



Full Length Article

Assessment of Pearl Millet Genotypes for Downy Mildew Resistance and Agronomic Performance under Field Conditions in Senegal

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Abstract

Sclerospora graminicola is a pathogen that causes important damage in pearl millet [*Pennisetum glaucum* (L.) R. Brown] fields in Asia and Africa. In order to identify new sources of resistance to this disease, a set of 20 genotypes was assessed during the rainy season 2015 at Bambey, Nioro, Kolda and Sinthiou Maleme research stations in Senegal. Comparative survey between locations showed that the highest downy mildew incidence (100%) was recorded in Kolda. The 7042S, an international susceptible check, showed downy mildew incidence of 91.12%, IP22441 line was susceptible at Kolda (incidence = 30.5%), resistant at Sinthiou Maleme (incidence = 6.5%), moderately resistant at Bambey (incidence = 18%) and highly susceptible at Nioro (incidence = 70%). IP22295 line was susceptible at Kolda (incidence = 24.5%) but resistant at Bambey (incidence = 0%), Nioro (incidence = 3.5%) and Sinthiou Maleme (incidence = 0%). A hierarchical cluster analysis of these tested genotypes integrating agronomic parameters and response to downy mildew has identified four genotype groups characterized by downy mildew resistance, vegetative growth, panicle yield and flowering time. This study has shown the variability of *S. graminicola* populations from different tested locations. Genotypes IP22315, IP2295, SOSAT-C88-Sadore and SOSAT-C88-Pantacheru presented stable resistance to downy mildew across the tested locations. These genotypes can be used as parents for breeding pearl millet varieties resistant to the downy mildew disease under Senegal environments. © 2018 Friends Science Publishers

Keywords: Downy mildew; Incidence; Pearl millet; Resistance; Senegal

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an important crop grown in Asia and Africa. It is a vital staple food crop for poor people in dry-land areas (Das, 2017). Climate change resilience, soil salinity and acidity tolerance are key characteristics of this crop in sustaining food security, nutrition and health in dry-land zones (Jukanti *et al.*, 2016). Approximately, 500 million people depend on pearl millet and it is currently grown on 28 million hectares throughout the world (Siddaiah *et al.*, 2017). In Senegal, this cereal is the most cultivated with approximately 922,008 hectares as harvested area and 749,874 tons produced in 2015 (Diagne *et al.*, 2017). Diourbel, Fatick, Kaolack, Kaffrine, Kolda and Tambacounda are the main pearl millet production regions in Senegal.

One of the major biotic constraints of pearl millet production is downy mildew disease (Sharma *et al.*, 2011). It is caused by an oomycete, *Sclerospora graminicola*, a

very destructive pathogen that can cause 80% yield loss (Sudisha *et al.*, 2011). Because of its genetic (Sastry *et al.*, 1995) and pathogenic variability (Pushpavathi *et al.*, 2006; Sudisha *et al.*, 2009), identification and monitoring of new sources of resistance are important in downy mildew management.

Several studies highlighted some host specific resistance (Hash *et al.*, 2006; Sharma *et al.*, 2011). But assessment of genotypes with interesting agronomic characteristic including resistance to downy mildew in Senegal field conditions is not clearly established. In addition, it is a great challenge to maintain durable stability in plant breeding when pathogen populations' variability risk exists (Leonard, 1977; Dangl and Jones, 2001). In fact, characterization of potential *S. graminicola* populations' virulence is currently unknown in Senegal. A multi-location monitoring of downy mildew virulence is one of the efficient ways to assess genotypes. It allows to finding out genotypes resistance status in natural conditions

(Wilson *et al.*, 2008). The objective of this study was to evaluate pearl millet genotypes provided by ICRISAT for downy mildew resistance and yield in four agro-ecological zones of Senegal. Sources of resistance identified in this study can be used in pearl millet breeding program in Senegal.

Materials and Methods

Plant Materials

Plant materials provided by the Genetic Resources Division, ICRISAT (Patancheru, India) were composed of twenty genotypes including twelve downy mildew resistant differential lines, six improved varieties and two local checks (Souna 3 and IBMV8402) (Table 1).

Experimental Set up

Field evaluation was conducted during the rainy season 2015 (from 1st July to 31st October) at four locations, Bambey, Niore, Kolda and Sinthiou Maleme corresponding respectively to North groundnut basin, South groundnut basin, Casamance and Oriental Senegal agro-ecological zones (Fig. 1). Genotypes were evaluated in each zone, in a randomized complete block design with two replications. Space between rows was 0.80 m and 0.40 m between plants within a row. Length of each row was 4.8 m. A NPK (15-15-15) fertilizer was applied before sowing at the rate of 150 kg ha⁻¹. Urea (100 kg ha⁻¹) was applied twice (50% at 15 days after sowing and 50% at 30 days after sowing). Sprinkler irrigation was used as needed in order to maintain favourable relative humidity for pathogen development. The Fig. 2 presents the relative humidity and temperature of the four locations. Temperature globally varied from location to another with daily means of 28.59±1.74°C, 29.99±1.90°C, 29.51±1.52°C, 31.60±2.24°C for Kolda, Bambey, Niore and Sinthiou Maleme, respectively. High levels of humidity were recorded at Kolda ranging from 58% to 93%. At Niore, Bambey and Sinthiou Maleme, total means of daily relative humidity were respectively 77.79±8.52%, 72.05±10.57% and 62.13±13.23%.

Data Collection

Number of infected plants per plot was counted at 30 and 40 days after sowing. Genotypes were classified as described by Sharma *et al.*, (2015) in different categories according to the following scale: resistant (downy mildew incidence ≤ 10%), moderately resistant (10.1 ≤ downy mildew incidence ≤ 20%), susceptible (20.1 ≤ downy mildew incidence ≤ 50% incidence) and highly susceptible (downy mildew incidence > 50%). Number of diseased plants at 40 days after sowing was considered for incidence calculation because of augmentation of this number from 30 to 40 days after sowing. Days from sowing to 50% flowering (FLO), plant height (HEI), panicle length (LEN), panicle weight

(WEI), the number of productive panicles harvested (NPPH) and number of productive tillers (NPT) and yield (YIELD) were recorded.

Data Analysis

To examine the effect of genotype, location and their interaction on downy mildew incidence, analysis of variance was carried out based on the following Poisson regression model:

$$\log\left(\frac{\text{Number of infected plants}}{\text{Total number of plants per plot}}\right) = \beta_0 + \beta_1 \text{ Genotype} + \beta_2 \text{ Location}$$

β_s are the parameters to be estimated from data. The generalized linear model (GLM) was used to fit the model with glm function of package stats (R Core Team, 2017). Relative variation was calculated as described by (Thakur *et al.*, 2004) to evaluate genotypes resistance stability across agro-ecological areas. This parameter was computed in statistical program R 3.4 (R Core Team, 2017) using the following formula:

$$\text{Relative variation} = \frac{\text{Standard deviation}}{\sqrt{\text{Mean of incidence} (100 - \text{Mean of incidence})}}$$

A principal component analysis was performed to describe agronomic data set (20 genotypes and 6 agronomic variables) using PCA function of FactoMineR package (Lê *et al.*, 2008). In order to classify genotypes according to the agronomic traits, hierarchical clustering was performed on the first two components of the previous principal component analysis using HCPC function of FactoMineR package (Husson *et al.*, 2010). Euclidean distance calculation and Ward clustering method were used.

Results

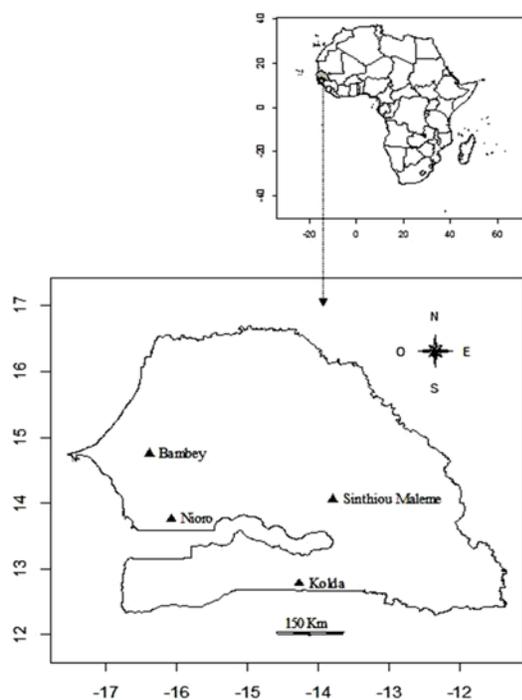
Reaction of Pearl Millet Genotypes to Downy Mildew

Significant differences among tested lines, locations and their interaction for downy mildew incidence were observed (Table 2). Differential responses to downy mildew between populations of the pathogen were observed in the tested lines. Differential downy mildew resistance lines IP22295 and IP22441 vary in their relative positions for downy mildew. IP22295 was susceptible at Kolda (incidence = 24.5%) but resistant at Sinthiou Maleme, Bambey and Niore. IP22441 was susceptible at Kolda (incidence = 30.5%), resistant at Sinthiou Maleme (incidence = 6.5%), moderately resistant at Bambey (incidence = 18%) and highly susceptible at Niore (incidence = 70%). The international susceptible check 7042S was highly susceptible in all the four locations Table 3. The improved varieties did not show variation in downy mildew responses. They were consistently resistant across locations. Downy mildew incidence was ranging from 0% to 91.12% across the tested locations

Table 1: Pearl millet material used in this study

Line	Description	Country of origin
7042 DMS	Check	India
852B	DMR Differential line	India
IBMV8402	Check	Senegal
ICMV-IS90309	Improved variety	India
ICMV-IS90311	Improved variety	India
ICMV-IS92326	Improved variety	India
IP22291	DMR Differential line	India
IP22295	DMR Differential line	India
IP22313	DMR Differential line	India
IP22315	DMR Differential line	India
IP22319	DMR Differential line	India
IP22439	DMR Differential line	India
IP22441	Improved variety	India
IP22442	DMR Differential line	India
IP22445	DMR Differential line	India
IP22446	DMR Differential line	India
IP5082	DMR Differential line	India
SOSAT-C88_Patancheru	Improved variety	India
SOSAT-C88_Sadore	Improved variety	India
Souna3	Check	Senegal

*DMR: Downy mildew resistant

**Fig. 1:** Map showing the different surveyed sites

(Table 4). Significant difference of incidence mean ($p < 0.001$) between 852B and IP22441 was noted with 4.25% and 31.25% respectively (Table 4). Across locations, the highest incidence (91.12%) was observed for 7042S and the lowest (0%) for IP22445, IP22442 and ICMV-IS92326 (Table 4). Relative variation analysis revealed that outside of genotypes with 0 as relative variation, IP22315, IP22295, SOSAT-C88-Sadore, SOSAT-C88-Pantacheru and local check Souna 3 presented stable resistance to downy mildew across the tested locations (Table 4).

Agronomic Performance of Pearl Millet Genotypes

Mean values of the agronomic traits of the tested genotypes in the four locations were presented in Table 5. Number of days from sowing to 50% flowering ranged from 44 days after sowing (IP22442) to 58 days after sowing (IP5082). Highest plant height and panicle length were respectively recorded for IP22313 (168.53 cm) and Souna 3 (36 cm) genotypes. SOSAT-C88_Patancheru produced the highest yield (3.766 t ha⁻¹) followed by ICMV-IS 90311 (3.581 t ha⁻¹), SOSAT-C88_Sadore (3.325 t ha⁻¹), ICMV-IS 90309 (3.251 t ha⁻¹), Souna 3 (3.138 t ha⁻¹) and IBMV 8402 (3.072 t ha⁻¹).

Principal component analysis was performed to identify discriminating agronomic traits according to 6 active variables (days from sowing to 50% flowering, plant height, panicle length, yield, the number of productive panicles harvested and number of productive tillers and 1 supplementary variable, downy mildew incidence recorded 40 days after sowing. Factorial axis 1 described 56% of overall variability (Fig. 3). Agronomic variables highly correlated to axis F1 were yield ($r = 0.91$, $p < 0.01$), plant height ($r = 0.86$, $p < 0.01$), number of productive panicles ($r = 0.85$, $p < 0.01$), number of productive tillers ($r = 0.77$, $p < 0.01$) and panicle length ($r = 0.65$, $p < 0.01$).

Second factorial axis explained 19.16% of overall variability. It was correlated with days to 50% flowering ($r = 0.84$, $p < 0.01$). Hierarchical ascendant clustering performed on factorial plan (axis 1, axis 2) revealed four clusters (Fig. 4). Cluster 1 contained three downy mildew susceptible genotypes (7042DMS, IP22295 and IP22315), characterized by low seed yield (v -test = -2.5976, $p < 0.001$). Cluster 2 is composed of six early maturing genotypes, resistant to downy mildew whereas cluster 3 grouped two extra-early and downy mildew susceptible genotypes. Nine genotypes with high yield (v -test = 3.5326, $p < 0.001$) and green fodder (v -test = 3.9232, $p < 0.001$) belonged to the cluster 4 (Fig. 4).

Discussion

The present study revealed variation in downy mildew incidence among the tested pearl millet genotypes. This variation can be due to intra-genetic variability of the tested plant materials. The variation in the responses of some genotypes to downy mildew across locations was also highlighted, suggesting that different populations of *S. graminicola* might be present in the various locations where the trials were conducted. IP22295 was susceptible at Kolda but resistant at Bambey, Nioro and Sinthiou Maleme. Furthermore, IP22441 was susceptible at Kolda, resistant at Sinthiou Maleme, moderately resistant at Bambey and highly susceptible at Nioro. This downy mildew variability across locations can be due to pathogenic variation of *S. graminicola* populations.

Table 2: Analysis of deviance table of fitted model

Source of variation	Df	Deviance Residual	Df Residual	Deviance	p (>Chi)	
Genotype	19	3647.1	140	916.8	<2.2.10 ⁻¹⁶	***
Location	3	42.3	137	874.5	0.002479	**
Genotype × Location	57	558.6	80	315.9	4.039.10 ⁻¹⁶	***

** p< 0.01; ***p < 0.001
Df: degree of freedom

Table 3: Reaction of genotypes to downy mildew in Bambe, Niuro, Kolda and Sinthiou Maleme

Genotypes	Bambe		Niuro		Sinthiou		Kolda		Mean
	DMI	Sensibility	DMI	Sensibility	DMI	Sensibility	DMI	Sensibility	
7042 S	91	HS	100	HS	73.5	HS	100	HS	91.12
852B	3.5	R	0	R	8	R	5.5	R	4.25
IBMV 8402	2	R	0	R	6.5	R	0	R	2.12
ICMV-IS 90309	0	R	3	R	0	R	0	R	0.75
ICMV-IS 90311	0	R	0	R	2	R	2	R	1
ICMV-IS 92326	0	R	0	R	0	R	0	R	0
IP22291	2.5	R	0	R	0	R	0	R	0.62
IP22295	3.5	R	0	R	0	R	24.5	S	7
IP22313	0	R	0	R	0	R	0	R	0
IP22315	12.5	MR	0	R	0	R	0	R	3.12
IP22319	7	R	0	R	0	R	5.5	R	3.12
IP22439	0	R	0	R	5	R	10	R	3.75
IP22441	18	MR	70	HS	6.5	R	30.5	S	31.25
IP22442	0	R	0	R	0	R	0	R	0
IP22445	0	R	0	R	0	R	0	R	0
IP22446	0	R	0	R	2	R	3	R	1.25
IP5082	0	R	0	R	0	R	2	R	0.5
SOSAT-C88-Sadore	2	R	2.5	R	2	R	9.5	R	4
SOSAT-C88-Pantacheru	2	R	7	R	6.5	R	3.5	R	4.75
Souna 3	10.5	MR	0	R	14.5	MR	9	R	8.5
Mean	7.73		9.13		6.33		10.25		

R: Resistant (Downy mildew incidence ≤ 10%, HS: Highly susceptible (Downy mildew incidence > 50%, MR: Moderately resistant (10.1% ≤ Downy mildew incidence ≤ 20%) and S: Susceptible (20.1% ≤ Downy mildew incidence ≤ 50%), DMI: Downy mildew incidence

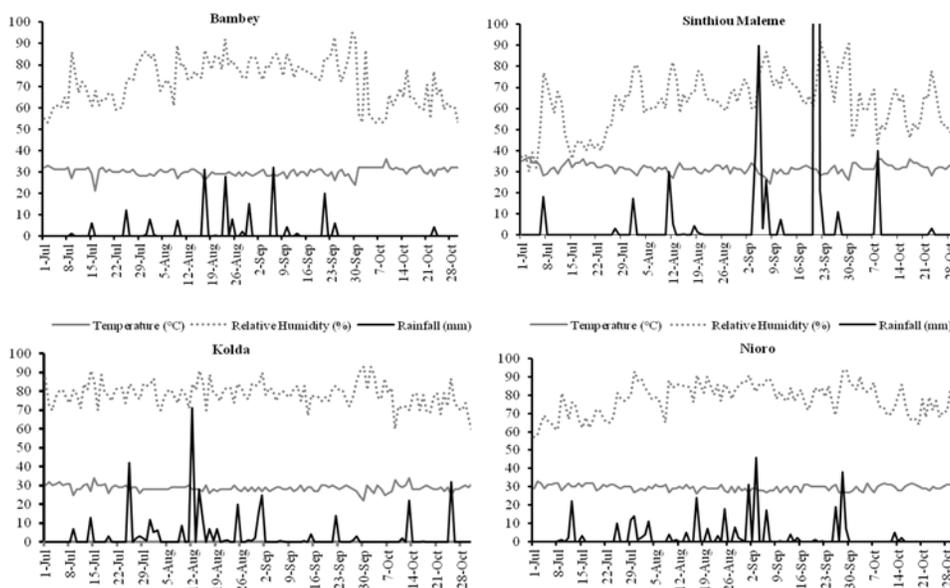


Fig. 2: Daily relative humidity and temperature at Kolda, Bambe Niuro and Sinthiou Maleme during rainy season 2015

Pathogen variability has already been proved through many studies using on-farm assessment (Sharma *et al.*, 2007, 2011), isolates virulence under greenhouse conditions (Thakur *et al.*, 2004; Pushpavathi *et al.*, 2006) and DNA

markers (Sastry *et al.*, 1995; Sharma *et al.*, 2010).

Our study revealed that the highest downy mildew incidence was observed at Kolda. It can be due to climatic difference between locations. In our study, the

Table 4: Mean of downy mildew incidence at four locations and relative variation

Genotypes	Downy mildew incidence mean	Sensibility	Relative variation
7042S	91.12 a	HS	0.45
852B	4.25 d	R	0.24
IBMV8402	2.12 efg	R	0.19
ICMV-IS90309	0.75gh	R	0.00
ICMV-IS90311	1.00 h	R	0.00
ICMV-IS92326	0.00 h	R	-
IP22291	0.62 gh	R	0.45
IP22295	7.00 c	R	0.19
IP22313	0.00 h	R	-
IP22315	3.12 def	R	0.04
IP22319	3.12 def	R	0.24
IP22439	3.75 de	R	0.00
IP22441	31.25 b	S	0.39
IP22442	0.00 h	R	-
IP22445	0.00 h	R	-
IP22446	1.25fgh	R	0.00
IP5082	0.50 gh	R	0.00
SOSAT-C88-Pantacheru	4.75 d	R	0.14
SOSAT-C88-Sadore	4.00 de	R	0.13
Souna3	8.50 c	R	0.17

R: Resistant (Downy mildew incidence \leq 10%, HS: Highly susceptible (Downy mildew incidence $>$ 50%, S: Susceptible (20.1% \leq Downy mildew incidence \leq 50)

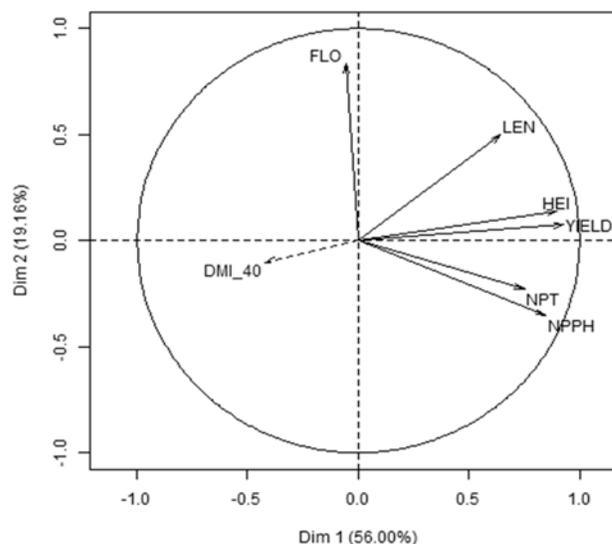
highest relative humidity was observed at Kolda. Kumar *et al.* (2012) explained that with 85–90% of relative humidity and temperature of 20–30°C, downy mildew development is important. Environmental factors as weather and wind also influence spread of inoculum (Thakur *et al.*, 2011).

Clustering revealed diversity in the tested lines according to downy mildew sensibility, seed yield and green fodder potentials and number of flowering day. Most of pearl millet lines possess desirable agronomic traits. Breeding lines have been generally found to be morphologically, genetically, and geographically quite diverse (Hu *et al.*, 2015; Sehgal *et al.*, 2015).

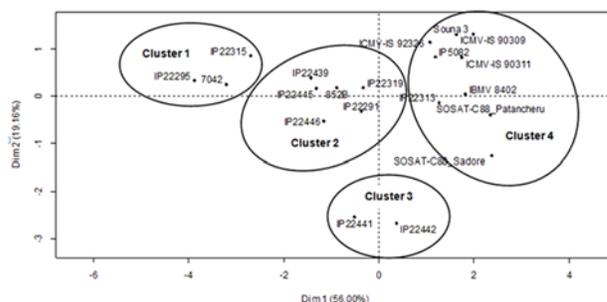
Table 5: Mean values of agronomic traits across four locations

Genotypes	FLO (days)	HEI (cm)	LEN (cm)	NPT	NPPH	WEI (g)	Yield (tha ⁻¹)
7042S	53.62*	105.85	14.12	10.62	38.12	0.39	1.03
852B	55.62	102.3	21.25	16.75	79.25	0.49	1.27
IBMV 8402	53.62	144.41	27.12	18.25	98.5	1.17	3.07
ICMV-IS 90309	56.87	142.01	29.37	18.87	77.75	1.24	3.25
ICMV-IS 90311	55.75	141.11	27.37	17.87	87.87	1.37	3.58
ICMV-IS 92326	54.87	158.28	31.5	19	64.25	0.75	1.96
IP22291	54.37	101.4	18.5	17.12	80	0.91	2.37
IP22295	53.87	72.78	16.75	12.5	27.5	0.15	0.40
IP22313	55.62	168.53	18.87	17.87	104.87	0.80	2.10
IP22315	57.37	75.45	17.87	14.12	51.87	0.38	0.99
IP22319	57.5	117.9	15.87	19	78.12	0.69	1.81
IP22439	57	93.87	17.62	15.75	73.62	0.55	1.45
IP22441	49.12	95.57	12.87	21.25	81.37	0.59	1.55
IP22442	44.37	124.08	22.37	15.62	100.37	0.79	2.07
IP22445	55.5	89.4	19.62	18.25	58.62	0.54	1.42
IP22446	53.5	110.05	16.12	15.75	73	0.60	1.57
IP5082	58.25	145.45	22.12	17.75	98.87	1.01	2.64
SOSAT-C88_Patancheru	53.5	152.2	22.75	19.87	99.37	1.44	3.76
SOSAT-C88_Sadore	52.62	154.81	18.62	21.12	111.37	1.27	3.32
Souna 3	54	160.05	36	16.75	79.25	1.20	3.13

*Means with 8 replications (2 replications \times 4 locations), FLO: days to 50% flowering, HEI: plant height, LEN: panicle length, WEI: panicle weight, NPPH: number of productive panicles harvested, NPT: number of productive tillers

**Fig. 3:** Variables factor map

FLO: Days from sowing to 50% flowering, HEI: plant height, LEN: panicle length, WEI: panicle weight, NPPH: the number of productive panicles harvested, NPT: number of productive tillers, DMI_40: Downy mildew incidence recorded 40 days after sowing

**Fig. 4:** Hierarchical ascendant classification analysis of the tested genotypes

Results of this study clearly highlighted the variability of downy mildew incidence among breeding lines and agro-ecological areas of Senegal. Furthermore, a pathogenic variability has been inferred across the different locations. Genotypes possessing desirable agronomic traits and showing stable resistance to downy mildew can be used in pearl millet breeding programs. Accurate identification of *S. graminicola* pathotypes present in Senegal by genetic tools should be explored as key issue for further study.

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