Evaluating Sheath Blight Resistance in Rice Using Detached Tiller and Field Screening Methods

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ABSTRACT

In present study physical resistance test on 12 rice varieties against sheath blight (ShB) caused by *Rhizoctonia solani* was examined. A detached tiller test was used to measure components of ShB physiological resistance at Regional Agricultural Research Station (RARS), Tarahara, Nepal. The varieties were evaluated in a randomized complete block design (RCBD) with 3 replications in year 2010-12. A sclerotium was inserted below the leaf collar of individual tillers maintained in tubes filled with water. Only the 56-day old susceptible plants were inoculated. After 7 days of inoculation, number of lesions, dead leaves, vertical sheath colonization and disease severity were measured. In 2011, field experiments were conducted with three replications to address morphological resistance in 28 rice genotypes. Disease incidence was recorded on 14, 21 and 28 days after inoculation. The number of dead leaves differed only numerically while disease variables significantly (*P=0.004*) varied among varieties. The other variables were highly correlated with each other. Cluster analysis of variables formed three varietal groups; *Sabitri* with lowest, and *Jasmine-85* and *Betichikon* having highest values. Rice genotypes differed significantly (*P=0.05*) for disease incidence and area Under Disease Progress Curve (AUDPC). *Tetep* and *IAC-165* had the lowest and highest terminal disease incidences and AUDPC, respectively.

*Sabitri*, *MTU-1010* and *IR-26* recorded lower AUDPC values. Hence, *Tetep* and *Sabitri* could be used as donors in hybridization and their plant morphology could be considered as selection guide for improving ShB resistance in rice.

Key words: Detached tiller, field screening, morphological, physiological resistance and sheath blight

INTRODUCTION

Sheath blight (ShB) disease caused by fungus *Rhizoctonia solani* (Kuhn), occurs throughout the rice growing areas in Nepal (Manandhar 1987, Manandhar et al 1992) having high potential threat to rice yield (NARC 1997, Gharti et al 2004). This rice disease can reduce yield up to 28% (Parajuli 1997) and deteriorate grain quality by breaking grain during milling (NRRP 2000). The fungus has a wide host range, thus known to infect more than 33 plant families of 188 genera (Srinivasachary et al 2011, Sattari et al 2014). This pathogen does not produce spores instead lesion multiplication in a rice canopy occurs through the growth of runner hyphae from mother lesion at below the leaf collar of individual tillers maintained in tubes filled with water. Onl y the 56-day old susceptible plants were inoculated. After 7 days of inoculation, number of lesions, dead leaves, vertical sheath colonization and disease severity were measured. In 2011, field experiments were conducted with three replications to address morphological resistance in 28 rice genotypes. Disease incidence was recorded on 14, 21 and 28 days after inoculation. The number of dead leaves differed only numerically while disease variables significantly (*P=0.004*) varied among varieties. The other variables were highly correlated with each other. Cluster analysis of variables formed three varietal groups; *Sabitri* with lowest, and *Jasmine-85* and *Betichikon* having highest values. Rice genotypes differed significantly (*P=0.05*) for disease incidence and area Under Disease Progress Curve (AUDPC). *Tetep* and *IAC-165* had the lowest and highest terminal disease incidences and AUDPC, respectively. *Sabitri*, *MTU-1010* and *IR-26* recorded lower AUDPC values. Hence, *Tetep* and *Sabitri* could be used as donors in hybridization and their plant morphology could be considered as selection guide for improving ShB resistance in rice.

Key words: Detached tiller, field screening, morphological, physiological resistance and sheath blight
Breeding for ShB resistance in rice has been difficult, mainly because of the lack of identified donors in cultivated rice varieties (Bonmann et al. 1992, Srinivasachary et al. 2011, Liu et al. 2013, Zou et al. 2014). To date, no variety has been found to be immune to R. solani, although cultivars with varying levels of resistance were reported (Khush 1977, Groth and Novick 1992, Zou et al. 2000, Shrestha et al. 2008, Hossain et al. 2014, Zou et al. 2014). Resistance to ShB is a complex, quantitative trait controlled by polygenes (Sha and Zhu 1990, Li et al. 1995, Pinson et al. 2005). The accurate measurement of ShB resistance under field conditions depends on a range of environmental factors (Ou 1985, Castilla et al. 1996, Eizenga et al. 2002) and plant morphological traits, such as plant height (Li et al. 1995, Zou et al. 2000, Pinson et al. 2005, Eizenga et al. 2013, Sattari et al. 2014), which interact, resulting in the variation in resistant phenotypes.

Disease intensification and spread are favored by long duration of tissue wetness (Castilla et al. 1996), which depends on canopy microclimate. Therefore, morphological attributes of rice genotypes can influence strongly disease intensification and spread, by providing an environment to disease development with varying levels of contact frequency and canopy wetness duration. Morphological traits allowing a reduction of disease epidemics can, thus be associated to disease escape mechanisms (Poland et al. 2009, Zou et al. 2014). Rice ShB is predominantly canopy-borne in terms of spread, even though the pathogen is often soil-borne (Savary and Mew 1996). The diversity of genotypes in terms of morphological traits and maturity duration are two important factors for the development of sheath blight epidemics. The genetic diversity of rice may incorporate genes that directly contribute to physiological host resistance to ShB (Srinivasachary et al. 2011, Zou et al. 2014), genes that determine the architecture of crop canopies, as well as genes from these different groups that collectively confer resistance through interactions. Therefore, the objective of the study was to assess the diversity of rice genotypes in terms of susceptibility to sheath blight, according to genetic group and morphological traits under field conditions.

MATERIALS AND METHODS

The components of physiological resistance to ShB were quantified using the detached tiller test during 2010. The test was undertaken in Plant Pathology Laboratory (PPL) at Regional Agricultural Research Station (RARS), Tarahara, Nepal to minimize the effect of disease escape. Attempt was made to maintain leaf wetness and negate disease spread at the canopy level among the tillers as only one tiller was used. In 2011, an experiment was conducted including 28 entries received from IRRI to evaluate morphological resistance to ShB under field conditions at RARS, Tarahara.

The experimental site is situated in eastern terai region of Nepal at 136 m above sea level and at 26º42'16.85" North latitude and 87º16'38.43" East longitude (Figure 1) The climate of the area is sub-tropical with cool winter (8°C) and hot summer (37°C). The research station has six command districts where rice is the number one food crop (RARS 2014). Majority of the area is under irrigation but recently adversely affected by occasional flood and drought. The ShB susceptible varieties such as Sona Masuli, Ranjet and others are popularly grown and are being infected each year by this pathogen. As a result, inoculums build up in the soil year after year in an alarming way to cause the disease in other recommended varieties.

Plant Materials

Twelve popular rice varieties among farmers in Nepal susceptible to diseases were used (Table 1). The material consisted of tillers with roots remained attached to the tillers were detached from the selected rice varieties and grown in a screen house. The tiller collection was made at 42 days after transplanting of 14-day old seedlings. The roots were washed under tap water, and each tiller was placed in a test tube filled with 10 ml of distilled water. Seedlings of 28 entries for field trial were raised in dry seedbeds during 2011.

Inoculation Method, Disease Assessment and Experimental Design

The inoculum consisted of 7-day-old sclerotia, obtained by placing at the centre of each 9.5 cm diameter petri dish filled with PDA (extract from 200 g of potato L⁻¹, 20 g L⁻¹ dextrose, 20 g L⁻¹ agar) and incubating at 28ºC in the darkness. The local isolate, collected at the RARS experimental farm, was used to prepare the inoculum. This isolate, when inoculated on rice, produced typical sheath blight symptoms on sheath and leaves. Mycelial growth and sclerotia production was also typical of R. solani. To standardize the inoculum, only sclerotia with a diameter size of 1.5 ± 0.3 mm were used. Tillers were inoculated by transferring a single sclerotium just below the leaf collar, on the inner side of the sheath, of the second top-most collar of the tiller, thus corresponding to the second or third leaf of the tiller from the top.

The inoculated tillers were grouped in tube racks. Each group of tillers was sprayed with distilled water and placed into a transparent plastic bag to provide an environment, where air was saturated with humidity and tissue surface was moist. The racks were transferred into a growth chamber at 25ºC, with a 12 h photoperiod, until disease assessment 7 days after inoculation (DAI).

The following disease variables were measured for each tiller: number of lesions; disease severity; and distance between the tips of the lowest and highest lesions on the sheaths (Vertical Sheath Colonization, VSC). Four independent experiments were performed. Each experiment was arranged according to a randomized complete block design, with three blocks including each of the 12 rice varieties assigned at random. Disease severity and VSC values were arc-sine transformed before analyses of variance.
Figure 1. Location of the experimental site in RARS, Tarahara, Nepal

Table 1. Varieties used in the detached tiller test to measure sheath blight resistance components in rice in 2010.

| Varieties       | Basis of selection                              |
|-----------------|-------------------------------------------------|
| Jasmine-85      | Reported to be resistant (Zou et al 2000)        |
| Dudruchi        | Reported to be resistant (Shrestha et al 2008)   |
| Khato-chalani   |                                                  |
| Teqing          | Reported to be resistant (Li et al 1995, Pinson et al 2005) |
| Betichickon     | Reported to be resistant (Shrestha et al 2008)   |
| BR-11           | Varieties popularly grown in a larger areas in Nepal |
| Hardinath-1     |                                                  |
| Sabitri         |                                                  |
| Radha-12        |                                                  |
| Radha-4         |                                                  |
| Kanchhi Masuli  |                                                  |
| Anandi          | Reported to be resistant (NARC 1997)             |

The field trial was laid out according to randomized complete block design with three replications. Two rows of each entry were transplanted when seedlings were 25 day old. Five hills per row spaced at 15 cm plant to plant and 20 cm row to row, the middle hill was planted with susceptible genotype i.e. Swarna (source hill) and either side of the source hill was planted with two hills of the entry. Each entry was surrounded by the buffer hills i.e. Sabitri, a variety with physiological resistance to sheath blight (Gharti et al 2004). The trial block was fertilized with 100:50:0 (N:P₂O₅:K₂O) kg ha⁻¹. Water level of 5 cm was maintained from 10 days after transplanting to grain filling stage. Weed management was done manually before top dressing.

For field experiment, the sclerotium with a small piece of PDA was inoculated to sterilized rice stem beats kept in a conical flask to prepare inoculum. The flasks were incubated at 25±2°C until the beats were colonized with fungal mycelium. Fifty six day old source hill was inoculated with 3-5 pieces of R. solani colonized rice stem beats, inserting them in the middle of the hill just above the water surface (IRRI 1992). Natural spread of the disease was allowed to the either side of the source hill.

Disease scoring was begun from 14 days after inoculation and continued for three times at weekly interval. At first scoring, only four hills, one side of the source hill was considered. Total number of tillers and infected tillers were counted to calculate disease incidence. On the second date, the remaining four hills were scored. During the final third date, total hills of the entry were scored. Area under disease progress curve (AUDPC) was calculated using the formula outlined by Shanner and Finney (1977) to estimate the horizontal disease spread among the tillers as follows:
Where,
\[ y_i = \text{disease severity at the } i^{th} \text{ observation}, x_i = \text{time at the } i^{th} \text{ observation}, \text{ and } n = \text{total number of observations} \]

Data of the detached tiller test and field trial was analyzed using MSTATC. Means were separated to compare the effect of genotypes, experiment and their interaction on disease variables.

**RESULTS**

**Relationships between Disease Variables**

The different variables measured were significantly \( P<0.01 \) correlated (Table 2), with correlation coefficients above 0.8 for the variable pairs (number of lesions tiller\(^{-1} \) and disease severity), (number of lesions tiller\(^{-1} \) and VSC) and (disease severity and VSC).

Figure 2A showed the ranking of varieties according to increasing number of lesions tiller\(^{-1} \). The same ranking of varieties was used to display the other disease variables (Figure 2B and C). The number of lesions tiller\(^{-1} \) ranged between 3.8 and 8.9. Sabitri and Betichikon had the lowest and highest number of lesions tiller\(^{-1} \), respectively. Anandi and Kanchhi Masuli had the next lowest number of lesions (4.9). The number of lesions progressively increased from 5.2 to 8.9 among the 8 other varieties. Jasmine 85 had more than 8 lesions tiller\(^{-1} \). Disease severity (and the corresponding LSD) ranged from 6.07\% (Sabitri) to 24.13\% (Jasmine 85; Figure 2B).

**Effect of Varieties on Sheath Blight Variables**

The varieties Hardinath 1, Radha 4 variety had the largest effect on the disease severity \( (P = 0.004) \), and vertical sheath colonization \( (P = 0.03) \). Experiments had no effect on all variables. The interaction of variety \( \times \) experiment was not significant, and was associated with \( P \) values larger than 0.34 for all variables except for the disease severity for which \( P \) value was 0.01.

Anandi, Radha 12, BR 11 and Khato-chalani formed one group with a disease severity lower than 14\%. Betichikon had the next highest disease severity (23.9\%) followed by Dudruchi (18.14\%), Kanchhi Masuli (17.68\%) and Teqing (15.39\%). The ranking of varieties was in general similar with respect to VSC (Figure 2C). VSC was highest for Jasmine 85 (22.74 cm). Sabitri and Hardinath-1 showed the lowest values for both disease severity and VSC variables.

When considering the different disease variables measured (Figure 2), Sabitri consistently had the first lowest values. Hardinath-1 was among the varieties with lowest disease severity and VSC next to Sabitri, but had intermediate values for number of lesions. Kanchhi Masuli had medium-high levels of disease severity, but showed lower values for number of lesions and VSC. Anandi had the second lowest values for number of lesions, but had intermediate values for disease severity and VSC. Betichikon was the most susceptible in terms of number of lesions, and was also the second most susceptible variety next to Jasmine 85 with regard to disease severity and VSC. Dudruchi and Teqing had medium-high levels lesions, disease severity and VSC. Khato-chalani, BR 11 and Radha 12 were varieties with medium levels of number of lesions, disease severity and VSC. Radha 4 was less susceptible with respect to disease severity, but had medium-high levels of number of lesions and VSC.
Table 2. Correlation coefficients among the different rice sheath blight disease variables measured at 7 days after inoculation on detached tillers in 2010.

| Disease variables          | Number of lesions tiller \( r \) | Disease severity (%) | Vertical sheath colonization (VSC, cm) |
|----------------------------|-----------------------------------|----------------------|---------------------------------------|
| Number of lesions tiller 1 | 0.88                              | 0.90                 |                                       |
| Disease severity (%)       | 0.83                              |                      |                                       |

Figure 3. Clusters of rice varieties based on SBD variables measured after inoculation on detached tillers. Clusters were generated from hierarchical clustering analysis using the Euclidian distance of 12 rice varieties according to number of lesions, disease severity and vertical sheath colonization (VSC) in 2010.

**Variety Groups According to the Components of Resistance**

Cluster analysis was conducted with three variables: number of lesions, disease severity, and VSC. The three variables captured the main components of resistance measured, with respect to physiological resistance. Three main groups were identified from the hierarchical cluster analyses: a first group with *Sabitri*, a second with *Jasmine-85* and *Betichikon*, and the third, larger group, including all the other varieties (Figure 3). The first group corresponded to lowest average values in number of lesions (3.8), disease severity (6.07%) and VSC (10.16 cm). The second group had the highest average number of lesions, highest disease severity and maximum values for VSC.

Figure 4. Proportion of dead leaves for 12 rice varieties, 7 days after inoculation by *R. solani* on detached tillers.
Effect of Variety on Leaf Blade Senescence and Occurrence of Dark Margin

Proportion of dead leaves at 7 DAI ranged from 0.20 (Radha-4) to 0.80 (Jasmine-85; Figure 4). The next lowest values were observed forhardinath-1, Khato-chalani, Sabitri and Kanchhi Masuli (0.30), and BR-11 and Anandi (0.40). The highest proportion of dead leaves was observed for Betichikon and Dudruchi (0.70).

Field Screening 2011

During 2011, genotypes differed significantly (P<0.05) for the final disease development and AUDPC values (Table 3). The disease incidence varied from 13 to 90%, while AUDPC values ranged from 46 to 515 among the genotypes. Out of 28 genotypes, seven showed moderate level of resistance to sheath blight. Among them, Tetep showed the lowest disease incidence and AUDPC values. The disease incidence was up to 17.7% and AUDPC value was recorded below 100 in such genotypes. On the contrary, disease spread was faster in susceptible genotypes. Two weeks after inoculation, susceptible lines attained up to 90% disease incidence with the highest AUDPC values of 515. Disease spread in moderately resistant (MR) genotypes was slower than others. Over two weeks after inoculation, increase in disease incidence was up to 17.7% and AUDPC value was recorded below 100 in such genotypes. On the contrary, disease spread was faster in susceptible genotypes. Two weeks after inoculation, susceptible lines attained up to 90% disease incidence with the highest AUDPC values of 515.

N22 (IRGC-19379) and Tomoe Moch had statistically similar disease incidence with MR genotypes, but were put under susceptible group based on higher AUDPC values, which indicated that these lines might record higher disease incidence over time beyond 28 DAI. Tetep had the lowest values for disease incidence (13.3) and AUDPC (46.6). The genotypes Sabitri, IR-26, SHB-134-11-23, MTU-1010 were also less susceptible based on these variables (Table 3).

DISCUSSION

A range of complementary variables were used to describe the disease cycle. The number of lesions was used to quantify the infection efficiency of the pathogen, i.e., its capacity to infect the host tissue and produce lesions. VSC was the most discriminating variable, and showed consistent results, as no significant experiment x variety interaction was detected (Figure 2C). Significant (P = 0.03) effect of variety on VSC was detected from the present experiments. The VSC represents key component of resistance, reflecting the capacity of the pathogen to progress and the lesion to expand. It should, therefore be measured when comparing rice varieties, because differences may be detected, and contribute to identifying overall differences in resistance level.

Disease severity is also an important variable to measure ShB resistance in rice. The variable was substantially different among the varieties, and thus reflected different mechanisms of partial resistance in rice. Disease severity and VSC variables provided an insight on the ability of runner hyphae of the fungus to progress spatially over plant tissues.

Many varieties were ranked differently, according to the disease variable measured (Figure 2). For example, Kanchhi Masuli had medium-high values for the number of lesions, whereas it was associated with low and intermediate values for the other disease variables. Differential response according to components of resistance has been reported for many other diseases, such as barley leaf rust, wheat leaf rust, groundnut rust, and potato late blight (Parlevliet 1979, Savary et al 1988). In the same way, the study indicates that several components of resistance need to be measured in order to provide an overall assessment of the level of resistance to sheath blight.

The most resistant variety from the present study, Sabitri, was reported to have a relatively high level of resistance (Gharti et al 2004). Jasmine-85 (Srinivasachary et al 2011) and Betichikon (Shrestha et al 2008) were reported to have higher level of resistance to sheath blight, but had high levels of disease intensity with respect to the number of lesions per tiller, disease severity and VSC. Betichikon was also reported to be susceptible with respect to physiological resistance (Willocquet et al 2011). Dudruchi also showed medium-high level of susceptibility to ShB, whereas it was also known for relatively higher level of ShB resistance (Shrestha et al 2008). Teqing and Khato-chalani, which are considered as varieties with high levels of partial resistance, were in general, associated to intermediate to medium levels of disease intensity in the study, which may indicate a larger tolerance to the disease (Zadoks et al 1979).

In general, difference between results in present work and other studies may have several such as differences in methods and types of disease variables measured, which are associated with differences in resistance mechanisms expressed. For example, the expression of some defense mechanisms may be higher in the case of the detached tiller tests than in the tests performed with detached leaves (eg, IRRI 1987, Prasad and Eizenga 2008), corresponding to plant organs which are surviving in isolation of other tiller and root tissues. Another example is comparison of tests performed in the field (eg, Li et al 1995, Pinson et al 2005) with detached tiller: the former type of test includes the combined effects of physiological resistance and disease escape, while the second type of test focuses on physiological resistance. A third, intermediate example, corresponds to the micro-chamber tests (Jia et al 2007), which are performed at the plant scale at a very early stage, and partly incorporates a fair level of disease escape mechanisms (associated with plant height and contact between tillers), as compared to the detached tiller test.

Considering results from detached tiller test and field screening, Sabitri, earlier reported to be resistant to blast (Chaudhary 1999) and bacterial blight (Adhikari 2004), confers higher level of physiological and morphological resistance to ShB. Tetep is the most resistant to ShB in the field screening. Morphological resistance (disease escape) in rice may vary over locations, seasons and genotypes; and a variety with physiological resistance may show susceptibility in the field conditions (Srinivasachary et al 2011, Zou et al 2014). Sabitri is a medium-tall variety and its tillers are up-right straight with minimum angle among themselves. Its leaves form minimum angle with
tillers allowing penetration of sunlight and good aeration. The flag leaves are straight up-right with length of more than 20 cm above panicles. These morphological characters allow less contact among tillers, thus negate disease spread. This is probably the reason that Sabitri and Tetep were extensively used as donor parents in breeding program considering plant morphology as a model in selecting the genotypes with similar plant stature for improving ShB resistance in rice.

**Table 3.** Resistance in rice genotypes to sheath blight under field conditions in 2011 wet season

| IRRI accession | Genotype     | Disease incidence (%) | AUDPC$^1$ |
|---------------|--------------|------------------------|-----------|
|               |              | 14 DAI  | 21 DAI  | 28 DAI$^1$ |           |
| IRGC-32576    | TETEP        | 0      | 0      | 13.33 G    | 46.66 H   |
| IECG-24154    | IR 26        | 0      | 0      | 16.13 FG   | 56.45 GH  |
| IRGC7-17-15-11-23 | SHB-134-11-23 | 0      | 1.852  | 19.16 FG   | 80.04 F-H |
| MTU-1010      | MTU-1010     | 1.852  | 1.515  | 20.69 FG   | 89.50 E-H |
| IRGC39723     | RNR 57979    | 0.952  | 5.119  | 16.07 FG   | 95.40 D-H |
| IR6861-17-5-18-45 | SHB-129-18-45 | 1.667  | 4.227  | 17.72 FG   | 97.46 D-H |
| IRGC5656      | SABITRI      | 1.389  | 4.568  | 17.56 FG   | 98.31 D-H |
| IRGC39932     | SI 51        | 2.279  | 3.262  | 21.98 E-G  | 107.8 C-H |
| IRGC19907     | IR 24        | 0      | 5.681  | 20.39 FG   | 111.1 C-H |
| IR6861-17-15-11-44 | HSB-134-11-44 | 3.03   | 8.73   | 12.73 G    | 116.3 C-H |
| IR6861-17-15-11-42 | SHB-134-11-42 | 2.646  | 4.884  | 22.66 E-G  | 122.8 C-H |
| IRGC77301     | RNP74229     | 0.813  | 6.389  | 22.29 E-G  | 125.6 C-H |
| RTP11793      | BR 3         | 2.464  | 7.898  | 21.48 E-G  | 139.1 C-H |
| IRGC40020     | LANKANDA 41  | 3.171  | 6.624  | 24.89 C-G  | 144.6 B-H |
| IRGC47111     | RAJESHWARI   | 0      | 5.808  | 33.09 B-G  | 156.5 B-H |
| IRGC117278    | SWARNA       | 1.235  | 10.345 | 23.72 D-G  | 159.7 B-H |
| IRGC59244     | TARA         | 1.235  | 11.765 | 28.37 B-G  | 186.0 B-H |
| IRGC53200     | MATAHAMBRE   | 3.889  | 13.041 | 40.52 B-G  | 246.7 B-H |
| IRGC6264      | N-22         | 1.389  | 16.186 | 41.86 B-G  | 264.7 B-G |
| IRGC77471     | YUEFU        | 5.635  | 15.588 | 40.92 B-G  | 272.1 B-F |
| IRGC116438    | AZ(C)        | 0      | 16.667 | 44.74 B-G  | 273.3 B-F |
| IRGC117398    | VANDANA      | 2.564  | 9.804  | 63.09 A-D  | 298.4 B-E |
| IRGC4819      | N-22         | 4.312  | 9.283  | 63.72 A-C  | 303.1 B-D |
| IRGC74540     | TOMOE MOCH   | 3.935  | 11.997 | 61.11 A-E  | 311.6 BC  |
| IRGC19379     | N 22         | 0      | 16.987 | 56.02 A-F  | 315.0 BC  |
| IRGC62172     | KAMMRA       | 0      | 4.762  | 90.20 A    | 349.0 AB  |
| IRGC82775     | IAC 165      | 6.278  | 16.84  | 67.42 AB   | 515.8 A   |
| LSD$_{null}$  |             | NS     | NS     | 33.47      | 172.3     |

$^1$ Values followed by different letters are significantly different at (P = 0.05)

$^2$ Values followed by different letters within the column are significantly different at (P = 0.05)

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