# A comprehensive bioinformatics study on Tea (Camellia sinensis) Genome for WRKY transcription factor

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Abstract- Tea (Camellia sinensis; CS) is one of the world's most popular aromatic and non-alcoholic beverages, with great economic, health, & cultural importance. It is one of the major evergreen commodities grown in over 50 nations across the world. Tea leaves contain a variety of bioactive substances, particularly caffeine, theophylline, catechins, and flavonoids, which have been proven to have antioxidant, anti-inflammatory, and anti-cancer activities. Nowadays, the major concern is that the quality of tea leaves is getting deteriorated due to various environmental stresses. Fascinatingly, a transcription factor named as 'WRKY' has been found to play a key role in the development and growth of plants in response to these stresses. However, research on the role of WRKY in tea plants is limited. Therefore, we focused on the identification and characterization of this transcription factor (CsWRKY) in Tea plants through bioinformatics analyses. Our in silico study has found a total of 92 WRKY transcription factors. Out of the 46 were found to be basic in nature, 2 were neutral, and 44 were acidic in nature. As per our chromosomal distribution study, the CsWRKY genes were mostly located at chromosome numbers 1 to 10, 12, and 13. The chromosome number 1 was found to contain the maximum number of CsWRKY genes and the least were present on Chr. 12 and 13. Further, our phylogenetic study found that most of the CsWRKY genes were orthologous to AtWRKY (Arabidopsis thaliana). Our expression analysis reported that most of these genes are expressed in bud, flower, leaf, ovary, root, seed, and shoot of the Tea plant. This study also revealed the highly conserved motifs and structures of CsWRKY. Considering together, this study provides in-depth information regarding the CsWRKY gene family, and further research is required to validate the stress-tolerant activity of CsWRKY.

Keywords: Camellia sinensis, CsWRKY, Biotic stress, Abiotic stress, Phylogenetic analysis.

#### 1. INTRODUCTION

Most of the plant cells respond differently with respect to environmental stresses. At the molecular level, it affects gene expression where some genes are upregulated and some are downregulated. WRKY is one such transcription factor (TFs) that regulates the gene expression against these external stimuli. They are a huge group of regulatory molecules in plants (1). The name is WRKY because of the presence of the conserved amino acid sequence 'WRKYGQK'. They play the role of both activator and repressor in plant signaling. The WRKY domain is 60 amino acid-long with a conserved heptapeptide of WRKYGQK at the N-terminus and unique zinc-finger-like motifs at the C-terminal region. Both of these conserved regions are necessary for the specific binding to the W box (TTGACT/C) cis-acting sites present in the promoter of target genes (2). A majority of proteins that have one WRKY domain belonged to group II, however, only those with two WRKY domains do. Putative zinc ligands for the two groups I and group II protein followed a similar structure (C-X4-5-C-X22-23-H-X1-H). Because WRKY proteins only have one WRKY domain, they are classified as Group III. The C2H2 pattern is replaced with WRKY domains from group III. Group II proteins are further divided into IIa, IIb, IIc, IId, and IIe based on their primary amino acid sequence (3).

The perennial evergreen plant known as tea (*Camellia sinensis* L.) has significant commercial value and is a member of the Theaceae family. There are several different bioactive substances in tea, including polyphenols, catechins, flavonoids, and alkaloid compounds that contribute to its flavor, scent, and possible health advantages. Tea is drunk all over the world. It is rich in various secondary metabolites, which are important for human health. Tea is rich in beneficial metabolites, such as tea polyphenols, theanine, and polysaccharides, and has been widely reported to prevent cancer, cardiovascular, and neurodegenerative diseases (4–8). Catechins (flavan-3-ols), a group of polyphenolic compounds produced via flavonoid biosynthesis, have a main contribution to protect cells against oxidative stress (9,10). Tea plant can grow in moist climate and in acidic soil of tropical, subtropical, and temperate regions (11). However, tea plants have been subjected to varied climates and complex geography that affect tea yield and quality (8). In plants, relevant mechanisms have been established to demonstrate growth and reproduction in diverse environments (12). WRKY proteins are one of the most important transcription factor families that regulate various signaling pathways of several biological processes related to plant stress defenses, development, and metabolism (13). However, the WRKY transcription factors in tea plant have a profound significance. Our *in silico* study has provided a thorough analysis of the tea plant (*Camellia sinensis*) WRKY family transcription factors. This research also laid the groundwork for further molecular identification and genetic breeding by providing a comprehensive scan of *CsWRKY* transcription factors.

## 2. MATERIALS AND METHODS

## 2.1. Identification of CsWRKYs gene family

The information on the genomic sequences of tea plants and proteins was amassed in a worldwide network of integrated plant databases. The plant transcription factors database, including all 92 sequences of tea proteins, was used to produce the data annotation. The presence of a WRKY domain in an accessed protein sequence was predicted using the online TeaCon database, and the chemical and physical characteristics of putative CsWRKYs were confirmed using a pay-program tool that gathered amino acid numbers determining each protein sequence's length. the molecular weight of the protein (MW), and an isoelectric point(pI) (14).

## 2.2. Phylogenetic analysis of CsWRKY genes

The MEGA7.0 program was used to create a disconnected evolutionary tree of recognized sequences of protein of CsWRKY in the species *C. sinensis & A. thaliana*. The following parameters were used to generate the phylogenetic tree: a phylogenetic test using 1000 bootstrap replicates, gap/missing information treatment: partially deletion, the rate across sites: gamma distribution with invariant (G) models/method: LG model, and the maximum likelihood technique (ML), respectively (15).

## 2.3. Analysis of CsWRKY Expression of Genes in Various Tissues and Organs

The TeaCon database was used to acquire DNA sequence data regarding the CsWRKY families in order to analyze the patterns of gene regulation of the CsWRKY families in various tissues and organs. The gene id was used to calculate the genome-wide transcription frequencies of the CsWRKY genes.

## 2.4. Exon-intron structure, conservation domains, and motif descriptions are provided

The newly found *CsWRKY* gene structure was investigated by mapping the CDS to the DNA sequences using the online GSDS2.0 database. *C. sinensis* genomic DNA and CDS sequences were downloaded using the Tea genome database (16).

## 2.5. Pathways analysis of CsWRKY

KEGG pathway (17) is a collection of manually drawn pathway maps representing our knowledge of the molecular interaction, reaction and relation networks. Here, Organism-specific versions were created for KEGG pathway maps, BRITE hierarchies and KEGG modules through the KEGG Orthology (KO) system, either as static files in the daily database update procedure (for the well-annotated genomes in KEGG GENES) or as temporary files on the fly (for GhostKOALA-annotated MGENES). The organism-specific pathway maps and module maps are colored in green, which is a KEGG convention.

## **3. RESULTS**

## 3.1. Identification of WRKY transcription factor in Tea plant

First of all, from the tea plant genome, 92 probable associates of WRKY transcription factor genes were identified after removal of redundant and incomplete protein sequences. The identified 92 WRKY were renamed as TEA015194.1 to TEA020191.1 on the bases of the regularity order of the WRKY TF genes on chromosomes (1-13) of tea. The length of *CsWRKY* protein sequences ranges from 29 (TEA008808.1) to 735 (TEA030233.1) amino acids. The isoelectric point ranged from 4.77 (TEA020545.1) to 10.071 (TEA028765.1), suggesting the operation of the different *CsWRKYs* in various microenvironments (18). Out of them, 46 were found to be basic in nature, 2 were neutral, and 44 were acidic in nature. Interestingly composition of the total proteins (87.09 %) of *CsWRKYs* showed higher proportion of WRKY superfamily protein (average ~715 %) amino acid. The parameters used to describe have been shown in (Table 1).

#### 3.2. Chromosomal location of WRKY in tea plant

Identified CsWRKY genes were found to be distributed in the thirteen tea chromosomes with uneven distribution (Table 2). The study found that relatively more CsWRKY genes are distributed on chromosome 3 (25.80 %), and least on chromosome 5 (8.06 %). This paradox suggested that the mapped CsWRKY genes on all the chromosomes had a significantly uneven distribution and non-random. As per our chromosomal distribution study, the CsWRKY genes were mostly located at chromosome numbers 1 to 10, 12, and 13. The chromosome number 1 was found to contain the maximum number of CsWRKY genes and the least were present on Chr. 12 and 13. The chromosomal location of TEA020545.1, TEA022377.1, TEA006586.1, TEA026188.1, TEA001873.1, and TEA011588.1 were not found in the analysis.

Table 1. List of putative CsWRKY genes and their physicochemical properties, variants, and number of WRKY domains.

Sl. No.	Gene ID	Protein Name	TF ID	Taxon omic ID	Proteins properties			WRKY Domai	ľ n
					Length (aa)	MW (Da)	pI	Start	End
1	TEA0 15194. 1	WRKY DNA- binding protein 72, putative	XP_028051760. 1	542762	541	58403. 5	7.3916	211	270
2	TEA0 28099. 1	WRKY transcription factor 1-like isoform	XP_028051846. 1	542762	468	51752.	6.4626	195 369	252 427

3	TEA0	WRKY	XP_028051918.	542762	557	61469.	8.8917	221	278
	02467.	DNA-	1			3		389	447
	1	binding							
		protein 33							
		isoform							
4	TEA0	FMN-linked	XP_028051958.	542762	346	38234.	10.136	275	332
	02471.	oxidoreducta	1			3	9		
	1	ses							
		superfamily							
		protein							
5	TEA0	WRKY	XP_028052697.	542762	29	33397.	5.0936	130	190
	08808.	DNA-	1			2			
	1	binding							
		protein 70,							
		putative							
		isoform							
6	TEA0	WRKY	XP_028053974.	542762	296	32041.	6.3139	151	208
	01162.	DNA -	1			2			
	1	binding							
		domain							
7	TEA0	Translation	XP_028054239.	542762	165	18114.	4.8841	128	147
	30998.	initiation	1			9			
	1	factor 6							
8	TEA0	WRKY	XP_028055071.	542762	325	36726.	6.9482	131	191
	07370.	DNA-	1			6			
	1	binding							
		protein 70,							
		putative							
•		isoform	ND 020055055	5407.00	220	25045	6.0500	1.61	010
9	TEA0	WRKY	XP_028057975.	542762	328	35845.	6.2539	161	219
	14384.	transcription	1			6			
	1	factor 22-							
10		like	ND 020050704	540760	200	227.40	7 (0(0	150	010
10	1EA0	WRKY18	XP_028059704.	542762	290	32749.	/.6868	156	213
	12365.	transcription	1			9			
11		WDVV19	VD 028050727	512762	202	22512	7 6202	120	100
11	12360	transcription	AF_020039727.	542702	293	32312. 2	1.0292	150	100
	12300.	factor	1			2			
12		WRKV	XP 028061050	542762	227	25080	8 6764	130	107
14	10167		AF_028001039.	542702	221	23909.	0.0704	139	197
	17107.	binding -	1			1			
	1	domain							
13	TEA0	WRKY	XP 028062068	542762	186	20611	5 6583	117	175
10	33820	transcription	1	512702	100	7	5.0505	11,	175
	1	factor	-			,			
14	TEA0	WRKY	XP 028064109.	542762	613	66861	6.6157	352	410
	05142.	transcription	1						
	1	factor 6-like							
15	TEA0	Dihydrofolat	XP 028066881.	542762	117	12659.	8.4876	9	32
	29093.	e	1			7			
	1	synthase/fol							
		ylpolygluta							
		mate							
		synthase							
16	TEA0	Putative	XP_028067460.	542762	589	63901.	7.0207	322	380
	31948.	WRKY1a	1			2			
	1	transcription							
		factor							
17	TEA0	WRKY	XP_028071264.	542762	442	48385.	5.1083	230	288
	04305.	transcription	1			5			
	1	factor							

18	TEA0	WRKY	XP_028072625.	542762	475	52431.	8.7562	197	253
	04132.	family	1			2			
	1	transcription							
		factor family							
10	TEAO	protein	VD 029072777	540760	260	20602	5 6250	76	124
19	1EA0 23777	WKKY	XP_028072777.	542762	269	29692. 8	5.6358	/6	134
	1	factor	1			0			
20	TEA0	WRKY	XP 028075202.	542762	287	30358.	9.6215	33	91
-	05334.	family	1			1			-
	1	transcription							
		factor							
21	TEA0	Putative	XP_028077060.	542762	324	35493.	9.8054	253	310
	06754.	WRKY	1			3			
	1	transcription							
		factor 15 -							
22	TEAO	WRKY	XP 028077318	542762	330	37/08	5 2501	162	220
	27312	transcription	1 AF_020077518.	542702	550	57490.	5.2501	102	220
	1	factor 22-	1			5			
	1	like isoform							
23	TEA0	WRKY	XP_028077632.	542762	280	31314.	5.8787	75	133
	28913.	transcription	1			5			
	1	factor 5							
	<b>TTT</b> + 0	isoform		F 10 F 10	22.6	0544		100	101
24	TEA0	WRKY	XP_028077914.	542762	336	37641	5.0619	120	181
	11602.	DNA- binding	1						
	1	binding protein 30							
		protein 50,							
25	TEA0	WRKY	XP 028078525.	542762	388	40986.	6.3118	182	242
	27100.	transcription	1	0.2702	200	2	0.0110	102	
	1	factor 55							
		isoform							
26	TEA0	WRKY	XP_028079096.	542762	523	57335.	5.7849	211	266
	27106.	transcription	1			2		380	436
27		factor	XD 020070122	540760	212	24460	4 7205	125	105
21	1EA0 27083		XP_028079122.	542762	313	34468. 7	4.7385	135	195
	27005.	binding	1			1			
	1	protein 70.							
		putative							
		isoform							
28	TEA0	WRKY	XP_028079124.	542762	220	23669.	6.6659	14	74
	27100.	transcription	1			1			
	1	factor 55							
20	TEAO	1SOIOTM	VD 029090001	542762	576	62026	7.050	242	208
29	1EA0 08318	transcription	AF_020000001.	342702	570	03920. 6	1.939	242 408	290 466
	1	factor	1			0		400	400
30	TEA0	Plant zinc	XP 028081295.	542762	322	35992.	10.262	257	314
	04792.	cluster	1			7	7		
	1	domain							
31	TEA0	WRKY	XP_028081368.	542762	486	53307.	6.1507	229	288
	07721.	DNA-	1			1			
	1	binding							
		protein 9,							
		putative							
33	TEAO	WRKV18	XP 028081784	542762	312	34821	7 4044	153	212
	27058	transcription	1	5 12102	512	1	,	155	212
			1 .	1	1	-	1	1	1

34	TEA0	WRKY	XP_028082795.	542762	303	33595.	5.9643	162	219
	22377.	DNA-	1			8			
	1	binding							
		protein 57							
25	TEAO	1soform WDVV	VD 02002200	542762	221	25502	7 0020	145	205
35	1EA0 08812	WKK I	AP_028085209.	342702	521	55502. 8	1.9828	143	203
	1	factor 55	1			0			
	-	isoform							
36	TEA0	WRKY	XP_028083248.	542762	296	33397.	5.0936	130	190
	08808.	DNA-	1			2			
	1	binding							
		protein 70,							
		putative							
27	TEAO	isoform	XD 020004020	540760	102	20000	0.0002	101	150
37	1EA0 27108	WKKY	XP_028084020.	542762	182	20808.	9.9083	101	159
	2/190.	factor	1			5			
	1	putative							
38	TEA0	WRKY	XP 028084310.	542762	193	22072.	8.1188	70	128
	25613.	DNA-	1			7			
	1	binding							
	TEA0	protein 27,							
	11326.	putative							
	1	<b>D</b>	ND 00005500	5 10 5 10	0.5.5	40.440	4 5004	100	100
39	TEA0	Putative	XP_028085508.	542762	355	40449.	4.7801	128	188
	05213.	WRKY	1			9			
	1	factor							
40	TEA0	WRKY	XP_028087006.	542762	336	38060.	6.3161	127	187
	31299.	transcription	1			1		-	
	1	factor 41							
		family							
		protein							
41	TEA0	Putative	XP_028089465.	542762	307	34434.	7.15	158	215
	0/19/.	WRKY	1			/			
	1	factor							
42	TEA0	WRKY	XP 028089628	542762	337	38034.	10.417	270	327
	11330.	transcription	1	0.2702	007	4	8		0-1
	1	factor 39							
		family							
		protein							
43	TEA0	WRKY	XP_028091443.	542762	522	57343	7.6458	232	288
	28167.	transcription	1					408	466
44		WPKV	VD 028001585	542762	337	37060	6 6786	197	244
	23233	super family	1 AF_020091303.	542702	557	9 9	0.0780	107	244
	1	protein	1			,			
45	TEA0	WRKY	XP_028091903.	542762	478	52138	9.0901	199	255
	04132.	family	1					397	454
	1	transcription							
		factor family							
11		protein	VD 020002544	540750	222	05241	7.0050	140	201
40	1EA0	WKKY	AP_028092641.	542762	222	25341.	1.2358	148	206
	30336. 1	transcription	1			/			
	1	factor							
47	TEA0	P-loop	XP 028093134	542762	99	10689	9,2266	71	93
	30312.	containing	1	2.2.02		8			
	1	nucleoside							
		triphosphate							
		hydrolases							

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		superfamily							
		protein							
		isoform							
48	TEA0	WRKY	XP_028093640.	542762	186	21087.	9.6582	107	165
	06586.	transcription	1			5			
	1	lactor,							
49	TEAO	WRKY	XP 028094020	542762	255	29081	1 8/193	55	113
47	23297	super family	1	542702	233	8	4.0495	55	115
	1	protein	1			0			
50	TEA0	WRKY	XP_028094353.	542762	433	48408.	9.2572	155	212
	05358.	transcription	1			7		329	387
	1 J	factor 1-like							
		isoform							
51	TEA0	WRKY	XP_028094837.	542762	331	36724.	6.8805	125	185
	26188.	DNA-	1			8			
	1	binding							
		protein 70,							
		julative							
52	TEAO	WRKY	XP 028100462	542762	322	35693	6 8/196	177	234
54	01873	super family	1	572702	522	6	0.0490	1//	234
	1	protein	1			0			
53	TEA0	WRKY	XP 028100469.	542762	243	28316.	9.0231	139	163
	02329.	DNA -	1			6		187	227
	1	binding							
		domain							
54	TEA0	Plant zinc	XP_028100648.	542762	319	35482	9.9554	247	304
	23720.	cluster	1						
	1	domain							
55	TEA0	WRKY	XP_028101411.	542762	715	77797.	7.1857	248	305
	30233.	super family	1			6		452	510
	1	protein	ND 020102260	540560		<000 <b>7</b>	6 6 4 1 0	220	207
56	1 EA0	WRKY	XP_028103268.	542762	222	60997. 5	6.6413	230	287
	11300.	factor	1			3		202	441
57	TEAO	WRKY	XP 028106608	542762	569	63061	8 282	227	284
57	28505	DNA-	1	542702	507	7	0.202	392	450
	1	binding				-			
		protein 33							
		isoform							
58	TEA0	WRKY	XP_028106713.	542762	364	39755.	5.0625	129	190
	29100.	DNA-	1			8			
	1	binding							
		protein 30,							
50	TEAO		VD 028107205	542762	363	40371	7 5/37	163	221
39	16074	transcription	AF_020107293.	542702	505	9	7.5457	105	221
	1	factor	1						
60	TEA0	WRKY	XP 028107356.	542762	173	19830.	10.035	93	151
	23807.	DNA -	1			3	2		
	1	binding							
		domain							
61	TEA0	WRKY	XP_028107506.	542762	577	63049.	6.5784	330	388
	10793.	family	1			6			
	1	transcription							
0	TEAO	tactor	VD 020100417	540750	100	22457	10.107	100	170
02	1EA0 13771		AP_028109415.	542762	199	22457. 5	10.107	120	1/8
	15771.	binding -	1			5	/ ·		
	1	domain							

63	TEA0	WRKY	XP 028109416	542762	316	34951	6 5837	151	209
00	13772	transcription	1	0.2702	510	4	0.2027	101	207
	1	factor 22-	1						
	1	like							
64	TEAO	WPKV	XP 028100764	542762	310	35605	7 3257	156	214
04	1 LAU 28/73		1	342702	519	7 7	1.5251	150	214
	20475.	binding -	1			/			
	1	domain							
65	TEAO	WDVV	VD 029110002	512762	162	51219	7 6727	267	225
05	1 EAU	W KK I fomily	AF_020110002.	342702	405	<i>J</i> 1210.	1.0151	207	525
	09907.	transportion	1			0			
	1	factor							
((	TEAO	WDVV	VD 020111112	5407(0)	526	57256	0.0045	226	202
00	1 EAU		AP_028111115.	342702	320	27550.	0.2243	230	292
	05587.	franscription	1			3		411	469
		Tactor	XD 020112541	540760	005	0((11	0.5004	150	016
67	IEA0	WRKY	XP_028112541.	542762	235	26611.	9.5994	158	216
	06/55.	transcription	1			1			
	1	factor 13							
		family							
(0)		protein	ND 020112001	5407.00	220	05710	10.170	0.5.1	200
68	TEA0	WRKYII	XP_028113091.	542762	328	35713.	10.179	251	309
	01484.	transcription	1			4	8		
(0)	1	factor	ND 020110256	<b>5</b> 10 <b>7</b> 60				211	250
69	TEA0	WRKY	XP_028113356.	542762	520	57061.	6.1166	214	270
	27106.	transcription	1			3		384	440
	1	factor						1.70	
70	TEA0	Putative	XP_028115186.	542762	311	34324.	7.0322	159	217
	08513.	WRKY	1			3			
	1	transcription							
		factor 48 -							
		like protein				10=01		1.00	100
71	TEA0	WRKY	XP_028115466.	542762	363	40781	4.9772	130	190
	31299.	transcription	1						
	1	factor 41							
		family							
50		protein	ND 020115574	540760	107	22227	0.6224	110	176
12	1EA0	WRKY	XP_028115574.	542762	197	22227.	9.6324	118	1/6
	07789.	transcription	1			3			
72		Tactor 25-1	ND 020115570	540760	226	26799	4 00 40	150	217
13	1 EAU	WKKY	AP_028115579.	542762	330	30/88.	4.8849	159	217
	0/804.	factor 22	1			/			
	1	like isoform							
74	TEAO	Dutativa	VD 029116770	510760	227	27674	4 7701	116	175
/4	1 EAU 20545		AF_020110770.	342702	337	57074	4.//21	110	175
	20345.	wnni	1						
	1	factor							
75	TEAO	WDKV	VD 028117015	542762	735	70548	6 3 6 5 1	300	366
15	20222	wKKI	AF_020117015.	542702	133	0	0.5054	509	570
	30233. 1	super family	1			0		521	519
76		WEEV	VD 020117015	512762	275	21056	1 1017	52	111
70	17542		AF_020117013.	342702	213	51050.	4.4042	55	111
	1/342.	DNA- binding	1			9			
	1	onding protoin 25							
		protein 55,							
77	TEAO		VD 020117024	545076	221	27046	0 577	140	212
//	1EAU	WKKY	$AP_{02\delta11/\delta54}$	545276 2	331	37046. 7	8.377	149	212
	1/544.	super family	1	2		/			
70		Translat	<b>VD</b> 020110070	540750	124	14500	0.4004	100	126
/8	1EA0	I ranslation	AP_028118079.	542762	154	14560.	8.4804	106	126
	30998.	initiation	1			6			
1	1	factor 6		1	1	1	1	1	1

70	TEAO	WRKV	YP 028118081	542762	235	26582	8 1111	100	158
17	00122	transprintion	1	542702	235	20302.	0.1111	100	150
	00152.		1			1			
	1	lactor 18-							
		like							
80	TEA0	FMN-linked	XP_028118126.	542762	340	37988.	6.525	313	332
	08134.	oxidoreducta	1			8			
	1	ses							
		superfamily							
		protein,							
		partial							
81	TEA0	Plant zinc	XP 028119049.	542762	282	31177.	9.8933	250	276
	23720.	cluster	1		-	8			
	1	domain							
82	TEA0	WRKY	XP 028119210	542762	559	60702	8 162	305	363
	05334	family	1	0.2702	557	6	0.102	202	505
	1	transcription	1			Ū			
	1	factor							
92	TEAO	WDVV	VD 029110425	512762	226	27640	5 2655	120	101
05	1 EAU 20297		AF_020119433.	542702	550	370 <del>4</del> 0.	5.5055	120	101
	20387.	DNA- hinding	1			1			
	1	billiding							
		protein 50,							
0.4			XD 020120001	540760	(11	((70)	7.4406	262	221
84	IEA0	WRKY	XP_028120991.	542762	611	66726.	7.4496	263	321
	03077.	DNA-	1			9			
	1	binding							
		protein 72,							
		putative							
85	TEA0	WRKY	XP_028121796.	542762	547	60193	6.4311	247	306
	26850.	DNA-	1						
	1	binding							
		protein 72,							
		putative							
86	TEA0	WRKY	XP_028122384.	542762	318	35125.	8.0069	164	223
	11154.	transcription	1			3			
	1	factor							
87	TEA0	Plant zinc	XP_028124442.	542762	345	38960.	10.387	279	337
	22842.	cluster	1			9			
	1	domain							
88	TEA0	Putative	XP_028124717.	542762	303	33786.	4.8139	114	170
	19607.	WRKY	1			8			
	1	transcription							
		factor							
89	TEA0	WRKY	XP 028124928.	542762	181	20467	10.071	102	160
	28765.	transcription	1				8		
	1	factor,							
		putative							
90	TEA0	WRKY	XP 028125074.	542762	192	21654.	6.504	104	162
	05255	transcription	1			8			
	1	factor 51	-			Ť			
	-	family							
		protein							
91	TEA0	Transcriptio	XP 028127034	542762	604	65595	6 6741	244	301
/ <b>1</b>	20117	n termination	1	512102	00-	4	0.07-11	417	475
	1	factor	1			-		71/	475
	1	MTERFQ							
		chloroplastic							
92	TEAO	WRKV	XP 028127260	512762	320	35075	8 8022	160	210
74	20101	transcription	1	J+2702	520	3	0.0722	100	217
	1	factor	1			5			
1	1	Tactor	1	1	1	1	1	1	

Table 2. Chromosomal distribution of CsWRKYs.

Sl no.	Gene ID	Protein Name	TF ID	Taxonomi	Chromosomal location
				c ID	
1	TEA015194. 1	WRKY DNA- binding protein 72, putative	XP_028051760. 1	542762	Chr05:776392-781485(-)
2	TEA028099.	WRKY	XP_028051846.	542762	Chr01:1882448-
	1	transcription factor 1-like isoform	1		1885976(-)
3	TEA002467. 1	WRKY DNA- binding protein 33 isoform	XP_028051918. 1	542762	Chr03:299883- 302756(+)
4	TEA002471. 1	FMN-linked oxidoreductases superfamily protein	XP_028051958. 1	542762	Chr03:1688743- 1716217(+)
5	TEA008808. 1	WRKY DNA- binding protein 70, putative isoform	XP_028052697. 1	542762	Chr04:430848-433334(-)
6	TEA001162. 1	WRKY DNA - binding domain	XP_028053974. 1	542762	Chr03:686293-690246(-)
7	TEA030998. 1	Translation initiation factor 6	XP_028054239. 1	542762	Chr06:1079774- 1096574(-)
8	TEA007370. 1	WRKY DNA- binding protein 70, putative isoform	XP_028055071. 1	542762	Chr01:307023- 309236(+)
9	TEA014384. 1	WRKY transcription factor 22-like	XP_028057975. 1	542762	Chr02:1272608- 1274342(+)
10	TEA012365. 1	WRKY18 transcription factor	XP_028059704. 1	542762	Chr01:732103-745499(-)
11	TEA012360. 1	WRKY18 transcription factor	XP_028059727. 1	542762	Chr01:823531-826145(-)
12	TEA019167. 1	WRKY DNA - binding domain	XP_028061059. 1	542762	Chr01:1575674- 1578863(-)
13	TEA033820. 1	WRKY transcription factor	XP_028062068. 1	542762	Chr01:1844883- 1846475(-)
14	TEA005142. 1	WRKY transcription factor 6-like	XP_028064109. 1	542762	Chr02:601835-605813(-)
15	TEA029093. 1	Dihydrofolate synthase/folylpoly glutamate synthase	XP_028066881. 1	542762	Chr07:723455-732491(-)
16	TEA031948. 1	Putative WRKY1a transcription factor	XP_028067460. 1	542762	Chr01:638294- 642060(+)
17	TEA004305. 1	WRKY transcription factor	XP_028071264. 1	542762	Chr03:997322- 1000207(-)
18	TEA004132[ .1	WRKY family transcription factor family protein	XP_028072625. 1	542762	Chr05:714276- 718356(+)

19	TEA023777.	WRKY	XP 028072777.	542762	Chr05:262202-
	1	transcription	1		263401(+)
		factor			
20	TEA005334.	WRKY family	XP_028075202.	542762	Chr05:366521-
	1	transcription	1		369321(+)
		factor			
21	TEA006754.	Putative WRKY	XP_028077060.	542762	Chr05:311583-318065(-)
	1	transcription	1		
		factor 15 -like			
		protein			
22	TEA027312.	WRKY	XP_028077318.	542762	Chr02:404328-
	1	transcription	1		405790(+)
		factor 22-like			
		isoform			
23	TEA028913.	WRKY	XP_028077632.	542762	Chr05:811788-
	1	transcription	1		812900(+)
		factor 5 isoform			
24	TEA011602	WRKY DNA-	XP 028077914	542762	Chr13:64541-66364(+)
	1	hinding protein	1	512702	
	1	30 nutative	1		
25	TEA027100.	WRKY	XP 028078525.	542762	Chr01:1666955-
	1	transcription	1	0.2702	1670001(-)
	-	factor 55 isoform	-		()
26	TEA027106.	WRKY	XP 028079096.	542762	Chr01:242198-254828(-)
-	1	transcription	1		
		factor			
27	TEA027083.	WRKY DNA-	XP_028079122.	542762	Chr01:1641047-
	1	binding protein	1		1643714(+)
		70, putative			
		isoform			
28	TEA027100.	WRKY	XP_028079124.	542762	Chr01:1666955-
	1	transcription	1		1670001(-)
		factor 55 isoform			
29	TEA008318.	WRKY	XP_028080001.	542762	Chr01:783601-794873(-)
	1	transcription	1		
		factor			
30	TEA004792.	Plant zinc cluster	XP_028081295.	542762	
21	I	domain	1 VD 020001260	540760	C1 01 1229575
31	1EA007721.	WKKY DNA-	XP_028081368.	542762	Chr01:1338565-
	1	binding protein 9,	1		1340946(-)
22	TE 4 027059		VD 020001704	540760	Chr01,1920700
33	1 EA027058.	WKKY18	AP_028081784.	542762	Cnr01:1850700-
	1	factor	1		1855870(-)
		Tactor			
34	TEA022377.	WRKY DNA-	XP_028082795.	542762	-
	1	binding protein 57	1		
		isoform			
35	TEA008812.	WRKY	XP_028083209.	542762	Chr04:314891-
	1	transcription	1		322381(+)
		factor 55 isoform			
36	TEA008808.	WRKY DNA-	XP_028083248.	542762	Chr04:430848-433334(-)
	1	binding protein	1		
		70, putative			
27	TE 4 027100	1SOIOFM	<b>VD</b> 020004020	540760	Ch::01:705400
31	1 EAU2/198.	WKKY	AP_028084020.	542762	Cnr01:/95490-
	1	factor putotion	1		191030(+)
20	TEA025(12	WDKV DNA	VD 020004210	540760	Chr02.2049972
30	1 EAU23013.	winding protoin	лг_020004310. 1	342702	2050150(+)
	т ТЕΔ011326	27 putative	1		2030130(+)
	1 1	21, putative			

39	TEA005213.	Putative WRKY	XP 028085508.	542762	Chr08:766533-
	1	transcription	1		770719(+)
		factor			
40	TEA031299	WRKY	XP 028087006	542762	Chr05:552276-554060(-)
••	1	transcription	1	512762	01100.002270 00 1000()
	-	factor 41 family	1		
		protein			
41	TEA007197	Putative WRKY	XP 028089465	542762	Chr12.255889-258533(-)
••	1	transcription	1	512762	01112.200000 2000000()
	1	factor	1		
42	TEA011330	WRKY	XP 028089628	542762	Chr02·272636-275687(-)
	1	transcription	1	512702	CIII 02.272030 273007()
	1	factor 39 family	1		
		nrotein			
43	TEA028167	WRKY	XP 028091443	542762	Chr01:676453-
	1	transcription	1	542702	$680728(\pm)$
	1	factor	1		000720(+)
11	TE A 022222	WDKV super	VD 020001505	542762	Chr05:255240,256006()
	1 EA025255.	family protain	AF_020091303.	342702	CIII05.555249-550900(-)
45	TEA004122	WDKV family	T VD 028001002	542762	Chr05:714276
45	1EA004152.	transarintian	AF_020091903.	342702	718256(+)
	1	factor family	1		/18550(+)
		nactor family			
16	TEA020226	WDKV family	VD 02002641	542762	Chr04:1006727
40	1 EAU30330.	WKKI lainiiy	AP_028092041.	342762	1000288(+)
	1	transcription	1		1009288(+)
47	TE 4 020212	Tactor	VD 020002124	540760	C1-01-502284 520724()
4/	1EA030312.	P-loop containing	XP_028093134.	542762	Chr01:508284-529724(-)
	1	nucleoside	1		
		tripnosphate			
		hydrolases			
		superfamily			
40	TE 100(59)	wpw	VD 020002640	540760	
48	1 EA006586.	WKKY	XP_028093640.	542762	
	1	transcription	1		
40	TE 4 02 22 0 7	Tactor, putative	VD 020004020	540760	C1.07.79025.05792()
49	1 EA023297.	formily protoin	AP_028094020.	542762	Chr07:78925-95783(+)
	1	ranning protein	1		
50	TEA005358.	WRKY	XP_028094353.	542762	Chr03:180957-
	1 J	transcription	1		185253(+)
		factor 1-like			
		isoform			
51	TEA026188.	WRKY DNA-	XP_028094837.	542762	
	1	binding protein	1		
		70, putative			
		isoform			
52	TEA001873.	WRKY super	XP_028100462.	542762	
	1	family protein	1		
53	TEA002329	WRKY DNA -	XP 028100469	542762	Chr02:152376-155299(-)
	1	binding domain	1	512762	011020102070 100200()
54	-	Di	ND 000100640	5 407 50	CI 01 1220077
54	1EA023720.	Plant zinc cluster	XP_028100648.	542762	Chr01:1329877-
	1	domain	1		13316/2(+)
55	TEA030233.	WRKY super	XP_028101411.	542762	Chr03:856995-
	1	family protein	1		860560(+)
56		-		<b>.</b>	
50	TEA011500	WRKV	XD 000102060	5/10/160	
	TEA011588.	WRKY	XP_028103268.	542762	
	TEA011588. 1	WRKY transcription	XP_028103268. 1	542762	
57	TEA011588. 1	WRKY transcription factor	XP_028103268. 1	542762	Chr01:409529
57	TEA011588. 1 TEA028505.	WRKY transcription factor WRKY DNA- binding protein 22	XP_028103268. 1 XP_028106608.	542762	Chr01:408528-

58	TEA029100.	WRKY DNA-	XP_028106713.	542762	Chr07:650747-
	1	binding protein 30, putative	1		653443(+)
59	TEA016074.	WRKY	XP_028107295.	542762	Chr09:1649386-
	1	factor	1		1650694(+)
60	TEA023807. 1	WRKY DNA - binding domain	XP_028107356. 1	542762	Chr04:59114-62001(+)
61	TEA010793.	WRKY family	XP_028107506.	542762	Chr01:1434099-
	1	factor	1		1440574(+)
62	TEA013771. 1	WRKY DNA - binding domain	XP_028109415. 1	542762	Chr02:892500-906216(-)
63	TEA013772.	WRKY	XP_028109416.	542762	Chr02:916853-919668(-)
	1	factor 22-like	1		
64	TEA028473.	WRKY DNA -	XP_028109764.	542762	Chr02:2772424-
<i>(</i> <b>-</b>	1	binding domain	1	5.407.60	2773965(+)
65	TEA009907. 1	transcription	XP_028110002.	542762	Chr08:538218-540/13(-)
		factor			
66	TEA003387.	WRKY transcription	XP_028111113.	542762	Chr01:3039767- 3046181(+)
	-	factor	-		
67	TEA006755.	WRKY	XP_028112541.	542762	Chr07:816877- 821297(+)
	1	factor 13 family	1		0212)7(1)
68	TE A 001/19/	protein WPKV11	VD 028112001	542762	Chr04.2844474
00	1 EA001464.	transcription	1	542702	2845743(+)
(0)	TE 1027107	factor	ND 020112256	540760	C1-01-040109-054909()
09	1 EA02/106.	transcription	XP_028113356.	542762	Cnr01:242198-254828(-)
		factor		5 10 5 10	C1 00 100 50 55
70	TEA008513.	Putative WRKY transcription	XP_028115186.	542762	Chr02:1085977- 1087276(-)
	-	factor 48 -like	-		100/2/0()
71	TEA031299	protein WRKY	XP 028115466	542762	Chr05:552276-554060(-)
/ <b>1</b>	1	transcription	1	512702	Cinco:352270 551000()
		factor 41 family			
72	TEA007789.	WRKY	XP_028115574.	542762	Chr04:1024979-
	1	transcription	1		1027228(-)
73	TEA007804.	WRKY	XP_028115579.	542762	Chr04:1057597-
	1	transcription	1		1059281(-)
		isoform			
74	TEA020545.	Putative WRKY	XP_028116770.	542762	-
		factor	1		
75	TEA030233. 1	WRKY super family protein	XP_028117015. 1	542762	Chr03:856995- 860560(+)
76	TEA017542.	WRKY DNA-	XP_028117815.	542762	Chr03:1315202-
	1	binding protein 35, putative	1		1316756(+)
77	TEA017544.	WRKY super	XP_028117834.	5452762	Chr03:1777201-
	1	family protein	1		1779660(+)

78	TEA030998.	Translation	XP_028118079.	542762	Chr06:1079774-
	1	initiation factor 6	1		1096574(-)
79	TEA008132.	WRKY	XP_028118081.	542762	Chr01:1171624-
	1	transcription	1		1180172(-)
90	TE 4 009124	factor 18-like	VD 020110126	542762	Chr01.426612
ðU	1EA008134.	oxidoreductases	AP_028118120.	542762	Cnr01:420012- 433580(+)
	1	superfamily	1		455500(1)
		protein, partial			
81	TEA023720.	Plant zinc cluster	XP_028119049.	542762	Chr01:1329877-
	1	domain	1		1331672(+)
82	TEA005334.	WRKY family	XP_028119210.	542762	Chr05:366521-
	1	transcription	1		369321(+)
		factor			
83	TEA020387.	WRKY DNA-	XP_028119435.	542762	Chr01:625156-626983(-)
	1	binding protein	1		
84	TEA003077.	WRKY DNA-	XP 028120991.	542762	Chr10:1022993-
-	1	binding protein	1		1044947(-)
		72, putative			
85	TEA026850.	WRKY DNA-	XP_028121796.	542762	Chr03:1407223-
	1	binding protein	1		1412123(+)
86	TEA011154	VPKV	XP 028122384	542762	Chr03:7/1152
00	1	transcription	1	542702	750066(+)
	-	factor	-		
87	TEA022842.	Plant zinc cluster	XP_028124442.	542762	Chr03:997123-999071(-)
	1	domain	1		
88	TEA019607.	Putative WRKY	XP_028124717.	542762	Chr06:746509-749310(-)
	1	transcription	1		
00	TE 4029765	factor	XD 020124020	540760	01-10-169505
89	1 EA028705.	WKK I	AP_028124928.	542762	Cnr10:108505- 172993( $\perp$ )
	1	factor, putative	1		172775(1)
90	TEA005255.	WRKY	XP_028125074.	542762	Chr11:669585-674439(-)
	1	transcription	1		
		factor 51 family			
01		protein		E 10E (0	
91	TEA020117.	Transcription	XP_028127034.	542762	Chr02:534002-544254(-)
	1	MTFRF9	1		
		chloroplastic			
92	TEA020191.	WRKY	XP_028127360.	542762	Chr06:2601588-
	1	transcription	1		2609578(+)
		factor			

#### 3.3. Phylogenetic analysis of CsWRKY

An evolutionary tree is a schematic diagram or tree that depicts the predicted evolutionary connections and phylogeny of distinct biological organisms or other entities based on genetic similarities and differences. The taxa connected in the tree are thought to be descended from an ancestral lineage. The multiple sequence alignment was performed for all sequences of CsWRKY proteins and the construction of phylogenetic tree was done using the maximum-likelihood (ML) mode in MEGA 7.0 with 1,000 bootstrap replicates. The tree of phylogeny was built using neighbor-joining. For 1,000 encounters, the bootstrap parameters were determined.



**Figure 1. Phylogenetic tree demonstrating interactions among Tea WRKY proteins.** The multiple sequence alignment was per- formed for all sequences of *CsWRKY* proteins and the construction of phylogenetic tree was done using the maximum-likelihood (ML) mode in MEGA 7.0 with 1,000 bootstrap replicates.

## 3.4. Expression of WRKY in different plant parts

*CsWRKY* gene expression levels in Tea plant tissues (Axillary buds, buds, leaves, flowers, ovaries, roots, seeds, & stems) were studied. Interestingly, TEA015194.1 was found to be overexpressed in roots.





Figure 2. WRKY expression profile in different parts of Tea plant.

#### 3.5. Co-expression analysis of different CsWRKY in various regions of the Tea Plant

DNA-seq data of CsWRKY families were obtained from the TeaCon databases, which include the expression levels across root, leaf, blossom, stem, and seed to analyze the expression profile, of the *CsWRKY*. The overall expression level of *CsWRKY* transcripts was estimated utilizing standardized analysis and visualization of gene expression information for each gene id of a transcript was performed (Figure 3, 4, and 5). Red (dark) denotes strong expression, yellow shows medium expression, and blue indicates lower expression of gene in the figure 3, 4 and 5.



Figure 3. Co-expression of *CsWRKY* genes (TEA002467.1, TEA002471.1, TEA015194.1, and TEA028099.1) in different parts of Tea plant. Red (dark) denotes strong expression, yellow shows medium expression, and blue indicates lower expression



**Figure 4.** Co-expression of *CsWRKY* genes (TEA001162.1, TEA007370.1, TEA008808.1, and TEA030998.1) in different parts of Tea plant. Red (dark) denotes strong expression, yellow shows medium expression, and blue indicates lower expression. Almost all the *CsWRKY* genes are expressed in all parts of the plant except TEA008808.1.



**Figure 5.** Co-expression of *CsWRKY* genes (TEA003077.1, TEA005334.1, TEA020387.1, and TEA026850.1) in different parts of Tea plant. Red (dark) denotes strong expression, yellow shows medium expression, and blue indicates lower expression. Expression of TEA026850.1, TEA020387.1, and TEA003077.1 are negligible.

#### 3.6. WRKY mediated signalling Pathway in Tea Plant

The MAP kinase pathway is related to stress adaptive pathways (19). Interestingly, WRKY is found to be associated with the MAPK pathway representing its role in stress responses (Figure 6).



Figure 6. CsWRKYs have a role in controlling stress responses through the MAP kinase pathway.

#### 3.7. Analysis of Tea plant motifs

The motifs present in *CsWRKY* protein were also analysed (Figure 7). Different coloured boxes represent the motif conservation in the *CsWRKY* protein. The size of the box indicates the overall length of the motif, whereas grey lines represent non-conserved sequences. Motifs 1-8 were identified as a total of 10 distinct motifs. Motifs one to three contain the WRKYGQK sequence. The length of the motif is indicated by the length of the box.

#### 3.8. CsWRKY gene structure analysis

The *CsWRKY* gene's exon-intron distribution from many main groups and their related subgroups are shown by boxes in figure 8. There are no upstream or downstream sequences in the majority of WRKY.



**Figure 7.** The conserved motif in *CsWRKY* in Tea plant protein is represented by different coloured boxes using MEME software. The length of the motif is represented by the size of the box, and non-conserved sequences are indicated by grey lines.



**Figure 8.** Diagram of the *Camellia sinensis* WRKY gene organisation. Distribution of exon-intron of CsWRKY genes from dissimilar major groups boxes using the GSDS software the blue boxes, yellow boxes, and black lines indicate upstream of initiation codon/downstream of stop codon, CDS, and introns, respectively.

## 4. CONCLUSION

Studies on Tea WRKYs were carried out on progression, characterization and functional explanation of *CsWRKYs* (20). Our present studies improved the understanding the WRKY genes in Tea and provide a comprehensive awareness into the tea genome. The *in silico* investigation for the WRKY transcription factor in the tea species (*Camellia sinensis*) genome is a crucial step toward identifying the molecular processes driving tea plant growth, advancement, and stress response (21). The evolutionary categorization of *WRKY* genes alongside Arabidopsis *WRKY* genes gives a foundation for studying the functional variety of *WRKY* genes in tea. In the current study, 92 *CsWRKY* genes were acknowledged from the complete genome of Tea plant. Phylogenetic relationships, classification, structure of gene, physical and chemical properties of proteins, chromosomal location, conserved motif of proteins and cis-acting element composition, were thoroughly investigated (22). Considering together, our outcomes will also offer a more widespread understanding on *CsWRKY* genes under growth, developmental and stress environments (1).

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## Author contributions

M.N and A.N conceived the study and designed the approach for execution. M.N and A.N. accomplished the analyses and construed the data. M.N and M.D prepared the figures and tables. A.N. transcribed the main manuscript text with M.N. and all the authors finally reviewed this.

#### **Competing interests**

The author(s) state no competing interests.

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