RESEARCH ARTICLE

Genetic diversity analysis of wheat and triticale using transferable simple sequence repeat markers of barley

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ABSTRACT
Simple sequence repeat (SSR) markers are powerful tools for the determination ofrelationships between genotypes and genetic diversity because of advantages such as high polymorphism, codominance nature, multiallelism and distribution on encoding and non-encoding regions, This study investigated the transferability and polymorphism of 102 barley SSR markers in 41 bread and durum wheat and triticale genotypes. A total of 50 pair primers were transferable, and 40 pair primers producing 118 alleles (on average 2.95 alleles per locus) were polymorphic. The Polymorphism Information Content and genetic diversity for these markers were estimated to be 29% and 33%, respectively. The average of Shannon and Nei indices for the studied genotypes were 34% and 23%, respectively. Cluster analysis, using a Minimum Evolution algorithm and P-distance coefficient, assigned the genotypes into five groups. In principal coordinates analysis, the first principal component explained 74.69% of the total molecular variation. Analysis of molecular variance based on the genome structure of the genotypes using molecular data revealed a high level of variation within groups. In conclusion, this study has identified a set of 50 polymorphic SSR markers of barley as being useful for diversity analysis of common wheat, durum wheat, and triticale. These microsatellites could be used for diversity analysis, molecular mapping/QTL analysis, molecular marker-assisted selection, and comparative genome analysis.

Key words: Crop genetics, Marker-assisted breeding, Microsatellite, SSRs

Introduction

Wheat, the third most important crop in the world, contributes to the diets of human and livestock and has essential economic importance. The most common species of wheat grown widely around the world is *Triticum aestivum*, usually called "common" or "bread" wheat. "Durum wheat" or "pasta wheat" (*T. turgidum* var. *durum*) is another species of wheat adapted to mostly hot regions (Shewry & Hey, 2015). Triticale (X *Triticosecale* Wittmack) is the first synthetic cereal to be created by merging wheat and rye together. This crop has the positive trait of wheat for food production and rye traits of adaptability to harsh environmental conditions (Arendt & Zannini, 2013). The increasing global need for food and feed derived from crops including common wheat, durum wheat, and triticale requires crops with high yield and performance. One of the ways of improving crops is molecular breeding in which genetic markers play an important role in the rapid implementation of breeding programmes (Arendt & Zannini, 2013). SSR (simple sequence repeat) markers, also known as short tandem repeats, are now widely used for gene mapping, analysis of genetic diversity, and marker-assisted selection in breeding programmes (Mohammadi et al., 2002; Arendt & Zannini, 2013; Shewry & Hey, 2015). It has been shown that SSR markers are universal across species and have a high level of transferability between closely related species (Wang et al., 2015; Yan et al., 2017).

Many reports indicate the transferability of SSR markers (Varshney et al., 2005; Zhang et al., 2005; Castillo et al., 2008; Castillo et al., 2010; Mahmoudi et al., 2019). A study of interspecies and intergene transferability of barley and wheat SSRs has shown that microsatellite markers are polymorphic (Castillo et al., 2010). Our study showed that SSR markers from wheat and barley are valuable resources for the genetic characterization of *H. chilense*, for analysis of its genetic variability, and as a tool for wheat introgression. An examination of 165 EST-SSR markers from barley in a study by Varshney et al., (2005) showed that barley SSR markers have a transferability of 78.2% in wheat, 75.2% in

rye, and 42.4% in rice. In silico analysis of SSR markers from barley with related species has shown a range of homology with closely related species, more than 90% in wheat, and about 57% in rice (Varshney et al., 2005). Castillo et al. (2008) reported that 21 of 82 barley SSR markers studied in their work showed polymorphism and transferability in *Hordeum chilense*, a native South American diploid wild barley. They showed that identified SSRs are useful for diversity analysis of *H. chilense*, related wild barleys like *H. murinum*, and wheat marker-assisted introgression breeding. They also showed across-genera transferability of the barley EST-SSR within the Triticeae (Castillo et al., 2008).

Studies have shown that SSR markers in legumes are more transferable between intra-generic than in inter-generic legumes (Sim et al., 2009; Chen et al., 2010). However, it has been shown that SSR markers are transferable in closely related species and even in closely related genera (Gutierrez et al., 2005). The development of SSR markers for a particular crop involves a costly and labour-intensive process. One of the ways to overcome this problem is to use transferable SSR markers from other species. The conserved nature of the genetic material and arrangement in the regions flanking SSRs have been found among close species (Castillo et al., 2008; Kalia et al., 2011). These findings have shown that SSR markers developed for one species could be used to detect marker loci in other related species (Gutierrez et al., 2005; Varshney et al., 2005; Castillo et al., 2008; Mohammadi, 2011; Wang et al., 2015; Yan et al., 2017). In this study, the main goal was to investigate a number of SSR markers from barley to find the highest polymorphism and a large number of transferable SSRs for genetic studies in common wheat, durum wheat, and triticale. A total of 102 SSRs of barley were tested, and 40 polymorphic SSR markers were used to study genetic relationships among 41 genotypes.

Materials and Methods

Plant materials and DNA isolation

In this study, a total of 41 genotypes from common wheat (32 genotypes), durum wheat (seven genotypes), and triticale (two genotypes) were used (Table 1).

To isolate DNA from plant materials, seeds were provided from the Seed and Plant Improvement Institute, Karaj, Iran, and grown under greenhouse conditions in the research greenhouse at the University of Tabriz. When plants started to produce several real leaves, young leaves were harvested and frozen at -80°C. Genomic DNA from fresh and young leaves was extracted and purified using the CTAB method (Saghai-Maroof et al., 1984). The quality of DNA was checked on 0.8 % (w/v) agarose gel and concentrations were determined using a spectrophotometer. A portion of DNA was diluted in molecular grade water to a concentration of 25 ng/ml.

Table 1. *List of common wheat, durum wheat, and triticale genotypes used in the study for transferability evaluation and genetic diversity analysis.*

No.	Name of the genotype	Scientific name	No.	Name of the genotype	Scientific name
1	MV17	*T. aestivum*	22	*T. aestivum*	Karim
2	No. 49	*T. aestivum*	23	*T. aestivum*	Zareh
3	Regaw	*T. aestivum*	24	*T. aestivum*	Pishgam
4	UN-11	*T. aestivum*	25	*T. aestivum*	Rasad
5	URwyt 6	*T. aestivum*	26	*T. aestivum*	101
6	Urwyt 8	*T. aestivum*	27	*T. aestivum*	Pishtaz
7	Urwyt 9	*T. aestivum*	28	*T. aestivum*	Sardari
8	Urwyt 15	*T. aestivum*	29	*T. aestivum*	Shahriyar
9	870 Zhong 291	*T. aestivum*	30	*T. aestivum*	Ohedi
10	PTZ	*T. aestivum*	31	*T. aestivum*	Homa
11	Norstar	*T. aestivum*	32	*T. aestivum*	Homa-4
12	Supper wheat	*T. aestivum*	33	*T. turgidum subsp. Durum*	Kndru
13	Chinese Spring	*T. aestivum*	34	*T. turgidum subsp. Durum*	Pgs
14	Yecora Rajo	*T. aestivum*	35	*T. turgidum subsp. Durum*	G.1252
15	Azar 2	*T. aestivum*	36	*T. turgidum subsp. Durum*	61-130
16	Zagrous	*T. aestivum*	37	*T. turgidum subsp. Durum*	Gardish
17	Takab	*T. aestivum*	38	*T. turgidum subsp. Durum*	Chehel Daneh
18	Hoorani	*T. aestivum*	39	*T. turgidum subsp. Durum*	Zardak
19	Ug99	*T. aestivum*	40	*Triticale*	Triticale 1
20	Sayson	*T. aestivum*	41	*Triticale*	Triticale 2
21	Mihan	*T. aestivum*			

PCR amplification

A total of 102 barley SSR markers were used to study the transferability of these SSRs in common wheat, durum wheat, and triticale. Of 102 SSRs, 41 polymorphic barley SSR markers were used to analyze genetic relationships among genotypes. PCR amplifications were performed in a final volume of 10 µL containing 25 ng/µL genomic DNA, 4 µL (2x) Master Mix (dNTPs, Tris–HCl, mM $MgCl_2$, Taq DNA polymerase, Tween 0.2), 0.5 µL of each primer, and 3 µL of double-distilled water. For barley SSR markers, PCR cycling conditions were five min at 94 °C, 4 cycles of three min at 94 °C, 1 min at the annealing temperature, two min at 72 °C and a final extension step of 10 min at 72 °C. For wheat SSR markers, PCR cycling conditions were five min at 94 °C, three cycles of two min at 94 °C, 2 min at the annealing temperature, 20 s at 72 °C and a final extension step of seven min at 72 °C. The PCR products were subjected to electrophoresis on 4% polyacrylamide gels and stained using Ethidium bromide. In addition, the MS0371 DNA marker with the size of 50-1000 base pairs was used to determine the sizes of the PCR products.

Data analysis

The patterns of bands were scored based on the presence or absence of bands and used to construct? a binary data matrix of one and zero for each genotype that was analyzed. Allelic polymorphic information content (PIC) was calculated using the formula:

$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\sum_{i-1}^{n}\sum_{j=i+1}^{n} p_i^2 p_j^2$ (Botstein et al., 1980), where Pi and Pj are the frequency of the ith and jth allele, calculated for each SSR locus.

Nei genetic diversity was calculated using:

$He = 1 - \sum_{i=1}^{n} p_i^2$ (Nei, 1973), where Pi is the frequency of the ith allele. The software PowerMarker was used to calculate PIC and He (Liu & Muse, 2005), and the correlation between them was assessed with SPSS V.20.00.

n order to cluster genotypes, the software MEGA 4.0.2 based on the Minimum evolution algorithm (Tamura et al., 2007) and GenALEx 6.4 software based on Nei genetic distance (Peakall & Smouse, 2006) were used. In addition, GenALEx 6.4 was used for AMOVA (Analysis of Molecular Variance) analysis. The estimation of population structure was based on effective allele number (Ne), Shannon diversity index (I), Nei genetic diversity index (He), and unbiased Nei genetic diversity index (UHe) as in the following formulae:

$Ne = 1/(\sum p_i^2)$ (Peakall & Smouse, 2006),

$I = -1\sum(pi\ln(pi))$ (Maguran, 2013),

$He = 1 - \sum p_i^2$ (Nei, 1973),

$UHe = \left(\frac{2N}{2N-1}\right) He$ (Peakall & Smouse, 2006);

Where Pi is the frequency of ith allele and N is the population size.

Results

Polymorphism of barley SSR markers

A total of 102 primer pairs from two primer groups including GBM and GBMS were used to determine the polymorphism of barley SSRs in the genotypes studied. Of the 102 SSR primer pairs tested, 50 produced clear and repeatable PCR products in wheat (common and durum) and triticale (Figure 1). Of the 50 primer pairs, 40 were polymorphism (Table 2). The polymorphic SSRs produced 118 alleles with an average of 2.95 alleles per locus. The primers GBM1166, GBM1214, GBM1215, GBM1227, GBM1232, GBM1288, GBM1300, GBM1356, GBM1359, GBM1364, GBM1388, GBM1469, GBM1483, GBM5008, and GBM5210 detected at least two fragments. Two primers (GBM1420 and GBM1121) detected seven and six fragments, respectively. The PIC for the polymorphic SSR markers ranged from 0.08 (GBM1227, GBM1232, and GBM5008) to 0.73 (GBM1420) with an average of 0.29 (Table 2). GBM1420, with genetic diversity of 0.76, had the highest diversity while the primers GBM1227, GBM1232, and GBM5008 showed the lowest. An average genetic diversity of 0.33 was calculated. The frequency of prevalent allelic was estimated at 0.95 for GBM1227, GBM1232, GBM1270, GBM5008, and 0.38 for GBM1420 with an average of 0.76 for all primers.

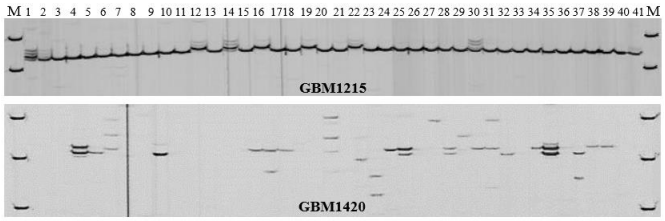

Figure 1. *Amplification of two barley SSRs in 41 genotypes (common wheat, durum wheat, and triticale). M; DNA marker (MS0371) with a size of 50-1000 base pairs. Numbers indicate corresponding genotypes listed in table 1.*

Cluster analysis

Clustering based on Minimum evolution and P. distance algorithms revealed the genetic relationship of 41 genotypes (Figure 2). It showed that 41 genotypes were classified into five groups. The first group was 25 genotypes of common wheat, the second five genotypes of common wheat, the third only one genotype of common wheat, the fourth four genotypes of durum wheat, and fifth and the last were three genotypes of durum wheat and two of triticale.

The relationships observed in the cluster analysis were studied in the principal coordinate analysis.

Table 2. *The allele number, genetic diversity, prevalent allelic frequency and polymorphism information content for polymorphic barley SSR markers.*

Primer	Allele number	Genetic diversity	Prevalent allelic frequency	PIC (Polymorphism Information Content)
GBM1121	6	0.50	0.68	0.48
GBM1153	2	0.10	0.94	0.10
GBM1166	2	0.44	0.65	0.34
GBM1214	2	0.33	0.78	0.28
GBM1215	2	0.24	0.85	0.21
GBM1226	3	0.34	0.79	0.31
GBM1227	2	0.09	0.95	0.08
GBM1231	3	0.22	0.87	0.20
GBM1232	2	0.09	0.95	0.08
GBM1270	3	0.09	0.95	0.09
GBM1274	4	0.13	0.92	0.13
GBM1275	3	0.18	0.90	0.17
GBM1288	2	0.34	0.78	0.28
GBM1297	3	0.49	0.61	0.39
GBM1300	2	0.13	0.92	0.12
GBM1336	4	0.52	0.58	0.43
GBM1356	2	0.18	0.90	0.16
GBM1359	2	0.28	0.82	0.24
GBM1364	2	0.13	0.92	0.12
GBM1388	2	0.48	0.58	0.36
GBM1413	3	0.11	0.94	0.10
GBM1419	3	0.33	0.80	0.30
GBM1420	7	0.76	0.38	0.73
GBM1437	3	0.52	0.48	0.41
GBM1446	3	0.20	0.88	0.19
GBM1451	3	0.38	0.75	0.34
GBM1461	4	0.41	0.74	0.38
GBM1469	2	0.46	0.63	0.35
GBM1480	4	0.26	0.85	0.25
GBM1482	3	0.56	0.53	0.48
GBM1483	2	0.43	0.67	0.34
GBM1490	3	0.15	0.91	0.14
GBM1501	3	0.61	0.50	0.53
GBM1506	3	0.48	0.68	0.43
GBM1508	4	0.41	0.75	0.38
GBM1509	3	0.51	0.63	0.44
GBM1523	3	0.34	0.79	0.30
GBM5008	2	0.09	0.95	0.08
GBM5210	2	0.24	0.85	0.21
GBMS183	5	0.68	0.39	0.61
Average	**2.95**	**0.33**	**0.76**	**0.29**

The first components explained 40% of the variation in the estimates. This analysis enabled the grouping of all genotypes based on their genetic structure, in which two genotypes of triticale were in the same group, seven genotypes of durum wheat in the other group, and all the common wheat genotypes in the same group (Figure 3). Complementary to this, clustering based on Nei index, using the Neighbor-joining algorithm, divided genotypes into two groups including Triticale and Triticum (*T.aestivum* and *T. durum*).

Genetic diversity analysis of genotypes

The effective allele number (Ne), Shannon diversity index (I), Nei genetic diversity index (He), and unbiased Nei genetic diversity index (UHe) were used to study the genetic diversity of the genotypes. One hundred and eighteen alleles were detected at the 40 polymorphic SSR loci on 41 genotypes studied. As reported in Table 3, the highest Nei index was observed in T. aestivum genotypes with Nei of 0.45, and the lowest in triticale with Nei of 0.15. The results

for the Shannon diversity index showed the highest for T. aestivum and the lowest for triticale genotypes. In the case of Ne, the highest Ne was for T.durum (Ne of 1.54) and the lowest for triticale (Ne of 1.2).

AMOVA variation

AMOVA analysis was performed on three groups (common wheat, durum wheat, and triticale). The results showed 35% and 65% variation among and within groups, which large variation within groups (Table 4).

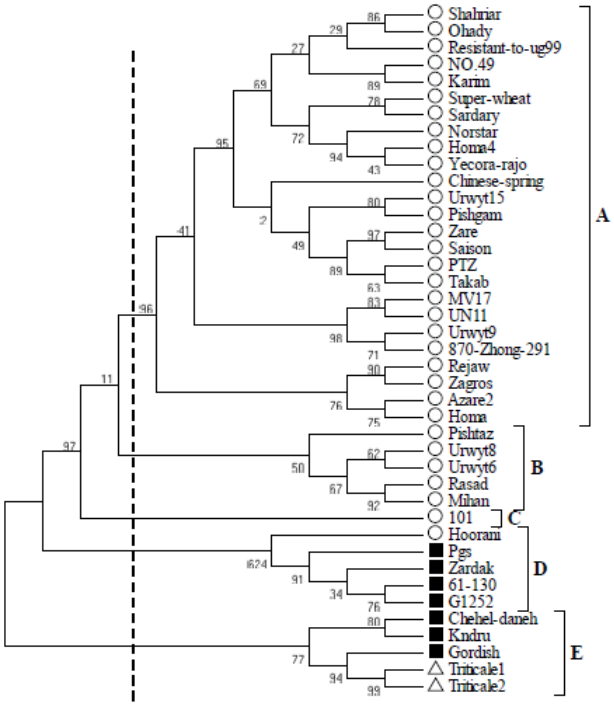

Figure 2. *Clustering of common wheat, durum wheat, and triticale genotypes based on barley SSR markers.*

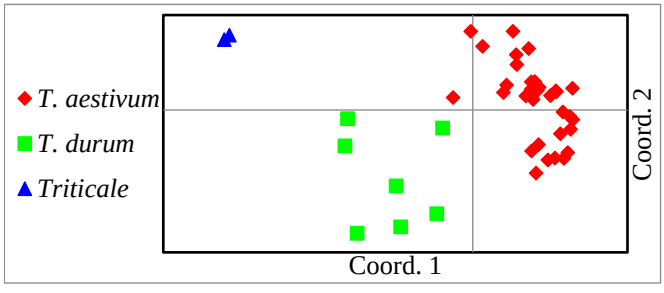

Figure 3. *Principal coordinates analysis estimated for common wheat, durum wheat, and triticale genotypes based on barley SSR markers.*

Discussion

Due to their advantages of codominance, easy manipulation, and low cost, SSR markers have been widely used for genetic diversity analysis, gene mapping, QTL analysis, and marker-assisted selection (Botstein et al., 1980; Zhang et al., 2005). However, the development of SSR markers is expensive and labour-intensive. To overcome problems associated with SSR development for particular species, transferable SSR markers among closely related species and even close genera were used for genetic studies in other species. In recent years, many studies investigated the transferability of SSR markers between species in major crops (da Silva et al., 2017; Babu et al., 2018a; Babu et al., 2018b; Du et al., 2019). However, there are few studies that investigate the transferability of SSRs from close species such as barley into wheat and triticale. In this study, a total of 102 SSRs from barley were tested from which 40 SSR markers identified as polymorphic and transferable in 41 genotypes from common wheat, durum wheat, and triticale. The SSR markers identified were then used to study the genetic diversity.

The results of this study showed that 50 SSR markers of barley (31.25%) could produce successful and clear amplification with polymorphic bands in 41 genotypes studied. This supports previous results in cereal crops. A study by MacRitchie and Sun (2004) reported that 52% of barley microsatellite markers are successfully amplified in species like Elymus trachycaulus. The transferability of barley SSR markers have been indicated in other species such as Hordeum chilense (Castillo et al., 2008; Castillo et al., 2010), wheat (Gupta et al., 2003; Varshney et al., 2005), rye (Varshney et al., 2005), and rice (Varshney et al., 2005).

Table 3. *Average and standard deviation (SD) of the effective allele number (Ne), Shannon diversity index (I), Nei genetic diversity index (He), and unbiased Nei genetic diversity index (UHe) for 41 genotypes (common wheat, durum wheat, and triticale) studied in this work.*

Genotypes	I	He	UHe	Ne
T. aestivum	0.457 (0.063)	0.254 (0.035)	0.259 (0.036)	1.519 (0.107)
T. durum	0.427 (0.065)	0.267 (0.038)	0.292 (0.042)	1.544 (0.093)
Triticale	0.156 (0.046)	0.113 (0.033)	0.150 (0.045)	1.200 (0.073)

*Numbers in parenthesis indicate SD.

Table 4. *Analysis of molecular variance (AMOVA) of three groups including common wheat, durum wheat, and triticale.*

Source of variation	Degree of freedom	Sum of squares	Variance component	Total variation (%)	p-value
Among group	2	272.748	14.734	35	<0.001
Within group	38	1058.179	27.847	65	<0.001
Total	40	1330.927	42.581	100	

Varshney, Sigmund (Varshney et al., 2005) in a part of their study on interspecific transferability of barley EST-SSR markers in wheat, rye, and rice, reported that a higher proportion of barley markers (78.2%) had amplification in wheat. Some studies have shown the transferability of SSR markers from other species to wheat (Kuleung et al., 2004; Yıldırım et al., 2009; Kavanagh et al., 2013) and triticale (Kuleung et al., 2006; Costa et al., 2007; Yıldırım et al., 2009) in agreement with the results of this study. Studies have found that the transferability of SSR markers depends on differences in the DNA sequence, genome size, evolution rate, and PCR conditions used in an experiment (Gupta et al., 2003; Kuleung et al., 2004; Wang et al., 2005). Compared to other SSR transfer studies mentioned above, in this study, the low transferability rate can be attributed to the limited number of selected SSR markers. Of 50 SSRs, 40 SSR markers were polymorphism. The average number of alleles per SSR marker was only 2.95, which was lower than those reported for other species (Wang et al., 2005; Zhang et al., 2005; Sim et al., 2009; Yıldırım et al., 2009). Thus, the low level of polymorphism detected by these SSR markers might not suggest the narrow genetic diversity of the accessions used but may reflect the possibility that these transferable SSRs amplified sequences with a high level of conservation (Wang et al., 2005). This possibility would limit the efficient use of SSR markers. Genetic diversity was measured by the polymorphic information content (PIC). Polymorphic loci can be considered high, medium or low if PIC>0.5, 0.5>PIC>0.25 and PIC<0.25. In 41 genotypes, SSRs showed an average of 0.29, indicating that all the SSR markers might provide substantive information for the genetics and breeding research of common wheat, durum wheat, and triticale.

The genetic diversity estimated by barley SSR loci was supported by the high values of I, He, UHe, and Ne. In this study, the highest values of I, He, UHe, and Ne were 0.45, 0.26, 0.29, and 1.54, respectively. Yu et al. (2013), in their study on the transferability of rice SSR to 20 genotypes of Miscanthus sinensis, reported that the Nei index varies from zero to 0.72 with an average of 0.35, and Shannon diversity index from zero to 1.49 with an average of 0.56. Another study on genetic diversity analysis and transferability of cereal SSR markers to orchardgrass showed that 74 genotypes of orchardgrass had 0.52 of the Shannon index. Fan et al. (2013) in pears showed that pear SSR markers have good transferability to Rosaceae with an average Ne of 1.51, Nei index of 0.31, and Shannon index of 0.46. In this study, these values were lower than those seen in the studies mentioned above. This may be because the expressed sequences, from which EST-SSR are derived, are highly conserved. EST-SSR markers detected a lower rate of polymorphism than the genomic SSRs (Cho et al., 2000). PCA analysis based on the SSR data showed that the genotypes tended to be in the same group according to their genetic structure (Figure 3). As shown in Figure 2, some of the common and durum genotypes were grouped together. However, based on the Neighbor-joining algorithm, all genotypes were correctly clustered in three distinctive groups. These results showed that genetic distance may not be the only criterion for the genetic differentiation of populations. Moreover, the genetic structure revealed that some species show admixture between group I and group II. It is therefore important to use more EST-SSR loci and more individual plants.

Conclusions

Our study shows the transferability and utility of barley SSR for genetic analysis of common wheat, durum wheat, and triticale, with a remarkably high level of polymorphism within groups. The transferred markers have been shown to be useful for genetic diversity studies. Application of these SSR markers will undoubtedly accelerate the genetic study of common wheat, durum wheat, and triticale related to diversity analysis, molecular mapping/QTL analysis, molecular marker-assisted selection, and comparative genome analysis.

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