



Yield Stability of Groundnut Cultivars in *Ralstonia* Wilt Endemic Areas in Indonesia

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Abstract: Bacterial wilt caused by *Ralstonia solanacearum* (Smith) is an important production constraint production of groundnut (*Arachis hypogaea* L.) in some countries of Asia including Indonesia. Seventeen wilt resistant lines, including 11 breeding lines, developed from the germplasm obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), five improved cultivars, and a susceptible check cultivar (i.e., MLGG 0627) were tested for their pod yield and stability of resistance to bacterial wilt in five bacterial wilt endemic areas in Indonesia. The plant wilt intensity across all locations was high on the susceptible check cultivar, indicating severe incidence of the disease. Among the improved cultivars, only Gajah exhibited resistance to the disease and its resistance was stable across locations, whereas the other five improved cultivars were susceptible to the disease. Eight out of the 11 breeding lines were highly resistant to this bacterial wilt, comparable or even higher than Gajah's resistant level. All the resistant genotypes produced average pod yield of 2.23 t ha^{-1} , ranging from 1.01 to 3.28 t ha^{-1} , which was higher compared to pod yield of the susceptible lines. Only two breeding lines (i.e., ChiIc-8 and LPTr-12) exhibited high yield potential (i.e., $>3.0 \text{ t ha}^{-1}$). Average pod yield of susceptible genotypes ranged from 0.09 to 2.5 t ha^{-1} (mean, 0.87 t ha^{-1}).

Keywords: *Arachis hypogaea* L., bacterial wilt, germplasm, high yield, resistance

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important legume crop in Indonesia. However, its production is much less than required to meet the national requirement. Therefore, Indonesia is constrained to import about 242,800 t groundnut annually [1]. The national average productivity of groundnut in Indonesia is quite low, i.e., 1.66 t ha^{-1} , compared with yield potential of the improved cultivars which can produce up to $>4 \text{ t ha}^{-1}$.

Bacterial wilt, caused by *Ralstonia solanacearum* (Smith), is an important production constraint for groundnut over large areas in some countries of Asia, including China, Indonesia, and Vietnam [2]. In Indonesia, the bacterial wilt disease has long been existed on groundnut planting areas. It was reported that since 1920 all soil in Java has

been contaminated by the bacterium [3]. In a survey conducted in 1990, high disease intensity was found in groundnut crop in West Sumatra, Lampung, West Java, Central Java, East Java, Bali and South Sulawesi [4]. These areas contribute almost 70 % of the total groundnut production in Indonesia [5]. Yield loss caused by the disease ranges between 15 % to 35 % for resistant varieties and 60 % to 100 % for susceptible varieties [6, 7] when planted in high disease intensity areas.

Farmers in wilt endemic areas, still plant old varieties which were released around 1950, such as Macan, Jepara, Gajah and Kidang. The lower level of resistance to wilt is the main reason of farmers in the area for not planting new varieties. Breeding for wilt resistance has attempted to address the disease problem and many resistant

cultivars have been developed. However, the source of resistance used in the breeding is limited. During 1950 to 2013, as many as 39 high yielding varieties of groundnut were released, 26 of them were declared as bacterial wilt resistant. Of those 26 high yielding bacterial wilt resistant varieties, 20 were derived from Schwarz 21 resistant variety, either directly or indirectly. Schwarz 21, a bacterial wilt resistant variety, was the first bacterial wilt resistant variety released in 1925 in Indonesia [8]. However, resistance expressions of those high yielding varieties were not on the expected level when planted in wilt endemic areas. For the reason, broadening the genetic base for wilt resistance and adaptation to the environments in diseased areas should be a priority.

Bacterial wilt has the potential to spread into new areas as the disease can spread through seed (*seed borne*), though the rate is low, 4 % to 8 % [9], through irrigation water [10–12], and the presence of disease as latent infection in resistant varieties [13]. Although many high yielding resistant varieties are available, the bacterial wilt remained a serious problem in most of the groundnut production centers in Indonesia. *Ralstonia* wilt is recently reported from areas that are not formerly reported as endemic areas, *i.e.*, Malang, Probolinggo, Pasuruan, Tuban, and Borneo [14]. Allegedly there has been a decrease in the resistance of the old high yielding varieties [15], whereas the level of resistance of new high yielding varieties is lower than the existing local varieties [16]. The high yielding varieties, *i.e.*, Komodo and Biawak, grown in Malang wilted up to 80 %. Domba and Singa varieties showed wilt incidence up to 60 % when grown in Banjarnegara. These studies suggested that resistance to *Ralstonia* bacterial wilt is critical in adoption of new groundnut varieties in Indonesia.

Therefore, a breeding program was initiated to develop new groundnut varieties with bacterial wilt resistance and high yield potential by employing new sources of wilt resistant, *i.e.*, Turangga-s, Local Pati-s, and ICGV 93370. The new wilt resistant germplasm, obtained from an extensive screening, were crossed with high yielding genotypes. Genetic analysis in these three new resistant lines showed that resistance governed by a few genes with additive effect and narrow-and

broad-sense heritabilities was high, suggested that employing pedigree breeding should be successful in developing resistant lines [17]. Development of the segregating populations (F2 to F5) was conducted in endemic areas of Banjarnegara, Central Java. The susceptible genotypes were completely wilted. Selected resistant lines were further tested for their yield and a number of promising resistant lines have been obtained. The aim of the present study was to evaluate the resistance stability and pod yield of these promising lines against *Ralstonia* wilt in endemic areas.

2. MATERIALS AND METHODS

2.1 Planting Material

Field experiments were conducted during dry season of 2013 at five locations in Java, Indonesia which are known to be endemic areas for the bacterial wilt, *i.e.*, Tayu, Ngetuk, Blingoh, Tulakan, and Wonogiri. Seventeen groundnut genotypes, including 11 breeding lines developed from the ICRISAT germplasm, five improved cultivars (*i.e.*, Gajah, Bison, Kancil, Hypoma 1, and Tuban), and a susceptible check (*i.e.*, cv. MLGG 0627) were tested. These varieties exhibited high yield potential, ranging from 2.4 t ha⁻¹ and 3.7 t ha⁻¹, and thus have farmers' preference. Pre-planting bacterial enumeration indicated that *Ralstonia* population in the soils at experimental locations in Tayu and Ngetuk were quite high (2.1×10^6 cfu and 2.6×10^6 cfu g⁻¹, respectively), whereas bacterial population were high in Blingoh, Tulakan, and Wonogiri (*i.e.*, $0.75 \text{ cfu} \times 10^6$ cfu g⁻¹, 1.36×10^6 cfu g⁻¹, and 1.88×10^6 cfu g⁻¹, respectively).

2.2 Experimental Design

The experiment at each location was arranged in a randomized block design, repeated three times. Each genotype was planted in a plot of 2.4 m × 5 m, plant spacing was 40 cm × 10 cm. Basal fertilizers, *i.e.*, Phonska (N, P and K) @ 300 kg ha⁻¹ and SP36 @ 100 kg ha⁻¹, were applied entirely at planting time.

2.3 Data Collection

Wilt disease was observed at weekly intervals after

planting, until harvesting time. At the end of the field trial, the percentage of wilted plants were counted to assess bacterial wilt incidence. Bacterial wilt reactions of the test genotypes were categorized as: (i) resistant ($\leq 15\%$ wilted plants); (ii) moderately susceptible ($> 15\%$ to 25%); (iii) moderately susceptible ($> 25\%$ to 35%); (iv) susceptible ($> 35\%$) [18]. Plants were harvested after 90 days. Fully matured pods were separated from plants, sundried, cleaned and weighed in grams, and then the dry pod yield per plot (12 m^2) were converted to $t \text{ ha}^{-1}$. Seed sample for 100 seed weight measurement was taken randomly from plot yield. Plant height, branch number plant^{-1} , and number pods plant^{-1} were measured on 10 plant samples plot^{-1} .

2.4 Data Analysis

Analysis of variance was conducted for yield and wilt incidence data in each location. Pooled analysis of variance across five locations was performed on the two characters. In case of significant genotype \times environment interaction, further analysis for yield data was done to assess genotype's stability. Stability analysis parameters were estimated following Eberhart and Russell model [19] based on a linear model as Eq. 1:

$$Y_{ij} = U_i + B_j + I_j + d_{ij}, i = 1, 2, \dots, g \quad (1)$$

Where Y_{ij} = yield average of line i at test site j , U_i = overall mean, B_j = slope of response of lines on locations, I_j = location index, d_{ij} = deviation of the regression i th lines on j th location. A genotype with high mean seed yield, regression coefficient (b_i) close to unity and deviation from regression (s_{di}^2) near to zero was defined as a stable cultivar [19]. Simple correlation coefficients were estimated to determine the relationship between yield and wilt incidence and yield related traits.

3. RESULTS AND DISCUSSION

Reaction differences to bacterial wilt were evident among genotypes and locations, either on days to visible symptoms and disease incidence. First symptoms of bacterial wilt were observed one week after planting. Disease symptoms initially occurred on a few leaves, and then the plants die suddenly while the leaves were still green. The

presence of dark brown color in xylem and the flow of bacteria mass from incised stems in the water is a diagnostic characteristic of this disease [20]. In resistant genotypes wilt symptoms stopped after 3 wk to 4 wk. The highest wilt incidence was recorded in Blingoh-Jepara, followed by Tulakan, Tayu, Wonogiri, and Ngetuk (Table 1). Average wilt incidence was not in line with the level of bacterial population in soil prior to planting.

The wilt incidence on susceptible check (MLGA0627) was high (53.23 % to 82.33 %) with an average of 68.14 %. Whereas the wilt incidence of Gajah and the 11 promising lines ranged from 0.33 % to 11.99 %, those wilt incidence levels were belong to resistant classification (Table 1). All high yielding varieties except Gajah were found susceptible with wilt incidence ranging from 40.73 % to 50.21 %. All the genotypes, except Bison in Tayu and Hypoma 1 in Wonogiri, were reacted consistently to the bacterial wilt across the test sites (Table 1). The eleven breeding lines were consistently resistant across the five sites, indicated their wilt resistant stability. Average bacterial wilt incidence of the resistant lines across locations was less than 5 % (Table 1). Bacterial wilt in groundnut is caused by *R. solanacearum* race 1 biovar 1, 3 and 4. Ralstonia biovar 1 which can infects groundnut is only found in the USA, whereas biovar 3 and 4 were reported to infect groundnut in Asia and Africa. Biovar 3 is dominant in Asia [20], and biovar 3 is more virulent compared to biovar 1 or 4 [6]. Isolates obtained from groundnut in Indonesia and China were mostly belongs to biovar 3 [4, 6], biovar 4 was only found in Manokwari, Papua province [6]. This report suggested that Ralstonia which existed in the five test sites was most likely belong to the same biovar, and this is explained by the stability of the genotypes's reactions against the bacterium.

Negative correlation was found between dry pod yield and wilt incidence (Table 2). High wilt incidence was correlated with relatively larger grain size and higher number of pods (Table 2). As wilt incidence increased the plant harvest and yield also decreased although pod number and seed mass per plant increased. This increase was likely attributed to a reduction in competition from adjacent peanut plants for water, nutrients, and light [21]. However, the increase of pod number and seed size were not

Table 1. The wilt incidence and reaction in groundnut genotypes at five test sites during dry season (DS) of 2013.

S. No.	Genotype	Blingoh		Ngetuk		Tayu		Tulakan		Wonogiri	
		WI ¹⁾ (%)	R ²⁾	WI (%)	R	WI (%)	R	WI (%)	R	WI (%)	R
1	ChiIc -1	8.97	R	2.68	R	1.24	R	3.05	R	3.37	R
2	ChiIc -3	3.82	R	0.60	R	2.32	R	3.51	R	0.84	R
3	ChiIc -8	5.18	R	2.75	R	1.57	R	1.80	R	3.22	R
4	LPTR -10	4.90	R	1.20	R	1.04	R	3.24	R	2.53	R
5	LPTR-12	2.83	R	1.82	R	1.63	R	0.67	R	1.08	R
6	ChiLP 14	2.61	R	4.92	R	1.71	R	3.20	R	2.10	R
7	LPTr -21	2.15	R	1.12	R	1.44	R	1.06	R	0.68	R
8	IcLP-24	8.21	R	1.28	R	1.35	R	0.53	R	0.33	R
9	IcLP -25	8.68	R	2.62	R	1.56	R	4.91	R	1.91	R
10	IcLP-27	6.10	R	0.89	R	3.37	R	3.57	R	0.47	R
11	Chico-s	7.80	R	2.36	R	1.57	R	2.53	R	0.91	R
12	Bison	54.67	S	26.67	S	28.89	MS	58.32	S	35.11	S
13	Hypoma 1	64.71	S	37.99	S	49.10	S	59.00	S	15.64	MR
14	Kancil	43.79	S	36.90	S	45.33	S	59.20	S	33.08	S
15	Tuban	63.18	S	33.68	S	40.11	S	67.71	S	46.40	S
16	MLGA0627	82.33	S	53.23	S	64.29	S	77.32	S	63.55	S
17	Gajah	11.19	R	0.75	R	1.46	R	0.80	R	0.37	R
	Average	22.42		12.44		14.59		20.61		12.45	

¹⁾ WI : Wilt Incidence; ²⁾ Classified according to Machmud and Rais [18]**Table 2.** Correlations coefficients (r-values) between agronomic traits and *Ralstonia* wilt incidence in groundnut genotype tested at five endemic bacterial wilt locations during dry season of 2013.

Characters	Dry pod yield (t ha ⁻¹)	Wilt incidence (%)	100 seed weight (g)	Plant height (cm)	Branches plant ¹
Wilt incidence (%)	-0.835**				
100 seed weight (g)	-0.316**	0.404**			
Plant height (cm)	0.232*	-0.328**	-0.041ns		
Branch no./plant	0.067ns	0.144ns	-0.161ns	-0.330**	
No. pods/plant	-0.113ns	0.252*	-0.092ns	-0.517**	0.395**

¹⁾ * and **= significant at P = 0.05 and P = 0.01 respectively; ns = non-significant

able to compensate plant harvest decrease due to susceptible reaction to the wilt which lead to the lower yield of the susceptible genotypes compared to the yield of the resistant ones.

Mean yield of the tested genotypes at all field locations is presented in Table 3. Average yield in each location representing environment productivity. The most productive environment on this test, also indicated by I_j value [19], is Tayu, followed by Wonogiri, Ngetuk, Tulakan and Blingoh (Table 5). Productivity of the environment

was related to the genotypes' average wilt incidence at the respective location as indicated by b negative correlation between dry pod yield and wilt incidence (Table 2). The highest average pod yield across the locations obtained by line LPTR-12 with average dry pod yield 2.58 t ha⁻¹, while the highest pod yield was achieved by ChiIc-8 at Tayu location with dry pod yield reached 3.28 t ha⁻¹. ChiIc-8 derived from single cross between Chico and ICGV 93370, both of the parents were introduced from ICRISAT, India, a non wilt endemic area. Most of groundnut

Table 3. Dry pod yield of groundnut genotypes in adaptation trials at five wilt endemic locations, DS 2013.

S. No.	Genotype	Dry pod yield ($t ha^{-1}$)					Mean	Min	Max
		Blingoh	Ngetuk	Tayu	Tulakan	Wonogiri			
1	ChiIc -1	1.31	2.15	2.95	2.30	2.42	2.22	1.31	2.95
2	ChiIc -3	1.41	1.97	2.54	2.32	2.70	2.19	1.41	2.70
3	ChiIc -8	1.51	1.99	3.28	2.40	2.44	2.33	1.51	3.28
4	LPTR -10	1.60	2.15	2.79	2.22	2.46	2.24	1.60	2.79
5	LPTR-12	1.67	2.57	3.11	2.64	2.92	2.58	1.67	3.11
6	ChiLP 14	1.38	1.48	2.48	2.18	2.62	2.03	1.38	2.62
7	LPTr -21	1.53	2.40	2.83	2.11	2.72	2.32	1.53	2.83
8	IcLP-24	1.56	2.39	2.96	2.19	2.78	2.38	1.56	2.96
9	IcLP -25	1.52	1.80	2.66	2.03	2.49	2.10	1.52	2.66
10	IcLP-27	1.85	1.99	2.80	2.44	2.74	2.36	1.85	2.80
11	Chico-s	1.53	1.94	2.60	1.01	2.20	1.86	1.01	2.60
12	Bison	0.57	1.05	2.04	0.65	2.50	1.36	0.57	2.50
13	Gajah	1.55	2.09	2.72	1.92	2.63	2.18	1.55	2.72
14	Hypoma 1	0.34	0.91	1.05	0.67	1.48	0.89	0.34	1.48
15	Kancil	0.85	0.86	1.24	0.41	1.06	0.88	0.41	1.24
16	Tuban	0.79	0.82	1.29	0.48	0.92	0.86	0.48	1.29
17	MLGA0627	0.11	0.28	0.56	0.09	0.67	0.34	0.09	0.67
	Mean	1.24	1.70	2.35	1.65	2.22			
	Ij	-0.59	-0.13	0.52	-0.18	0.39			

Table 4. Mean squares of pooled analysis of variance of pod yield and wilt incidence of 17 groundnut genotypes evaluated at five locations.

Source of variation	df	Mean square	
		Dry pods ($t ha^{-1}$)	Wilt incidence (%)
Genotype (G)	16	7.00**	1.397**
Location (E)	4	10.44**	0.242**
G x E	64	0.21**	0.016**
Error	168	0.06	0.005

**= significant at $P = 0.01$

bacterial wilt resistance sources are of Chinese or Indonesian origin [22]. This result suggested that the wilt resistance could be found in genotypes that were introduced from wilt non-endemic area, and a chance to get genotypes resistant to wilt disease and high pod yield at once. Generally, resistant cultivars have lower yield potentials due to the presence of latent infection [23].

Pooled variance analysis revealed significant location (environment) effects for dry pod yield

and wilt disease incidence. Similarly, there were significant genotype and genotype x location (G x E) interaction effects for the two characters (Table 4). Existence of G x E interaction on agronomic traits in crops, including groundnut, have been widely reported [24–27], likewise, the G by E interaction on the incidence of wilt disease [28, 29]. The G by E interaction indicated a differential response among the tested genotypes across the five locations which cause changes in relative ranking

Table 5. Relative ranking of pod yield of 17 genotypes at five locations, DS 2013.

S. No.	Genotype	Rank No.					Average rank no.
		Blingoh	Ngetuk	Tayu	Tulakan	Wonogiri	
1	ChiIc -1	12	5	4	5	12	7.6
2	ChiIc -3	10	9	11	4	5	7.8
3	ChiIc -8	9	8	1	3	11	6.4
4	L PTR -10	3	4	7	6	10	6.0
5	L PTR-12	2	1	2	1	1	1.4
6	ChiLP 14	11	12	12	8	7	10.0
7	L PTR -21	6	2	5	9	4	5.2
8	IcLP-24	4	3	3	7	2	3.8
9	IcLP -25	8	11	9	10	9	9.4
10	IcLP-27	1	7	6	2	3	3.8
11	Chico-s	7	10	10	12	13	10.4
12	Bison	15	13	13	14	8	12.6
13	Gajah	5	6	8	11	6	7.2
14	Hypoma 1	16	14	16	13	14	14.6
15	Kancil	13	15	15	16	15	14.8
16	Tuban	14	16	14	15	16	15.0
17	MLGA0627	17	17	17	17	17	17.0

Table 6. Stability parameters for 17 groundnut genotypes estimated by Eberhart and Russel model

S. No.	Genotype	Average pod yield ($t \text{ ha}^{-1}$)	Pod yield range ($t \text{ ha}^{-1}$)	Regression coefficient (b_i)	Regression deviation (s_{di}^2)
1	ChiIc -1	2.22	1.31 to 2.95	1.20ns	0.050ns
2	ChiIc -3	2.19	1.41 to 2.70	1.03ns	0.037ns
3	ChiIc -8	2.33	1.51 to 3.28	1.29ns	0.095ns
4	L PTR -10	2.24	1.60 to 2.79	0.93ns	-0.004ns
5	L PTR-12	2.58	1.67 to 3.11	1.15ns	0.030ns
6	ChiLP 14	2.03	1.38 to 2.62	1.09ns	0.083ns
7	L PTR -21	2.32	1.53 to 2.83	1.12ns	0.001ns
8	IcLP-24	2.38	1.56 to 2.96	1.19ns	-0.007ns
9	IcLP -25	2.10	1.52 to 2.66	1.02ns	-0.009ns
10	IcLP-27	2.36	1.85 to 2.80	0.88ns	0.018ns
11	Chico-s	1.86	1.01 to 2.60	1.05ns	0.183ns
12	Bison	1.36	0.57 to 2.50	1.76**	0.136ns
13	Gajah	2.18	1.55 to 2.72	1.08ns	-0.020ns
14	Hypoma 1	0.89	0.34 to 1.48	0.82ns	0.034ns
15	Kancil	0.88	0.41 to 1.24	0.44**	0.052ns
16	Tuban	0.86	0.48 to 1.29	0.44**	0.037ns
17	MLGA0627	0.34	0.09 to 0.67	0.53**	-0.007ns

*= significant at $P = 0.01$, ns = non-significant

of the tested genotypes. Pod yield of line no. 1, ChiIc-1, rank fifth in Ngetuk and Tulakan, but it turned to rank twelfth in Blingoh and Wonogiri (Table 5). Likewise for other genotypes, their relative yield rank changes over locations. Such genotypes' response variation may be due to inoculum pressure, pathogen virulence, environment, and interactions between host and those factors that affected genotypes' resistance level and yield. The varied reactions of some accessions were also found on ICGs 5272, 5273, and 5276 when they evaluated in Indonesia and China [30]. Changes in ranking complicate genotypes's evaluation and make it difficult to select the best genotype over the locations. Stability analysis developed by Eberhart and Russel [19] can be employed to evaluate genotypes' performance across locations when genotype by environment interaction exist and can be used as a tool to select the best genotype.

Stability refers to the behavior of a crop to varying environments. Different approaches to assessing stability were used [31], one of them is regression approach suggested by Eberhart and Russel [19]. Eberhart and Russel [19] used regression coefficient (b_i) and the deviation of the regression (s^2_{di}) as stability parameter. A genotype is stable if it has a regression coefficient (b_i) of unity and the deviation of the regression (s^2_{di}) equal to zero. Genotypes that have regression coefficient (b_i) > 1 will adapt well to the productive environments and genotype with the regression coefficient (b_i) < 1 will adapt well in marginal environments. Regression coefficient values (b_i) ranged from 0.44 to 1.76 and the amount of deviation from regression (s^2_d) ranged from -0.001 to 0.183 (Table 6). All the genotypes have a coefficient equal to unity, except Bison, Kancil, Tuban, and MLGA 0627; while the deviation of the regression is not significant for all the genotypes. These results suggested that all the promising lines, except Chico-s, classified as ideal cultivars, i.e., stable and have high average dry pod yield ($> 2 \text{ t ha}^{-1}$) (Table 6). Characteristics which are recommended for increasing productivity of groundnut planted under endemic areas which is accounted for almost 70 % of the total groundnut production in Indonesia. Among the improved varieties, only Gajah belongs to that criterion. Bison belongs to below average stability, which

means that the variety gave high yield in only productive environments. In this case, productive environment means low wilt disease incidence. Improved varieties were susceptible to the disease and relatively unstable (Table 6). The results were in agreement with previous reports [16] that wilt resistance level of most groundnut improved varieties were lower compared to that of local varieties when planted in endemic areas.

4. CONCLUSIONS

High incidence of bacterial wilt disease, varying from 53.2 % to 82.3 % on the susceptible genotypes, verified that the trial locations were wilt endemic. Some breeding lines exhibited resistant to bacterial wilt disease consistently across the endemic areas. Eight of the 11 tested lines exhibited comparable or even better resistance compared with cv. Gajah, which is the most resistant improved cultivar of groundnut. The wilt disease intensity on the breeding lines ranged from 1.2 % to 2.9 %. Stable genotypes are characterized by bacterial wilt disease resistance and high yielding. Thus, the level of resistance to bacterial wilt disease contributes greatly in high pod yield when planted in the bacterial wilt endemic areas. Average pod yield of resistant genotypes ranged from 1.86 t ha^{-1} to 2.58 t ha^{-1} , while that of susceptible genotypes ranged from 0.34 t ha^{-1} to 1.56 t ha^{-1} . Only two breeding lines (i.e., ChiIc-8 and LPTr-12) exhibited high yield potential, i.e., $> 3.0 \text{ t ha}^{-1}$; thus, these two lines are classified as stable and high yielding.

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