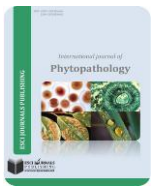

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MOLECULAR MARKERS IDENTIFICATION OF LEAF RUST RESISTANT GENES LR19, LR21, LR24, LR47 AND LR51 IN SELECTED EGYPTIAN WHEAT CULTIVARS

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A B S T R A C T

Leaf rust, caused by *Puccinia triticina* is a common and widespread disease of bread wheat (*Triticum aestivum L.*), in Egypt. Host resistance is the most economical, effective and ecologically sustainable method for controlling the disease. Molecular markers help to determine leaf rust resistance genes (*Lr* genes) that may be present in a large group of wheat germplasm. The objective of this study was to evaluate and detect leaf rust resistance genes in Egyptian wheat cultivars. Ten out of fifteen cultivars were resistance to leaf rust disease in four locations i.e., Dakahlia, Kafr el-Sheikh, Beheira and Sharqia during seasons 2011/2012 and 2012/2013. As for, using specific SSR primers proved that Lr19 was present in five cultivars i.e., Sakha-95, Gemmeiza-9, Gemmeiza-10, Misr-1 and Misr-2. *Lr21. Lr24, Lr47,* and *Lr51* were detected in all tested cultivars. These genes should be taken into consideration in wheat breeding programs for successful rust resistance. Furthermore these materials can be used as a parent for plant breeders to add new effective resistance genes to their breeding materials because of the dynamic change of leaf rust races which can breakdown the resistance.

Keywords: wheat, leaf rust, *Puccinia triticina,* wheat cultivars, resistance genes, molecular markers.

INTRODUCTION

Wheat leaf rust is one of the most important diseases resulting in high yield losses and reduced grain quality (Cloutieret *et al*., 2007). Resulting in the use of resistant cultivars offers the most effective and ecologically sustainable method of control of the disease; therefore, incorporating genetic resistance to this pathogen into adapted germplasm is a major goal in most wheat breeding programs.

Plant disease resistance can be classified into two categories: qualitative resistance, conferred by a single resistance gene (also termed as major, seedling, or race specific resistance) and quantitative resistance, mediated by multiple genes or quantitative trait loci (QTLs) (also termed as adult plant, race non-specific or slow rusting resistance) with each providing a partial increase in resistance (Kou and Wang, 2010). More than sixty genes for leaf rust resistance (*Lr*), most of them major, seedling or race specific genes, have been catalogued to date in wheat (McIntosh *et al*., 2008 and Samsampour *et al.,* 2010). However, the gene-for-gene interaction between host resistance genes and pathogen virulence genes combined by virulence shifts in pathogen populations have reduced the effectiveness of a significant number of major leaf rust resistance genes (Johnson, 2000; Bulos *et al.,* 2006). Replacement of highly variable land races by higher yielding, pure-line varieties in many parts of the world has further reduced the wheat gene pool and favored virulence shifts events in pathogen populations.

In this context, a better knowledge on the identity of effective *Lr* genes present in adapted cultivars that can be used as donors of resistance in wheat breeding programs could greatly improve the efficiency of developing resistant cultivars by using these genes or by stacking different resistant genes in a given cultivar, a process also known as gene pyramiding (Messmer *et al.,* 2000); thereby helping to avoid the release of cultivars that are genetically uniform (Mebrate *et al.,* 2008).

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Before gene pyramiding is practiced, it is advisable to identify effective and genetically different sources of resistance. Alternatively to gene postulation, presence of *Lr* genes can be determined by testing host cultivars with molecular markers linked to resistance genes. This approach overcomes some of the problems associated with traditional gene postulation, such as gene interactions and plant stage of gene expression. Recently there have been advances in the mapping and development of molecular markers of several leaf rust resistance genes (Helguera *et al*., 2000; Prins *et al.,* 2001; Helguera *et al.,* 2003, Helguera *et al*., 2005; Gupta et al., 2006; Lagudah *et al*., 2006; Bansal *et al.,* 2008; Mebrate et al., 2008; Kuraparthy *et al*., 2009; Sun *et al*., 2009; Samsampour *et al.,* 2010). Once these genetic factors are mapped, they can be controlled by molecular markers and the corresponding genotypes of individuals can be assessed easily. As a consequence, the identification of cultivars carrying favourable alleles at these loci will provide valuable genetic material for the development of new improved varieties. The objective of this study was to identify leaf rust resistance in ten bread Egyptian wheat cultivars using molecular markers.

MATERIALS AND METHODS

Evaluation of 15 Egyptian Wheat Cultivars and Four Monogenic Lines Under Field Condition: A total of 15 wheat cultivars i.e., Sakha-61, Sakha-69, Sakha-93, Sakha-94, Sakha-95, Gemmeiza-7, Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Sids-1, Sids-12, Sids-13, Giza-168, Misr-1 and Misr-2 and four resistance monogenic lines (Lr genes) *Lr19, Lr21, Lr24 and Lr47* were evaluated under field condation at four locations: Dakahlia, Kafr el-Sheikh, Beheira and Sharqia during two seasons 2011/12 and 2012/13 for leaf rust resistance**.** These cultivars were sown in 3m long rows, with 30cm apart and 5g seed rate for each row. The experiment was surrounded by 1.5m belt of highly susceptible varieties i.e., Morocco and Triticum spleta saharences, served as a spreader of leaf rust. This spreader was artificially inoculated using a mixture of races in addition to the natural infection during late tillering and early booting. Rust reaction was expressed in five types i.e., Immune = (0), resistant = (R), moderately resistant = (MR), moderately susceptible = (MS) and susceptible = (S) (Stakman *et al*., 1962). Then rust reaction was transformed to Average Coefficient of Infection (ACI) values according to the methods adopted by Saari and Wilcoxson (1974).

Molecular Markers

Laboratory studies: This part of the investigation was carried out at the molecular biology laboratory, Faculty of Agriculture Research Park (FARP), Faculty of Agriculture, Cairo University.

Plant Material: Resistance Egyptian wheat cultivars: Sakha-94, Sakha-95, Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Sids-12, Sids-13, Giza-168, Misr-1 and Misr-2 and four resistance monogenic lines: *Lr19*, *Lr21*, *Lr24* and *Lr47* were selected as plant materials for detection of leaf rust resistance genes using molecular markers.

DNA Extraction: A modified method based on the protocol of Dellaporta *et al*. (1983) was conducted for extraction of total genomic DNA.

PCR Amplification: Polymerase chain reaction was performed in thermocycler (Rocorbett-Research, CG1-96) in 25µl reaction volume containing: 2.5µl 50ng/µl of genomic DNA, lµl each primer (10 pmol, F & R) and 8µl MQ H_2O (Devos and Gale, 1992). The specific SSR primers used to verify the presence of *Lr19, Lr21, Lr24, Lr47* and *Lr51* genes are listed in Table 1.

Amplification products were electrophoresed at 100V/1h. After electrophoresis, the gel was stained with ethidium bromide and bands were visualized using UV light and photographed with a Syngen UV visualizer (gel documentation system, G:BOX). The Mid-Range DNA Ladder 100bp-3kbp linear saele (Jena Bioscience) was used to detect the molecular weight of the tested samples.

RESULTS

Evaluation of 15 Egyptian Wheat Cultivars and Four Resistance Monogenic Lines Against Leaf Rust Under Field Conditions: The aim of this work was to study the response of 15 wheat cultivars i.e., Sakha-61, Sakha-69, Sakha-93, Sakha-94, Sakha-95, Gemmeiza-7, Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Sids-1, Sids-12, Sids-13, Giza-168, Misr-1 and Misr-2 and four resistance monogenic lines (Lr,s) *Lr19, Lr21, Lr24* and *Lr46* against leaf rust under field condition in four locations Kafr el-Sheikh, Beheira, Dakahlia and Sharqia during growing seasons 2011/12 and 2012/13.

Table 1. Primer names, sequences, PCR annealing temperature and references for Lr gene associated markers used in this study.

No.	Gene	Name	Primer sequences (5'-3')	Annealing Temp.	References
1	*Lr19*	SCS73719-1 SCS73719-2	TCG TCC AGA TCA GAA TGT G CTC GTCGATTAGCAGTGAG	55°C	Prins *et al.,* 2001
2	*Lr21*	F R	CCA AAG AGC ATC CAT GGT GT CGC TTTT ACC GAG ATT GGT C	57°C	Huang and Gill, 2001
3	*Lr24*	J9/1 J9/2	TCT AGT CTG TAC ATG GGG GC TGG CAC ATG AAC TCC ATA CG	58°C	Schachermayr *et al*., 1995
4	*Lr47*	PS10L PS10L2	TCT TCA TGC CCG GTC GGG T GGG CAG GCG TTT ATT CCA G	60°C	Helguera *et al*., 2000
5	*Lr51*	S30-13L AGA7-759R	GCA TCA ACA AGA TAT TCG TTA TGA CC TGG CTG CTC AGA AAA CTG GAC C	59°C	Helguera e*t al.*, 2005

The first growing season 2011/12: Data presented in Table (2) revealed that the wheat cultivars Giza-168, Sakha-94, Misr-2, Misr-1, Sakha-95, Sids-13, Gemmeiza-9, Sids-12, Gemmeiza-10 and Gemmeiza-11 showed high resistance where the rust severity values were 0.50 %, 1.00 %, 1.10 %, 1.30 %, 1.30 %, 1.50 % 4.75 %, 4.90 %, 6.50 % and 6.50 % respectively. On the other hand, the wheat cultivars Gemmeiza-7, Sakha-93, Sakha-61, Sakha-69 and Sids-1 showed high levels of rust severity i.e., 67.50 %, 55.00 %, 52.50 %, 52.50 % and 42.50 respectively. Therefore, these cultivars were considered highly susceptible to leaf rust disease. Likewise, the monogenic line *Lr19* showed highly resistance (0 DS) to leaf rust disease in the four locations followed by *Lr47* (7.00 %), *Lr21* (13.25 %) and *Lr24 (*14.00 %).

Table 2. Leaf rust severity on 15 wheat cultivars and four monogenic lines grown in four locations during seasons 2011/12 and 2012/13.

Cultivar	Rust severity 2011/2012				Mean	Rust severity 2012/2013				Mean
	Kaffr el-Sheikh	Beheira	Dakahlia	Sharqia		Kaffr el-Sheikh	Beheira	Dakahlia	Sharqia	
Sakha-61	50S	30S	60S	70S	52.5	70S	50S	60S	50S	57.5
Sakha-69	70S	60S	20S	60S	52.5	30S	40S	30S	40S	35
Sakha-93	60S	50S	40S	70S	55	60S	70S	50S	70S	62.5
Sakha-94	0	0	5MS	0	1	5R	0	TrMR	5R	0.8
Sakha-95	5R	5MR	5R	TrMR	1.30	TrMR	5MR	0	5R	1.05
Gemm.-7	70S	80S	40S	80S	67.5	80S	60S	70S	70S	70
Gemm.-9	5MR	10S	5MR	5S	4.75	5S	10MS	10MS	5S	6.5
Gemm.-10	10MR	10S	10S	5MR	6.5	20MS	5MS	5S	5S	7.5
Gemm.-11	10MR	10S	10S	5MR	6.5	30MS	10S	2OMS	10MS	14.5
Giza-168	0	0	10R	0	0.5	5R	0	0	5MR	0.75
Sids-1	60S	70S	10S	30S	42.5	80S	80S	50S	90S	75
Sids-12	TrMR	0	TrMS	20MS	4.9	10MS	5S	10MR	20MR	6.25
Sids-13	5MR	0	10MR	0	1.5	5S	5MS	10S	10MR	5.75
Misr-1	0	TrMS	0	5R	1.3	0	0	0	5MR	0.5
Misr-2	0	TrMS	0	5MR	1.1	0	TrMR	0	5MR	0.8
Lr19	0	0	0	0	0	0	0	0	0	0
Lr21	10MS	5S	20MS	30MS	13.25	5S	TrS	5S	5S	4.5
Lr 24	20S	10MR	20MS	20MS	14	5S	20MS	0	5S	6.5
Lr47	10MR	0	30MS	0	7	5S	10MS	5MS	20MS	8.25

The second growing season 2012/13: The lowest response of rust severity was found on the cvs. Misr-1 (0.50 %), Giza-168 (0.75), Misr-2 (0.80 %), Sakha-94 (0.80 %), Sakha-95 (1.05 %), Sids-13 (5.75 %), Sids-12 (6.25 %), Gemmeiza-9 (6.5 %), Gemmeiza-10 (7.5 %) and

Gemmeiza-11 (14.5 %). On the other hand, the wheat cultivars Sids-1, Gemmeiza-7, Sakha-93, Sakha-61 and Sakha-69 showed the highest response of rust severity. They were 75.00 %, 70.00 %, 62.50 %, 57.50 % and 35.00 %, respectively. Furthermore data showed that *Lr19* was highly resistance to leaf rust in the four locations followed by *Lr21* (4.50 %), *Lr24* (6.50 %) and *Lr47* (8.25 %) (Table 2).

Molecular markers: The polymorphic survey revealed that the marker for *Lr19* was identified as a fragment of 130bp in five cultivars namely: Sakha-95, Gemmeiza-9, Gemmeiza-10, Misr-1 and Misr-2, while five cultivars; Sakha-94, Gemmeiza-11, Giza-168, Sids-12 and Sids-13 did not show the presence of *Lr19* (Fig. 1). On the other hand, the diagnostic PCR fragments associated with *Lr21* and *Lr47* were detected in all tested cultivars (Fig. 2 and 3). Likewise markers for resistance genes *Lr24* and *Lr51* were found in the ten tested cultivars (Table 3).

Table 3. *Lr* genes detected with PCR based markers in ten Egyptian wheat cultivars.

No.	Cultivar	*Lr* gene				
		Lr19	*Lr21*	*Lr24*	*Lr47*	*Lr51*
1	Sakha-94	_	+	+	+	+
2	Sakha-95	+	+	+	+	+
3	Gemmeiza-9	+	+	+	+	+
4	Gemmeiza-10	+	+	+	+	+
5	Gemmeiza-11	_	+	+	+	+
6	Sids-12	_	+	+	+	+
7	Sids-13	_	+	+	+	+
8	Giza-168	_	+	+	+	+
9	Misr-1	+	+	+	+	+
10	Misr-2	+	+	+	+	+

(+) = presence of Lr gene in wheat cultivars and (-) =absence of Lr gene in wheat cultivars

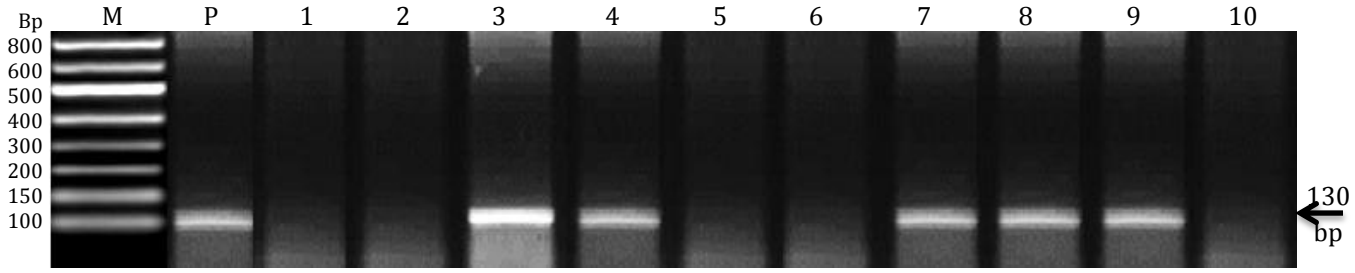

Figure 1. Electrophoretic amplified pattern of DNA extracted from 10 cultivars using the specific primer for *Lr19* (TCG TCC AGA TCA GAA TGT G-F, CTC GTCGATTAGCAGTGAG -R). M= DNA Ladder (DNA Marker), P= Positive, Lane 1= Giza-168, Lane 2= Sids-12, Lane 3= Misr-2, Lane 4= Sakha-95, Lane 5= Sakha-94, Lane 6= Sids-13, Lane 7= Gemmeiza-10, Lane 8= Gemmeiza-9, Lane 9= Misr-1= Lane 10= Gemmeiza-11.

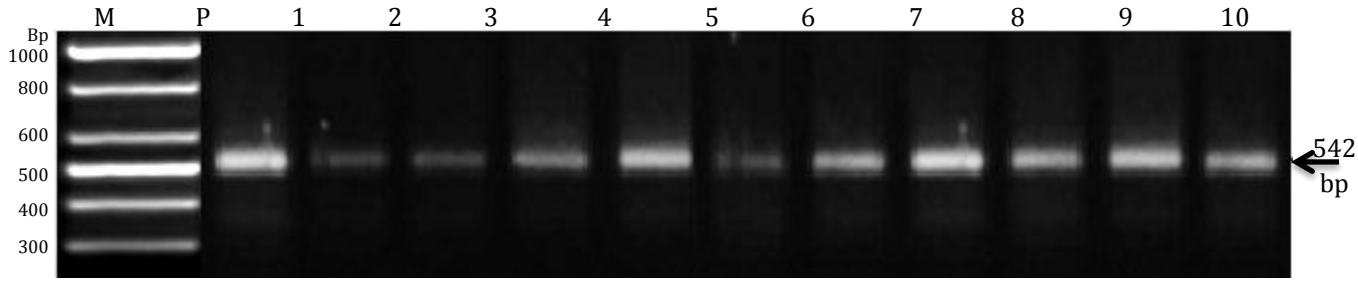

Figure 2. Electrophoretic amplified pattern of DNA extracted from 10 cultivars using the specific primer for *Lr21* (CCA AAG AGC ATC CAT GGT GT-F, CGC TTTT ACC GAG ATT GGT C-R). M= DNA Ladder (DNA Marker), P= Positive, Lane 1= Giza-168, Lane 2= Sids-12, Lane 3= Misr-2, Lane 4= Sakha-95, Lane 5= Sakha-94, Lane 6= Sids-13, Lane 7= Gemmeiza-10, Lane 8= Gemmeiza-9, Lane 9= Misr-1= Lane 10= Gemmeiza-11.

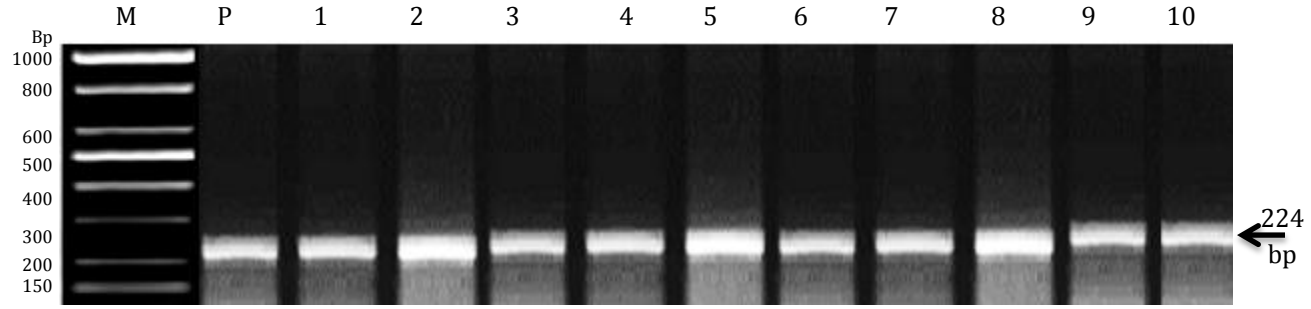

Figure 3. Electrophoretic amplified pattern of DNA extracted from 10 cultivars using the specific primer for *Lr47* (TCT TCA TGC CCG GTC GGG T-F, GGG CAG GCG TTT ATT CCA G-R). M= DNA Ladder (DNA Marker), P= Positive, Lane 1= Giza-168, Lane 2= Sids-12, Lane 3= Misr-2, Lane 4= Sakha-95, Lane 5= Sakha-94, Lane 6= Sids-13, Lane 7= Gemmeiza-10, Lane 8= Gemmeiza-9, Lane 9= Misr-1= Lane 10= Gemmeiza-11.

DISCUSSION

Leaf rust of wheat was the cause of eliminating many cultivars i.e., Giza 139, Super X, Mexipak 69 and Chenab 70 because of their susceptibility under field conditions. Moreover, some wheat genotypes were discarded very shortly after their release such as Giza 139. The failure of such cultivars was mainly due to the dynamic nature, in population, of the causal organism, which produces new virulence having the ability to breakdown their resistance. Thus, we evaluated 15 Egyptian wheat commercial cultivars under field condition in four locations: Dakahlia, Kafr el-Sheikh, Beheira and Sharqia during two seasons 2011/12 and 2012/13 for leaf rust resistance. We found ten out of fiteen cultivars: Sakha-94, Sakha-95, Gemmeiza-7, Gemmeiza-9, Gemmeiza-10, Gemmeiza-11, Sids-12, Sids-13, Giza-168, Misr-1 and Misr-2 showed high level of resistance against leaf rust in four locations during the two seasons. These results supported by Nazim *et al*. (1990) and Boulot (2007) showed that final rust severity (%) and area under disease progress curve (AUDPC) of wheat varieties Giza 168, Sakha 94, Gemmeiza 9 and Gemmeiza 10 were low compared to susceptible varieties.

These results are agree with Liatukas 2003; Tariq *et al*., 2003; Masar *et al*., 2004; Martinez *et al*., 2005 and Hanzalova and Bartos 2006; Hanzalová 2010; Hanzalová *et al*., 2010 and Hanzalová *et al*., 2012. Elyasi-Gomari (2010) showed that no leaf rust damage occurred on *Lr9, Lr25, Lr28* and *Lr29* in the field, and lines with *Lr19, Lr16, Lr18, Lr35, Lr36, Lr37* and the combination *Lr27 + Lr31* showed less than 15% severity.

In this context, gene pyramiding of effective *Lr* genes is probably the faster strategy to develop leaf rust resistant wheat cultivars. Gene pyramiding can be greatly facilitated with associated markers through marker assisted selection programs (MAS), this is particularly true in the field of wheat breeding for leaf rust resistance where PCR-based markers are already available for almost half of the 80 or more designated resistance genes and alleles (Samsampour *et al*., 2010, Herrera-Foessel *et al.,* 2011 and McIntosh *et al.,* 2012). Many authors conclude there is a greater predictive ability of molecular markers than pedigree data (Błaszczyk *et al*., 2008 and Stępień *et al*., 2003). Our results clearly indicate the advantage of molecular markers for evaluating the presence of Lr genes in wheat cultivars compared to pedigree data and are in accordance with numerous studies and reviews (Stępień *et al*., 2003; Ordon et al. 2004). We selected 10 out of 15 cultivars for molecular markers identification and explained their resistance to leaf rust resistance. The Results obtained proved that resistance in the tested cultivars was due to the presence of resistance genes i.e., *Lr19*, *Lr21, Lr24, Lr47*, *and Lr51.* On a global scale, *Lr19* is probably the most widely distributed gene for resistance to *P. triticina* (McIntosh *et al*., 1995 and Winzeler *et al*., 2000). Therefore, it is still considered important gene because it is present in several bred cultivars in CIMMYT in combination with other adult plant resistance genes which continue to give excellent leaf rust protection (Huerta-Espino *et al*., 2011). In Egypt, this gene is important gene for resistance and detected in five cultivars, Sakha-95, Gemmeiza-9, Gemmeiza-10, Misr-1 and Misr-2 (present study). We advise especially the planting cultivars Misr-1 and Misr-2 because they carry many resistant genes (*Lr19*, *Lr21, Lr24, Lr47*, *and Lr51)* for leaf rust and resistant genes *Sr2* and *Sr25* for stem rust (Singh *et al*., 2011). Therefore, they considered very resistance to leaf and stem rusts (especially Ug99 race) and they can be grown in different environmental conditions. In addition the results showed that the genes *Lr21, Lr24, Lr47* and *Lr51* were identified by molecular

markers in the ten tested cultivars. Thus, the good resistance of these cultivars is due to the complementary effect between these major genes which enhance the response of a variety and give its higher levels of resistance.

Finally, in the future studies we recommend the genes pyramiding as a method to achieve more durable resistance against pathogens with low genetic diversity, high gene flow and asexual mating systems (McDonald & Linde, 2002; Hysing et al., 2006). The combination of several effective resistance genes into a single cultivar should extend the period of resistance and this is called horizontal resistance.

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