



ISSN (E): 2277-7695

ISSN (P): 2349-8242

NAAS Rating: 5.23

TPI 2023; 12(7): 996-1001

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www.thepharmajournal.com

Received: 23-04-2023

Accepted: 24-05-2023

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In silico analysis of C2 domain gene in triticale family (*Triticum aestivum*, *Hordeum vulgare*, *Secale cereale*)

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Abstract

Plant C2 domain proteins play essential biological functions in numerous plants. By sensing intracellular Ca²⁺ signals, Plant C2 domain proteins play crucial roles in numerous cellular processes, including growth, development, membrane targeting, and abiotic and biotic stress adaptations. In this study, a novel C2 domain protein gene was discovered in the Triticale family. This study provides a genome-wide analysis of the C2 domain family in *Triticum aestivum*, *Hordeum vulgare*, and *Secale cereale*. Ca²⁺ and +dependent C2 domain proteins are reportedly implicated in a variety of processes in plants, including abiotic stress, pollen fertility, membrane targeting, and leaf senescence.

Keywords: C2 Domain, *Triticum aestivum*, abiotic stress, biotic stress, membrane targeting

Introduction

Environmental stress caused by components such as Heat and drought has a substantial impact on plant development and metabolic processes, threatens plant viability, and prevalently reduces crop yield. (Bohnert *et al.*, 1995) [1] Developing crop cultivars that can tolerate biotic and abiotic stress has been an ongoing research topic for decades. (Kumar *et al.*, 2019; Dhankher and Foyer, 2018) [7, 6]. Abiotic stress influences include components such as heat, cold, salt, and drought, whereas biotic factors include various fungi, bacteria, and viruses.

Many intracellular and extracellular proteins are partially or entirely composed of protein modules. The C2 domain is a typical intracellular protein module (Chapman *et al.*, 1996) [3]. Being a Ca²⁺-dependent membrane targeting domain, this domain shows Ca²⁺-dependent phospholipid binding activity and a higher sensitivity to salt and drought stresses (Banci *et al.*, 2002) [4]. The Ca²⁺ binding sites of most C2 domain proteins are associated with the presence of five aspartic acid residues, which are highly conserved in plants and animals (Lopez-Nicolas *et al.*, 2006) [2] Ca²⁺ is a ubiquitous secondary messenger in plants that are involved in plant growth, development, hormone signaling, and environmental response. Most of the recent research has revealed that Ca²⁺ signaling has a role in plant responses to abiotic stresses such as salinity and drought. When Ca²⁺-binding proteins bind to Ca²⁺, they release a signal to a downstream signaling pathway, promoting the production of stress-response genes. Wheat is a widely cultivated staple grain crop that is grown on 217 million hectares annually and meets the nutritional needs of 2.5 billion people globally. Wheat is a widely cultivated staple grain crop that is cultivated on 217 million hectares annually and satisfies the nutritional requirements of 2.5 billion people worldwide. Cereals are the primary source of nutrition for humans worldwide. Wheat (*Triticum aestivum* L.) is the most widely farmed cereal on the planet and the second most important food source after rice. It provides 20% of the world's total calorie intake and a similar proportion of total protein, making it one of the primary pillars of food security.

Thus, scientists have been introducing molecular biology and genetic techniques to improve the yield and quality of wheat by increasing their tolerance to stress. Through genome-wide identification and analysis, and the advancement of genomics it is now feasible to predict the gene function of a large gene family, since the advent of biotechnology (Agarwal *et al.*, 2019) [5]. Not only does the study of genomics contribute substantially to the fields of comparative, structural, and functional biology, but it also enables the identification of members of gene families associated with signal transduction pathways. Comparative genomic analysis involves comparing the genomes of various organisms. A wide range of in silico methods can be used to manage and functionally annotate biological data effectively.

Considering the significance of the C2 domain family in plant stress tolerance and the availability of wheat genomic data, we will attempt to systematically examine the C2 domain family in wheat.

This study will identify wheat C2 domain genes and examine their evolutionary relationships, chromosomal locations, motif compositions, cis-elements, and expression patterns in different treatments.

Keeping in view the above aspects, the proposed research objectives are:

1. Identification of C2 domain gene family in *Triticum aestivum*, *Hordeum Vulgarae*, and Sequence retrieval.
2. In silico expression of C2 domain genes in wheat.
3. Gene enrichment analysis of c2 domain genes in *Triticum*.

Materials & Methods

Gene sequence analysis

The family of C2 domain gene sequences will be obtained and analyzed by retrieving coding DNA sequences (CDSs) of Arabidopsis genes (AtC2Dp) and rice genes (OsC2Dp) from the EnsemblPlants database (<http://plants.ensembl.org/>) in order to obtain wheat genome sequences encoding C2 domain genes. Individually, these gene sequences will be utilized in a tBLASTx search against a widely available wheat genome assembly (IWGSC RefSeq v1.1). HMMER will be used to obtain any additional genes (<http://plants.ensembl.org/>). The query sequences will be examined for the presence of the

specified domain(s) using the conserved domain database (CDD) batch search utility at NCBI (<https://www.ncbi.nlm.nih.gov>). Two criteria will be used to identify genuine orthologs in wheat: (i) high sequence identity (>60%) and query coverage along the length of the protein; and (ii) the presence of all domains and motifs detected in the query sequences. Transcripts, splice variants, cDNA, and proteins associated with wheat C2 domain family genes are also available for release. Using TBtool (<https://github.com/CJ-Chen/TBtools/>), the genes will be assigned to chromosomes (Chen *et al.*, 2020). Homoeologous relationships between genes can be identified using chromosome assignment and the frequency of similar protein sequences (greater than 90 percent).

Results & Discussions

Genome-wide identification of C2 Domain in different Triticale species.

Table 1 lists the 100 full-length c2 domain genes that were discovered in wheat along with information on their affinity to rice genes, *Hordeum vulgar*, and *Secale cereale*.

The present study is the first study of the c2 domain gene family in the Triticale family, which was facilitated due to the availability of the whole genome sequence of bread wheat and its other species in the form of pseudomolecules representing individual chromosomes. In this study, 70 family members of the C2DP family were identified in wheat and named the TaC2DP gene family.

Table 1: Detailed information on Rice orthologue and wheat orthologue

RAP-ID	<i>Triticum aestivum</i>	<i>Hordeum vulgare</i>	<i>Secale cereale</i>
Os01g0128800	TraesCS3A02G043600	HORVU.MOREX.r3.3HG0222090	SECCE3Rv1G0149860
	TraesCS3B02G040000		
	TraesCS3D02G037200		
Os01g0172400		HORVU.MOREX.r3.3HG0241720	SECCE3Rv1G0164510
Os01g0172400			
Os01g0242600		HORVU.MOREX.r3.3HG0251310	SECCE3Rv1G0170190
Os01g0369500	TraesCS4D02G324700	HORVU.MOREX.r3.4HG0409680	
Os01g0587300	TraesCS7A02G491900	HORVU.MOREX.r3.7HG0739610	SECCE2Rv1G0064500
	TraesCS7B02G397000		
	TraesCS7D02G478500		
Os01g0819150			
Os01g0819300	TraesCS5B02G330500		
	TraesCS5B02G330600		
	TraesCS5D02G336100		
	TraesCS5D02G336200		
	TraesCS5A02G330400		
Os01g0841700	TraesCS3A02G359500	HORVU.MOREX.r3.3HG0302620	SECCE3Rv1G0197890
	TraesCS3A02G359700		SECCE3Rv1G0197960
	TraesCS3B02G392100		SECCE3Rv1G0197910
	TraesCS3B02G392200		SECCE3Rv1G0197900
	TraesCS3B02G392300		
	TraesCS3B02G392500		
	TraesCS3D02G353600		
	TraesCS3D02G353700		
	TraesCS3D02G353800		
	TraesCS3D02G353900		
Os01g0853800	TraesCS3A02G367800	HORVU.MOREX.r3.3HG0301390	SECCE3Rv1G0198850
	TraesCS3B02G399500		
	TraesCS3D02G360800		
Os01g0934100	TraesCS3A02G454300	HORVU.MOREX.r3.3HG0313780	SECCE3Rv1G0211030
	TraesCS3B02G492900		
	TraesCS3D02G446700		
Os01g0951100	TraesCS3B02G548000	HORVU.MOREX.r3.6HG0549950	SECCE6Rv1G0432060

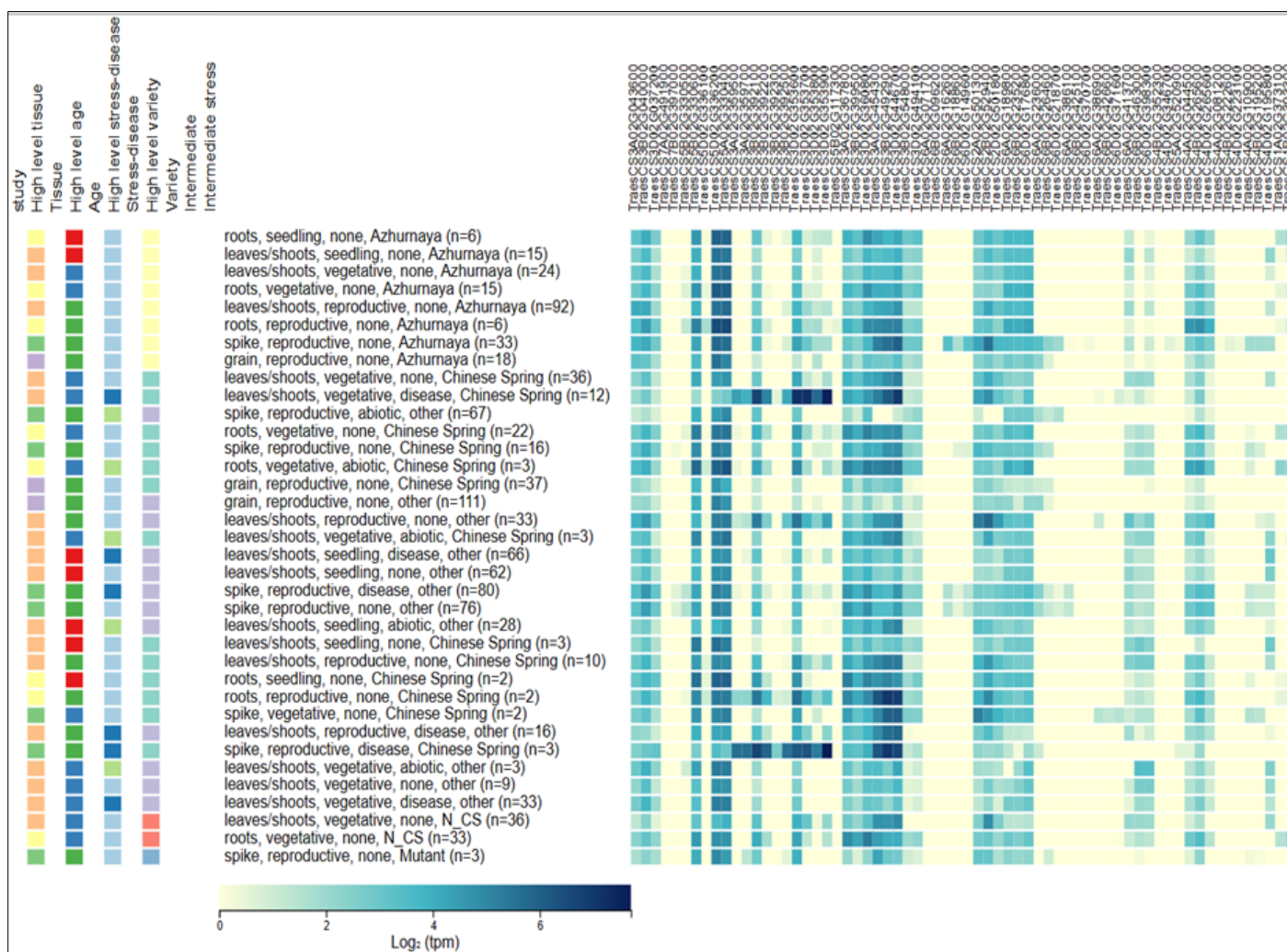
	TraesCS3D02G494100		
Os02g0120200	TraesCS6A02G071700	HORVU.MOREX.r3.6HG0549950	SECCE6Rv1G0432060
	TraesCS6B02G096200		
Os02g0198300	TraesCS6A02G160700	HORVU.MOREX.r3.6HG0572820	SECCE6Rv1G0387750
Os02g0199800	TraesCS6A02G162600	HORVU.MOREX.r3.6HG0572300	SECCE6Rv1G0387590
	TraesCS6B02G188600		
	TraesCS6D02G149600		
Os02g0313700	TraesCS2A02G144400	HORVU.MOREX.r3.2HG0119520	SECCE2Rv1G0079130
Os02g0327000	TraesCS2A02G501300		
	TraesCS2B02G529400		
	TraesCS2D02G501800	HORVU.MOREX.r3.2HG0202460	SECCE2Rv1G0127750
Os02g0448400			
Os02g0521300	TraesCS6A02G189800	HORVU.MOREX.r3.6HG0581160	SECCE6Rv1G0392040
	TraesCS6B02G235200		
	TraesCS6D02G176800		
Os02g0605400			
Os02g0640000	TraesCS6A02G236000	HORVU.MOREX.r3.6HG0598440	SECCE6Rv1G0400900
	TraesCS6B02G264600		
	TraesCS6D02G218700		
Os02g0663900		HORVU.MOREX.r3.6HG0601830	
Os02g0665100			
Os02g0722450			
Os02g0815100	TraesCS6A02G386100	HORVU.MOREX.r3.6HG0628870	SECCE6Rv1G0421490
	TraesCS6B02G425100		
	TraesCS6D02G370700		
Os02g0816000	TraesCS6A02G386900	HORVU.MOREX.r3.6HG0629000	SECCE6Rv1G0421620
	TraesCS6B02G426600		
	TraesCS6D02G371600		
Os02g0829200	TraesCS6A02G413700	HORVU.MOREX.r3.6HG0632990	SECCE3Rv1G0212880
	TraesCS6B02G463000		
	TraesCS6D02G398300		
Os03g0119100	TraesCS4B02G352300	HORVU.MOREX.r3.4HG0413350	SECCE5Rv1G0371000
	TraesCS4D02G346700		
	TraesCS5A02G520900		
Os03g0194100	TraesCS4A02G044500	HORVU.MOREX.r3.4HG0400060	SECCE7Rv1G0469970
	TraesCS4B02G265600		
	TraesCS4D02G265600		
Os03g0251600	TraesCS4A02G081200	HORVU.MOREX.r3.4HG0391490	SECCE7Rv1G0475570
	TraesCS4B02G222600		
	TraesCS4D02G223100		
Os03g0289300	TraesCS4A02G109000	HORVU.MOREX.r3.4HG0385050	SECCE7Rv1G0479260
	TraesCS4B02G195200		
	TraesCS4D02G195800		
Os03g0391400	TraesCS1A02G373100	HORVU.MOREX.r3.1HG0083620	SECCE1Rv1G0053460
	TraesCS1B02G393300	HORVU.MOREX.r3.1HG0083580	
	TraesCS1D02G379900		
Os03g0652000			
Os03g0840800	TraesCS4A02G347800	HORVU.MOREX.r3.1HG0061100	SECCE7Rv1G0459250
	TraesCS5B02G525600		
	TraesCS5D02G524600		
Os04g0472900	TraesCS2A02G329400	HORVU.MOREX.r3.2HG0174880	SECCE2Rv1G0107140
	TraesCS2B02G352900	HORVU.MOREX.r3.2HG0174880	
	TraesCS2D02G333300		
Os04g0476600			
Os04g0531100	TraesCS2A02G372000	HORVU.MOREX.r3.2HG0183890	SECCE2Rv1G0111750
	TraesCS2B02G389100		
	TraesCS2D02G368300		
Os04g0644900			
Os04g0682100	TraesCS2A02G584700	HORVU.MOREX.r3.2HG0213430	SECCE2Rv1G0138570
	TraesCS2B02G598600		
	TraesCS2D02G601500		
Os04g0683800	TraesCS2A02G582000	HORVU.MOREX.r3.2HG0214090	SECCE2Rv1G0139540
	TraesCS2B02G605000		
	TraesCS2D02G598200		
Os04g0691800	TraesCS1A02G014300	HORVU.MOREX.r3.2HG0217090	SECCE2Rv1G0142520
	TraesCS1D02G012500	HORVU.MOREX.r3.1HG0002100	SECCE1Rv1G0001510
	TraesCS2A02G557900		SECCE1Rv1G0001530

	TraesCS2A02G558300		SECCE2Rv1G0142680
	TraesCS2A02G558400		SECCE1Rv1G0001350
	TraesCS2B02G621600		SECCE7Rv1G0458960
	TraesCS2B02G622500		
	TraesCS2D02G571700		
	TraesCSU02G020600		
Os05g0127200	TraesCS1A02G069300	HORVU.MOREX.r3.1HG0015110	SECCE1Rv1G0010580
	TraesCS1D02G071800		
Os05g0149100	TraesCS1A02G105000	HORVU.MOREX.r3.1HG0021810	SECCE1Rv1G0014030
	TraesCS1B02G116500		
	TraesCS1D02G097200		
Os05g0171000	TraesCS1A02G115300	HORVU.MOREX.r3.1HG0026950	SECCE1Rv1G0016770
	TraesCS1B02G135200		
	TraesCS1D02G116700		
Os05g0370600	TraesCS5A02G265400	HORVU.MOREX.r3.5HG0490810	SECCE5Rv1G0334120
	TraesCS5B02G265100		
	TraesCS5D02G273300		

In silico expression analysis of C2 domain

The expression data for the 70 TaC2Dp was available in the Wheat Exp database. The expression of these genes was examined in five different tissues (root, stem, leaf, spike and grain) sampled at different growth stages (according to

Zadoks growth scale (Z00 to Z95) and under conditions of heat and drought. The summary data in terms of level of expression (up-regulation and down regulation) is presented in Fig. 2; more details are available in Supplementary Table S1.



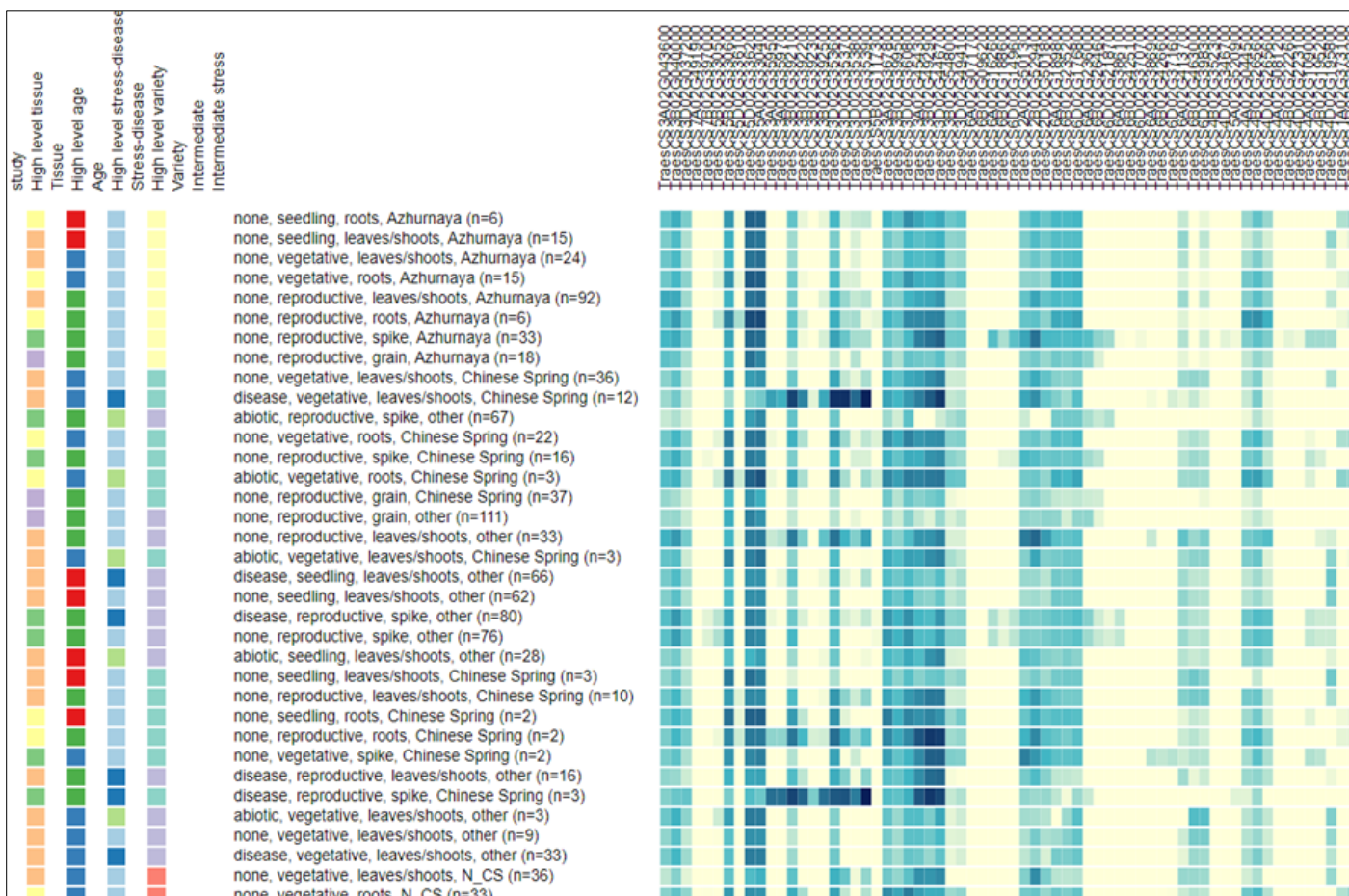


Fig 1: Heat map (generated using the online software tool showing in silico expression profile of 70 TaC2 Domain belonging to six classes at different developmental stages of wheat

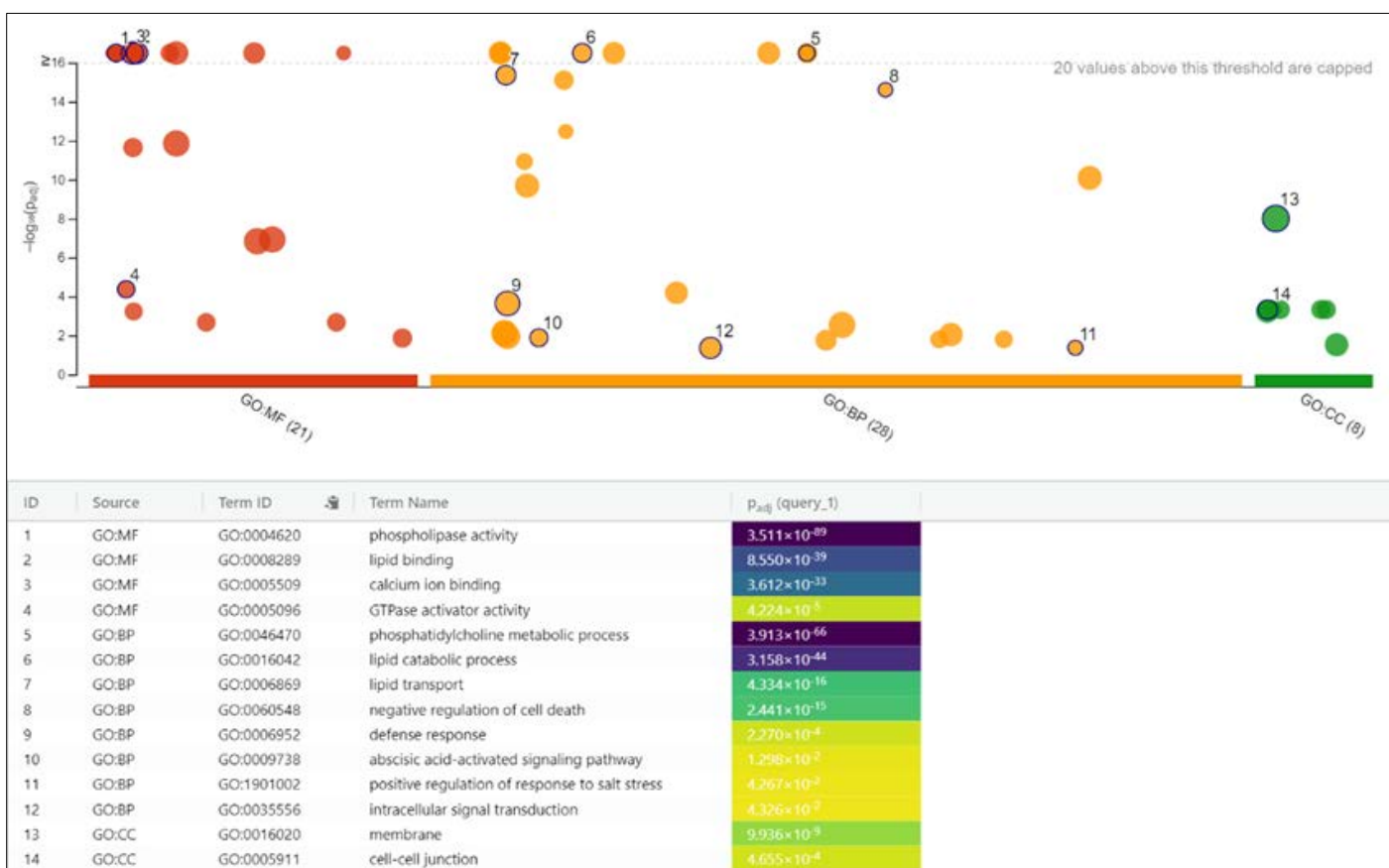


Fig 2: Manhattan Plot depicting Gene enrichment analysis

Gene enrichment Analysis

Gene enrichment analysis is performed with an g: Profiler is a web-based bioinformatics tool used for functional enrichment analysis of gene lists. It helps in the interpretation of large-scale genomics and proteomics data by identifying the biological processes, pathways, and molecular functions that are overrepresented in a given gene or protein list. According to the GO annotation results, the C2 domain proteins were annotated in various biological processes, including response to stress, phospholipase activity, lipid binding, calcium ion binding, GTPase activator activity, positive regulation of response to salt stress, negative regulation of cell death, intracellular signal transduction, phosphatidylcholine metabolic process. (Sun Y, 2021) ^[8] These studies further supported that C2 Domain play role in calcium binding, salt and heat & drought stress in Soyabean.

Conclusion

Thus, the present study provides useful information about the genetic control of C2 domain which may be utilized for fruitful future studies for developing climate resilient Wheat and Hordeum.

The presence of high-quality genome. High-quality genome sequencing and bioinformatics toolkits can help identify distinct genes or gene families and clarify their functional roles. The need to develop climate-smart cultivars in response to environmental challenges. The results provide a theoretical foundation for enhancing Wheat gene family identification and characterization. The discovery of a gene family has significant implications for crop plant improvement and agricultural productivity. To develop genetically modified crops with better attributes, scientists must first identify the gene families responsible for desirable traits such as disease resistance, drought tolerance, or increased yield. This may help to ensure global food security and sustainability.

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