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Tech ID: 18.638

Patent Literature

Published: WO/2020/065331; EP 3856912; US-2025-0092411-A1;

Granted: US 12/209,248

Novel Plant Starches

Flexible starch modification by simple mutation

Mutant wheat lines with altered starch granule size profile

Multiple high value applications in a range of crops

David Seung at the John Innes Centre has found that specific mutations in the MRC1 gene of wheat cause a shift in the particle size distribution of endosperm starch. Loss of function mutants cause a shift to on average smaller granules (fewer A-type granules and relatively more B-type granules). In contrast, certain other mutants can increase the activity of MRC, causing a shift to on average more large granules. The choice of MRC mutation therefore can result in starches of very different physico-chemical properties, as required for different uses and industrial applications. MRC is centrally responsible for controlling starch granule initiation in plant storage organs and is conserved across plant species, thus affording broad application for tailoring starch to particular end-uses.

Starch granule size, and the overall size distribution profile of plant starches, has a major influence on the physico-chemical behaviour of the starch, particularly during the gelatinisation process (the swelling and disintegration of the starch granule when heated in the presence of water - a process that occurs during cooking and industrial processing of starch). Larger starch granules tend to have high swelling power and viscosity, while small granules tend to provide smoother paste textures for food applications. Smaller granules and narrower (non-bimodal) granule size distributions are also desirable outside the food industry, in particular for use in papermaking and plastics, and also for use as a binder or carrier material in the pharmaceutical and cosmetics industries. Small granule starch is also more effectively digested than larger granules, due to their larger surface area. Thus, smaller starch granules may be desirable for applications where complete and efficient starch digestion is required (e.g.: glucose syrup or bioethanol production, or use as animal feed). **Illustrating the potential of MRC modifications, the JIC group has found that the mrc loss-of-function mutants have a higher onset and peak gelatinisation temperatures, 3x increase in swelling power, significantly reduced viscosity (RVA test) and accumulate significantly more glucose during germination.**

MRC encodes MYOSIN-RESEMBLING CHOROPLAST PROTEIN and its function in starch granule initiation in Arabidopsis chloroplasts has been recently described (Seung et al, Plant Cell 2018, doi:10.1105/tpc.18.00219). Mutants of MRC in Arabidopsis leaf chloroplasts have a single large starch granule, and so the phenotype of wheat loss-of-function *mrc* mutants studied by the JIC team - smaller A-type granules and increased B-granules - is completely unexpected from the Arabidopsis work. While granule size distribution is widely recognized as an important parameter for end-use quality of cereal starch, the mechanism of starch granule initiation has not been understood and there has been no genetic method to enhance the granule size profile of crops such as wheat and barley in a controlled manner.

MRC was discovered by the inventor in a search for proteins that interact with PTST (Protein Targeting To Starch) proteins, which in Arabidopsis chloroplasts are required for normal starch granule initiation. MRC has long coiled coils, which are specialized α-helices that interact with each other, and commonly found in proteins involved with starch metabolism. They can mediate protein-protein interactions, but also act as scaffolds, organise membrane networks, and exert physical force by acting as springs or levers. MRC also interacts with Starch Synthase 4. Although the number of starch granules per chloroplast is greatly reduced in Arabidopsis mutants defective in MRC, the total amount of starch synthesised in these mutants is identical to wild-type plants.

The wheat *MRC* genes are located on the Group 6 chromosomes. The inventor has found *MRC* homeologs on 6A and 6D in hexaploid wheat (cv. Cadenza) and on 6A, only, in tetraploid wheat (cv. Kronos). Only parts of the MRC gene were found on 6B, suggesting that this copy has been lost or inactivated during wheat evolution. The 6A and 6D homeologs show similar patterns of expression, with expression higher in the early stages of grain development (6-9 dpa) than in the later stages (12-30 dpa). The peak in expression during grain development corresponds to when the synthesis of A-type granules is initiated.

EMS-mutants (leading to premature stop codons) in these *MRC* loci were isolated and used to study the novel starch phenotype, alone and in combination after pyramiding the mutants on the different genomes. The starch granules from the Kronos *mrc-1 aa bb* mutant were noticeably different in size from the wild-type (WT) Kronos (AA BB) starch (Figs 1 and 2). The starch in the mutant contained moreB-type granules than the starch from the WT, and the A-type granules were smaller in the mutant, reflecting increased granule initiation due to loss of MRC function. A separate Kronos *mrc* mutant line containing different *mrc* mutations, *mrc-2 aa bb*, also had smaller granules than the WT, but to a lesser extent than *mrc-1 aa bb*. In both WT and mutant starch, the A-type granules had their typical lenticular shape, while the B-type granules were round. Aside from the difference in starch granule size, the *mrc* mutants were indistinguishable from the WT in terms of plant growth and grain size (Fig 3).

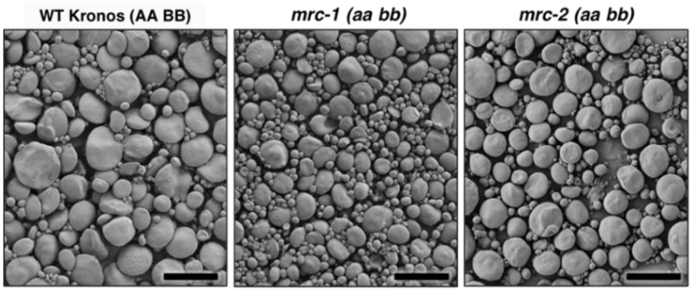


Fig 1 Granule size and morphology of endosperm starch in *mrc* mutants. Starch was purified from mature grains of wild type (WT) and *mrc* mutants and observed using scanning electron microscopy. All panels are shown at the same scale. Bars = 40 µm.

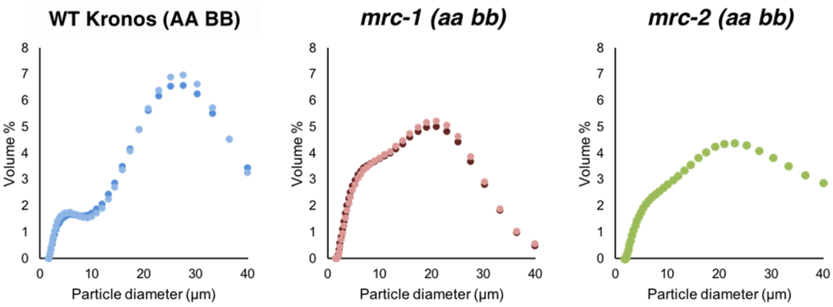


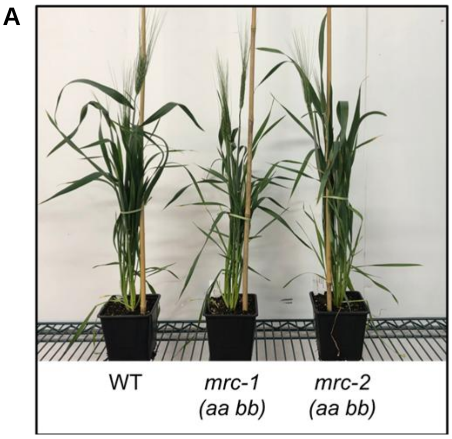
Fig 2 Wheat *mrc* mutants have overall reductions in granule size and more unimodal size distributions. Size distributions were measured using laser scattering. Two biological replicates from different plants were analysed for WT and *mrc-1*, and both replicates are plotted on the same graph. One replicate of the *mrc-2* starch was analysed.

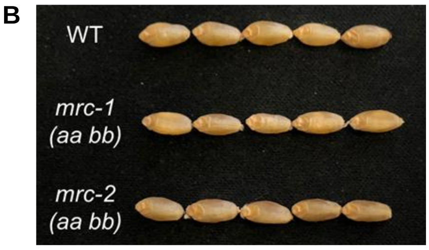
Differential Scanning Calorimetry was used to monitor the gelatinisation of starch over the temperature range 10°C to 150°C at a rate of 1°C/min. The *mrc-1 aa bb* starch had significantly higher onset and peak gelatinisation temperatures compared to the WT (Table 1). The higher peak temperature was expected from the higher proportion of B-type granules in *mrc-1 aa bb* starch, since it is known that B-type granules have a higher peak gelatinisation temperature than A-type granules. No significant difference between the mutant and WT was detected for gelatinisation enthalpy, or in the temperature difference between the measured peak and onset temperatures.

Table 1. Gelatinisation temperature of *mrc-1 aabb* starch

	Onset (°C)	Peak (°C)	Peak-Onset (°C)	Enthalpy (J/g)
WT	**50.2 ± 0.5**	**56.3 ± 0.3**	6.2 ± 0.3	6.5±0.3
mrc-1 aa bb	**52.2 ± 0.3***	**57.8 ± 0.3***	5.7 ± 0.1	7.0±0.1

Values are the mean ± SE for three biological replicates (grains harvested from different plants). Values marked with an asterisk (*) are significantly different ($p < 0.05$) from the WT value under a two tailed *t*-test.





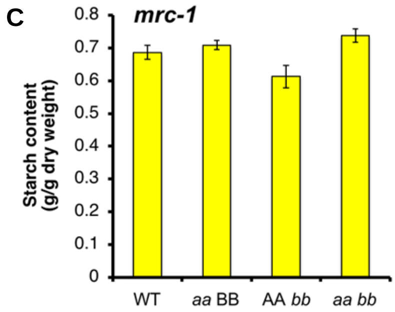


Figure 3: *mrc* mutants have normal phenotypes. (A) 7-week-old wild type (WT) Kronos and *mrc-1 aa bb* and *mrc-2 aa bb* mutants. No visible differences in plant size, morphology, time to heading or spike number were observed. (B) Mature grains from the *mrc* mutants compared to those of WT. No differences in grain size or morphology were observed. Moreover, starch content of grains from *mrc* mutants is not different from WT (C).

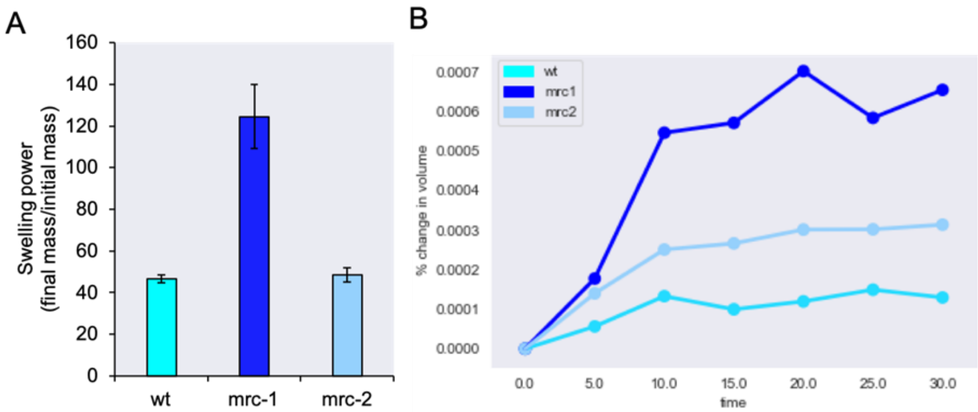


Figure 4: Swelling Power. Panel A shows that the *mrc-1* mutant has a swelling power at 100°C that is almost **three-fold higher than the wild-type**. The Panel B shows the change in the average volume of granules over time at 60°C, quantified with a Coulter counter, with the starch from *mrc-1* **swelling more and faster** than starch from the wild type.

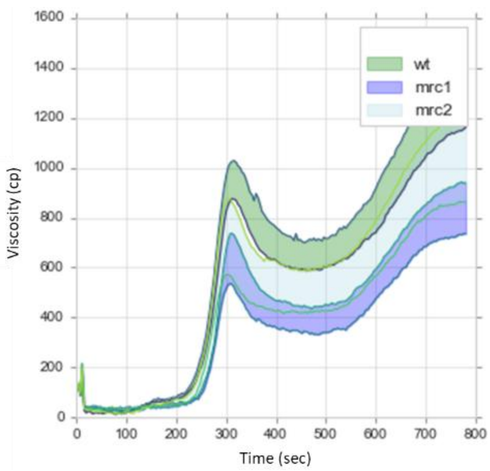


Figure 5: Rapid Visco Analysis (RVA) of mrc wheat starch. RVA was carried out to determine the viscosity during the gelatinisation of starch from the mrc mutants compared to wild type. Two runs were carried out per sample and the shading indicates the area between the two replicate curves.

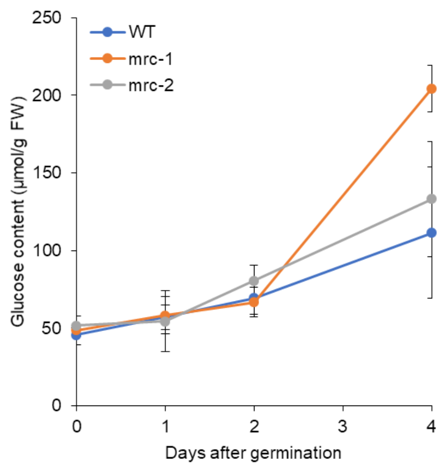


Figure 6: Germination. Four days into germination, grains of the *mrc-1* mutant accumulated significantly more glucose than the wild type and the *mrc-2* mutant grains, under a two-tailed *t*-test ($p < 0.05$).

The germination experiment (Fig 6) mimics malting, which involves the controlled germination of grains for 4-6 days, and suggests that malt from the *mrc* mutant grains will have more starting sugars available for fermentation. The more efficient breakdown of starch *in vivo* may also translate to the later stages of brewing, where the remaining starch in the malted grain is digested during mashing.

Mutants enhancing MRC function cause more, larger granules

As well as loss-of-function / reduced-function mrc mutants, the JIC team has isolated and characterized certain other MRC mutants that confer increased functional activity of MRC. This is likely because these particular mutations promote more stable interactions of MRC. This has the opposite effect on the process of starch granule initiation (reduced initiation) resulting in more, larger granules and a consequent “rightwards” shift in the granule particle size distribution.

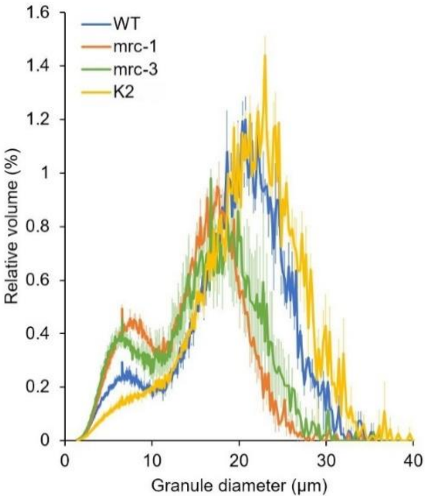
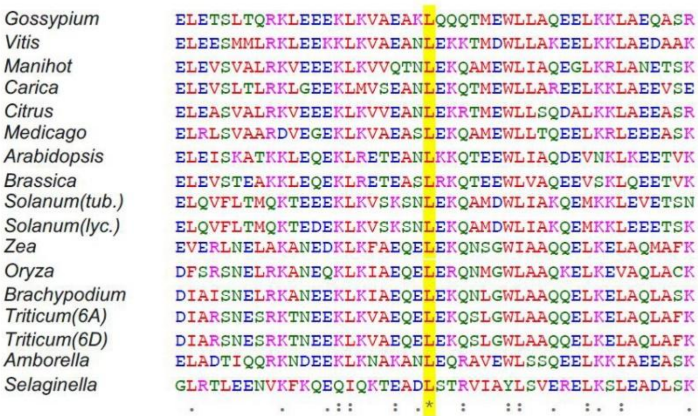


Fig 7 shows the granule size distribution of one of these “increased activity” mutants, K2, compared with WT and two reduced-function mutants, mrc-1 and mrc-3.

Fig 8 shows a multiple sequence alignment of MRC orthologs generated with Clustal O, showing the region around one of the target mrc mutation sites, and the universally conserved Leu289 residue.



The wheat mrc mutants described here are EMS mutants. The JIC team is exploring with partners the wide range of novel physico-chemical properties of these new starches. MRC is conserved and orthologues are easily identifiable in many crop species.

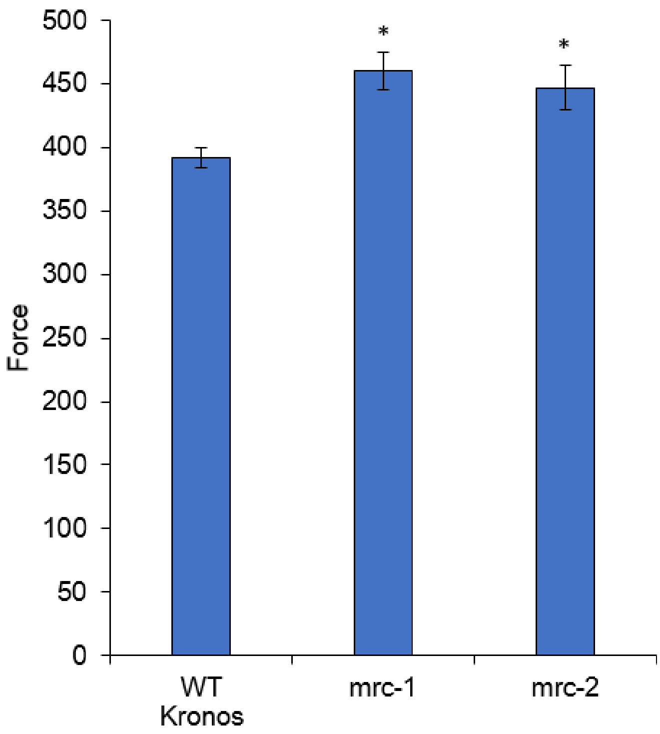
Applications:

- Novel starch in wheat, pasta wheat and other starch crops.
- Simple mutation breeding approach to altered plant starch with novel physico-chemical properties for diverse applications in food industry, brewing, pharmaceuticals, papermaking, feed and biofuels.
- Choose as required between smaller average granule size (mrc disruption) or larger average granule size (mrc enhancement).

UPDATE November 2020: Enhanced pasta firmness - premium quality trait feature

With increased quantities of MRC1 mutants becoming available in both Durum (Kronos origin) and bread wheat (Cadenza origin), the JIC team have been obtaining new information about the physico-chemical properties of the MRC1 mutant starches.

An evaluation of pasta cooking behaviour was conducted with a partner laboratory. The results show that mrc mutants confer a **significant increase in firmness, which is a highly desirable feature that commands a price premium**. There was no difference in cooking time compared to the WT Kronos control.



Other features such as colour and cook loss were within a commercially acceptable range.

For more information or licensing interest, please contact PBL

References:

Seung et al (2018). Two Plastidial Coiled-Coil Proteins are Essential for Normal Starch Granule Initiation in Arabidopsis. *Plant Cell*; 30(7): 1523-1542 doi:10.1105/tpc.18.00219