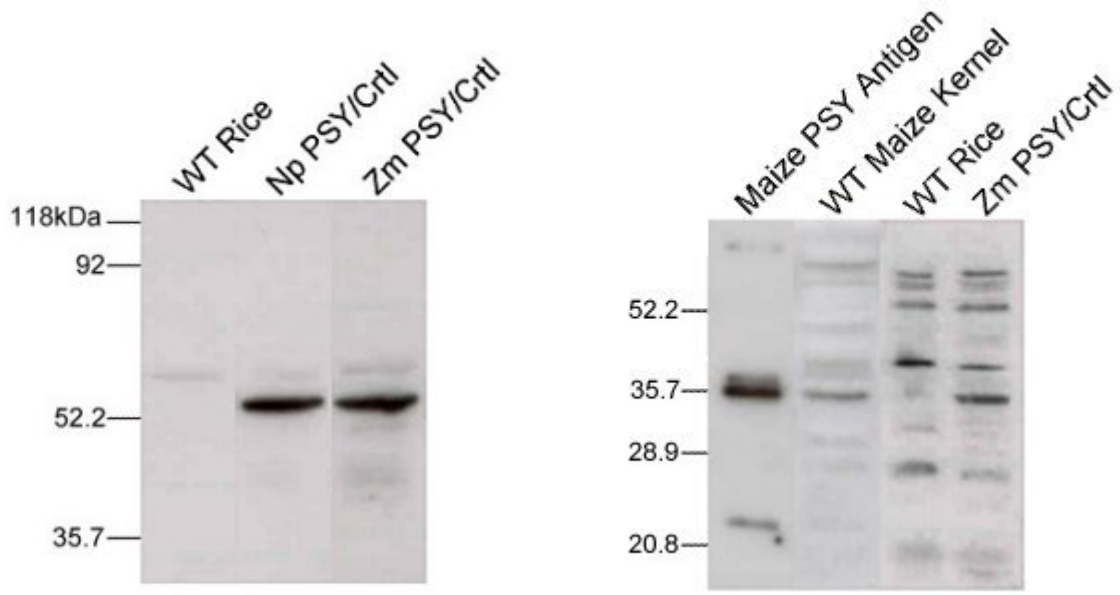
**Supplementary Figure 2 Expression of CRTI and maize PSY proteins in rice endosperm**

**a** **b**




Immunoblots of grain protein extracts from wild-type (WT) and transgenic plants. The blots were immunodecorated with polyclonal antisera raised against the CRTI **(a)** or maize PSY **(b)** proteins. Analysis was performed on transgenic rice plants containing the daffodil *Psy* and *crtI* genes (Np PSY/crtI) and the maize *Psy* and *crtI* genes (Zm PSY/crtI). The maize PSY antigen and an extract from mature wild-type maize kernels were included in **(b)** as controls.

The CRTI antiserum gave a strong reaction with a protein of 57 kDa in endosperm containing the daffodil *Psy/crtI* and maize *Psy/crtI* gene pairs (Fig. 2a) with no equivalent band in the untransformed sample. The CRTI protein has a predicted molecular mass of 55 kDa assuming accurate processing of the RUBISCO small subunit plastid transit peptide. Despite cross-reacting bands, the maize PSY antiserum detected one protein of approximately 36 kDa which was unique to transgenic rice seed containing the maize *Psy* transgene (Fig. 3b). Given the limitation of SDS-PAGE for estimating protein size especially for hydrophobic proteins, it is likely that this protein band represents the processed maize PSY protein (predicted molecular mass, assuming transit peptide cleavage after Tyr62, of 39.8 kDa). A band of a very similar molecular mass was also detected in the protein extract of maize kernels most probably representing the endogenous maize PSY protein.

**Methods for production of antigen, antibody and western blot analysis**

The *crtI* coding sequence was inserted into the *XmnI/HindIII* site of a pBR322-based vector containing the *tac* promoter and the coding sequence for six His residues 5' to the *XmnI* cloning site resulting in an N-terminal tag on the CRTI protein. The tagged-CRTI was expressed in *E. coli* DH5α and purified from the supernatant on a Ni-NTA agarose column (Qiagen) according to the manufacturers protocol. The maize *Psy* coding sequence, lacking the first 354 bp from the 5' end of the coding sequence, was cloned into the *NcoI/BamHI* site of pET24a (Novagen) under control of the *T7*

promoter. The truncated maize PSY was expressed in *E. coli* BL21(DE3) RP cells and purified from pelleted inclusion bodies.

Protein (100 μg) in Freunds Complete Adjuvant was administered subcutaneously to New Zealand White rabbits. A further 100 μg of protein was administered in Freunds Incomplete Adjuvant 28, 56 and 84 days after the initial immunisation and antibody was collected at 98 days.

Polished rice endosperm was homogenised in Laemmli sample buffer. Protein was collected in the supernatant by centrifugation at 13 000 rpm for 30 min. SDS-PAGE[40] and western blotting[41] were performed using standard protocols. Gel lanes contained 100 ng maize PSY antigen, 100 μg maize kernel protein extract or 40 μg polished rice endosperm protein extract respectively. Detection of antibody-reactive proteins was carried out using the ECL system and manufacturers protocol (Amersham).