About OMICS Group

OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

About OMICS Group Conferences

OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

A proteomic approach to investigate specific traits in Tunisian barley accessions with contrasting salinity tolerance

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Cereals

Human nutrition: more than half of the caloric intake is related to cereal production

Growing of human population

Climate change

Cereal crop productivity

Barley (Hordeum vulgare)

One of the most important crops, ranking fifth in term of production all over the world and usually used as human food, animal feed, malt and cosmetic production



Human consumption



Animal Feed



Brewing malts



Cosmetic product



Barley (Hordeum vulgare L.)

Amongst the most salinity tolerant crop species



Well-studied in terms of genetics, genomics and breeding :

- **Diploid self-pollinating crop specie s**
- **A low chromosome number (2n = 14)**
- **Genomic size: 5,5 pg DNA/nucleol**
- **A relatively short life cycle**
- **Its genome is recently completely sequenced: the German partner institute (IBSC, http://www.barleygenome.org).**

A model plant for Triticeae research

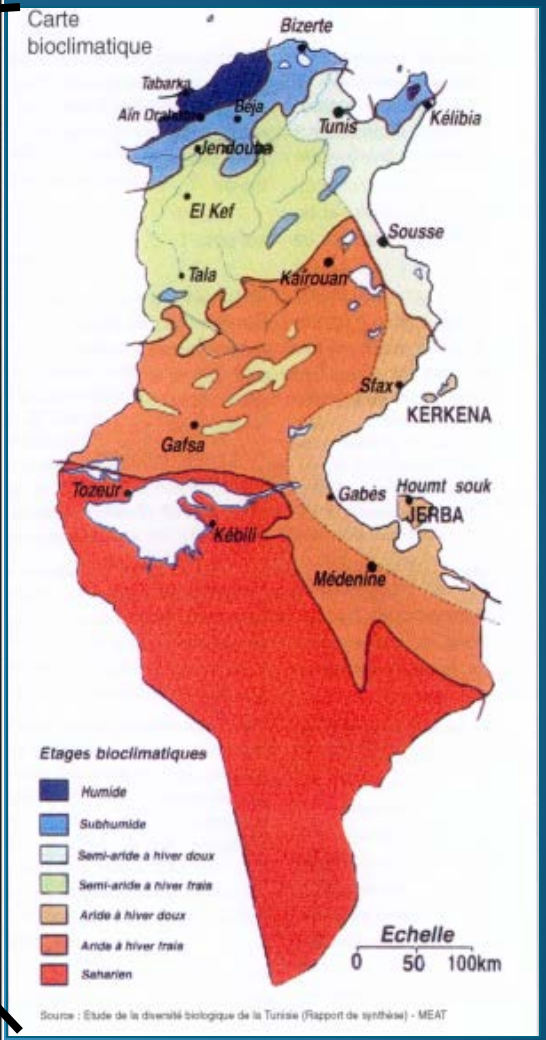
Genetic variation	Physiological performance	Biochemical performance

Identification of significant traits in Barley

cereal salt tolerance improvement by means of conventional and molecular approaches

TUNISIA


ALGERIA
LIBYA
EGYPT
SAHARA
MAURITANIA
NIGER
MALI
CHAD
SUDAN
NIGERIA
ETHIOPIA
SOMALIA
CONGO BASIN
DEM. REP. OF THE CONGO
ANGOLA
ZAMBIA
MOZAMBIQUE
BOTSWANA
NAMIBIA
SOUTH AFRICA
MADAGASCAR
TUNISIA
Scale 1:51.400.000


Carte bioclimatique
Bizerte
Tabarka
Aïn Draham
Béja
Tunis
Kélibia
Jendouba
El Kef
Sousse
Tala
Kairouan
Sfax
KERKENA
Gafsa
Tozeur
Gabès
Houmt souk
JERBA
Kébili
Médenine
Etages bioclimatiques
Humide
Subhumide
Semi-aride à hiver doux
Semi-aride à hiver frais
Aride à hiver doux
Aride à hiver frais
Saharien
Echelle
0 50 100km
Source : Etude de la diversité biologique de la Tunisie (Rapport de synthèse) - MEAT



Barley in Tunisia



The most important cereal crop cultivated with the 2ed rang in terms of cereal production and occupies between 34% and 38% of the cereal cultivated area

* 10 à 15 % for human consumption

* 85 % for feed animals

Salinity



In the water-limited areas of Tunisia, more than 30% of subsurface resources used for irrigation are saline, leading to a long-term salinization and degradation processes.

Plant growth inhibition

Salt stress

Plants: Development of various strategies that increase induced tolerance or adaptation by altering genes or proteins expression

Identification of the genes and/or proteins responsible for tolerance and their role is important to design effective breeding strategies

Genomics/Transcriptomics

Correlation between mRNA and protein is not always observed!! Post-transcriptional modifications!!

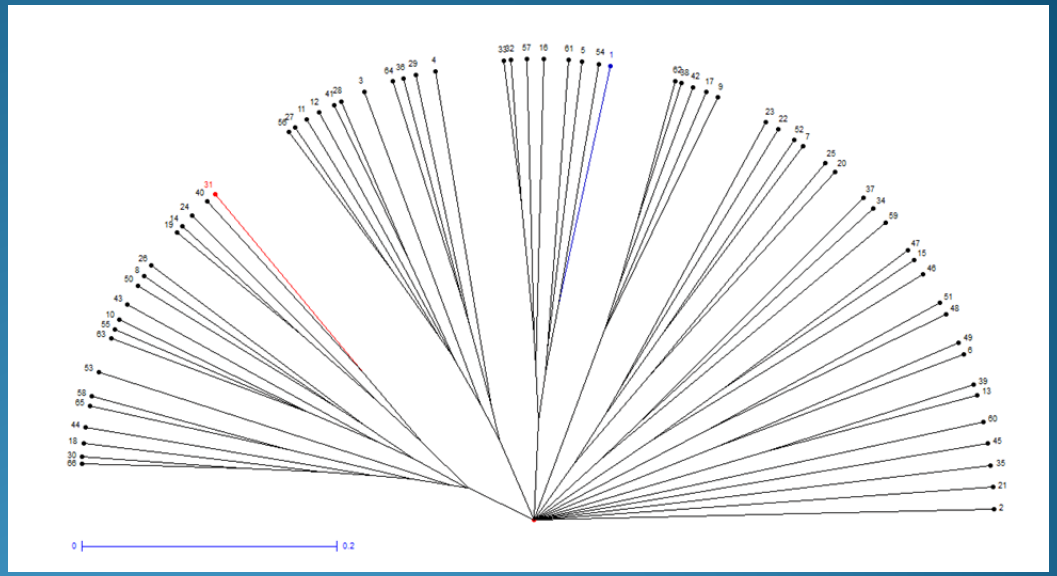
Recent improvement in proteomics

Investigation on specific traits in Tunisian barley accessions with contrasting salinity tolerance

Correlations between proteomes from different developmental stages in barley under salt treatment

Tunisian Barley accessions

Discrimination of 66 tunisian barley accessions using 44 SSR loci



Genetic diversity

Microsatellites (Zoghlami et al. 2011)

Physiological salt assays

Identification of 3 contrasting barley pairs from different developmental stages

Germination



Tillering

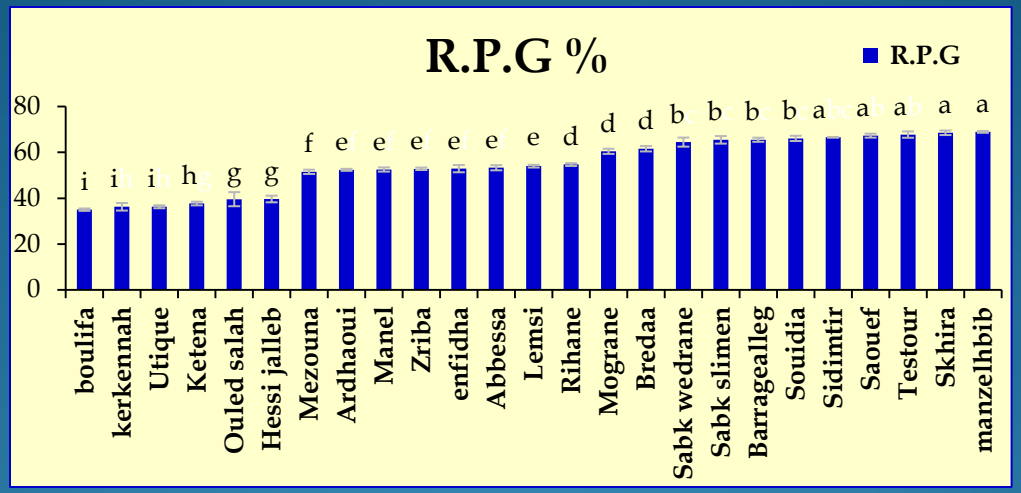




Maturation

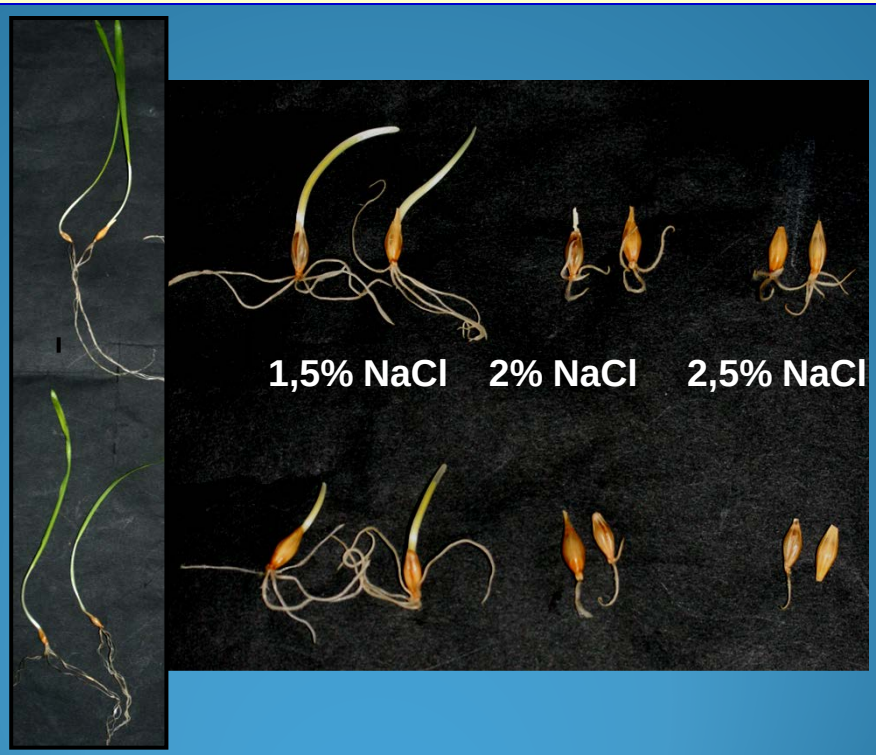
Germination

R.P.G: Percentage of germination decrease



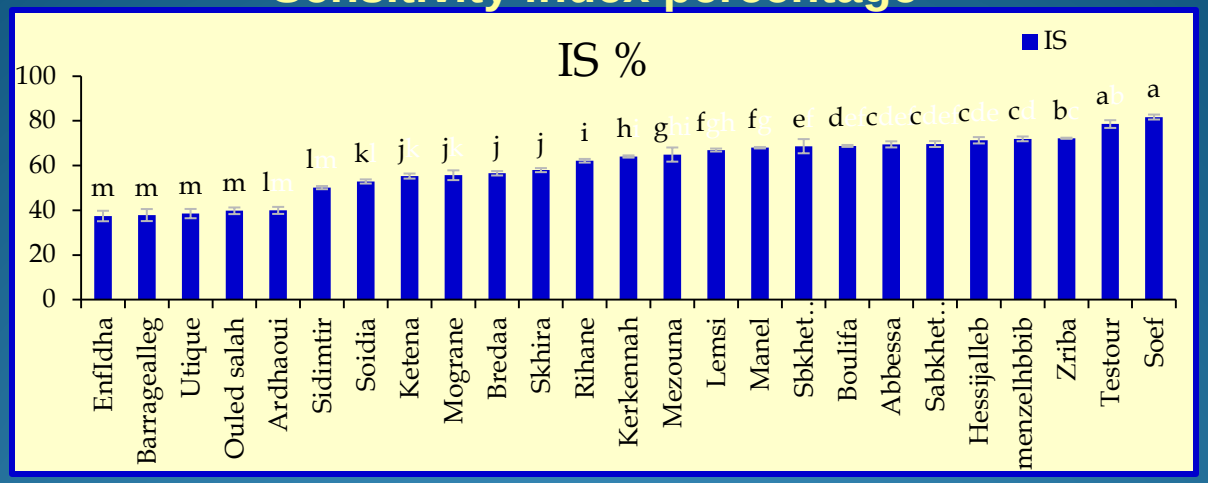
Boulifa
Tolerant

Menzel Hbib
Sensitive



Tillering stage

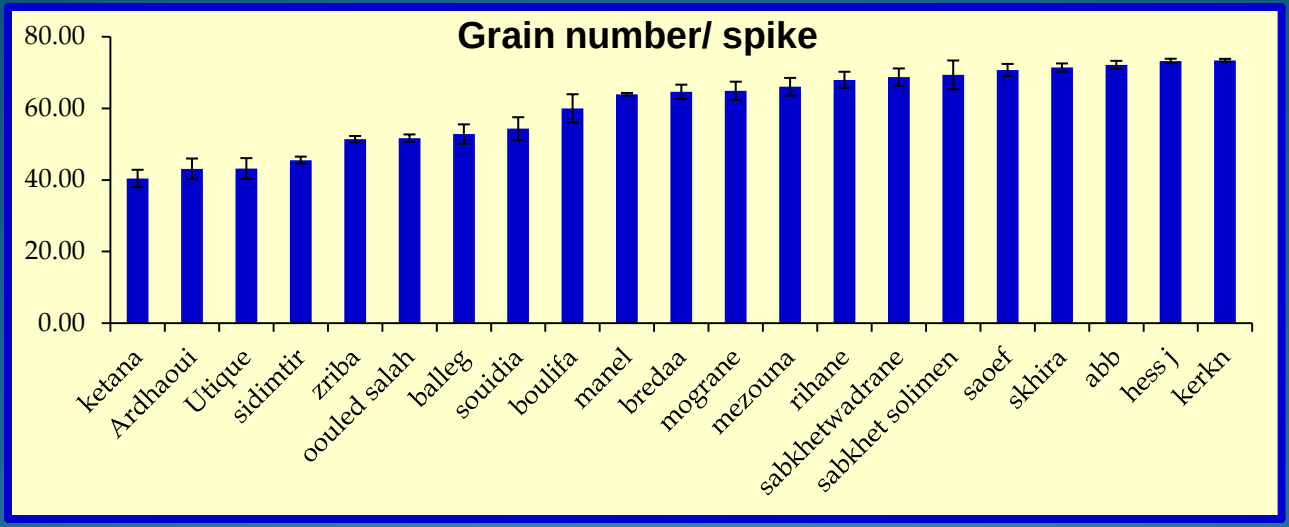
Sensitivity index percentage







Maturation


Grain number/ spike
80.00
60.00
40.00
20.00
0.00
ketana
Ardhaoui
Utique
sidimtir
zriba
ouled salah
balleg
souidia
boulifa
manel
bredaa
mograne
mezouna
rihane
sabkhetwadrane
sabkhet solimen
saoef
skhira
abb
hess j
kerkn


Control
Kerkena Tolerant
Ketena Sensitive


Salt stress
Kerkena Tolerant
Ketena Sensitive

Proteome analysis of the contrasting pair at germination stage

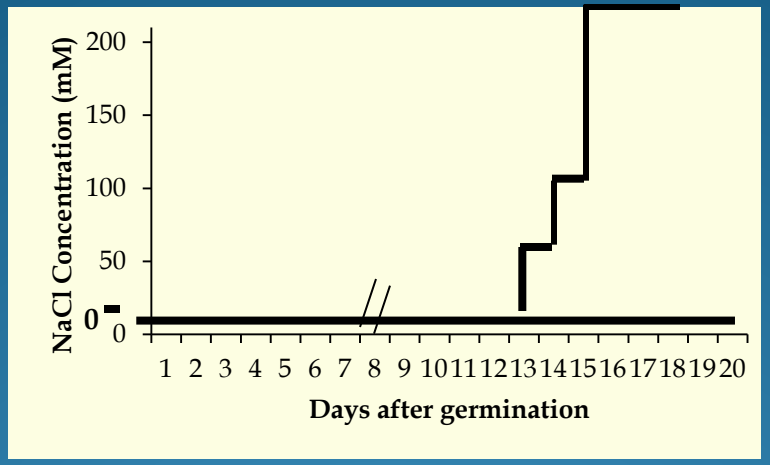
Mature grains



Boulifa (Tolerant)



Menzel Hbib (Sensitive)



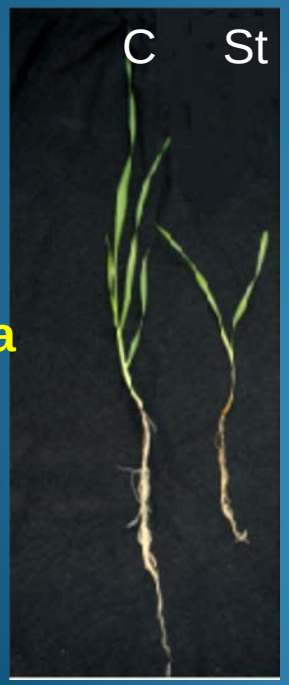
Roots

Boulifa (Tolerant)

Testour (Sensitive)

Hydroponic salt assay (200 mM NaCl)

The Roots were harvested after an hydroponic salt assay imposed on 12-day-old barley seedlings at 200 mM NaCl concentration which was increased in a step- wise manner until the desired salinity range. The harvesting for proteomic analysis was done at 3 days salt stress



Boulifa (T)





Testour (S)

C: control- St: stress

Approach

Protein extraction

Grains: Hydrosoluble proteins (Globulins and albumins): Witzel et al., 2010

Extraction buffer: 5 mM Tris pH 7,5 ; 1 mM $CaCl_2$
Precipitation: Acetone 2h (-20°c)
Solubilisation: 8 M urea (Roth), 2% CHAPS (Roth), 20mM DTT (Biochemica), 0,5% IPG-buffer (Healthcare)

Roots : Witzel et al., 2009

- **Extraction buffer**: 10% TCA/ 0.07% ß- mercaptoethanol/ aceton
- **Precipitation**: Aceton/0.07% ß -Mercaptoethanol (-20°c); 30min
- **Solubilisation**: 8 M urea (Roth), 2% CHAPS (Roth), 20mM DTT (Biochemica), **0,5%** IPG-buffer (Healthcare)
- **Filtration:** Centrifugation through Amicon Ultrafree-MC 0,45 µm Filter; 12000g, 10min
- **Roots dialysis:** PlusOne™ Mini Dialysis Kit kDa cut-off



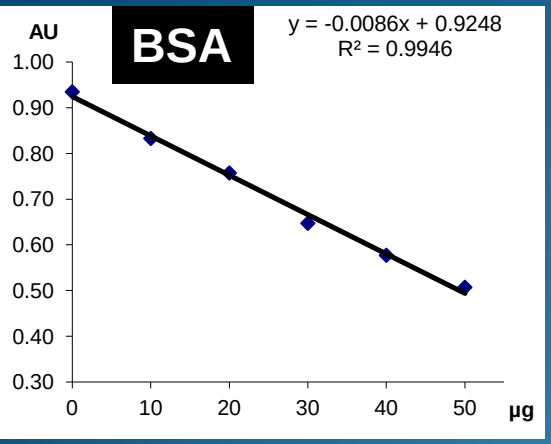
Protein quantification

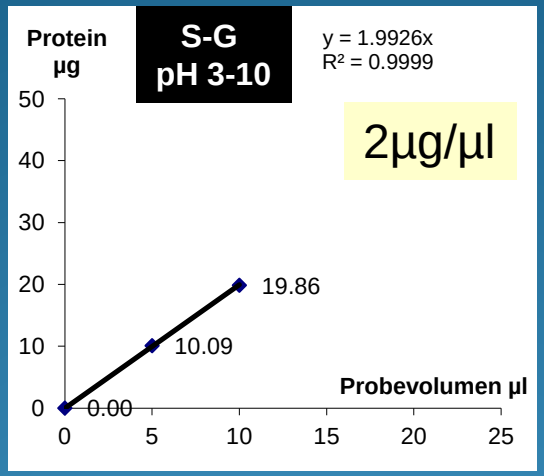
2-D Quant Kit (GE Healthcare)

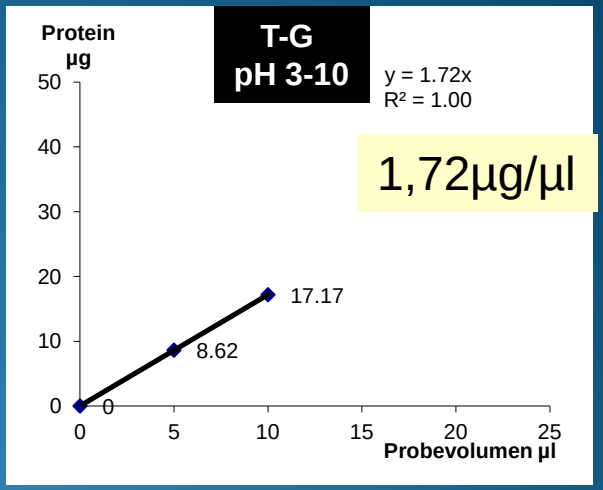
Protein quantification

2-D Quant Kit (GE Healthcare)

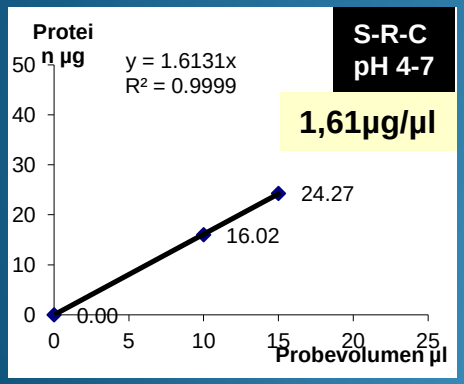
Grain proteins

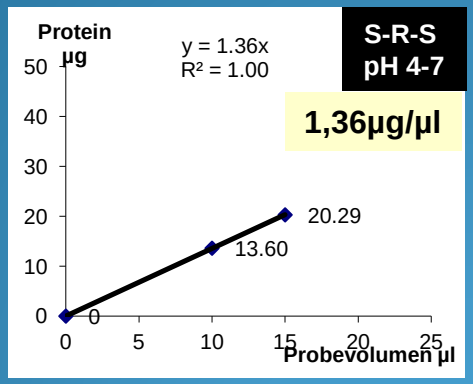


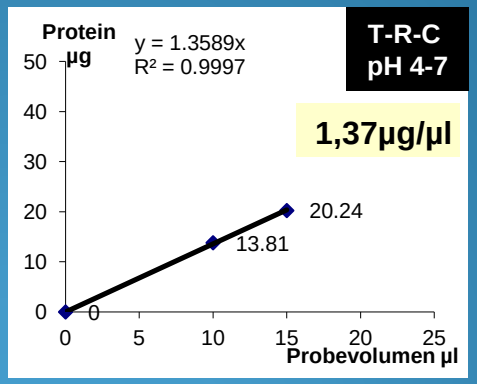


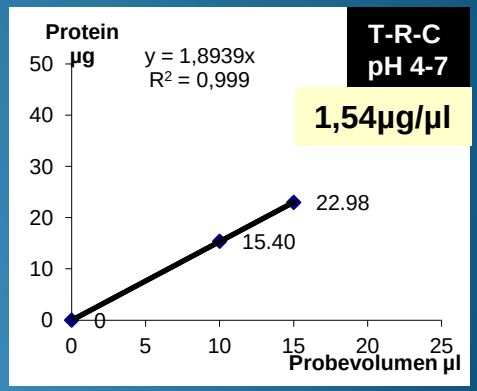


Root proteins









2-D GE and protein staining

Isoelectric_Focusing

- IPGphor (GE Healthcare) with printer DPU-414 (Seiko)
- Immobiline DryStrips
- IPG buffer: 3-10 for grains and 4-7 for roots

-7 cm - IPG – Strip
Rehydration: 14 h 20°C
IEF:50 µA 20 °C

Step	*Volt*	*h : min*	*Vh*	*SnH / G*
S1	250	0:30		G
S2	500	0:30		G
S3	3000	0:30		G
S4	3000	4:40		SnH

Run finish 3000V 15120Vh
28 µA 6:10h

Equilibration of the IPG-Strip

EquiBuffer:
1,5 M Tris-HCl pH 8,8: 50 mM
Urea 6 M
Glycerol 30 %(v/v)
SDS 2 %(w/v)
Bromphenolblue 0,5 ml 1% BPB
18MΩ - water
2.5ml EquiBuffer+ 162,5 µl 1M DTT
15min shake

SDS-PAGE

MiniproteanII BioRad:
Sparating gel (11,25% Aa) Aa/Bis 30/08 (Rotiphorese Gel 30 (37,5:1)
Stacking Gel (6 % Aa)
Agarose (0,5%)
(Electrode distance 12 cm)
1X Running Buffer Leammli 8,8 (RT)
30min 75v
50min 150v

protein staining:

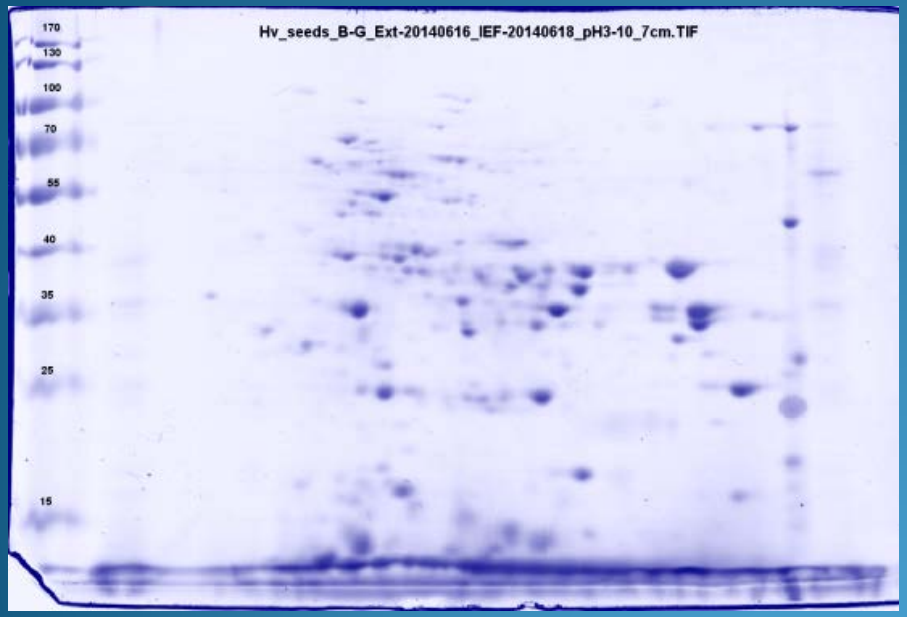
Comassie blue

Image acquisition and analysis of the grain proteome

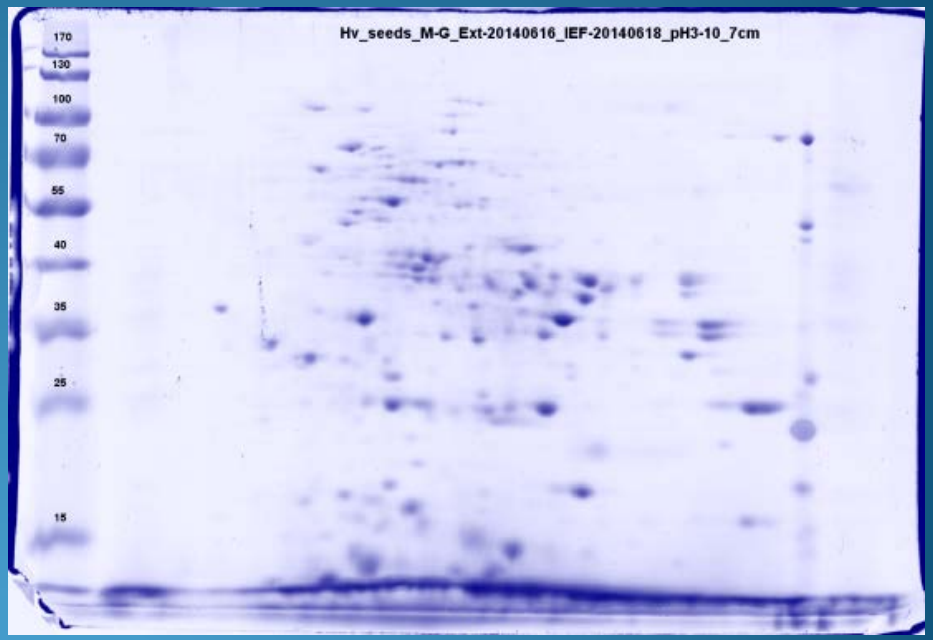
A Fuji FLA-5100

The Progenesis PG220 v2006 software (Nonlinear, Dynamics)

Detection of 248 spots per line and per gel among the water-soluble grain proteomes



Tolerant genotype (T)

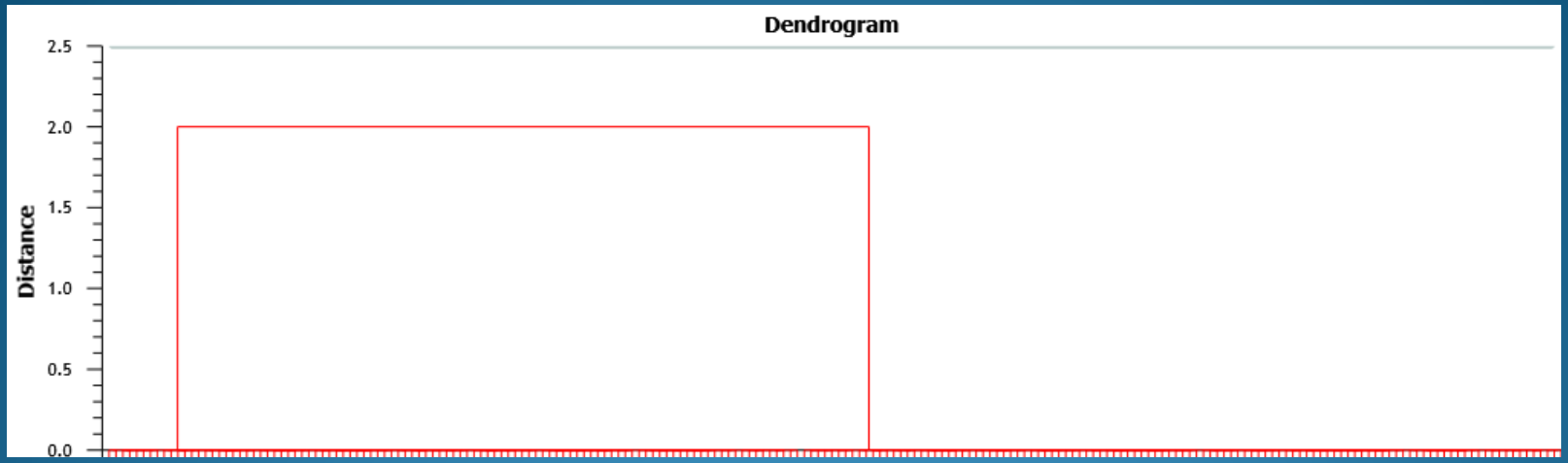


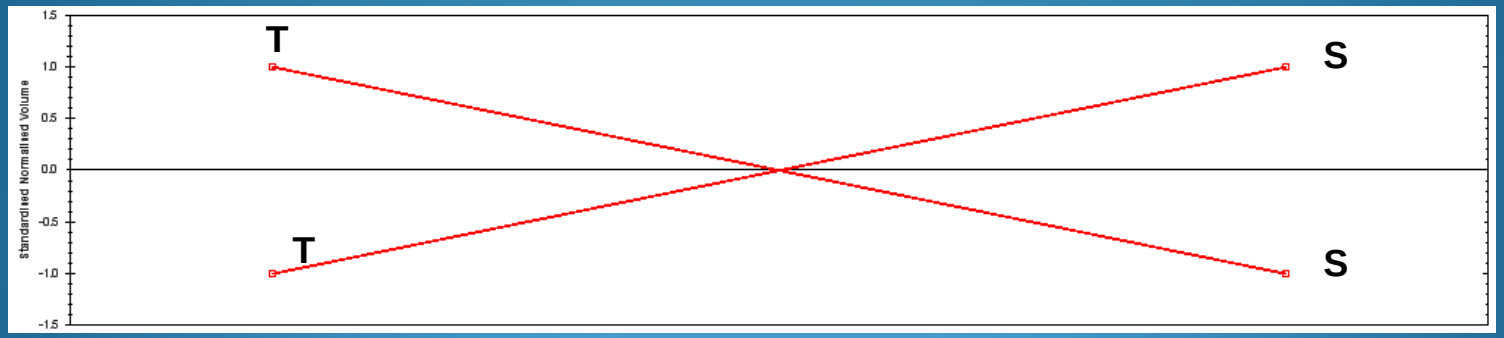
Sensitive line (S)

The water-soluble grain proteomes of salinity sensitive and tolerant Tunisian barley accessions

Image acquisition and analysis

The Progenesis PG220 v2006 software (Nonlinear, Dynamics)
212 protein spots out of 248 spots are analyzed





2 Groups of spots in T and S lines:

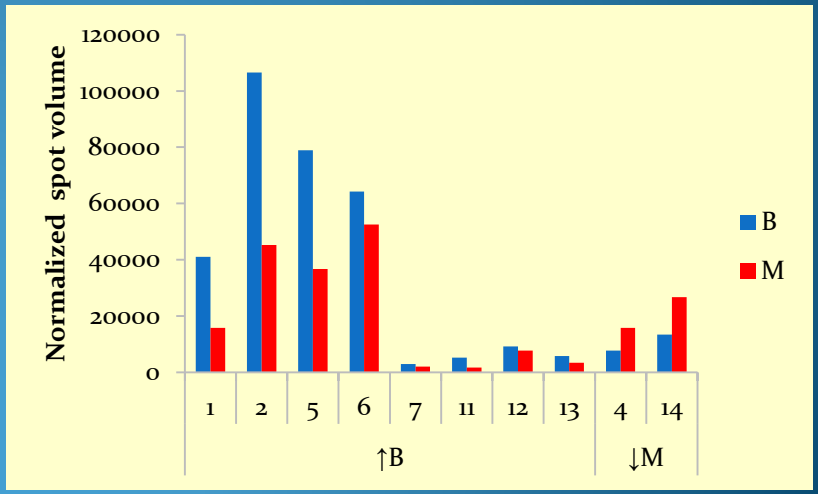
*** 1st group correspond to protein spots that are over-expressed in T and down regulated in S**
*** 2ed group correspond to protein spots that are down regulated in T and up-regulated in S**

Image acquisition and analysis

10 selected protein spots

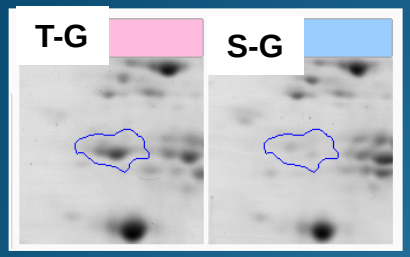
#	Anova (p)	Fold	Included	Notes	Max CV (%	Highest Me	Lowest Me	X Position	Y Position	Area	B/G Normli	Normalized Volume B-G Hv_seeds_	Normalized Volume M-G Hv_seeds_	Volume B-G Hv_seeds_	Volume M-G Hv_seeds_	Background B-G Hv_seeds_	Background M-G Hv_seeds_	Peak Height B-G Hv_seeds_	Peak Height M-G Hv_seeds_	
13=50		1.687996	Yes		0	B-G	M-G	390	195	368	1.687996	5840	3459.724	5840	3453	3309	3310	51.00815	21.00543	↑B
1=110		2.593052	Yes		0	B-G	M-G	413	305	1522	2.593052	41050	15830.77	41050	15800	21307	19786	121.0007	32	↑B
11=114		3.116656	Yes		0	B-G	M-G	787	310	453	3.116656	5318	1706.316	5318	1703	2718	2265	30	19	↑B
2=127		2.35045	Yes		0	B-G	M-G	818	326	2061	2.35045	106532	45324.09	106532	45236	10305	10304	163	87.00049	↑B
14=133		1.991234	Yes		0	M-G	B-G	726	334	1031	0.502201	13454	26790.07	13454	26738	5155	7217	38	111	↓B
3=174		1.612558	Yes		0	M-G	B-G	524	393	812	0.620133	9847	15878.86	9847	15848	5684	8120	30	79	↓B
12=208		1.188527	Yes		0	B-G	M-G	563	479	463	1.188527	9310	7833.224	9310	7818	5556	5093	54	47	↑B
4=230		2.017199	Yes		0	M-G	B-G	404	575	728	0.495737	7832	15798.7	7832	15768	5824	5096	27	67	↓B
6=236		1.223494	Yes		0	B-G	M-G	482	592	1813	1.223494	64296	52551.13	64296	52449	16317	16317	123	103	↑B
7=242		1.413099	Yes		0	B-G	M-G	678	605	414	1.413099	3037	2149.177	3037	2145	7038	5795	15	28.00242	↑B
5=251		2.149549	Yes		0	B-G	M-G	652	657	1898	2.149549	78971	36738.4	78971	36667	34163	34164	96.00053	41	↑B

8 up-regulated spots in the T and 2 up-regulated spot s in the S were selected

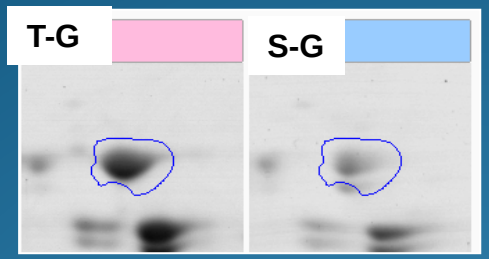


Selected up-regulated spots in the tolerant accession (T)

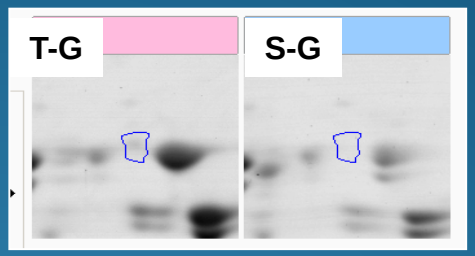
Spot110= 1



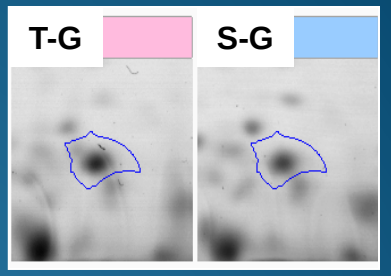
Spot 127= 2



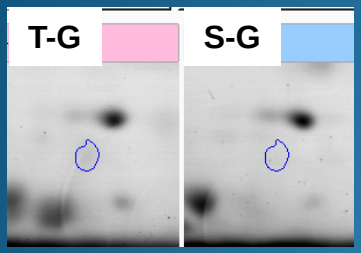
Spot 114= 11



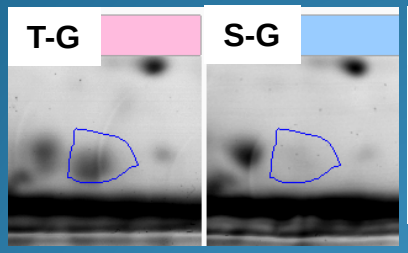
Spot 236= 6



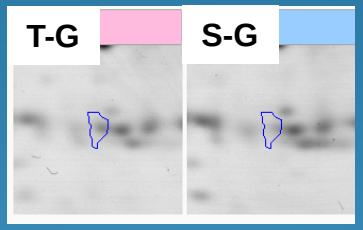
Spot242 = 7



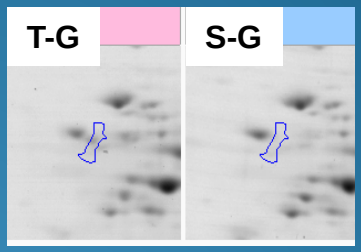
Spot 251=5



Spot 208= 12

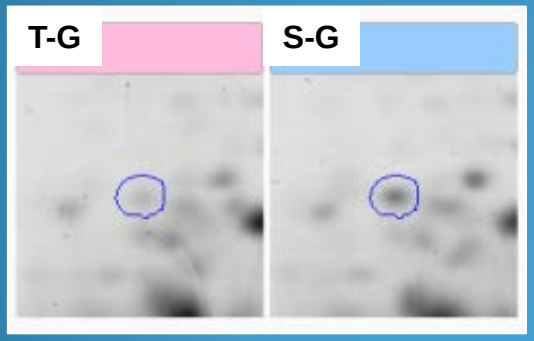


Spot 50= 13

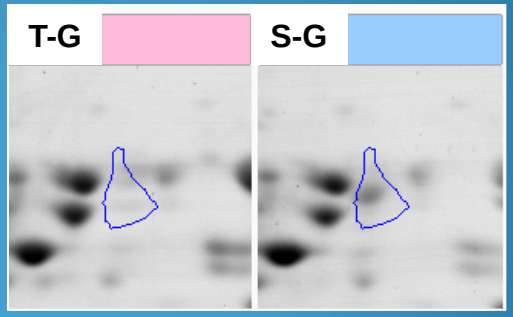


Selected up-regulated-spots in the sensitive accession (S)

Spot 230= 4



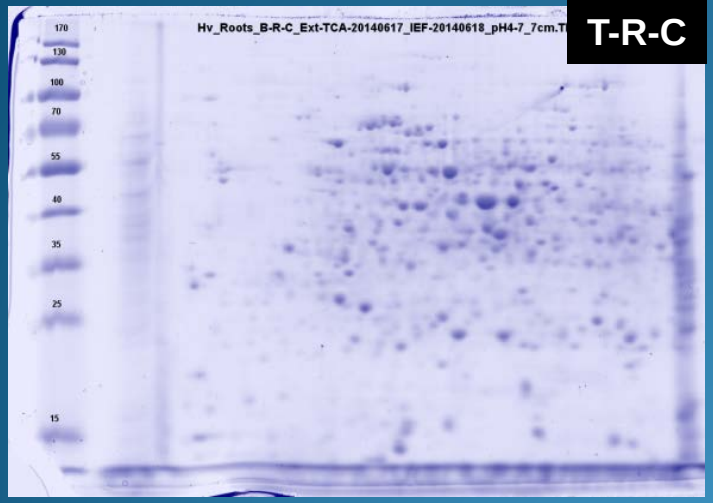
Spot 133=14

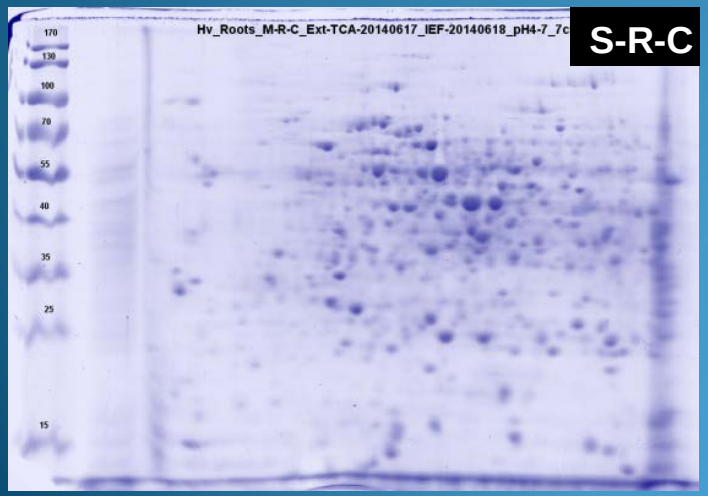


The root proteomes of salinity sensitive and tolerant Tunisian barley accessions

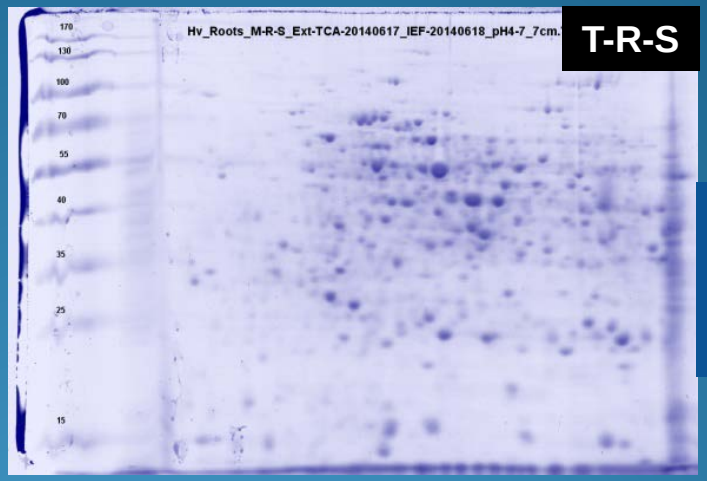
Detection of approximately 500 protein spots on the 2-D gels, of which 390 were matched between groups of lines and treatments.

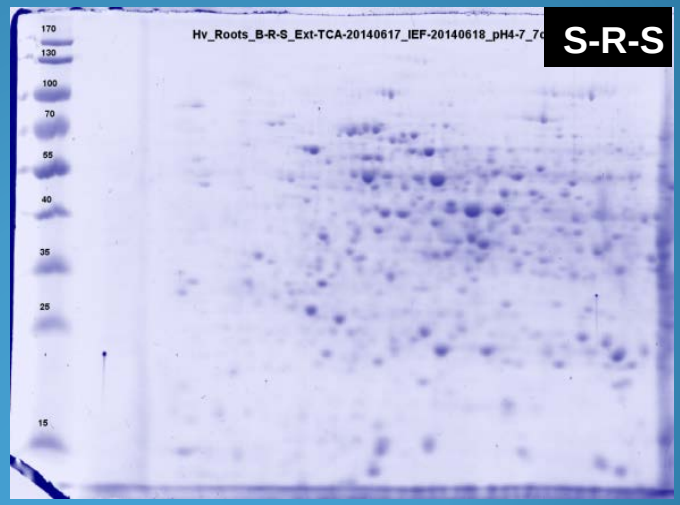
Control conditions (C)





Salt stress (S)





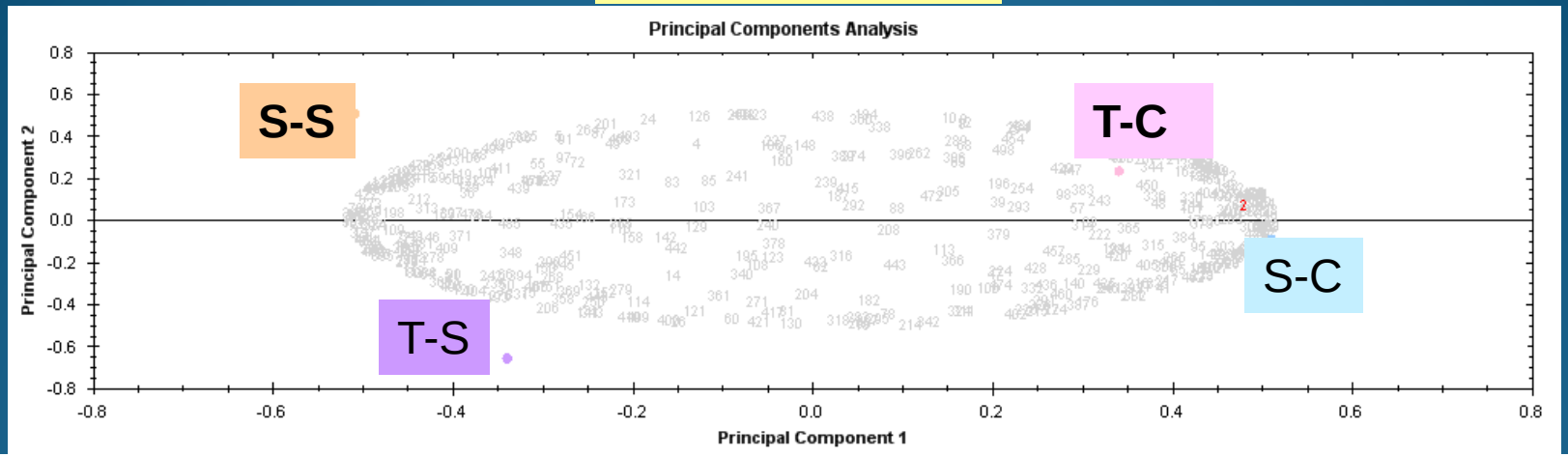
Tolerant accession (T)

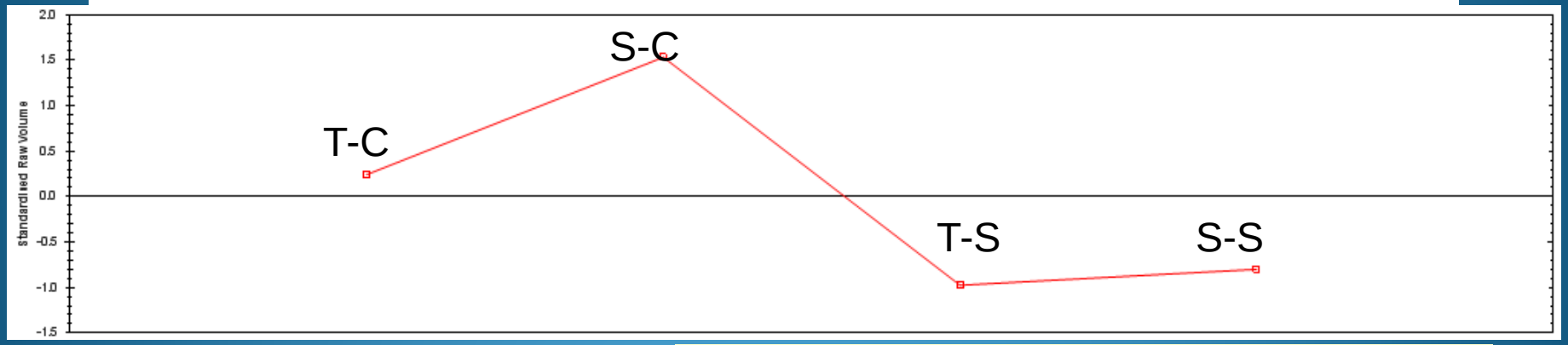
Sensitive accession (S)

Image acquisition and analysis

Contol	T-C	S-C
Stress	T-S	S -S

Lines and stress



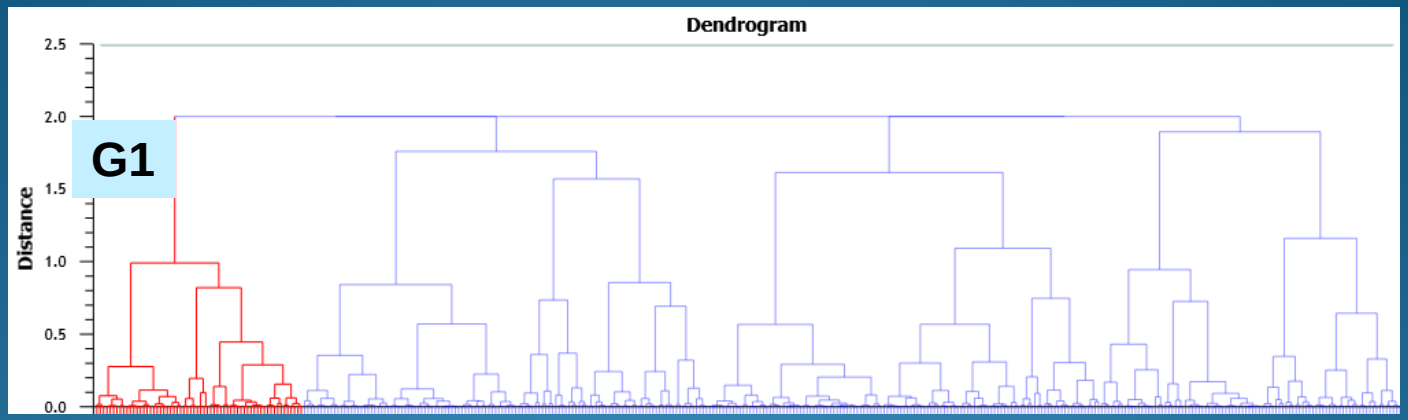


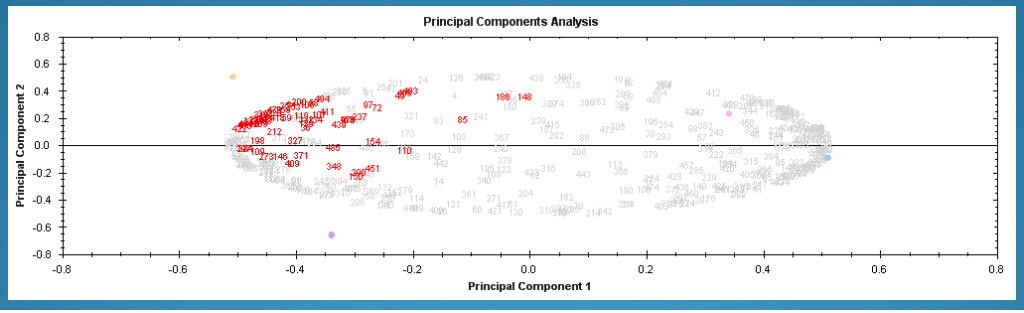
Principle component (PC1) → **Genotype-specific expression regarding salt stress treatment and accounted for 48,5% of the variation,**

Principle component (PC2) → **Genotype variation and accounted for 28,3% of the variation**

Image acquisition and analysis

G1 corresponds to spots that are higher in S during salinity and unchanged in T





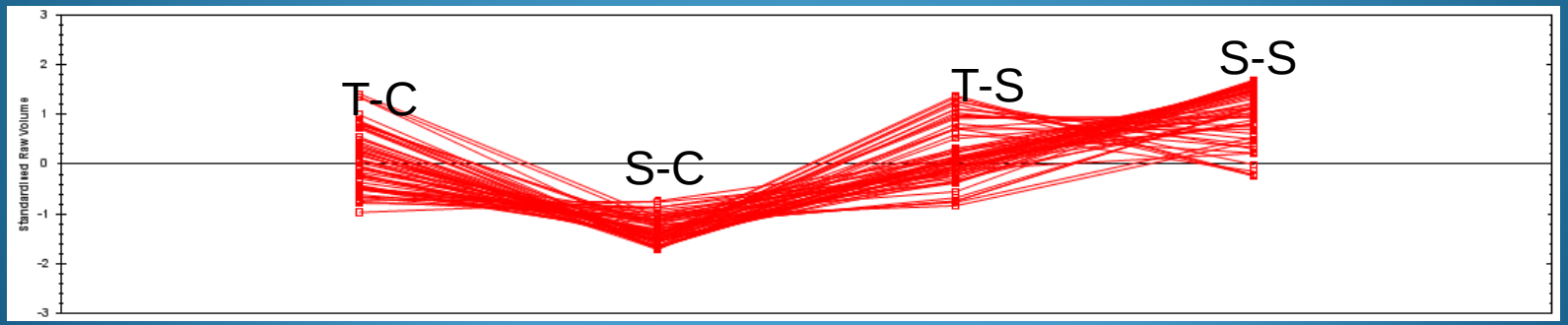
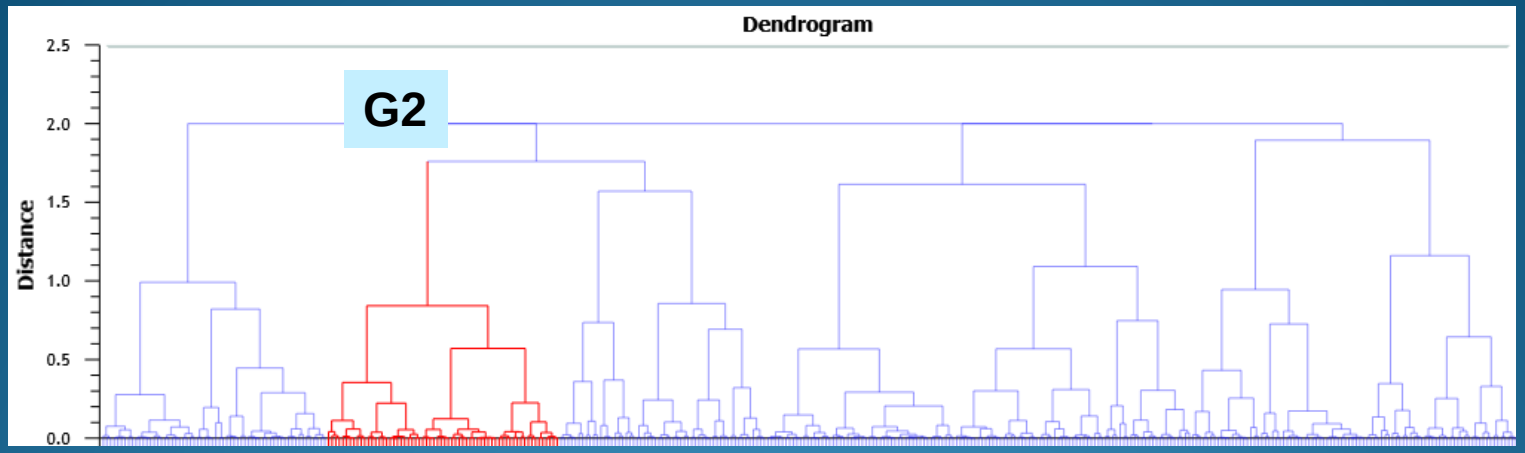
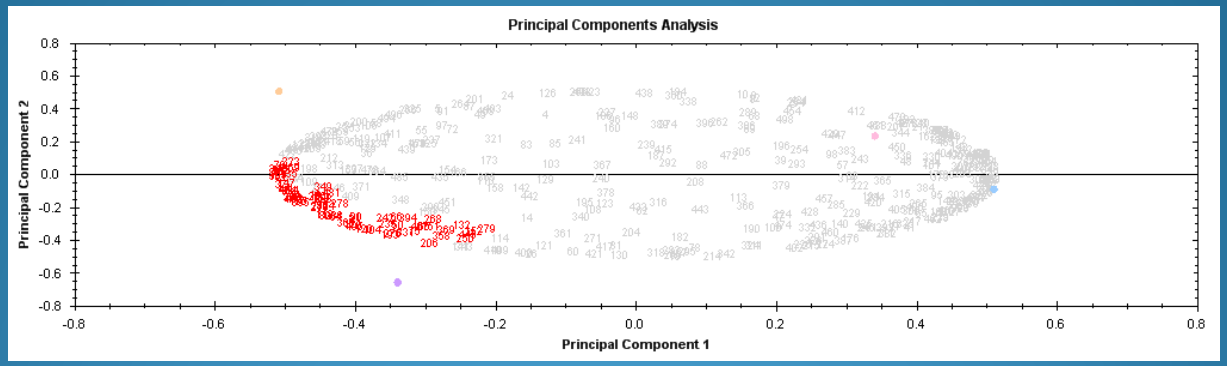


Image acquisition and analysis

G2: corresponds to spots that are higher in S and T during salinity





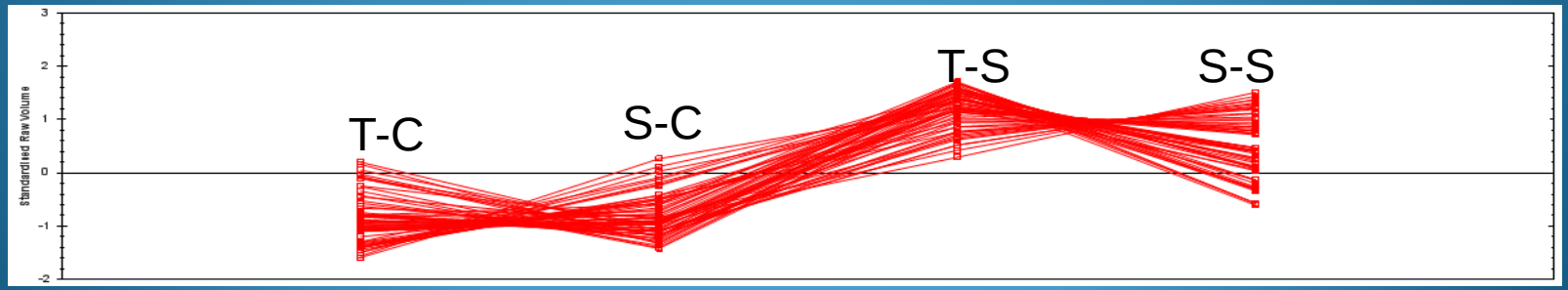
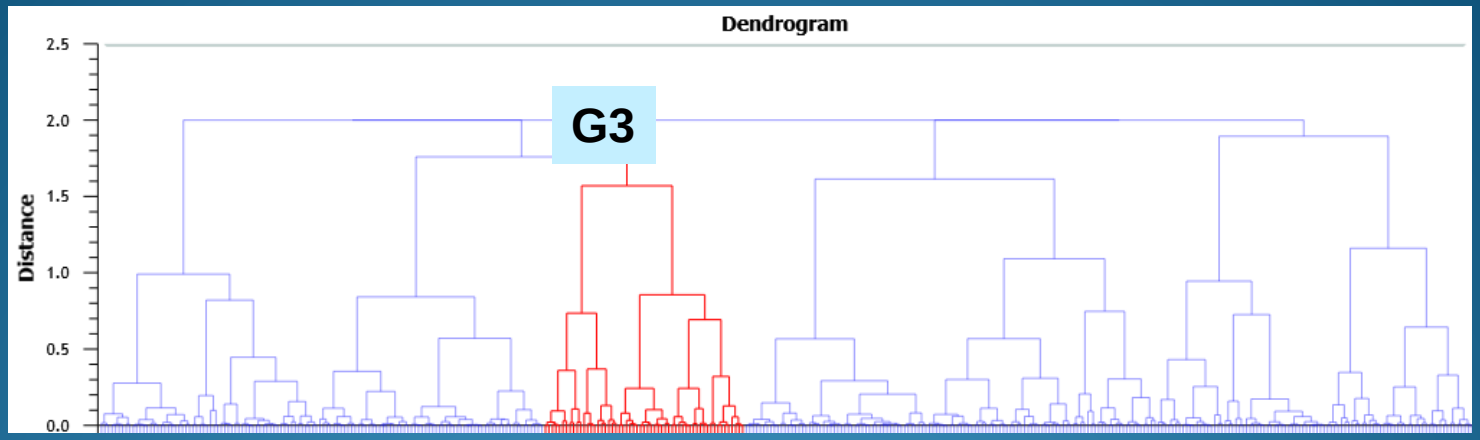
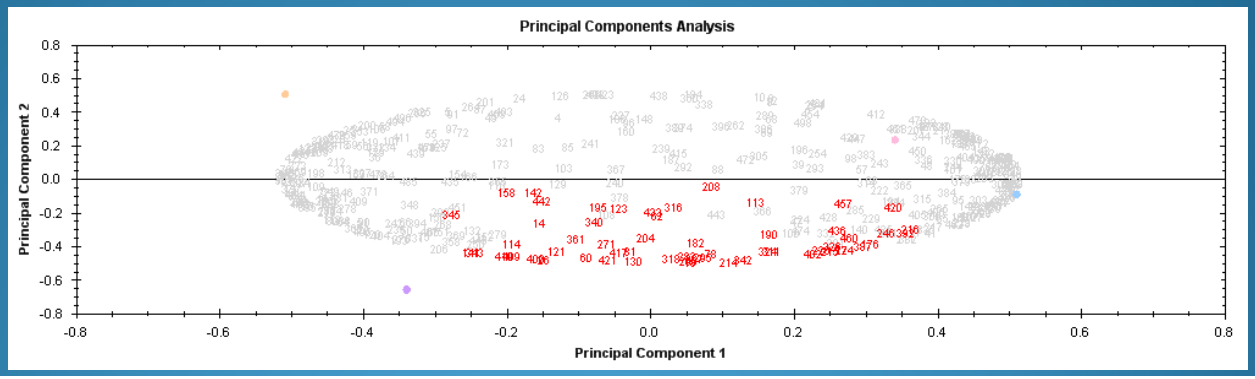


Image acquisition and analysis

G3: corresponds to spots that are higher in T during salinity and less abundant in S





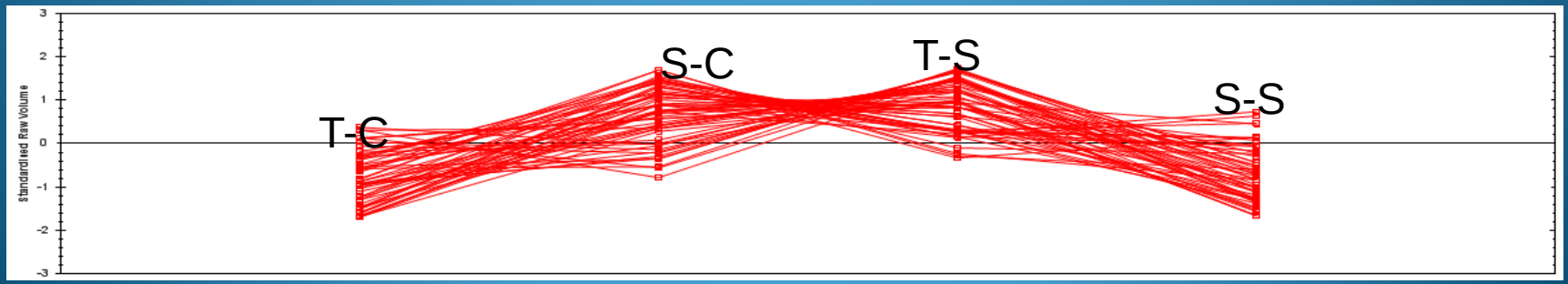
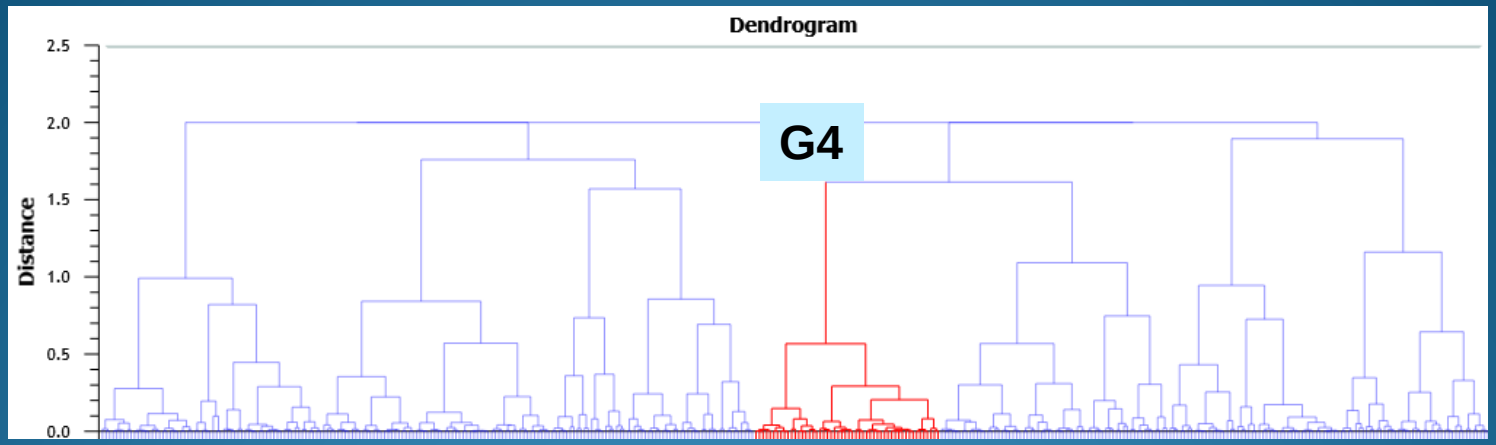
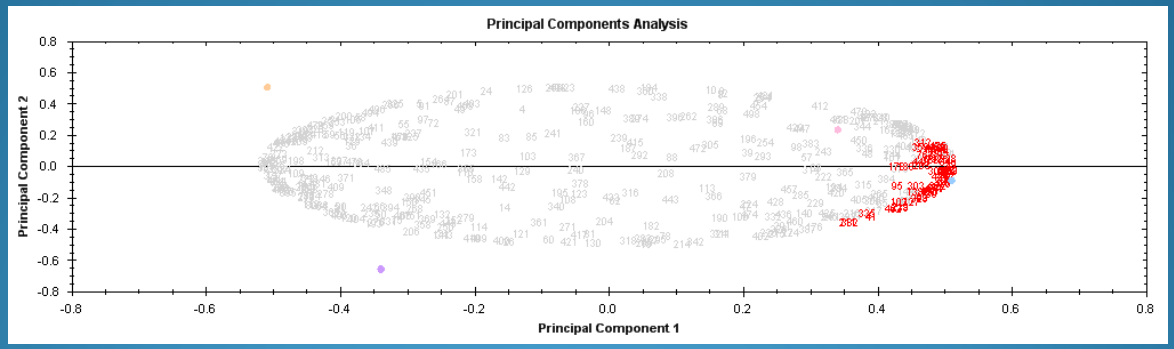


Image acquisition and analysis

G4: Spots are more down regulated in S than in T during salinity





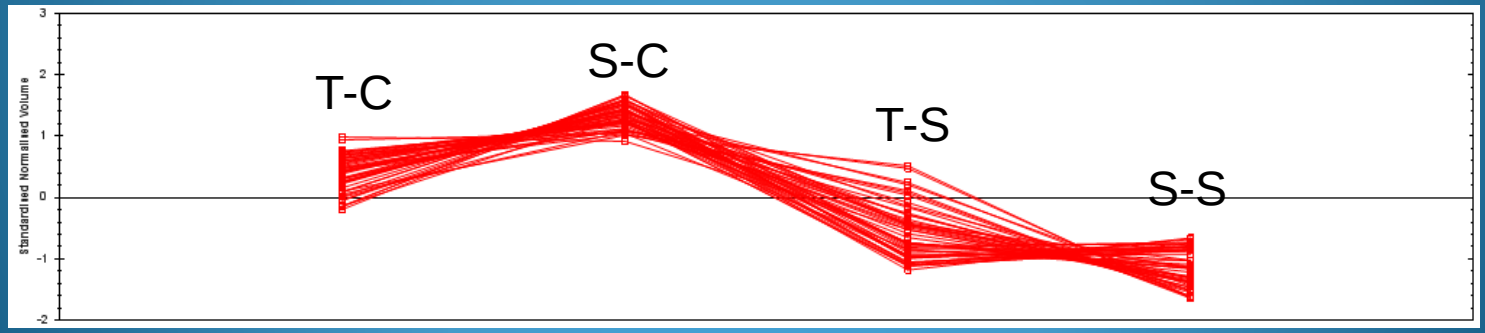
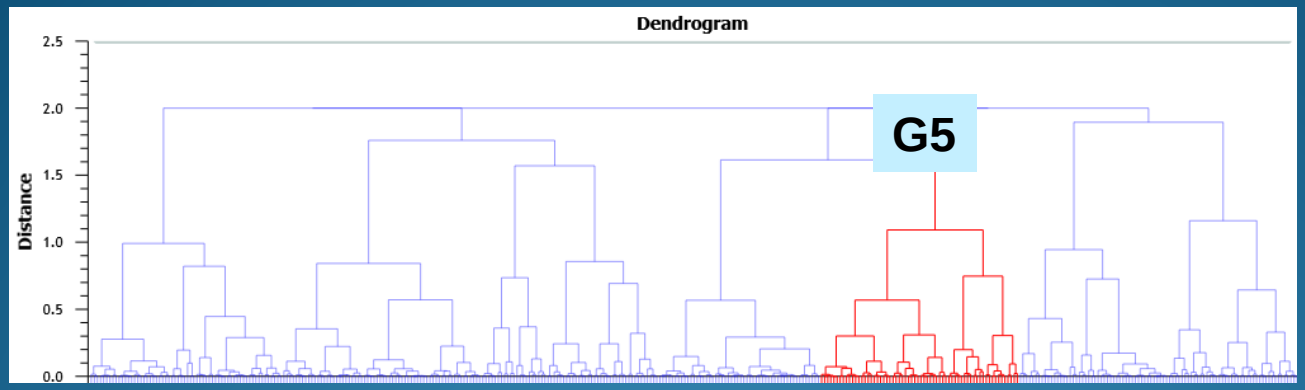
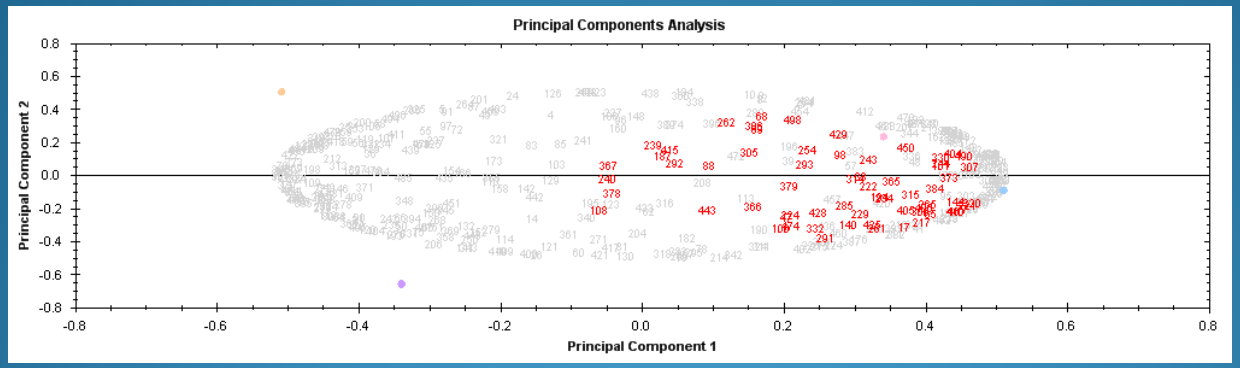


Image acquisition and analysis

G5: Spots are down similarly in S and T during salinity





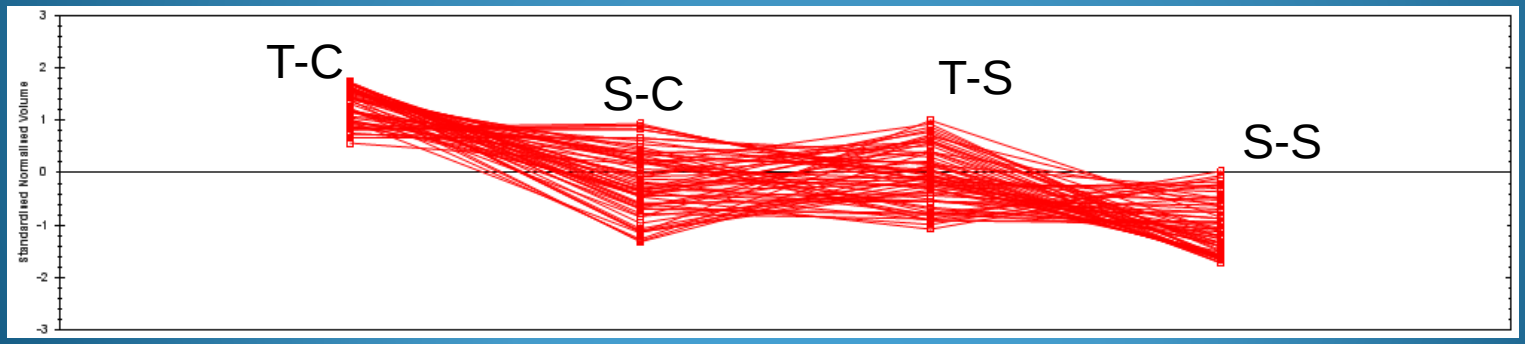
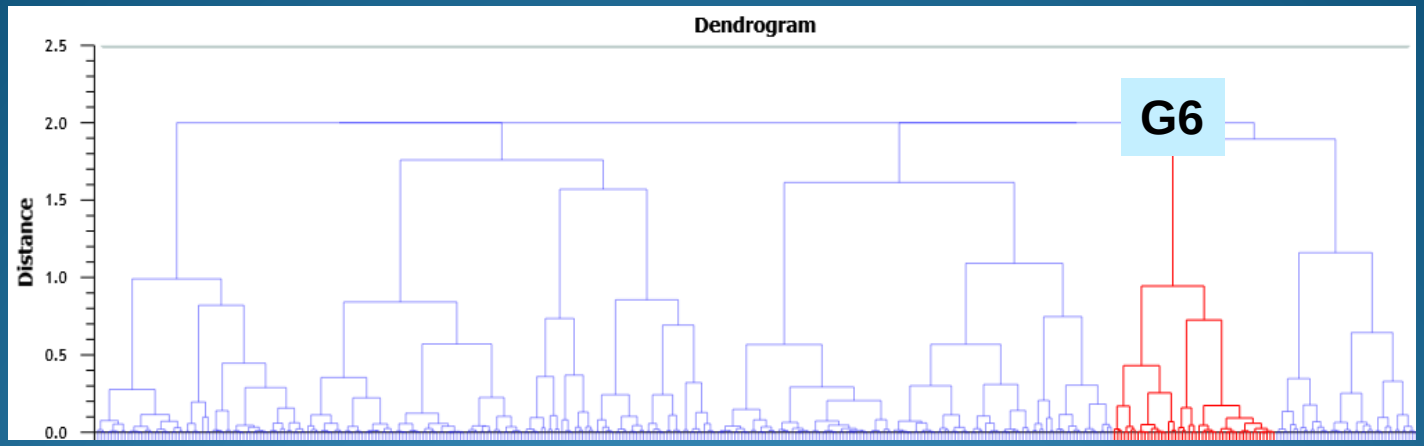
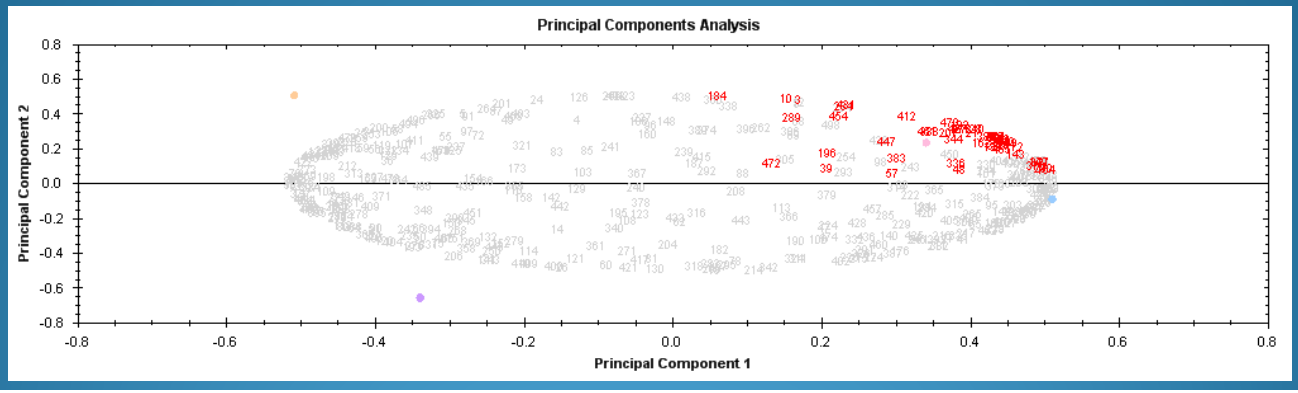


Image acquisition and analysis

G6: spots are down regulated in B during salinity and unchanged in M





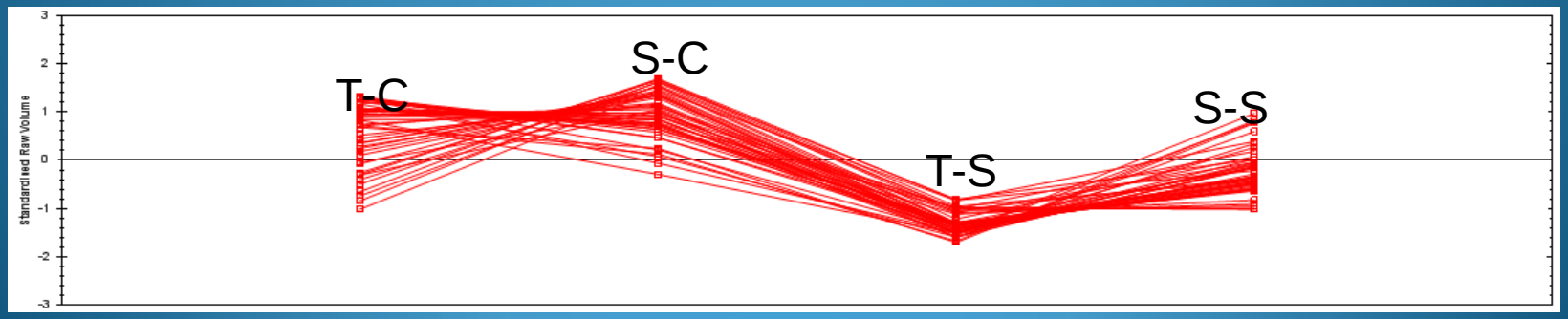
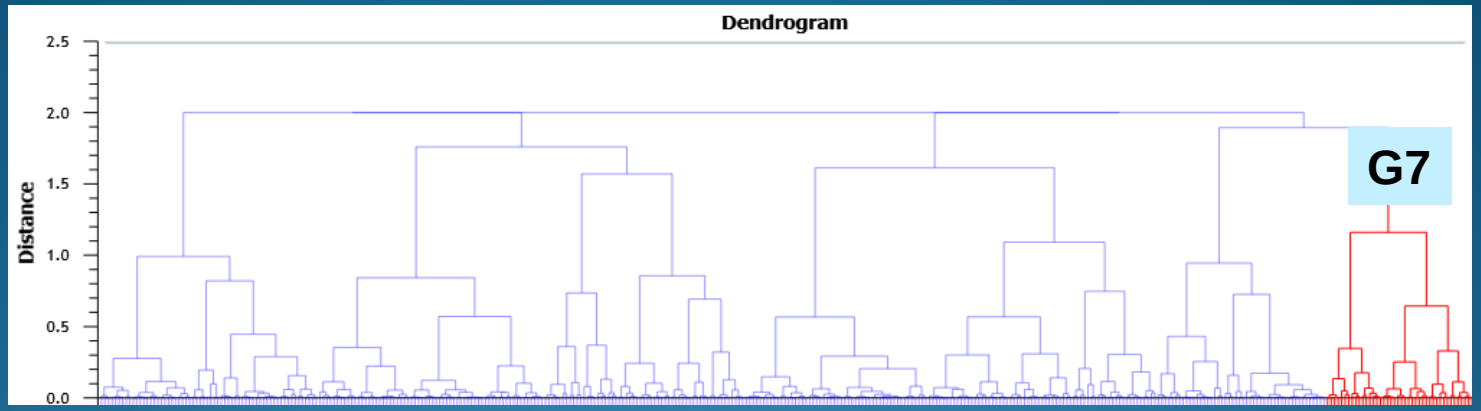
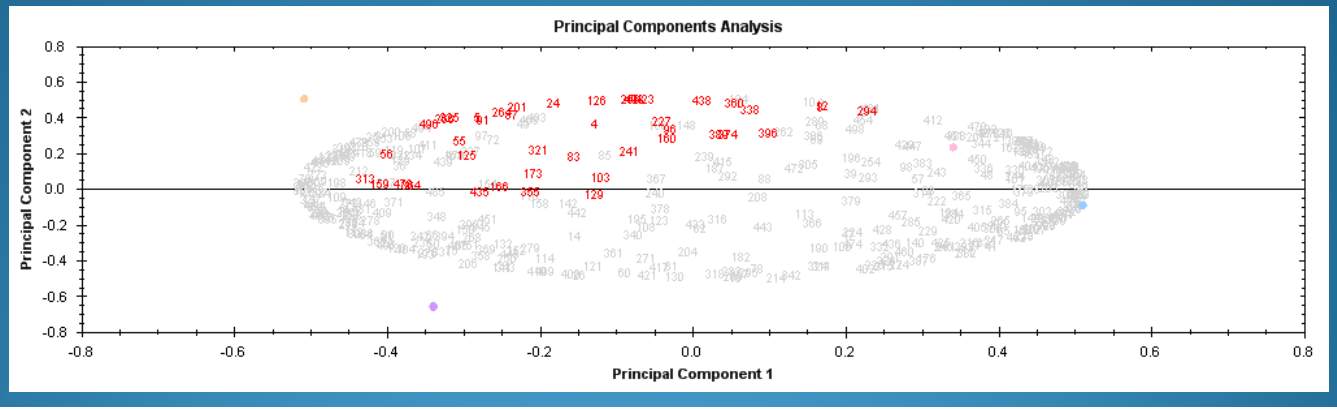


Image acquisition and analysis

G7: spots are unchanged in T and higher in Sduring salinity





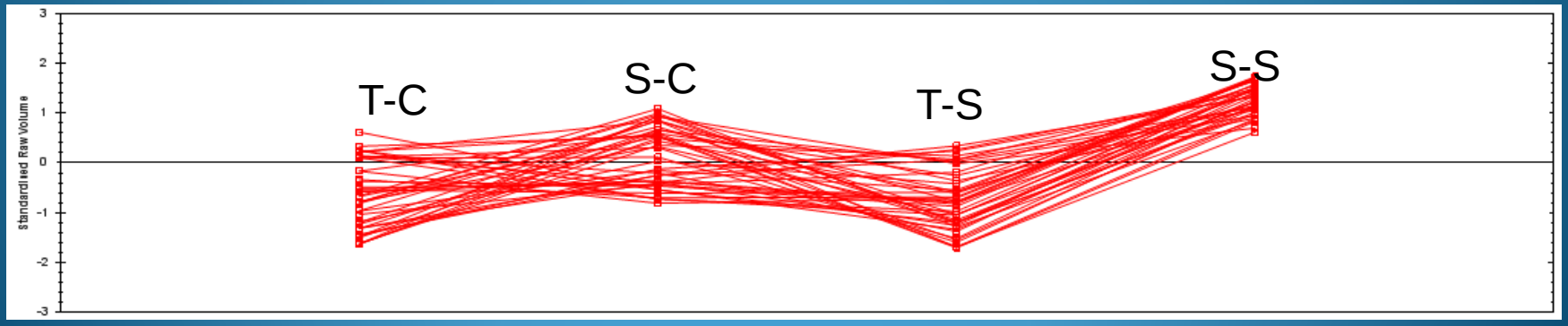
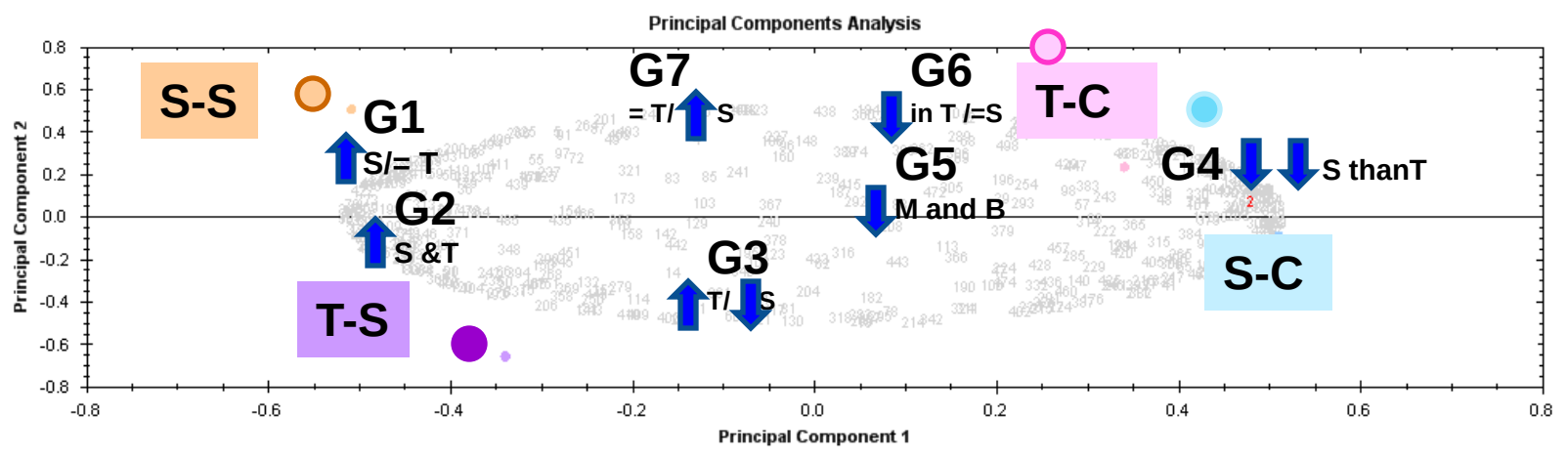


Image acquisition and analysis



Control conditions

Salt stress treatment

T

More unchanged protein spots
Less dow-regulated protein spots

S

More up-regulated protein spots

Image acquisition and analysis

18 up-regulated spots from T

								Normalized Volume								
								control		salt stress		control	salt stress			
Spot numb	Anova (p)	Fold	Included	Max CV (%	X Position	Y Position	Area	Hv_Roots	Hv_Roots	Hv_Roots	Hv_Roots	B/M	B/M	BC/BS	MC/MS	
62	0.890221	1.021536	Yes	26.3609	407	167	407	4863	7091.273	6302.772	5908.944	0.685772	1.066649	0.771565	1.200091	down in M
121	0.711759	1.09381	Yes	13.03677	553	212	475	11385	13677.85	14970.56	12443.43	0.832368	1.203089	0.760493	1.099202	down in M
193	0.336029	1.22758	Yes	15.35336	814	264	640	11741	11909.08	16092.12	12940.23	0.985886	1.243573	0.729612	0.920314	**more than M
235	0.386759	1.25709	Yes	12.9491	944	300	482	14929	17938.48	22072.48	19244.89	0.832233	1.146927	0.676363	0.932116	**more than M
282	0.941385	1.027784	Yes	20.09664	731	343	300	3911	4933.563	5191.038	3899.264	0.792733	1.331287	0.753414	1.265255	**down in M
295	0.984636	1.012842	Yes	25.8695	645	350	715	20438	19982.74	24214.36	16725.45	1.022782	1.447756	0.844045	1.194751	**down in M
311	0.908577	1.114448	Yes	59.62089	504	368	505	4305	9778.379	8982.335	3654.75	0.440257	2.457715	0.479274	2.675526	***dowm in M
318	0.932847	1.037155	Yes	26.38961	889	372	1304	25304	24751.16	30801.21	21113.74	1.022336	1.458823	0.821526	1.172277	***down in M
326	0.791669	1.086614	Yes	19.49725	823	379	884	16012	20146.35	18931.91	14344.24	0.794784	1.319826	0.845768	1.40449	***down in M
340	0.763897	1.036656	Yes	11.40577	485	394	1027	23239	27316.34	26718.96	25689.52	0.850736	1.040072	0.869757	1.063326	***down in M
345	0.46615	1.371366	Yes	36.91208	664	399	369	2865	4888.804	5568.497	5064.809	0.586033	1.099449	0.514501	0.965249	****simil in M
361	0.741844	1.066794	Yes	14.42002	798	419	416	4596	5639.681	5769.022	5150.346	0.81494	1.120123	0.796669	1.09501	****down in M
387	0.715937	1.078143	Yes	14.89663	664	452	528	7713	8687.948	8407.3	6804.919	0.887782	1.235474	0.917417	1.276716	down in M
421	0.800193	1.038136	Yes	9.497055	779	486	996	16029	17506.32	18576.07	16238.15	0.915612	1.143977	0.862884	1.078098	**** down in M
435	0.308227	1.301326	Yes	33.43023	798	505	678	10561	17099.62	16392.91	19602.59	0.617616	0.836262	0.644242	0.872314	****more than M
436	0.77121	1.077282	Yes	16.44217	899	505	580	11585	14633.23	13015.44	11321.95	0.791691	1.149576	0.890097	1.292465	***down in M
442	0.612369	1.121244	Yes	28.67562	361	524	409	3731	5628.877	5187.107	5307.595	0.662832	0.977299	0.719283	1.060532	***down in M
445	0.313941	1.188971	Yes	23.14784	881	532	884	14396	12321.17	18482.69	13283.24	1.168395	1.391429	0.778891	0.927573	***more than M

13 down-regulated spots from T

								Normalized Volume								
								control		salt stress		control	salt stress			
Spot numb	Anova (p)	Fold	Included	Max CV (%	X Position	Y Position	Area	Hv_Roots	Hv_Roots	Hv_Roots	Hv_Roots	B/M	B/M	BC/BS	MC/MS	
3	0.62045	1.122255	Yes	22.96187	593	70	210	1981	1882.98	1442.01	2001.04	1.052056	0.72063	1.373777	0.941001	up in M
12	0.578823	1.11141	Yes	23.01363	517	100	190	1606	1642.978	1223.791	1699.502	0.977494	0.720088	1.312315	0.966741	similar inM
69	0.644754	1.330978	Yes	41.79002	277	175	122	1070	581.8718	567.1708	673.9255	1.838893	0.841593	1.886557	0.863407	up in M
88	0.673827	1.140851	Yes	32.95235	881	189	166	3233	2011.085	2414.162	2182.482	1.60759	1.106155	1.339181	0.921467	similar inM
97	0.747512	1.060544	Yes	13.21081	731	197	412	7056	5850.358	6476.757	7211.003	1.20608	0.898177	1.089434	0.81131	up in M
184	0.794547	1.058897	Yes	19.1254	514	258	497	10800	9623.265	8339.475	10947.83	1.12228	0.761747	1.295045	0.879011	up inM
187	0.818324	1.100686	Yes	34.00432	291	260	374	8652	5297.812	6458.08	6215.667	1.633127	1.039	1.339717	0.852332	up in M
254	0.487497	1.077251	Yes	10.08339	737	317	783	19557	16953.77	16985.64	16906.89	1.153549	1.004658	1.151385	1.002773	similar in M
292	0.775744	1.11404	Yes	35.09239	753	348	366	8527	5136.524	6399.102	5865.744	1.660072	1.090928	1.332531	0.875682	up in M****
305	0.606214	1.083182	Yes	15.58009	396	360	716	17393	13941	14563.61	14364.12	1.247615	1.013888	1.194278	0.970544	similar in M
412	0.442296	1.283548	Yes	18.72115	282	479	277	2331	1987.933	1459.703	1905.136	1.172574	0.766194	1.5969	1.04346	similar in M
429	0.473888	1.189024	Yes	17.09362	561	500	671	17435	13674.76	12667.47	13496.65	1.274977	0.938564	1.37636	1.013196	***similar in
498	0.588983	1.111591	Yes	12.65008	559	655	1094	35276	29483.31	27764.83	30493.4	1.196474	0.910519	1.270528	0.966875	similar in M

Image acquisition and analysis

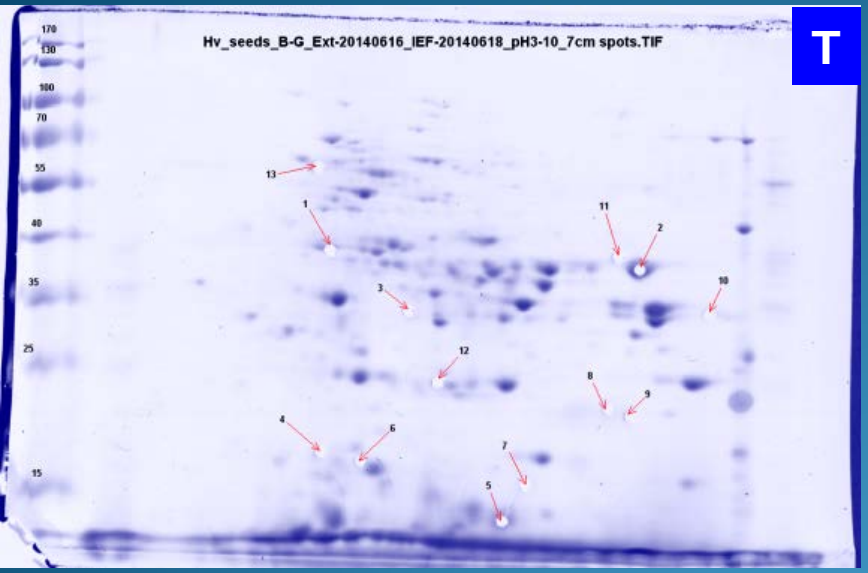
15 up-regulated spots from S

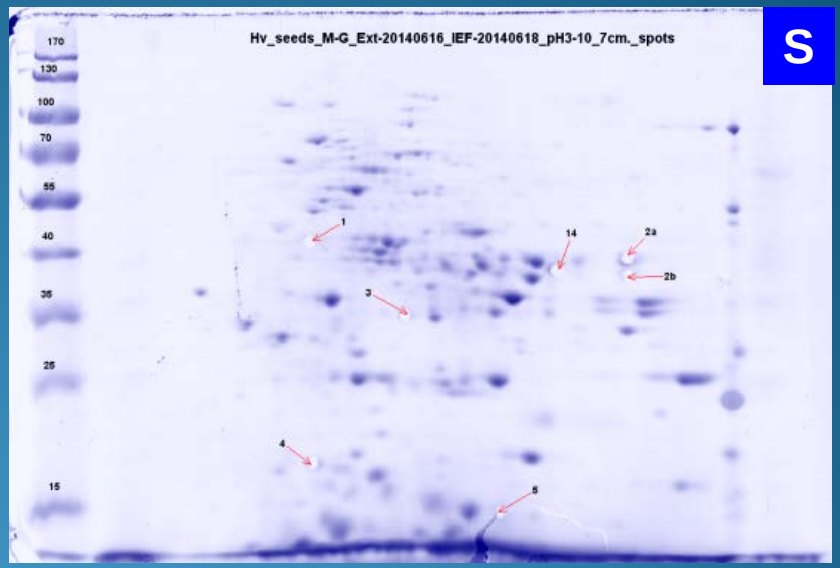
								Normalized Volume								
								control		salt stress		control	salt stress			
spot numb	Anova (p)	Fold	Included	Max CV (%	X Position	Y Position	Area	Hv_Roots_	Hv_Roots_	Hv_Roots_	Hv_Roots_	B/M	B/M	BC/BS	MC/MS	
25	0.446935	1.243119	Yes	19.55241	857	114	614	12174	11297.88	12572.12	16606.22	1.077547	0.757073	0.968333	0.680341	similar in B
55	0.298371	1.298086	Yes	32.08074	624	161	405	4102	5102.568	4618.953	7329.372	0.803909	0.630198	0.88808	0.696181	**more than B
56	0.196144	1.349157	Yes	23.0066	641	162	351	4874	5631.964	5934.16	8240.035	0.865418	0.720162	0.821346	0.683488	more tan B
107	0.603306	1.250624	Yes	31.83284	838	200	533	11128	7038.797	10115.69	12604.13	1.580952	0.802569	1.100073	0.558451	similar in M
119	0.55138	1.214379	Yes	21.73186	830	211	300	4475	3282.868	4318.165	5102.826	1.363137	0.84623	1.03632	0.643343	**similar in B
122	0.318434	3.770003	Yes	63.06709	434	213	226	818	553.3184	1432.18	3737.694	1.478353	0.383172	0.571157	0.148037	**more tan B
205	0.4304	1.613712	Yes	29.82945	461	277	376	5501	3584.608	6056.048	8605.51	1.534617	0.703741	0.908348	0.416548	**similar in B
225	0.158007	2.0654	Yes	15.27599	860	293	943	18278	14714.26	31482.4	36659.82	1.242197	0.858771	0.580578	0.401373	more than B
255	0.364264	2.338232	Yes	38.47787	352	318	304	1795	1027.15	2430.873	4167.97	1.747553	0.583227	0.738418	0.246439	**more than B
257	0.413223	1.281035	Yes	13.73635	556	318	876	17197	14365.44	18252.68	22179.92	1.197109	0.822937	0.942163	0.647678	**similar in B
301	0.097711	1.49195	Yes	5.365133	929	356	421	8806	8395.469	12345.06	13318.67	1.048899	0.926899	0.713322	0.630353	more in M than B
323	0.13492	2.002025	Yes	23.90762	377	376	320	1553	1630.63	2648.108	3725.598	0.952393	0.710787	0.586456	0.437683	more in M
418	0.475604	1.421516	Yes	28.081	846	484	742	12791	8552.898	13138.31	17202.38	1.495516	0.76375	0.973565	0.497193	**similar in B
468	0.425573	1.562271	Yes	28.52398	339	584	575	6079	4038.376	6689.077	9117.002	1.505308	0.733693	0.908795	0.44295	**more than B
494	0.634648	2.043411	Yes	72.42009	375	647	723	7281	2852.252	5051.457	15654.94	2.552719	0.322675	1.441366	0.182195	down in B

12 spots down-regulated from S

								Normalized Volume								
								control		salt stress		control	salt stress			
spot numb	Anova (p)	Fold	Included	Max CV (%	X Position	Y Position	Area	Hv_Roots_	Hv_Roots_	Hv_Roots_	Hv_Roots_	B/M	B/M	BC/BS	MC/MS	
27	0.69637	1.080616	Yes	20.6283	567	118	576	12427	13022.66	13493.16	10057.91	0.95426	1.341548	0.920985	1.294769	up in B
41	0.5506	1.272571	Yes	26.62719	269	138	543	7181	8889.365	7502.972	5125.29	0.807819	1.463912	0.957087	1.734412	similar in B
63	0.378919	1.553349	Yes	25.75734	898	167	214	2029	2582.153	1754.593	1213.93	0.785778	1.445382	1.156394	2.127102	more than B
102	0.478867	1.265558	Yes	15.81782	373	199	264	2406	3011.997	2369.929	1911.184	0.798806	1.240032	1.01522	1.575985	similar in B
138	0.411238	1.51672	Yes	25.336	275	223	420	4742	6811.913	4288.677	3329.019	0.696133	1.28827	1.105702	2.046222	**more than B
196	0.29456	1.284733	Yes	43.85763	835	268	329	5438	10327.07	4865.677	7405.404	0.526577	0.657044	1.117625	1.394531	more than B
272	0.183407	1.752624	Yes	17.35116	270	330	632	6075	6645.995	4074.39	3183.866	0.914084	1.279699	1.491021	2.087398	*more than B
291	0.595607	1.135809	Yes	33.94867	677	347	254	5864	5494.599	6200.544	3799.903	1.06723	1.631764	0.945724	1.445984	**similar in B
324	0.779239	1.030545	Yes	16.76515	775	376	412	7617	7367.547	8132.07	6408.34	1.033858	1.268982	0.936662	1.149681	up in B
359	0.095381	1.628869	Yes	20.25199	250	416	786	15722	20977.48	10458.75	12071.91	0.74947	0.866371	1.503239	1.737711	more than B
392	0.631675	1.068606	Yes	7.387151	617	457	897	24174	26691.25	25043	22556.63	0.90569	1.110228	0.9653	1.183299	***up in B
434	0.327346	1.277935	Yes	9.811733	483	505	343	3631	4168.795	3263.444	2839.991	0.870995	1.149104	1.112628	1.46789	**more than B

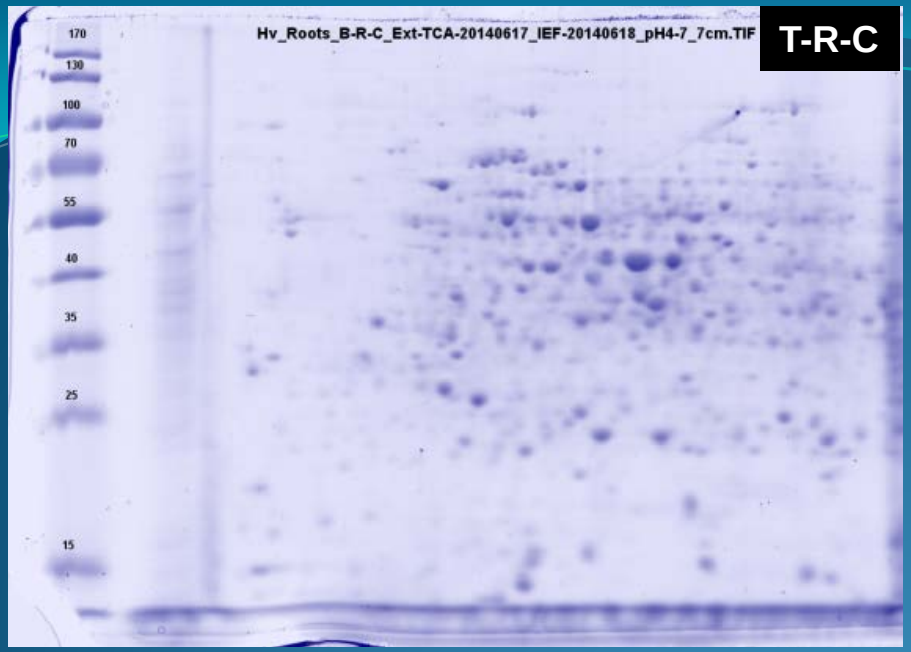
Spot cutting
(manually)

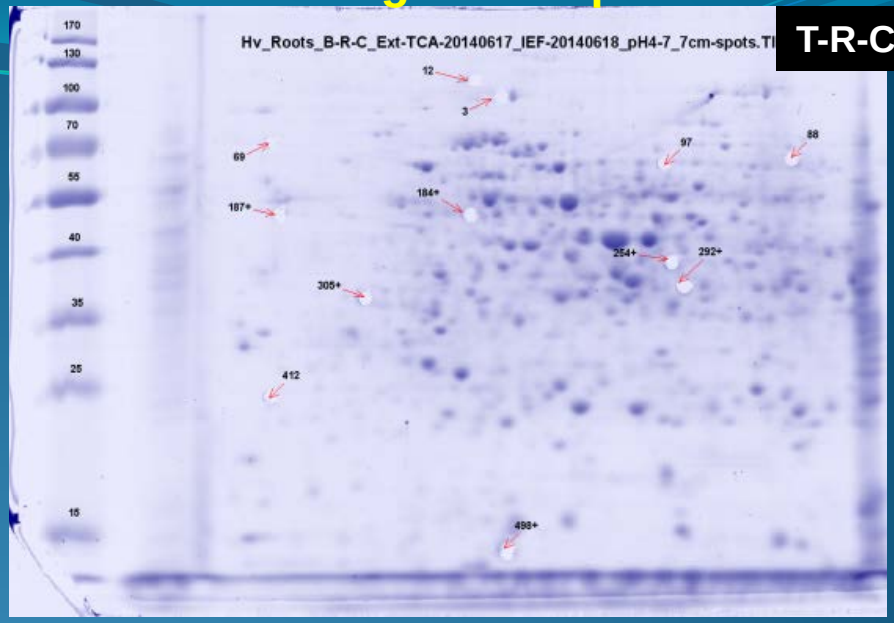




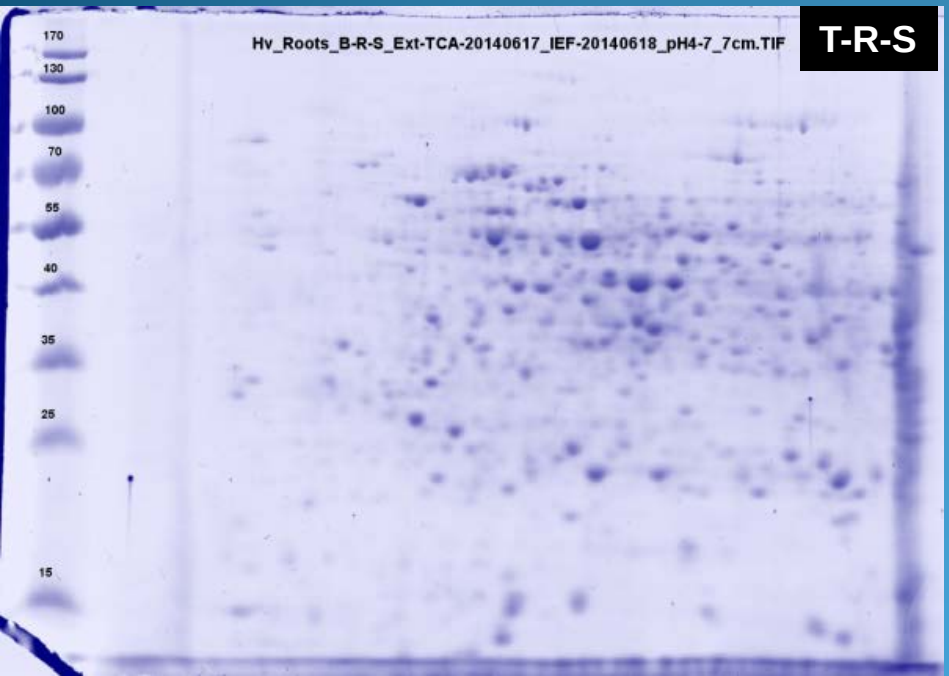
Representative two-dimensional gel electrophoresis separation of the water-soluble protein fraction from mature barley grain T and S showing the excised spots.

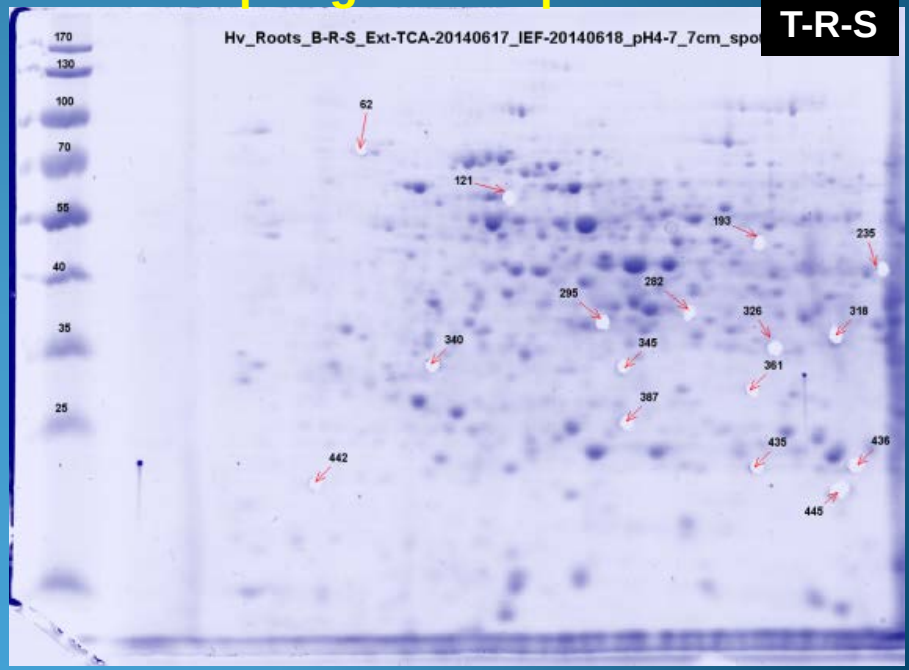
Down-regulated spots in T



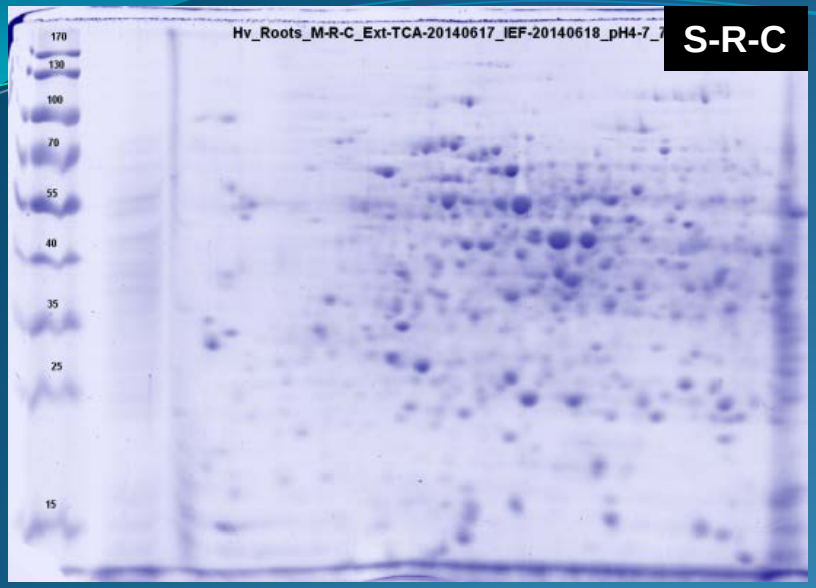


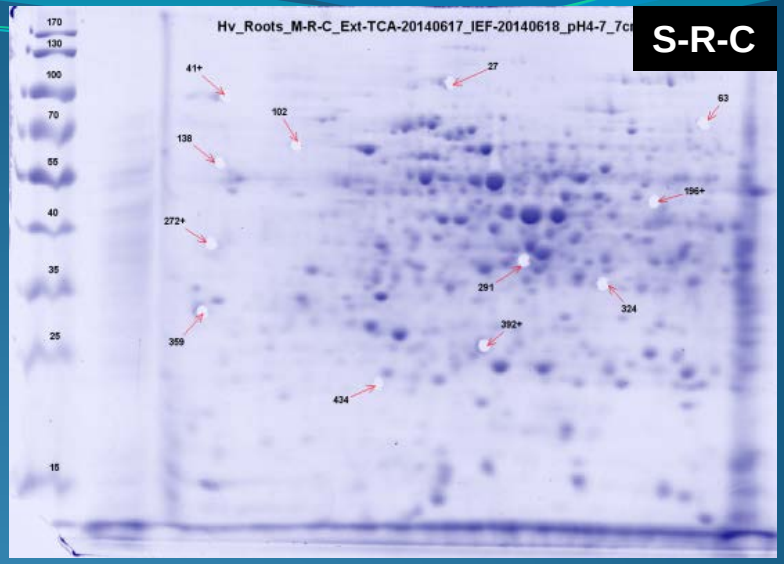
Up-regulated spots in T



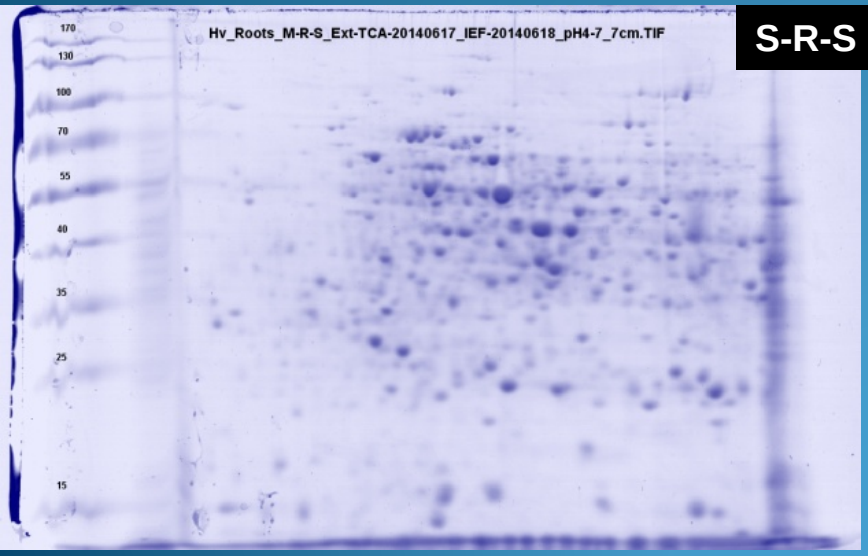


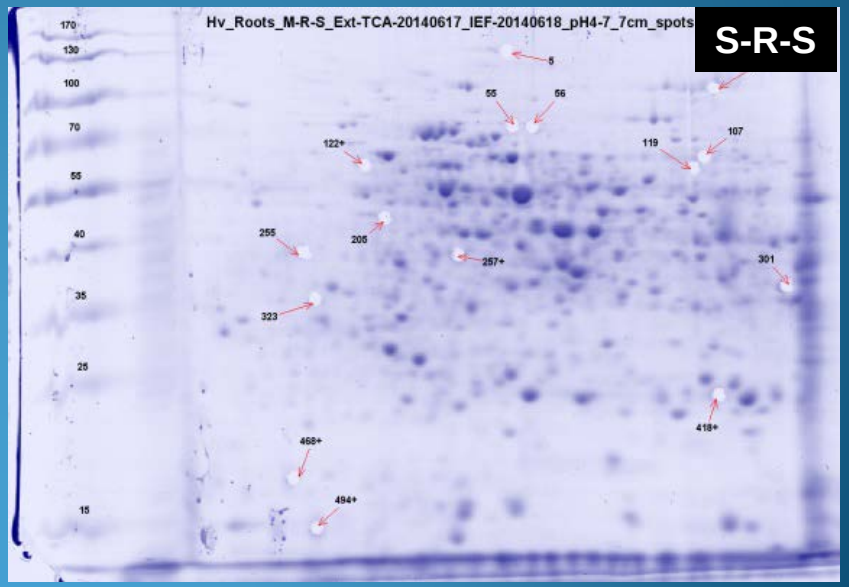
Down-regulated spots in S





Up-regulated spots in S





Protein identification

Digestion with Trypsin
(Witzel et al. 2007)

Acquisition of peptide mass fingerprint data: MALDI MS; Ultraflextreme (Bruker)

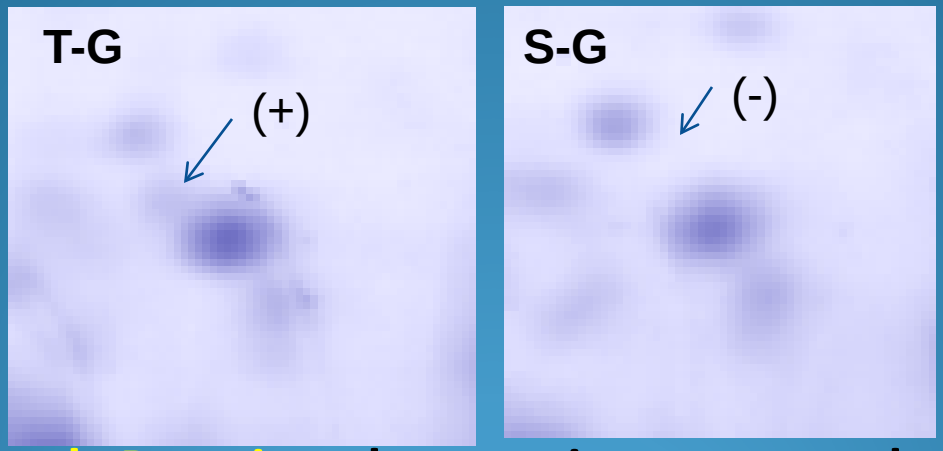
Protein identification was performed with the MASCOT search engine searching the barley genomic database and for the EST Gene Index in the TIGR database (Poacees family)

De novo sequencing,
***Database* searches for homologous sequences from related plant species**

Protein identification & Database search

Grain

Spot number	Expression in salt tolerant line	Protein name	Accession number	Prot score	Seq. coverage	Theorical Mr/pI
I1: 236 (BG6)	↑	Class I Heat Shock Protein	MLOC_2337.1	62	41%	16754/ 5,47



Class I Heat Shock Protein: plays an important role in membrane quality control and potentially contribute to the maintenance of membrane integrity especially under stress conditions

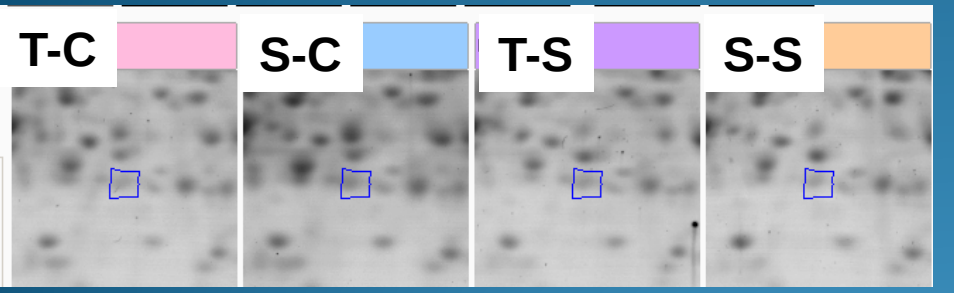
Protein identification & Database search

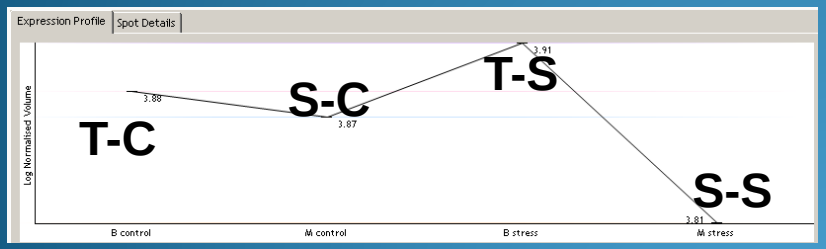
Spot number	Exp. in salt tolerant line	Protein name	Accession number	Sequence coverage	Theoretical Mr/pI
MC324(O3)	↓	NADPH-Thioredoxin - reductase	HCLC: MLOC_2337-1	6%	34.745 /5,83
B-S 318 (D5)	↑	Anionic Peroxidase	HCLC: MLOC_54892-1	7%	36.381/5,67
B-S 326 (D6)	↑	Guanine nucleotide binding protein subunit beta-like protein I	HCLC: MLOC_54892-1	8%	36.142/5,97
B-S340 (C6)	↑ (↓ in S)	Drepp 4 protein (Developmentally Regulated Plasma membrane Polypeptides) Salt stress root protein RS1 – (Oryza sativa subsp. indica (Rice), partial (97%)	HCLC: MLOC_12553.1 TIGR: HV_TC199652	32% 35%	31.098/5,76
B-S 435 (I6)	↑	Gluthathione S-Transferase 3like	HCLC : MLOC-68101.1	49%	23.557
BS436 (K5)	↑ (↓in S)	Manganese Superoxide dismutase	HCLC: AK372029	45%	24.874/7,89
MC434 (O4)	↓T less than S	Thioredoxin like protein1	HCLC: AK368636	34%	21.019/5,17
B-S 193 (B5)	↑	Arginino-succinate synthase	HCLC: MLOC_11947-2	24%	52.186 / 5,15

Protein identification & Database search

5 proteins up-regulated in the tolerant and down-regulated in the sensistive

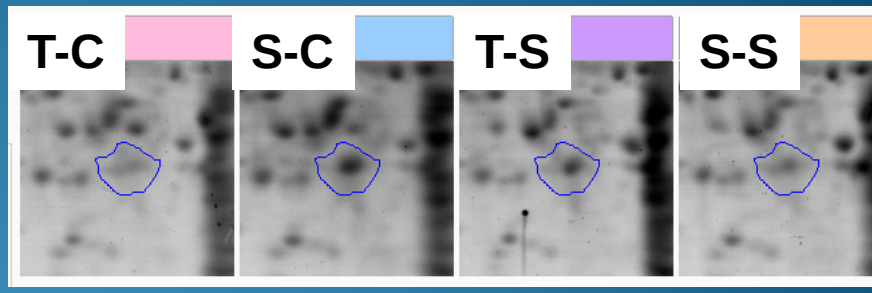
S-C 324: NADPH-Thioredoxin reductase

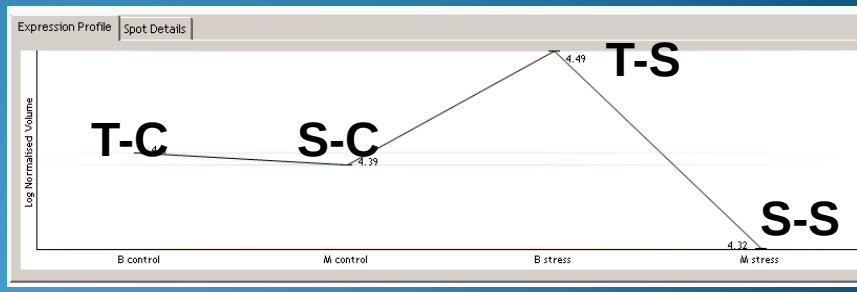




-Regulation of cell metabolism through redox regulation of disulfide bridges or through removal of H_2O_2.

T-S 318: Anionic peroxidase

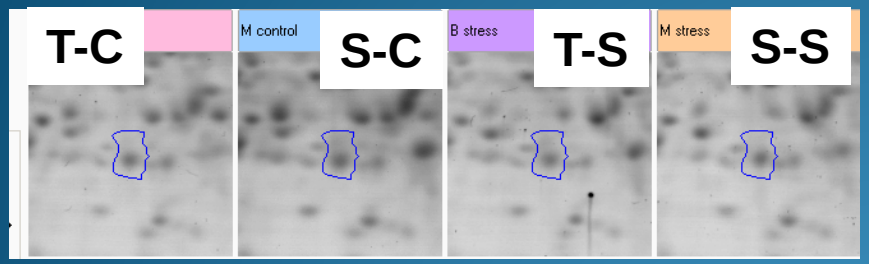


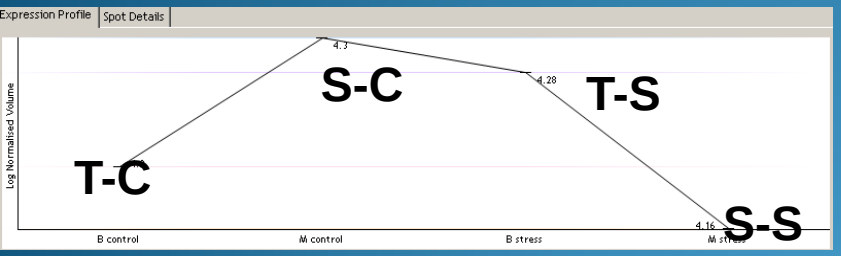


Removal of H_2O_2, oxidation of toxic reductants and in environmental stresses such as oxidative stress.

Protein identification & Database search

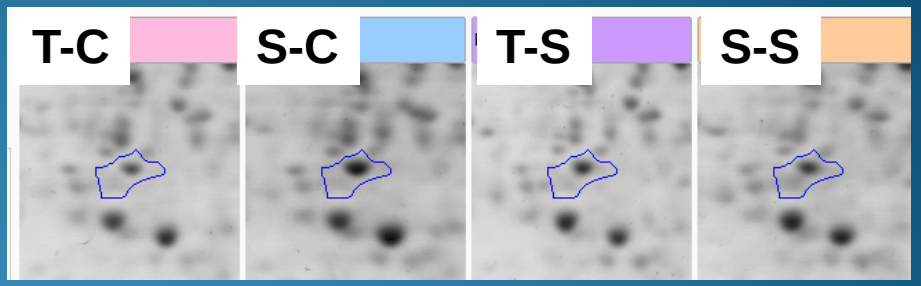
B-S 326: Guanine nucleotide binding protein beta-like

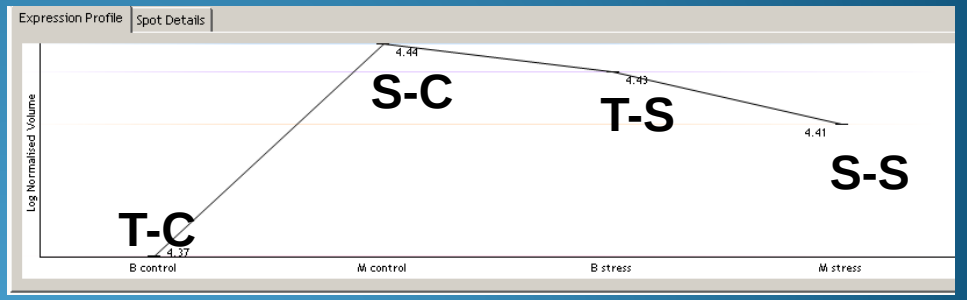




-Serves as a scaffold protein that can recruit other proteins to the ribosome
- Involved in the negative regulation of translation of a specific subset of proteins

BS 340: Drepp protein (RS1) Developmentally Regulated Plasma membrane Polypeptide

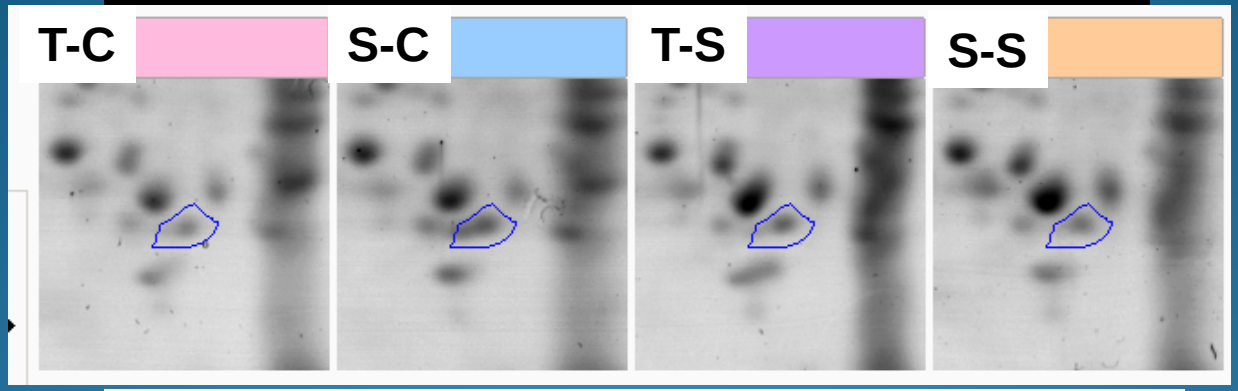


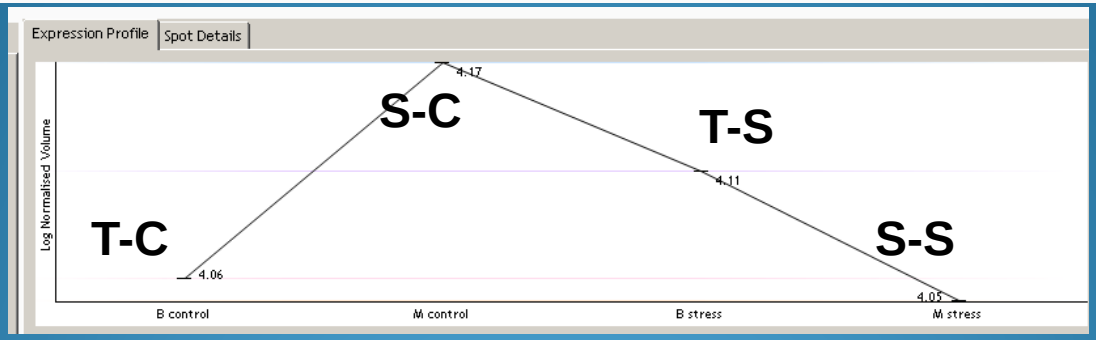


Could serve as a plant-specific signaling interface between the plasma membrane and the cytoskeleton.

Protein identification & Database search

B-S 436: Manganese Superoxide Dismutase



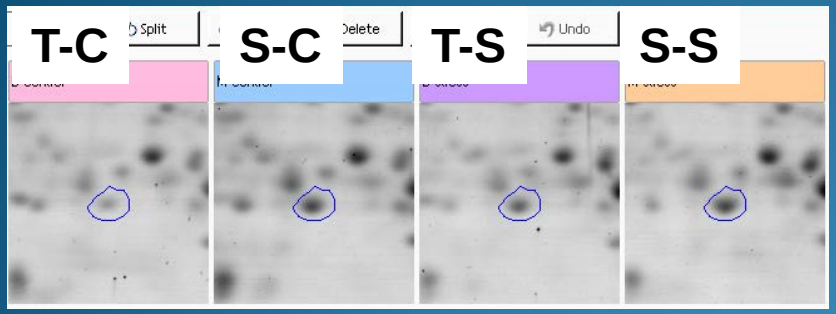


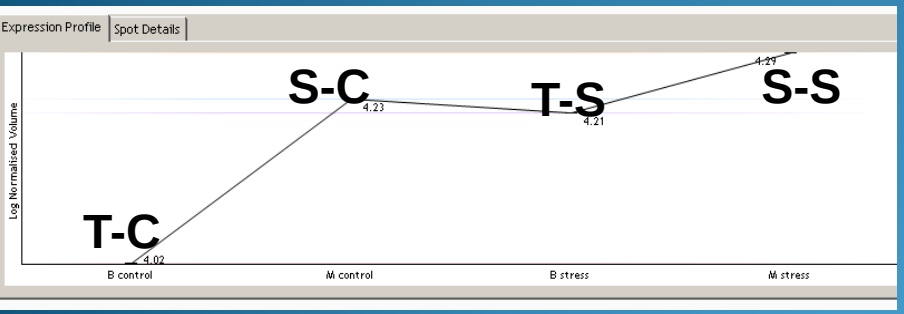
Destroys superoxide anion radicals which are toxic to biological systems

Protein identification & Database search

2 proteins more up-regulated in the tolerant than in the sensitive

B-S 435: Gluthathione S-Transferase 3 like

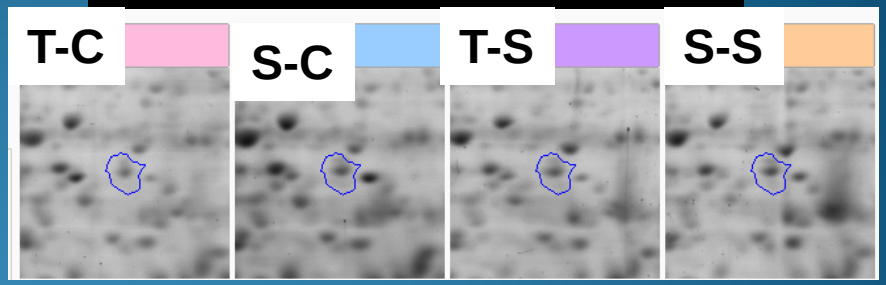


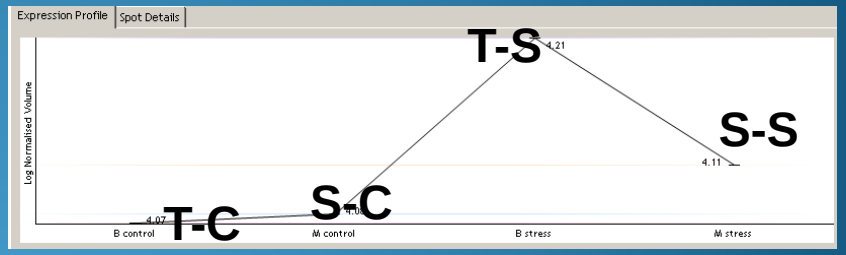


- Catalyses the conjugation of reduced glutathione with a variety of target compounds (Detoxification process)

- Enhances salt tolerance during germination

B-S 193: Argininosuccinate synthetase



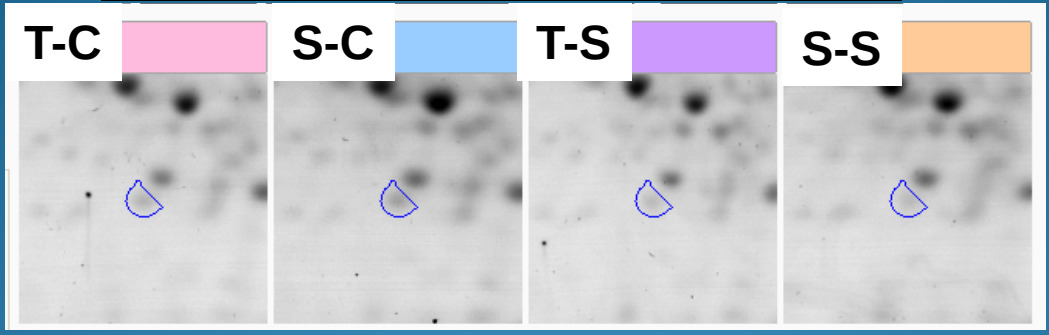


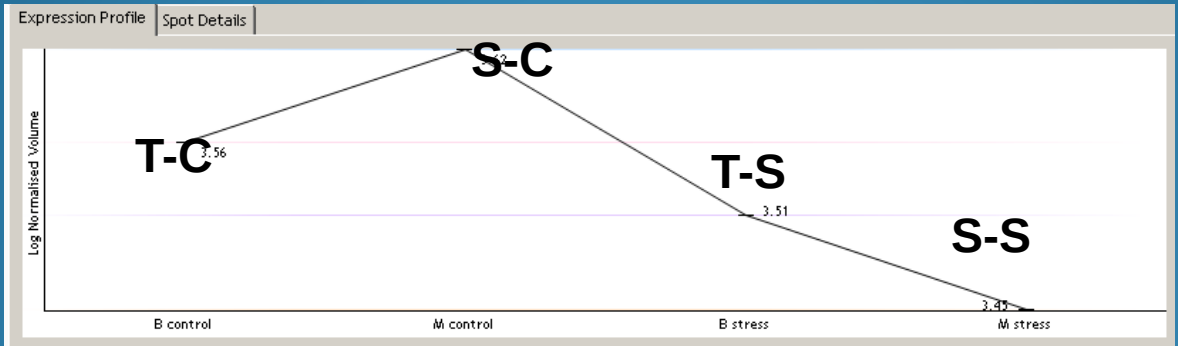
Involved in the polyamine biosynthesis

Protein identification & Database search

1 protein more down-regulated in the sensitive than in the tolerant

MC434: Thioredoxin like protein1

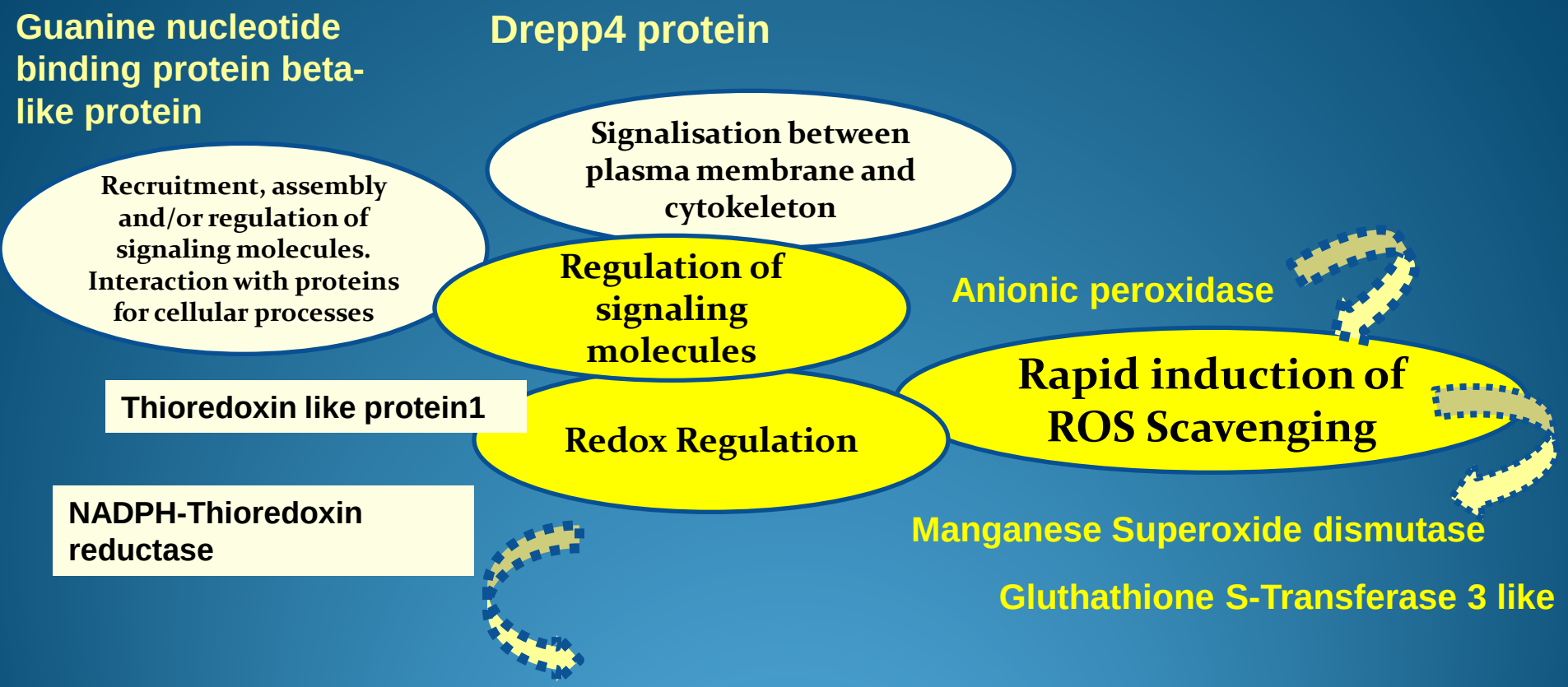




- **Plant redox signaling network**

- **Root ROS homeostasis**

Hypothesis



Enhanced Tunisian barley salt stress tolerance at germination stage

Outlook

- Biological replicates: Germination stage

- Proteome analysis of contrasting pairs at the tillering and the maturation stages

-Analysis of salt tolerance of Tunisian barley mapping populations

- Analysis of the proteome of contrasting genotypes from the mapping population

Efficient selection of potential candidate protein involved in barley salt stress tolerance

Barley Genetic Engineering

Genetic transformation: Protein over-expression

Production of salt tolearnt barley genotypes



Dr. Rahma Jardak Jamoussi
Prof. Assistant
E-mail: rjardak@yahoo.fr

RESEARCH AREA OF INTEREST

-Abiotic stress research and functional genomics making use of complementary approaches: Proteomics/Metabolomics
-Plant tissue culture and genetic transformation.
-Biotic stress research (Genetic engineering; Gene silencing).

Laboratory of Plant Molecular Biology

- **GENETIC DIVERSITY OF GRAPEVINE, CERELAS AND CITRUS**
- **IMPROVEMENT OF CEREAL, GRAPEVINE AND CITRUS TOLERANCE TO BIOTIC AND ABIOTIC STRESSES**
- **GENOMICS & PROTEOMICS OF CEREALS AND GRAPEVINE**
- **FUNCTIONAL GENOMICS**

Thank you
for your attention



Smelling
the **jasmine** in
Tunisia















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