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Virulence Spectrum of Wheat Stem Rust (*Puccinia graminis* f.sp.*tritici*) in the Eastern Showa of Central Ethiopia

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Abstract

Wheat stem rust caused by *Puccinia graminis* f.sp. *tritici* is amongst the biotic factors which causes up to 100% yield loss during epidemic years. Ethiopia is considered as a hot spot area for the development of stem rust complex; and hence, this study was carried out to investigate the distribution and intensity of wheat stem rust, and to detect the virulence spectrum of *P. graminis* f.sp. *tritici*. The survey result showed that 30 (38.9%) of the fields were affected with stem rust. The overall incidence of the disease was 5.2% and the mean severity of the disease was below 5%. The lowest incidences and severity were noted at Gimbichu while the highest were at Adeaa District. *P. graminis* f. sp. *tritici* variability study was resulted in the identification of 16 races of which race SRKSF was the most prevalent with a frequency of 14.3% followed by the races such as TTKSK and TTTTF with frequencies of 7.1% and 3.6%, respectively. Differential host carrying *Sr24* was effective to all races considered, while gene *Sr36* gene was resistant to 85.7% of the races. These two genes could be used in resistance breeding program of wheat.

Keywords: Stem rust; *Puccinia graminis* F.Sp; Tritici; Differentials; Race; Sr Gene

Introduction

Wheat (*Triticum aestvium* L.) is among the major cereal crops cultivated in Ethiopia. Ethiopia is the second largest producer of wheat in sub-Saharan Africa [1]. The crop has considered as the main staple food of Ethiopian population particularly in highlands of the country [2], where it has produced in a large volume and 95% of the total production is produced by small scale farmers. Currently it ranks second both in terms of volume of production and productivity after Maize and third in terms of area coverage after Maize and Tef [3]. The production and productivity of wheat in Ethiopia has increased in the last decades, though the national average yield has not exceeded 21.25 tons/ha [4]. This is by far below the world's average yield/ha which is about 33.3 tones/ha [5]. This low yield is attributed to multi-faced abiotic and biotic factors such as cultivation of unimproved low yielding varieties, low and uneven distribution of rainfall, poor agronomic practices, insect pests and serious disease like rusts [6].

Rust fungal pathogens are among the major stresses that cause high yield losses in wheat crop. Over 30 fungal wheat diseases are identified in Ethiopia, stem rust caused by *Puccinia 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% during epidemic years [7]. Several hot spot area exists worldwide where stem rust has been more severe [8]. Nevertheless, as a result of the world wide reduction of major epidemics of stem rust, the disease has largely been under control worldwide except in East Africa where it has still a major problem. As the result of epidemics, the world wide important Sr genes such as Sr29 and Sr30 [9] has been defeated by virulent stem rust races developed in Ethiopia [10] and the long lived resistant genes of wheat such as Sr31 has been defeated for the first time in Eastern Africa after it has been resistant for over 30 years [11,12]. These area might be estimated for 50 million hectare of wheat grown globally i.e. about 25% of the world's wheat area [13] and germplasm with resistance to Ug 99 is available for many parts of the world [14].

The high lands of Ethiopia are considered to be one of the hot spot areas where an estimated loss due to stem rust of wheat ranges from 40% in endemic areas to 100% where epidemics occur on susceptible varieties [15]. Recent studies in the country showed that most previously identified races were virulent on most of varieties grown in the country [10,16]. There is potential danger of resistance breakdown in Ethiopian released Bread, Durum and Emmer wheat varieties [10]. According to Naod, [10] comparison of Durum and Emmer Wheat, Durum wheat is less resistant to stem rust races than emmer wheat. Stem rust races prevalent in the central highlands of Ethiopia are among the most virulent in the world [17]. This study was, therefore, carried out to investigate the distribution and intensity of wheat stem rust, and to detect the virulence spectrum of *P. graminis* f.sp. *tritici* in wheat growing areas of Eastern Showa of Central Ethiopia.

Materials and Methods

Survey of wheat stem rust in wheat growing areas of eastern showa zone of central ethiopia

In the main growing season (June to October) of 2009, stem rust (*P. graminis* f.sp. *tritici)* survey was carried out in the major durum and bread wheat growing areas of Eastern Showa zone of Central Ethiopia (Figure 1) The survey routes/areas were Adeaa, Akaki, Gimbichu, Lomea and Liben Districts. In each survey route, peasant associations that were located along the main and accessible road sides were selected at five kilometer intervals. 15 Durum wheat fields which were grown in the altitude range of 1856-2139 m.a.s.l and 62 bread wheat in the altitude range of 1856-2436 m.a.s.l., a total of 77 wheat fields in

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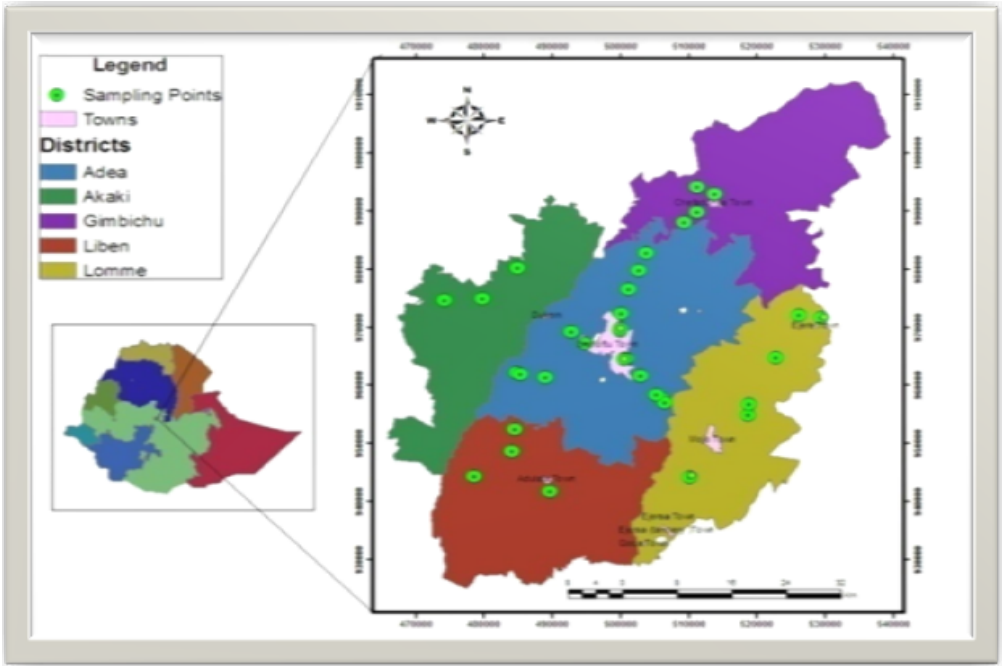

Figure 1: Map of the survey area.

the area were surveyed after heading growth stage of the crop (early October to mid October, 2009). During which stem rust disease was at its maximum severity level [18].

Rust assessments were made along the two diagonals of the field at five points using 0.5 m^2 quadrants. In each farm, plants within the quadrant were counted and recorded as diseased and healthy. The incidence of wheat stem rust was calculated by using the number of infected plants and expressed as a percentage of the total number of plants assessed. The severity of disease was examined as the percentage of plant part (tissue) affected, by observing visually on the whole plants within the quadrants and was recorded using scoring scale of rust disease as described by Peterson et al. [19]. The prevalence was also expressed as percentage of diseased fields of the total fields inspected. In addition, for each surveyed wheat field, supplementary information was collected using the survey report format which includes place of collection, variety, plant growth stages as well as description of the field (altitude and grid references). The prevalence, incidence and severity data were analyzed by using the means over districts and altitude range.

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Collection of stem rust samples, isolation of single-pustule and multiplication

To determine the range of variability within wheat stem rusts and to detect virulent race, stem rust samples were collected mainly from subsistence wheat farms of Eastern Showa, trial plots and trap nurseries of Debrezeit Agricultural Research Center (DZARC) and Dembi substation of DZARC. Stems of wheat plants infected with stem rust were cut into small pieces of 5-10 cm and placed in paper bags. The samples collected in the paper bags were labeled with the name of the zone, District, and variety, GPS (altitude, latitude and longitude) data and date of collection and then transported to Ambo Plant Protection Research Center's Laboratory.

Steam sterilized 2:1:1 soil, sand and manure mixture were filled in suitable 10 cm diameter clay pots and 6 seedlings of the universally rust susceptible variety "Morocco" were raised in each pot. When the primary leaf was fully expanded (it took about a week) the leaves were rubbed gently with clean moistened fingers to remove the waxy layer from the surface and, part of the stem rust infected sample was scrubbed with scalpels on a watch glass and suspended in distilled water to make rust spore suspension, and then it was rubbed on the susceptible seedlings of Morocco. The plants were then moistened with

Actual percentage	visual percentage
0.37	1
1.87	5
3.7	10
7.4	20
11.1	30
14.8	40
18.5	50
22.2	60
25.9	70
29.6	80
33.3	90
37.0	100

Table 1: The actual percent and visual percentage through considering combination of pustule size and distribution of severity of rust disease [19].

fine droplets of distilled water produced with an atomizer and placed in an incubation/dew chamber for 18 hr dark period at 18-22°C and 3 hr of light to provide condition for infection. Thereafter, the seedlings were transferred from dew chamber to glass compartments/growth chamber in the greenhouse where conditions were regulated at 12 hr photoperiod, at temperature of 18-25°C and RH of 60-70%. The remaining of the rust samples were kept in the refrigerator at 4°C and were used to substitute samples which failed to produce infection on the universally susceptible variety Morocco in the greenhouse. Two weeks later, leaves containing two isolated single pustules from each location were collected separately, and multiplied on the variety Morocco. Pots containing inoculated seedlings were covered with cellophane bags (145×235 mm) and tied up at the base with a rubber band to avoid cross contamination. Spores from single pustules were collected with test tubes until sufficient amount of spores were produced to inoculate the set of stem rust differential hosts.

Inoculation of wheat differentials and race designation

The seedlings of 20 wheat differential hosts with known stem rust resistance genes and a susceptible variety Morocco were grown in 10 cm diameter pots. Each rust isolate was suspended in distilled water. The suspension was sprayed onto seedlings of the differentials using atomizers. The plants were then moistened with fine droplets of distilled water produced with an atomizer and placed in an incubation/dew chamber for 18 hr dark period at 18-22°C and 3h of light. Upon removal from the dew chamber, plants were placed in separate glass compartments in a greenhouse to avoid contamination and produce infection. Greenhouse temperatures were maintained between 18°C and 25°C Natural day light was supplemented for additional 4 hr/day with 120µ $E.M^2$ S^1 photo synthetically active radiations emitted by cool white fluorescent tubes arranged directly above plants

Data collection and analysis

Races were determined by inoculating urediniospores onto 20 wheat differential lines. The differential wheat lines possessed resistance genes Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr21, Sr24, Sr30 Sr31, Sr36, Sr38, SrTmp, SrMcN and the susceptible control variety Morocco (with no Sr gene) [20].

Infection types were scored 14 days after inoculation using 0 to 4 scoring scales (Table 2). Pathotypes designation were based on the infection phenotypes of the pathogen isolate on the 20 differential wheat hosts where Low (L)=incompatibility (infection phenotype 0, 1 or 2) and High (H)=compatibility (infection phenotype 3 & 4)

Race designation was done by grouping the differential lines into five subsets in the following order: (i) Sr5, Sr21, Sr9e, Sr7b, (ii) Sr11, Sr6, Sr8a, Sr9g, (iii) Sr36, Sr9b, Sr30, Sr17, (iv) Sr9a, Sr9d, Sr10, SrTmp, and (v) Sr31, Sr24, Sr38, SrMcN. Based on its reaction on the differential lines, Each isolate was assigned using a combination of a three letter code of Roelfs and Martens [21] and an additional two letter race code of Jin et al. [22], which finally give a five letter of designation.

Result and Discussion

Survey of wheat stem rust

Eastern Showa is durum and bread wheat growing zone in Central Ethiopia. In view of this, wheat stem rust surveys were carried out during early to mid October 2009 in five districts of Eastern Showa (Table 3). In these Districts, 77 wheat fields between flowering and dough development growth stage were assessed and within this growth stages, the importance of the disease was increasing with mounting in growth stage of the crop, and therefore, the prevalence and intensity of the disease were highest during dough development growth stage, which is in favor of reports of Roelfs et al. [23], they mentioned as the late growth stage of the crop is the important period to reach stem rust disease of wheat at its maximum severity level**.**

Stem rust was observed on 30 (38.9%) of the 77 wheat fields inspected. Of these the disease was prevalent and its intensity was relatively higher in Adeaa and Lomea Districts of Eastern Showa (Table 4). In contrast, stem rust disease was not recorded in any of wheat fields of Gimbichu District, this might be due to lower temperature at higer

Class	it	Description of symptom
Immune	0	No sign of infection to the naked eye
Very resistant	0:	No uredia, but distinct flakes of varying sizes, usually a chlorotic yellow but occasionally necrotic
Resistant	1	Small uredia surrounded by yellow chlorotic and necrotic area
Moderately resistant	2	Small to medium sized uredia, typically in a dark green island surrounded by a chlorotic area
Mesothentic or Heterogeneous	X	A range of infection type from resistant to susceptible scattered randomly on a single leaf caused by a single isolate not a mixture
Moderately Susceptible	3	Medium sized uredia. Usually surrounded by a light green chlorotic
Susceptible	4	Large uredia with limited amount of chlorosis: may be diamond shaped
Modified character		
Lower uredinia	=	Uredia much smaller than than typical and at the lower limit of the infection type
Small uredinia	-	Uredia smaller than normal
Larger uredinia	+	Uredia larger than normal
Largest uredinia	++	Uredia much larger than typical and at the upper limit for the infection type

Table 2: Description of infection types used in classifying the reactions to stem rust on seedling wheat leaves [20].

District	Altitude range (m)	NFI	Mean %		
			P	I	S
Adeaa	1856-2221	23	69.6	13.3	7.7
Akaki	1856-2261	4	25	1.25	0.25
Gimbichu	2281-2436	12	0	0	0
Lomea	1975-2263	20	40	3.3	1.7
Liben	1854-1964	18	27.8	1.1	1.1
Total	**1854-2436**	**77**	**38.9**	**5.2**	**3**

NB: NFI: Number of Fields Inspected; P: Prevalence; I: Incidence; S: Severity

Table 3: Intensity of stem rust in the five Districts of Eastern Showa zone in 2009.

Variety	Altitude range (m)	NFI	Mean %		
			P	I	S
Kubssa	1862-2436	41	46.3	3.7	2.1
Pavon-76	1856-2436	20	15	0.6	o.2
Dariel*	1877	1	100	75	40
Local mixture	2139	1	0	0	0
Ude*a	1856-1975	5	20	2.2	0.2
Yerer*b	1902-1964	5	60	4.2	1.6
Boohie	1941	1	0	0	0
Worer*	1877	1	100	20	10
DWNVT**	1902	2	100	52.5	40

NB: DWNVT: Durum Wheat National Variety Trial, * 1 Varity has taken from research trial plots, ** 2 varieties has taken from research trial plots, a1variety has taken from seed multiplication fields, b2 varieties has taken from seed multiplication fields.The first three varities are bread wheats, while the last 6 are Durum wheats

Table 4: Reaction of wheat varieties to stem rust in Eastern Showa zone in 2009.

altitude, above 2300 m, where stem rust disease is not a threat to wheat crop [24].

The prevalence and intensity of stem rust under small scale farmers' field of durum wheat growers were negligible, but it was significant in seed multiplication and trial plots of Dembi research substation and DZARC. Similar survey result was also reported by Worku, [25]. Moreover, the survey results of this study supports the reports of Mengistu and Yeshi, [26] they mentioned as the large proportion of Ethiopian durum wheat accessions are resistant or moderately resistant to stem rust [27].

Using Fetch and Dunsmore, [28] method of designation, Belayneh et al., [16] was identified the virulent races TTTTR and TTKSR from samples collected during 2006 and 2007 cropping season in Central Ethiopia, which were more closely related with races TTTTF and TTKSK, identified in this study. According to Belayneh et al., [16], race TTKSR was virulent on the wheat differential lines carrying the resistance genes Sr31 and Sr38, and it was the world wide feared new strain of stem rust, Ug99. This race was widely distributed in the country and the predominant race with frequencies of 18.5%, 30.8% and 45.4% in Showa, Arsi and Bale regions of Ethiopia, respectively. In the same way, the more closely related race which defeats the wheat differential lines carrying the resistance genes Sr31 and Sr38 (TTKSK) is the second most important race in this study with a frequency of 7.1%. This might be due to the aggressiveness of the race on the commercial varieties grown in the area. This worldwide feared new strain of stem rust, which appeared first in Uganda in 1998 [29], it was subsequently detected in Kenya in 2002 and Ethiopia in 2003, in Sudan and Yemen in 2006, and in Iran in 2007 [30] and is becoming the main threat of wheat production in Ethiopia and therefore, it needs special attention before it causes great loss in the commercial varieties grown in the country.

The virulence spectrum of the races was variable (Table 5). About 63% of the races identified showed virulence in the range of 55% of the Sr genes for races DRJTC and JKJSC to 90% for race TTTTF. The remaining 37% of the races were virulent on less than 50% of the 20 Sr genes included in the test. In this study, race SRKSF defeated 70% of the Sr genes in the wheat differential lines including Sr 30 which were not defeated by the most prevalent race TTR identified from isolates of Debrezeit during 2004 cropping season using a three letter designation of Roelfs & Martens, [21] Belayenh and Embet, [7].

Based on the twenty differential lines, the race TTTTF was virulent to all differential lines except Sr24 and Sr31. Races TTKSK and PTKTK were virulent to all differential lines except Sr24, Sr36, SrTmp and Sr21, Sr24, Sr36, respectively. The virulence pattern observed in this study confirmed the presence of wider range of virulence in Ethiopian races as reported by Belayneh A, Naod B, Worku Denbel [7,10,16,26].

Virulence to Sr resistance genes

More than 60% of the Sr genes were ineffective to more than 50% of the races identified in this study. The differential hosts carrying the resistance gene McNair (Sr McN) was ineffective to all isolates tested (Table 6). Similar findings were reported by Belayneh et al. [16]. Four differential hosts carrying resistance genes Sr9g, Sr10, Sr11, and Sr30 were ineffective to more than 75% of races identified. Sr7b which was ineffective for 92.6% of races identified from Showa regions of Ethiopia in the previous findings of Belayneh et al. [16] was effective for more than 50% of races isolated in this study. This might be for

Race	Virulence spectrum (ineffective Sr resistance genes)	Frequency (%)
BLFFK	10,11,30,31,38,Tmp,17,McN	7.1
BMFJK	9d,9g,10,11,30,31,38,17,McN	7.1
DKJJC	6,8a,9b,9d,9e,9g,10,30,McN	3.6
DRJTC	6,9a,9b,9d,9e,9g,10,11,30, Tmp,McN	7.1
JHHNC	6,9a,9b,9e,9g,10,21,17,McN	3.6
JKJSC	6,8a,9a,9b,9d,9e,9g,10,21,30,McN	3,6
KCGSC	7b,9a,9b,9d,9e,9g,10,21,McN	3.6
KRJSC	6,7b,9a,9b,9d,9e,9g,10,11,21,30,McN	7.1
PTKTK	5,6,7b,8a,9a,9b,9d,9e,9g, 10,11,30,31, 38,Tmp, 17, McN	7.1
PTQTH	5,6,7b,8a,9a,9b,9d,9e,9g,10,11,31,36,Tmp.McN	7.1
RTSTH	5,7b,8a,9a,9b,9d,9g,10,11,21,30,31,36,Tmp.McN	3,6
SCHNC	5,9a,9b,9e,10,21,17,McN	7.1
SRKSF	5,6,9a,9b,9d,9e,9g,10,11,21,30,38,17,McN	14.3
TRJSC	5,6,9a,9b,9d,9e,9g,10,11,21,30,McN	7.1
TTKSK	5,6,7b.8a,9a,9b,9d,9e,9g,10,11,21,30,31,38,17,McN	7.1
TTTTF	5,6,7b,8a,9a,9b,9d,9e,9g,10,11,21,30,36,38,Tmp, 17,McN	3.6

Table 5: Virulence of *P. graminis* f.sp *tritici* collected from Eastern Showa, Central Ethiopia in 2009 growing season.

Differential hosts	Sr genes	Frequency (%))
LcSr24Ag	24	100
W2691SrTt-1	36	85.7
ISr7b-Ra	7b	64.3
ISr8a-Ra	8a	64.3
CnSSrTmp	Tmp	64.3
Sr31(Benno)/6*LMPG	31	60.7
T.mono.deriv	21	57.4
RL6081	38	53.6
ISr9a-Ra	9a	46.4
ISr9d-Ra	9d	46.4
LC/Kenya Hunter	17	46.4
ISr5-Ra	5	42.9
ISr6-Ra	6	42.9
W2691Sr9b	9b	42.9
Vernstein	9e	42.9
W2691Sr10	10	25
BtSr30Wst	30	21.4
CnsSr9g	9g	17.9
ISr11-Ra	11	17.9
McNair	McN	0

Table 6: Percentage of avirulent *P. graminis* f.sp. *tritici* isolates to the twenty wheat differential hosts.

most of the rust samples used for race analysis were collected from durum wheat varieties and trials rather than from the susceptible bread wheat varieties Kubssa and Galama as in Belayneh et al. [16] and it is evident that the wheat types and varieties in the study area could be considered as a source of resistance for the specific race which defeats the resistance gene Sr7b.

Virulence for Sr5, Sr6, Sr9a, Sr9d, Sr9e, and Sr17 are common worldwide [23]. Likewise, more than 50% of the races identified in this study were virulent to these genes (Table 6). In contrast, Sr 30 which was ineffective for only 38.9% of the races in Showa regions of Ethiopia [16] is ineffective for 75% of races identified this year. Such difference in the virulence distribution over time is common; as the races prevalence in a specific season depends on the predominant environmental condition especially temperature [23] and the type of wheat varieties grown in the season.

Resistance gene Sr24 was found to be effective to the twenty eight isolates analyzed in this study; and hence, this confirms Roelfs et al. [23] and Borlaug (2005), they mentioned as this gene, is amongst the effective genes which have an adequate and some immediate values to almost all races in the world. Although there is a historical damage of Sr36 by the race developed in Ethiopia in the variety Enkoy in 1993/1994 [30], it has found to be effective to 85.7% of the races analyzed this year. Similarly, it was effective to more than 90% of races identified during 2006-2007 cropping season in Showa regions of Ethiopia [16]. This might be for the race type which causes to dispose the variety Enkoy might be changed with the unavailability of that specific susceptible host variety and there is no wider variation in the breakage of the Sr genes for the races of the near past and this year. Therefore, effective genes like Sr24 and Sr36 (Table 6) may be useful for developing resistant varieties for the currently available races in the country.

Conclusion

Stem rust survey was carried out along five routes, in five Districts of Eastern Showa during the main season of 2009 growing season. These were Akaki, Adeaa, Lomea, Liben and Gimbichu. The prevalence and intensity of the disease was variable with location, crop type, variety, altitude range and the growth stage of the crop. The highest and lowest stem rust incidence and severity were observed in Adeaa and Gimbichu Districts respectively. Stem rust samples were taken at altitude ranges of 1856-2436 m.a.s.l. in which the severity of the disease was relatively high at altitude below 2200 m.a.s.l.

The proportion of durum wheat in the survey area was about 12%, which was by far below the bread wheat grown by small scale farmers. Stem rust disease was also relatively lower in durum wheat than bread wheat grown in small scale farms. The highest stem rust intensity was observed after dough development growth stage than at earlier growth stages.

The variability of the pathogen resulted in sixteen races. Of these races, SRKSF was the most prevalent race which accounts for 14.3% of the races identified. Race TTKSK with a frequency of 7.1% and the highly virulent race TTTTF with a proportion of 3.6% were also amongst the important races which were identified in the area.

Differential host carrying Sr24 was an effective gene which confers resistance to 100% of the races identified in the area followed by Sr36 which was lightly affected by the races identified. In contrast, a differerential host carrying SrMcN was ineffective gene to all races identified. Sr9g and Sr11 were ineffective for 82.1% of the races.

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