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Tech ID: 22.698

Patent Literature

Published: WO/2024/208896

Healthy Triticeae Starches

Increased starch granule size, by simple mutation

Applications for starch with lower Glycaemic Index and novel processing properties

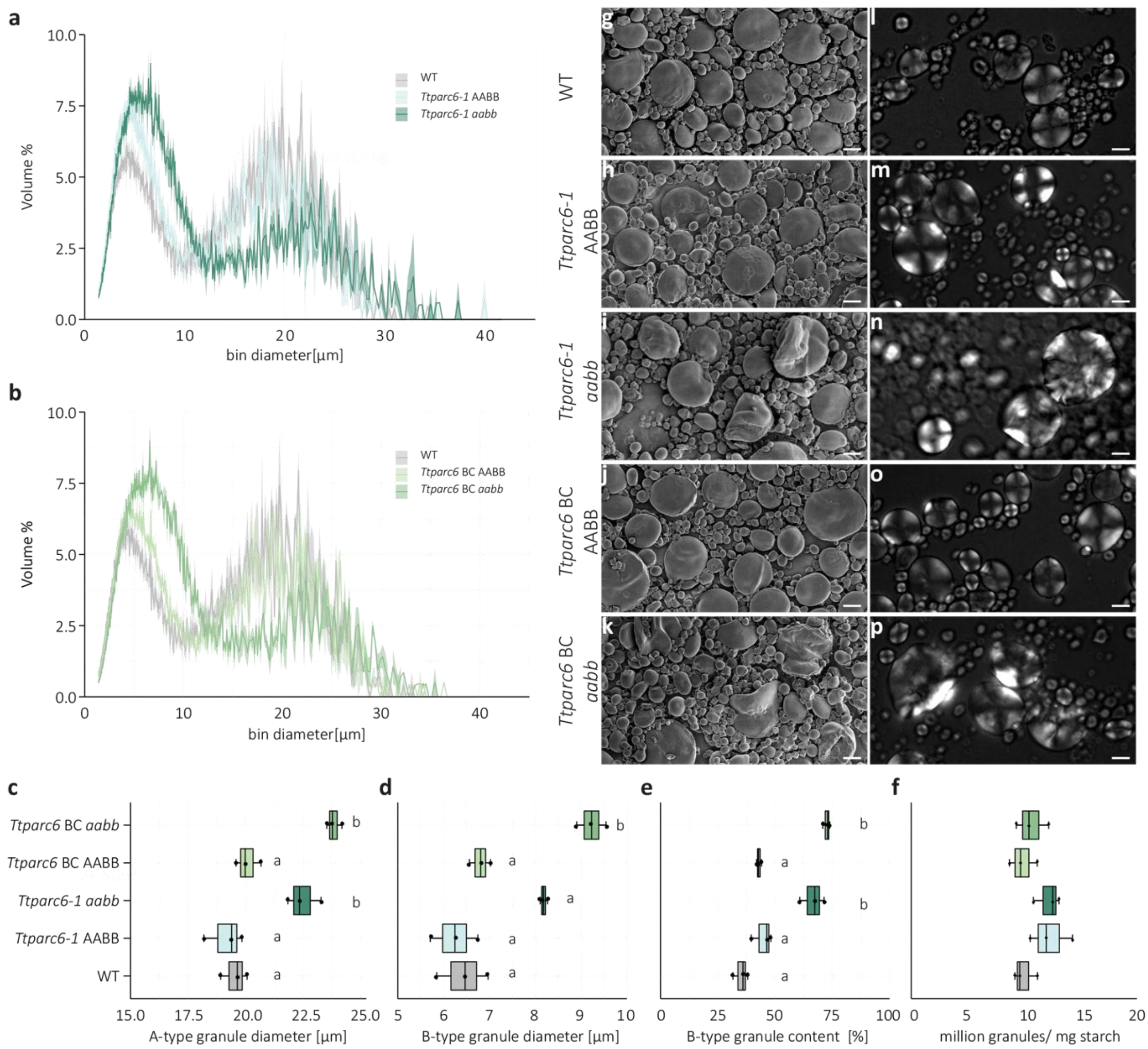
There is significant economic value in manipulating plant starch granule morphology and size. These parameters largely determine the physical performance and processing features of starch. For example, nutritional properties - such as susceptibility to digestion - and physicochemical properties of great significance to processing are all influenced by starch granule morphology and size. Cereal crops of the Triticeae tribe (wheat, rye and barley) differ from other grain crops in having a bimodal distribution of granule size, with larger, discoidal A-type granules and smaller, spherical B-type granules. A-type granules account for more than 70% of wheat endosperm starch by weight, but less than 10% of the granules by number.

Increased starch granule size may result in greater resistance to digestion in the gut, and act as a form of 'resistant starch'. Health benefits from consuming more resistant starch include a reduction in glycaemic index, and a prebiotic effect that promotes healthy gut microbiota. The surface area of granules is also relevant to starch digestion in cooked foods, since in many wheat products, including bread and pasta, starch granules do not fully gelatinise during the cooking process. Thus, increasing starch granule size may lower the glycaemic index of these foods.

Plastid size and morphology are greatly influenced by plastid division. The division machinery consists of ring-shaped protein complexes on the inner and outer envelope membranes, which contract and divide the plastids by binary fission. ARC6 and PARC6 are paralogous proteins that coordinate these contractile rings, in the inner envelope membrane, together with PDV1 and PDV2 in the outer envelope membrane.

David Seung and colleagues at the John Innes Centre have identified a mutant in durum wheat (both A and B genome homoeologues), in cv Kronos, that is defective in the plastid division protein PARC6.They find that the loss-of-function double mutant (Ttparc6 aabb) has **increased plastid size in both leaves and endosperm**. More significantly, the A- and B-type **starch granules of mature grains were significantly larger** in the mutant compared to the wild type, and the surface morphology of A-type granules was not smooth and discoid like wild type but irregular and more lobate. These phenotypes were not at all predicted from studies of PARC6 and ARC6 in other species. This striking change in morphology is already evident at the early stages of grain development, when granule size is identical between the mutant and the wild-type, and occurrs without alterations in starch polymer structure and composition. The PARC6 double mutants also have very markedly increased chloroplast size in leaves. Despite the large changes in plastid size and starch granule morphology, the growth, development and photosynthetic efficiency is not altered by the *Ttparc6* mutants. Similarly, grain size, number and starch content are not affected. Interestingly, a mutant in the PARC6 paralog, ARC6, does not have increased plastid or starch granule size. This is presumably due to the ability of PARC6 to complement disrupted ARC6 function, since wheat PARC6 can interact with both PDV1 paralogs as well as PDV2, the outer plastid envelope protein that typically interacts with ARC6 to promote plastid division. Thus, increasing plastid compartment size and available stromal volume seems to result in increased starch granule size as well as more starch granules per amyloplast.

In studies of starch gelatinisation properties, the viscographs of the different mutant/WT *T. durum* genotypes generally are very similar, and there is no obvious difference in peak viscosity or the holding strength during cooling. Similarly there are no consistent differences observed during cooling (retrogradation) or in final viscosity. This indicates that the underlying crystalline structure of the starch granules is unlikely to be altered in the mutants, since changes in crystallinity are usually associated with altered gelatinisation temperature.



Above: Size distribution and morphology of purified starch granules from mature grains of Ttparc6 mutants
(a-b) Size distribution plots from Coulter counter analysis. (c-f) Granule size and number parameters obtained from fitting a log-normal distribution to the B-type granule peak and a normal distribution to the A-type granule peak in the granule size distribution data presented in (a – b). (g-k) Scanning Electron Microscopy of starch granules from mature grain. Bar = 10 μm. (l-p) Polarised light microscopy of starch granules from mature grain.

There are many examples of genes affecting starch granule morphology through starch polymer biosynthesis, and **MRC1 (PBL Tech Id 18.638**; Seung et al (2018) *Plant Cell*, 30;7) acts through effects on granule initiation, but PARC6 is the first example of modification of amyloplast architecture in Triticeae via components of plastid division, and the only example in wheat where granules are made larger.

Large starch granule size may have diverse applications such as better milling efficiency and enhanced nutritional quality (lower GI). High B-type granule content, such as seen here, is known to be associated with better pasta quality.

Further characterisation of the PARC6 mutant starch is ongoing.The work is published in a BioRxiv Preprint March 2023. For more information or licensing interest, please contact PBL.

References:

Esch L *et al* (2023). Modification of amyloplast size in wheat endosperm through mutation of PARC6 affects starch granule morphology. BioRxiv preprint https://doi.org/10.1101/2023.04.03.535339

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