Supporting Information

Novel insights into understanding the molecular dialogues between *Bipolaroxin* and Gα and Gβ subunit of the wheat heterotrimeric G-protein during host-pathogen interaction

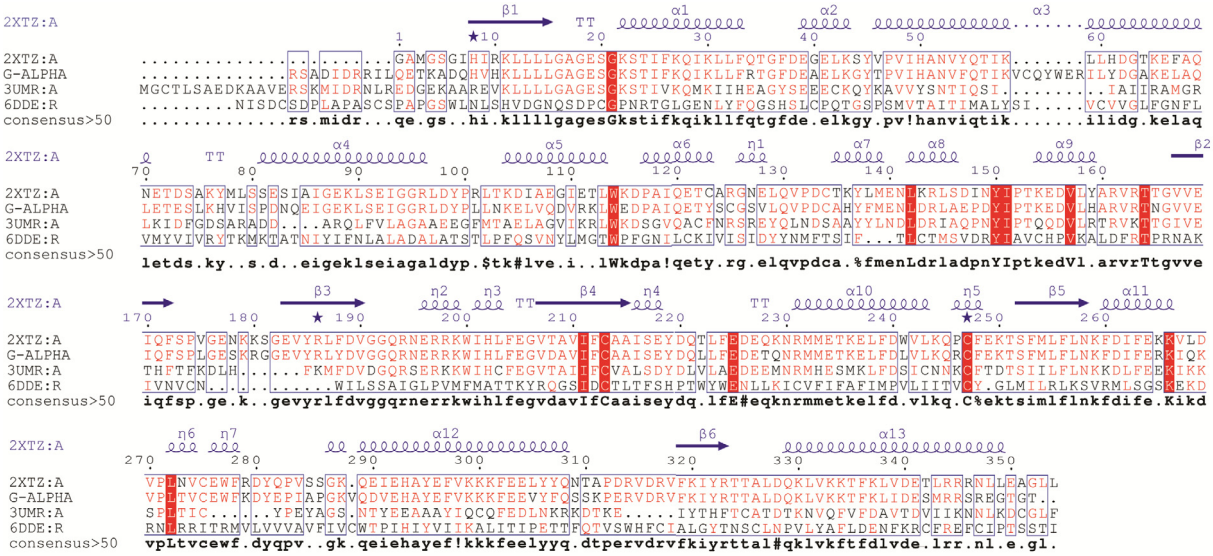
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Table S1 Inter-molecular contact analysis of the top ranked cluster of G-alpha and G-beta system with Biploaroxin

Protein-ligand system	Pairs	Distance	Type	Category of bonds
G-alpha-subunit with Biploaroxin	ARG75:HH21 - UNK371:O3	2.45614	Hydrogen Bond	Conventional Hydrogen Bond
	ARG75:HH21 - UNK371:O3	2.45614	Hydrogen Bond	Conventional Hydrogen Bond
	ARG178:HH11 - UNK371:O2	3.04711	Hydrogen Bond	Conventional Hydrogen Bond
	ARG178:HH12 - UNK371:O2	2.7013	Hydrogen Bond	Conventional Hydrogen Bond
	UNK371:H15 - LEU146:O	2.73565	Hydrogen Bond	Conventional Hydrogen Bond
	UNK371:O1 - LYS68:O	2.9578	Hydrogen Bond	Conventional Hydrogen Bond
	UNK371:O3 - CYS70:SG	3.38649	Hydrogen Bond	Conventional Hydrogen Bond
	UNK371:H15 - LEU146:O	2.73565	Hydrogen Bond	Conventional Hydrogen Bond
	UNK371:C18 - LYS68:O	3.63798	Hydrogen Bond	Carbon Hydrogen Bond
	UNK371:C18 - LYS68:O	3.63798	Hydrogen Bond	Carbon Hydrogen Bond
	LYS68 - UNK371	4.64792	Hydrophobic	Alkyl
	UNK371:C11 - VAL148	5.11052	Hydrophobic	Alkyl
	TYR116 - UNK371	4.92656	Hydrophobic	Pi-Alkyl
Protein-ligand system	Pairs	Distance	Type	Category of bonds
G-beta-subunit with Biploaroxin	UNK381:O1 - ASP73:OD2	4.81706	Electrostatic	Attractive Charge
	LYS256:HZ2 - UNK381:O4	2.06657	Hydrogen Bond	Conventional Hydrogen Bond
	LYS256:HZ2 - UNK381:O4	2.06657	Hydrogen Bond	Conventional Hydrogen Bond
	PHE306:H - UNK381:O3	2.37025	Hydrogen Bond	Conventional Hydrogen Bond
	PHE306:H - UNK381:O3	2.37025	Hydrogen Bond	Conventional Hydrogen Bond
	LEU352:H - UNK381:O3	2.75029	Hydrogen Bond	Conventional Hydrogen Bond
	LEU352:H - UNK381:O1	2.56318	Hydrogen Bond	Conventional Hydrogen Bond
	LEU352:H - UNK381:O3	2.75029	Hydrogen Bond	Conventional Hydrogen Bond
	UNK381:H15 - LEU352:O	2.2291	Hydrogen Bond	Conventional Hydrogen Bond
	UNK381:O1 - LEU352:O	2.6625	Hydrogen Bond	Conventional Hydrogen Bond
	UNK381:H15 - LEU352:O	2.2291	Hydrogen Bond	Conventional Hydrogen Bond
	UNK381:O3 - PHE306	4.13086	Hydrogen Bond	Pi-Donor Hydrogen Bond
	UNK381 - PRO76	4.61919	Hydrophobic	Alkyl
	UNK381 - ILE308	4.60817	Hydrophobic	Alkyl
	UNK381:C13 - LEU352	5.21716	Hydrophobic	Alkyl
	PHE306 - UNK381	5.32001	Hydrophobic	Pi-Alkyl
	PHE306 - UNK381:C13	4.1251	Hydrophobic	Pi-Alkyl

UNK: Biploaroxin

Figures



Figures S1. The multiple sequence-structure alignment of G-alpha subunit of wheat with its close structural homologs from Arabidopsis, and Human aligned using ESPript software. The secondary structure displayed on the top was obtained from the crystal structure G alpha protein AtGPA1 from Arabidopsis thaliana (PDB ID: 2XTZ_A).

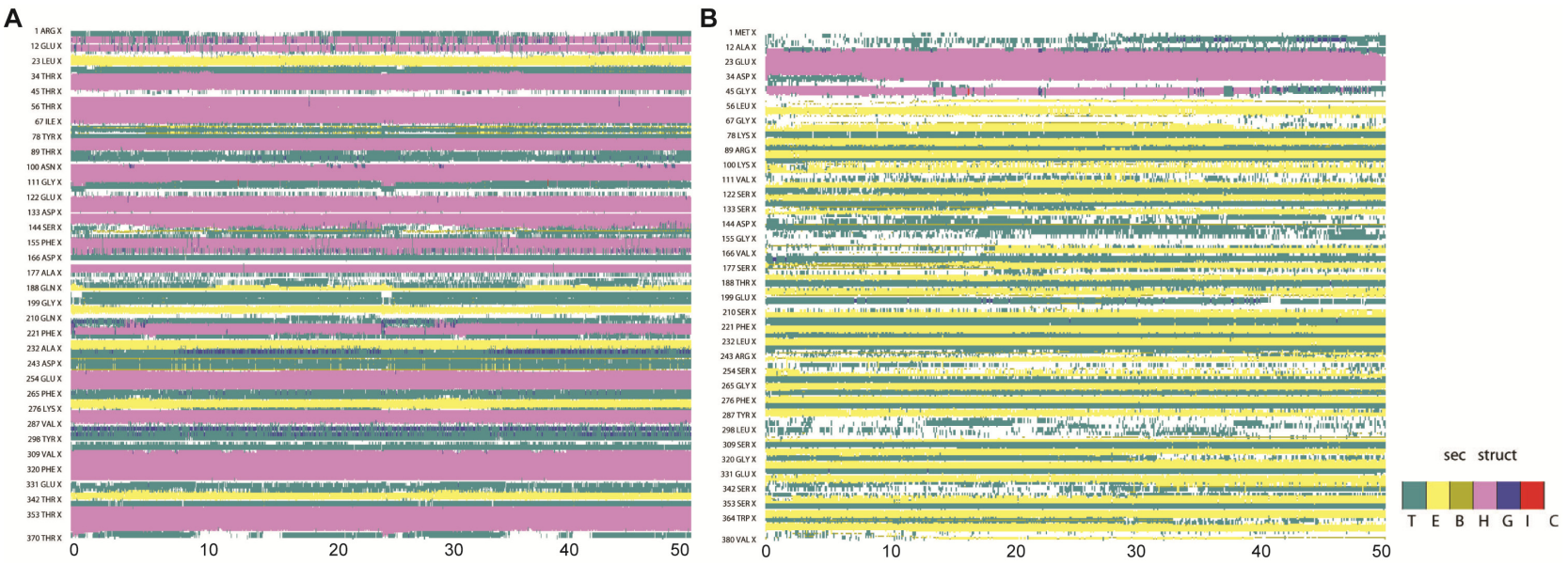


Figure S2 The time dependent evolution of secondary structure elements of the G-α and G-β sub-unit complex systems during 50 ns MD simulation. The image was plotted using timeline viewer of VMD.

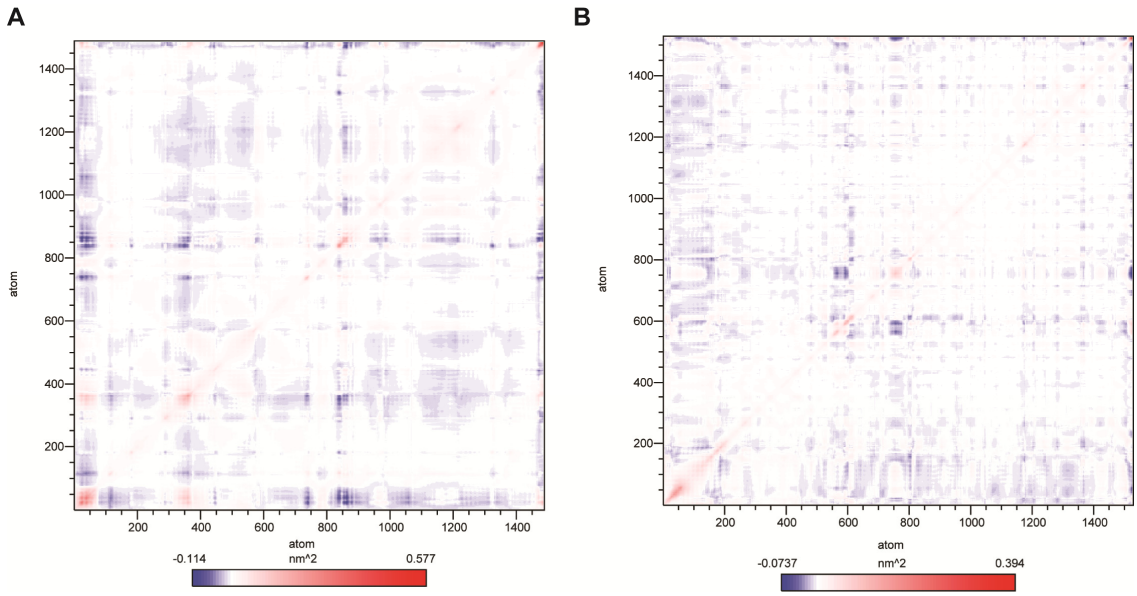


Figure S3 The cross-correlation matrix of the C-α displacement of G-alpha (A) and beta (B) complex systems. Highly positive regions (red) designate a strong correlation in the movement of residues, whereas negative regions (blue) are associated with strong anti-correlated motion of the residues.

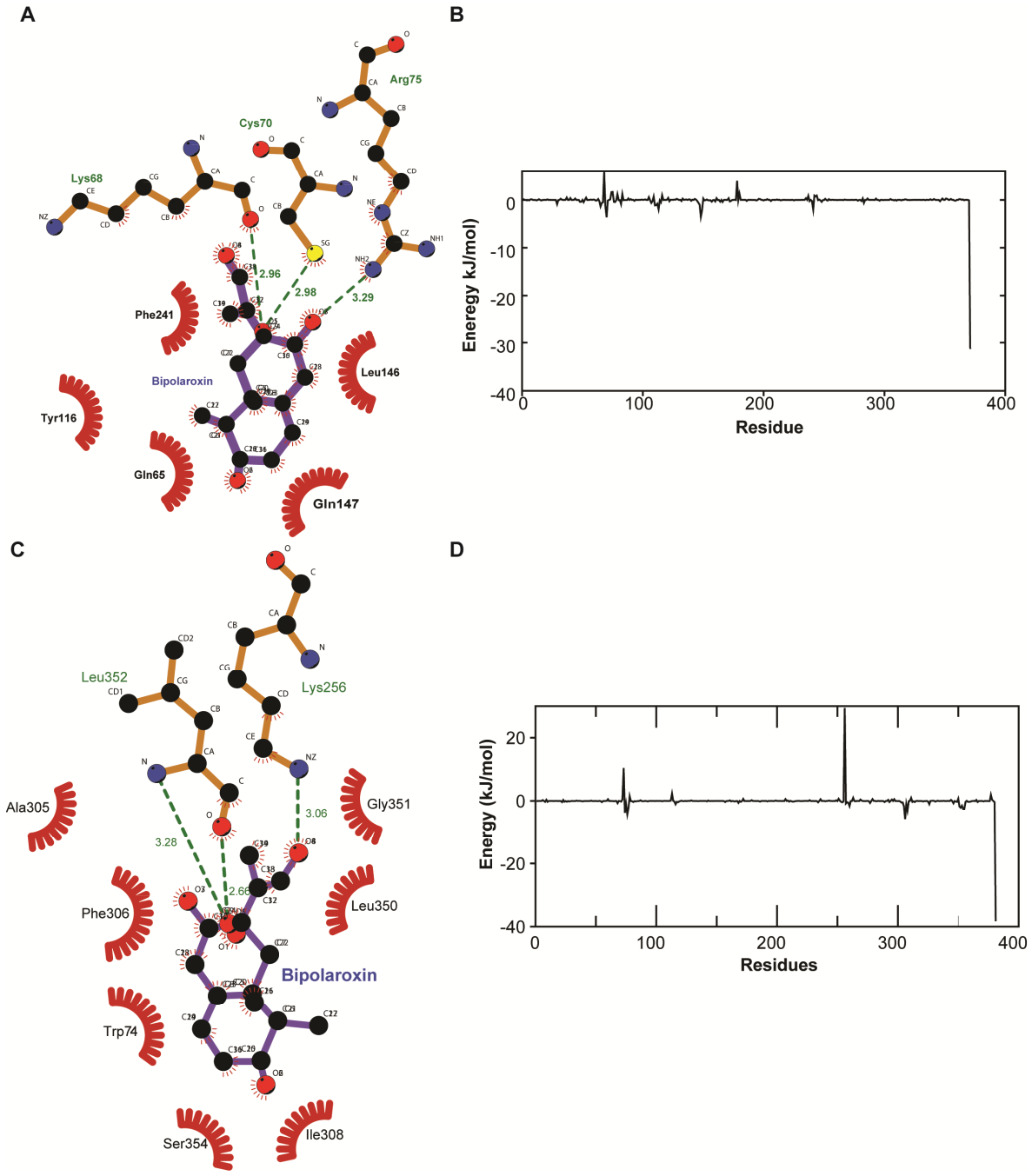


Figure S4 Molecular representation of the top ranked clusters G alpha (**A**) and G-beta (**C**) subunit with Bipolaroxin rendered using LigPlot^{+}, where the green dotted lines show the hydrogen bonds, residues with dark-red semi-circles forming hydrophobic contacts and residues labeled in green portrays the H-bond forming amino acids. Binding free energy per residue decomposition analysis of G-alpha (**B**) and G-beta sub-unit (**D**) complex used for binding free energy analysis. Per residue decomposition map displaying the contribution of each amino acids to the binding free energy of each complex system. The amino acids with a positive energy value impair the binding and vice versa.

S. Table 2. qPCR primers of Gα and Gβ subunit of heteromeric G proteins

S. No.	Gene	Primer sequences
1.	Triticum aestivum G protein beta subunit	F-TCCCTGATGGTCAGAGGTTC R-AGCGTGTCCCACACATAACA
2.	Triticum aestivum G protein beta subunit	F-TCCCTGATGGTCAGAGGTTC R-GCGTGTCCCACACATAACAG
3.	Triticum aestivum G protein beta subunit	F-AGACCAGGAAACTCGCTTGA R-CGAACTGCCCGACTTGTAAT
4.	Triticum aestivum G protein beta subunit	F-GACCAAACGTGTGTCCTGTG R-CGAACTGCCCGACTTGTAAT
5.	Triticum aestivum G protein beta subunit	F-TGACCAAACGTGTGTCCTGT R-CGAACTGCCCGACTTGTAAT
6.	Triticum aestivum G protein alpha subunit	F-ACTCGTGTGGAAGTGTGCTG R-TCTCTCCAAGGGGGCTAAAT
7.	Triticum aestivum G protein alpha subunit	F-AAGGTTCCTTTGACCGTGTG R-TCCCTGGAGCGTCTCATACT
8.	Triticum aestivum G protein alpha subunit	F-AAGGTTCCTTTGACCGTGTG R-CCCTGGAGCGTCTCATACTC
9.	Triticum aestivum G protein alpha subunit	F- AAGGTTCCTTTGACCGTGTG R- CTCCCTGGAGCGTCTCATAC
10.	Triticum aestivum G protein alpha subunit	F- AATGGGGTTGTGGAAATTCA R- CGAACAGTTCCTTCGTCTCC

S. Table 3. qPCR primers of key genes of MAPKs pathways used in expression analyses.

S. No.	Gene	Primer sequences
1.	*TaWRKY23*	F- GAGCGTAGACGTCAGCACCA
		R- CACGGATGCTAATGGCCA
2.	*TaWRKY29*	F- ATCCAACGGATCAAGAGCTG
		R- GATGGGTTTCTGCCCGTATT
3.	*TaWRKY70*	F- TCGACGACGCTGCTGCGACG
		R- GCAGAGCTCCCTGGCGTGC
4.	*TaMKK1*	F- GCGAGGCTCCTCCTATCAAG
		R- CCATCTGCTTGCGTATGCTT
5.	*TaMKK4*	F- GACATCAAGCCGTCCAACCT
		R- CGTTGTAGTTGCCGTCGTTG
6.	*TaMKK5*	F- CGCATCAACACCGACATCAA
		R- GGAGTAGCAGATGGCGACC
7.	*TaMKK6*	F- CAGCTTATCTCCGAGGAAAACG
		R- TTGTGCCGCACTAGTTGGA
8.	*TaMPK3*	F- GGAGATCAAGCTCCTCAGGC
		R- ACTGGCAGTGTTCTTCCGAG
9.	*TaMPK4*	F- TCGAGCCTGGGATTTCTTCG
		R- GTCAACAGTGATGCGTCTGC
10.	*TaMPK6*	F- CAGCTTATCTCCGAGGAAAACG
		R- TTGTGCCGCACTAGTTGGA
11.	*TaMPK20*	F- ATAAGCCTTCGCCTGACGAG
		R- TTCGTAAGGCAGAACTGGACC
12.	*TaMPK24*	F- CTCCGCCACCCAAACATAGT
		R- CCTGCGGTGAGGTTCTCATT
13.	*TaPR1*	F- GAACCAGGAAGTAATGGAGACGCCC
		R- CAGGTGTTGGACCCGTAGTTGTAGTC
14.	*TaPR-2*	F- AAGATGTTGCCTCCATGTTTGCAG
		R- AAGTAGATGCGCATGCCGTTGAT
15.	*TaPR-5*	F- GTTAATTAAGGTGCTCCTTCAATGGCG
		R- TTGCGGCCGCTCATGGACAGAAGGTGATCTGGTA
16.	*TaPR-5*	F- GTTAATTAA ATGGCGACCTCCGCGGTGCTC
		R- TTGCGGCCGCCTACCGCCATTGAAGGAG
17.	*TaPR-10*	F- AGTGCAAGTCCACCCTCATC
		R- CTTGGCCTTGGTGATCTCAT
18.	*TaTGA*	F- AAGCATTTCCAGTTGCTGCT
		R- TCCAGGACAAGGAGATACCG
19.	*TaNPR-1*	F- CCAAAACAGTCGAACTCGGCAA
		R- GACGATGAGGAAGATGAAAGGGTTG
20.	*TaAPx*	F- GGCTAATGCTGGTCTTGTGA
		R- GCACTGGCAAGTTGAAACAG
21.	*TaCAT*	F- GAGTCGCTCCACATGTTCAC
		R- TTCACCAGCGTGTAGGTGTT
22.	*TaSOD*	F- GCCCATTTTGGCGAGTGTTT
		R- GACAAGAGACAAGCCCGGAA
23.	*TaPOx*	F- GGCATGGAACAAAACGCTAT
		R- TGATACTCTTACGGCGACGA
24.	*Beta-tubulin*	F- GCTCACATCTCGTGGGTCACAGA
		R- CGCCAGTGTACCAATGCAAGAAA
25.	*β-actin*	F- TGCTATCCTTCGTTTGGACCTT
		R- AGCGGTTGTTGTGAGGGAGT
26.	*TEF1*	F- GGTGATGCTGGCATAGTGAA
		R- GATGACACCAACAGCCACAG

S. Table 4. qPCR primers of key genes of phenylpropanoid pathways used in expression analyses.

S. No.	Gene	Primer sequences
1.	*Triticum aestivum* phenylalanine ammonia-lyase [EC:4.3.1.24]	F- TCATGTTTGCCCAATTCTCA R- CTGGACATGGTTGGTCACAG
2.	*Triticum aestivum* phenylalanine ammonia-lyase [EC:4.3.1.24]	F- GCTCATGTTTGCCCAATTCT R- CTGGACATGGTTGGTCACAG
3.	*Triticum aestivum* phenylalanine ammonia-lyase [EC:4.3.1.24]	F- CTGTGACCAACCATGTCCAG R- TGTCTTCCTAGCCACCGTCT
4.	*Triticum aestivum* phenylalanine/tyrosine ammonia-lyase [EC:4.3.1.25]	F- CTGTTCTCTCCGAGGTCCTG R- TTGAGCTGCGTGTCAATCTC
5.	*Triticum aestivum* phenylalanine/tyrosine ammonia-lyase 7 (PAL7) [EC:4.3.1.25]	F- CCACTGTTGACGGGAAGAAT R- CAGGACCTCGGAGAGAACAG
6.	*Triticum aestivum* phenylalanine/tyrosine ammonia-lyase 6 (PAL6) [EC:4.3.1.25]	F- CCACTGTTGACGGGAAGAAT R- CAGGACCTCGGAGAGAACAG
7.	*Triticum aestivum* cinnamoyl-CoA reductase [EC:1.2.1.44]	F- GTTCACCCCTGTGCAAGAAT R- TGCGGAAGCCTATCAAAGTT
8.	*Triticum aestivum* cinnamoyl-CoA reductase [EC:1.2.1.44]	F- ACCTTGGCATGAAGTTCACC R- TGCGGAAGCCTATCAAAGTT
9.	*Triticum aestivum* cinnamyl-alcohol dehydrogenase [EC:1.1.1.195]	F- ACACCGACCTCCACTTCATC R- CCCAGAAGATGCCGTTGTAT
10.	*Triticum aestivum* cinnamyl-alcohol dehydrogenase [EC:1.1.1.195]	F- GTCTCTACCGACGGGATCAA R- TGGGCTGAAAATGAAGAAGG
11.	*Triticum aestivum* 4-coumarate--CoA ligase [EC:6.2.1.12]	F- TACTCCTGTCCCATCCTTCG R- AGCATCCGGAGCTTCTTGTA
12.	*Triticum aestivum* 4-coumarate--CoA ligase [EC:6.2.1.12]	F- CTGGAGGCCATACTCCTGTC R- AGCATCCGGAGCTTCTTGTA
13.	*Triticum aestivum* ferulate-5-hydroxylase [EC:1.14.-.-]	F- TCGACGAGTTCATCGACAAG R- GCCAAACATCACATCCATGA
14.	*Triticum aestivum* ferulate-5-hydroxylase [EC:1.14.-.-]	F- TCGCCTACCTCACCTACGAC R- CGGAAGATGACGTTCTTGGT
15.	*Triticum aestivum* class II peroxidase-like [EC:1.11.1.7]	F- TAGCACGCTGCACAAACTTC R- CCATTGAAGAGCTCCTGGTC
16.	*Triticum aestivum* class II peroxidase-like [EC:1.11.1.7]	F- ATTGCTGTCCTCTCGCTCAT R- AGCAGTCGTGGAAGAAGAGG
17.	*Triticum aestivum* peroxidase [EC:1.11.1.7]	F- CTCTCCGGATCTCACACCAT R- CTTGGCAACCAGGTTCTTGT
18.	*Triticum aestivum* peroxidase [EC:1.11.1.7]	F- ACACGCGCGCTCTATAGTCT R- GGCACGAGCTCGAGTAGAAC