Available Online at eSci Journals
eSci Journal of Plant Pathology
ISSN: 2305-106X (Online), 2306-1650 (Print)
http://www.escijournals.net/EJPP

PHYSIOLOGICAL RACES AND VIRULENCE DIVERSITY OF *PUCCINIA GRAMINIS* PERS. F. SP. *TRITICI* ERIKS. & E. HENN. ON WHEAT IN TIGRAY REGION OF ETHIOPIA

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A B S T R A C T

Wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* is a disease that causes complete annihilation of wheat crops over wide areas during epidemic years. The highland of Ethiopia is considered as a hot spot area for the development of stem rust complex. Hence, this study was carried out to detect the virulence diversity of *P. graminis* f. sp. *tritici* in Southern Tigray. The findings of this paper were based on race analysis through inoculation of stem rust populations, isolation and multiplication of single-pustule of the pathogen and race determination by inoculating on stem rust differential hosts. The phenotypic characterization of *P. graminis* f. sp. *tritici* resulted in identification of 20 races from 32 isolates, which included the most prevalent races TTSNK, RRJJC and HRJJC with a frequency of 9.4% each and the most virulent races TTKSK and TTSSK each making 85% of Sr genes ineffective. Three important races (TTSSK, TTSNK and RRTTF) are new to the study area and the country (Ethiopia) as a whole putting a significant wheat proportion at risk. Among 20 wheat stem rust differential hosts, four were found effective for 75% and more of the races identified. Differential host carrying Sr24 was effective to all, while gene SrTmp was effective to 90% of the races followed by Sr17 and Sr31 each effective for 75%. In contrast, differential hosts carrying SrMcN, Sr9b, Sr9g and Sr10 were ineffective to 96.9, 93.8, 87.5 and 81.2% of the isolates tested, respectively. Thus, use of effective Sr genes such as Sr24 and SrTmp in single cultivar through gene pyramiding has paramount importance as the additive effects of several genes gives the cultivar a wider base stem rust resistance along with periodic race survey.

Keywords: Wheat stem rust, *Puccinia graminis* f. sp*. tritici*, race analysis, virulence diversity.

INTRODUCTION

Wheat (*Triticum* spp) is among the most important crops grown in Ethiopia. It is among the cereal crops that contribute significantly to food security in the country. Wheat ranks second both in terms of volume of production and productivity after maize with the total volume of production of 2.54 million tons at the national level and it ranks third in terms of area coverage with the total area of 1.5 million ha after maize and tef (CSA, 2009). It is produced largely in the South, Central, Northwest and the remaining small amount is produced in the rest of the North and South regions of the country (MoARD, 2009). It is the main staple food for about 36% of the Ethiopian population (CIMMYT, 2005). Wheat in Ethiopia is represented by hexaploid (bread wheat) and tetraploid (durum and emmer wheats) types (Belayneh *et al.,* 2009).

Though the productivity of wheat has increased in the last few years in Ethiopia, it is still very low i.e. about 1.7 t/ha (CSA, 2009). This is by far below the world's average (3.3 t/ha) (FAO, 2007). Over 30 fungal wheat diseases have been identified in Ethiopia, stem rust caused by *P. graminis* f. sp. *tritici* is one of the major production constraints in most wheat growing areas of the country, causing yield losses of up to 100% at times of epidemics (Belayneh and Emebet, 2005). Several epidemics have been recorded in different parts of the country. For instance, in 1975 and subsequent two years stem rust epidemics were reported to occur throughout the country (CIMMYT, 2005). Stem rust received even more attention when the popular bread wheat cultivars Enkoy and Kubsa became susceptible in 1992/93 & 1994, respectively (Temesgen *et al.,* 1996).

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Several other bread wheat cultivars succumbed to this disease and frequent epidemics have been reported (CIMMYT, 2005). The biggest risk to wheat production is the defeat of the long lived resistance gene *Sr31* by physiological race designated Ug99 for the first time after it has been resistant for over 30 years (Singh *et al*., 2008). Generally, studies that were carried out in the country showed that most previously identified races were virulent on most of varieties grown in the country (Belayenh and Embet, 2005; Belayneh *et al.,* 2009) and are among the most virulent in the world (Van Ginkel *et al.,* 1989). Although, investigations on stem rust physiologic race variability & their virulence on *Sr* genes have been going on for several years in Ethiopia, which was not intensive and did not cover all regions like Tigray. Hence, the aim of this study was therefore to examine the virulence diversity of *P. graminis* f. sp*. tritici* in South Tigray.

MATERIALS AND METHODS

Collection of wheat stem rust samples: Samples of infected stems (one sample per field) were collected at 5-10 km interval from wheat fields and trial plots (66 surveyed fields) Southern zone of Tigray (Figure 1). The Southern zone of the region includes five districts viz; Emba-Alaje, Enda-Mekoni, Ofla, (highland areas reached up to 3935masl) Raya-Azebo, and Alamata (mid to lowland areas b/n 1400 to 2200masl). Stems and/or leaf sheath of wheat plants infected with stem rust were cut into small pieces of 5-10 cm in length using scissors and placed in paper bags after the leaf sheath was separated from the stem in order to keep stem and/or leaf sheath dry.

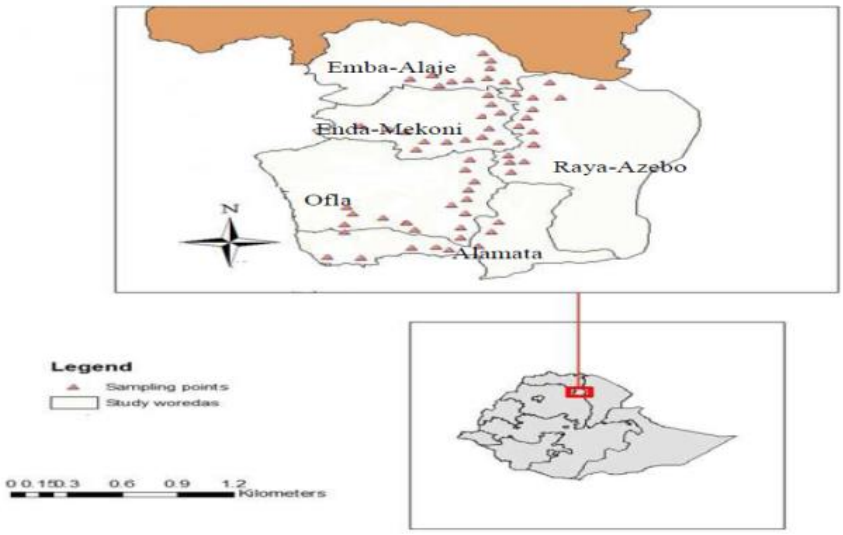

Figure 1: Map of Ethiopia showing wheat stem rust survey districts in Southern zone of Tigray.

This technique helps the samples easily air dry (reduced moisture) so as the spores cannot germinate before processing in the greenhouse. The samples were transported to Ambo Plant Protection Research Center's (APPRC) Laboratory for analysis within two to three days after collection putting with portable refrigerator.

Isolation and multiplication of single-pustules: Seedlings (5-6 seedlings) of the universally rust susceptible variety "Morocco" which does not carry known stem rust resistance genes (Roelfs *et al*., 1982) were raised in suitable 8 cm diameter pots. Leaves of seven-day-old seedlings or seedlings with fully expanded primary leaves and second leaves beginning to grow, were rubbed gently with clean moistened (with sterile distilled water) fingers. By this way the waxy layer that hinders the penetration of the spores were removed from the surface of the leaves. Greenhouse inoculations were done using the methods and procedures developed by Stakman *et al*. (1962). Spores from the stem rust infected sample were scraped off with scalpels on to a watch glass and suspended in distilled water to make rust spore suspension (approximately 3-5 mg of spores per ml of liquid suspension), which was rubbed on the seedlings of Morocco. The plants were then moistened with fine droplets of distilled water produced with an atomizer and placed in an incubation chamber for 18 hours dark at 18-22ºC followed by exposure to light for 3-4 hours to provide condition for infection and seedlings were allowed to dry their dew for about 1-2 hours. Then, the seedlings were transferred from the dew chamber to glass compartments in the greenhouse where conditions was regulated at 12 hours photoperiod, at temperature of 18–25ºC and relative humidity (RH) of 60-70%. The

remaining rust spore samples were kept in the refrigerator at 4°C and were used to substitute for samples which failed to produce infection on the universally susceptible variety in greenhouse. After seven to ten days of inoculation (when the flecks/symptoms was clearly visible) leaves containing a single fleck that produce single pustule was selected from the base of the leaves and the remaining seedlings within the pots were removed. The leaves with single a pustule were separately covered with cellophane bags (145 × 235mm) and tied up at the base with a rubber band to avoid cross contamination (Fetch and Dunsmore, 2004).

After two weeks of inoculation (when the pustule was well developed) spores from each pustule were collected using power operated vacuum aspirator and stored separately in gelatine capsules . A suspension, prepared by mixing urediospores with lightweight mineral oil (Soltrol 130), was inoculated on seven-day-old seedlings of the susceptible variety 'Morocco' for multiplication purpose for each of the single pustules on separate pots. Immediately after inoculation, the seedlings were placed in a humid chamber in dark condition at 18-22ºC for 18 hours and light for 3-4 hours, after which they were transferred to a greenhouse (18-25°C and RH of 60-70%). About 14-15 days after inoculation, the spores of each single pustule were collected in separate test tubes and stored at 4°C until they were inoculated on the standard differential sets. This spore multiplication procedure was repeated until sufficient spores were produced to inoculate the set of stem rust differential hosts.

Inoculation of wheat stem rust differential hosts: Five seeds of the twenty wheat stem rust differentials with known resistance genes (*Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr11*, *Sr31*, *Sr17*, *Sr21*, *Sr30*, *Sr36*, *Sr38*, *Sr24*, *SrTmp*, and *SrMcN*) and one susceptible variety Morocco were grown in 3 cm diameter pots separately in greenhouse. The susceptible variety Morocco (without *Sr* gene) was used to ascertain the viability of spores inoculated to the differential hosts. The single pustule derived spores (approximately 3-5 mg of spores per ml of liquid suspension) was suspended in distilled water and sprayed/inoculated onto seven-day-old seedlings using atomizers and/or an air pump. After inoculation, plants were moistened with fine droplets of distilled water produced with an atomizer and placed in an incubation chamber for 18 hours dark period at 18-22ºC and 3-4 hours of light and seedlings were allowed to remove their dew for about 1-2 hours. Upon removal from the dew chamber, plants were placed in separate glass compartments in a greenhouse to avoid contamination and produce infection. Greenhouse temperature was maintained between 18ºC and 25ºC. Natural day light was supplemented for additional 4 hours/day with 120 μ $E.M^{-2}$ S^{-1} photo synthetically active radiations emitted by cool white fluorescent tubes arranged directly above plants.

Phenotyping differential sets and designation of races: Phenotypc differential sets were based on the reaction of the inoculated urediospores. Infection types (IT) were scored after 14 days using the 0–4 scale of Stakman *et al.* (1962). Infection types were categorized as either being 'Low' (incompatible or resistant; ITs of 0, 0;, 1, 1+, 2 and 2+) or 'High' (compatible or susceptible; ITs of 3-, 3+ & 4). Race designation was done by grouping the differentials into five subsets: (i) *Sr5, Sr21, Sr9e, Sr7b*, (ii) *Sr11, Sr6, Sr8a, Sr9g*, (iii) *Sr36, Sr9b, Sr30, Sr17*, (iv) *Sr9a, Sr9d, Sr10, SrTmp*, (v) *Sr24, Sr31, Sr38* and *SrMcN* (Roelfs and Martens, 1988; Jin *et al*., 2008). The results were based on experiments in two replications.

RESULTS AND DISCUSSION

Virulence and physiologic race composition of *P. graminis* f. sp. *Tritici:* Of 22 rust samples collected from farmers' field and research experimental plots of Southern zone of Tigray in 2010 growing season, six samples did not yield viable spores at the time of inoculation in the laboratory. From 16 field samples from the Sothern zone of Tigray, 32 single pustule-derived isolates (two isolates per sample) were developed using the methods and procedures developed by Stakman *et al*., (1962). Hence, 32 isolates were used for final race analysis. Using the international system of nomenclature for *P. graminis* f. sp. *tritici* (Roelfs and Martens, 1988; Jin *et al*., 2008), 20 races were identified based on their reaction on 20 differential hosts. Most of the single pustules from individual fields varied in their race groups, and only some belonged to the same race group. This indicated a high level of variation both in quantity and virulence spectrum. The highest level of race variation was detected from Raya-Azebo district accounting for 65% of the races identified. Out of the total 20 races, 13 were identified from 22 isolates in this particular district. Likewise, the worldwide important races like RRTTF, TTSNK, TTKSK and TTSSK were detected from Raya-Azebo district on variety Dashen making wheat production jeopardy in the area. Not only the number

of races detected in this particular district was high, but also the virulence spectra of the races had broadest than other districts. The majority of the wheat fields assessed in Raya-Azebo have lower to mid elevation (1500-2300m.a.s.l) as compared to the elevation in the other districts. Therefore, this elevation range had warmer climate, which is suitable for the development of stem rust. This epidemiological zone could be probably creating conducive environment for the development and diversity of stem rust races (Roelfs *et al*., 1992).

The remaining 35% of the races were detected from Raya-Alamata, Ofla and Enda-Mekoni districts, individually contributes three, two and two races from four, two and four single pustules, in that order (Table 1). In these districts, large of samples were collected but most of the samples were unable to germinate in the greenhouse race analysis.

Table 1: Prevalence of races of *Puccinia graminis* f. sp. *tritici* from isolates obtained in districts of southern Tigray.

District	Race	Isolate	Remark
Raya-Alamata	BBBBC, HHSTF and JRGSC	4	-
Raya-Azebo	BBBLC, BHJBC, CCGBC, GMHJC, HRJJC, JTGDB, RRTTF, SKQNH, SPSSF, TCQJH , TTKSK, TTSNK and TTSSK	22	-
Ofla	DBHQC and DBHSC	2	-
Enda-Mekoni	GKJSF and RRJJC	4	-
Emba-Alaje	-	-	No viable spore

The frequency of each race was calculated as a percentage from the total number of isolates analyzed. Of the 20 races, the most frequent and predominant races identified were TTSNK, RRJJC and HRJJC with a frequency of 9.4% each. The second most frequent and dominant races were BHJBC, GMHSC, HHSTF, RRTTF, SPSSF, and SKGNH, with a frequency of 6.3% each. On the other hand, the remaining 11 including the worldwide most important races such as TTKSK and TTSSK were detected all only once each with frequency of 3.1% (Table 2).

The 20 races identified from wheat grown areas in Southern Tigray zone had wide virulence spectra (Table 2). The broadest virulence spectra were recorded for races TTKSK and TTSSK making 17 stem rust resistance genes ineffective. The most devastating stem rust race TTKSK (commonly known as Ug99) virulence on gene *Sr31* was first detected in Uganda in 1999 (Pretorius *et al*., 2000), and had spread to most of the wheat growing areas of Kenya in 2002 and Ethiopia in 2003. In 2005, Ethiopian reports confirmed its presence in six dispersed locations (Singh *et al*., 2008), and was spread to most wheat growing regions of the country and is becoming the main threat of wheat production (Belayneh *et al*., 2009). The present study also detected the race at additional one location, indicating the race is getting spreads in the region. TTKSK (Ug99) was virulent to 17 *Sr* genes except *Sr36, Sr24,* and *SrTmp*. Furthermore, the new Ug99 variant TTSSK, which is identified in this study, was also detected in Kenya in 2006 and 2007 with virulence to gene *Sr36* indicating Ug99 is evolving (Singh *et al*., 2008). This race was virulent to all the resistance genes except *Sr17, SrTmp* and *Sr24.* In the same way, TTSNK and RRTTF were equally virulent to 80% of the stem rust resistance genes. Except Ug99, the three important races (TTSSK, TTSNK and RRTTF) were new to the study area specifically and the country (Ethiopia) as a whole. These virulent races could be important challenges for wheat production in the country and the rest of the world as pathogen is rapidly migrating and evolving. On the other hand, eight races or 40% of the races identified were virulent on less than 50% of the 20 *Sr* genes included in the test. Race BBBBC was the least virulent, producing susceptible reaction on only monogenic gene, *SrMcN*. Races such as BBBLC, CCGBC, BHJBC, DBHQC and DBHSC were also the least virulent, producing susceptible reactions on only two, four, five, six, and seven wheat differential hosts, respectively (Table 2).

In general, the virulence spectrum of the pathogen in this study confirmed the presence of wider range of virulence in the study area. Most of the race composition and virulence diversity of the pathogen in southern zone of Tigray was different from races detected in other parts of Ethiopia. In the same way, previously a considerable number of different virulent races (including Ug99) was detected in Ethiopia (Belayenh and Emebet, 2005; Belayneh *et al*., 2009). A comparison of the races identified in the present study with these earlier reports revealed differences. This could be due to variation over location and time, as the

races prevalent in a specific season and region depend on the type of wheat cultivars grown and to some extent on the predominant environmental conditions, especially temperature (Roelfs *et al*., 1992).

Most of the races in Ethiopia varied from one another by single-gene changes (Belayneh *et al*., 2009). In this study, 8 races or 40% of the races identified varied by single-gene changes. For instance, race TTSSK was similar to TTSNK with additional virulence to *Sr9d*. In the same way, races BBBLC, DBHSC, and RRJJC were similar to BBBBC, DBHQC, and HRJJC with additional virulence to *Sr9a, Sr10* and *Sr5*, following the same order mentioned (Table 3). Such single-step changes in virulence were reported to be the main process of evolutionary change in *P. graminis* f. sp. *tritici* populations (Green, 1975; Belayneh *et al*., 2009).

Table 2: Virulence spectrum (based on ineffective Sr genes) of races of *Puccinia graminis* f. sp. *tritici* from southern Tigray collected in 2010.

Race	Virulence spectrum (ineffective *Sr* genes)	No. of isolates	Frequency (%)
BBBBC	*McN*	1	3.1
BBBLC	*9a, McN*	1	3.1
BHJBC	*6, 9g, 9b, 30, McN*	2	6.3
CCGBC	*7b, 9g, 9b, McN*	1	3.1
DBHQC	*9e, 9b, 17, 9a, 9d, McN*	1	3.1
DBHSC	*9e, 9b, 17, 9a, 10, 9d, McN*	1	3.1
GKJSF	*21, 6, 8a, 9g, 9b, 30, 9a, 9d, 10, 38, McN*	1	3.1
GMHJC	*21, 11, 6, 9g, 9b, 17, 9d, 10, McN*	2	6.3
HHSTF	*21, 7b, 6, 9g, 36, 9b, 30, 9a, 9d, 10, Tmp, 38, McN*	2	6.3
HRJJC	*21, 7b, 11, 6, 9g, 9b, 30, 9d, 10, McN*	3	9.4
JRGSC	*21, 9e, 11, 6, 9g, 9b, 9a, 9d, 10, McN*	1	3.1
JTGDB	*21, 9e, 11, 6, 8a, 9g, 9b, 10,*	1	3.1
RRJJC	*5, 21, 7b, 11, 6, 9g, 9b, 30, 9d, 10, McN*	3	9.4
RRTTF	*5, 21, 7b, 11, 6, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN*	2	6.3
SKQNH	*5, 21, 9e, 6, 8a, 9g, 36, 9b, 9a, 10, 31, McN*	2	6.3
SPSSF	*5, 21, 9e, 11, 8a, 9g, 36, 9b, 30, 9a, 9d, 10, 38, McN*	2	6.3
TCQJH	*5, 21, 9e, 7b, 9g, 36, 9b, 9d, 10, 31, McN*	1	3.1
TTKSK	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 31, 38, McN*	1	3.1
TTSNK	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 36, 30, 9a, 10, 31, 38, McN*	3	9.4
TTSSK	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 36, 30, 9a, 9d, 10, 31, 38, McN*	1	3.1
Total		32	100

Virulence frequency of *P. graminis* f. sp. *tritici* isolates to *Sr* resistant genes: It was evident that the majority of the resistance genes were found ineffective against most of the isolates tested in this study. About 55% of the *Sr* genes were ineffective to more than 60% of the isolates. The differential host carrying the resistance gene McNair 701 (*SrMcN*) was ineffective to 96.9% of the isolates tested. Similarly, six differential hosts carrying resistance genes *Sr9d, Sr21, Sr6, Sr10, Sr9g* and *Sr9b* were ineffective, with virulence frequency of 65.6, 78.1, 75, 81.2, 87.5 and 93.8% to the isolates tested, in that order (Table 3). Belayneh *et al*., (2009), reported similar finding, McNair 701 (*SrMcN*) was susceptible to all of the races identified. In the same way, according to the same authors seven stem rust resistance genes; *Sr9a, Sr9g, Sr10, Sr7b, Sr9b, Sr9d* and *Sr8b* were ineffective for more than 96% of the isolates collected during 2006-2007 cropping season from Shewa, Arsi, Bale and northwest regions of Ethiopia. Earlier studies indicated that virulence to *Sr6, Sr8b, Sr9a, Sr9d* and *Sr11* is common worldwide (Roelfs *et. al.,* 1992).

On the other hand, the stem rust resistance gene *Sr24* was found effective to all stem rust isolates collected from Southern Tigray region. This confirms the report of Roelfs *et al*., (1992), that stated this gene is amongst the effective genes, which have an adequate and some immediate values to almost all races in the world. But, virulence to *Sr24* was reported in Kenya in 2006. A variant of Ug99 that added virulence on stem rust gene

Sr24 (Ug99+*Sr24* virulence, called TTKST) has further increased the vulnerability of wheat to stem rust worldwide (Jin *et al*., 2008). Furthermore, the most important gene *Sr24* was also defeated by another race PTKST which is detected in Ethiopia in 2007, Kenya and South Africa in 2009. This represents the first confirmed occurrence of Ug99 variant with virulence to *Sr24* in Ethiopia (FAO, 2011).

Five resistance genes, *SrTmp, Sr17, Sr31, Sr36* and *Sr38* were found to be effective against most of the stem rust races detected. Of these *Sr* genes, differential hosts carrying *SrTmp Sr31* and *Sr17* were was resistant to 87.5, 75 and 78.1% of the isolates tested, respectively. Correspondingly, gene *Sr38* was effective for about 62.5% of the isolates analyzed in this year followed by *Sr36* which was effective against 59.4% (Table 4). Belayneh *et al*., (2009) reported similar finding that *Sr36* and *SrTmp* were effective for 81.6 and 76.3% of the isolates, respectively for the samples collected from Shewa, Arsi, Bale and northwest regions of Ethiopia.

Table 3: Virulence frequency of isolates of *Puccinia graminis* f. sp. *tritici* from southern Tigray on 20 individual Sr genes

***Sr* gene**	**Virulence frequency (%)**	***Sr* gene**	**Virulence frequency (%)**
5	46.9	*30*	62.5
21	78.1	*17*	21.9
9e	43.8	*9a*	56.25
7b	53.1	*9d*	75.0
11	59.4	*10*	81.5
6	75.0	*Tmp*	12.5
8a	31.3	*24*	0.0
9g	87.5	*31*	25
36	40.6	*38*	37.5
9b	93.8	*McN*	96.9

CONCLUSION

Wheat stem rust is the most important disease of wheat in Ethiopia. The pathogen is able to produce new races that can attack previously resistant varieties and develop rapidly under optimal environmental conditions which results in a serious yield loss. Hence, monitoring the disease and its races is of great importance for sustainable wheat management programs. The identification of 20 races from 32 isolates is a clear indication of the presence of high virulence diversity of the pathogen in the region. The worldwide important races TTSNK, RRTTF, TTSSK (new races) and TTKSK are amongst the most important races that were identified in the region. Differential hosts carrying *Sr24* and *SrTmp*, which confers resistance to most of the races. Thus, use of effective *Sr* genes such as *Sr24* and *SrTmp* in single cultivar through gene pyramiding (breeding) has paramount importance as the additive effects of several genes offer the cultivar a wider base stem rust resistance along with periodic race survey.

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