expressed), *LOC_Os02g40890.1* (GLTP domain containing protein, putative, expressed) and *LOC_Os02g40900.1 (*RNA recognition motif containing protein, putative, expressed). The polymorphic marker RM13611 on this chromosome is related to grain size QTL (*qGS7.1*) according to the previous study by Pao, X. et al. [34]. There is a significant difference between Sakha 101 and Sakha 108 in grain width and grain thickness as well as 1000- grain weight suggesting that RM13611 marker gave polymeric pattern between the studied varieties based on grain size of these two varieties. We also identified 2 polymorphic markers (RM3839 and RM17377) out of 42 SSR markers on chromosome 4 with two target regions. The first region around marker RM3839 and within 23,865,630 - 23,875,962 kb including eight candidate genes, such as *LOC_Os04g40070.1 (*GRAM and C2 domains containing protein, putative, expressed), *LOC_Os04g40080.1 (*Leucine rich repeat containing protein, expressed), *LOC_Os04g40090.1* (zinc finger, ZZ type family protein, expressed), *LOC_Os04g40100.1* (BTBN11 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with non-phototropic hypocotyl 3 NPH3 domain, expressed), *LOC_Os04g40110.1* (Hypothetical protein), *LOC_Os04g40120.1* (Expressed protein), *LOC_Os04g40130.1* (*Rf1*, mitochondrial precursor, putative, expressed) and *LOC_Os04g40140.1* (Expressed protein). Previously, SSR genomic region RM3839 mapped on chromosome 4 was significantly associated with QTL controlling shoot dry weight [10]. On the other hand, the same marker allele was significantly associated with root length [3]. A series of researchers reported a highly significant correlation between root length trait and shoot dry weight using different populations in their studies [57, 5, 6, and 18]. Dropping the words on the two varieties under study, we find that the rice cultivar Sakha 108 is characterized by a relatively stronger shoot than Sakha 101, and therefore it is likely that it contains root length, and maybe is reflected on its yield. Increasing root and shoot traits by utilizing favorable allele at this locus would be useful for increasing water and nitrate uptake as well as photosynthetic area of plant. On the same chromosome, we have a second region around marker RM17377 and within 28,926,144 - 28,936,312 kb. This region involved seven predicted genes like; *LOC_Os04g48500.1* (Expressed protein), *LOC_Os04g48510.1* (Growth regulating factor protein, putative, expressed), *LOC_Os04g48520.1* (ZOS4-12 - C2H2 zinc finger protein, expressed), *LOC_Os04g48530.1* (C4-dicarboxylate transporter/malic acid transport protein domain containing protein, expressed), *LOC_Os04g48540.1* (Dihydrodipicolinate synthase, chloroplast precursor, putative, expressed), *LOC_Os04g48550.1* and *LOC_Os04g48560.1* with the same putative function (Retrotransposon protein, putative, unclassified, expressed). The polymorphic marker RM17377 was used in only one further linkage map construction study to identify the QTLs associated with rice grain quality (starch paste viscosity attributes) [44]. This marker was used to detect the QTLs for yield related traits in RILs population and unlinked with any studied traits [23]. Among the six predicted genes in the mapped region on chromosome 9 within 19,783,247 - 19,793,377 kb around the marker RM160, four genes *LOC_Os09g33520.1*, *LOC_Os09g33530.1*, *LOC_Os09g33540.1 and LOC_Os09g33555.1* expressed protein with unknown functions. One gene, *LOC_Os09g33550.1* has been reported previously as a CCT/B-box zinc finger protein, while, *LOC_Os09g33559.1* has been reported as a proline-rich family protein. Strikingly, *LOC_Os09g33559.1* with a putative function as a proline-rich protein slows plant's defense responses and blast resistance genes expression [13].

We further identified the candidates within the target region on chromosome 11 around marker RM27154. As shown in table 8 and figure 8, *LOC_Os11g41210.1* (Disease resistance protein RPM1, putative, expressed), *LOC_Os11g41230.1* and *LOC_Os11g41240.1* as they were predicted as (ATBPM6, putative, expressed), *LOC_Os11g41250.1* (Expressed protein with unknown functions), *LOC_Os11g41260.1* (snRNP protein, putative, expressed), *LOC_Os11g41270.1* and *LOC_Os11g41280.1* with the same function (Retrotransposon protein, putative, unclassified, expressed). Rice chromosome 11 is rich in disease-resistance genes and has at least 201 putative *R* genes [35, 59]. In fact, it has been reported that there are many genes resistant to blast that are located on the long arm of chromosome 11, such as *Pi1(t)* [29], *Pik* [30], *Pi-lm2* [49], *Pi-18* [2], *Pi44(t)* [9], *Pi-30(t)* [39], Pi34 [53, 54], Pi-*y(t)* [56], *Pik-h* [45], *Pif*, and *Pi7* [12]. Recently, *Pik-m* and *Pi-hk1(t)* were reported to be localized near to our target region [25, 26]. Based on these above information, the genetic diversity of the studied cultivars (Sakha 101 and Sakha 108) can be utilized to predict approaches such as association analysis, classical mapping population development; parental line selection in breeding programs and hybrid development for exploiting the natural genetic variation exists in the rice

populations.

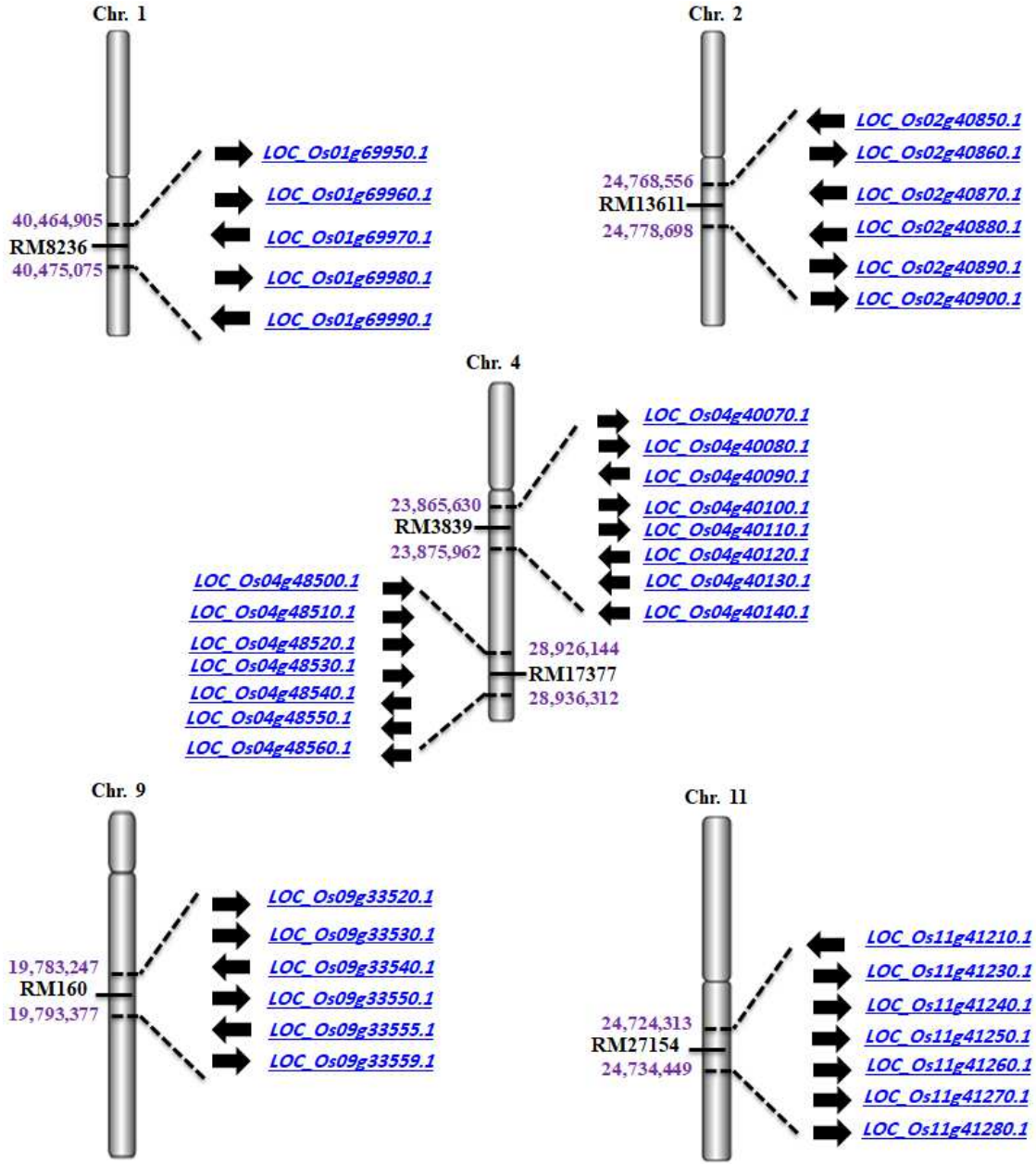

Figure 8. *Mapping results and predicted selected genes found within mapping regions on five chromosomes.*

Table 8. *Candidate genes located within the target regions on each chromosome.*

Chr.	Gene ID	Gene start (bp)	Gene end (bp)	Putative function
Chromosome 1	*LOC_Os01g69950.1*	40453829	40456679	Ribosomal protein L27, putative, expressed
	LOC_Os01g69960.1	40460001	40461483	Expressed protein
	LOC_Os01g69970.1	40467759	40463007	WD domain, G-beta repeat domain containing protein, expressed
	LOC_Os01g69980.1	40477480	40478907	TCP family transcription factor, putative, expressed
	LOC_Os01g69990.1	40489660	40483305	GYF domain containing protein, putative, expressed
Chromosome 2	*LOC_Os02g40850.1*	24765292	24762747	Retrotransposon protein, putative, unclassified
	LOC_Os02g40860.1	24766807	24772750	CK1_CaseinKinase_1.5 - CK1 includes the casein kinase 1 kinases, expressed
	LOC_Os02g40870.1	24775040	24773421	Phosphatidylinositol N-acetylglucosaminyltransferase subunit C, putative, expressed
	LOC_Os02g40880.1	24779637	24777727	Ribosomal protein L14, putative, expressed
	LOC_Os02g40890.1	24780566	24782489	GLTP domain containing protein, putative, expressed
	LOC_Os02g40900.1	24786565	24783195	RNA recognition motif containing protein, putative, expressed

Chr.	Gene ID	Gene start (bp)	Gene end (bp)	Putative function
Chromosome 4 (region 1)	*LOC_Os04g40070.1*	23859220	23863737	GRAM and C2 domains containing protein, putative, expressed
	LOC_Os04g40080.1	23864150	23867322	Leucine rich repeat containing protein, expressed
	LOC_Os04g40090.1	23872309	23867457	zinc finger, ZZ type family protein, expressed
	LOC_Os04g40100.1	23876327	23878958	BTBN11 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with non-phototropic hypocotyl 3 NPH3 domain, expressed
	LOC_Os04g40110.1	23881016	23881351	Hypothetical protein
	LOC_Os04g40120.1	23882332	23881847	Expressed protein
	LOC_Os04g40130.1	23885227	23882661	Rf1, mitochondrial precursor, putative, expressed
	LOC_Os04g40140.1	23887923	23885498	Expressed protein
Chromosome 4 (region 2)	*LOC_Os04g48500.1*	28917831	28921401	Expressed protein
	LOC_Os04g48510.1	28921895	28923696	Growth regulating factor protein, putative, expressed
	LOC_Os04g48520.1	28924932	28929265	ZOS4-12 - C2H2 zinc finger protein, expressed
	LOC_Os04g48530.1	28929773	28932034	C4-dicarboxylate transporter/malic acid transport protein domain containing protein, expressed
	LOC_Os04g48540.1	28932886	28932065	Dihydrodipicolinate synthase, chloroplast precursor, putative, expressed
	LOC_Os04g48550.1	28942064	28940424	Retrotransposon protein, putative, unclassified, expressed
	LOC_Os04g48560.1	28945666	28943285	Retrotransposon protein, putative, unclassified, expressed
Chromosome 9	*LOC_Os09g33520.1*	19769695	19775102	Expressed protein
	LOC_Os09g33530.1	19780676	19775475	Expressed protein
	LOC_Os09g33540.1	19782226	19782513	Expressed protein
	LOC_Os09g33550.1	19786777	19783524	CCT/B-box zinc finger protein, putative, expressed
	LOC_Os09g33555.1	19788719	19789621	Expressed protein
	LOC_Os09g33559.1	19796192	19798778	Proline-rich family protein, putative, expressed
Chromosome 11	*LOC_Os11g41210.1*	24,712,661	24,719,904	Disease resistance protein RPM1, putative, expressed
	LOC_Os11g41230.1	24724484	24725050	ATBPM6, putative, expressed
	LOC_Os11g41240.1	24727308	24727862	ATBPM6, putative
	LOC_Os11g41250.1	24730266	24730709	Expressed protein
	LOC_Os11g41260.1	24734244	24737370	snRNP protein, putative, expressed
	LOC_Os11g41270.1	24737698	24741366	Retrotransposon protein, putative, unclassified, expressed
	LOC_Os11g41280.1	24749285	24743260	Retrotransposon protein, putative, unclassified, expressed

4. Conclusion

The Egyptian rice cultivars (Sakha 101 and Sakha 108) were displayed a close range of their characters due to their same genetic background. New physiological races of *P. oryzae* play an important role for breakdown of the promising lines and new released cultivars; especially in case of expansion the growing areas of one or two cultivars. The forty nine isolates were infected Sakha 101 rice cv. while, only one isolate (9) as group ID-3 and gave reaction on Sakha 108 as moderately resistance (MR). By SSR we used Sakha 101 and Sakha 108 and, only six markers exhibited polymorphism between the two cultivars and found there are many genes resistant to blast that are located on the long arm of chromosome 11. The genetic background of Sakha 108 is somehow similar to Sakha 101 but Sakha 108 has displayed very good yield potential, resistance to blast disease and its superiority over Sakha 101 in some other traits.

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