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Genetic Diversity of Wild Rice (*Oryza longistaminata*) in Ghana

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Abstract
Oryza longistaminata is a wild rice taxon and an excellent source of genetic variation that remains largely unexploited. This study was conducted to understand the genetic diversity between and within *O. longistaminata* populations, collected from different geographic locations in Ghana. In this study, SSR markers were used to determine the intra-specific variability in *O. longistaminata,* and how the variation is partitioned within and between different populations. Analysis of molecular variance revealed high levels of polymorphism (95.9%) within the populations. The overall genetic diversity for all the loci in the six populations was high (Shannon's Information Index $I = 0.579$, Nei's unbiased expected heterozygosity, $He = 0.405$). Hierarchical partitioning also indicated a high genetic diversity between and within populations, with some level of relatedness between samples taken from the Savannah agroecological area. The high genetic diversity observed in this study offer a great opportunity for screening useful traits among the populations of *O. longistaminata* in Ghana for rice breeding programs.

Keywords: Genetic variation, *He, Oryza longistaminata,* SSR markers

Diversité génétique du riz sauvage (*Oryza longistaminata*) au Ghana

Résumé
Oryza longistaminata est un taxon de riz sauvage et une excellente source de variation génétique qui reste largement inexploitée. Cette étude a été menée pour comprendre la diversité génétique entre et au sein des populations *d'O. longistaminata*, recueillies à différents endroits géographiques au Ghana. Dans cette étude, des marqueurs SSR ont été utilisés pour déterminer la variabilité intraspécifique de *l'O. longistaminata* et la façon dont la variation est répartie au sein des différentes populations et entre elles. L'analyse de la variance moléculaire a révélé des niveaux élevés de polymorphisme (95,9 %) au sein des populations. La diversité génétique globale pour tous les loci dans les six populations était élevée (indice d'information de Shannon I = 0,579, hétérozygotie attendue sans biais de Nei, He = 0,405). Le partage hiérarchique indique également une grande diversité génétique entre et au sein des populations, avec un certain niveau de relation entre les échantillons prélevés dans la zone agroécologique de la savane. La grande diversité génétique observée dans le cadre de cette étude offre une excellente occasion de dépister les caractères utiles parmi les populations *d'O. longistaminata* au Ghana pour les programmes de sélection du riz.

Mots-clés: Variation génétique, *Oryza longistaminata, marqueurs* SSR*, He*

Introduction

Rice (*Oryza sativa*) is one of the most important food crops in the world (Mohanty, 2013;Maurer *et al.,* 2018). Increasing world population, especially in the developing countries, is estimated to require about 122 million metric tons of additional rice by 2035 to feed the world (Seck *et al.,* 2012). Worsening climatic conditions and urbanization will mean that rice will be produced with less resources in the future (Wang *et al.,* 2018). This necessitates developing new cultivars that are robust and high yielding to meet the expected demand.

The genus *Oryza* belongs to the family poaceae, and *Oryza* can be classified into about 26 species based on modern classifications (Vaughan *et al.,* 2008). Two species *O. sativa* and *O. glaberrima* are cultivated species, while the remaining 24 are wild species. Some of the wild species have different ploidy levels being diploids or tetraploids (Vaughan *et al.,* 2008;Wambugu *et al.,* 2013). The genus *Oryza* can also be grouped into genomes based on the level of chromosome pairing among their hybrid plants during meiosis. Based on this classification, previous studies have identified ten groups: AA, BB, BBCC, CC, CCDD, EE, FF, GG, HHJJ and HHKK (Ge *et al.,* 1999; Nonomura *et al.,* 2010). The AA genome is of significance to scientists because the two cultivated rice species *O. sativa* and *O. glaberrima* (home in West Africa) as well as six wild relatives belong to the AA genome. All the eight species are diploid species (Vaughan *et al.,* 2003). The West African region, of which Ghana forms a part, has a long history of cultivated rice co-inhabiting with wild rice. The region is of great importance when it comes to the genetic diversity within the *Oryza* genus (Nuijten and Richards, 2013). This is due to the presence of four AA genome species in the region. Wild taxa *O. bartii* and *O. longistaminata* have been growing closer to cultivated species *O. sativa* and *O. glaberrima* for a long time, and has resulted in significant hybridization over time (Wambugu *et al.,* 2015).

Continuous cultivation of a few modern varieties on commercial scale has led to limited diversity and makes these varieties susceptible to biotic and abiotic stressors in the environment (Kottapalli *et al.,* 2010). Broadening of germplasm resources, through exploring the diversity of wild relatives, is important to tackle these problems. The wild relatives of cultivated plants serve as a rich reservoir for studying the evolutionary processes of domesticated crops (Vaughan *et al.,* 2003). Understanding the extent and structure of the genetic variability in wild populations is significant for collecting and conserving wild populations for their efficient use (Kiambi *et al.,* 2005;Nonomura *et al.,* 2010). Fortunately, international conservation of *Oryza* species in the past has paid off. The genus *Oryza* has one of the most extensive collections of ex situ germplasm worldwide(Olufowote *et al.,* 1997).

The wild relatives of cultivated rice are an essential component of the rice gene pool, and have contributed immensely to rice improvement over the years (Vaughan *et al.,* 2008). Resistance traits for biotic and abiotic stressors continue to be discovered in wild population(Wambugu *et al.,* 2013). Researchers have asserted that *O. longist-aminata* has traits that confer resistance to bacterial blight, nematodes, drought tolerance and rhizomatous (Khush *et al.,* 1990;Brar and Khush, 2002;Jena, 2010; Hu *et al.,* 2011). *O. longistaminata* is a great genetic resource that

offers a very good potential for improvement in cultivated rice(Wambugu *et al.,* 2013).

Currently, there is limited knowledge on the inter-and intra-population genetic diversity of *O. longistaminata* in Ghana. The objectives of this study were to investigate the intra-specific variation as well as the intra-populations diversity in *O. longistaminata* populations collected from six locations in Ghana.

Materials and Methods

Plant material and DNA extraction

Six thousand (6,000) *O. longistaminata* individuals' plants were sampled from six different populations in six locations across the two ecological zones of Ghana: the transitional belt and Guinea savannah (Figure 1). One thousand (1,000) *O. longistaminata* individual plants were sampled from each location. Coordinates of sample locations were recorded by a global positioning system (GPS) (Table 1). *O. longistaminata* plants were carefully identified by using their morphological characteristics as described by Vaughan (1994). The genetic structure and diversity of the populations were determined using six highly polymorphic SSR markers selected from 20 SSR markers (Table S1).

DNA extraction was performed by bulking 50 young leaf samples together. In all a total of 6,000 *O. longistaminata* plants were genotyped with 120 pools of 50. The samples of leaves collected for DNA extraction were quickly placed on ice, transported to the laboratory, and stored at -80°C until DNA extraction. Genomic DNA was extracted from frozen leaf tissue of about 200 mg at the CSIR - Crops Research Institute Kumasi, Ghana, as per Egnin *et al.* (1998), and later used for SSR based PCR.

The quality of DNA was determined by aliquoting 1 µl of the extracted DNA, running on a 0.8% agarose gel electrophoresis, stained with 0.05 µg/ml ethidium bromide, and visualized under a UV transilluminator (Voytas, 2000). To determine the purity and concentration of the DNA samples, a Nanodrop D-1000 spectrophotometer was used (Gallagher and Desjardins, 2008). The concentrations of DNA were diluted to 50 ng/µl for SSR marker analysis.

PCR amplification using SSR markers

The diluted DNA samples were amplified in a polymerase chain reaction using six SSR markers, selected from 20 SSR markers, based on their higher polymorphism levels (Table S1) (Chakravarthi and Naravaneni, 2006; Dramé *et al.,* 2013). The sequences of these molecular markers were obtained from Gramene website(Gramene, 2016).
PCR was performed in 25 µl reaction volume.

Table 1: Sample locations across the different geographical location and their GPS coordinates

Sample location	Ecological zone	Longitude (W)	Latitude (N)
Nobewam	Transitional	-1◦26'6"	6°63'3"
Mabang	Transitional	-2◦21'7"	6°98'3"
Nkawie	Transitional	-1◦48'59.99"	6°39'59.99"
Afife	Savannah	.9◦16'4"	6°06'32"
Nyankpala	Savannah	9◦39'65"	0°98'92"
Golinga	Savannah	9◦21'0"	0°57'0"

Table S1: Sequences of 20 SSR primers used, underlined markers were used for the genetic diversity study; numbers in parenthesis represents chromosome number. Supplementary information

Markers	Forward	Reserve	Allele Size Range (bp)	Repeat Motif
RM11 (7)	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	121-147	$(GA)_{17}$
RM223 (8)	GAGTGAGCTTGGGCTGAAAC	GAGGCAAGTCTTGGCACTG	110-180	$(CT)_{25}$
RM234 (7)	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	136-164	$(CT)_{25}$
RM206 (11)	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	110-190	$(GA)_{21}$
RM249 (5)	GGCGTAAAGGTTTTGCATGT	ATGATGCCATGAAGGTCAGC	100 - 160	$(GA)_{14}$
RM 333 (10)	GTACGACTACGAGTGTCACCAA	GTCTTCGCGATCACRCGC	120-190	(TAT)n
RM455	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC		(CTT)n
RM20A (12)	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG	200- 280	$(TAA)_{14}$
RM287	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC		
RM154 (2)	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC	110 - 190	(CTT)n
RM1 (1)	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	67-113	
RM178	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCCTGC		
RM215 (9)	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG	138-152	$(CT)_{16}$
RM284 (8)	ATCTCTGATACTCCATCCATCC	CCTGTACGTTGATCCGAAGC	139 - 159	19
RM252 (4)	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	216	
RM242 (9)	GGCGTAAAGGTTTTGCATGT	ATGATGCCATGAAGGTCAGC	225	26
RM231 (3)	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG	186	16
RM5704 (11)	AGTGCGCTACTACTCGGTCC	GTATCATTTCGTCGAACGGG	184-210	$(AAT)_6$
RM7356 (8)	CCAAGGACACATATGCATGC	GCAATTCATGGCGCTGTTC	158	5(AATT)5AACT
Rm60 (3)	AGTCCCATGTTCCACTTCCG	ATGGCTACTGCCTGTACTAC	165	(AATT)

Each 25 µl reaction volume contained 5 µl of 5× One Taq buffer (New England BioLabs Inc., USA), 0.5 µl of 10 mM DNTPs, 1 µl of 10 mM forward and reverse primer, 0.125 µl of 0.625 units/25 µl Taq polymerase, 17.375 µl of nuclease free water and 1 µl of DNA template. The PCR was carried out in a Gene Touch Thermal Cycler, and was programmed as follows; 94°C for initial 2 minutes, followed by 37 cycles for 30 seconds at 94°C, 30 seconds for 50°C, and an extension time of 2 minutes at 68°C. The amplified DNA products were stained with bromophenol blue and then separated by electrophoresis on 3% agarose gels at a voltage of 100 mv for 30-45 minutes. SYBR® safe gel stain (10,000 ×) (Invitrogen Life Technologies USA) was used to stain the gel and photographed using UV trans illumination.

Data collection

Genotypic data was generated by scoring the amplified DNA of a given locus as different alleles to represent different O. longistaminata samples, based on the molecular weight (bp) of SSR alleles.

Statistical analysis

Genetic diversity and cluster analysis were estimated based on the genetic distances

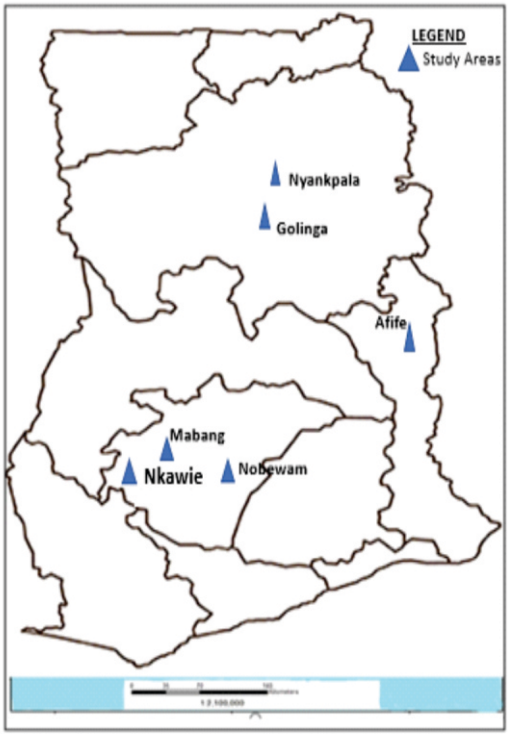

Figure 1. Geographical locations of the six populations in Ghana from which *O. longistaminata* samples were collected.

between the different populations and ecological zones studied (Excoffier *et al.,* 2006) using GenAlEx 6.5 (Peakall and Smouse, 2006). Genetic diversity between the O. longistaminata populations was quantified by calculating Nei's genetic diversity (He) and the molecular analysis of variance (AMOVA).

A dendogram was constructed based on the Unweighted Pair Group Method with Arithmetic mean (UPGMA) at 10% threshold equality. The genetic relationship between the different *O. longistaminata* samples was estimated using the dissimilarity analysis and representation software for windows DARwin version 6.0.19 (Perrier, 2006).

Results

Genetic diversity

The results of this study indicated that the overall genetic variation as revealed by Nei's genetic diversity was high (H_e = 0.405) among the different *O. longistaminata* samples in the six populations (Table 2). The value of within population genetic diversity (H_e) ranged from 0.393 to 0.420 (Table 2). The highest within population genetic variability (H_e = 0.420) was observed in the Golinga population in the savannah agroecological zone, while the lowest diversity (He = 0.393) was recorded in the Nyankpala population in the same agroecological zone.

The AMOVA analysis clearly revealed a high overall within population genetic differentiation in this study, which accounts for 95.90% of shared genetic variation within the populations (P <0.042) (Table 3). The analysis showed a low among population genetic differentiation (4%), and negligible genetic differentiation (0.1%) among the different ecological zones of the wild rice populations (Table 3).

Genetic relationship and cluster analysis

A dendogram was constructed based on the Unweighted Pair Group Method with Arithmetic mean (UPGMA) at 10% threshold equality. This was done using the polymorphisms of 15 SSR loci to reveal the genetic relationship among 30 different *O. longistaminata* samples, randomly taken from the six populations (Figure 2). The results of the cluster analysis did not indicate a clear pattern of grouping based on geographic locations (Figure 2). A 10-group cluster was revealed. The first three clusters were separate groups made up of samples from the Nobewam, Mabang and Nkawie populations, respectively. The fourth cluster consisted of samples from the Afife and Nyankpala.

Table 2: Genetic diversity parameters of the wild rice population

Pop	Na	Ne	I	Ho	He
Nobewam	2	1.649 (0.036)	0.577 (0.015)	0.389 (0.014)	0.389 (0.014)
Mabang	2	1.721 (0.057)	0.597 (0.023)	0.409 (0.021)	0.409 (0.021)
Nkawie	2	1.683 (0.069)	0.573 (0.031)	0.390 (0.027)	0.390 (0.027)
Afife	2	1.655 (0.057)	0.56 (0.025)	0.385 (0.022)	0.385 (0.022)
Nyankpala	2	1.671 (0.070)	0.563 (0.038)	0.383 (0.032)	0.383 (0.032)
Golinga	2	1.709 (0.041)	0.599 (0.016)	0.409 (0.015)	0.409 (0.015)
Total	2	1.68 (0.023)	0.579 (0.010)	0.394 (0.009)	0.394 (0.009)

Number of observed alleles; Ne: number of effective alleles; I: Shannon's Information Index, Ho: observed heterozygosity; He: Nei's unbiased expected heterozygosity. Numbers in parentheses represents standard error of mean.

Table 3: Analysis of molecular variance (AMOVA) of six *O. longistaminata* populations, based on 6 SSR markers

Source	df	SS	MS	Est.Var.	%
Among Regions	1	2.902	2.932	0.007	0.10%
Among Pops	5	32.125	6.425	0.139	4.00%
Within Pops	133	483.750	3.637	3.637	95.90%
Total	139	518.807		3.777	100%

P-value (<0.042) estimates are based on 9999 permutations. df: degree of freedom; SS: sum of squared deviations; MS: mean of squared deviations Var. comp.: variance component estimates; %: percentage of total variation.

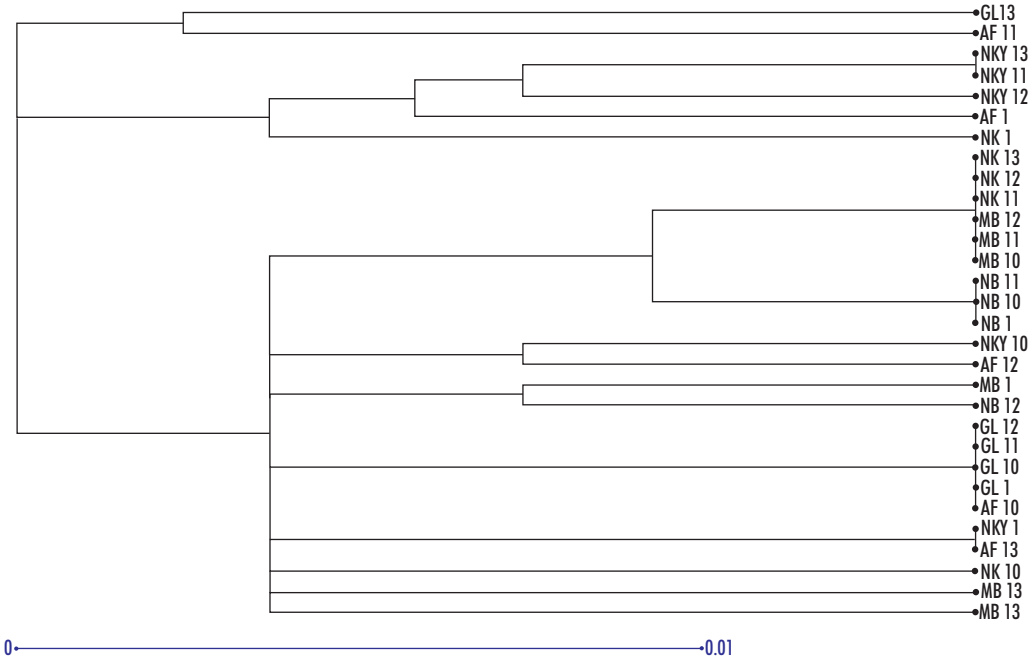

Figure 2: A dendrogram of the genetic relationship among 30 different *O. longistaminata* samples (Each sample is made up of 50 individual plants bulked together) from 6 populations based on 15 SSR loci. Using the UPGMA algorithm method of DARwin Version 6.0.19 (Perrier, 2006). NB: Nobewam population, NK: Nkawie Population, AF: Afife population, MB: Mabang population, GL: Golinga population, NKY: Nyankpala population

Accessions from the Golinga population dominated the fifth group, with four samples, while one sample was from the Afife population. The sixth cluster comprised of samples from Nobewam and Mabang populations, and the seventh group consisted samples from Nyankpala and Afife. The eighth cluster contained the highest number of samples (nine samples). These samples came from three populations, Nkawie, Mabang and Nobewam which are all found in the transitional belt of the agroecological zone. The ninth cluster consisted of 3 populations with samples from Nkawie, Afife and Nyankpala populations. The tenth group contained 2 populations, Golinga and Afife. While Mabang and Nobewam formed separate groups.

Discussion

Understanding the genetic variation and structure among populations of *O. longistaminata* is essential for tapping into the genetic reservoir of *O. longistaminata* germplasm for useful traits utilization.

The results showed a high genetic diversity among the populations of *O. longistaminata* in Ghana. The high genetic diversity among population observed in this study suggest that environmental factors had a major impact in shaping the diversity of *O. longistaminata* in Ghana. The overall genetic diversity recorded in this study (H_e = 0.405) was higher than the average genetic diversity (H_e = 0.302) reported by Kiambi *et al.* (2005). *O. glaberrima* is indigenous to West Africa

(Jusu, 1999) and thus, the higher genetic diversity observed in this study, may be as a result of the long history of rice cultivation in the West African region. In addition, the distribution of different *Oryza* species, both wild and cultivated, and their proximity to each other, in the sub region, make gene flow a possibility (Nuijten *et al.,* 2009; Nuijten and Richards, 2013). The genetic constituent of wild rice taxa populations can be significantly altered by continuous allelic introgression with time, increasing the genetic diversity (Xia *et al.,* 2011; Jiang *et al.,* 2012; Sun *et al.,* 2013). This may account for the high genetic variation seen in the present study. A higher genetic diversity among *O. glaberrima* accessions (H_e = 0.608) was reported by Doku *et al.* (2013) in Ghana. The high genetic variation seen in this study is a potential that needs to be exploited to the fullest capacity to augment rice breeding activities. Having a high genetic variability is a crucial tool to improve crops (Ravi *et al.,* 2003; Wambugu *et al.,* 2013). We also observed from the results that genetic diversity varied among the populations (H_e = 0.393-0.420). The range of genetic variations among the populations seen in this study was low compared to genetic diversity ranges (H_e = 0.145-0.453) and (H_e = 0.37-0.69) reported by Xia *et al.* (2011) and He *et al.* (2014), respectively. Our present result suggests that genetic diversity among *O. longistaminata* in Ghana are to some extent, fairly distributed among the populations. However, this observation is different from the findings of Song *et al.* (2015) and Wang *et al.* (2019) who reported that genetic diversity was not fairly distributed across different locations in China. The findings of the present study may be as a result of the slight latitudinal differences among where the populations were collected, compared to the large area of coverage considered in the previous studies (Vik *et al.,* 2010). The AMOVA analysis further supports these findings, as genetic diversity was strongly distributed within the populations (95.9%) rather than among the population (4 %), and the ecological zones (0.1%) (Table 3). Similar observation was made in Italy among weedy rice species by Jiang *et al.* (2012) but the among population variation recorded in that study was higher (25%) than the present study. Generally, one of the main factors that cause genetic differentiation within populations is outcrossing (Parsons *et al.,* 1999; Xia *et al.,* 2011). The high within population diversity seen in the present study may arise from a substantial level of gene flow, a significant factor of low differentiation among populations (Slatkin, 1987; He *et al.,* 2014; Wang *et al.,* 2019).

The results of the cluster analysis in this study showed a wide genetic variation among *O. longistaminata* samples throughout the populations in Ghana. The results of cluster analysis are in congruous with the results of the Nei's genetic diversity, as evidenced by the number of branches observed which is an indication of higher genetic diversity. The first three clusters formed separate groups (Figure 2). These show how distinct these samples are from each other, indicating a higher genetic variation (Kiambi *et al.,* 2005).

The results showed some level of affinity between the Afife and Golinga populations, and the Afife and Nyankpala populations, to some extent. This is noteworthy since the Afife, Nyankpala and Golinga populations are all in the savanna agroecological zone. This result suggests that, agroecological zone may have played a role in the relatedness of the samples in the study populations. However, the agroecological zone did not significantly affect the genetic diversity in this study. The present study agrees with earlier research that suggests that environmental differences play a role in shaping the genetic distinctiveness of *O. longistaminata* populations (Kiambi *et al.,* 2005; Wambugu *et al.,* 2013).

Conclusion

In summary, the genetic indicators estimated from the SSR data have shown that there is a high level of genetic variation in the *O. longistaminata* populations in Ghana. We also found that the diversity within populations is higher than between populations. The high genetic diversity of *O. longistaminata* can be exploited for rice breeding activities in West Africa. Conservation strategies should be put in place in Ghana to preserve and make readily available *O. longistaminata* germplasm for crop improvement.

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