

Experimental *in vitro* characterization of TATA-binding protein to TATA-boxes affinity of *Arabidopsis thaliana* real promoter genes

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Motivation and Aim: There are practically no works on studying the interaction of TBP with TATA boxes of real plant genes. In this work, we have for the first time determined the equilibrium dissociation constants characterizing the affinity of TBP to the TATA boxes of the model plant *Arabidopsis thaliana* genes promoters located in different plant tissues.

Methods: DNA sequences of *Arabidopsis t.* promoter genes TATA boxes took from the original works of the authors; used the EMSA method to determine the equilibrium dissociation constants.

Gene	Sequence ODN (5' – 3')	K _D , nM
<i>TT8</i>	TCTTCTCACATATTAATAACAACCCCT	19 ± 3
<i>β-amylase</i>	TCTACTTCTATATAAAGAGCTCGTGA	1,3 ± 0,3
<i>Pur7</i>	AATTCGTCTCTACACTTCCCCCACC	60 ± 10
<i>PFL</i>	CTCGGTGTCTTATATAACTCAACCTC	5 ± 1
<i>TFL1</i>	GGAAAACCCCTATAAATAGATGTCTC	0,9 ± 0,2
<i>Cer2</i>	CTCAATCCTTATATAACACATCCCAT	6 ± 1
<i>AMY1</i>	GGGTCTCAATATAAACACACAAAGCA	23 ± 2
<i>EDD1</i>	AAAATCATATAGTTTAGGTGCAACCG	24 ± 4
<i>MS5</i>	GTGTTTCATCTATAAACTCAAAGCTC	8 ± 4
<i>AtMLH1</i>	GATGACAAATTATAACCCTAAATAAA	2,5 ± 0,7

Results: As can be seen from the above sequences, they all contain similar TATA elements, more or less homologous to the TATA-box consensus. If we compare the influence of SNP in the TATA box of arabidopsis and human – T1A2T3A4A5A6A7, then the substitutions in positions 2, 3 and 4 (A and T to G or C) are the ones with the greatest influence on transcription and expression, which unites them [1]. Substitutions in positions 1, 6 and 7 have a less significant impact. From the data given in the Table, it can be seen that despite the preservation of the most conservative nucleotides in positions 2, 3 and 4, the affinity of TBP/TATA differs from 3.8 to 26 times. The lowest affinity (60 nM) was determined for the *Pur7* gene, in the TATA box of which the conservative 2T was replaced with C. From the presented data, it can also be seen that the highest affinity of TBP/TATA is observed in the genes of the apical meristem of the roots, stem, shoots and inflorescences of the plant (*PFL*, *TFL1*), and *CER2*, the expression of which was observed only in the epidermis [2].

Conclusions: The expanded data obtained can be an important aid for the widely used genome editing technology of food-important agricultural plants to regulate their yield.

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References

1. Mukumoto F. et al. DNA sequence requirement of a TATA element-binding protein from Arabidopsis for transcription *in vitro*. *Plant Mol Biol.* 1993;23:995-1003.
2. Haslam T.M. et al. Arabidopsis ECERIFERUM2 is a component of the fatty acid elongation machinery required for fatty acid extension to exceptional lengths. *Plant Physiol.* 2012;160:1164-1174.