Meta Gene 2 (2014) 606–615

Contents lists available at ScienceDirect

Meta Gene

Genome wide analysis of *Arabidopsis thaliana* reveals high frequency of $AAAG_{N7}CTTT$ motif

Rajesh Mehrotra [a,b,*], Vishesh Jain [a], Chandra Shekhar [c], Sandhya Mehrotra [a]

[a] *Department of Biological Sciences, Birla Institute of Technology & Sciences, Pilani, Rajasthan 333031, India*
[b] *Okinawa Institute of Science and Technology, Onna son, Okinawa, Japan*
[c] *Department of Mathematics, Birla Institute of Technology & Sciences, Pilani, Rajasthan 333031, India*

ARTICLE INFO

Article history:
Received 4 March 2014
Accepted 14 May 2014
Available online 29 August 2014

Keywords:
Arabidopsis thaliana
Cis element
Designer promoter
Dof
Genome

ABSTRACT

Sequence specific elements in DNA regulate transcription by recruiting transcription factors. The Dof proteins are a large family of transcription factors that share a single highly conserved zinc finger. The core to which Dof proteins bind has a consensus AAAG or ACTTTA sequence. These motifs have been over represented in many promoters. We performed a genome wide analysis of AAAG repeat elements increasing the spacer length from 0 to 25. Similar analyses was done with AAAG-CTTT motifs. We report unusual high frequency of $AAAG_{N7}CTTT$ in *Arabidopsis thaliana* genome. We also conclude that there is a preference for A/G nucleotides in spacer sequence between two AAAG repeats.

Introduction

Promoters frequently contain multiple functional regulatory elements (Wray et al., 2003). This has an inherent question. How do redundancy and the evolution of cis element multiplicity take place. Cis elements are non coding DNA sequences present upstream of a gene and is required for proper spatio-temporal expression of the gene present downstream of it. It contains binding sites for transcription factors. The Dof domain proteins are typical example of plant specific transcription factors (Riechmann et al., 2000; Yanagisawa and Sheen, 1998; Yanagisawa, 2002). Dof transcription factor binds to a core sequence AAAG as shown by Vicente-Carbajosa et al. (1997) in a pull down assay. Dof domain proteins

* Corresponding author at: Department of Biological Sciences, Birla Institute of Technology & Sciences, Pilani, Rajasthan 333031, India. Tel.: +91 159 624 4183; fax: +91 159 624 5073.
E-mail address: rajmeh25@hotmail.com (R. Mehrotra).

http://dx.doi.org/10.1016/j.mgene.2014.05.003

have been shown to interact with another class of transcription factors (Zhang et al., 1995). We are very much interested in knowing how transcription factors select their target-like sequences which are scattered on the entire chromosome and how they function at this site. We have shown earlier that the minimal core sequences of the commonly occurring cis elements can enhance promoter expression, even when used out of their native contexts (Mehrotra and Mehrotra, 2010; Mehrotra and Panwar, 2009; Mehrotra et al., 2005; Sawant et al., 2005). Using ACGT core sequence we showed that probabilistic model is not followed when we look for the evolution of cis element multiplicity (Mehrotra et al., 2012, 2013). In this study we searched for the multiplicity of AAAG core sequence in the genome of *Arabidopsis thaliana* and reported that $AAAG_{n7}$ CTTT is a preferred sequence in the genome. This information will be useful for designer promoters where specific interactions could be directed.

Methodology

The objective was to find out the frequency of the recurring sequences. Sequences of chromosomes were downloaded form the NCBI website (www.ncbi.nlm.nih.gov) and converted to a single line sequence using Notepad++. An ANSI C code was generated and later a code in Python 2.6.5 was used to find the results. The code written is as follows:

1. Code to find frequency of AAAG(A/G/C/T)AAAG as in Table 1

2. Python codes to find frequency of two AAAG/CTTTs separated by 0–25 nt. Spacer

```
#include<stdio.h>
#include<stdlib.h>
int main ()
{
char *p,*x;
int j, singlemotif= 0;

int nA = 0;


int nG = 0;


int nC = 0;

int nT = 0;


if(( x = (char*) malloc(25000000)) == NULL)
{
printf("No space available \n");
exit(1);
}
FILE *fp=fopen("chr1_Ied.txt","r");
if (fp == NULL)
{
puts ("Cannot open file");
exit(1);
}
while(!feof(fp))
{
for(p=x;p<=x+25000000;p=p++)
{
fscanf(fp,"%c",p);
} }
for (p=x;p<=x+25000000;p=p++)
{

for (p=x;p<=x+25000000;p=p++)
{


if ( (*p == 'A') && (*(p+1) == 'A') && (*(p+2) == 'A') && (*(p+3) == 'G') &&
(*(p+4) == 'A') && (*(p+5) == 'A') && (*(p+6) == 'A') && (*(p+7) == 'A')   &&
(*(p+8) == 'G') ) nA = nA + 1;

if ( (*p == 'A') && (*(p+1) == 'A') && (*(p+2) == 'A') && (*(p+3) == 'G') &&
(*(p+4) == 'G') && (*(p+5) == 'A') && (*(p+6) == 'A') && (*(p+7) == 'A')   &&
(*(p+8) == 'G') ) nG = nG + 1;

if ( (*p == 'A') && (*(p+1) == 'A') && (*(p+2) == 'A') && (*(p+3) == 'G') &&
(*(p+4) == 'C') && (*(p+5) == 'A') && (*(p+6) == 'A') && (*(p+7) == 'A')   &&
(*(p+8) == 'G') ) nC  = nC + 1;

if ( (*p == 'A') && (*(p+1) == 'A') && (*(p+2) == 'A') && (*(p+3) == 'G') &&
(*(p+4) == 'T') && (*(p+5) == 'A') && (*(p+6) == 'A') && (*(p+7) == 'A')   &&
(*(p+8) == 'G') ) nT = nT + 1;


}

printf("No. of matches of single motif are %d\n",singlemotif);

printf("count of AAAGAAAAG = %d\n",nA);

printf("  count of AAAGGAAAG = %d\n",nG);

printf("  count of  AAAGCAAAG = %d\n",nC);

printf("count of AAAGTAAAG = %d\n",nT);

}
```

```
•   f = open( "C:\Users\Ujjwal\Downloads\chromosome1.txt", "r" )
buff = f.read( )

values = {}


for i in range( 0, 27 ):
    values[ i ] = 0
    for x in range( 0, len( buff ) -7 - i ):
        if ( buff[ x:x+4 ] == "AAAG") and ( buff[ x+4+i:x+4+i+4 ] == "AAAG" ):
            values[ i ] = values[ i ] + 1


print( 'printing frequency below:\n' )

for i in range( 0, 27 ):
    print( 'Nucleotiedes AAAGnAAAG separated by ' + str( i ) + ' : ' + str( values[ i ] ) )
```

```
•   f = open( "C:\Users\Ujjwal\Downloads\chromosome1.txt", "r" )
buff = f.read( )

values = {}
```

```
for i in range( 0, 27 ):
    values[ i ] = 0
    for x in range( 0, len( buff ) -7 - i ):
        if (  buff[ x:x+4 ] == "CTTT" ) and (  buff[ x+4+i:x+4+i+4 ] == "CTTT" ):
            values[ i ] = values[ i ] + 1


print( 'printing frequency below:\n' )

for i in range( 0, 27 ):
    print( 'Nucleotiedes CTTTnCTTTseparated by ' + str( i ) + ' : ' + str( values[ i ] ) )


  •  f = open( "C:\Users\Ujjwal\Downloads\chromosome1.txt", "r" )
buff = f.read( )

values = {}

for i in range( 0, 27 ):
    values[ i ] = 0


    for x in range( 0, len( buff ) -7 - i ):
        if ( buff[ x:x+4 ] == "AAAG"  ) and (  buff[ x+4+i:x+4+i+4 ] == "CTTT" ):
            values[ i ] = values[ i ] + 1

print( 'printing frequency below:\n' )

for i in range( 0, 27 ):
    print( 'Nucleotiedes AAAGnCTTTseparated by ' + str( i ) + ' : ' + str( values[ i ] ) )


  •  f = open( "C:\Users\Ujjwal\Downloads\chromosome1.txt", "r" )
buff = f.read( )

values = {}

for i in range( 0, 27 ):
    values[ i ] = 0
    for x in range( 0, len( buff ) -7 - i ):
        if (  buff[ x:x+4 ] == "CTTT" ) and ( buff[ x+4+i:x+4+i+4 ] == "AAAG" ):
            values[ i ] = values[ i ] + 1

print( 'printing frequency below:\n' )

for i in range( 0, 27 ):
    print( 'Nucleotiedes CTTTnAAAG separated by ' + str( i ) + ' : ' + str( values[ i ] ) )
```

Results and discussions

AAAG$_{n7}$CTTT sequence is highly preferred in A. thaliana genome

Dof proteins, which are typically composed of 200–400 amino acids, are defined as DNA-binding proteins that have a highly conserved Dof domain. The strong similarity among Dof DNA-binding domains suggested that all Dof proteins display similar DNA-binding specificity. Indeed, an AAAG sequence or its reversibly oriented sequence, CTTT, is always found in the binding sequences of individual Dof proteins (Chen et al., 1996; dePaolis et al., 1996; Kang and Singh, 2000; Mena et al., 1998; Plesch et al., 2001;

Table 1
Frequency of two AAAG motifs separated by all possible distances (till 25 bp), across the five chromosomes. ‘n’ represents the intervening distance between the motifs. The second column displays the value of ‘n’.

		chr1	chr2	chr3	chr4	chr5	Total
	0	3224	2171	2501	1934	2908	12,738
AAAGnAAAG	1	2951	1873	2282	1711	2499	11,316
	2	3314	2088	2546	2302	3215	13,465
	3	2635	1755	2112	1693	2390	10,585
	4	2732	1751	2038	1594	2377	10,492
	5	2577	1746	2076	1570	2256	10,225
	6	2529	1792	2107	1541	2373	10,342
	7	2407	1663	2278	1589	2278	10,215
	8	2533	1644	2134	1548	2341	10,200
	9	2201	1454	1720	1330	2026	8731
	10	2148	1518	1737	1390	2067	8860
	11	2308	1543	1719	1365	2073	9008
	12	2169	1454	1763	1274	2021	8681
	13	2194	1438	1671	1352	1939	8594
	14	2497	1501	1909	1428	2080	9415
	15	2172	1435	1738	1348	2022	8715
	16	2556	1507	1789	1439	2142	9433
	17	2482	1690	2028	1583	2331	10,114
	18	2154	1459	1888	1345	1925	8771
	19	2230	1476	1819	1378	1917	8820
	20	2338	1553	1776	1418	2105	9190
	21	2144	1430	1646	1296	1939	8455
	22	2129	1308	1609	1267	1881	8194
	23	2159	1467	1733	1435	2015	8809
	24	2254	1443	1689	1549	1997	8932
	25	2147	1504	1721	1400	1923	8695

Washio, 2001; Yanagisawa and Izui, 1993) except a pumpkin Dof protein (AOBP) that recognizes an AGTA motif (Kisu et al., 1998). In *A. thaliana*, two AAAGs separated by one neuclotide is a known binding site for the OBP-1 protein (Yanagisawa, 2002). Similarly clusters of AAAG sites have been shown to additively contribute to guard cell-specificity of *AtMYB60* promoter in guard cells (Cominelli et al., 2011). With an intention to discover potential new DOF binding sites in *A. thaliana*, the frequency of two AAAG or CTTT motifs separated by an increasing distance was carried out.

The frequency of AAAGAAAG without any spacer has a maximum occurrence of 12,738 as shown in Table 1 and Fig. 1 As we increase the spacer length, the frequency of occurrences started decreasing. There

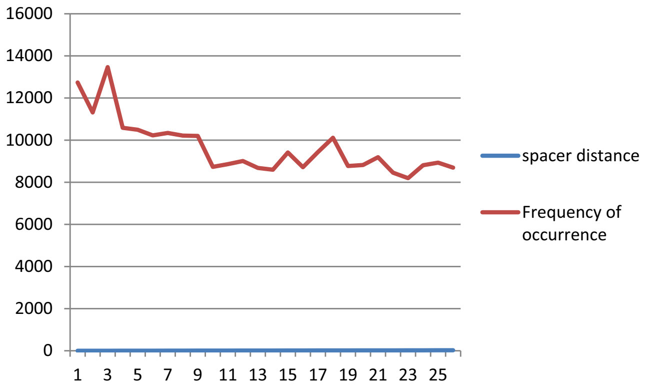

Fig. 1. Frequency of two AAAG motifs separated by all possible distances till 25 bp across the five chromosomes of *Arabidopsis thaliana*.

Table 2
Frequency of two CTTT motifs separated by all possible distances (till 25 bp), across the five chromosomes. 'n' represents the intervening distance between the motifs. The second column displays the value of 'n'.

		chr1	chr2	chr3	chr4	chr5	Total
CTTTnCTTT	0	3195	2086	2447	1896	2764	12,388
	1	3192	1910	2262	1755	2627	11,746
	2	3269	2168	2465	2139	3060	13,101
	3	2648	1706	2150	1677	2497	10,678
	4	2616	1688	2081	1547	2374	10,306
	5	2582	1723	2078	1606	2452	10,441
	6	2591	1740	2014	1612	2344	10,301
	7	2402	1708	2183	1664	2292	10,249
	8	2416	1729	2097	1576	2287	10,105
	9	2286	1448	1836	1390	2053	9013
	10	2260	1528	1698	1384	2108	8978
	11	2326	1531	1770	1381	2212	9220
	12	2231	1484	1683	1293	1939	8630
	13	2143	1484	1683	1407	1896	8613
	14	2360	1606	1837	1435	2136	9374
	15	2493	1523	1656	1431	1978	9081
	16	2227	1494	1829	1477	2327	9354
	17	2402	1673	2043	1568	2320	10,006
	18	2237	1482	1797	1318	1985	8819
	19	2240	1444	1657	1353	2001	8695
	20	2305	1555	1746	1402	2101	9109
	21	2180	1459	1610	1402	2045	8696
	22	2124	1401	1578	1278	1946	8327
	23	2218	1527	1747	1361	2014	8867
	24	2156	1428	1625	1321	1916	8446
	25	2219	1457	1703	1373	1986	8738

was a slight increase in frequency for the spacer length 14–17. Statistical analyses (data not shown) indicated them to be non significant as the deviation was essentially within 10–15%. Similar trend was observed for ($CTTT_nCTTT$) as shown in Table 2 and Fig. 2.

A very interesting observation was made when we looked for combination of AAAG and CTTT sequences. An unexpected high frequency was observed for $AAAG_{n7}CTTT$. The frequency of occurrence was observed as

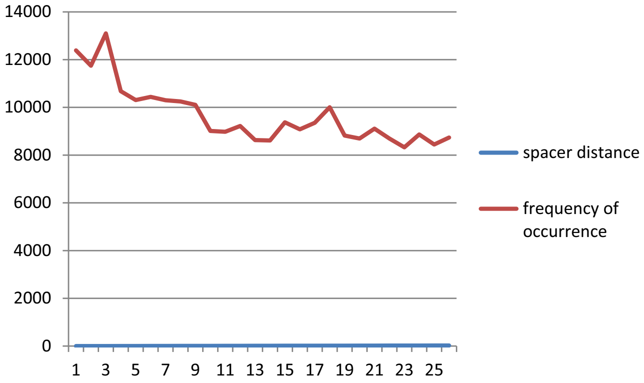

Fig. 2. Frequency of two CTTT motifs separated by all possible distances till 25 bp, across the five chromosomes of *Arabidopsis thaliana*.

Table 3
Frequency of a AAAG and a CTTT motif separated by all possible distances (till 25 bp), across the five chromosomes. 'n' represents the intervening distance between the motifs. The second column displays the value of 'n'.

AAAGnCTTT	chr1	chr2	chr3	chr4	chr5	Total
0	2379	1352	1437	1570	2320	9058
1	1504	910	1236	905	1384	5939
2	1187	792	921	736	1018	4654
3	1398	903	993	792	1205	5291
4	1199	842	992	957	1190	5180
5	1308	863	995	795	1221	5182
6	1853	1205	1396	1069	1654	7177
7	3827	2482	2854	2358	3456	14,977
8	1546	990	1201	922	1350	6009
9	1534	1026	1197	994	1405	6156
10	1674	1050	1183	968	1366	6241
11	1544	1006	1218	1083	1633	6484
12	1620	976	1201	977	1470	6244
13	1557	1033	1180	974	1358	6102
14	1660	1081	1245	1012	1385	6383
15	1687	1119	1309	1032	1479	6626
16	1664	1107	1335	1016	1575	6697
17	1715	1092	1251	1135	1871	7064
18	1685	1119	1508	970	1454	6736
19	1518	992	1189	1014	1388	6101
20	1649	1040	1231	925	1354	6199
21	1673	1111	1269	951	1412	6416
22	1635	1046	1298	955	1454	6388
23	1548	1066	1196	937	1485	6232
24	1631	1059	1243	969	1461	6363
25	1655	1081	1278	977	1521	6512

14,977 which is more than two times the predecessor whose frequency is 7177 as shown in Table 3 and Fig. 3. However, when we change the orientation to CTTT_{n7} AAAG this tendency was not observed as shown in Table 4. The other implication of this is that transcriptional factor binding is direction specific. Not all AAAG motifs in plant promoters are targets of the Dof domain proteins. However, since an AAAG and a CTTT motif separated by a distance of 7 bp is present in an exceptionally high frequency, we think it is highly likely that this sequence combination may have a functional significance yet to be discovered.

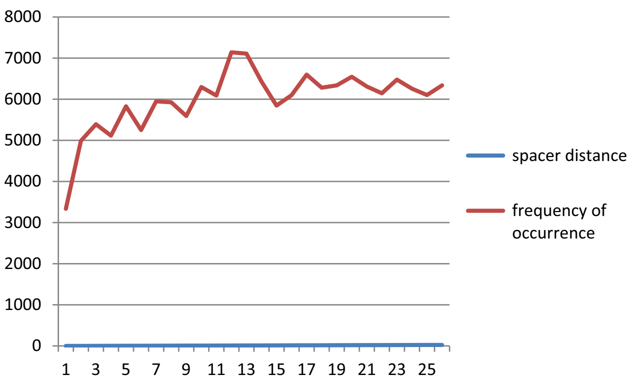

Fig. 3. Frequency of CTTT and AAAG motifs separated by all possible distances till 25 bp, across the five chromosomes of *Arabidopsis thaliana*.

Table 4
Frequency of a CTTT and a AAAG motif separated by all possible spacer distances (till 25 bp), across the five chromosomes. 'n' represents the intervening distance between the motifs. The second column displays the value of 'n'.

		chr1	chr2	chr3	chr4	chr5	Total
CTTTnAAAG	0	871	587	623	505	749	3335
	1	1206	878	1005	736	1162	4987
	2	1356	915	1097	833	1191	5392
	3	1289	866	1065	818	1076	5114
	4	1341	917	1381	871	1318	5828
	5	1354	861	1039	785	1212	5251
	6	1552	1003	1107	884	1403	5949
	7	1461	1021	1118	924	1402	5926
	8	1442	939	1100	832	1279	5592
	9	1659	1052	1235	975	1375	6296
	10	1635	941	1223	885	1406	6090
	11	1689	1144	1590	1082	1635	7140
	12	1697	1131	1279	1149	1854	7110
	13	1606	1054	1223	1003	1544	6430
	14	1433	1004	1117	878	1414	5846
	15	1553	1060	1191	853	1442	6099
	16	1703	1154	1214	1056	1471	6598
	17	1629	1037	1203	961	1450	6280
	18	1595	1016	1199	1002	1525	6337
	19	1680	1135	1271	976	1484	6546
	20	1579	1067	1256	957	1451	6310
	21	1545	1040	1239	929	1392	6145
	22	1632	1124	1251	1011	1459	6477
	23	1541	1096	1265	998	1356	6256
	24	1619	1002	1106	969	1406	6102
	25	1650	1120	1201	952	1414	6337

A and G are preferred as flanking nucleotides

We were interested to know which residues predominate in the flanking of AAAG sequence. Such studies are very important because many studies indicate that flanking sequences are very important for binding specificity (Foster et al., 1994; Izawa et al., 1993). We changed one nucleotide at a time following AAAG. As shown in Table 5, A and G predominate as flanking residues although there is an exception when (AAAG)_(AAAG) is separated by one nucleotide where the frequency of G flanking is 1918 which is less than C which is 2057. In all other cases G dominates as a flanking sequence over C and T.

Conclusions

The promoter region of many genes contain multiple binding sites for the same transcription factor. One possibility is that individuals with multiple, redundant binding sites have higher fitness. Cis regulatory element multiplicity has been correlated with several gene functionalities like Promoters containing multiple sites evolve more slowly. In this paper we focused on the multiplicity of AAAG sequence with varied spacer lengths and also in combination with CTTT sequence. We report that AAAG$_{n7}$ CTTT is a preferred sequence in the genome of *A. thaliana*. This information will be useful for designer promoters where specific interactions could be directed.

Acknowledgment

This work is part of UGC funded project of Dr Sandhya Mehrotra and Dr Rajesh Mehrotra. The authors are grateful to the administration of Birla Institute of Technology and Sciences, Pilani, Rajasthan for providing logistic support.

Table 5
Frequency of flanking nucleotide between two AAAG motifs separated by an increasing sequence length across five chromosomes.

AAAG?_AAAG	CHR1	chr2	chr3	chr4	chr5	Total
A	1480	962	1143	885	1264	5734
G	487	306	399	296	430	1918
C	551	330	413	286	477	2057
T	433	275	327	244	328	1607
AA	725	441	574	403	646	2789
AG	402	248	286	234	327	1479
AC	189	124	135	115	366	929
AT	196	118	178	123	173	788
AAA	286	170	189	156	250	1051
AAG	152	105	143	96	136	632
AAC	74	41	59	48	55	277
AAT	65	37	28	36	43	209
AAAA	187	98	109	81	141	616
AAAG	122	79	95	65	118	479
AAAC	52	35	43	23	31	184
AAAT	33	18	23	19	38	131
AAAAA	76	42	69	49	66	302
AAAAG	33	26	45	30	37	171
AAAAC	22	21	13	14	15	85
AAAAT	15	13	11	6	18	63
AAAAAA	55	37	45	28	43	208
AAAAAG	20	13	18	9	27	87
AAAAAC	4	6	5	6	14	35
AAAAAT	8	1	6	6	2	22
AGA	102	85	87	72	108	454
AGG	47	32	39	25	49	192
AGC	32	30	30	27	26	145
AGT	55	34	47	41	59	236

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mgene.2014.05.003.

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