Association Analysis of Candidate Quantitative Trait Loci for Resistance to Banded Leaf and Sheath Blight in Maize

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***Abstract*—Association mapping can be used for mapping and identifying genes control complex maize traits including diseases. In this study, the genetic structure of 198 inbred lines association population was conducted using 64 SSR markers by association mapping analysis, as well as the association for QTLs contribute resistance to banded leaf and sheath blight (BLSB). It could be referred to 6 subgroups including Lan, PA, PB, BSSS, SPT and LRC, and distributed uniformly. According to two years BLSB resistance identification, most of the inbred lines were high susceptible, few of them were resistance. 26 loci distributed in Chromosome 2, 3, 4, 5, 7, 8, 9, and 10 were discovered through the association mapping for resistance to BLSB using TASSEL, in which umc1202, umc2190 and umc1505 were significant related to the resistance ($p<0.05$), while umc214 was significant in two years association mapping for relative RHDS (relative height of disease spot). Compared with the previous mapping results, more than half of the 26 loci in our research were reported in previous disease-resistance studies while part of them were consistent in BLSB resistance-related traits. Among these consensus loci, dupssr06, umc2164 and umc2287 were associated with disease index, bnlg666 and umc1858 were associated with the EH (ear height), which were consistent with our previous results of linkage analysis for QTL controlling BLSB.**

***Index Terms*—Maize, banded leaf and sheath blight, QTL analysis, association mapping.**

I. Introduction

Rhizoctonia solani Kühn is one of the most severe diseases happened worldwide maize belts. It was first reported by Voorhees that Rhizoctonia disease in maize ear caused by Rho-ctonis zeae occurred in southern of the US [1]. During the 1960s and 1970s, the continuous occurrence of R. solani Kühn was also reported in India, Japan, South Africa and Russia. In China, the first report of BLSB was in Jilin Province in 1966 [2]. This disease spread rapidly due to the increasing maize planting area and high-density cultivation, with the nationwide occurrence and increasing severity in present days. Grain yield decreased with rising positions of infected sheathes and worsening infection conditions of ears, which might be attributed to the reduction of weight per thousand kernels [3]. The morbidity was about 40% per year, and could reach 70% when it is serious (it could reach 100% in some region or some cultivar) [4]. The yield reduction caused by BLSB ranged from 10% to 20%, and can reach 35% when it was serious [5]. With the advent of global warming, the lost caused by BLSB tends to be worse.

Lots of reports showed that BLSB was a quantitative trait controlled by minor polygenes. By using the maize crossing combination CML270 × Ye478 backcross population and combined with IM and CIM methods, some QTLs were found related to BLSB resistance traits including relative resistance index, height of disease spot (HDS), and relative height of disease spot (RHDS), and two QTLs increase the relative resistance index or lowering the relative/absolute height were mapped in the interval of 102.64-113.61 cM in Chromosome 1 [6]. According to the result of Zhao *et al.* [7], F_2 separation population of R15 × Ye478 were constructed and used for constructing a linkage map with 146 SSR markers and an average interval of 11.4 cM, 11 resistance QTLs were detected in Chromosome 1, 2, 3, 4, 5, 6, 10, and 4 QTLs distributed in Chromosome 2, 6, 10 were detected in two testing locations. 4 resistance QTLs were detected in Chromosome 6, 7, 10 by using a population of F_2 of CML246 (resistant) ×DM9 (susceptible), and two of them located in Chromosome 6 were linked to bnlg107 and umc1796 respectively, and 15.21% and 5.42% of the phenotype covariance could be explained by genetic effects respectively [8]. QTL mapping for BLSB resistance had been conducted widely in *Triticum aestivum* [9]-[11] and *Oryza stalia* [12], [13].

Most of the studies mentioned above were based on linkage genetic analysis which had shortcomings of long cycle length, requirement for high quality parents, and low mapping accuracy. Association mapping, based on linkage disequilibrium, greatly improved mapping effect and solution, had been widely used for gene mapping for complex traits in crops. 16 agronomy and quality traits of local soybean cultivars (393 representative materials) and wild populations (196 representative materials) was conducted by GWAS, 27 QTL related to traits in cultivated populations were identified [14]. Not only can association mapping complement the information of QTL linkage mapping, but also improve the breeding effect by providing allele variation information for parent selection, combination crossing and progency assisted selection. [15] glomerella resistance gene of lettuce was studied by association mapping and SNP marker Cntg10102 was significantly associated identified, and then the resistance materials could be isolated and applied to cultivar improvement. As for maize, Tharnberry [16] reported association mapping for flowering stage variations in 2001; association mapping for oil content [17], PH(plant height), smut resistance and water content of kernel [18] was conducted subsequently; Tian *et al.* (2010) identified genes

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related to the included angle of leaf and stem by GWAS. However, there was no report of analysis mapping for QTLs of BLSB resistance in maize [19].

II. Materials and Methods

A. Plant Materials and Phenotyping

Association mapping population consisted of 198 tropic, semi-tropic and temperate maize inbred lines came from China, USA, and CYMMIT (see Table I). Population materials were bred in the same experiment spot of Maize Research Institute of Sichuan Agricultural University, and a randomized complete block designed with two replications and single row was applied for two years. Wheat seeds infected by *R. solani* AG1-IA were inoculated into the sheath nearest to the ground and kept closed; then water was sprayed to the plant base to keep humidity of the small environment in order to facilitate *R. solani* infection. HDS (Height of disease spot), EH (ear height), and PH (plant height) was measured at maturity stage according to the following standards:

TABLE I: Inbred Lines for Testing

Zong31	Duo 212	DH29	CML326	ES40	B118	Shen135
Zhongzi03	D863F	Duo16/AH	CML325	EN46	B107	Qi319
Zhongzi 01	D387	Dong237	CML317	EN44	B105	MoP17
ZhongH204	D375	Dan599	CML316	EN40	ANL6	LXN
Zhong69	CMU3	Dan 598	CML306	EN28	ANL3	Liaogu001
Zhi41	CML495	Dan 360	CML297	EN25	ANL11	Liao785
Zheng36	CML494	Dan 340	CML292	EN12	A318	Liao5114
Zheng 35	CML493	Dan 3130	CML290	7327	975-12	Liao3180
Zheng 30	CML489	Chang7	CML278	7319	835-2	Liao2345
Zheng 29	CML487	Chang7-2	CML276	5311	698-3	Liao159
Zheng 28	CML484	Y731	CML261	5213	286-4	Liao138
Zheng 22	CML482	y272	CML256	4019	2002F34	Lian87
Yi1462	CML480	W138	CML249	3411	2002F32	JH96C
Ye478	CML479	V5	CML192	3394	2002F30	JH59
Xi502	CML473	V4	CML184	1058	18-599R	JF142
Tie9010	CML472	TZ18	CML183	835	18-599w	Ji853
Tie7922	CML470	TY30331-2	CML182	501	526018	Ji81162
Tian4	CML457	TY30331-1	CML181	268	414259	HTH
Taixi113	CML452	STL6	CML180	177	81565	Hai9-21
Si-273	CML450	STL5	CML178	150	9642	Shen137
CA34514	CA34501	CA14707	CA049Y01	CA03116	CA00308	CA00106
CA34502	CA3002	CA14520	CA03118	CA00390	CA00108	C8605-2
C ML442	CML435	C ML426	CML415	CML398	CML392	CML385
C ML438	CML429	C ML423	CML413	CML396	CML390	CML383
C8605	CML444	C ML328	CML348	CML365	CML376	CML379
Bt	IRF291	H95	STL18	STL17	SA24	S37
RP125	R15	R09	P138	O151	NC250	Mo17Ht
STL20	CML142	K10	KUI2007	L102	LX9801	L 9801
B73	B151					

The 6 levels of disease severity were established: 0, 1, 3, 5, 7, and 9, for which the RHDS was between 0, 0.1 to 0.25, 0.25 to 0.5, 0.5 to 0.75, 0.75 to 1.0 and.1, respectively, leve 9 refer to the dead plant. The disease index (DI) was calculated according to the following formula: DI= [∑ (severity class × plant numbers of this class)/ (the highest severity class × the total numbers of the investigated plants)] × 100. And the resistance type were classified as follows: disease index of grown plants:0 – immune (I); 0-5.0: high resistance (HR); 5.1-10.1: resistance (R); 10.1-30.0: middle resistance (MR); 30.1-50.0: susceptible (S); 50.1-100: high susceptible (HS) [20]. Statistical analysis was done by Excel 2003.

B. DNA Extraction and SSR Analysis

TABLE II: SSR PRIMERS

Name	Loci	Name	Loci	Name	Loci
phi022	9.03	umc1088	4.05	dupssr06	9
umc1231	9.05	phi328175	7.04	bnlg1017	2.02
umc1069	8.08	umc2324	6.08	umc1008	4.01
umc2322	6.06	bnlg1031	8.06	umc1019	5.06
bnlg2248	2.03	bnlg1525	9.07	umc2027	4.05
mmc0001	3.09	phi033	9.01	bnlg666	8.05
dupssr-5	3	umc1122	1.07	umc1014	6.04
nc007	5.01	umc1296	6.06	bnlg1538	6.01
bnlg1909	2.05	phi420701	8	umc1993	10.06
umc1143	6	bnlg2235	8.02	bnlg1805	7.03
umc2214	2.1	phi193225	3	phi396160	5
bnlg161	6	bnlg1940	2.08	bnlg1338	2.01
phi065	9.03	umc2137	4.08-4.09	bnlg2132	7
umc1202	8.03	mmc0022	3.05	umc2287	4.09
phi118	10	bnlg1178	1.02	umc1080	2.06
umc1384	8.08	umc1858	8.04	umc1154	7.05
phi021	4.03	bnlg1443	6.05	bnlg1583	9.01
phi052	10.02	umc2190	7.06	umc2136	5.08
bnlg108	2.04	bnlg2122	9.01	phi054	10.03
dupssr12	1.08	umc1789	9.06	phi094	1.09
phi109188	5.03	bnlg1258	2.08	umc1505	9.07
bnlg1714	9.04	bnlg1179	1.01	bnlg1808	7.02
bnlg1325	3.02	umc2164	5.05		

DNA was extracted via 2 × CTAB method [22], and its concentration and purity were measured by F-4500 spectrometer, then diluted to 20-40 ng/μL. The polymorphic SSR primers distributed uniformly in chromosomes and were selected and shown in Table II. The PCR reaction system was 15μl and its components were as follows: ddH_2O 10.2 μl, Taq-Buffer (10 ×Mg-free) 1.5 μl, $MgCL_2$ (25 $mmolL^{-1}$) 0.7 μl, dNTP-Mix (10 $mmolL^{-1}$ each) 0.8 μl, Taq-Enzyme (5U μl^{-1}) 0.12 μl, Primers (F+R 1.0 $\mu molL^{-1}$ each) 0.4 μl, Template（30 $ng\,\mu l^{-1}$） 1 μl. Products were amplified by the landing-type PCR procedures, detailed procedures are showed in Table III. The quality of the amplification products were checked on 3% (w/v) agarose gels.

TABLE III: PCR AMPLIFICATION PROCEDURES

Step	Temperature	Time	Step	Tempreature	Time
1	94℃	5min	7	94℃	30s
2	94℃	40s	8	57℃	30s
3	66℃	30s	9	72℃	40s
4	-1	Cycle	10	GO TO 7	30 times
5	72℃	40s	11	72℃	8 min
6	GO TO 2	10 times	12	12℃	preserving

C. Data Anlaysis

The software STRUCTURE was used for population structure analysis. The analyzing principle was: assuming type number of the allelic variation frequency characteristics in samples is K (subgroups obeying the Weinberger balance, where K is unknown), SSR loci of each subgroups were characterized by a set of allele variation frequencies, and materials in the sample were subsumed to (or estimated by Basyesian model) to the Kth subgroup, making loci frequencies within the subgroup follow the same Hardy-Weinberg equilibrium). Specific operating method in this experiment is as follows; First, K value was set range from 1 to 9, then conducting 6 iterative computation, bum-in time and MCMC repeat number in each operation were 500,00, the genetic relationship was mixed and frequency of allele was related. Association mapping for BLSB resistance was conducted by TASSEL using GML model and combined with the phenotype data with genetic similarity coefficient acquired by structure analysis.

III. RESULTS

A. Structure Analysis

Set the K value ranging from 1 to 9, then conducted 6 iterative computation, bum-in time and MCMC repeat number in each operation were set as 500,00, the genetic relationship in the model setting was mixed and the frequency of allele is related. Based on the LnP (D) value, K was 6. As can be seen from Fig. 1, the LnP (D) increased along with the increase of K, and

LnP (D) varies sharply among different K values before K reached 6; and the ongoing change of LnP (D) tent to slow down hand be stable when the K reached 6. Combined with previous results, the subgroup number was determined to 6, which were SPT (TangSipingtou and its derived lines), LRC (Lvda Red Cob and its derived lines), Lancaster, BSSS (including reid), PA, PB, respectively. In general, all materials in this study were evenly distributed among six groups; BSSS (including Reid) had maximum number of materials which account for 21% of the total materials; and the rest 5 accounted for approximately 15% each (see Fig. 2).

These results indicated that the abundant genetic structure of the mapping population was suitable for association analysis.

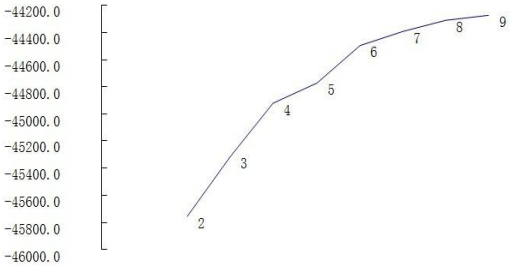

Fig. 1. Changing of K value.

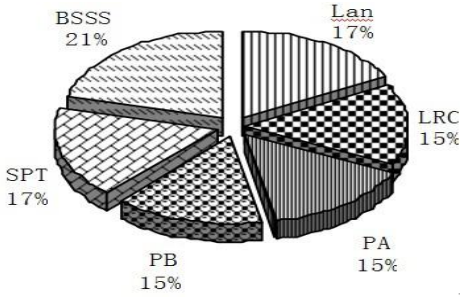

Fig. 2. Group distribution of materials.

B. *Association Mapping for BLSB Resistance in Maize*

A series of significant loci with p value <0.05 were detected in five traits HDS, EH, RHDS, PH and DI (disease index) by the association mapping for investigation results of BLSB resistance traits in 2008 and 2009, detailed information could be seen in Table IV. In 2008, loci were significant in multiple traits including phi021 in HDS, EH and DI, phi328175 in the HDS, EH, RHDS, PH and DI, phi033 in HDS, EH and PH, bnlg2235 in HDS, EH and PH, umc1858 in HDS and EH, umc1858 in HDS and EH, umc2190 in HDS and PH, umc1789 in HDS, EH, RHDS and DI, bnlg 666 in all the five traits. In 2009, few loci were detected and only 3 of them were significant in two traits relatively, they were bnlg1909 in HDS and PH, umc2287 in EH and RHDS, phi054 in PH and DI. By integrating the loci detected in the two years, umc2164 was significant in RHDS in two years; and phi 033 was significant in RHDS exclusively in 2009, while it was not significant in 2008 but significant in HDS, EH and PH in 2008. From the prospective of variation explanation, the maximum value was in 2009 in HDS where the value of phi328175 reached 0.58, while the maximum value was in HDS in 2009 where the value of dupssr06 reached 0.2853. The average value of explanation for trait variation in 2008 and 2009 respectively was 0.30 and 0.23 for HDS, 0.30 and 0.17 for EH, 0.30 and 0.12 for RHDS, 0.34 and 0.20 for PH, 0.27 and 0.14 for DI.

TABLE IV: RESULTS OF ASSOCIATION MAPPING FOR MAIZE RESISTANCE TO BLSB IN 2008 AND 2009

2008	Marker	p_Marker	Rsq_Marker	2009	Marker	p_Marker	Rsq_Marker
HDS	phi021	0.0469	0.1298	HDS	bnlg1909	0.0367	0.177
	phi328175	6.78E-06	0.58		dupssr06	0.002	0.2853
	phi033	0.0253	0.1094				
	bnlg2235	0.0034	0.2912				
	umc1858	0.0401	0.0831				
	umc2190	0.015	0.1631				
	umc1789	5.34E-05	0.4802				
	bnlg666	1.2E-08	0.4746				
	bnlg1805	2.16E-06	0.4002				
EH（Ear Height）	phi021	0.0493	0.1361	EH（Ear Height）	dupssr-5	0.0466	0.2177
	phi328175	2.16E-05	0.5625		nc007	0.024	0.1482
	phi033	0.0247	0.1099		umc1384	0.005	0.1388
	bnlg2235	0.0077	0.2723		bnlg1031	0.0407	0.2542
	umc1858	0.0442	0.0812		umc2287	0.011	0.0822
	umc2190	0.021	0.1552				
	umc1789	1.08E-04	0.4678				
	bnlg666	8.98E-09	0.4783				
	bnlg1805	2.03E-06	0.4025				
RHDS	phi328175	0.0424	0.4082	RHDS	phi052	0.0253	0.1221

	bnlg1525	0.0357	0.1597		phi033	0.0347	0.0999
	phi420701	0.0143	0.312		bnlg1258	0.0337	0.0629
	umc1789	0.0238	0.356		umc2287	0.0134	0.0792
	bnlg1017	0.0226	0.1269		umc2164	0.0356	0.2257
	bnlg666	1.47E-05	0.3802				
	bnlg1805	8.35E-04	0.2998				
	umc2164	6.31E-04	0.3373				
PH	phi328175	8.44E-05	0.5357	PH	bnlg1909	0.0448	0.1721
	phi033	0.0238	0.1105		phi054	0.0081	0.2557
	bnlg2235	0.0084	0.2681		bnlg1338	0.0395	0.1566
	umc2190	0.018	0.1576				
	umc1789	1.41E-04	0.4595				
	bnlg666	5.95E-08	0.4501				
	bnlg1805	4.73E-06	0.387				
DI	phi021	0.0471	0.1217	DI	phi052	0.0457	0.1069
	phi328175	0.0375	0.4117		umc2287	0.0067	0.0903
	bnlg1525	0.0431	0.1545		phi054	0.0154	0.2272
	phi420701	0.0101	0.3208				
	bnlg2235	0.0343	0.2383				
	umc1789	0.0173	0.3647				
	bnlg1017	0.0346	0.1171				
	bnlg666	1.58E-05	0.378				
	bnlg1805	0.001	0.2953				
	umc2164	7.97E-04	0.332				

Abbreviates: DI=Disease Index; PH=Plant Height; BLSB=Banded Leaf Sheath Blight; HDS=Height of Disease Spot; RDHS= Relative Height of Disease Spot; MRDV= Maize Rough Dwarf Virus; EH=Ear Height.

An association mapping was conducted after the identification of resistance levels by integrating two years' resistance identifications. 3 associated loci including umc1202, umc2190 and umc1505, were detected significant at $p<0.05$ level; most variations among these loci could be explained by umc2190. In addition, umc2190 was significant in the HDS, EH and PH, which corresponded to the results of loci detected in the traits.

IV. Discussion

In this research, half of the 26 loci were reported previously, and some of them were reported related with BLSB resistance traits, and detailed information could be seen in Table V. Dupssr06 in DI, bnlg666 in EH, umc1858 in EH, umc2164 in DI and umc2287 in DI, are accordance with our previous work on BLSB QTL mapping. Some loci were involved in resistance to other diseases; such as umc1202 and bnlg2235 were involved in resistance to *exserohilum turcicum* in maize [22], especially that umc1202 was directly involved in the closelinkage of *exserohilum turcicum* resistance gene Ht2 [23]; and further study could be conducted to investigate the Ht2 involvement in the formation of BLSB resistance; umc1505 involved in the formation of resistance to Maize Rough-Dwarf Virus Disease[24]; phi328175 might involve in the formation of resistance to Maize Silk Cut [25]; bnlg1258 involved in resistance to maize gibberellins [26]. These loci were detected in the BLSB resistance research, which may be attributed to the theory that they affect maize resistance to different disease via the same regulation mechanism such as the Programmed Cell Death and cell-wall thickening, and their specific was of involvements in the maize resistance can be further identified by physiology and biochemistry researches. The particular cases are as follows: the previous studies indicated that bnlg1909 involved in maize

photosynthesis via regulating the synthesis of chlorophyll a and b [27]; bnlg1017 involved in construction of maize plant type by affecting leaf angle, leaf direction, plant height and height of ear [28], [29]; bnlg1525 involved in drought tolerance [30]. The possible reason for their involvements in BLSB resistance might be as follows: bnlg1909 improved the efficiency of photosynthesis to provide energy for disease resistance [27]; bnlg1017 reduced the infection probability via regulating the angle to make it difficult for germs to invade sheath[31], this may be attributed to the phytopathology theory that BLSB germ infect the plant by invading the sheath initially, the it elevated the ear height to reduce the possibility of ear infection to reduce the effect on maize quality; bnlg1525 involved in the abiotic stress [25], the mechanism may be that according to the physiology and biochemistry researches for drought tolerance, osmotic pressure adjustments, including cell-wall thickening, were involved in the drought tolerance, and the BLSB infection also causes dehydration and degreasing, thus those loci involved in drought tolerance may get involved in maize resistance to BLSB via these methods.

TABLE V: QTL OF SHEATH BLIGHT DISEASE RESISTANCE COMPARED WITH THE PREVIOUS STUDIES

Loci	Position	Association results of this study	Putative traits in Previous results
phi054	10.03	DI and PH in 2009	DI for BLSB grey speck disease, Rust
phi052	10.02	in 2009 RHDS、DI	Not mentioned
bnlg1525	9.07	in 2008 RHDS、DI	Drought Tolerant Grain Yield
umc1505	9.07	Integrated resistance level	Maize MRDV
umc1789	9.06	in 2008 HDS,EH,RHDS,PH,DI	Not mentioned
phi033	9.01	in 2008 HDS、EH、PH in 2009 RHDS	Not mentioned
dupssr06	9	in 2009 HDS	BLSB DI
umc1384	8.08	in 2009 EH	Not mentioned
bnlg1031	8.06	in 2009 EH	Not mentioned
bnlg666	8.05	in 2008 HDS、EH、RHDS、 PH、DI	EH
umc1858	8.04	in 2008 HDS、EH	EH
umc1202	8.03	Integrated resistance level	BLSB gene*Ht2* close linkage
bnlg2235	8.02	in 2008 HDS、EH、 RHDS、DI	Maize BLSB
phi420701	8	in 2008 RHDS、DI	Not mentioned
umc2190	7.06	in 2008 HDS、EH、 PH、Integrated resistance level	PH
phi328175	7.04	in 2008 HDS、EH、 RHDS、PH、DI	Maize silk cut
bnlg1805	7.03	in 2008 HDS、EH、 RHDS、PH、DI	Not mentioned
umc2164	5.05	in 2008 RHDS、DI in 2009 RHDS	BLSB DI
nc007	5.01	in 2009 EH	Not mentioned
umc2287	4.09	in 2009 EH、RHDS、DI	BLSB DI
phi021	4.03	in 2008 HDS、EH、DI	Not mentioned
dupssr-5	3	in 2009 EH	Not mentioned
bnlg1258	2.08	in 2009 RHDS	Corn Fusarium head blight
bnlg1909	2.05	in 2009 HDS、PH	Chlorophy a,b
bnlg1017	2.02	in 2008 RHDS、DI	Angle and leaf direction PH、EH
bnlg1338	2.01	in 2009 PH	Not mentioned

Abbreviates: DI=Disease Index; PH=Plant Height; BLSB=Banded Leaf Sheath Blight; HDS=Height of Disease Spot; RDHS= Relative Height of Disease Spot; MRDV= Maize Rough Dwarf Virus; EH=Ear Height.

From the prospect of loci distribution in chromosomes, loci were detected in all the chromosomes except

Chromosome1 and 6. A relatively large number of 7 loci were detected in Chromosome 8; two loci, including bnglg1525 and umc1505, were detected in Chromosome 9.07; thus fine mapping for these QTLs, such as employing candidate gene strategy and increasing marker density, could be used to confirm whether there is key QTL in this loci. Detected loci may not be associated with only one trait usually, and the associations among traits can be discussed using date of this study. Among these traits, HDS and EH coexist usually, as well as DI and RHDS; thus it can be confirmed that there must be some associations between these traits.

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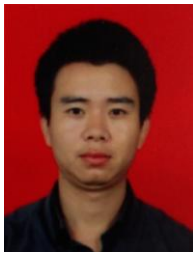

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