



ユーザー発表

jPOSTデータベースの利用

ストレス耐性作物の作出を目指した プロテオミクス解析

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ダイズ



枝豆



納豆



豆腐



醤油



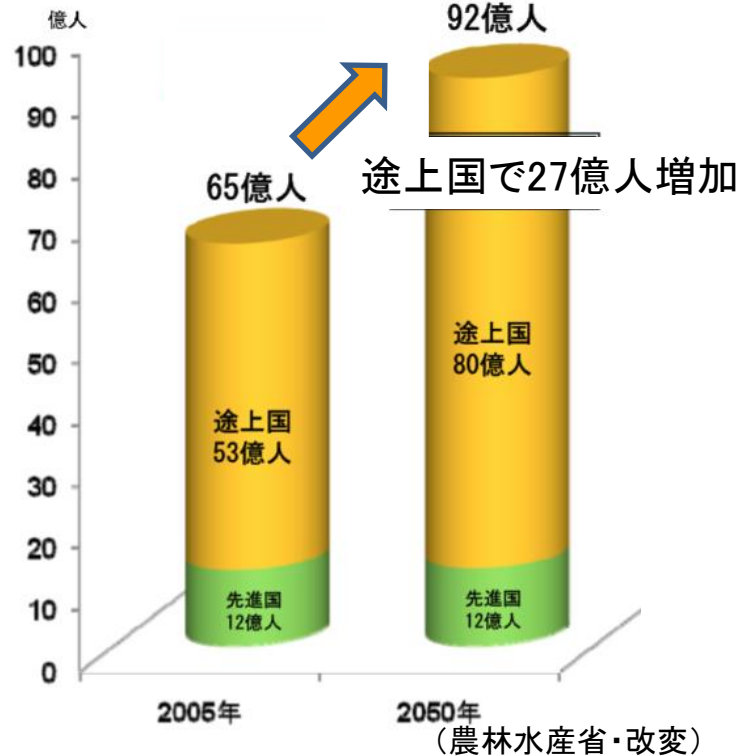
豆乳



初めに

(1) 食糧問題・環境問題

人口増加率

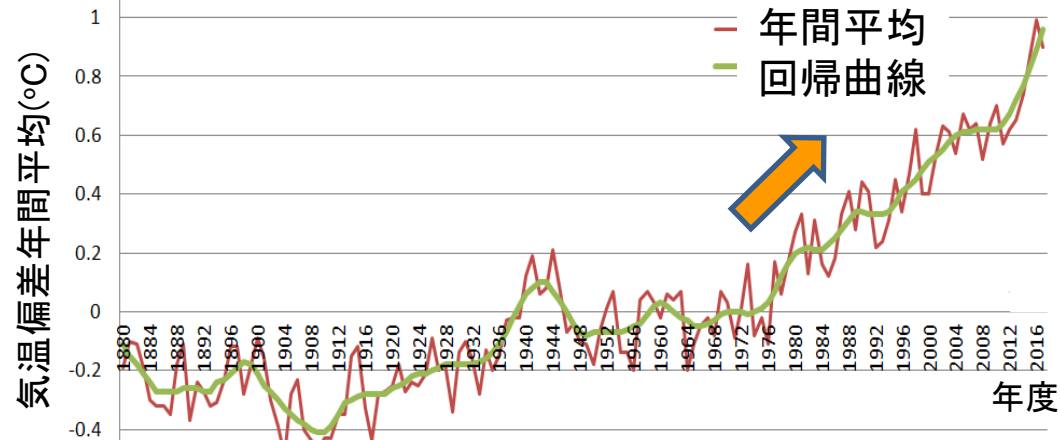


人口増加による食糧不足の懸念

食糧自給率の向上が重要

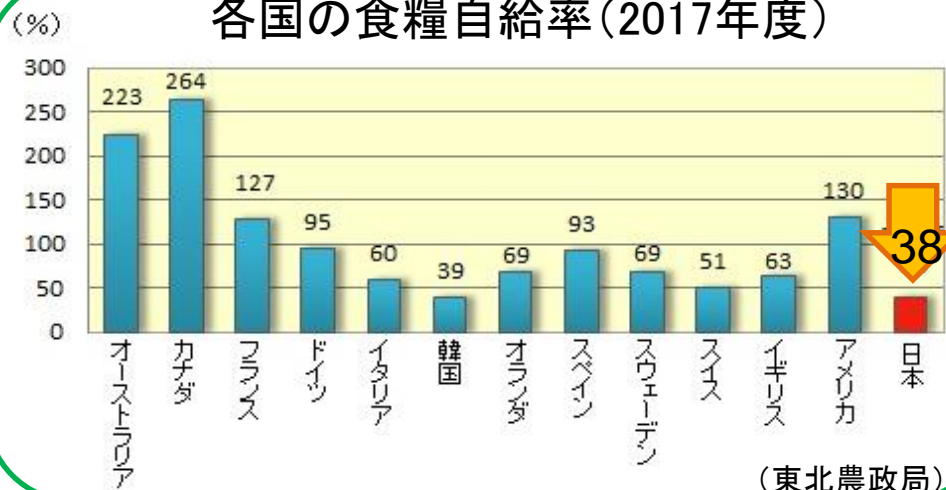
しかし

地球温暖化



地球温暖化による異常気象による農作物の生産条件の悪化

各国の食糧自給率(2017年度)



(2)なぜダイズの湿害耐性研究か？

ダイズの生産量が低い原因

ダイズ生産の85%を水田転換畑で栽培

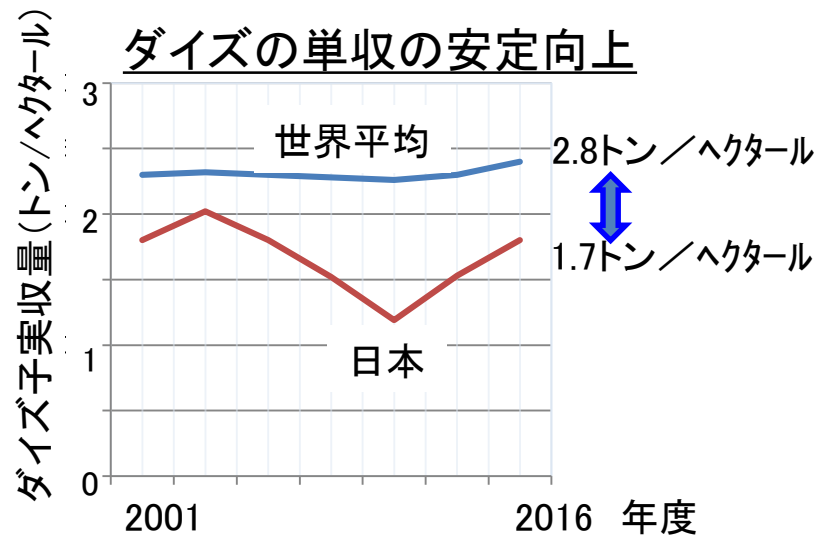
↓
湿害が生産性低下の原因

↓
生育初期の梅雨による湿害が問題
出芽不良による欠株
雑草との生育競合
土壌病原菌の感染



湿害を克服すれば。。。

ダイズの単収の安定向上



栽培面積の拡大

14. 7万ヘクタール & 9. 3万ヘクタール
現在 転作作物を作付け
していない水田

(農林水産省)



ダイズ自給率の向上



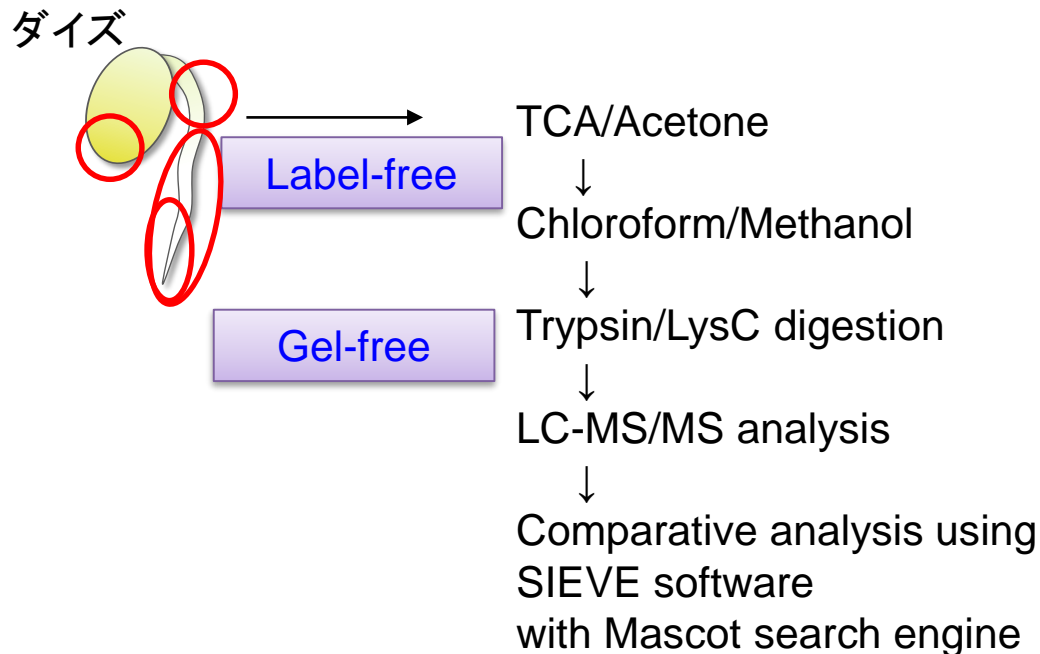
ダイズ耐湿性機構の解明



耐湿性ダイズ品種の開発

(3) プロテオミクス解析

gel free/label free proteomics (質量分析基盤無標識比較プロテオミクス解析)



生物学的: 3反復
技術的: 2反復

装置:

Ultimate 3000 (Dionex) with
Nano HPLC C18 capillary
column
(Nikkoy Technos)
LTQ-Orbitrap discovery
(Thermo Scientific)

液クロ:

Flow rate 200 nL/min
ACN gradient elution
10-30% for 100 min
30-50% for 10 min
80% for 10 min

質量分析計:

Data dependent Top10

1. ダイズプロテオームデータベース構築とデータ格納

Sakata et al. J Proteome Res. 8, 3539-3548 (2009)
 Ohyanagi et al. Front. Plant Sci. 3, 110 (2012)
 Komatsu et al. J Proteomics 163:52-66 (2017)

A

B

Seed	1 day	2 day	3 day	4 day	5 day	6 day	8 day	10 day	12 day
Cotyledon	Cotyledon	Cotyledon	Cotyledon	Cotyledon	Leaf	Leaf	Leaf	Leaf	Leaf
Embryo	Root	Root	Hypocotyl	Hypocotyl	Cotyledon	Cotyledon	Cotyledon	Cotyledon	Cotyledon
			Root	Root	Hypocotyl	Hypocotyl	Hypocotyl	Hypocotyl	Hypocotyl
					Root	Root	Root	Root	Root

[Mitochondria \(2day/root\)](#) [Nucleus \(2day/root\)](#) [Endoplasmic Reticulum \(2day/root\)](#)

C Search by accession(AC), description

Enter search keyword:

Search to: AC soybean (e.g. Glyma20g28550) AC arabidopsis (e.g. AT3G22490) Description

The data tables and search results are compatible with Microsoft Excel spread sheet. Please download the [How to use search results.](#)

[Download all data in spread sheet style \(size:11MB\).](#)

Web presentation of SPD

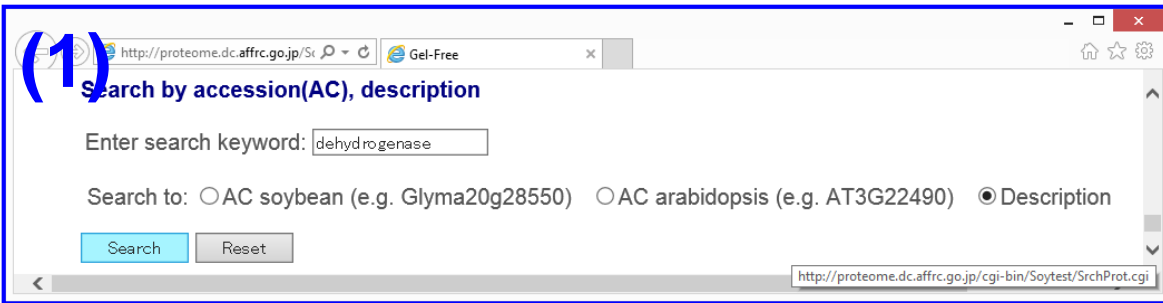
After opening the SPD homepage, the left hand menu includes options for “Gel-based Proteomics” and “Gel-free Proteomics”. In “Gel-free Proteomics”

A) images of soybean plants

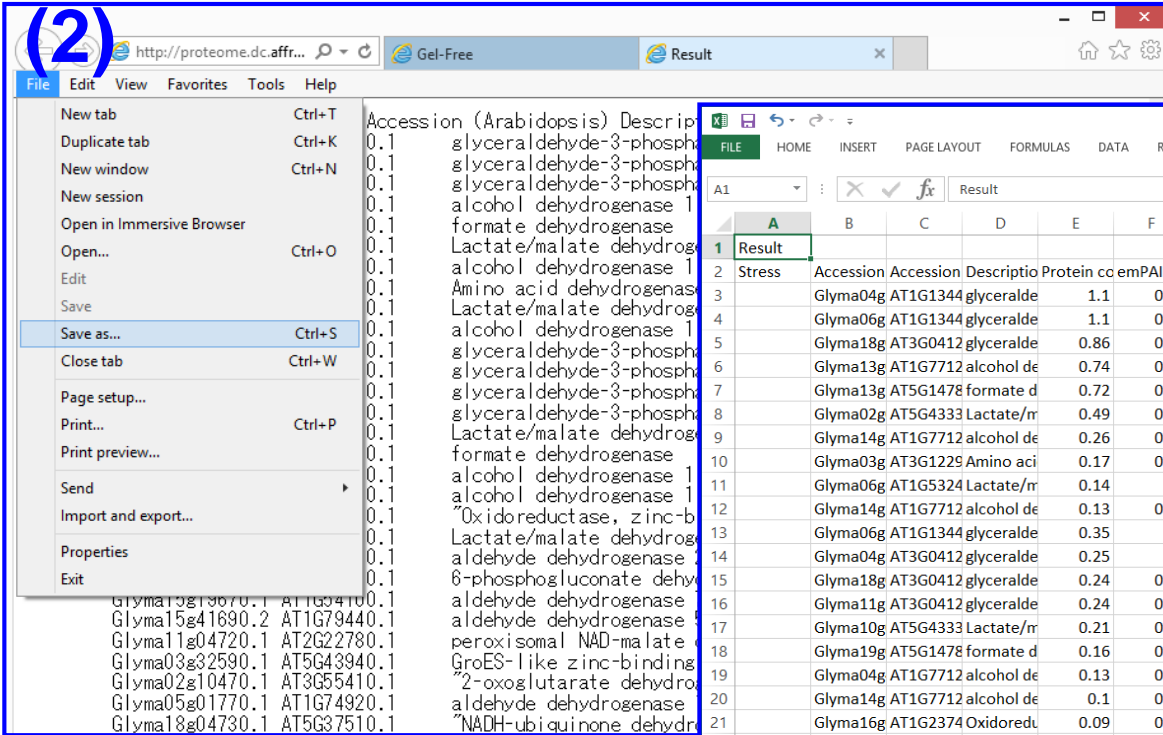
B) a list of organs or organelles over a sequence of time points

C) “Enter Search keyword” box allows the user to search for protein information

“Download all data in spread sheet style” is used to download all data.



Result_cgi.txt



(3)

Result_cgi.txt - Excel

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	
1	Result														
2	Stress	Accession	Accession	Descriptio	Protein	cc	emPAI	Score	Mass	Number	o	Stage	Organ	Organelle	Map on genome
3		Glyma04g	AT1G1344	glyceralde	1.1	0.79	208	36861	13	Seed	Cotyledon		Glyma04g36860.1		
4		Glyma06g	AT1G1344	glyceralde	1.1	0.79	246	36855	12	Seed	Cotyledon		Glyma06g18110.1		
5		Glyma18g	AT3G0412	glyceralde	0.86	0.62	259	37087	12	Seed	Cotyledon		Glyma18g01330.1		
6		Glyma13g	AT1G7712	alcohol de	0.74	0.53	306	41925	25	Seed	Cotyledon		Glyma13g09530.1		
7		Glyma13g	AT5G1478	formate d	0.72	0.52	267	43082	15	Seed	Cotyledon		Glyma13g23790.1		
8		Glyma02g	AT5G4333	Lactate/mr	0.49	0.35	169	35787	5	Seed	Cotyledon		Glyma02g00810.1		
9		Glyma14g	AT1G7712	alcohol de	0.26	0.19	159	41152	6	Seed	Cotyledon		Glyma14g24860.1		
10		Glyma03g	AT3G1229	Amino aci	0.17	0.12	24	31609	7	Seed	Cotyledon		Glyma03g37080.4		
11		Glyma06g	AT1G5324	Lactate/mr	0.14	0.1	32	36192	3	Seed	Cotyledon		Glyma06g34190.1		
12		Glyma14g	AT1G7712	alcohol de	0.13	0.09	47	41548	3	Seed	Cotyledon		Glyma14g27940.1		
13		Glyma06g	AT1G1344	glyceralde	0.35	1.4	805	36855	34	Seed	Embryo		Glyma06g18110.1		
14		Glyma04g	AT3G0412	glyceralde	0.25	1	798	46847	32	Seed	Embryo		Glyma04g36870.3		
15		Glyma18g	AT3G0412	glyceralde	0.24	0.97	785	37087	30	Seed	Embryo		Glyma18g01330.1		
16		Glyma11g	AT3G0412	glyceralde	0.24	0.97	744	37105	29	Seed	Embryo		Glyma11g37360.1		
17		Glyma10g	AT5G4333	Lactate/mr	0.21	0.83	702	35524	16	Seed	Embryo		Glyma10g00920.1		
18		Glyma19g	AT5G1478	formate d	0.16	0.65	664	43118	17	Seed	Embryo		Glyma19g01210.1		
19		Glyma04g	AT1G7712	alcohol de	0.13	0.54	284	41638	9	Seed	Embryo		Glyma04g41990.1		
20		Glyma14g	AT1G7712	alcohol de	0.1	0.41	229	41548	6	Seed	Embryo		Glyma14g27940.1		
21		Glyma16g	AT1G2374	Oxidoredu	0.09	0.36	206	34576	7	Seed	Embryo		Glyma16g08040.1		
22		Glyma12g	AT1G5324	Lactate/mr	0.08	0.34	87	36347	5	Seed	Embryo		Glyma12g19520.1		
23		Glyma08g	AT3G480C	aldehyde	0.07	0.28	225	58693	10	Seed	Embryo		Glyma08g39770.1		
24		Glyma08g	AT3G0236	6-phosph	0.05	0.22	320	53811	5	Seed	Embryo		Glyma08g28230.1		

How to use search result of SPD

- (1) Search data
- (2) Save "Result" as a text file
- (3) Open the file "Result cgi.txt" with Microsoft Excel.

List of proteins

	A	B	C	D	E	F	G	H	I	J	K	L	M
	Accession (Soybean)	Accession (Arabidopsis)	Description	Protein content (mol %)	emPAI	Score	Mass	Number of matched peptides	Stage	Organ	Organelle	Map on Genome	
1													
2	Glyma14g36850.1	AT2G36460.1	Aldolase superfamily protein	0.68	4.34	1747	38558	43	6 day	Root		Glyma14g36850.1	
3	Glyma08g24720.1	AT1G70890.1	MLP-like protein 43	0.6	3.84	394	17723	25	6 day	Root		Glyma08g24720.1	
4	Glyma12g07780.3	AT1G07890.1	ascorbate peroxidase 1	0.59	3.8	1035	27166	25	6 day	Root		Glyma12g07780.3	
5	Glyma15g04290.1	AT3G55440.1	triosephosphate isomerase	0.49	3.16	943	27409	35	6 day	Root		Glyma15g04290.1	
6	Glyma02g38730.1	AT2G36460.1	Aldolase superfamily protein	0.48	3.04	1728	38469	36	6 day	Root		Glyma02g38730.1	
7	Glyma20g38440.1	AT1G75270.1	dehydroascorbate reductase										
8	Glyma19g49070.1	AT3G01280.1	voltage dependent anion channel										
9	Glyma09g37570.1	AT3G01280.1	voltage dependent anion channel										
10	Glyma03g28850.1	AT3G57270.1	beta-1,3-glucanase 1										
11	Glyma13g41960.1	AT3G59480.1	pfkB-like carbohydrate kinase										
12	Glyma15g31520.1	AT1G70890.1	MLP-like protein 43										
13	Glyma06g14330.1	AT1G33140.1	Ribosomal protein L6 family										
14	Glyma04g36860.1	AT1G13440.1	glyceraldehyde-3-phosphate dehydrogenase										
15	Glyma09g04530.1												
16	Glyma19g01330.1	AT3G04120.1	glyceraldehyde-3-phosphate dehydrogenase										
17	Glyma19g40810.1	AT4G01850.1	S-adenosylmethionine synthase										
18	Glyma12g30800.3	AT3G44590.1	60S acidic ribosomal protein										
19	Glyma11g12170.2	AT1G17880.1	basic transcription factor 3										
20	Glyma15g30110.1	AT1G70890.1	MLP-like protein 43										
21	Glyma08g17600.1	AT1G79550.1	phosphoglycerate kinase										
22	Glyma08g24760.1	AT1G70890.1	MLP-like protein 43										
23	Glyma20g32850.1	AT1G66240.1	homolog of anti-oxidant 1										
24	Glyma15g21890.1	AT3G17390.1	S-adenosylmethionine synthase										
25	Glyma10g34880.2	AT1G66240.1	homolog of anti-oxidant 1										
26	Glyma04g01270.1	AT2G21580.1	Ribosomal protein S25 family										
27	Glyma06g01310.1	AT4G39200.1	Ribosomal protein S25 family										
28	Glyma04g37120.1	AT2G18110.1	Translation elongation factor										
29	Glyma01g03650.1	AT5G14670.1	ADP-ribosylation factor A1										

DAIZUbase
HOME GBrowse UnifiedMap BLAST

Chromosome >> 1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|17|18|19|20

Scaffold >>

Please be advised that the full-length sequence of each feature was deduced from the genomic sequence (Glyma scaffold sequence).

File - Help -

daizu: 3.035 kbp from Gm14:46,137,577..46,140,611

Browser Select Tracks Snapshots Custom Tracks Preferences

Search

Landmark or Region:
Gm14:46,137,577..46,140,611 Search

Annotate Restriction Sites

Save Snapshot Load Snapshot

Examples: Gm01:80,000..120,000, GMJENb0025B20.

Data Source
daizu

ScrollZoom: << < < Show 3.035 kbp > > >>

Overview

Region

Details

Gm14: 3.035 kbp 1 kbp

Glyma1 annotation
Glyma14g36850.1

Glyma1 TE annotation

Williams82_BAC

BACend
GHJENb0011G04
GHJENb0061A03

FLcDNA

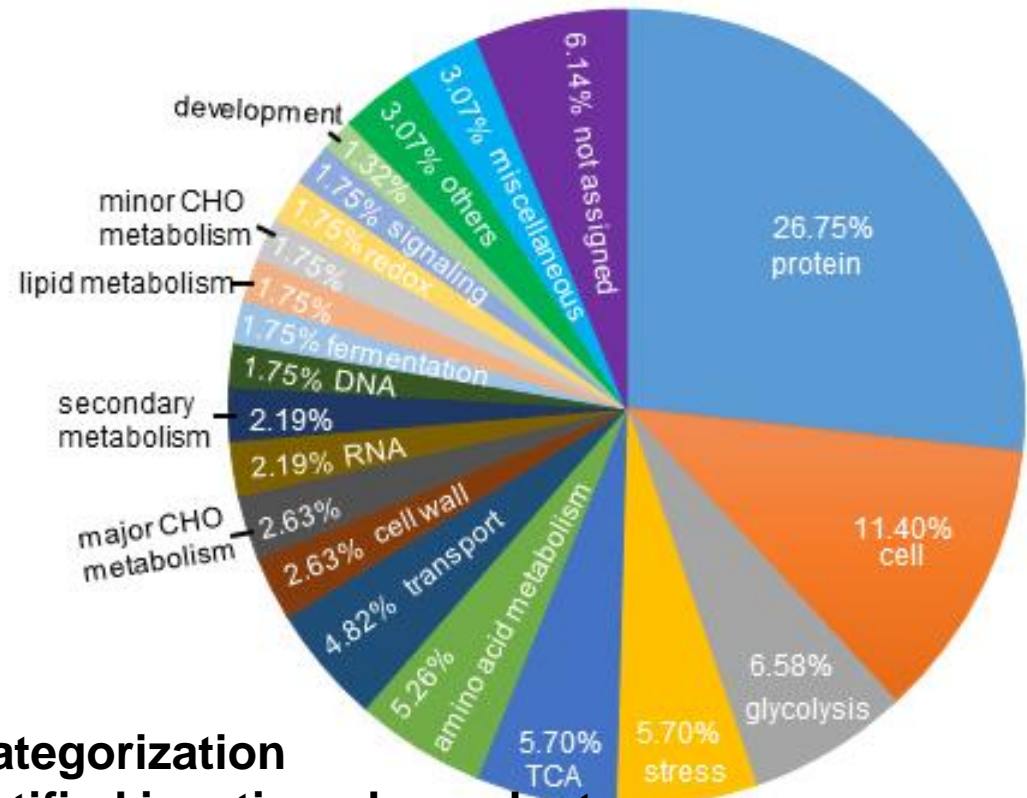
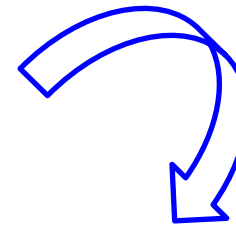
Example of a search for soybean protein information in SPD

The result file can be downloaded onto a personal computer, and dragged/dropped the file into a spreadsheet. When one of the proteins is selected, information on its chromosomal position from DAIZUbase (<http://daizu.dna.affrc.go.jp/>) is shown.

Seed	1 day	2 day	3 day	4 day	5 day	6 day
Cotyledon	Cotyledon	Cotyledon	Cotyledon	Cotyledon	Leaf	Leaf
Embryo	Root	Root	Hypocotyl	Hypocotyl	Cotyledon	Cotyledon
			Root	Root	Hypocotyl	Hypocotyl
					Root	Root

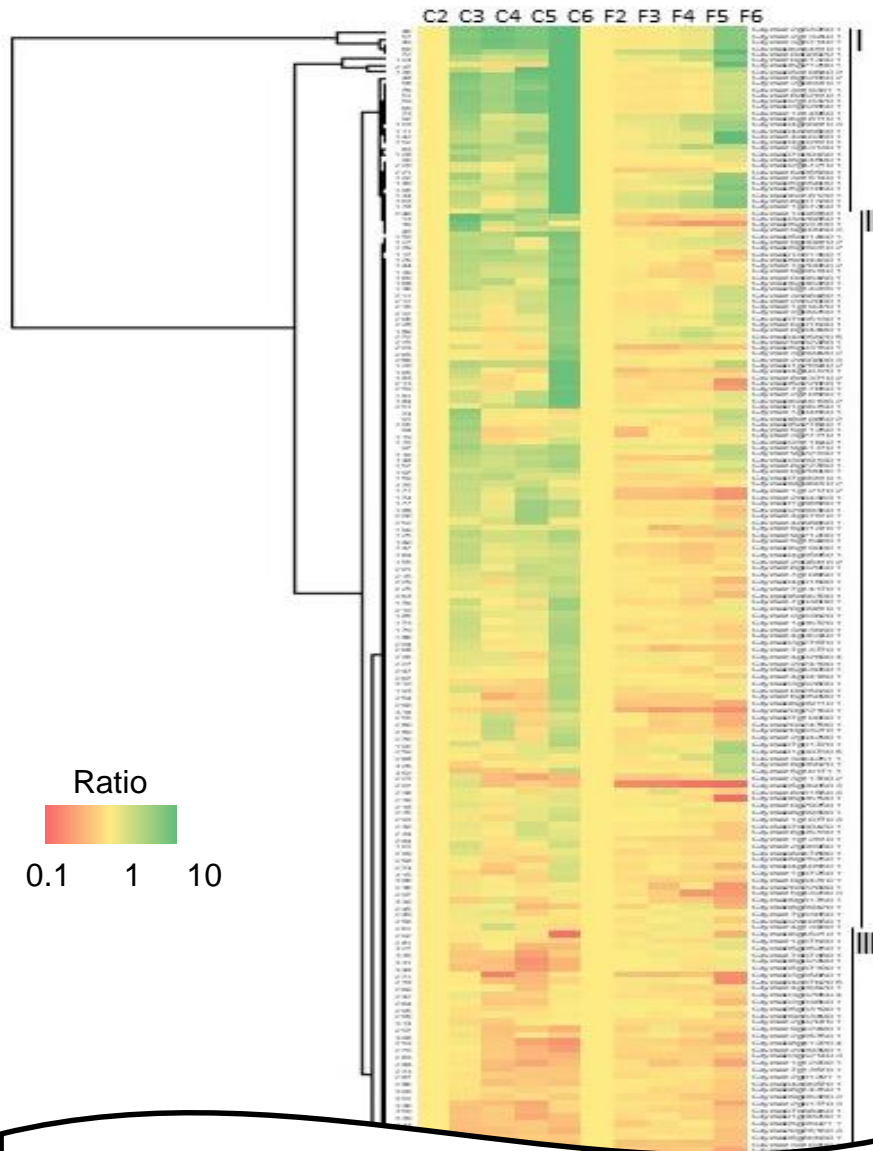
Mitochondria (2day,root)	Nucleus (2day,root)	Endoplasmic Reticulum (2day,root)
--------------------------	---------------------	-----------------------------------

2 day	3 day	4 day
0 day flood	1 day flood	2 day flood
Cotyledon	Cotyledon	Cotyledon
Root	Root	Root

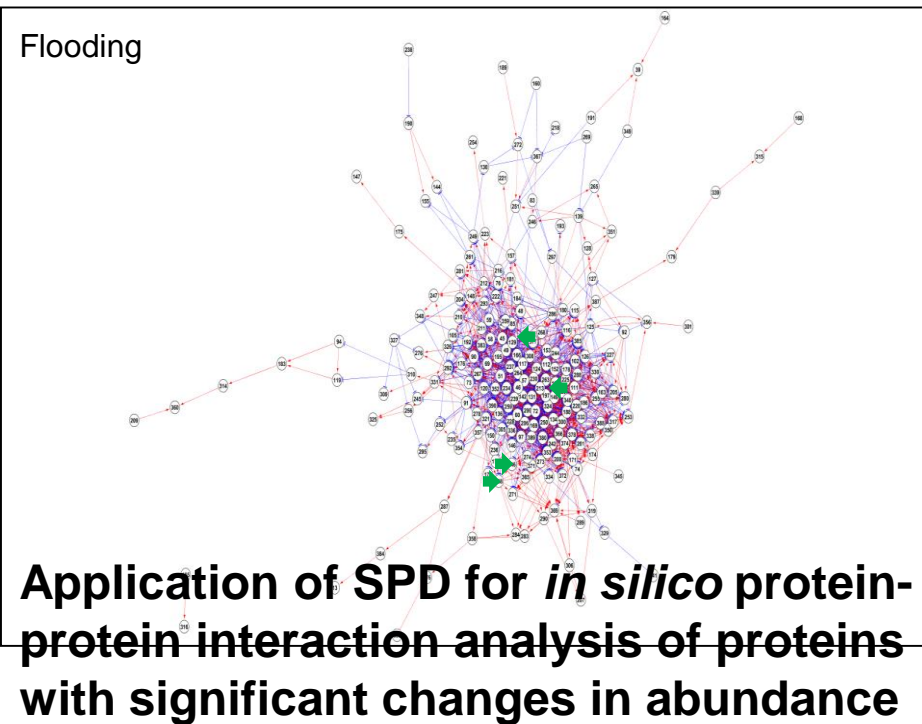
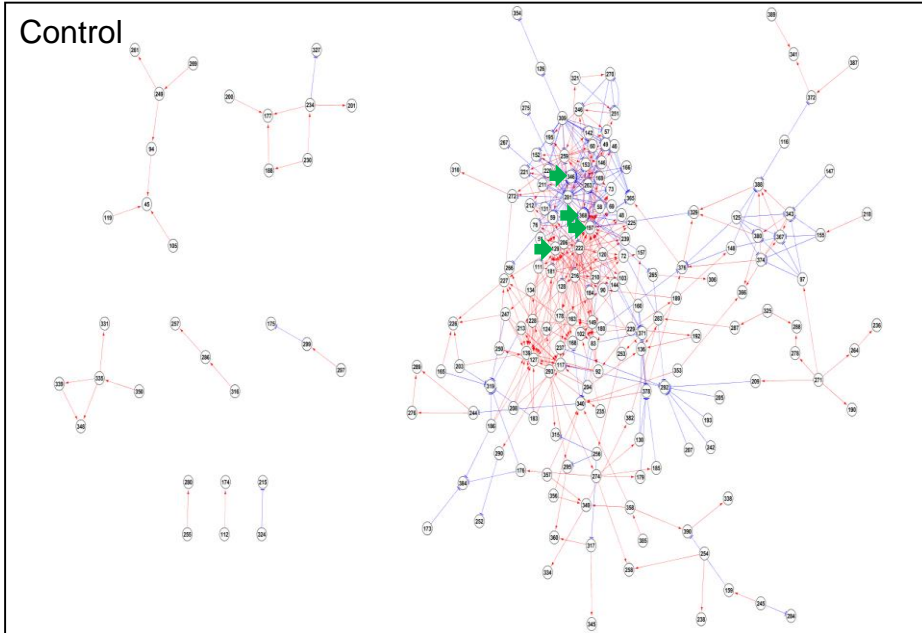


Application of SPD for functional categorization of proteins in the soybean root identified in a time-dependent manner

Using these data, a set of 228 proteins significantly changed in abundance in the root under flooding stress, compared with those of non-treated soybean, were detected and analyzed using MapMan bin codes.



Application of SPD for cluster analysis of significantly changed proteins in soybean during exposure to flooding



Application of SPD for *in silico* protein-protein interaction analysis of proteins with significant changes in abundance

まとめ 1

1. ダイズ遺伝子発現の包括的な研究の促進に資するため、機能解明研究に広く利用可能なダイズプロテオームデータベースを構築・公開。

(<http://proteome.dc.affrc.go.jp/Soybean/>)

2. データベースの特徴

(1)「電気泳動を基盤にしたプロテオミクス」と「質量分析計を基盤にしたプロテオミクス」解析技術を用いて得られたダイズタンパク質データ

(2)経時的生育時期の各種器官と細胞小器官から得られたタンパク質データ、さらに、ストレス下のダイズで変動するタンパク質データを提供。

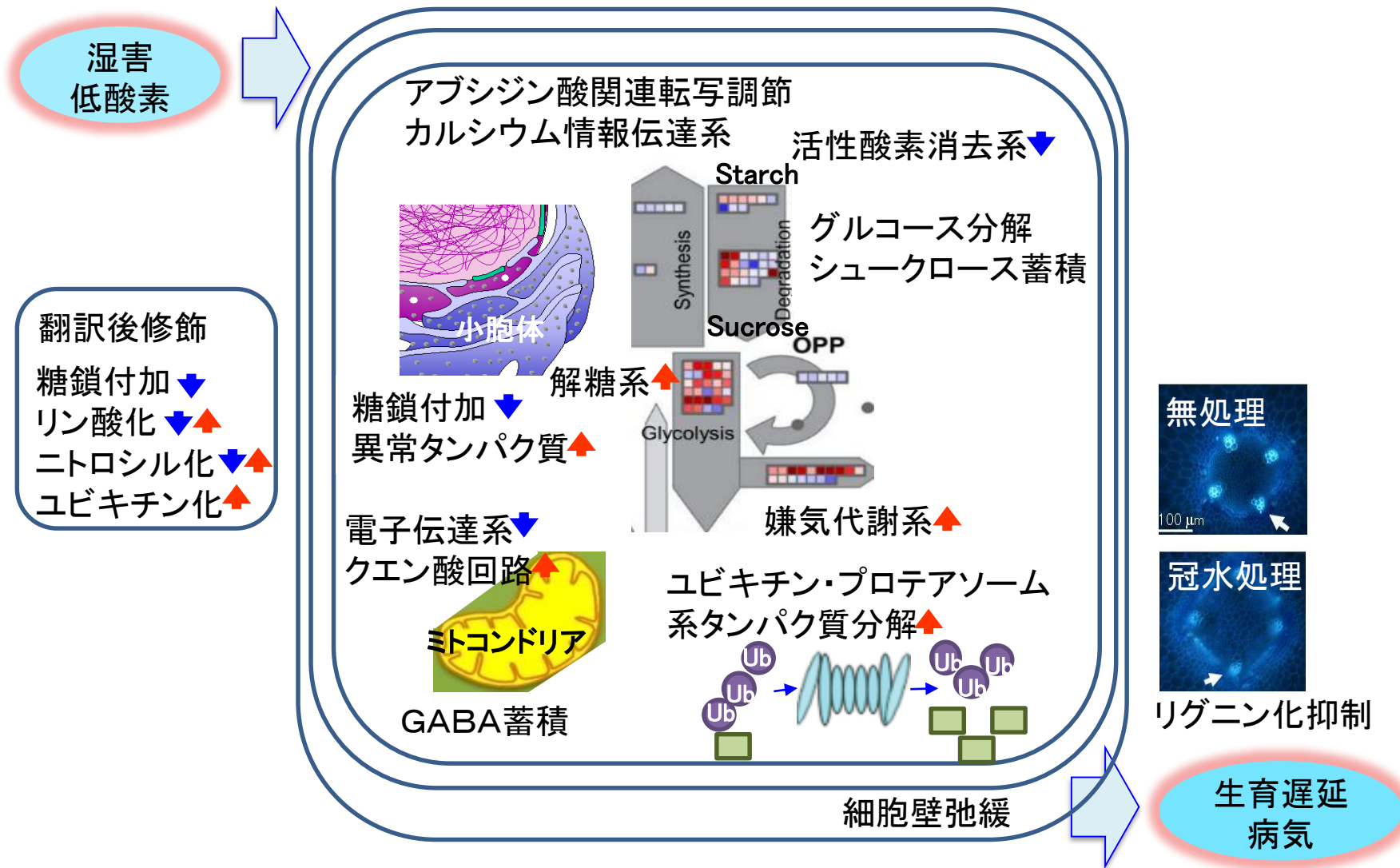
(3) DAIZUbase (<http://daizu.dna.affrc.go.jp/>)の染色体上の位置と連動。

(4)データのダウンロードが可能のため、使用者が加工し利用可能。

3. 得られた質量分析生データ等は「jPOSTプロテオームデータベース」へ格納し、閲覧・使用が可能。

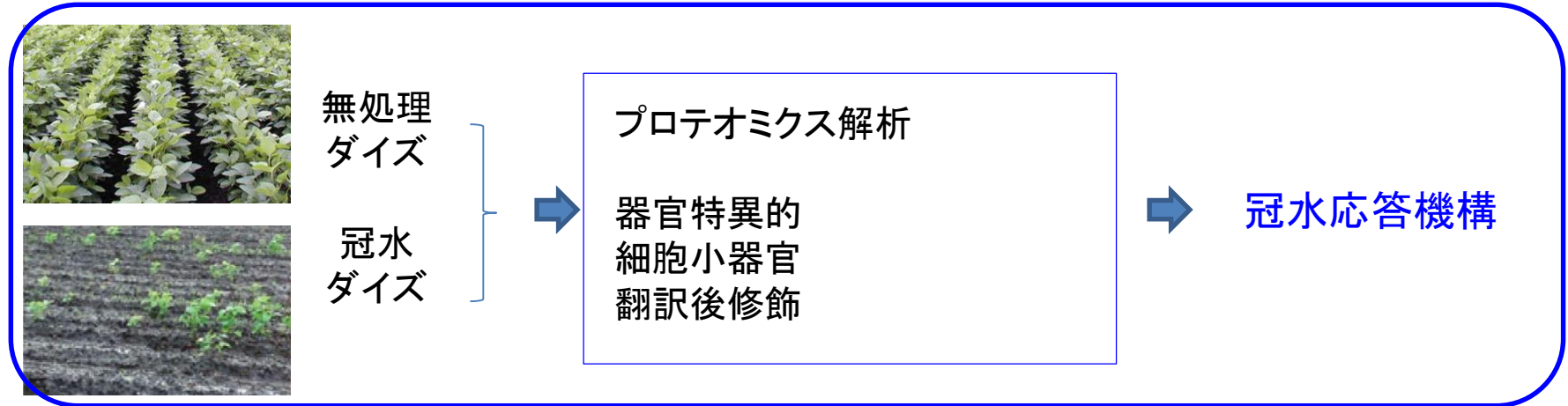
2. 湿害耐性ダイズの作出を目指したプロテオミクス解析

ダイズの冠水ストレス応答機構

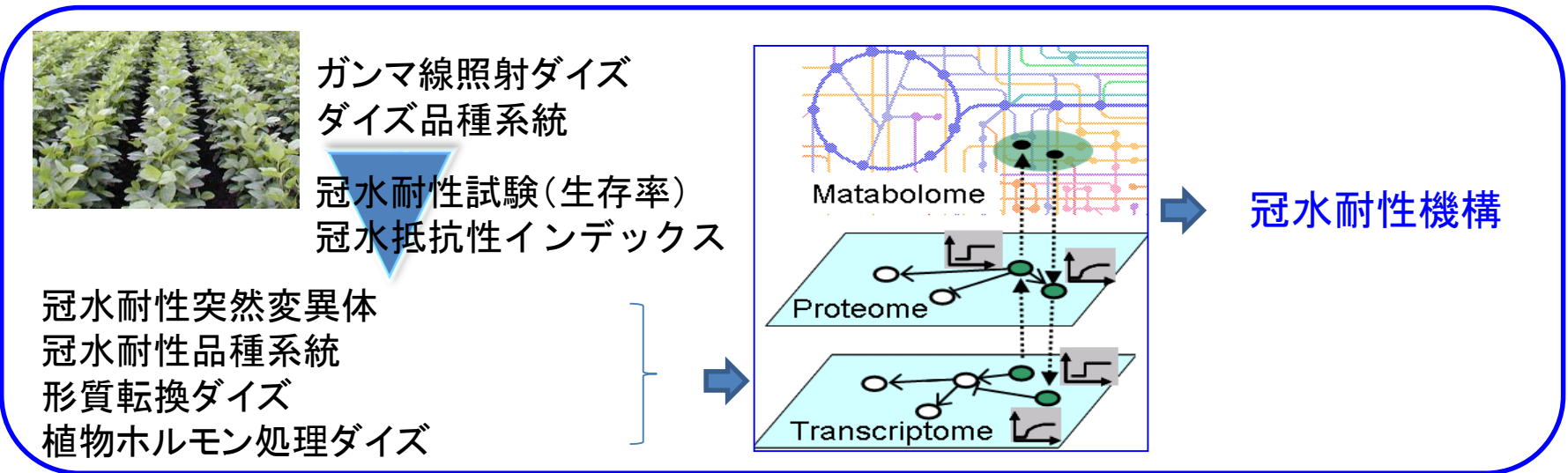


プロテオミクス解析で明らかになった生育初期のダイズの冠水ストレス応答機構

これまでの研究

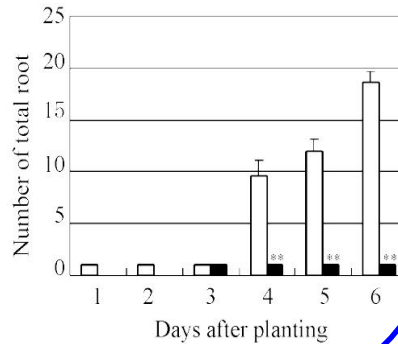
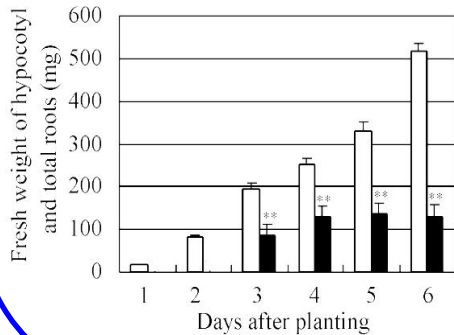
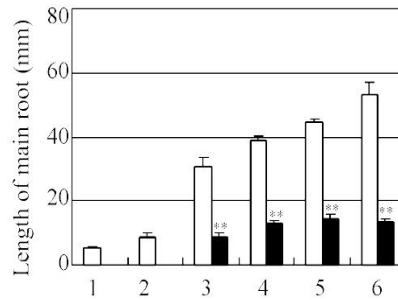
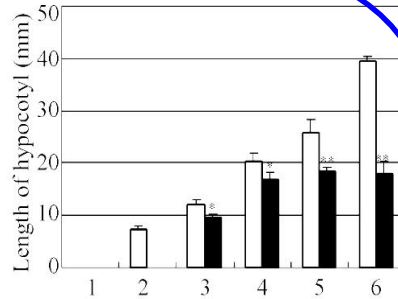
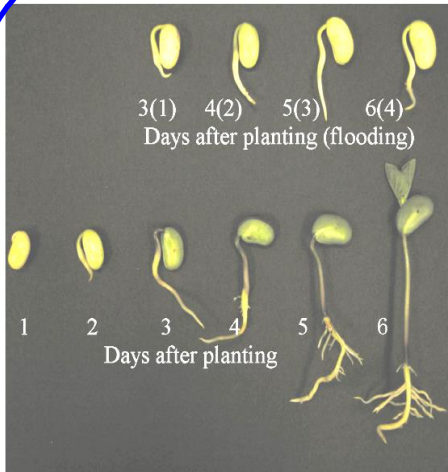


本研究

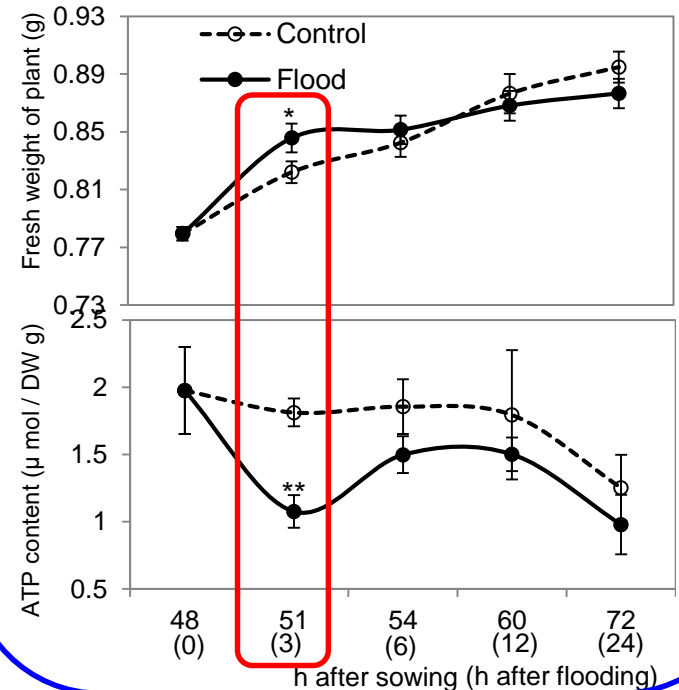
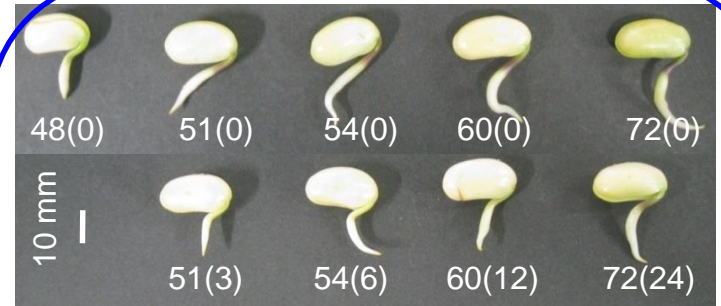


出芽期ダイズの湿害応答機構に関しては解明された。
しかし、湿害耐性機構に関しては未だ不明な点が多い。

これまでの研究

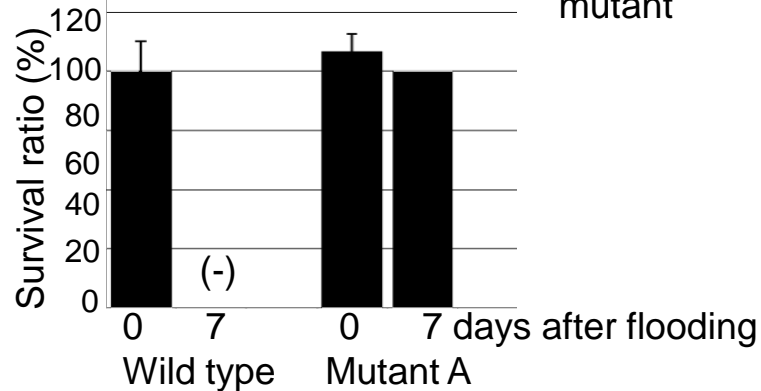
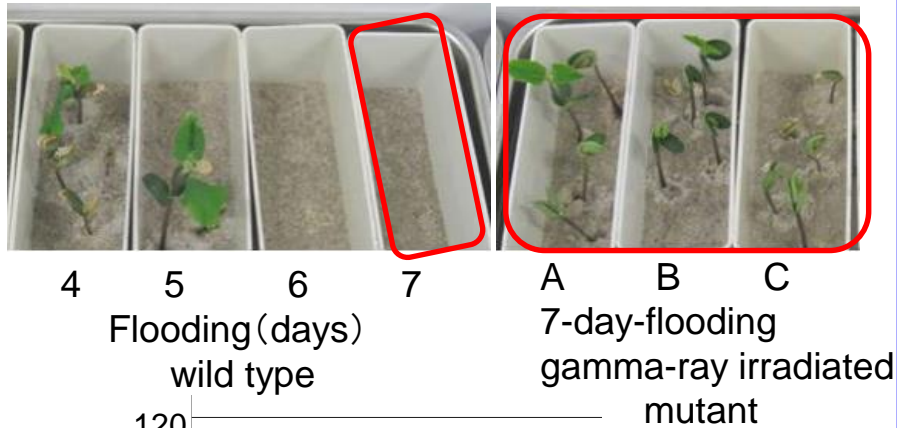


本研究



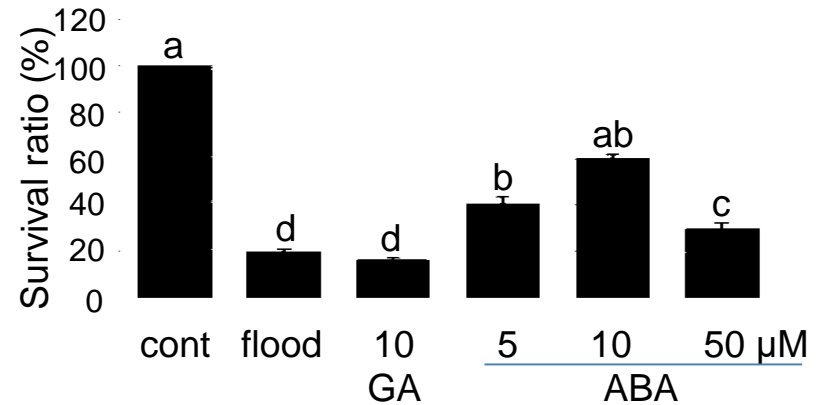
出芽期サイズにおいて、生育初期の湿害応答機構に関しては解明された。
しかし、湿害直後の湿害応答機構に関しては未だ不明な点が多い。

冠水抵抗性突然変異ダイズ



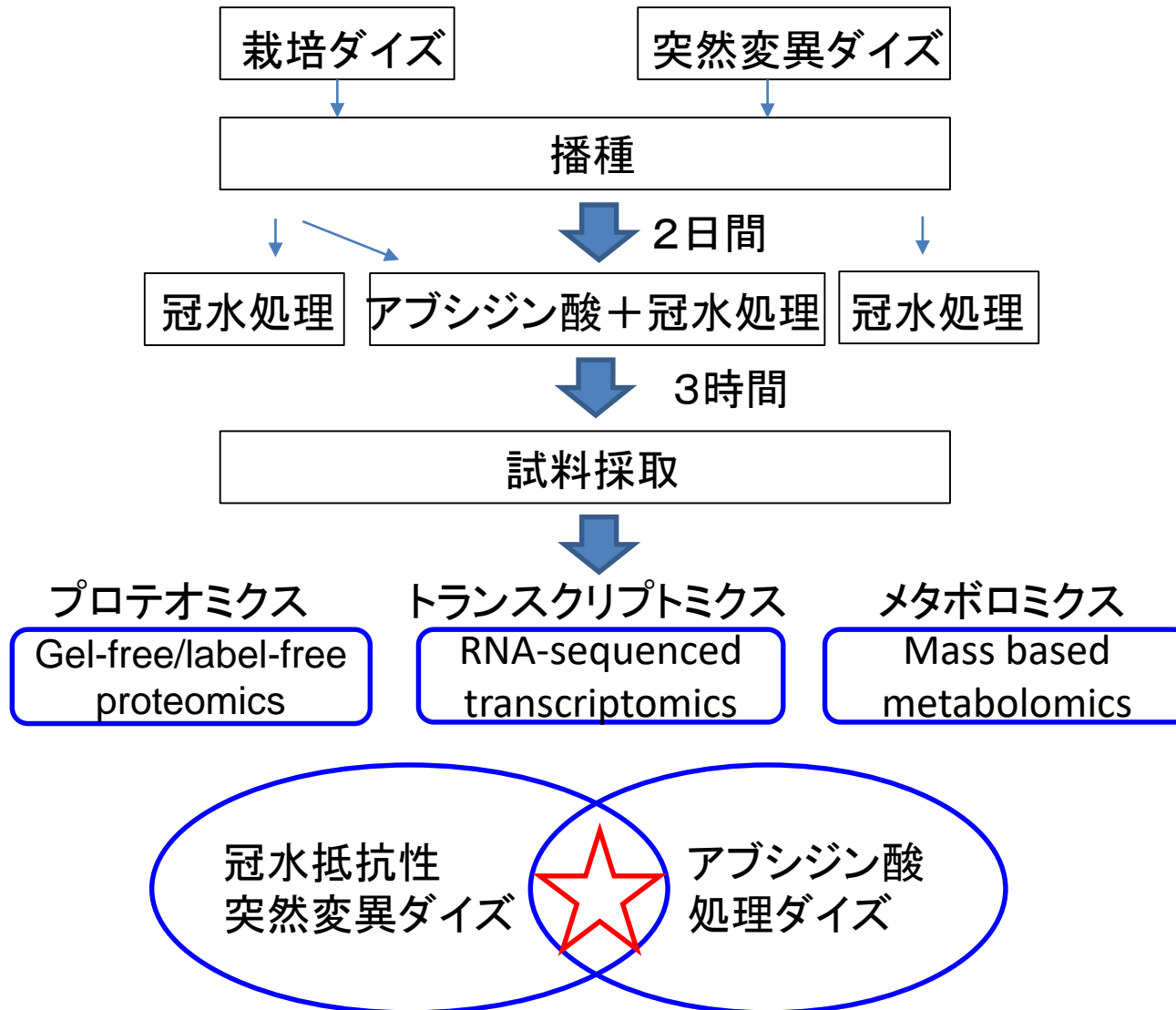
➤冠水抵抗性突然変異ダイズは、7日間の冠水後の水除去により生長を開始する。

植物ホルモン処理ダイズ



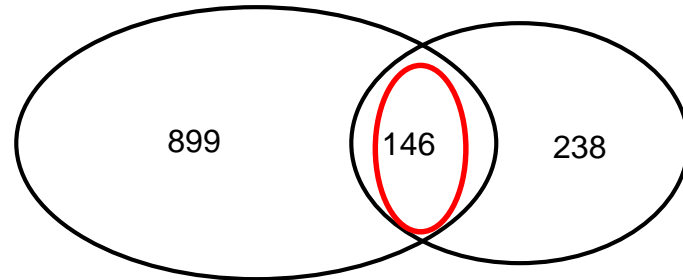
➤アブシジン酸処理ダイズは、7日間の冠水後の水除去により生長を開始する。

冠水抵抗性ダイズ素材を用いて、湿害抵抗性機構を解明する

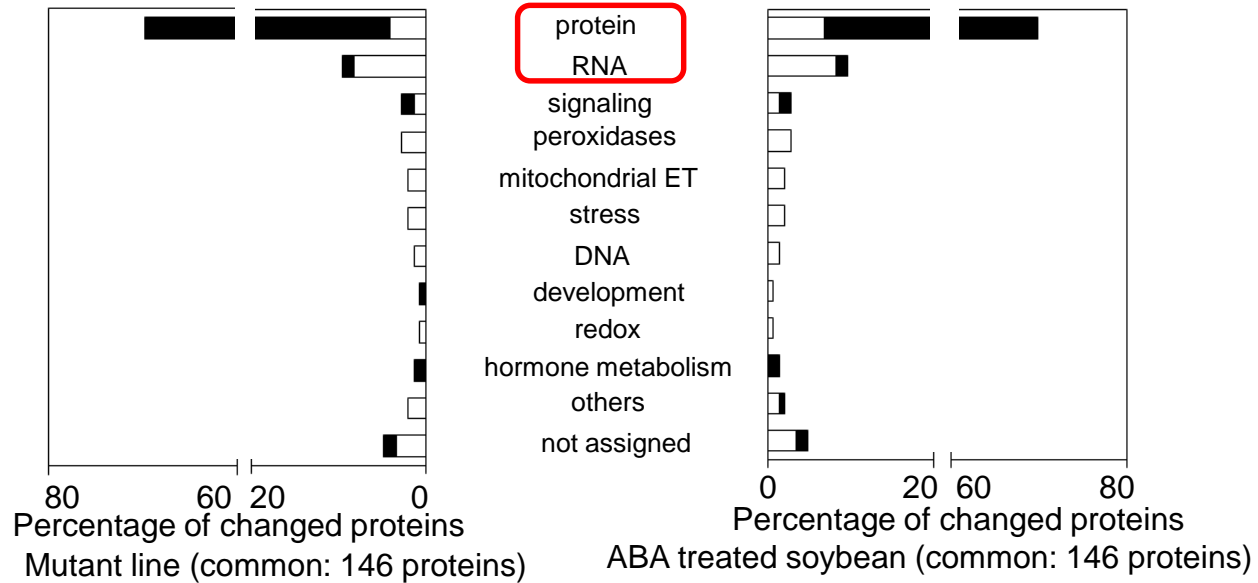


冠水抵抗性突然変異ダイズとアブシジン酸処理ダイズの両冠水抵抗性素材において共通に変動する因子が、湿害耐性機構に関与する鍵となる。

Proteomics



Mutant line/Wild type ABA treated soybean/Wild type



Functional distribution of proteins identified in mutant line and ABA-treated soybean at initial flooding stress.

The significantly changed flooding tolerance-related proteins were mainly involved in protein synthesis and RNA regulation.

protein synthesis

RNA regulation

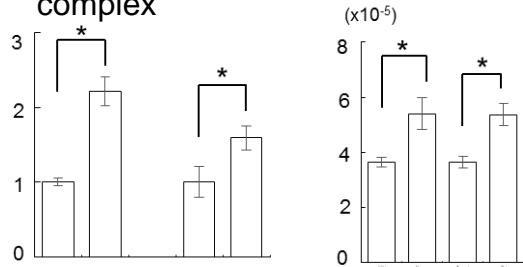
Nascent polypeptide associated complex
protects newly synthesized proteins

Chaperone
functions in the correct refolding of proteins.

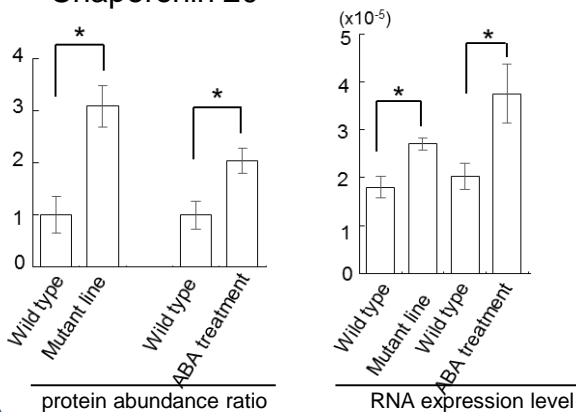
Glycine-rich RNA-binding protein
affects catalase/peroxidase activities under abiotic stresses.

Aspartyl protease
improves abiotic tolerance by increasing activity of antioxidants

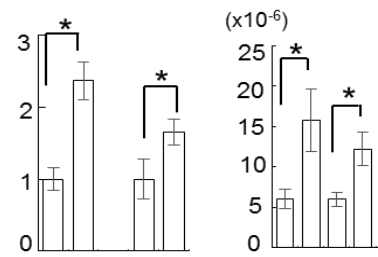
Nascent polypeptide associated complex



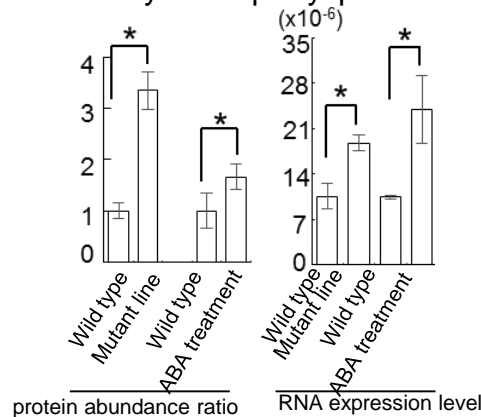
Chaperonin 20



Glycine rich RNA binding protein 3



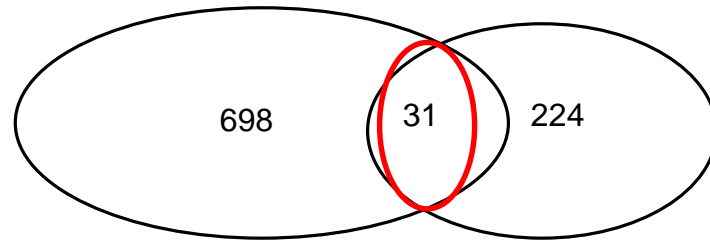
Eukaryotic aspartyl protease



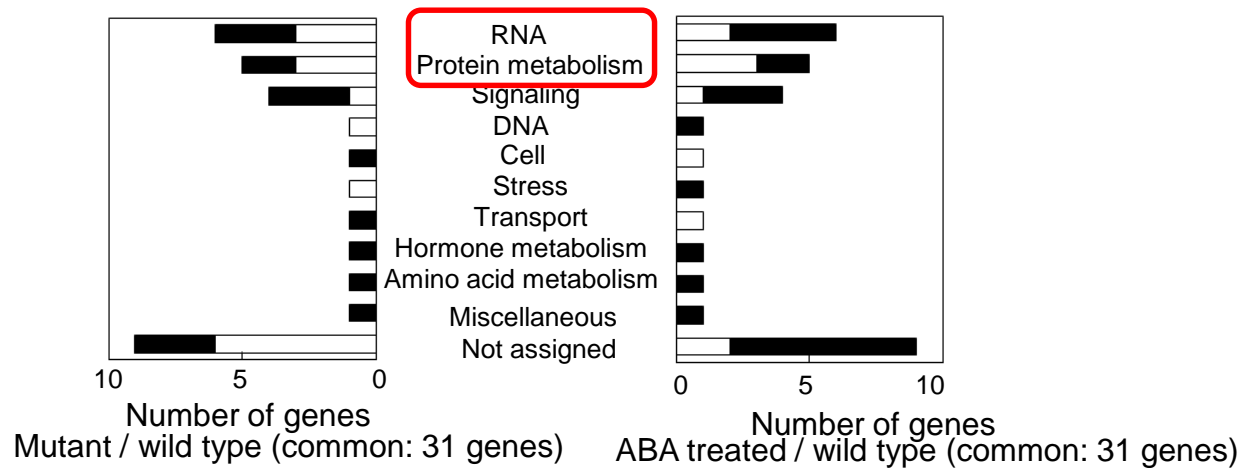
mRNA expression of genes related to protein synthesis and RNA regulation at initial flooding stress.

The mRNA expression level of *genes* related to refolding, assembly of newly synthesized proteins, and activity of antioxidants was up-regulated in mutant and ABA treated soybean.

Transcriptomics

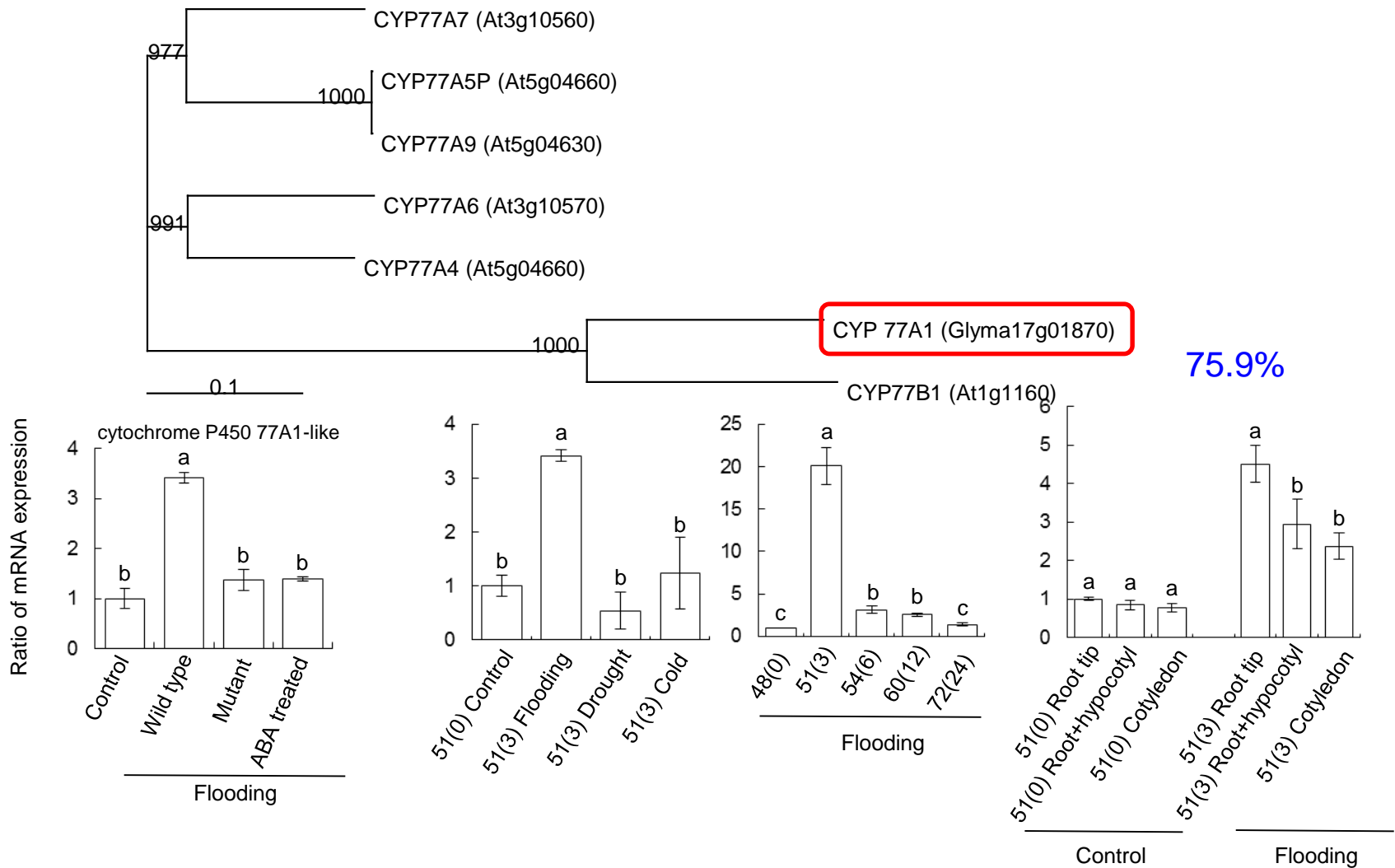


Mutant / wild type (729 genes) ABA treated / wild type (255 genes)



Functional categorization of differentially expressed genes in mutant line and ABA-treated soybean.

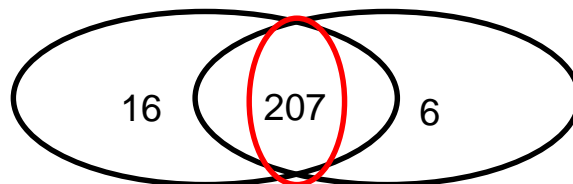
The significantly changed flooding tolerance-related genes were mainly involved in protein synthesis and RNA regulation.



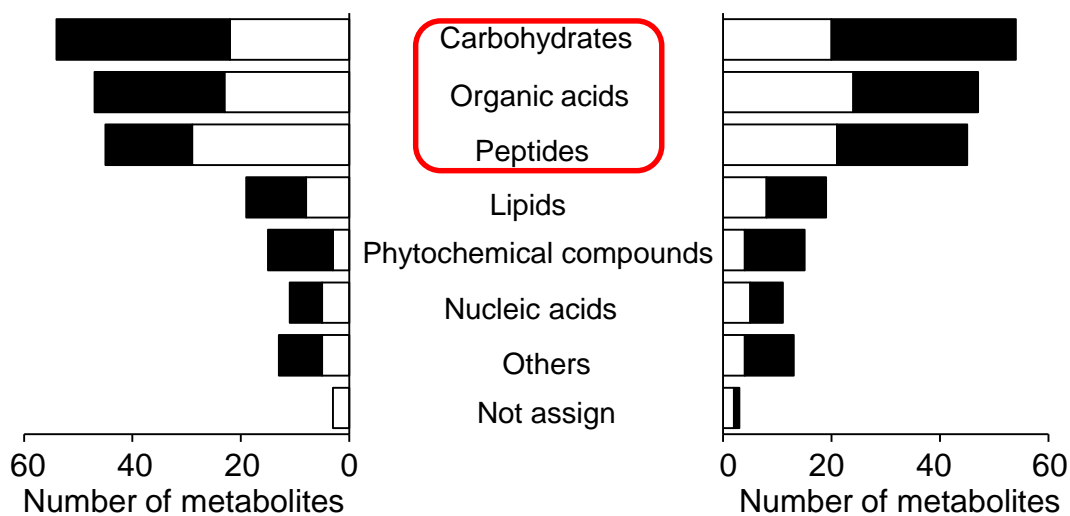
Stress, temporal, and organ specific mRNA expression level of cytochrome P450 77A1

Cytochrome P45077A1 exhibited high correlation with flooding tolerance in root tip of soybean at initial stage.

Metabolomics



Mutant / wild type (223 metabolites) ABA treated / wild type (213 metabolites)

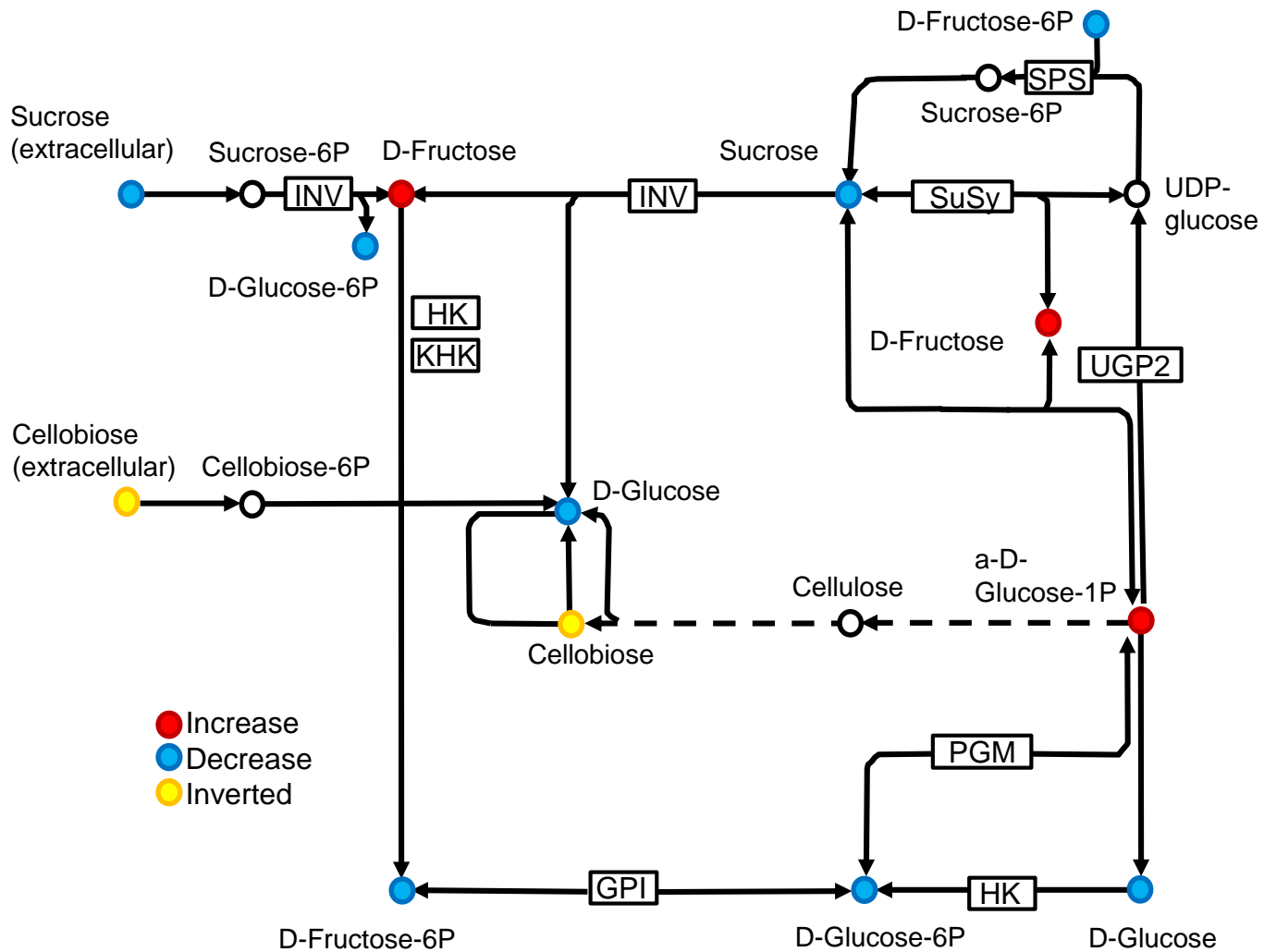


Mutant / wild type (common: 207 metabolites)

ABA treated / wild type (common: 207 metabolites)

Functional categorization of metabolites in flooding-tolerant mutant and ABA-treated soybeans at initial flooding stress

Significantly changed metabolites were carbohydrates, organic acids, and peptides between flooding-tolerant mutant and ABA-treated soybeans.

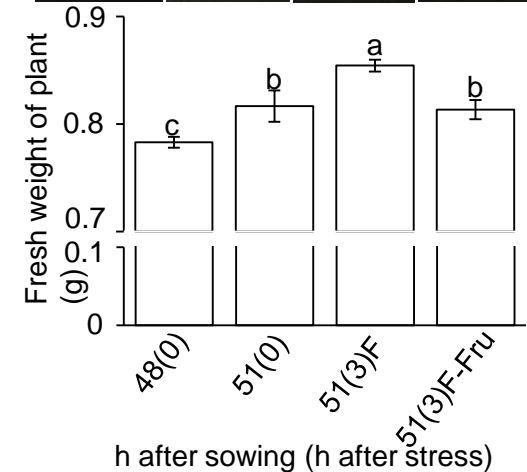
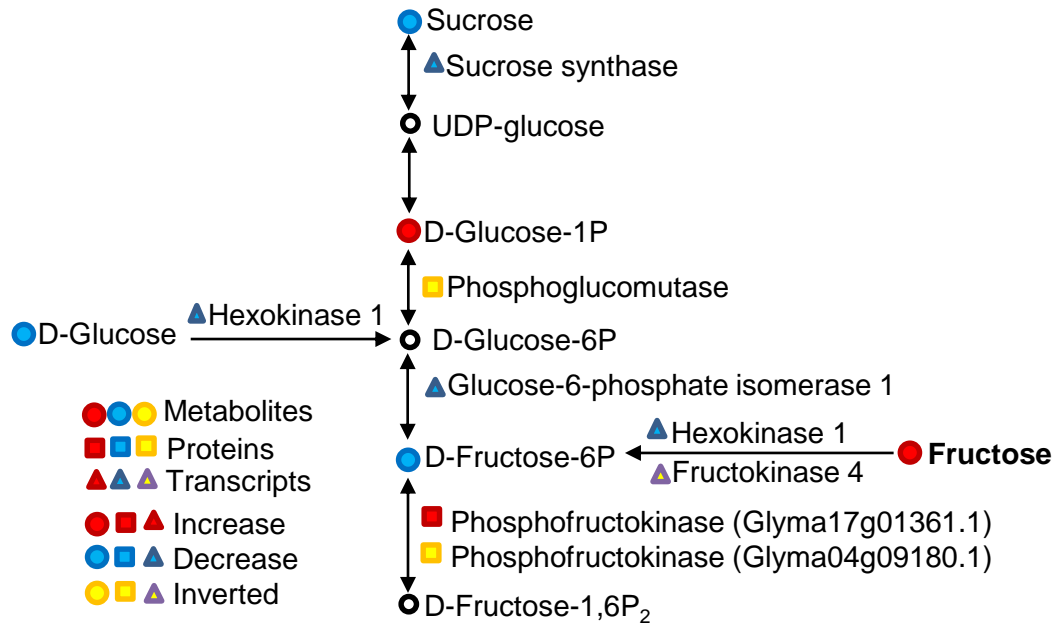


Sugar-metabolism pathway in flooding-tolerant mutant and ABA-treated soybeans at initial flooding stress.

Sucrose, glucose-6-phosphate, glucose, and fructose-6-phosphate were decreased, while fructose and glucose-1-phosphate were increased in both flooding-tolerant materials compared to flooded soybeans.

まとめ 2

オミクスデータの統合 (一部)



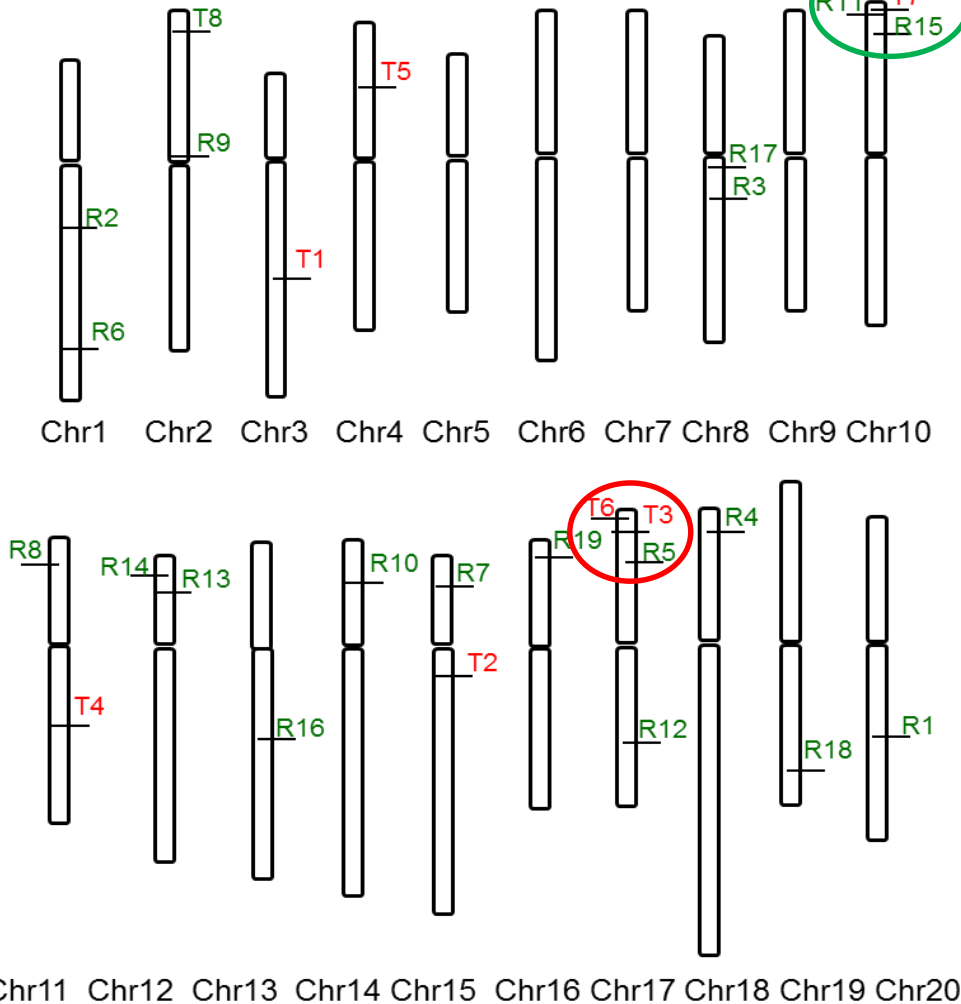
冠水耐性素材において共通に変動する因子

hexokinaseとphosphofructokinaseの制御下で糖代謝系が関与している。統合オミクス解析で重要因子とされたfructoseの添加により、冠水下でダイズの生長を制御できる。

オミクスデータの格納サイトに関しては要検討事項である。ダイズにおいては、未だ多くのmissing proteinsが存在する。

今後

結果の一部



冠水応答性タンパク質・遺伝子	
R1	Alcohol dehydrogenase
R2	Calreticulin 1b
R3	Xylosidase 1
R4	Eukaryotic translation initiation factor 4G
R5	NOP1
R6	NOP56
R7	THOC
R8	Zinc finger and BTB domain-containing protein 47
R9	Glycine rich protein
R10	rRNA processing protein Rrp5
R11	MAGOH
R12	Y14
R13	ACINUS
R14	Histone H1
R15	Histone H2A
R16	Histone H2B
R17	Histone H3
R18	Histone H4
R19	Sucrose phosphate synthase
R20	60S ribosomal protein
R21	UDP glucose 6 dehydrogenase
R22	40S ribosomal protein
冠水耐性タンパク質・遺伝子	
T1	Nascent polypeptide associated complex
T2	Chaperonin 20
T3	Glycine rich RNA binding protein 3
T4	Eukaryotic aspartyl protease family protein
T5	Matrix metalloproteinase
T6	Glucose-6-phosphate isomerase 1
T7	Cytochrome P450 77A1-like
T8	ATPase family AAA domain-containing protein 1

プロテオミクスおよびトランスクリプトミクス解析で同定されたタンパク質・遺伝子群をダイズ染色体へ貼付けることで、冠水耐性に関与する領域を絞込む。

→マーカー選抜育種

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(敬称略)

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基盤研究B(平成27～29年度)(25H04445)

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