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Functional analysis of drought tolerance QTLs in two barley populations using BLAST on associated SNP sequences

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Abstract: This study explored the relationship between QTLs associated with drought tolerance and functional SNPs using two populations: Vada × Susptrit (V × S) and Cebada Cappa × Susptrit (C. Cappa × S). Bioinformatics tools were employed to analyze significant SNPs within QTL regions, revealing markers on chromosomes 1H, 2H, 3H, 5H, and 7H for the V × S population, and on 4H and 6H for C. Cappa × S. In total, 24 proteins/enzymes related to drought tolerance were characterized in the V × S population, while 10 were identified in C. Cappa × S. Notable proteins, including phytochrome B and SAPK7, were located on chromosome 4H. The identified proteins are integral to the plant's adaptive response to drought stress, mediating essential regulatory mechanisms that enhance resilience. Gene ontology analysis delineated four primary cellular components—membrane, nucleus, chloroplast, and proteasome complex—linked to drought resistance pathways. Additionally, five critical biological processes, including oxidation-reduction and protein phosphorylation, were identified as pivotal in these adaptive responses. This comprehensive understanding underscores the potential application of these proteins in breeding strategies aimed at developing drought-tolerant barley cultivars. Overall, the study highlights potential functional SNP markers for validating QTLs related to drought tolerance in barley.

Key words: barley, bioinformatics, gene ontology, QTL validation, single nucleotide polymorphism

Funkcionalna analiza odpornosti na sušo (QTL) v dveh populacijah ječmena z uporabo BLAST analize na povezanih SNP zaporedjih

Izvleček: Raziskava preučuje razmerje med s toleranco na sušo povezanimi zaporedji (QTL) in funkcionalnimi SNP mesti v dveh populacijah ječmena: Vada × Susptrit (V × S) in Cebada Cappa × Susptrit (C. Cappa × S). Bioinformacijska orodja so bila uporabljena za analizo pomembnih SNP znotraj QTL območij za odkritje markerjev na kromosomih 1H, 2H, 3H, 5H in 7H za V × S populacijo in na kromosomih 4H in 6H za C. Cappa × S populacijo. Celokupno je bilo opisanih 24 proteinov/encimov, povezanih s tolerance na sušo v V × S populaciji med tem, ko jih je bilo v populaciji C. Cappa × S deset. Pomembni proteini, ki vključujejo fitokrom B in serein treonin kinazo (SAPK7) so bili locirani na kromosomu 4H. Ti proteini so sestavni del rastlinskega prilagoditvenega odziva na sušni stres, ki je bistven za sprožitev mehanizmov uravnavanja procesov, ki povečuje odpornost. Analiza delovanja genov je odkrila štiri glavne celične oddelke, ki so povezani s procesi odpornosti na sušo in sicer celično membrano, celično jedro, kloroplast in proteasom. Dodatno je bilo v tem prilagoditvenem odzivu prepoznanih pet ključnih bioloških procesov, ki so med drugimi obsegali oksidacijo, redukcijo in fosforilacijo proteinov. Takšno celostno razumevanje poudarja pomen potencilane uporabe teh proteinov v strategijah žlahtnenja z namenom vzgojiti na sušo toleratne sorte ječmena. Raziskava osvetljuje uporabo potencialnih funkcionalnih SNP markerjev za ovrednotenje QTL povezanih s toleranco na sušo pri ječmenu.

Ključne besede: ječmen, bioinformatika, genska ontologija, ovrednotenje QTLs, SNP (polimorfizem posameznih nukleotidov)

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1 INTRODUCTION

Drought stress is an unavoidable factor present in various environments, affecting plant biomass production, quality, and energy without regard for borders or providing clear warnings. Its pervasive nature hampers agricultural productivity by challenging plants' ability to thrive under water-limiting conditions.(Seleiman, 2021). Understanding the complex regulatory pathways guarantees in-depth consideration of a biological system. These challenges are closely related to informatics in biology, in other words "bioinformatics". The field of multi-omics has witnessed unprecedented growth, converging multiple scientific disciplines and technological advances. This surge is evidenced by a more than doubling in multi-omics scientific publications within just two years (2022–2023) since its first referenced mention in 2002, as indexed by the National Library of Medicine. (Moher et al., 2024). Bioinformatics actually manages the data collected through various techniques -omics, including genomics, transcriptomics, proteomics and metabolomics. Systems Bioinformatics is the framework in which systems approaches are applied to such data (oulas et al.,, 2017). Access to plant genome sequencing technology, the development of mapping populations, genetic diversity, and molecular markers with wide genomic coverage has led researchers to accelerate the identification of important QTLs (quantitative trait locus) and their responsible

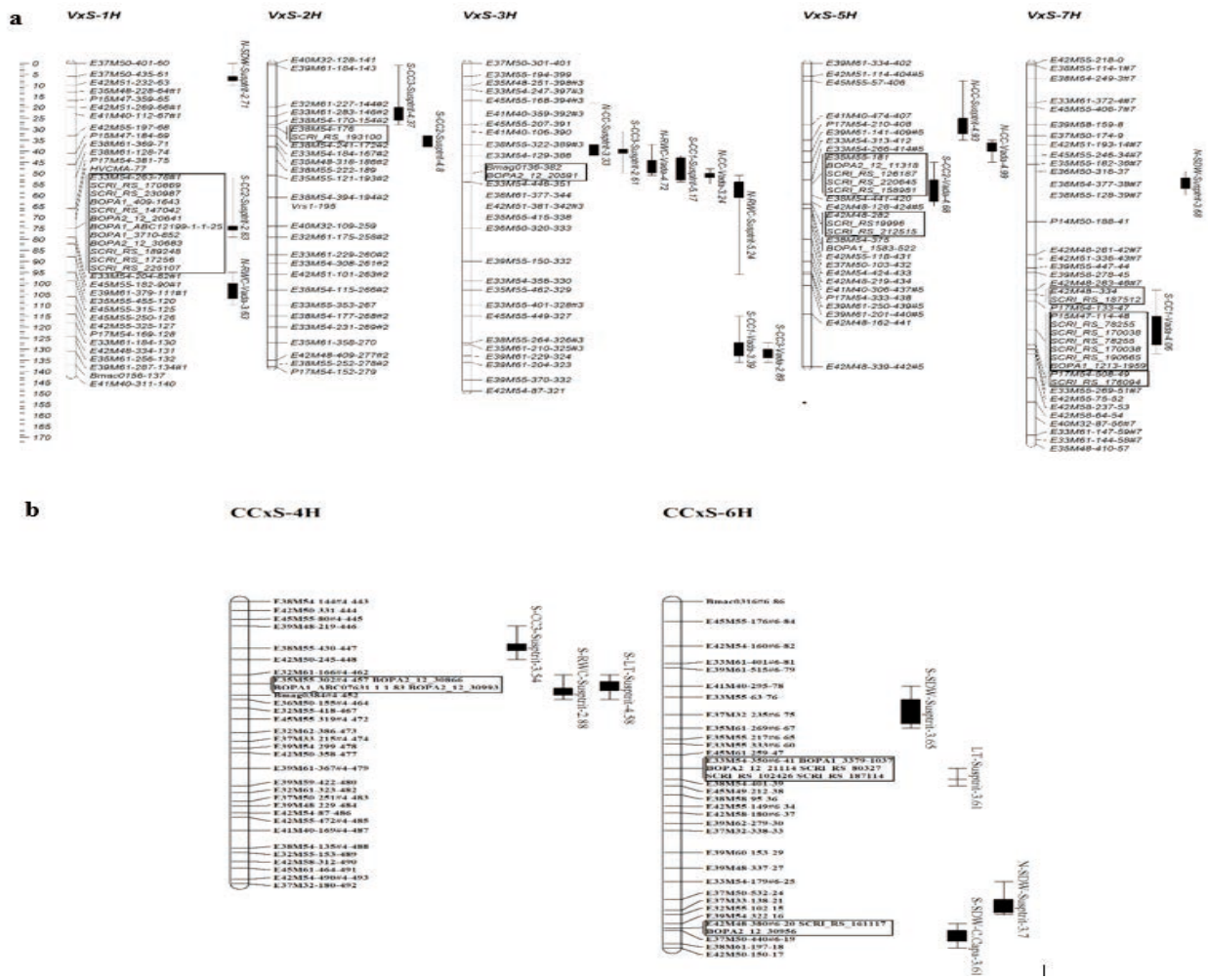


Figure 1: QTLs related to physiological traits under drought stress and normal irrigation in barley populations V × S (a) and C. Capa × S (b) and SNP markers associated with AFLP/SSR markers related to the QTLs. AFLP/SSR markers and the associated SNP markers are shown inside the rectangle. The letters S and N indicate drought and normal irrigation conditions, respectively. The numbers in the QTL area indicate the maximum LOD. Bold areas have significant LOD. CC1, CC2 and CC3 indicate chlorophyll content at 50 %, 30 %, and 20 % of field capacity, respectively. FC: field capacity; RWC: Relative leaf water content and SDM: Shoot dry mass and LT: Leaf temperature.

genes (Kurakawa et al., 2007). Among recent advances in -omics technology, the emergence of high-efficiency methods for genomic sequencing and high-saturation genotyping using DNA markers such as single nucleotide polymorphism (SNP), has proven to be very effective (Kuromori et al., 2009). Including the bioinformatics implements, are Sequence Analysis and Similarity Searching Tools. In bioinformatics, sequence alignment is a method of arranging DNA, RNA, or protein sequences to identify "similar regions" that can reveal functional, structural, or evolutionary relationships between sequences (Stormo, 2000). In the aligning method, there are strong evidences that two similar sequences have the same nucleotids (or identical amino acids). Due to the large number of gaps, multiple alignments can occur between the two sequences (Vassilev et al., 2005). Dynamic algorithms identify the optimal alignment. In the BLAST method, statistical techniques are used to assess the probability of a specific alignment between two sequences (Neumann et al., 2014). Alignments are widely used in bioinformatics to identify sequence similarity, prepare phylogenetic trees and homology models of protein structures (Dubey et al., 2010). The NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/), is the most popular tool to search for align sequences (Altschul et al., 1990). Bioinformatics method has been used in many studies to identify or predict the proteins or enzymes involved in the response of different plants to drought stress (Faghani et al., 2015; Landi et al., 2017; Neumann et al., 2014; Shaar-Moshe et al., 2015; Wehner et al., 2015). Barley, scientifically known as Hordeum vulgare L., ranks fourth in cereals in terms of production. The ability to grow barley in harsh and low-yield environments is higher than other cereals, and this crop is best adapted to environmental stresses such as drought, salinity and cold (Jogaiah et al., 2013). The present study was performed to BLAST analysis on SNP sequences have significant correlation with QTL regions related to drought tolerance identified in our previous study (Mohammadi et al., 2018) as well as prediction of related genes and proteins/enzymes and their ontology study.

2 MATERIALS AND METHODS

In our previous study (Mohammadi et al., 2018), drought tolerance chromosomal regions in seedling stage were identified and reported in two barley populations: 'Vada' ×' Susprit' (V × S) and 'Cebada Cappa' × 'Susprit' (C. Cappa × S). BLAST analysis was performed on SNP sequences have significant correlations with some AFLP/SSR markers associated with QTLs related to physiological traits under drought conditions. SNP markers were developed in the University of Wagningen, Netherlands. The QTLs and their associated SNP markers are shown in Figure 1. In order to study of functional genomics, BLAST analysis was conducted against the NCBI non redundant (nr) nucleotide collection (www.ncbi.nlm.nih.gov). The UNIPROT database (www.uniprot.org) was used to predict proteins and their function. Gene ontology information was also extracted through the European Bioinformatics Institute website (EBI = www.ebi.ac.uk). In order to plot three types of ontologies, including cellular components, molecular functions, and biological processes, EXCEL software was used. Bioinformatics pipeline, is shown in Figure 2.

3 RESULTS AND DISCUSSION

3.1 BLAST ANALYSIS SNPS ASSOCIATED TO QTL REGIONS IDENTIFIED IN V × S POPULATION

Summary of BLAST results SNP sequences with significant correlations with QTL regions identified in the V × S population are shown in Table 1. In this population, a total of 24 types of proteins / enzymes related to QTL regions were identified whose sequences of genes encoding them had very low FDR (near zero), which indicates

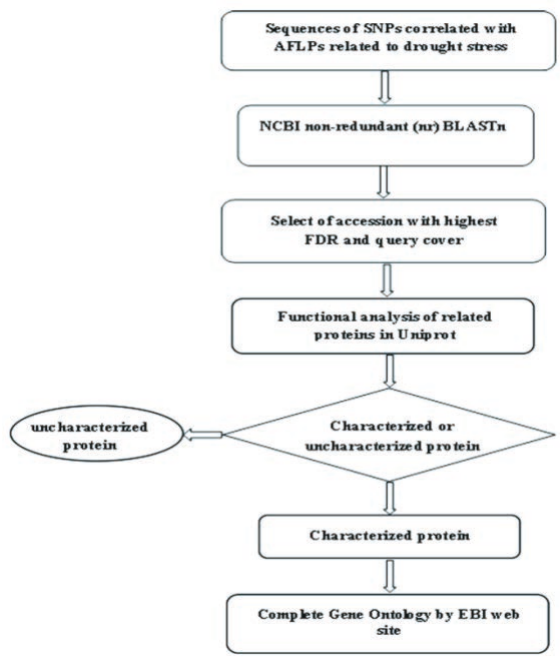


Figure 2: Bioinformatics pipeline used in this work

a very high similarity between SNP sequences and their alignments. These genes were identified on chromosomes 1H, 2H, 3H, 5H and 7H. On chromosome 1H, proteins/enzymes glycosyltransferases, protein dehydration-induced 19 homolog 5, dihydrolipoyl dehydrogenase, hexosyltransferase, salt tolerant protein-GSK-like kinase, peroxidase, glycine-rich RNA-binding protein RZ1B, mitogen-activated protein kinase, pectinesterase, cinnamoyl-CoA reductase-like SNL6, ribosomal protein, were identified. Two proteins/enzymes include S-adenosylmethionine decarboxylase proenzyme and ATP-dependent Clp protease proteolytic subunit, were predicted on chromosomes 2H and 3H, respectively. On chromosome 5H: pectinesterase, protoporphyrinogen oxidase, beta-carotene hydroxylase, zinc finger A20 and AN1 domain-containing stress-associated protein 1, chlorophyll a-b binding protein, probable anion transporter 5; and on chromosome 7H: proteasome subunit beta type, lactoylglutathione lyase, xyloglucan endotransglucosylase/hydrolase, phosphoglycerate kinase, D-3-phosphoglycerate dehydrogenase were identified. Pectin esterase and zinc finger proteins play crucial roles in plant responses to drought stress. Identifying these proteins can aid in developing new strategies for enhancing drought tolerance in barley and other crops. Given their involvement in key processes like water regulation and plant metabolism, a deeper understanding of their functions could assist farmers in improving crop yields under challenging environmental conditions, ultimately contributing to food security. Pectin esterase was detected on chromosomes 1H and 5H and also zinc finger A20 and AN1 domain-containing stress-associated protein 1 was identified in two regions on chromosome 5H. Based on BLAST results, it was observed that most genes were found in barley (Hordeum vulgare subsp. vulgare Spenn.) (Table 1). A summary of the results of former studies on the effect of identified proteins/enzymes on drought tolerance in different plants is given in Table 2. A review of the relationship between the identified proteins/enzymes and drought stress in different studies showed well, that all the proteins/enzymes identified in the present study, were directly involved in the drought stress response in previous studies conducted on different plants. The results confirm the QTLs reported in our previous study (Nadarajah and Sidek, 2010). In most studies, increase or induction of identified proteins, enhanced drought stress tolerance (Table 2). Rollins (2012) studied effect of heat stress in barley, that two dihydrolipoyl dehydrogenase proteins named F2E5U7 and F2E2T3 have been reported, which one of them, F2E5U7, was also identified in present study. As a result of BLAST on SNP marker named "BOPA2_12_20641" located on chromosome 1H correlated with AFLP marker E33M54-263, a glycogen synthase kinase enzyme called Q8LK43, which is the same protein reported by Talami et al. (2007) (Table 2). Qin et al. applied drought stress in rice and Arabidopsis and studied BLAST on sequence of genes related to chlorophyll a/b binding proteins in barley genomic database, that 17 genes expressing chlorophyll a/b binding proteins were reported. In this study, we identified MLOC_44755, which corresponds to the protein F2CRC1, through BLAST analysis of SNP "BOPA1_1583-522" located on chromosome 5. The identification of this protein suggests its potential role in biological processes related to the SNP. Understanding the functional implications of MLOC_44755 can provide insights into its contribution to phenotypic traits or disease susceptibility. Further exploration of this relationship may reveal important connections that enhance our understanding of the underlying genetic mechanisms involved (Table 1).

3.2 BLAST ANALYSIS OF CORRELATED SNP SEQUENCES OR QTL REGIONS IDENTIFIED IN C.CAPA × S POPULATION

Summary of BLAST analysis on SNP sequences have significant correlations with QTL regions identified in C.Cappa × S population are shown in Table 2. A total of 10 proteins/enzymes associated with QTL regions were identified, and their coding gene sequences exhibited a very low false discovery rate (near zero). This indicates a high level of similarity between the SNP sequences and the identified alignments. In other words, these findings suggest a strong and meaningful connection between the SNPs and the identified proteins. These genes were identified on chromosomes 4 and 6. On chromosome 4, proteins/enzymes phytochrome B, serine/threonine-protein kinase SAPK7, o-methyltransferase were identified. Proteasome subunit alpha type, nuclear cap-binding protein subunit 2, 3-ketoacyl-CoA synthase, heat shock protein 16.9C, calcium-dependent protein kinase 4, beta-carotene hydroxylase and HGWP repeats were detected on chromosome 6H.

3.3 REVIEW OF PREVIOUS STUDIES ON IDENTIFIED PROTEINS / ENZYMES AND THEIR RELATIONSHIP WITH DROUGHT STRESS IN DIFFERENT PLANTS

The results of this section in V × S and C. Capa × S populations are given in Tables 3 and 4, respectively. As can be seen, in both populations direct relationship of all

Table 1: Briefing of BLASTn analysis on SNP sequences having significant correlation with AFLP/SSR markers linked to QTLs related to drought stress in V × S population. Note. SNP: Single Nucleotide Sequence; AFLP: Amplified Fragment Length Polymorphism; SSR: Single Sequence Repeat; FDR: False Discovery Rate

Chromosome number, AFLP marker	SNPs correlated to associated AFLP marker	Correlation rate between SNPs and AFLPs	Accession	Species	FDR	Query cover	Gene	Uniprot name	Protein
1H,E33M54-263	SCRI_RS_170869	0.88435955	AK250861.1	*Hordeum vulgare* subsp. *vulgare*	2.00E-51	100 %	pglcat4	Q7XHJ7	Glycosyltransferases
	SCRI_RS_230987	0.90629731	AP014957.1	*Oryza sativa japonica*	4.00E-15	82 %	DI19-2	Q5JME8	Protein DEHYDRATION-INDUCED 19 homolog 5
	BOPA1_409-1643	0.92889292	AK371521.1	*Hordeum vulgare* subsp. *vulgare*	3.00E-116	100 %	N/A	F2E5U7	Dihydrolipoyl dehydrogenase
	SCRI_RS_147042	0.928893	AK362608.1	*Hordeum vulgare* subsp.. *vulgare*	7.00E-50	100 %	N/A	F2DFE1	Hexosyltransferase
	BOPA2_12_20641	0.9043956	AF525086.1	*Triticum aestivum (bread wheat)*	1.00E-40	89 %	N/A	Q8LK43 AAM77397	=salt tolerant protein-GSK-like kinase
	BOPA1_ABC12199-1-1-25	0.9043956	XM_006654903.2	*Oryza brachyantha*	4.00E-28	100 %	N/A	J3M3R4	Peroxidase
	BOPA1_3710-852	0.88129947	AK250786.1	*Arabidopsis thaliana (Mouse-ear cress)*			RZ1B	O22703	Glycine-rich RNA-binding protein RZ1B
	BOPA2_12_30683	0.88129947	AK356908.1	*Hordeum vulgare* subsp.. *vulgare*	2.00E-51	100 %	N/A	F2CZ52	Mitogen-activated protein kinase
	SCRI_RS_189248	0.83214722	AK371220.1	*Hordeum vulgare* subsp.. *vulgare*	2E-51	100%	N/A	F2E4Z6	Pectinesterase
	SCRI_RS_17256	0.80792947	XM_020338974.1	*Oryza sativa subsp. japonica (Rice)*			SNL6	Q0JKZ0	Cinnamoyl-CoA reductase-like SNL6
	SCRI_RS_225107	0.71110956	AK359627.1	*Hordeum vulgare* subsp. *vulgare*	2.00E-51	100 %	N/A	F2D6W5	Ribosomal protein
2H,E38M54-176	SCRI_RS_193100	0.47013575	AP014960.1	*Oryza sativa japonica*	7.00E-12	98 %	SAMDC	Q0JC10	S-adenosylmethionine decarboxylase
3H,Bmag0136	BOPA2_12_20591	0.95060956	AK249478.1	*Hordeum vulgare* subsp. *vulgare*	2.00E-51	100 %	clpP	P48883 (CLPP_HORVU)	ATP-dependent Clp protease proteolytic subunit
5H,E35M55-181	BOPA2_12_11318	0.711	AK365601.1	*Hordeum vulgare* subsp. *vulgare*	2.00E-49	100%	N/A	F2DNY3	Pectinesterase
	SCRI_RS_126187	0.711	AK356154.1	*Hordeum vulgare* subsp.. *vulgare*	7.00E-50	100%	N/A	F2CX01	Protoporphyrinogen oxidase
	SCRI_RS_220645	0.8	AP014959.1	*Oryza sativa japonica*	8E-22	96%	Os03g0125100	Q10SE7	Beta-carotene hydroxylase, putative, expressed
	SCRI_RS_158981	0.879	AP014965.1	*Oryza sativa japonica*	4.00E-34	98%	SAP1	A3C039	Zinc finger A20 and AN1 domain-containing stress-associated protein 1
5H,E38M54-375	BOPA1_1583-522	0.815	AK354173.1	*Hordeum vulgare* subsp.. *vulgare*	9.00E-116	100%	N/A	F2CRC1 :MLOC_44755, MLOC_44755.2	Chlorophyll a-b binding protein, chloroplastic
5H,E42M48-282	SCRI_RS_199964	0.7082	AK059357.1	*Oryza sativa japonica*	1E-14	89%	PHT4;5	Q0IZQ3	Probable anion transporter 5, chloroplastic
	SCRI_RS_212515	0.7082	AP014965.1	*Oryza sativa* subsp.. *japonica*	1.00E-28	90%	SAP1	A3C039	Zinc finger A20 and AN1 domain-containing stress-associated protein 1
7H,P15M47-184	SCRI_RS_78255	0.767	AK365413.1	*Hordeum vulgare* subsp.. *vulgare*	2.00E-36	79%	N/A	F2DNE5	Proteasome subunit beta type
	SCRI_RS_170038	0.921	AP014964.1	*Oryza sativa japonica*	4.00E-21	86%	GLYI-11	Q948T6	Lactoylglutathione lyase
	SCRI_RS_78255	0.767	AK365413.1	*Hordeum vulgare subsp. vulgare*	2.00E-36	79%	N/A	F2DNE5	Proteasome subunit beta type
	SCRI_RS_170038	0.921	AP014964.1	*Oryza sativa japonica*	4.00E-21	86%	GLYI-11	Q948T6	Lactoylglutathione lyase
	SCRI_RS_190665	0.948	AK371075.1	*Hordeum vulgare* subsp.. *vulgare*	2.00E-51	100%	N/A	F2DXV8	Xyloglucan endotransglucosylase/hydrolase
	BOPA1_1213-1959	0.817	FN179374.1	*Hordeum vulgare* subsp. *vulgare*	3.00E-116	100%	SSI	C3W8L3	Starch synthase, chloroplastic/amyloplastic
7H,E17M54-169	SCRI_RS_176094	0.757	AK354003.1	Hordeum vulgare subsp.vulgare	8.00E-49	100%	N/A	F2CQV1	Phosphoglycerate kinase
7H,E42M48-334	SCRI_RS_187512	0.751	AK371688.1	Hordeum vulgare subsp. vulgare	3.00E-48	99%	N/A	F2DG93	D-3-phosphoglycerate

N/A: Indicates that the gene name is not available in the database.

Table 2: Briefing of BLASTn analysis on SNP sequences having significant correlation with AFLP/SSR markers linked to QTLs related to drought stress in barley C. Cappa×S population.

Chromosome number, AFLP/SSR markers	SNPs correlated to associated AFLP/SSR markers	Correlation rate between SNPs and AFLPs/SSRs	Accession	Species	FDR	Query cover	Gene	Uniprot name	Protein
4H, E35M55-302	BOPA2_12_30866	0.623126467	DQ201144.1	*Hordeum vulgare* subsp. *vulgare*	2E-51	100 %	PhyB	Q2I7M0	Phytochrome B
	BOPA1_ABC07631-1-1-83	0.667539	AP014960.1	*Oryza sativa japonica*	6E-22	67 %	SAPK7	Q7XQP4	Serine/threonine-protein kinase SAPK7
	BOPA2_12_30993	0.712802	AY177404.1	*Secale cereale*	2e-19	77 %	N/A	Q84XW5	O-methyltransferase
6H, E33M54-350	BOPA1_3379-1037	0.655509896	AK360106.1	*Hordeum vulgare* subsp. *vulgare*	3.00E-116	100 %	N/A	F2D894	Proteasome subunit alpha type
	BOPA2_12_21114	0.724140479	AK355532.1	*Hordeum vulgare* subsp. *vulgare*	2.00E-51	100 %	N/A	F2CV80	Nuclear cap-binding protein subunit 2
	SCRI_RS_80327	0.704318358	AK362351.1	*Hordeum vulgare* subsp. *vulgare*	5.00E-51	100 %	N/A	F2DEN4	3-ketoacyl-CoA synthase
	SCRI_RS_102426	0.60858085	L14444.1	*Triticum aestivum*	2.00E-43	100 %	hsp16.9C	Q41561	Heat shock protein 16.9C
	SCRI_RS_187114	0.701762419	AP014958.1	*Oryza sativa japonica*	7.00E-31	99%	CPK4	Q6Z2M9	Calcium-dependent protein kinase
6H, E42M48-380	SCRI_RS_161117	0.575180587	AP014959.1	*Oryza sativa japonica*	7e-12	89 %	Os03g0125100	Q10SE7	Beta-carotene hydroxylase, putative, expressed
	BOPA2_12_30956	0.649579	AP008220.2	*Oryza sativa japonica*		96 %	OSJNOa246I10.1	Q6EP25	HGWP repeat containing protein-like

Table 3: Results of studies on proteins/enzymes identified in the population V × S under drought stress and other stresses.

Protein/enzyme	Species studied	Treated stress	Gene/peotein expressed	Expression type, effect	Reference
Glycosyltransferase	Arabidopsis	Drought	UDP-Glycosyltransferase	Down-regulation	[Li et al., 2015]
	Rice	Drought	Gene LOC_Os01g68324.3 encoding a glicosyltransferase named Dolichyl-diphosphooligosaccharide	Suppression	[Landi et al., 2017]
Protein DEHYDRATION-INDUCED 19 homolog 5	Arabidopsis	Drought	Gene DI19	Up-regulation, increasing drought tolerance	[Liu et al., 2013]
	Wheat	Drought	Gene TiDI19-2	Induction, enhancing drought tolerance	[Li & Chen, 2000]
Dihydrolipoyl dehydrogenase (LPD)	Sugarcane	Drought	Gene LPD	Up-regulation in tolerant cultivars	[da Silva et al., 2017]
	Barley	High temperature	Two LPD proteins: F2E5U7 and F2E2T3, that F2E5U7 identified in our study under drought stress	Induction, enhancing tolerance to high temperature	[Rollins, 2012]
Hexosyltransferase	Wheat	Drought	One hexosyltransferase gene	Induction, enhancing drought tolerance	[Ajigboye et al., 2016]
	Chickpea (Cicer arietinum)	Drought	11 hexosyltransferase genes related to drought tolerance QTLs included 7 sucrose synthase genes (SuSy) and 4 sucrose phosphate synthase (SPS)	Induction, enhancing drought tolerance	[Nagesh Nayak, 2010]
Salt-tolerant protein-GSK-like kinase	Rice	Drought and salinity	Mutation in gene OsGSK1 that was caused to enhanced expression of special stress respond genes and increased to drought and salinity	Up-regulation, increasing drought and salinity tolerance	[Koh et al., 2007]
	Barley	Dehydration and drought then rehydration	One glicosyle synthase kinase named AAM77397 that is same to Q8LK43 identified in our study.	Induction, enhancing drought tolerance	[Talamé et al., 2007]
Peroxidase	Barley	Drought	Peroxidase	Up-regulation in tolerante cultivares	[Hellal et al., 2018]
	Wheat	Osmotic stress	Peroxidase (TaPrx)	Up-regulation in tolerante cultivares	[Csiszár et al., 2012]
Glycine-rich RNA-binding protein RZ1B (GRP)	Transgenic tobacco	Salinity	GRP gene belong to Limonium bicolor	Up-regulation in tolerante cultivares	[Wang et al., 2014]
	Transgenic rice	Drought	Two GRP gene belong to Arabidopsis included AtGRP2 and AtGRP7	Enhancing drought tolerance	[Yang et al., 2012]
Mitogen-activated protein kinase	*Arabidopsis*	Drought	Two genes AtMPK4 and AtMPK6	Up-regulation, increasing drought tolerance	[Nadarajah and Sidek, 2010.]
	Rice	Drought	Two genes OsMAPK2 and OsMAPK5	Up-regulation, increasing drought tolerance	[Rohila & Yang, 2007]
Pectinesterase	Barley	Drought	Gene PME49	Up-regulation, increasing drought tolerance	[Wendelboe-Nelson et al., 2012]
	Rice	Drought and salinity	Three root genes that encoded pectinesterase	Up-regulation, increasing drought and salinity tolerance	[Koh et al., 2007]
Cinnamoyl-CoA reductase-like SNL6 (CCR)	Transgenic tobacco species *Nicotiana benthamiana*	Drought	Three CCR genes belong to sorghum including to SbCCR1, SbCCR2-1 and SbCCR2-2	Up-regulation, increasing drought tolerance	[Li et al., 2016]
	Tea	Drought	One CCR gene	Up-regulation, increasing drought tolerance	[Gupta et al., 2012]
Ribosomal protein	Maize	Drought	Ribosomal protein S18	Up-regulation, increasing drought tolerance	[Benešová et al., 2012]
	Bermuda grass	Drought	Two ribosomal protein S1 and L12	Down-regulation in sensitive cultivares	[Zhao et al., 2011]
S-adenosylmethionine decarboxylase proenzyme (SAMDC)	Transgenic *Arabidopsis* lines	Drought	One SAMDC gene from *Capsicum annuum*	Enhanced tolerance in transgenic plants than wiled types	[Wilkins et al., 2010]
	Wheat	Drought, salinity and external ABA treatment	TaSAMDC gene	Induction and increasing tolerance	[Li & Chen, 2000]

ATP-dependent Clp protease proteolytic subunit	Wheat	Drought	Two ATP-dependent chlroplastic protease proteolytic subunits	Up-regulation, increasing drought tolerance	[Cheng et al., 2016]
	Parthenium hysterophorus	Drought and salinity	ATP-dependent chlroplastic protease proteolytic subunit	Induction and increasing tolerance	[Ahmad et al., 2017]
Beta-carotene hydroxylase, putative, expressed	Mutant rice	Drought	Mutation in beta-carotene hydroxylase gene (BCH)	Decreased tolerance in mutant than wiled type	[Du et al., 2010]
	Transgenic tobacco	Drought	Overexpression of beta-carotene hydroxylase gene from arabidopsis (chyB)	Increasing beta-carotene and drought tolerance	[Zhao et al., 2014]
Probable anion transporter 5, chloroplastic	*Populus trichocarpa*	Drought	Two genes PtPHT1.2 and PtPHO9	Up-regulation	[Zhang et al., 2016]
	Apple	Drought	Genes MdPHT3;6, MdPHT3;7, MdPHT4;5, MdPHT1;12 and MdPHT4;7	Up-regulation in stressed plants than controls and enhanced drought tolerance	[Sun et al., 2017]
Zinc finger A20 and AN1 domain-containing stress-associated protein 1	Rice	Drought	Overexpression of OsiSAP1 gene	Enhanced drought tolerance	[Dansanaet al., 2014]
	Alfalfa	Drought	MtSAP1 gene	Enhanced drought tolerance	[Gimeno-Gilles et al., 2011]
Protoporphyrinogen oxidase	Transgenic rice	Drought	Overexpression of two Protoporphyrinogen oxidase genes (PPO) from *Arabidopsis thaliana* and *Myxococcus xanthus*	Enhanced drought tolerance	[Thu-Ha et al., 2011]
Chlorophyll a-b binding protein, chloroplastic	Rice and Arabidopsis	Drought, dark, high temperature and salinity	Based on BLAST analysis of sequences of light-harvesting complex (LHC) genes in barley genomic database, 17 LHC genes encoding Chlorophyll a-b binding proteins (HvLHC) were identified that one of them (MLOC_44755) is same to F2CRC1 characterized in our study.	Induction	[Qin et al., 2017]
	Morus indica L.	Drought	Two chloroplastic chlorophyll a-b binding protein	Up-regulation and increasing drought tolerance	[Guha et al., 2013]
Starch synthase, chloroplastic/ amyloplastic	Rice	Drought	LOC_Os07g22930 (AT1G32900) as a starch synthase gene	Up-regulation and increasing drought tolerance	[Basu & Roychoudhury, 2014]
	Sorghum	Drought	Starch synthase enzyme	Down-regulation and decreasing drought tolerance	[Yi et al., 2014]
Proteasome subunit beta type	Common bean	Drought	Proteasome subunit beta type-3-A	Down-regulation	[Zadražnika et al., 2013]
	Barley	Drought	Proteasome subunit beta type	Up-regulation	[Wehner et al., 2015]
Lactoylglutathione lyase	Eucalyptus	drought	Lactoylglutathione lyase gene	Down-regulation	[Ghosh & Dharanishanthi, 2017]
	Humulus lupulus L.	Drought	Lactoylglutathione lyase protein related to ROS pathwey		[Kolenc et al., 2016]
Xyloglucan endotransglucosylase/ hydrolase	Transgenic tomato	Drought	CaXEH3 gene from *Capsicum annum* L.	Overexpression	[Choi et al., 2011]
	Medicago truncatula	Drought	21 XEH genes	Induction	[Yi et al., 2014]
Phosphoglycerate kinase	Soybean	Drought and high temperature	Phosphoglycerate kinase protein	Up-regulation	[Das et al., 2016]
	Barley	Drought	Phosphoglycerate kinase protein	Down-regulation	[Pieczynski et al., 2012]
D-3-phosphoglycerate dehydrogenase	Arabidopsis	H2O2 and ABA treatment	D-3-phosphoglycerate dehydrogenase protein	Induction	[Cramer et al., 2013]
	Grapevine (*Vitis vinifera* L.)	Drought	D-3-phosphoglycerate dehydrogenase protein	Down-regulation	[Alqurashi et al., 2017]

identified proteins/enzymes with drought stress has been proven in previous studies.

As can be seen, all the proteins/enzymes identified in both populations were directly involved in the response to drought stress in several studies on different plants, which indicates the confirmation of the validity of the QTLs found in our previous study (Mohammadi et al., 2018). In order to identify genes related to drought stress in rice, Gorantla et al. (2007), prepared ESTs from the leaf tissue cDNA library of a rice cultivar under drought treatment, and one of the genes expressed in response to drought stress, was heat shock protein C16.9, which is consistent with the present research, and also Szűcs et al. (2006) reported 6 QTLs related to photoperiod in barley, and one of genes, HvPhyB, located on chromosome 4, i.e.

Table 4: Results of studies on protein/enzymes identified in the population C. Cappa × S under drought stress and other stresses.

Protein/enzyme	Species studied	Treated stress	Gene/protein expressed	Expression type, effect	Reference
Phytochrome B	Rice PhyB mutant	Drought	PhyB mutant protein	Increased drought tolerance	[Liu et al., 2012]
	Barley	Drought	The HvPhyB gene located on chromosome 4H, i.e. accession "DQ201144", which was also identified in the present study.	Increased drought tolerance	[Talamé et al., 2007]
Serine/threonine-protein kinase SAPK7	Rice	Dehydration	SAPK5 gene	Increased expression	[Basu & Roychoudhury, 2014]
	Groundnut	Drought	Serine/threonine-protein kinase HT1	Induced expression	[Ding et al., 2014]
O-methyltransferase	Sugarcane	Drought	O-methyltransferase 2	Increased expression and drought tolerance	[da Silva et al., 2017]
	Ocimum basilicum	Drought	Chavicol O-methyltransferase and eugenol O-methyltransferase genes	Increased expression	[Abdollahi Mandoulakanieet al., 2017]
	Tea	Drought	Caffeic acid 3-O-methyltransferase	Decreased expression	[Wang et al., 2017]
Proteasome subunit alpha type	Alfalfa	Drought and salt	Two proteins related to the proteasomal subunit, including the alpha-7 and beta-2-B subunits	Decreased expression	[Ma et al., 2016]
	Soybean	Drought	Proteasome subunit alpha type	Increased expression	[Pour Mohammadi et al., 2012]
	Common bean	Drought	Proteasome subunit alpha type	Decreased expression	[Zadražnika et al., 2013]
	Wheat	Drought	Proteasome subunit alpha type	Decreased expression	[Jiang et al., 2012]
Nuclear cap-binding protein subunit 2	Mutant barley in HvCBP20 gene	Drought	HvCBP20 gene	Increased drought tolerance	[Daszkowska-Golec et al., 2017]
	Potato CBP20 and CBP80 mutants	Drought	CBP20 and CBP80	Increased drought tolerance	[Pieczynski et al., 2012]
3-ketoacyl-CoA synthase	Cotton	Drought	3-ketoacyl-CoA synthase gene	Induction	[Wang et al., 2010]
	Two varieties of silver fir (Abies alba Mill.)	Drought	Two 3-ketosyl-CoA synthase genes	The expression of these two genes increased under drought conditions in one cultivar and decreased in the other cultivar	[Behringer et al., 2015]
Heat shock protein 16.9C	Rice	Drought	Heat shock protein 16.9C	Induction	[Gou et al., 2017]
	Wheat	Drought and high temperature	HSP proteins	Induction	[Guha et al., 2013]
Calcium-dependent protein kinase	Barley	Drought	HvCPK2a	Increased expression	[Ciésla et al., 2016]
	Rice	Drought	OsCPK4	Overexpressed	[Campo et al., 2014]
HGWP repeat containing protein-like	Maize, wheat and barley	Drought	Six genes encoding HGWP repeat containing protein-like	Increased tolerance	[Swamy et al., 2011]
	Two sensitive and drought-resistant varieties of barley	Drought	HGWP repeat containing protein-like	Increased expression in the sensitive variety	[Wendelboe-Nelson, 2012]

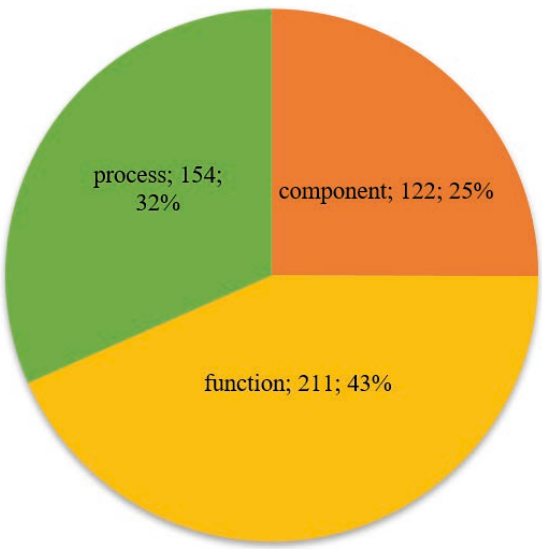
accession "DQ201144", which has been identified in the present study in the C. Cappa × S population on chromosome 4H (Tables 3 and 4).

3.4 GENE ONTOLOGY

3.4.1 Ontology results in V × S population

The results of gene ontology in population V × S are shown in Figures 3 and 4. The frequencies of three GO description, including cellular components, biological processes, and molecular functions, were 25 %, 32 %, and 43 %, respectively (Figure 3). Based on the results of complete gene ontology, four types of cell components including membrane, integral component of membrane, nucleus and chloroplast had the highest frequencies: 4.9 %, 12.4 %, 12.4 % and 9.1 % respectively (Fig. 4a). Two types of biological processes, including oxidation-reduction process and protein phosphorylation, had the highest frequencies: 11.5 % and 5.1 %, respectively (Figure 4b). The study of the frequency distribution of molecular functions showed that ATP binding, oxidoreductase activity, transferase activity, DNA binding, and metal ion binding, were most frequent: 9.0 %, 7.1 %, 5.69 %, 5.2 % and 4.74 % respectively (Figure 4c).

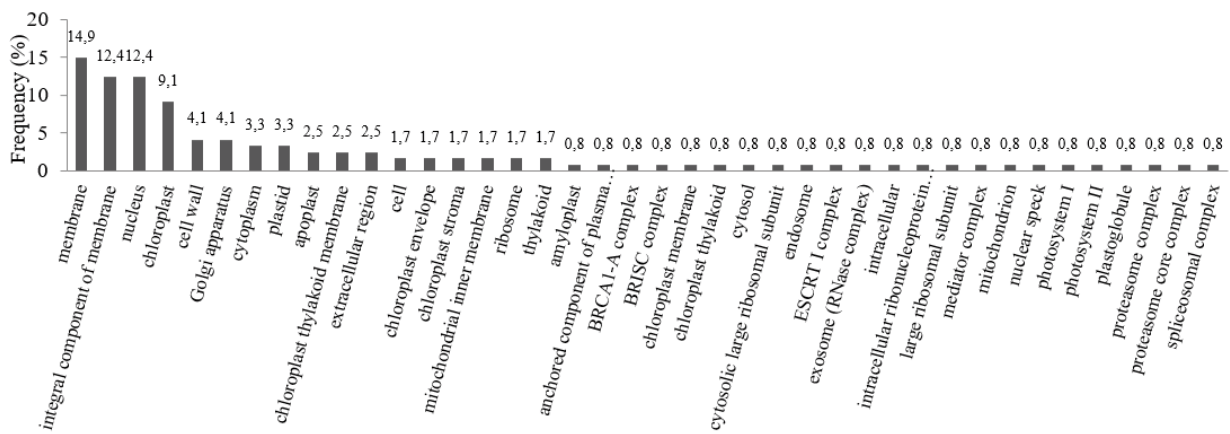
Wilkins et al. (2010) studied drought stress-induced transcripts in *Arabidopsis*, and based on ontology analysis, reported that transferase activity as a molecular function; chloroplast and membrane as cellular components had the highest frequency. Kokas et al. (2016) investigated the transcripts of wild barley in response to drought stress and as a result of the ontology study, they reported molecular functions such as binding, catalytic activity and binding to nucleic acid (Kokas et al., 2016). Similar results have been reported in other studies on different plants in drought conditions (Bedada et al., 2014; Zeng et al., 2016; Gou et al., 2017). All these studies confirm the results of the present research.



3.4.2 Ontology results in C. Capa × S population

The results of gene ontology in C. Capa × S population are shown in Figures 5 and 6. The frequency of GO descriptions including molecular functions, biological processes, and cellular components were 40.2 %, 30.4 %, and 29.4 %, respectively (Figure 5). Based on the analy-

Figure . Frequency distribution of GO description types (number, percentage) in population V×S



GO terms of Cellular Components (a)

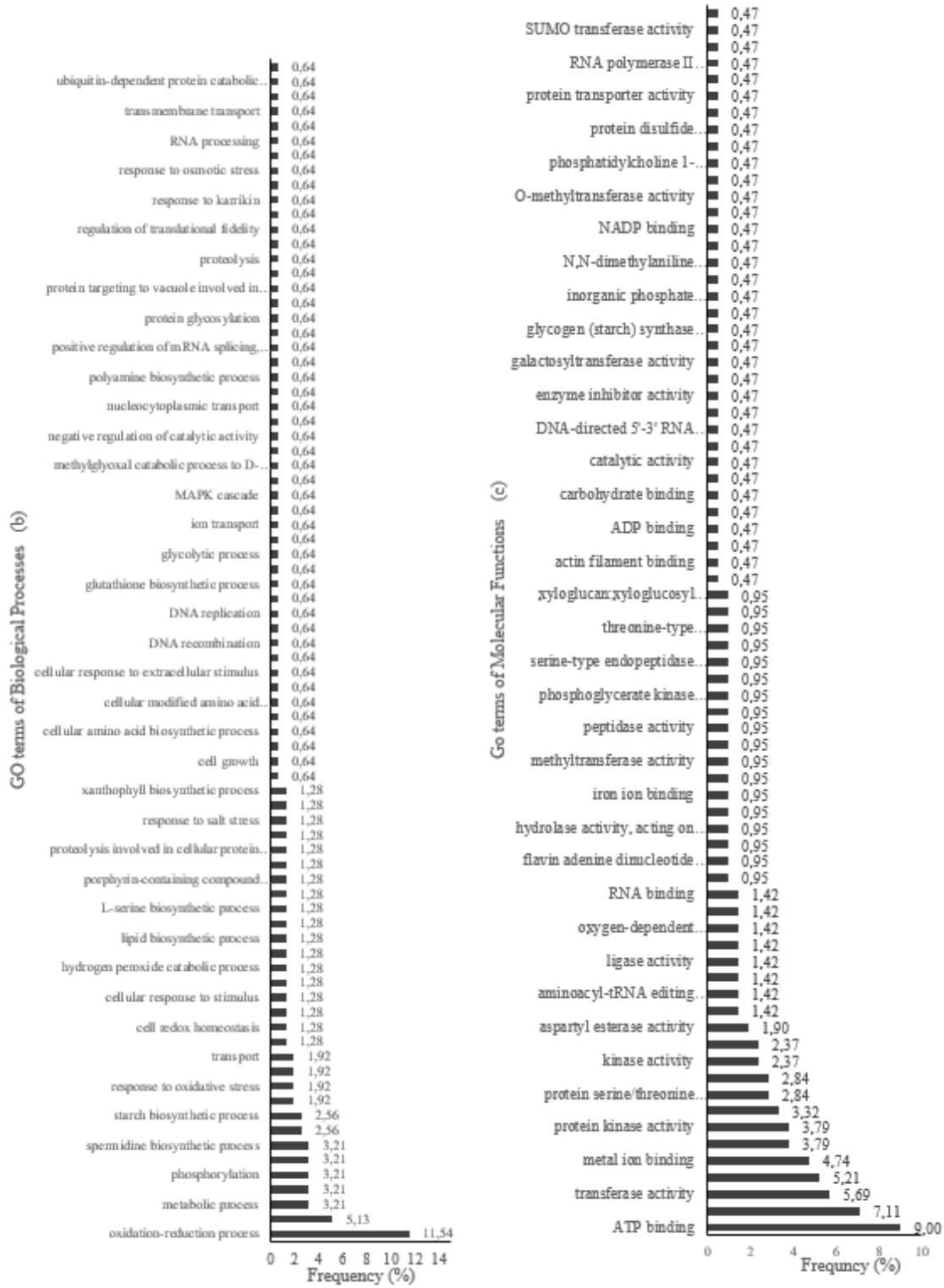


Figure 4: Frequencies of three types of GO descriptions characterized in V × S population; a: Cell Components, b: Biological Processes and c: Molecular Functions

sis results, the highest frequency of cellular components was associated with the nucleus, comprising 26.7 % of the total. Additionally, the membrane and cytoplasm accounted for 20 %, while the proteasome core complex and integral membrane components each represented 10 %. These findings highlight the predominant roles of these cellular structures in the context of the study, emphasizing their significance in cellular functions and processes (Figure 6a). Also 9 types of molecular functions including ATP binding and transferase activity (with a frequency of 9.8 %); thereonine-type endopeptidase activity and protein serine/thereonine kinase activity (with a frequency of 7.3 %); RNA binding, transferase activity, transferring acyl groups, kinase activity, protein kinase activity and methyltransferase activity (with a frequency of 4.9 %) had the highest frequency (Figure 6b). Among the biological processes, the highest frequencies were related to five types of biological processes, including protein phosphorylation (12.9 %), fatty acid biosynthetic process (6.5 %), proteolysis (6.5 %), intracellular signal transduction (6.5 %) and oxidation-reduction process (6.5 %) (Figure 6c). In Saavedra experience the highest frequencies were related to oxidation-reduction process (tree time of occurrence) (Saavedra et al., 2017).

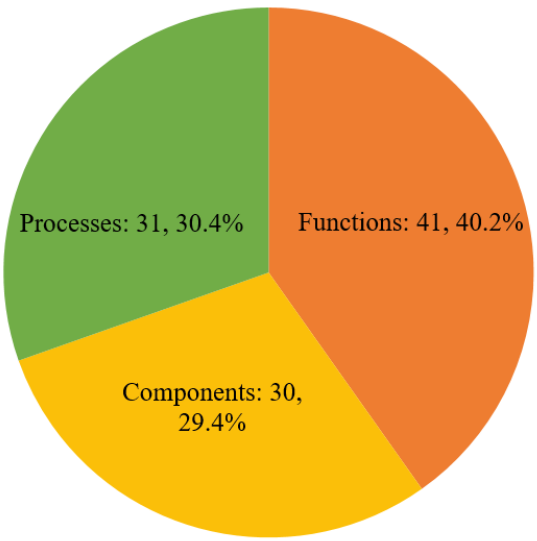


Figure 5: Frequency distribution of GO description types (number, percentage) in population C. Cappa × S

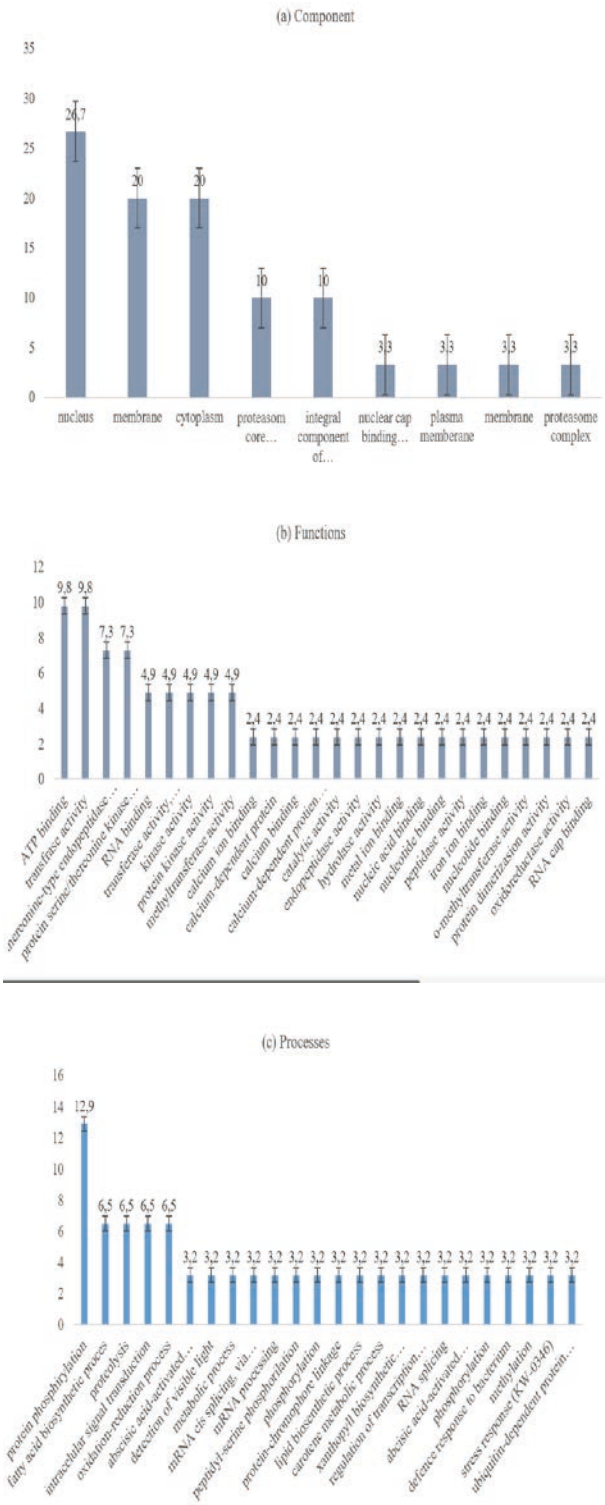


Figure 6: Frequencies of three types of GO descriptions characterized in C. Cappa × S population; a: Cell Components, B: Biological Processes and c: Molecular Functions

4 CONCLUSIONS

The results of this study, as well as previous similar

studies, showed that BLAST analysis on SNP sequences is a very effective tool for validating the identified QTLs. Based on the results of the present study, all proteins/enzymes identified in two populations are directly involved in the response to drought stress in different plants. Both up-regulation (increased expression) and down-regulation (decreased expression) have been reported for the identified proteins/enzymes in response to drought stress in different plants. The gene ontology results showed that the identified genes are significantly involved in drought stress. Practical result is that these QTLs, particularly those identified on chromosomes 1H, 4H, and 5H, along with related genes like glycosyltransferases, phytochrome B, and pectinesterase, can be very useful for developing drought tolerance in barley and other plants.

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