



Rodens Dag – onsdag d. 26. august 2020

Kan vilde planter blive fremtidens flerårige afgrøder?

Michael Broberg Palmgren
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Hvedegræs' lange flerårige rødder:

- Lager kulf i jorden
- Mindsker jorderosion
- Beskytter mod tørke
- Mindsker udvaskning af næringsstoffer

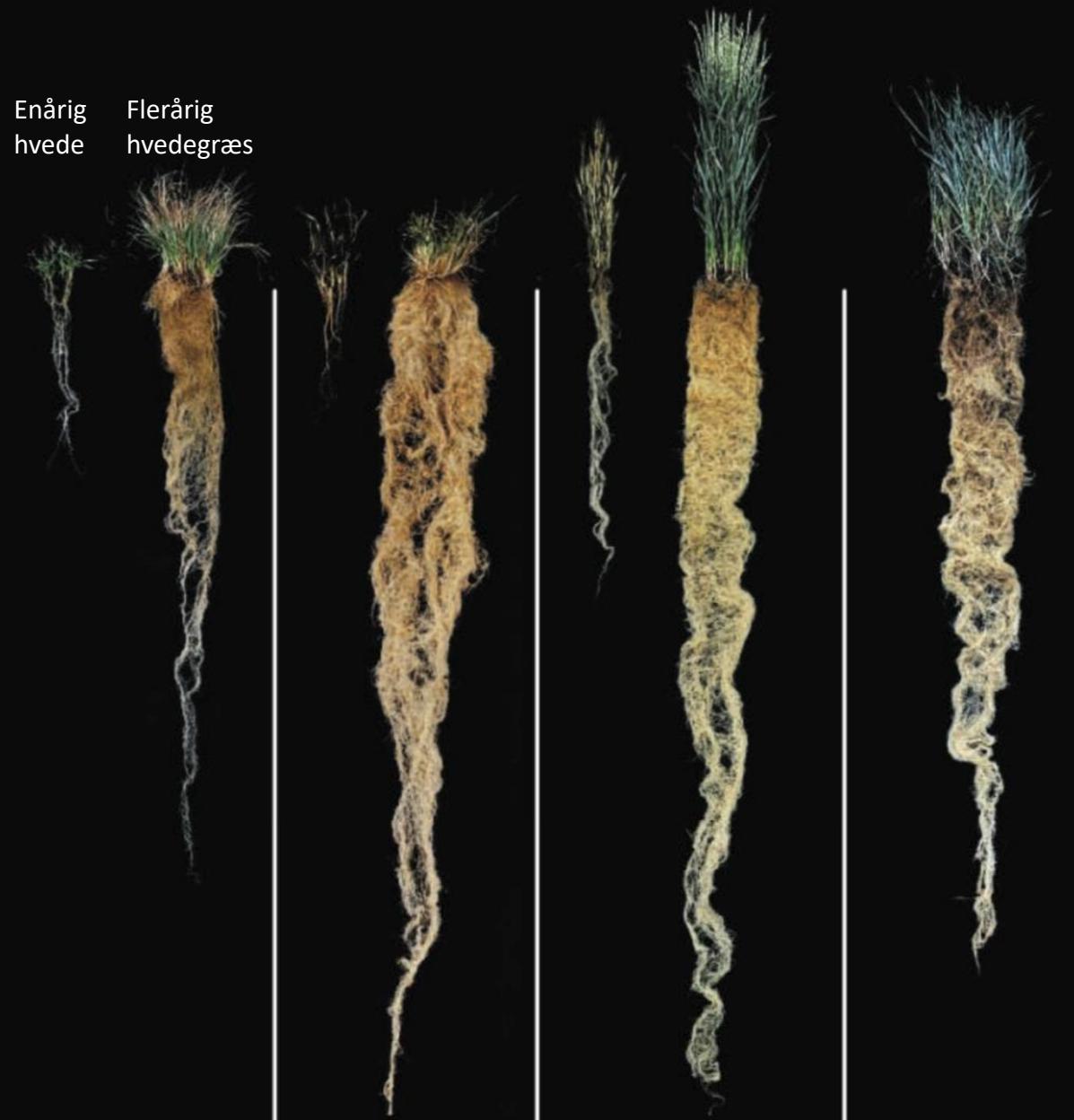


Figure 1. Annual wheat versus perennial intermediate wheatgrass (*Thinopyrum intermedium*). A seasonal comparison of annual winter wheat (left of each panel) and intermediate wheatgrass, its wild perennial cousin (right of each panel). From ref. [13]

Problem:

Hvedegræs er endnu langt fra fuldt forædlet.

Løsning:

Hvordan kan vi fremskynde forædlingsprocessen så den ikke tager urimeligt lang tid?

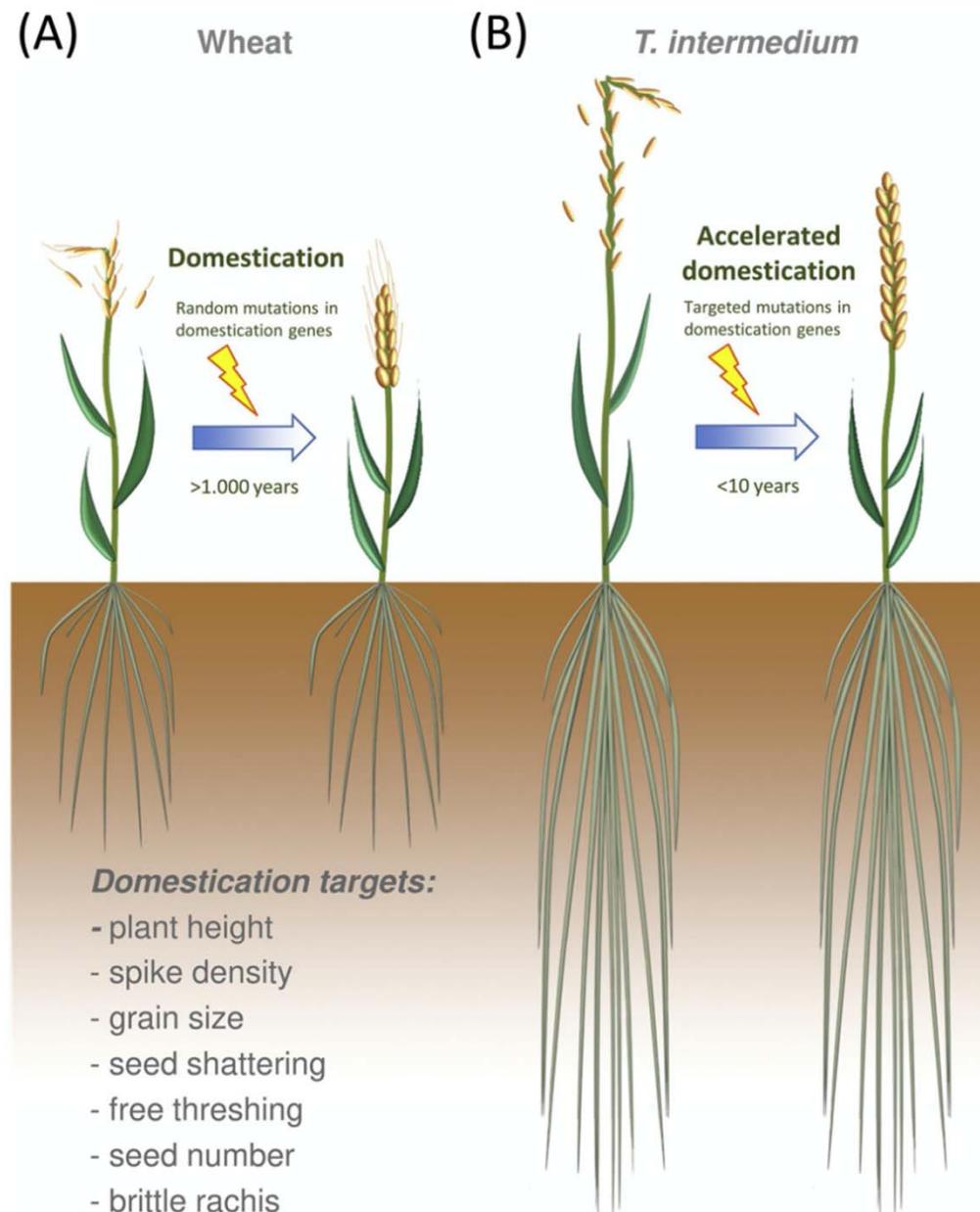


Figure 3. Domestication targets in perennial *T. intermedium*. Targeted screening for mutations in domestication genes is likely to accelerate domestication of *T. intermedium* (B), a relative to wheat, which is already domesticated (A). From ref. [14].

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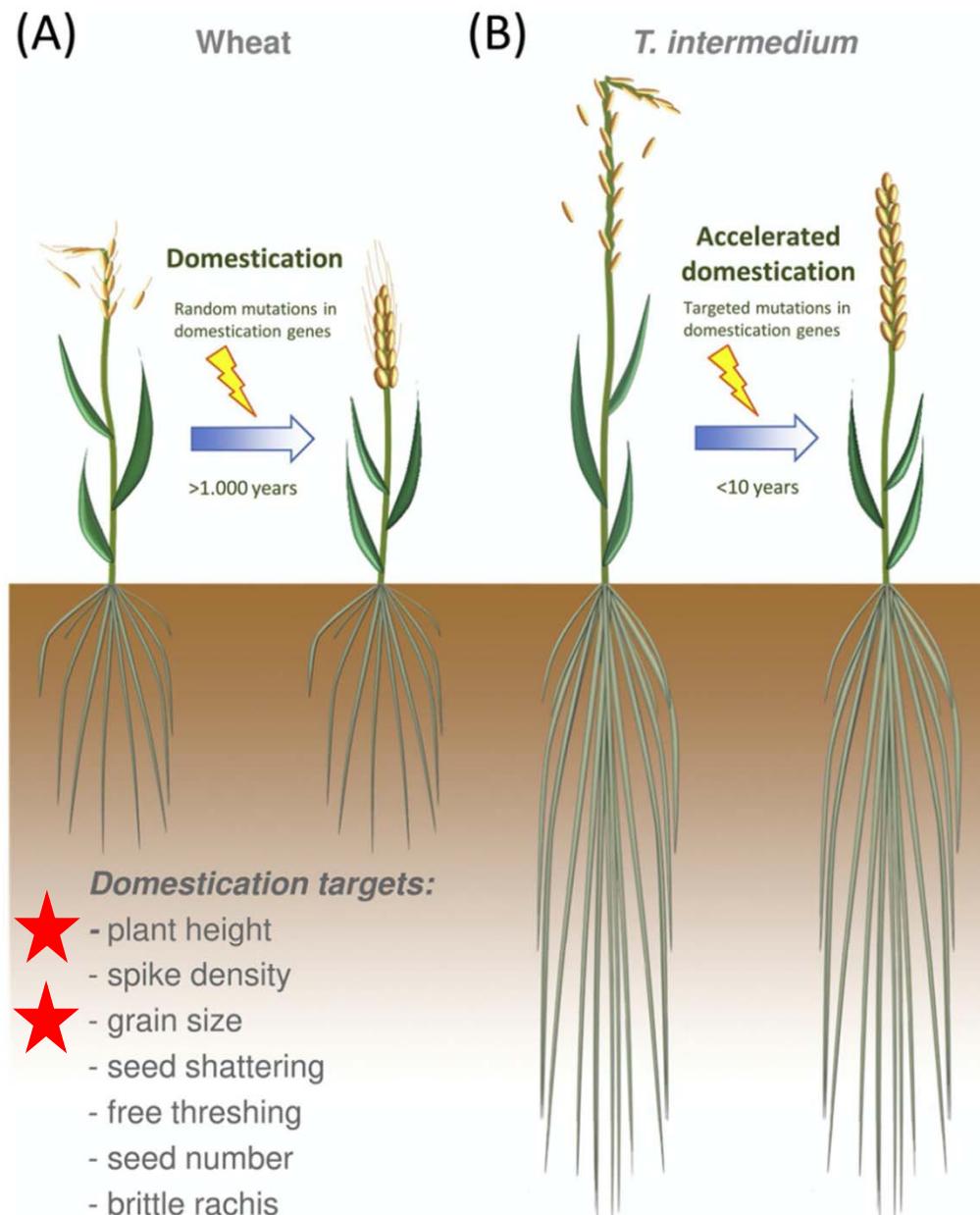


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Større kerner



Kernerne hos hvedegræs er betydeligt mindre end hvedens – men hvedens kerner var også små i jordbrugets barndom

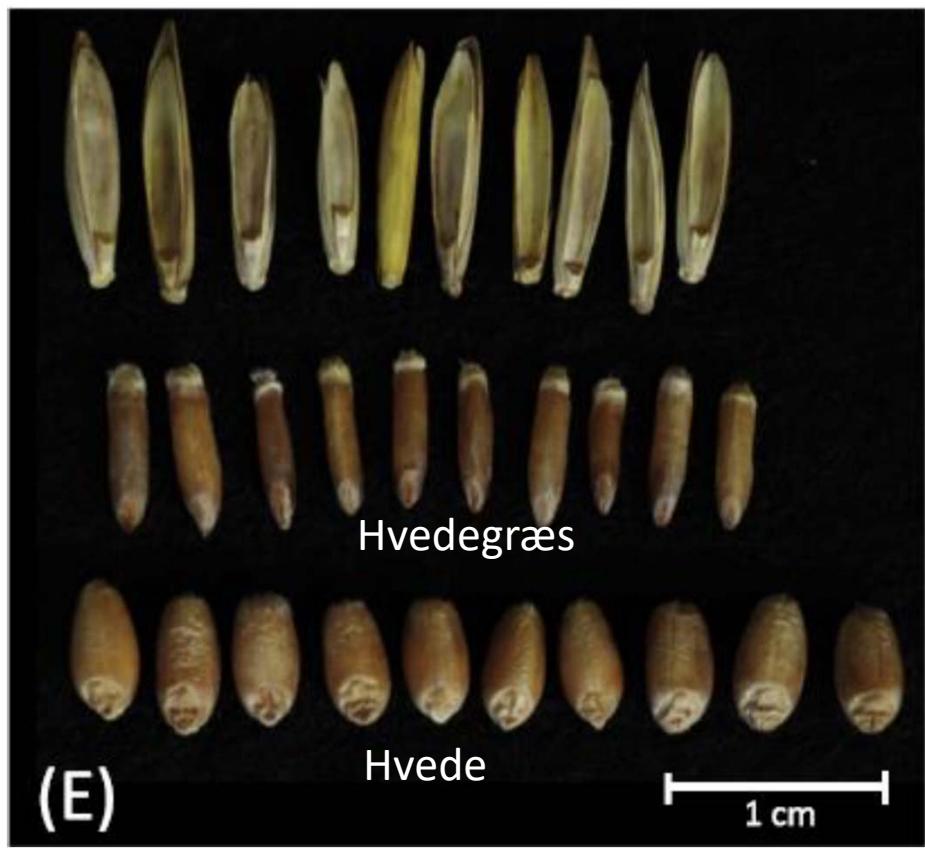


Figure 2. Some challenging traits in perennial *T. intermedium*. Perennial *T. intermedium* (D, left; E, top two rows), a close relative to annual wheat, which is already domesticated (D, right; E, bottom row), suffers from small seeds that shatters during maturation. From ref. [14].

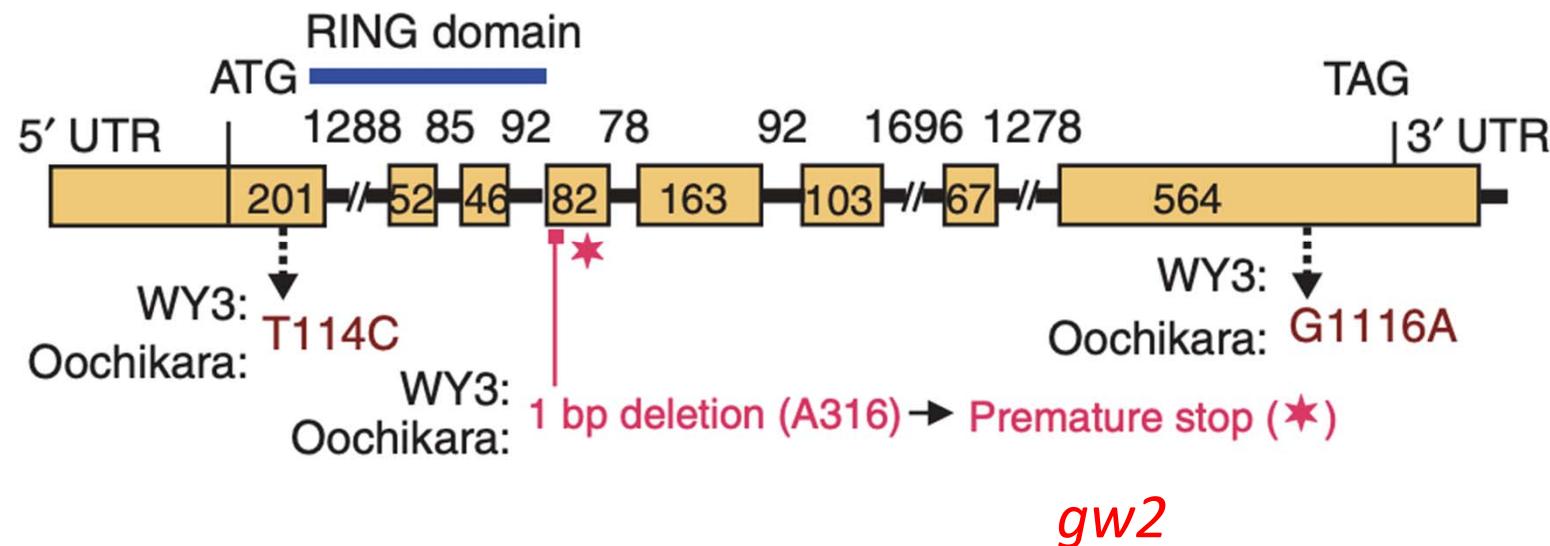
Grain Weight 2 (GW2)

A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase

Xian-Jun Song^{1,2}, Wei Huang^{1,2}, Min Shi¹, Mei-Zhen Zhu¹ & Hong-Xuan Lin¹

Grain weight is one of the most important components of grain yield and is controlled by quantitative trait loci (QTLs) derived from natural variations in crops. However, the molecular roles of QTLs in the regulation of grain weight have not been fully elucidated. Here, we report the cloning and characterization of *GW2*, a new QTL that controls rice grain width and weight. Our data show that *GW2* encodes a previously unknown RING-type protein with E3 ubiquitin ligase activity, which is known to function in the degradation by the ubiquitin-proteasome pathway. Loss of *GW2* function increased cell numbers, resulting in a larger (wider) spikelet hull, and it accelerated the grain milk filling rate, resulting in enhanced grain width, weight and yield. Our results suggest that *GW2* negatively regulates cell division by targeting its substrate(s) to proteasomes for regulated proteolysis. The functional characterization of *GW2* provides insight into the mechanism of seed development and is a potential tool for improving grain yield in crops.

Grain Weight 2 (GW2)



Store kerner hos ris er bl.a. et resultat af en mutation i genet *GW2*.

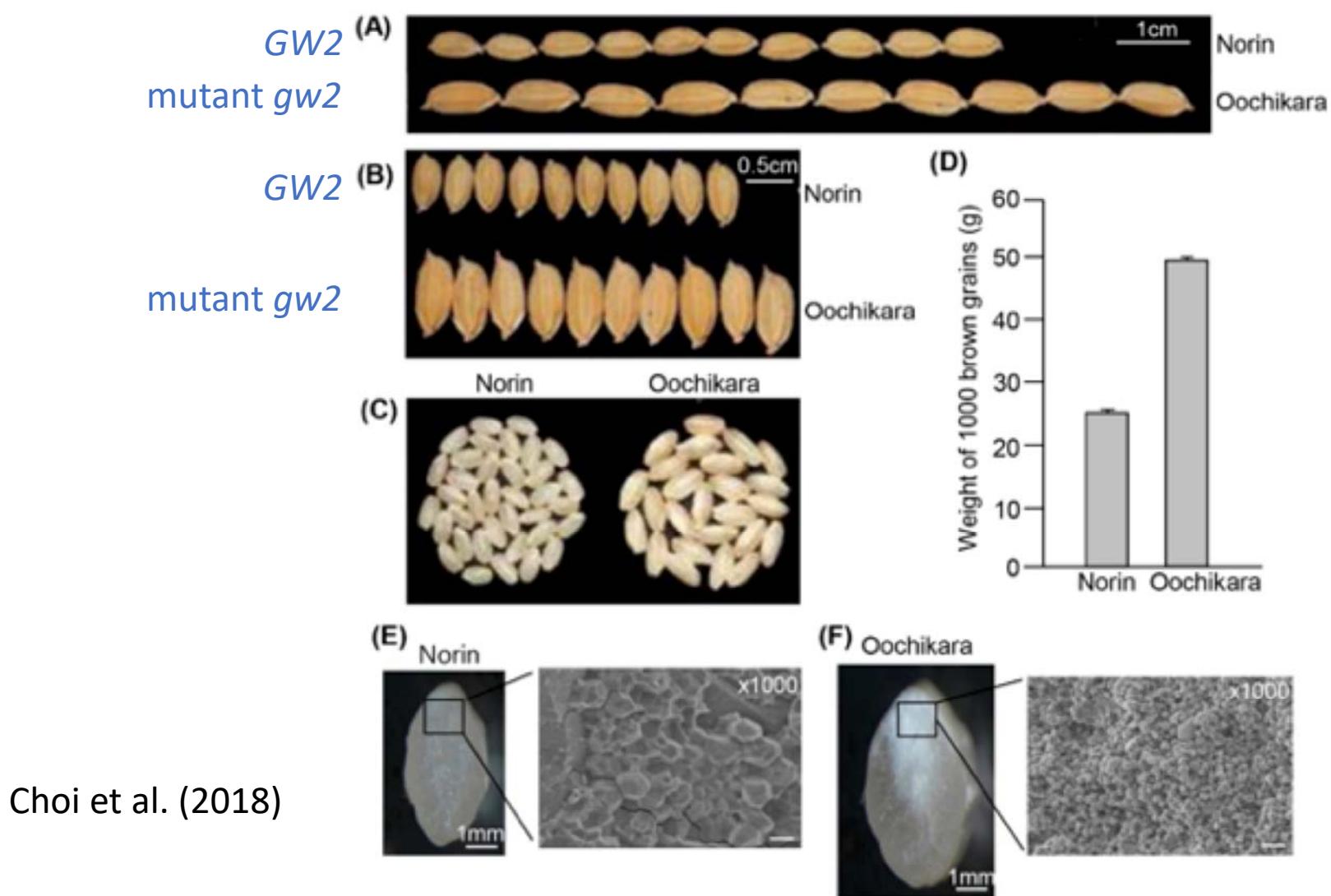
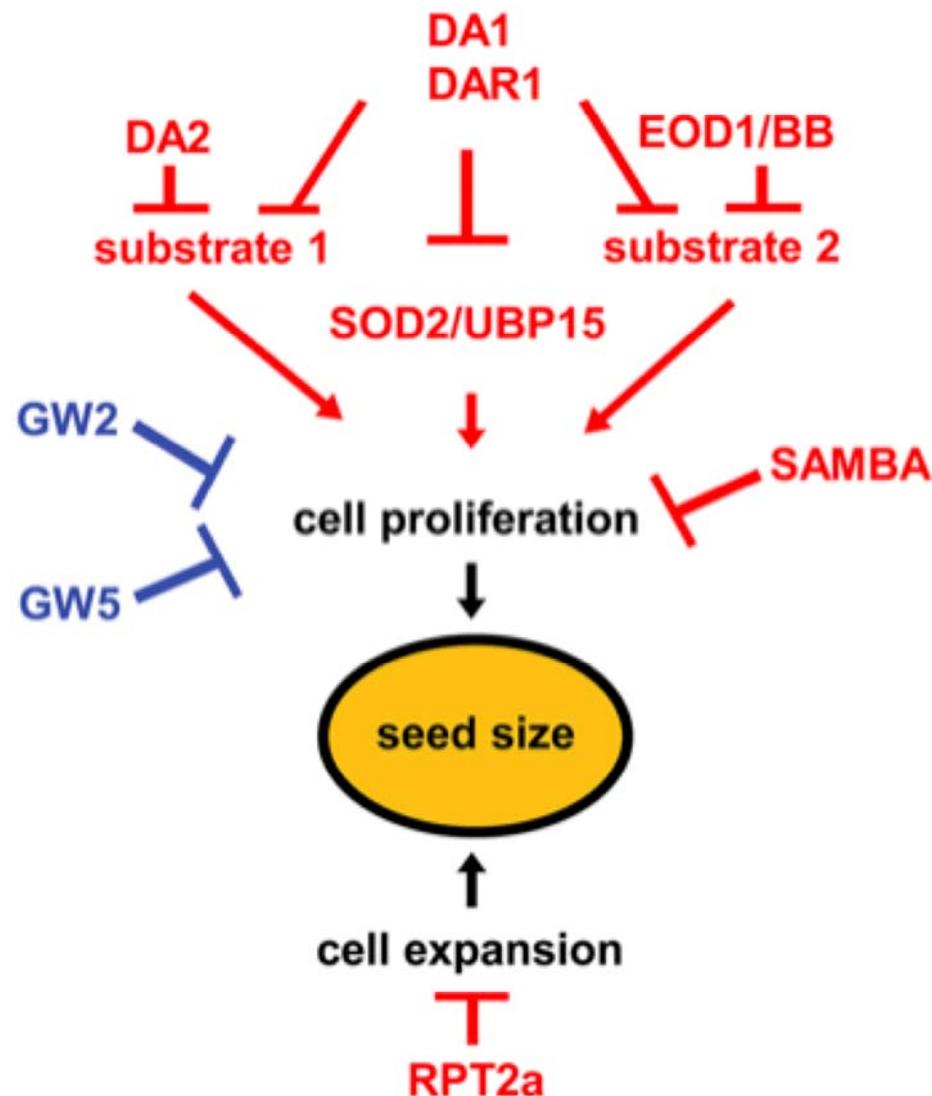


Figure 1. Phenotypic analysis of wild-type and *gw2* mutant grains. (A–C) Grain phenotypes of Norin and Oochikara. Grain length (A) and width (B) of ten seeds of Norin and Oochikara. Brown grains from which husks were removed (C); (D) Weight of 1000 brown grains of Norin and Oochikara. Error bars indicate standard deviations ($n = 3$); Palea and lemma of the grains of Norin (E) and Oochikara (F) were removed. The seeds were hand-sectioned with a razor blade transversely and then analyzed by

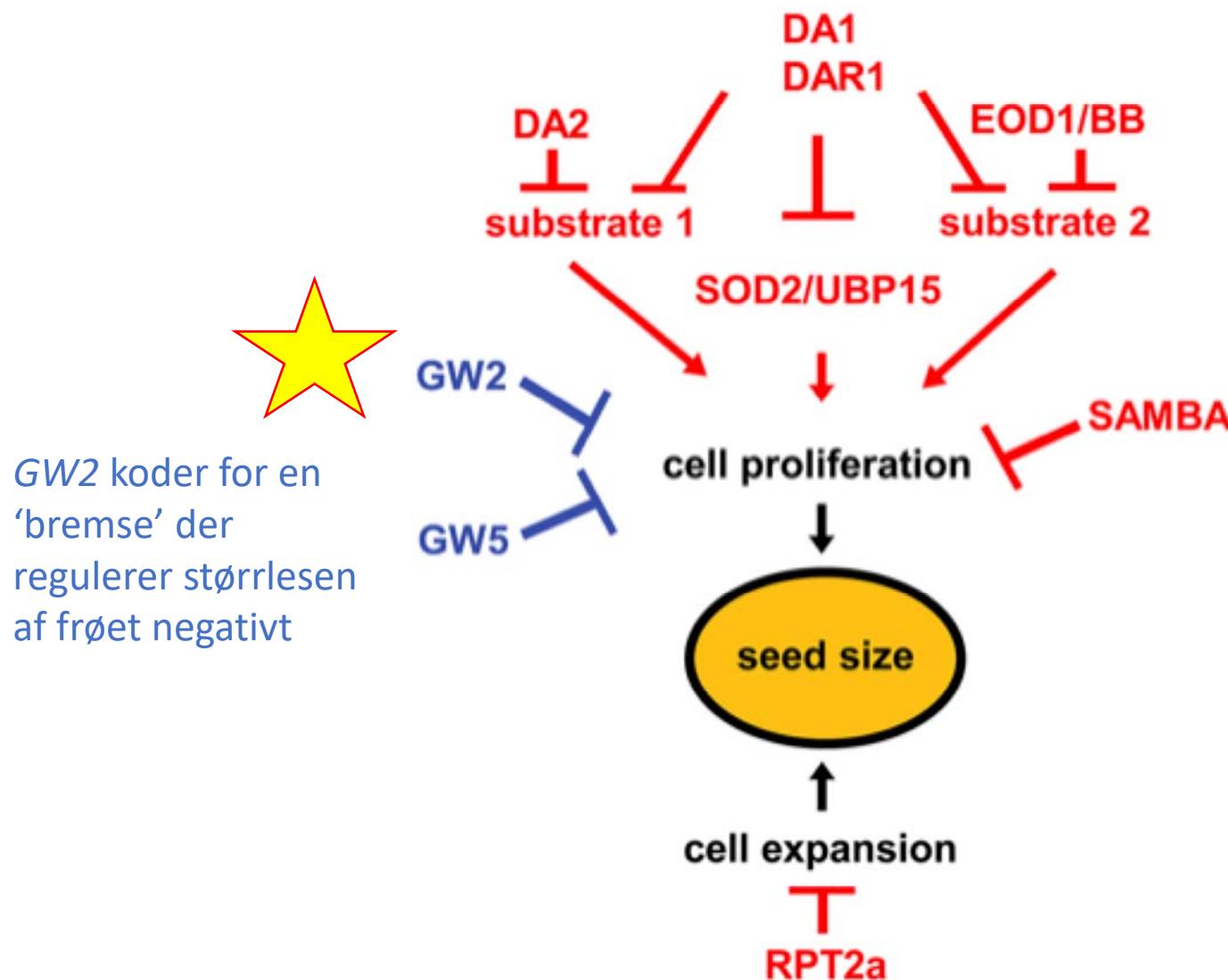
Ubiquitin-mediated control of seed size in plants.

Li N, et al. Front Plant Sci. 2014.



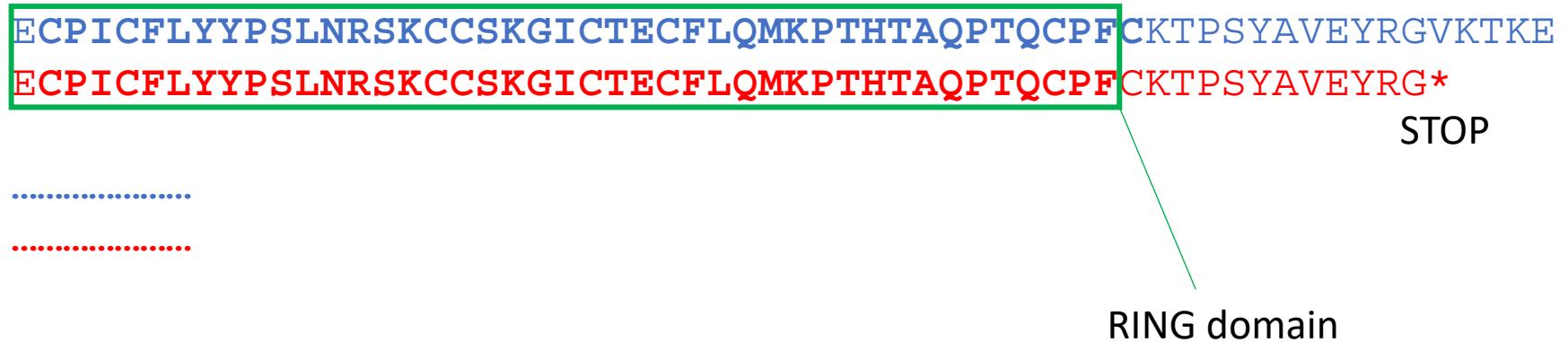
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>Ris GW2 (små kerner)

>Ris gw2 mutant (store kerner)



gw2 mutantgenet koder for et 'bremse'protein der er betydeligt kortere end GW2 og sandsynligvis mindre effektivt (men sandsynligvis stadig aktivt til en vis grad).

Hvedegræs koder for 3 proteiner der er næsten identiske med GW2 fra hvede

Table 1. Candidate Domestication Genes That Align with Intermediate Wheatgrass QTLs

					Gennavn i hvedegræs	% lighed med GW2	
Grain size	<i>GASR7</i>	<i>Gibberellic Acid-Stimulated Regulator</i>	Wheat (7)	7	Thint.19G0343500 Thint.20G0544200 Thint.21G0194900	94,95 98,02 93,68	[57]
Grain size	<i>GW2</i>	<i>Grain Width chr2</i>	Wheat (6D)	6	Thint.16G0144700 Thint.17G0207800 Thint.18G0258700	98,11 97,88 98,59	[60,61,85–87]
Grain size	<i>GW5 (qSW5)</i>	<i>Grain weight QTL chr5</i>	Rice (5)	1	Thint.02G0278200 Thint.03G0309500 Thint.01G0175600	75,52 74,12 75,10	[59]

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>Hvedegræs Thint.16G0144700.1.p
>Ris GW2 (små kerner)
>Ris gw2 mutant (store kerner)

ECPICTFLYYPSLNRSKCCSKGICTECFLQMKPTHTARPTQCPFCKTPNYAVEYRGVKTKE
ECPICTFLYYPSLNRSKCCSKGICTECFLQMKPTHTAQPTQCPFCKTPSYAVEYRGVKTKE
ECPICTFLYYPSLNRSKCCSKGICTECFLQMKPTHTAQPTQCPFCKTPSYAVEYRG*

RING domain

>Hvedegræs Thint.16G0144700.1.p
>Ris GW2 (små kerner)
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ECPICFLYYPSLNRSKCCSKGICTECFLQMKPTHTARPTQCPFCKTPNYAVEYRGVKTKE
ECPICFLYYPSLNRSKCCSKGICTECFLQMKPTHTAQPTQCPFCKTPSYAVEYRGVKTKE
ECPICFLYYPSLNRSKCCSKGICTECFLQMKPTHTAQPTQCPFCKTPSYAVEYRG*

RING domain

Vi skal finde en hvedegræs med en mutation der får GW2 proteinerne til at blive kortere – helst lige hertil!



Reduced height (Rht)

The Harvesters (1565)
Pieter Bruegel the Elder





Når kernerne bliver store slås kornet let ned af vejr og vind



Den grønne revolution i 1960'erne baserede sig på nye 'dværg'sorter af hvede og ris:
Stænglen var kortere, men akset var uforandret

Oprindelig sort

Reduced height (Rht)
varietet

Published: 15 July 1999

'Green revolution' genes encode mutant gibberellin response modulators

Jinrong Peng, Donald E. Richards, Nigel M. Hartley, George P. Murphy, Katrien M. Devos, John E. Flintham, James Beales, Leslie J. Fish, Anthony J. Worland, Fatima Pelica, Duraialagaraja Sudhakar, Paul Christou, John W. Snape, Michael D. Gale & Nicholas P. Harberd 

Nature **400**, 256–261(1999) | [Cite this article](#)

3236 Accesses | **1018** Citations | **13** Altmetric | [Metrics](#)

Abstract

World wheat grain yields increased substantially in the 1960s and 1970s because farmers rapidly adopted the new varieties and cultivation

Kort ris (og hvede) har mutationer i varianter af det samme gen: *Rht*

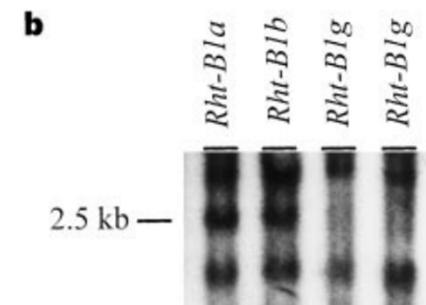
Mutanterne koder for proteiner der er kortere end det oprindelige protein

The *Rht-B1b* and *Rht-D1b* mutations are both nucleotide substitutions that create stop codons. In *Rht-B1b*, a T-for-C substitution converts the Q64 codon (CGA) to a translational stop codon (TGA; Fig. 3a). In *Rht-D1b*, a T-for-G substitution converts the E61 codon (GGA) to a translational stop codon (TGA; Fig. 3a). The similarity of the *Rht-B1b* and *Rht-D1b* mutations presumably explains why they confer very similar severities of dwarfism⁴.



Figure 3 Dominant mutant alleles encode proteins with mutant N termini. **a**, N-terminal segments of predicted proteins encoded by mutant alleles *gai*, *D8-1*, *D8-2023*, *D8-Mpl*, *Rht-B1b* and *Rht-D1b* are compared with those of their respective wild-type alleles (*GAI*, *d8*, *Rht-B1a* and *Rht-D1a*). For each locus, the wild-type sequence is shown above and below the mutant sequence(s). Differences between wild-type and mutant sequences (deletions and substitutions) are highlighted in white, the position of translational stop codons is represented by an asterisk, and the previously identified highly conserved regions I and II (Fig. 2a) are shown. All mutations alter the N-terminal region of their encoded proteins, and affect regions I and/or II. *D8-2023* also carries a 6-bp deletion that removes one G and one A residue from 510GAGA513, and a nucleotide substitution that converts

T519 to A519 (with respect to *d8* sequence; Fig. 2a, and data not shown). Because these altered residues are poorly conserved in *GAI/RGA/Rht-D1a/d8*, these changes are considered not to be of phenotypic significance. In wheat, Q64 of *Rht-B1a* is equivalent to Q62 of *Rht-D1a*, owing to a difference of two amino-acid residues in a poorly conserved N-terminal region (see text; data not shown). *gai* was isolated following X-irradiation mutagenesis⁸; all other mutant alleles shown are of spontaneous origin^{4,6,7}. **b**, Gel-blot hybridization of C15-1 with *Bam*HI-digested DNA from *Rht-B1a* (var. Mercia), *Rht-B1b* (Mercia near-isogenic line) and two *Rht-B1g* homozygotes. A hybridizing 2.5-kb *Bam*HI fragment (assigned to chromosome 4B by nullisomic-tetrasomic analysis; data not shown) is missing in the *Rht-B1g* samples.



Rht Natural Variants



>Hvede Rht-B1a (høj)
.....GEEVDELLAALGYK

>Hvede Rht-B1b (lav)GEEVDELLAALGYK

VRASDMADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHYN
VRASDMADVAQKLE^{STOP} MAMGMGGVGAGAAPDDSFATHLATDTVHYN

PTDLSSWVESMLS.....
PTDLSSWVESMLS.....

Rht-B1b mutantgenet koder for et ‘speeder’protein der er betydeligt kortere end Rht-B1a og sandsynligvis mindre effektivt (men sandsynligvis stadig aktivt til en vis grad).

Hvedegræs koder for 3 proteiner der er næsten identiske med Rht fra hvede

Table 1. Candidate Domestication Genes That Align with Intermediate Wheatgrass QTLs

Plant height	RHT	Reduced Height	Wheat (4)	4	Gennavn i hvedegræs	% lighed med Rht	
					Thint.10G0079300 Thint.12G0065200 Thint.V1841900	98,49 94,33 92,73	[51]

>Hvedegræs Thint.10G0079300.1.pEEEVDELLAALGYK
>Hvede Rht-B1a (høj)
.....GEEVDELLAALGYK
>Hvede Rht-B1b (lav)GEEVDELLAALGYK

VRASDMADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHYN
VRASDMADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHYN
VRASDMADVAQKLE* MAMGMGGVGAGAAPDDSFATHLATDTVHYN

PTDLSSWVESMLS.....

PTDLSSWVESMLS.....

PTDLSSWVESMLS.....

>Hvedegræs Thint.10G0079300.1.pEEEVDELLAALGYK

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VRASDMADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHYN

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Vi skal finde en hvedegræs
med en mutation der får Rht
proteinet til at blive kortere
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Hypotese:

Hvis hvedegræs muteres i gener, der ligner domesticeringsgener kendt fra hvede, vil resultatet blive det samme (fx større kerner og kortere vækst)

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Problem:

Hvordan finder vi hvedegræsplanter med de mutationer vi ønsker?

Lige nu arbejder vi med tre forskellige forsøg på at løse problemet:

- 1) Vi leder efter spontane mutationer der ligger bag naturlig genetisk diversitet i hvedegræs.
- 2) Vi forsøger at inducere mutationer i hvedegræs (non-gmo) og derefter lede efter ønskede mutationer i den muterede population.
- 3) Vi forsøger at anvende CRISPR-Cas9 genediterings-teknologi

Nye problemer:

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g **Hvedegræs er hidtil aldrig**
blevet transformert

**Vi krydser fingre for at mindst en af
strategierne vil bære frugt.**

Tak for opmærksomheden!