Original paper Open Access

Genetic structure change of selection cycles for reduced ASI of an Algerian maize population under drought conditions

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† In memory of Abderrahmane Djemel, who passed away after finishing the field work.

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Keywords: *Zea mays L., selection cycles, genetic structure, drought*

Abstract

Plant breeding involves a reduction of genetic diversity that could hinder the expected response to further selection, and the magnitude of that detrimental effect depends on both breeding population and breeding program design. The objective of the current study was to evaluate the effect of selection for reduced anthesis-silking interval in an Algerian maize (*Zea mays* L.) population in order to assess the potential value of this population and breeding strategy for subsequent breeding programs. We genotyped Cycle 0 and Cycle 3 from selection for reduced anthesis-silking interval under drought conditions of the Algerian maize population LOM, which was the best performing population in a previous breeding program, with 34 polymorphic simple sequence repeat markers. These selected markers were highly informative with Polymorphism Information Content values = 0.72 and genetic diversity was maintained over the selection cycles. The genetic distance (Nei's, 1978) between the two cycles was 0.47 and the genetic identity was 0.62. These results indicate that the population LOM was highly variable and that the genetic diversity was not significantly reduced for most genetic parameters by this selection program for reduced anthesis-silking interval. However, genetic selection has caused several genetic changes in Cycle 3 respect to Cycle 0, generating a large genetic divergence between the two cycles of selection. Variations in allelic frequency suggest that further studies could reveal markers associated with selection that could be useful for identifying quantitative trait loci.

Abbreviations

A: Number of alleles per locus
Ae: Number of effectives alleles
Au: Mean number of unique alleles
ASI : Anthesis silking interval
C0 : Original Cycle
C3: Cycle 3
CTAB: Cetyltrimethylammonium-bromide
DNA: Deoxyribonucleic Acid
dNTPs: Deoxynucleotide triphosphate
f: fixation index
Fis: Within-population inbreeding coefficient
Fit: total population inbreeding coefficient
Fst: among-population genetic differentiation coefficient
GMO: Genetically Modified Organism
H0: observed heterozygosity
He: expected heterozygosity
I: Shannon's Information index
MAF: Major allele frequency
Nm: gene flow
PCoA: Principal Coordinates Analysis
PCR: Polymerase chain reaction
PIC: Polymorphism Information Content
PVP: Polyvinylpyrrolidone
QTL: Quantitative trait locus
RNase: Ribonuclease
RPM: Revolutions per minute
SEC: Seconds
SSR: Simple Sequence Repeat

Introduction

Maize (*Zea mays* L.) is the third most important crop in the world, and is grown in most agricultural areas from temperate to tropical zones. In 2018, maize contributed more than 38% of global cereal production, which is equivalent to about 1,147 million tons of maize (http://www.fao.org/faostat/fr/#data/QC Accessed December 21,

2020). In most maize-producing regions, drought is the main factor limiting maize production, although the intensity of stress and its variability differ among temperate and tropical zones (Frova *et al.*, 1999).

Consequently, breeding maize for increased drought tolerance and the development of new breeding technologies are of paramount importance (Badicean *et al.*, 2011). Furthermore, drought tolerance is one of the most difficult traits to study and characterize due to its quantitative nature and polygenic determinism. Thus, the effectiveness of selection for different quantitative traits depends on the ability to develop effective screening methods for the traits individually and collectively. Indeed, the evaluation of selection cycles to determine the effectiveness of a selection scheme requires field experiments in which phenotypes are evaluated. Furthermore, in order to assess the effects of selection on the genetic diversity and structure of the improved populations, molecular markers are useful tools that allow appraising the genetics changes caused by selection (Butrón *et al.*, 2005). In this context, the identification of genetic changes associated with yield components as well as with the secondary morphological traits of interest, particularly anthesis-silking interval, allows the dissection of the genetic basis of drought tolerance (Guo *et al.*, 2008).

Four populations of Algerian maize (BTM, TAO, LOM and IGS) were previously studied by Djemel et al (2018) and Benchikh Lehocine et al (2021). These last authors carried out recurrent phenotypic selection for three successive cycles for reducing ASI, in order to improve their grain yields, under both well water and water stressed conditions for eventual integration into maize breeding programs for drought tolerance. A significant increase in yield was observed for BTM under well-water condition and a significant genetic gain in yield was observed for the LOM population under drought conditions. We concluded that these Algerian populations (BTM and LOM) may be used as new sources of favorable alleles for the reduction of the anthesis-silking interval (ASI) for increased earliness under drought conditions (Benchikh Lehocine *et al.*, 2021). However, the previous report shows that response to selection decreased in later cycles of selection, suggesting that the genetic diversity of these populations could have been exhausted with selection, potentially hampering further selection programs. Therefore, the objective of the current study is to evaluate changes in genetic diversity between cycle 0 and cycle 3 selected under drought conditions of the best performing population (LOM) in order to figure out the potential aptitude of this population for subsequent breeding programs.

Materials and methods

Plant materials

The experimental plant material used in this study was the maize population LOM, which was collected from the oases of the Algerian Sahara (Ouled Mahmoude Lamtarfa, Adrar. Algeria). (Djemel *et al.*, 2012) and is conserved at the High National School of Agronomy (ENSA) of El Harrach in Algiers. LOM was selected for drought tolerance for three consecutive cycles under both optimal and water stress conditions (Benchikh Lehocine et al, 2021). The original LOM population (C0) and its third cycle selected under drought conditions (C3) were used to study changes in genetic diversity and structure by using SSR (Simple Sequence Repeat) markers.

DNA extraction

The molecular analysis was carried out in the molecular biology laboratory of the Misión Biológica de Galicia (MBG), which belongs to the Spanish National Research Council (CSIC) and is located in the province of Pontevedra (Galicia) in Spain. DNA was extracted from 31 individuals from each selection cycle (C0 and C3) of the population LOM, randomly chosen, yielding a total number of 62 coleoptiles. DNA was extracted in accordance with the Maxwell® RSC PureFood GMO and Authentication Kit. The pre-treated solution (1000 µl) was mixed with 40 µl proteinase K and 20 µl of RNase A and 1000 µl of CTAB buffer with 2% of PVP, supplied with the Maxwell® RSC PureFood GMO and Authentication Kit (Promega). After incubation at 65 °C for 30 min with 20 µl of mercaptoethanol and centrifugation at 13200 rpm for 10 min, the aqueous phase (approximately 500 µl) with 300 µl lysis buffer was used for downstream extraction using Maxwell® RSC Cartridges (Promega). The quality and quantity of the extracted DNA was evaluated using a BioDrop µLITE spectrophotometer machine (Biochrom™).

PCR conditions and electrophoresis for SSR analysis

Based on previous experience with local germplasm, 34 SSR maize primers were used for PCR amplification of the 31 individuals from each both LOM population cycles (C0 and C3), which were chosen from the Maize Genetics Database (*https://www.maizegdb.org/data_center/ssr*) (MaizeGDB). The SSRs were selected based on the bin locations, which provides a uniform coverage of all the 10 chromosomes in the maize genome.

Primer names and chromosome loci (Bin number) of the SSR loci used in this study are included in Table 1. All SSR primers were diluted to a working concentration

Table 1 - Microsatellites (SSR) used for characterization of selection cycles of LOM, and position (bin)

SSR	Bin	SSR	bin	SSR	bin
phi109275	1.030	*umc1963*	4.040	*umc1545*	7.000
bnlg2295	1.040	*bnlg1755*	4.050	*umc1270*	7.010
umc1512	1.090	*umc1058*	4.110	*bnlg434*	7.030
umc1165	2.010	*umc1097*	5.000	*umc1327*	8.010
umc1422	2.020	*bnlg565*	5.020	*phi115*	8.030
phi083	2.040	*umc1221*	5.040	*bnlg1724*	9.010
umc1065	2.060	*umc1019*	5.060	*bnlg1688*	9.030
phi328189	2.080	*umc1225*	5.080	*umc1078*	9.050
bnlg1520	2.090	*umc1829*	5.090	*phi041*	10.000
phi101049	2.100	*umc1887*	6.030	*bnlg1028*	10.060
phi036	3.040	*umc1014*	6.040		
phi046	3.080	*umc2059*	6.080		

of 25 µM with sterile water and stored at -20 °C. The polymerase chain reaction (PCR) was performed in a 20 µl containing the following components: 50 ng µl-1 of genomic DNA (1 µl), 25 µM of each primer Forward and Reverse (2 x 0.25 µl), 40mM dNTPs (0.4 µl), 1 x PCR buffer (4 µl), and Taq polymerase (Promega) (0.08 µl) all dispensed into 14.02 µl of sterile water for one reaction. SSRs fragments were amplified in a 96-well PCR plate on a thermal cycler MyCycler™ (BIO-RAD Laboratories) using specific temperature of annealing for each primers pair. The thermal cycler profile included an initial denaturation step at 95°C for 5 min, followed by 39 cycles at 95°C for 30 sec, annealing temperature for 30 sec, and 72°C for 30 sec, and a final elongation step of 72°C for 10 min. DNA fragments were separated according to their size on a QIAxcel Advanced System (QIAGEN) using the QIAxcel DNA high resolution kit, the QIAxcel DNA screening, the QIAxcel DNA fast analysis kit, and the internal QIAxcel ScreenGel® Software (QIAGEN). The sizes of the DNA fragments were collected from electropherograms.

Statistical analysis

The 6.5.03 version of the GenAlex software (Peakall and Smouse, 2012) was used to evaluate genetic parameters Polymorphism Information Content (PIC), major allele frequency (MAF), number of alleles per locus (A), number of effectives alleles (A_e), mean number of unique alleles (Au), Shannon's Information index [Lewontin (1972)] (I), observed (H0) and expected (He) heterozygosity; and fixation index (f) for the selection cycles C0 and C3 of LOM maize population. This analysis allows the study of the genetic structure using Wright's F statistics (1978): Within-population inbreeding coefficient (F_{is}), total population inbreeding coefficient (F_{it}), among-population genetic differentiation coefficient (F_{st}), and gene flow (N_m) (Nei 1978). The Principal Co-ordinates Analysis (PCoA) of the two selection cycles was also carried out. For each polymorphic SSR marker, Polymorphism Information Content (PIC) was determined as described by Bantte and Prasanna (2003). PIC is a measure of allele diversity at a locus and is equal to $1\text{-}\sum f i^2$, where f is the frequency of i^{th} allele.

Results and Discussion

All individuals of the original LOM maize population (C0) and its third cycle selected under drought condition (C3) were successfully amplified using the 34 primers, and PCR products were clearly observed and unambiguously scored. Genetic diversity of LOM maize population (C0 and C3) and the SSR summary statistics are reported in table 2. The 34 SSR markers used were polymorphic for both maize population and the PIC (Polymorphism Information Content) value of the SSR loci ranged from 0.06 (*phi046*) to 0.90 (*phi036*) for C0 and from 0.19 (*phi046*) to 0.90 (*bnlg565*) for C3, while the mean PIC value estimated across all the polymorphic SSR loci was 0.72 for both LOM maize populations C0 and C3 (Table 2). The genetic diversity revealed by the mean PIC value (0.72) was greater than those observed (0.62 and 0.57) in Algerian maize previously studied by ACI *et al.* (2018) and Belalia *et al.* (2019). These parameters show that selection has not significantly reduced genetic diversity; similarly, other authors have reported that selection had non-significant reductions in genetic diversity when initial variability was high (Revilla *et al.*, 1997).

More than 70% of the SSR showed PIC values above 0.70 in C0 and C3 respectively. Among these, *phi036*

and *bnlg565* are noteworthy due to their relatively high polymorphism (18 and 15 alleles each, respectively), and high PIC values (0.90 for both SSR marker). PIC values, which are very useful for genotypes differentiation and genetic diversity analysis, reveal not only the presence of many alleles but also their frequencies. In fact, high mutation rates or the introduction of exotic germplasm can cause the presence of alleles with very low frequencies (Laborda *et al.*, 2005). Thus, observation of the allele frequencies of cycles C0 and C3 revealed that more than 50% of the alleles had frequencies below or equal than 0.1 (60.95% vs. 59.70%), more than 36% of the alleles had frequencies between 0.1 and 0.5, and only 2% had frequencies higher than 0.5% for both cycles. The overall PIC value could be influenced by various factors, particularly (a) the nature of germplasm used for the study; (b) number of alleles (allele richness) as well as distribution of these alleles across the genotypes; and (c) SSR loci assayed, in terms of the nature and type of repeats (Queller *et al.*, 1993 ; Bantte and Prasanna, 2003; Dubey *et al.*, 2009).

The 34 SSR markers used in the present study revealed a total of 274 different fragments for LOM C0 and 263 for LOM C3 and the different selection cycles have reduced the genetic diversity of the LOM population where the mean number of alleles per locus observed in the original population (C0) was 8.05 and it was 7.73 for the third selection cycle (C3) with values ranging from 2 (*phi041*) to 18 (*phi036*) and from 2 (*phi041*) to 15 (*bnlg565*) alleles per locus respectively. The mean allele per SSR marker in this study was lower than those detected by previous SSR studies on Algerian maize landraces from Sahara where Aci *et al.* (2018) and Belalia *et al.* (2019), who reported an average of 10.94 and 10.61 allele per locus for 47 and 46 maize populations respectively. Similar number of alleles per locus compared to our study were detected in maize landraces from Switzerland, Ghana, CIMMYT and Turkey with 8, 7.3, 7.4 and 6.21 alleles per locus, respectively (Xia *et al.*, 2005 ; Cömertpay *et al.*, 2012; Freitag *et al.*, 2012; Oppong *et al.*, 2014). Furthermore, Adeyemo *et al.* (2011) recorded a mean number of alleles per locus of 3.96 in 38 tropical maize inbred lines.

The mean number of effective alleles (A_e), the Shannon's information index, and the observed heterozygosity (H_0) were slightly higher in C0 than in C3, while expected (H_e) heterozygosity were equal and the fixation index (f) was higher in C3. The highest values of effective alleles were given by the marker *phi036* for C0 and by the marker *bnlg565* for C3 (Ae = 10.06; I = 2.57 vs. Ae = 9.75; I = 2.46). The *phi046* marker showed the lowest effective allele values for both selection cycles (A_e = 1.07; I = 0.16 *vs.* A_e = 1.23; I = 0.42).

Unique alleles were 120 and 109 for C0 and C3, respectively. Unique alleles were detected in 30 different SSRs loci for both cycles C0 and C3 of the LOM population respectively and the highest number was recorded by *umc1221* (C0) and *umc1829* (C3), both with 12 unique alleles. The presence of unique alleles could be useful to effectively differentiate specific genotypes and may be an indication of relatively high variability of this population that was not revealed in this study; suggesting that larger samples would be necessary for identifying residual variation not fully detected here. Other authors have suggested that the presence of unique alleles might be due to high mutation rate (Henderson and Petes, 1992) or introgression of alleles from certain exotic germplasm (Senior *et al.*, 1998); but these processes are unlikely in this selection program. Although the two cycles studied were from the same LOM population (the original cycle and its third cycle selected under water stress conditions), the reasons for this high presence of unique alleles could not be established in the present study. Nevertheless, the singular variation supports our previous conclusion indicating that this population has wide genetic base and can be used for further selection programs.

For both cycles of the LOM population (C0 and C3), the mean value of the expected heterozygosity (H_e) was 0.72 and it was higher than the observed one (H_0) (0.50 *vs.* 0.39) which indicates an excess of homozygotes. The mean value of the fixation index (f) of the original population (C0) was lower than that of C3 (0.30 vs. 0.46) but remains below 1. It is known that any selection program carried out within a given population for one or more selection criteria and over several selection cycles may alter and reduce existing genetic variability (Romay *et al.*, 2012). Therefore, as shown by our molecular data, the selection for reducing ASI carried out in the framework of our previous study (Benchikh Lehocine *et al.*, 2021) using the LOM population, allowed to reduce genetic diversity within the third selection cycle under drought conditions (C0: H_0 = 0.50 vs. C3: H_0 = 0.39). However, the levels of variability of C3 after the intrapopulation selection are still large enough for standing further selection programs. Nevertheless, the expected heterozygosity values (He= 0.72 for both selection cycles) demonstrate the maintenance of genetic variability throughout the three selection cycles. The progress of selection in the medium and long term is strongly linked to the maintenance of genetic diversity during multiplication processes and the presence of significant heterozygosity probably indicates a strong adaptive genetic variation in the population (Da Silva *et al.*, 2015). Furthermore, expected heterozygosity values (He = 0.72) for both selection cycles were higher

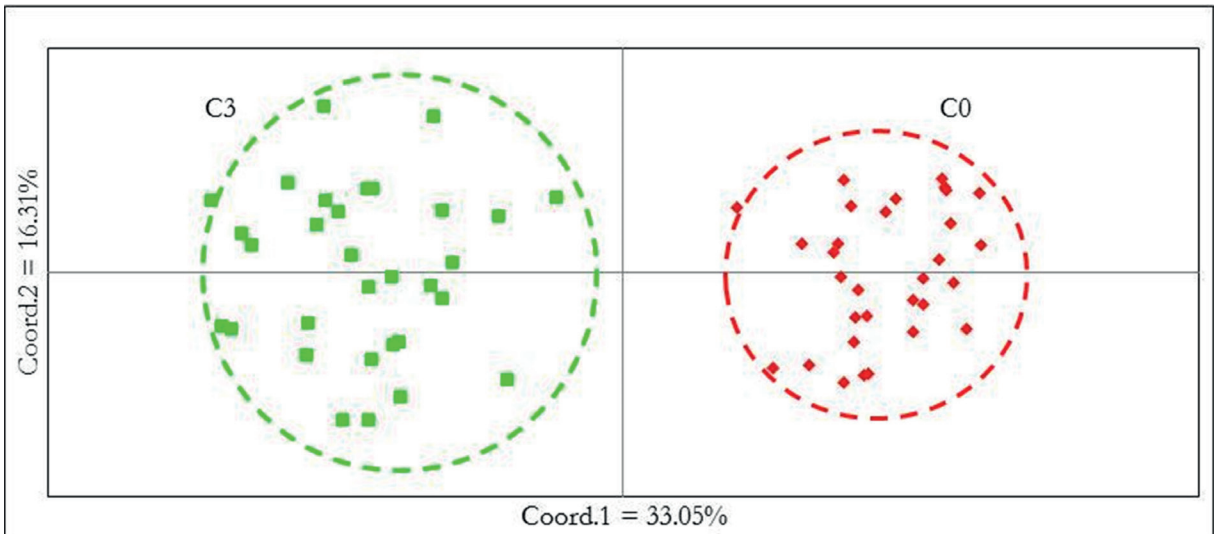

Fig. 1 - Principal coordinates analysis (PCoA) of LOM population C0 and its third cycle selected under drought condition (C3) based on SSRs characterization.

than those reported by Aci *et al.* (2013) (He = 0.44) and Bellalia *et al.* (2019) (0.51) for other Algerian maize populations, respectively.

The genetic distance (Nei's, 1978) between the original LOM population (C0) and its third cycle of selection under drought condition was 0.47 and the genetic identity was 0.62 between them. Evidence for the genetic distribution in C0 and C3 was obtained using Principal Coordinates analysis (PCoA) performed in GENALEX (Figure 1). The eigenvalues obtained from the PCoA showed that the three first components explained a 63% of the total variation (the first and second coordinates accounted for nearly 33.05% and 16.31% of the molecular variation, respectively). It could also be observed that principal coordinate analysis (PCoA) based on SSR genetic distance could differentiate the two selection cycles C0 and C3.

Conclusions

Our results indicate that the population LOM was highly variable and that the genetic diversity was not significantly reduced for most genetic parameters by this selection program for reduced ASI. However, genetic selection has caused some genetic changes in C3 respect to C0, generating a large genetic divergence between the original population and the third cycle of selection. Variations in allelic frequency suggest that further studies could reveal markers associated with selection that could be useful for identifying QTL.

Author Contributions

MBL data recording and draft preparation, AD and PR manuscript correction and final writing, conceptualization, materials, experimental design. MDE data analyses, draft preparation and final redaction. All authors have read and agreed to the published version of the manuscript

Funding

This research was funded by École Nationale Supérieure Agronomique d'Alger, and the Spanish Ministerio de Innovación y Universidades (MCIU), the Agencia Estatal de Investigación (AEI) and the European Fund for Regional Development (FEDER), UE (project code PID2019-108127RB-I00).

Conflict of interest

The authors declare no conflict of interest.

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Table 2 - Genetic diversity and summary of statistics of 34 SSR loci of LOM maize population: original (C0) and its third cycle selected under drought condition (C3). Polymorphism Information Content (PIC); Major allele frequency (MAF); number of alleles per locus (A); number of effectives alleles (Ae); mean number of unique alleles (Au); Shannon's Information index [Lewontin (1972)] (I); observed (H0) and expected (He) heterozygosity; and fixation index (f).

Marker	bin	LOM C0									LOM C3								
		PIC	MAF	A	A_e	A_u	I	H_0	H_e	f	PIC	MAF	A	A_e	A_u	I	H_0	H_e	f
phi083	2.04	0.71	0.38	5.00	3.44	1.00	1.34	0.55	0.71	0.22	0.66	0.46	5.00	2.92	1.00	1.22	0.36	0.66	0.45
phi036	3.04	0.90	0.21	18.00	10.06	9.00	2.57	0.80	0.90	0.10	0.76	0.37	9.00	4.12	-	1.68	0.45	0.76	0.40
phi046	3.08	0.06	0.97	3.00	1.07	2.00	0.16	0.03	0.06	0.49	0.19	0.90	4.00	1.23	3.00	0.42	0.16	0.18	0.10
phi115	8.03	0.53	0.50	3.00	2.13	-	0.81	0.46	0.53	0.12	0.84	0.29	10.00	6.10	7.00	2.02	0.35	0.83	0.57
umc1165	2.01	0.81	0.30	8.00	5.29	1.00	1.83	0.58	0.81	0.28	0.81	0.34	9.00	5.21	2.00	1.88	0.38	0.81	0.52
umc1422	2.02	0.79	0.35	10.00	4.74	5.00	1.82	0.58	0.79	0.26	0.77	0.38	8.00	4.43	3.00	1.73	0.57	0.77	0.26
bnlg1520	2.09	0.71	0.50	10.00	3.49	6.00	1.71	0.50	0.71	0.30	0.68	0.50	9.00	3.11	5.00	1.50	0.29	0.68	0.56
bnlg1755	4.05	0.84	0.27	9.00	6.09	4.00	1.96	0.29	0.83	0.65	0.79	0.24	8.00	4.84	3.00	1.70	0.31	0.79	0.61
umc1097	5.00	0.75	0.38	10.00	4.08	1.00	1.71	0.48	0.75	0.36	0.84	0.27	11.00	6.38	2.00	2.08	0.67	0.84	0.19
bnlg565	5.02	0.86	0.25	13.00	7.14	7.00	2.21	0.70	0.86	0.18	0.90	0.19	15.00	9.75	9.00	2.46	0.51	0.90	0.42
umc1225	5.08	0.83	0.28	11.00	6.01	4.00	2.04	0.60	0.83	0.27	0.82	0.33	10.00	5.46	3.00	1.93	0.41	0.81	0.49
umc1014	6.04	0.78	0.34	7.00	4.48	1.00	1.67	0.51	0.77	0.33	0.78	0.33	8.00	4.52	2.00	1.69	0.56	0.78	0.27
umc1545	7.00	0.62	0.43	7.00	2.60	3.00	1.16	0.61	0.61	0.00	0.52	0.64	4.00	2.06	-	0.92	0.19	0.51	0.62
bnlg434	7.03	0.88	0.23	14.00	8.29	5.00	2.33	0.50	0.88	0.43	0.87	0.19	12.00	7.68	3.00	2.20	0.34	0.87	0.60
umc1327	8.01	0.69	0.39	4.00	3.26	-	1.25	0.77	0.69	-0.11	0.73	0.34	5.00	3.65	1.00	1.39	0.64	0.72	0.11
bnlg1724	9.01	0.55	0.63	6.00	2.22	2.00	1.09	0.38	0.55	0.29	0.67	0.42	5.00	3.03	1.00	1.28	0.00	0.67	1.00
umc1078	9.05	0.81	0.27	9.00	5.17	3.00	1.82	0.60	0.80	0.24	0.80	0.36	10.00	5.05	4.00	1.91	0.34	0.80	0.57
phi041	10.00	0.45	0.65	2.00	1.82	1.00	0.64	0.06	0.45	0.86	0.28	0.83	2.00	1.38	1.00	0.45	0.00	0.28	1.00
bnlg1028	10.06	0.86	0.20	10.00	7.26	8.00	2.12	0.33	0.86	0.61	0.77	0.29	5.00	4.37	3.00	1.53	0.05	0.77	0.93
phi109275	1.03	0.81	0.30	7.00	5.16	3.00	1.76	0.76	0.80	0.06	0.71	0.45	7.00	3.46	3.00	1.48	0.38	0.71	0.46
umc1065	2.06	0.79	0.35	11.00	4.85	4.00	1.88	0.64	0.79	0.18	0.79	0.34	9.00	4.78	2.00	1.83	0.56	0.79	0.29
umc1963	4.04	0.70	0.42	5.00	3.38	-	1.35	0.71	0.70	-0.01	0.78	0.31	7.00	4.47	2.00	1.64	0.48	0.77	0.37
umc1058	4.11	0.84	0.21	10.00	6.45	8.00	1.99	0.74	0.84	0.12	0.83	0.27	9.00	5.86	7.00	1.93	0.80	0.83	0.03
umc1221	5.04	0.79	0.40	12.00	4.69	12.00	1.96	0.47	0.78	0.39	0.75	0.38	7.00	3.94	7.00	1.54	0.60	0.74	0.19
umc1019	5.06	0.80	0.30	9.00	5.12	5.00	1.82	0.80	0.80	-0.002	0.83	0.26	8.00	5.72	4.00	1.87	0.71	0.82	0.14
umc1887	6.03	0.65	0.48	4.00	2.84	3.00	1.15	0.48	0.65	0.25	0.54	0.50	3.00	2.16	2.00	0.83	0.00	0.54	1.00
bnlg2295	1.04	0.87	0.22	13.00	7.61	5.00	2.23	0.31	0.87	0.64	0.80	0.38	14.00	5.06	6.00	2.07	0.40	0.80	0.50
umc1512	1.09	0.65	0.42	4.00	2.82	4.00	1.16	0.32	0.64	0.50	0.60	0.50	5.00	2.49	5.00	1.09	0.10	0.60	0.83
umc2059	6.08	0.75	0.43	8.00	3.97	2.00	1.68	0.67	0.75	0.09	0.84	0.24	9.00	6.30	3.00	1.96	0.71	0.84	0.15
phi328189	2.08	0.79	0.36	7.00	4.75	2.00	1.74	0.60	0.79	0.23	0.72	0.41	5.00	3.54	-	1.38	0.36	0.72	0.49
phi101049	2.10	0.85	0.15	8.00	6.70	3.00	1.97	0.55	0.85	0.35	0.77	0.30	5.00	4.25	-	1.51	0.70	0.76	0.08
umc1829	5.09	0.69	0.46	6.00	3.26	3.00	1.40	0.14	0.69	0.79	0.86	0.26	15.00	7.33	12.00	2.28	0.29	0.86	0.66
bnlg1688	9.03	0.78	0.32	8.00	4.57	3.00	1.73	0.26	0.78	0.66	0.75	0.41	7.00	4.02	2.00	1.61	0.31	0.75	0.58
umc1270	7.01	0.49	0.68	3.00	1.95	-	0.84	0.38	0.48	0.20	0.66	0.29	4.00	2.90	1.00	1.20	0.45	0.65	0.31
Total	-	-	-	-	274	156.76	120	-	-	-	-	-	-	263	151.57	109	-	-	-	-
Means	-	0.73	0.38	8.05	4.61	3.52	1.61	0.50	0.72	0.30	0.72	0.38	7.73	4.46	3.20	1.59	0.39	0.72	0.46