

A Molecular Tool to Increase Protein Content and Broad Disease Resistance in Soybeans

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Soybean Breeders Workshop

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Protein and world health

Protein deficiency is a major health problem—especially affects children.

Consumption of proteins from plants have far less environmental impact than consumption of animal-derived protein.



→ Ability to optimize protein productivity in plants could have far-ranging impacts to world health and sustainability.

Combating plant diseases is key for sustainable crops

Climate change: plants more vulnerable to infectious disease, threaten crop yield and impact on the price and availability of food.

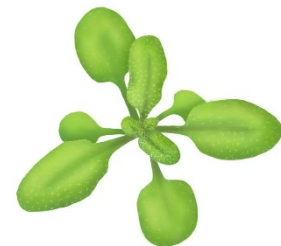
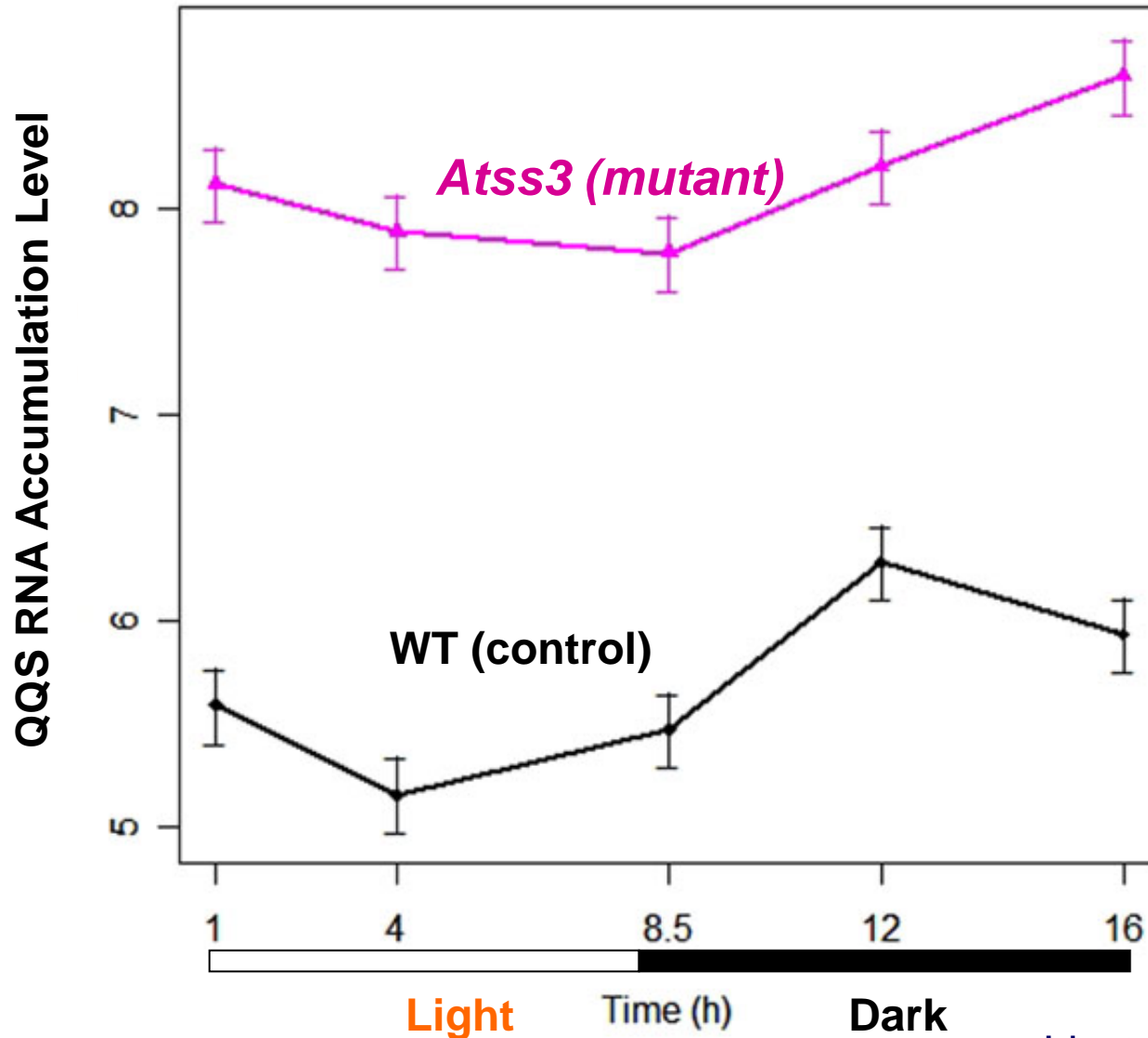
Less reliance on pesticide: essential to ensure sustainable crops and green environment.

→ Increase plant disease resistance.

Research

- Identification of QQS as a component to regulate carbon and nitrogen allocation
- A molecular tool to increase protein in soybeans
- Plant genes mediate pathogen/pest resistance
- A molecular tool for broad disease resistance in soybeans

QQS (Qua Quine Starch) is up-regulated in *Atss3* (starch synthase 3 knock out)

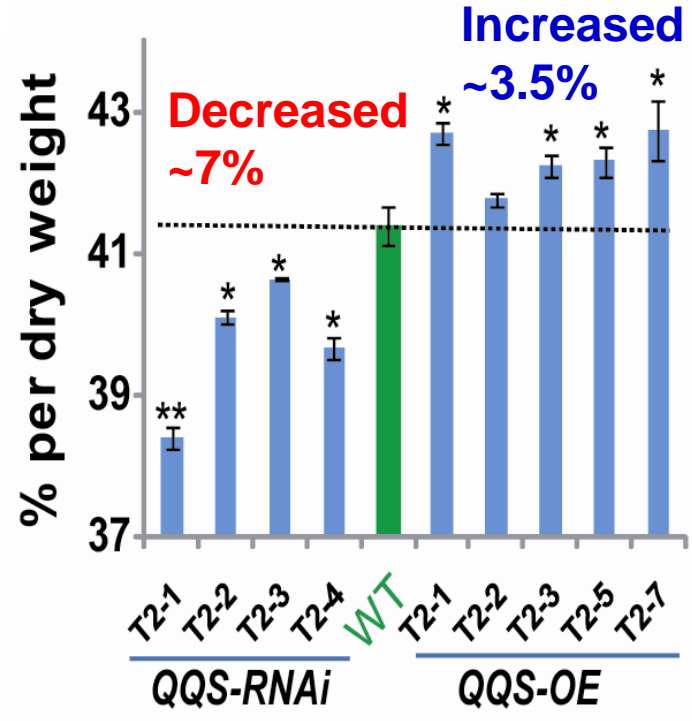
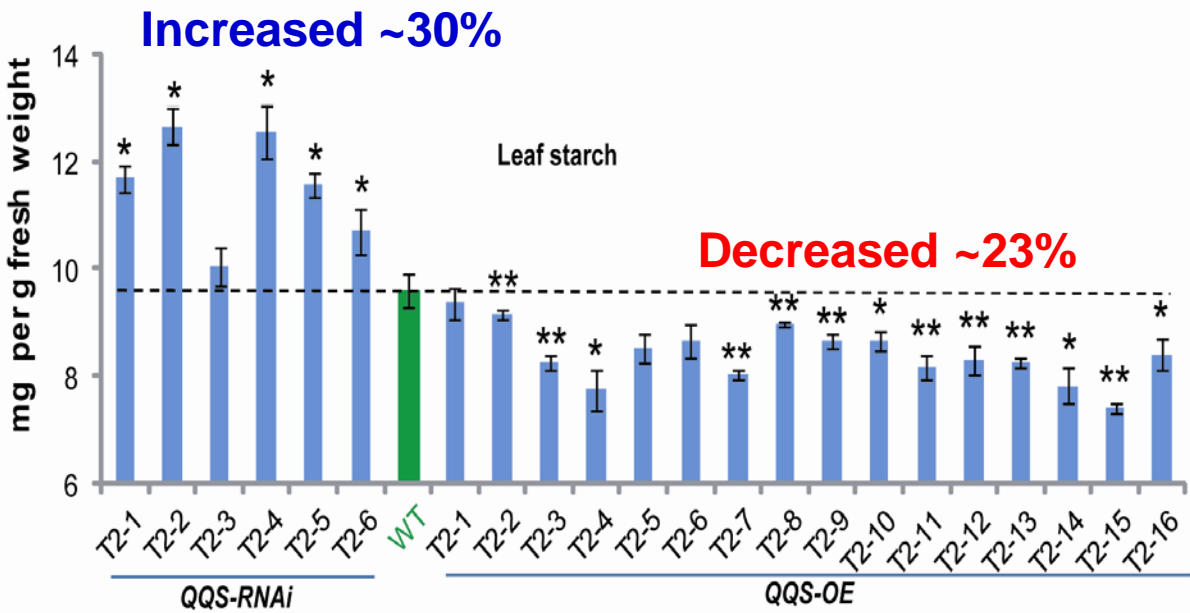


Plants with altered QQS expression have similar visual phenotype



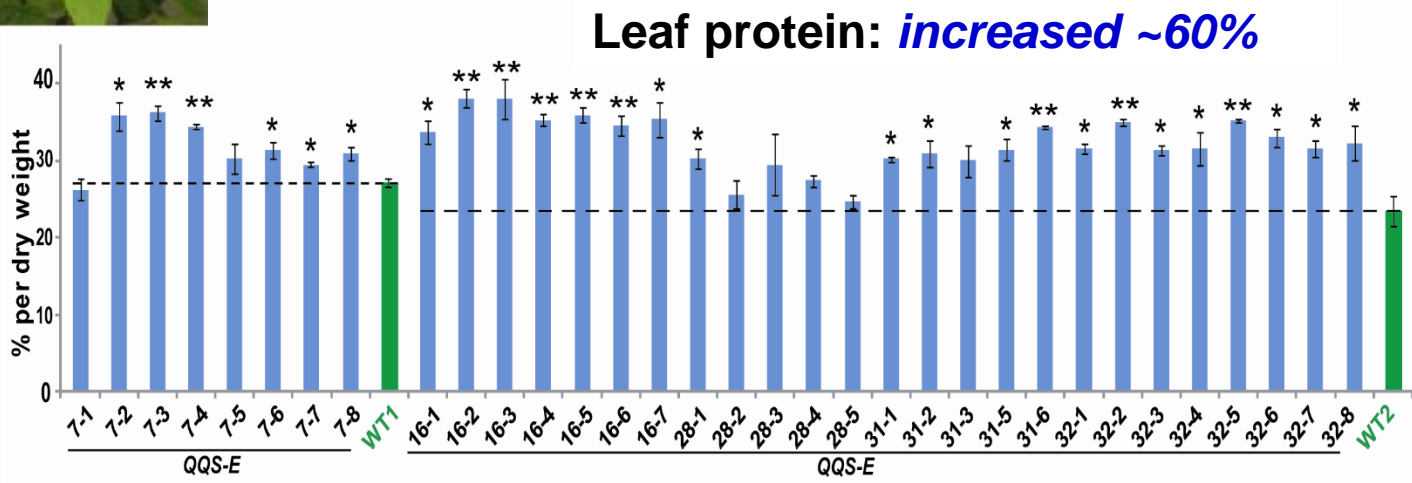
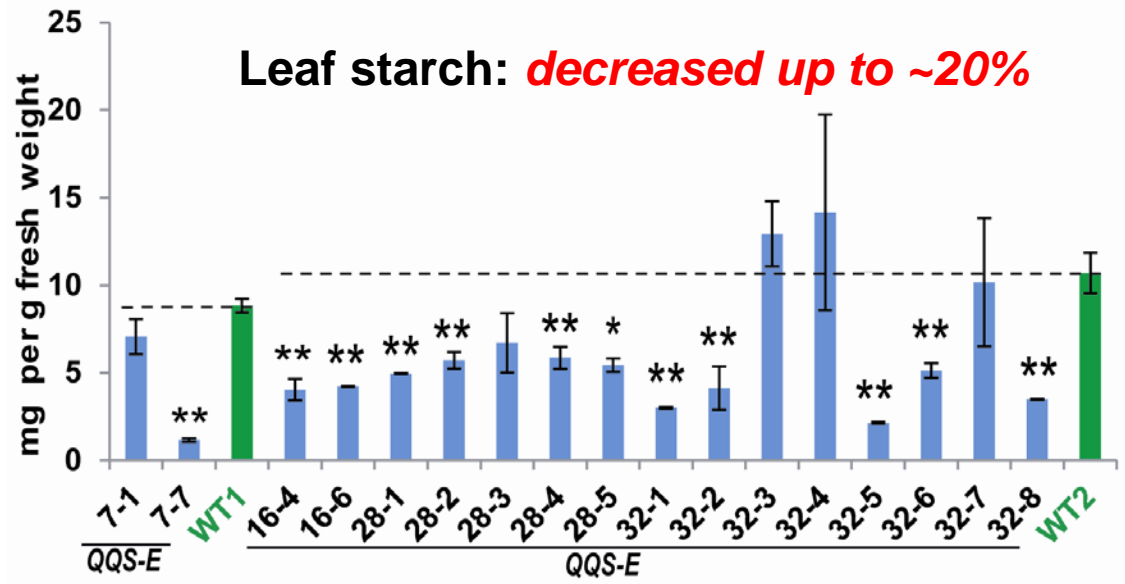
altered leaf starch content

altered leaf protein

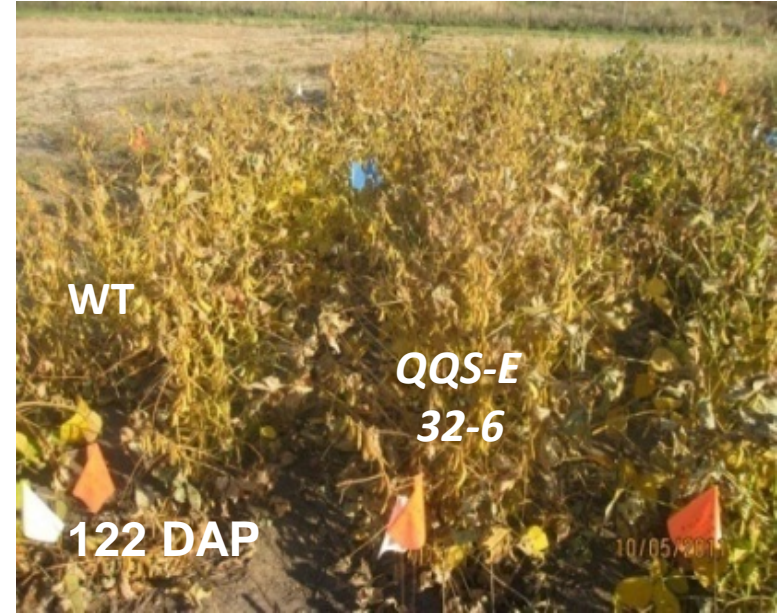
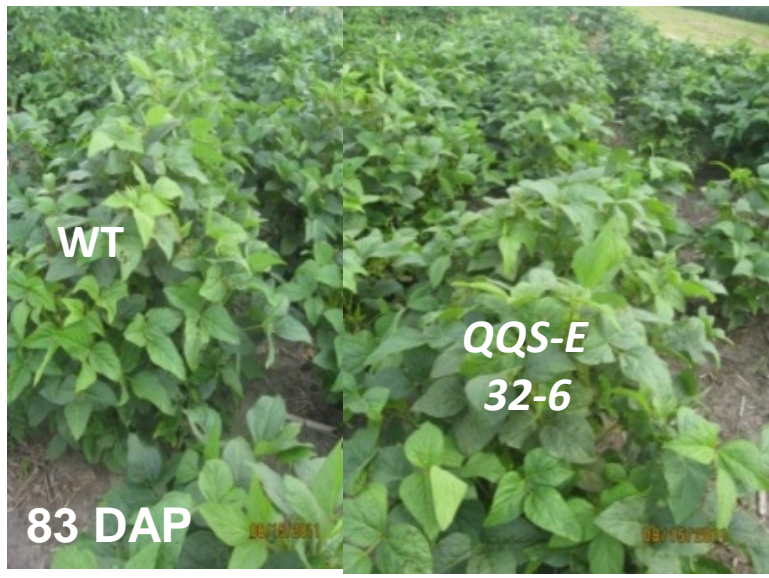
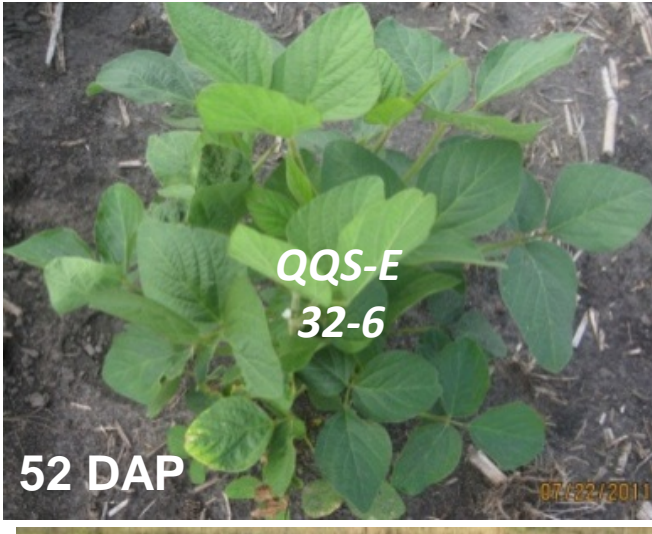


End of light, long day, mean ± sem, n=3, Student's t-test, *P < 0.05; **P < 0.01.

QQS-expressing soybeans have similar visual phenotype to WT but altered composition



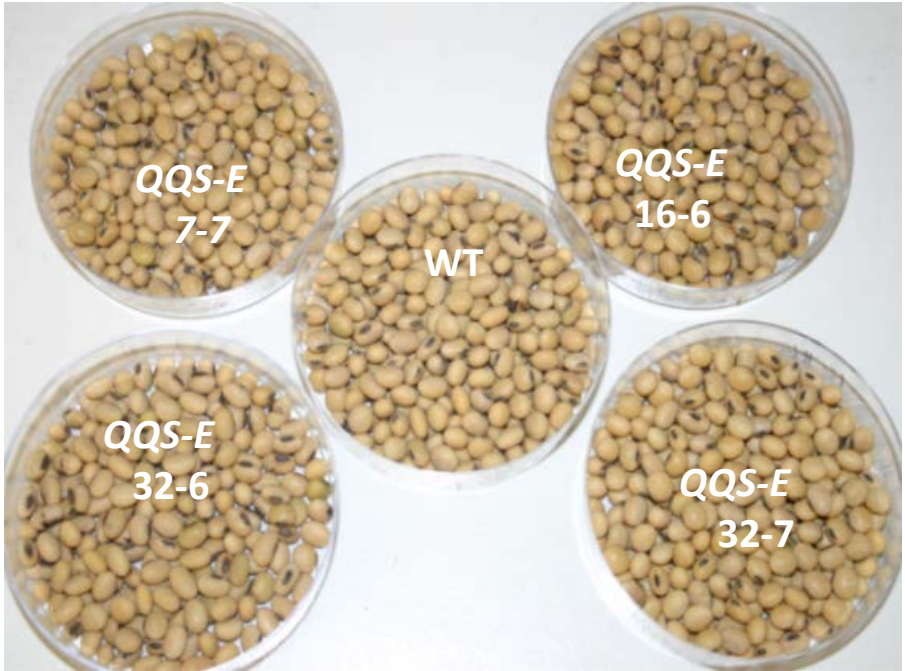
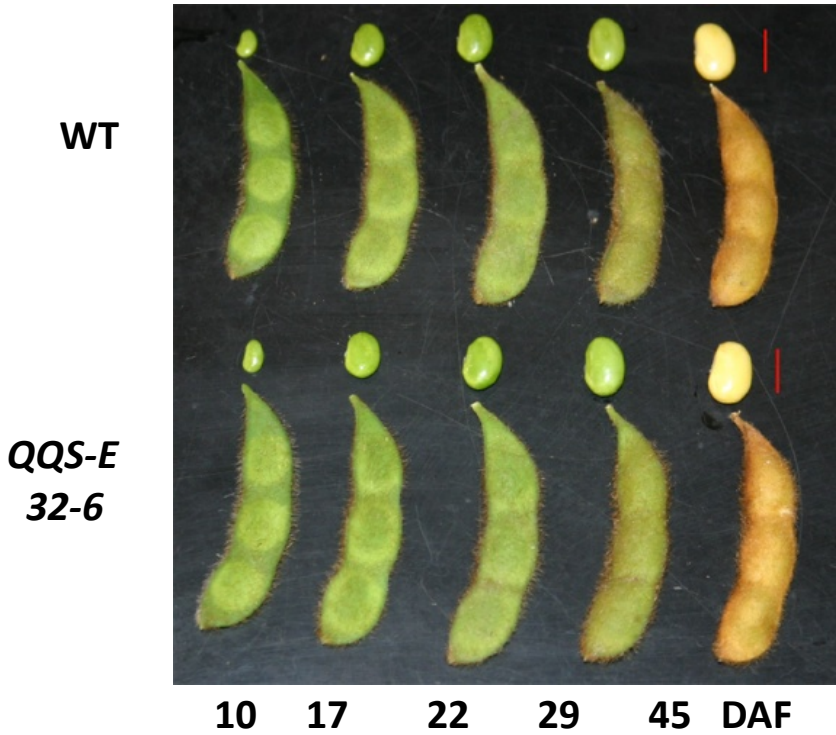
QQS-expressing soybeans have similar visual phenotype



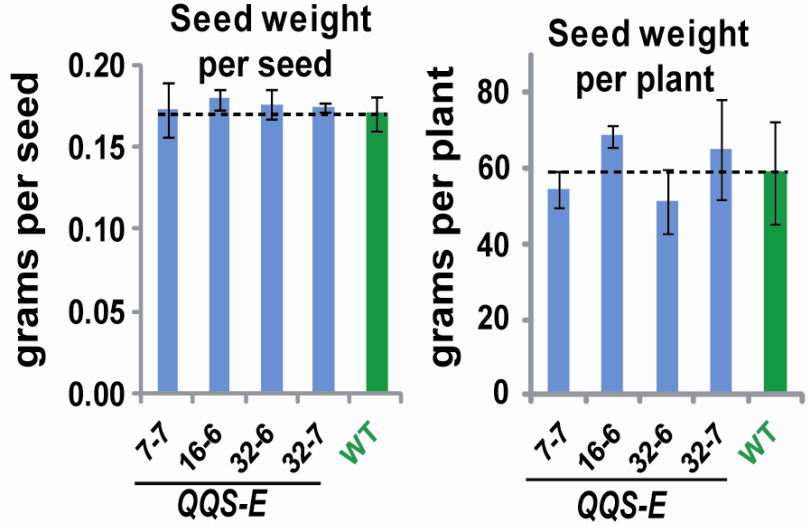
Field, T3 plants

Li and Wurtele, 2015, PBJ

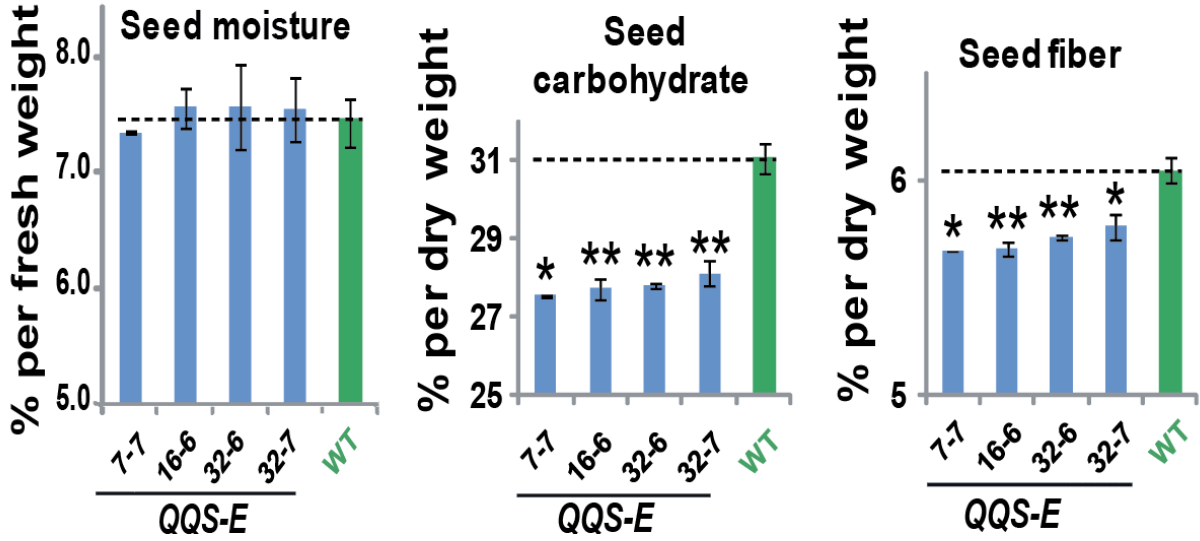
QQS-expressing soybeans have indistinguishable seed development, seed size and shape



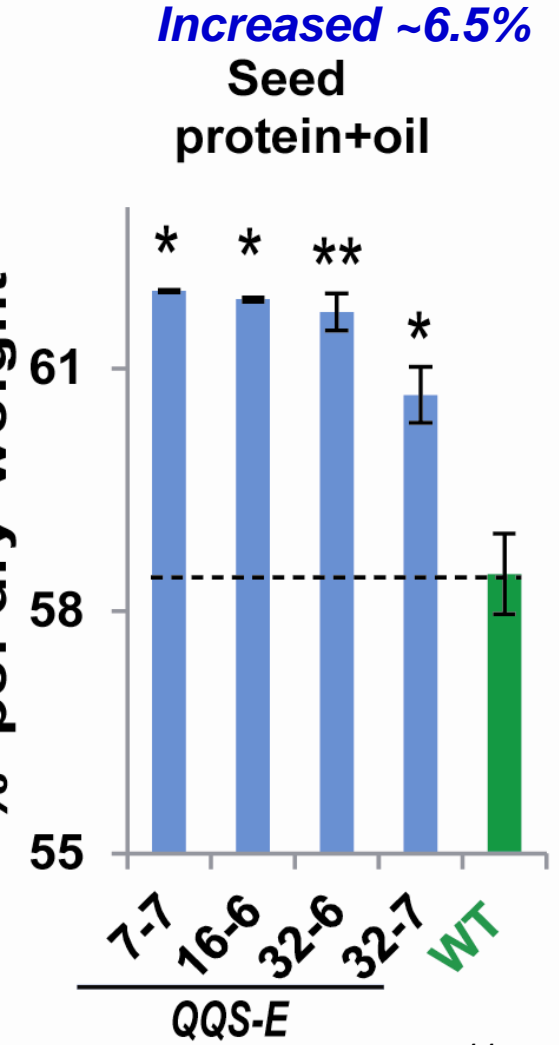
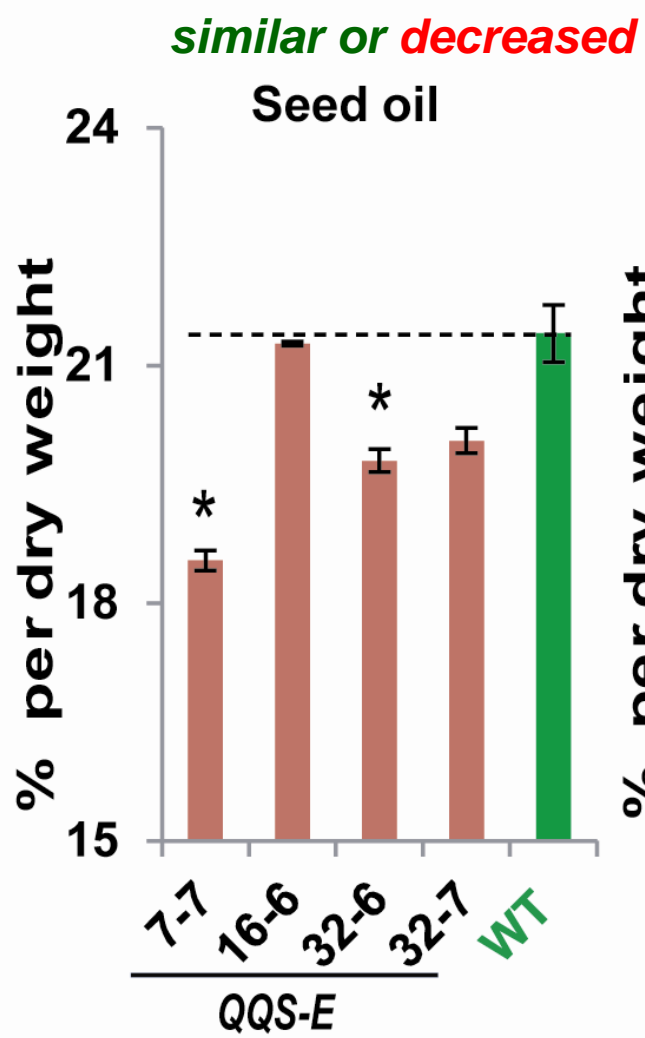
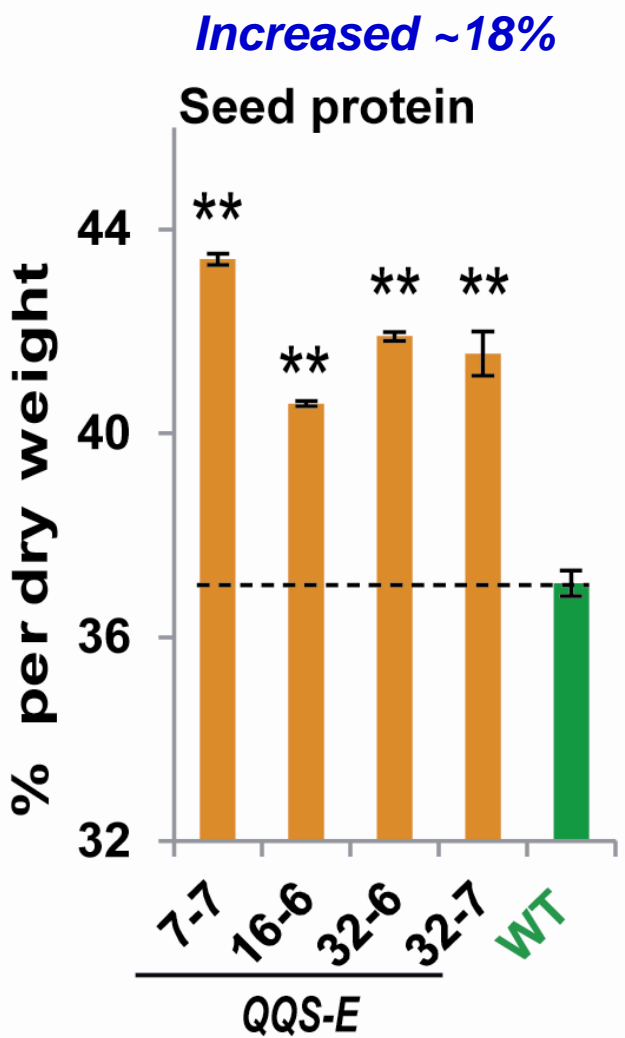
No obvious effect on seed yield



Decreased seed carbohydrate and fiber



Increased seed protein+oil



Free Lysine and Arginine are increased in seed



| | WT (% per dry weight) | QQS-E mutant ^{1,3} (% per dry weight) | Changes of QQS-E mutant vs WT (% relative to WT) ⁶ | P-value of t-test of QQS-E mutant vs WT |
|-----------------------------|-----------------------|---|---|---|
| Total protein | 39.30 ± 0.34 | 42.25 ± 0.83 | 8 | 0.03 |
| Glutamic acid (free) | 0.071 ± 0.007 | 0.051 ± 0.004 | -27.9 | 0.02 |
| Lysine (free) | 0.043 ± 0.006 | 0.054 ± 0.002 | 25.7 | 0.03 |
| Threonine (free) | 0.043 ± 0.012 | 0.044 ± 0.004 | 4.2 | 0.43 |
| Aspartic acid (free) | 0.039 ± 0.004 | 0.051 ± 0.005 | 31.1 | 0.03 |
| Arginine (free) | 0.039 ± 0.016 | 0.156 ± 0.062 | 301.5 | 0.04 |

Field, T4 seeds, from 3 transformation events, **Essential amino acids**

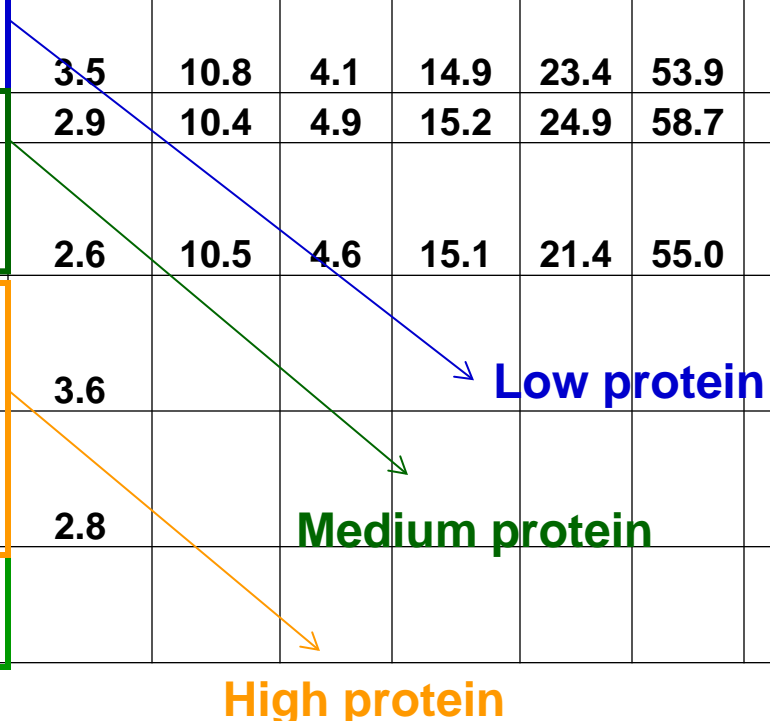
Almost all amino acids from hydrolyzed seed proteins are increased

| | Williams 82 (% per dry weight) | QQS-E mutant (% per dry weight) | Changes of QQS-E vs WT (% relative to WT) | P-value of t-test (QQS-E vs WT) |
|----------------------------------|--------------------------------|---------------------------------|---|---------------------------------|
| Total protein² | 39.39 ± 0.21 | 42.66 ± 0.54 | 8.3 | 0.000 |
| Glutamic Acid⁴ | 6.80 ± 0.07 | 7.35 ± 0.08 | 8.1 | 0.000 |
| Aspartic Acid | 4.39 ± 0.03 | 4.75 ± 0.06 | 8.3 | 0.000 |
| Leucine | 3.06 ± 0.03 | 3.25 ± 0.03 | 6.4 | 0.000 |
| Arginine | 2.82 ± 0.02 | 3.19 ± 0.08 | 12.9 | 0.001 |
| Lysine | 2.56 ± 0.02 | 2.75 ± 0.03 | 7.1 | 0.000 |
| Phenylalanine | 1.98 ± 0.02 | 2.08 ± 0.03 | 5.4 | 0.004 |
| Valine | 1.97 ± 0.03 | 2.10 ± 0.03 | 6.4 | 0.008 |
| Isoleucine | 1.88 ± 0.02 | 1.98 ± 0.03 | 5.7 | 0.008 |
| Proline | 1.85 ± 0.02 | 2.02 ± 0.02 | 9.3 | 0.000 |
| Serine | 1.78 ± 0.07 | 1.95 ± 0.04 | 9.2 | 0.044 |
| Alanine | 1.70 ± 0.02 | 1.81 ± 0.02 | 6.8 | 0.000 |
| Glycine | 1.68 ± 0.01 | 1.80 ± 0.02 | 7.2 | 0.000 |
| Threonine | 1.50 ± 0.02 | 1.61 ± 0.02 | 7.6 | 0.001 |
| Tyrosine | 1.47 ± 0.02 | 1.53 ± 0.02 | 4.4 | 0.027 |
| Histidine | 1.03 ± 0.01 | 1.12 ± 0.01 | 8.2 | 0.000 |
| Methionine | 0.55 ± 0.00 | 0.58 ± 0.01 | 6.0 | 0.000 |
| Cysteine | 0.54 ± 0.01 | 0.58 ± 0.01 | 7.6 | 0.004 |
| Tryptophan | 0.46 ± 0.05 | 0.51 ± 0.01 | 10.5 | 0.179 |
| Hydroxyproline | 0.0638 ± 0.003 | 0.0643 ± 0.005 | 0.7 | 0.471 |
| Taurine | 0.057 ± 0.004 | 0.055 ± 0.004 | -4.8 | 0.329 |
| Ornithine | 0.026 ± 0.003 | 0.028 ± 0.002 | 9.0 | 0.244 |
| Hydroxylysine | 0.023 ± 0.004 | 0.028 ± 0.003 | 18.9 | 0.206 |



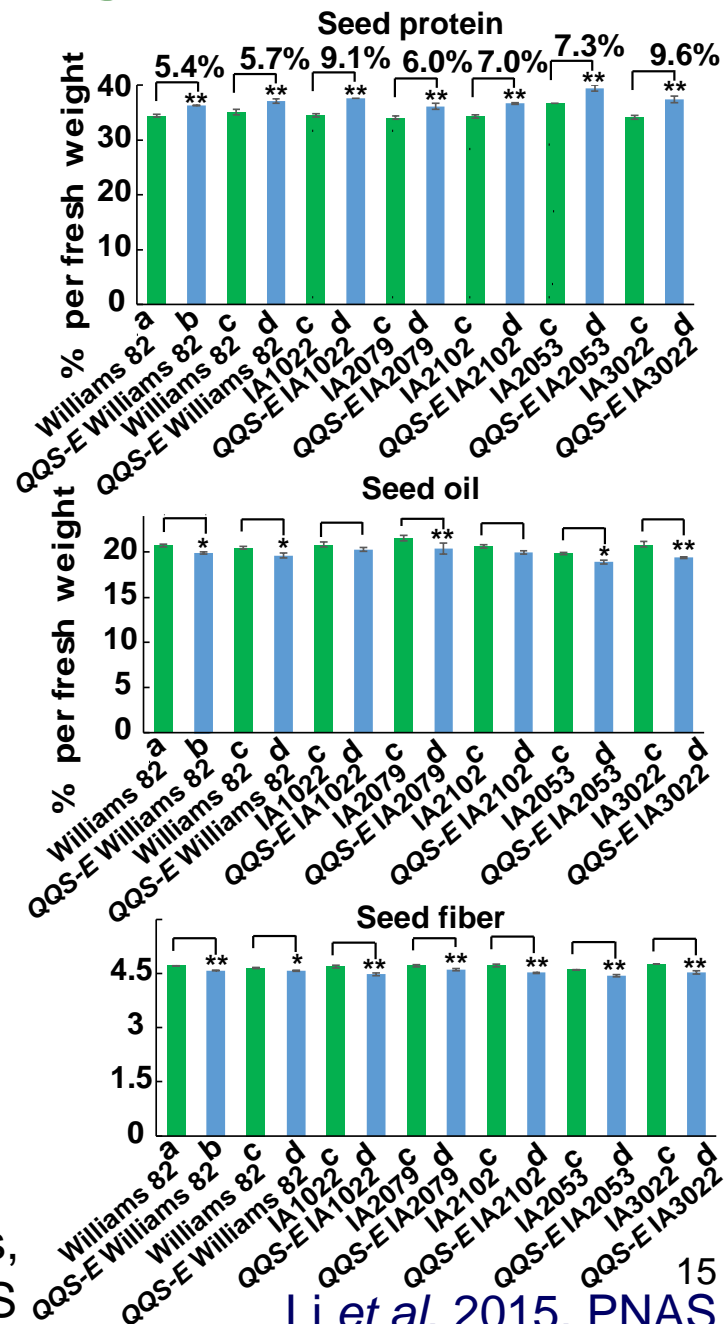
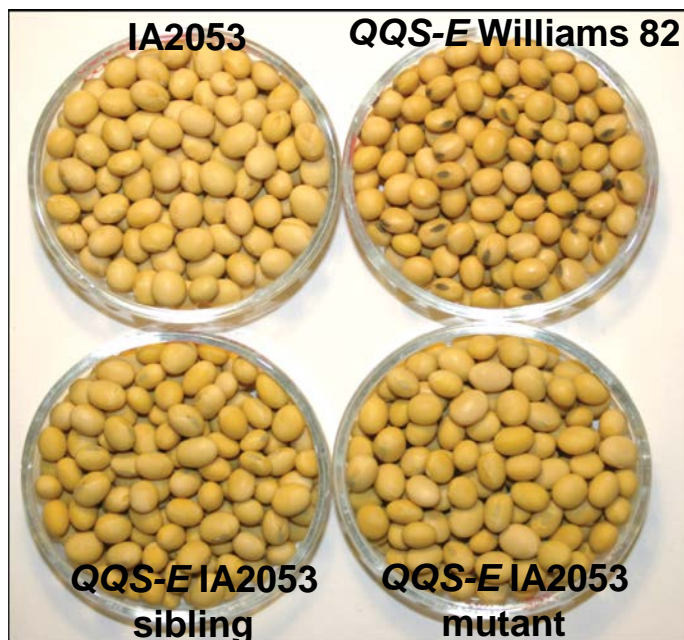
Does QQS increase seed protein in cultivars with different protein levels?

| Entry | Yield bu/a | Height inches | Seed weight | | Protein (NIRS) % | Oil % | Chlorosi s score | Palmiti c % | Steari c % | Palmiti c + Stearic % | Oleic % | Linolei c % | Linoleni c % | Character |
|-------------|---------------|------------------|-------------|------------|------------------------|----------|------------------------|-------------------|------------------|--------------------------------|------------|-------------------|--------------------|-----------------------------|
| | | | mg/s d | sds/ lb | | | | | | | | | | |
| IA1022* | 63.9 | 34 | 143 | 3180 | 31.5 | 20.5 | 3.5 | 10.8 | 4.1 | 14.9 | 23.4 | 53.9 | 7.8 | SCN resistant, yellow hilum |
| IA2079* | 59.9 | 38 | 144 | 3140 | 34.2 | 18.4 | 2.9 | 10.4 | 4.9 | 15.2 | 24.9 | 58.7 | 1.2 | 1% linolenic |
| IA2102* | 69.4 | 40 | 144 | 3140 | 34.3 | 18.5 | 2.6 | 10.5 | 4.6 | 15.1 | 21.4 | 55.0 | 8.5 | Commodity, yellow hilum |
| IA2053* | 54.4 | 40 | 195 | 2330 | 37.3 | 17.8 | 3.6 | | | | | | | Large seed & high protein |
| IA3022* | 52.7 | 38 | 204 | 2230 | 38.5 | 17.3 | 2.8 | | | | | | | Large seed & high protein |
| Williams 82 | | | | | 34 | 18.5 | | | | | | | | |



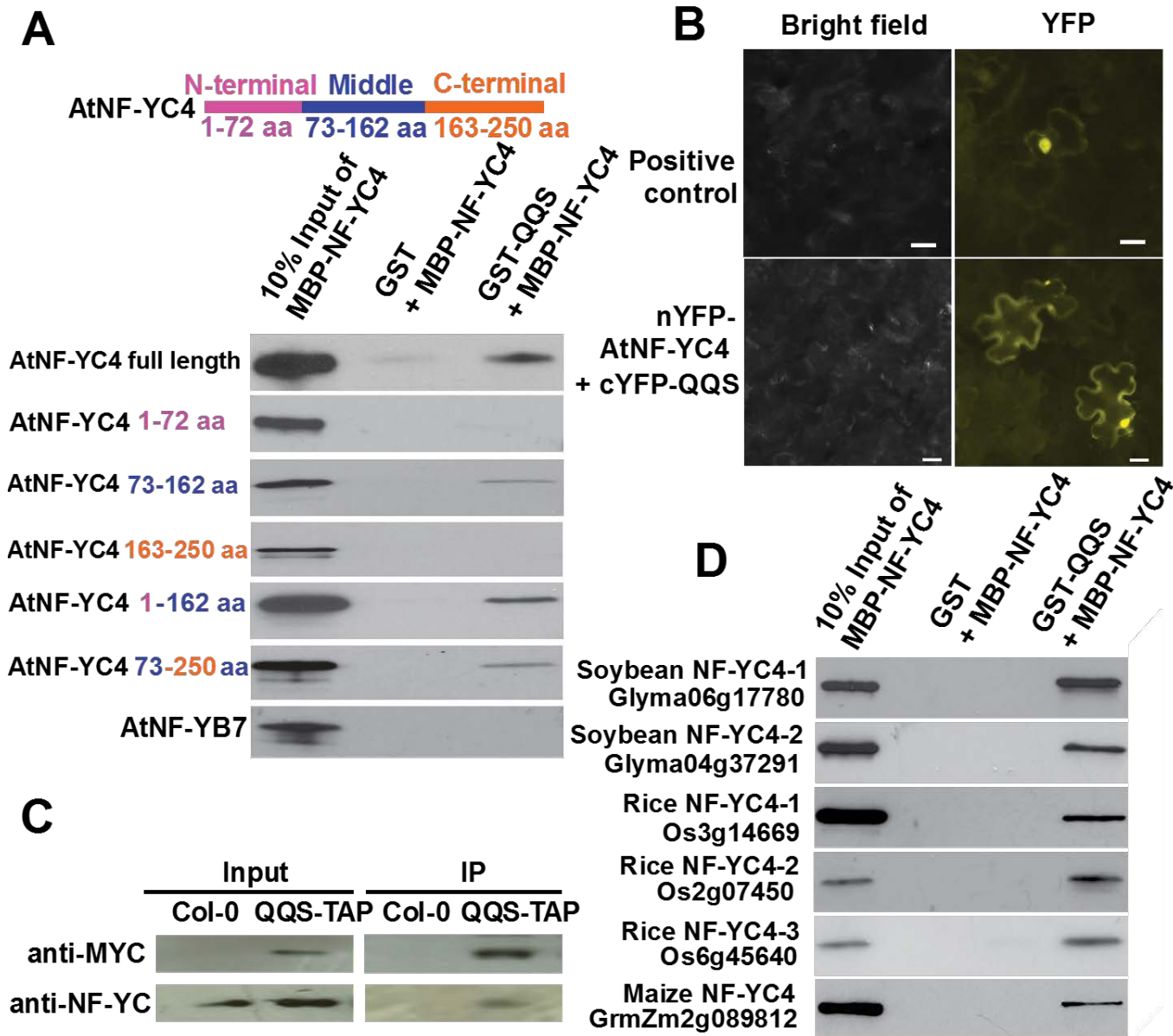
Data from Dr.Fehr, field, 2011

QQS increases soybean seed protein regardless of initial protein composition



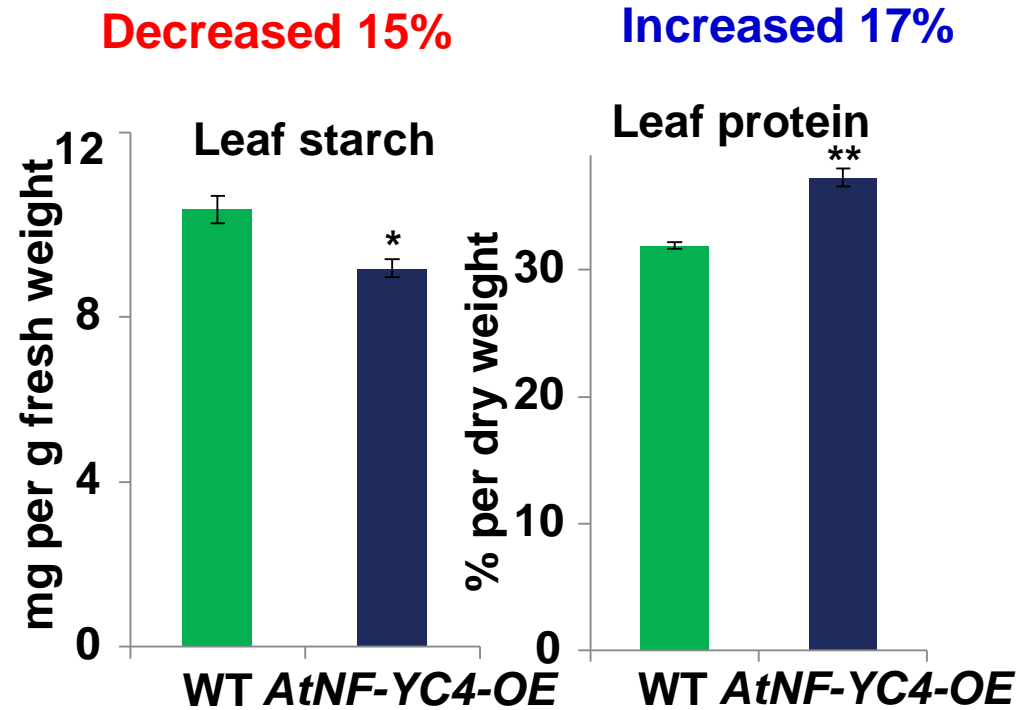
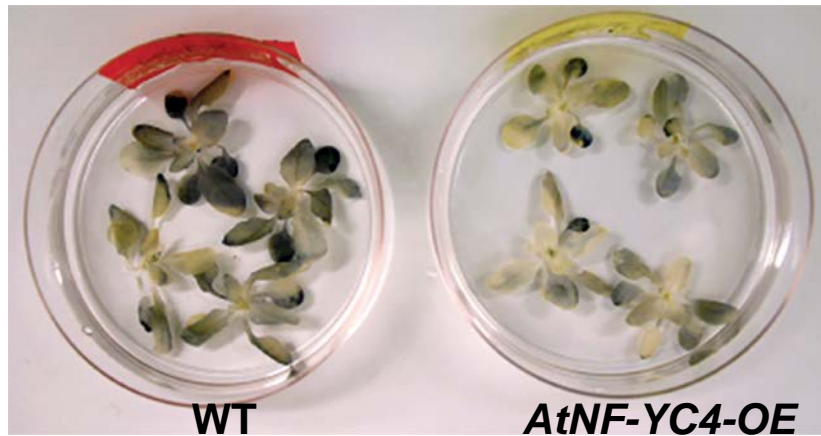
Field, F2 plants,
F3 seeds, NIRS

What does QQS interact with?



NF-YC:
widely conserved transcription factor

Overexpressing *NF-YC4* in *Arabidopsis* decreases leaf starch; increases leaf protein



Growth chamber, T2 plants, from 3 transformation events

Li *et al*, 2015, PNAS

Soybean *NF-YC4-OE* seeds (Williams 82) have increased seed protein and protein+oil, decreased seed fibers and similar yield per plant



Seed protein:
Increased 6~12%

Seed oil:
Similar or *decreased*

Seed Protein+oil:
Increased 5~7%

Seed fiber:
Decreased 3.4-5.6%

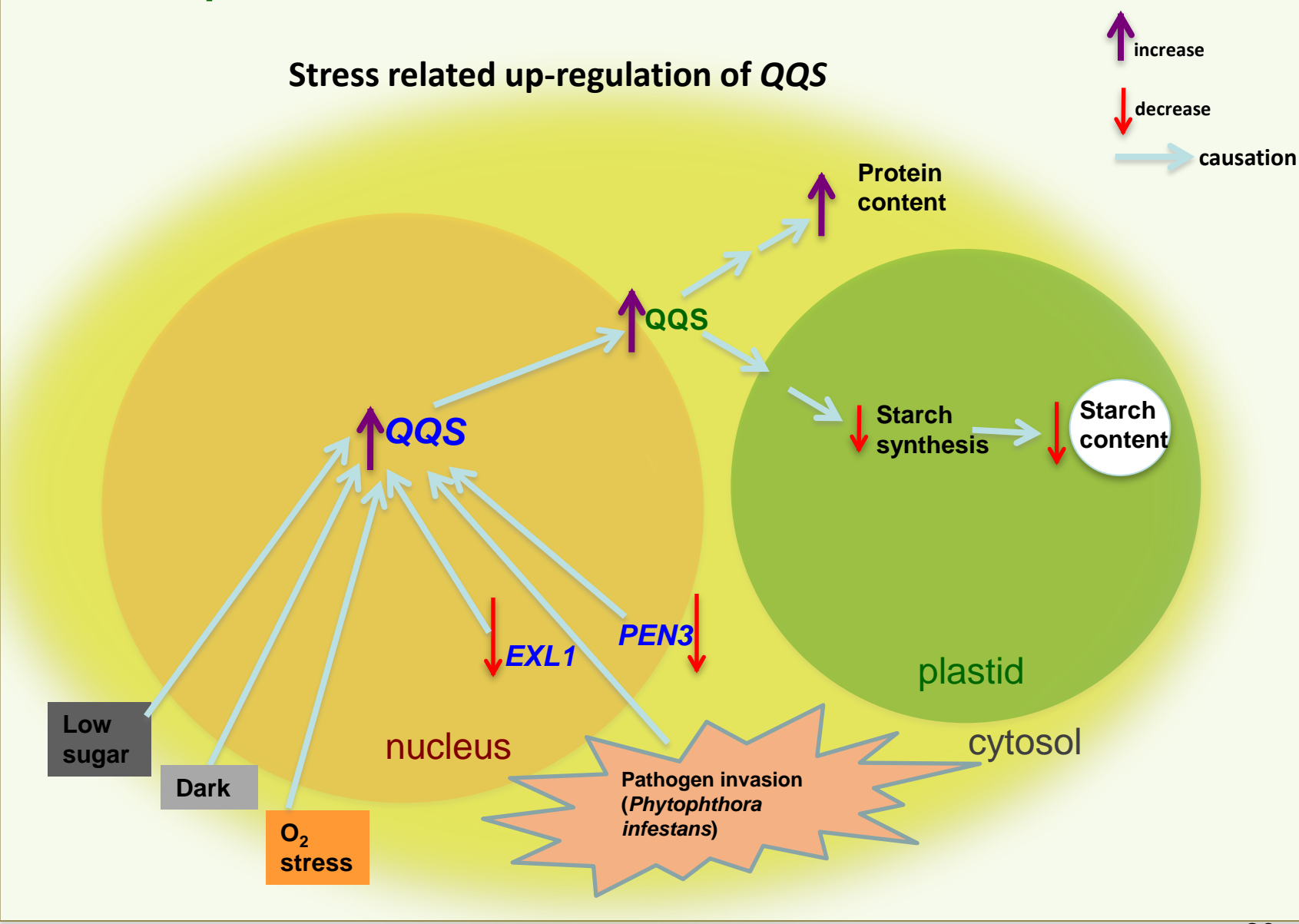
Seed weight per plant:
Similar

Non-transgenic approach to increase the soybean protein content

Soybean NF-YC4 expression is up-regulated when some promoter region is deleted.

Targeted mutagenesis, via TAL effector nucleases (TALEN) or CRISPER/Cas9 technologies, to increase soybean protein.

QQS may act to mediate cross talk between primary metabolism and environmental perturbations



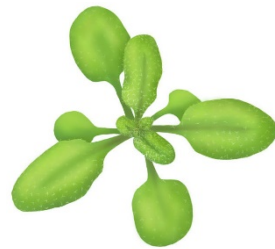
Both foci number and focus size of viral infection are decreased in transgenic Arabidopsis mutants overexpressing genes in NFY-related network

Foci number:

Decreased up to 88% in OE mutants

Foci size:

Decreased up to 51% in OE mutants



Turnip mosaic virus carrying GFP: TuMV-GFP,
120 hr after infection, growth chamber

Drs. Wenguang Zheng, 21
Mingsheng Qi, Steve Whitham

Bacterial growth in planta is similarly decreased in transgenic soybeans (Williams 82) of GmNFY-R-G2-OE



Soybean Cyst nematode female count numbers were decreased in transgenic soybeans of GmNFY-R-G2-OE (Williams 82)

Aphid numbers were decreased soybean mutants of GmNFY-R-G2-OE (Williams 82)

Transgenic and non-transgenic approach to reduce soybean susceptibility to pathogens and pests

Molecular tool of both Transgenic and Non-transgenic approaches

- Normal growth and development,**
- Increase soybean seed protein,**
- Reduce soybean susceptibility to pathogens and pests,**
- Increase sustainability.**

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