Seeds must maintain a constant level of nitrogen in order to germinate. When recombinant proteins are produced while endogenous seed protein expression is suppressed, the production levels of the foreign proteins increase to compensate for the decreased synthesis of endogenous proteins. Thus, exchanging the production of endogenous seed proteins for that of foreign proteins is a promising approach to increase the yield of foreign recombinant proteins. Providing a space for the deposition of recombinant protein in the intracellular compartment is critical, as this would lessen any competition in this region between the endogenous seed proteins and the introduced foreign protein. The production yields of several recombinant proteins have been greatly increased by this strategy.

Plants provide a promising platform for the production of recombinant proteins in terms of scalability, safety and cost-effectiveness and thus serve as an alternative to conventional fermentation systems that use bacteria, yeast or mammalian cells. Specifically, seeds provide an environment for high levels of recombinant protein production, as well as a stable, ample deposition site for recombinant proteins, which allow the proteins to avoid degradation even when stored at ambient temperatures for several years. Seed storage proteins are stored in cereal seeds at a level of 7–15% of dry seed weight, whereas dicotyledonous seeds accumulate these proteins at a level of 20–40% of dry seed weight. Prolamins are the major seed storage proteins in all cereals except rice and oat, and genes encoding prolamins are specifically expressed in the endosperm tissue. On the other hand, in dicotyledonous plants such as soybean, pea and sunflower, 7S and 11S globulins are specifically and predominantly expressed in the embryos of maturing seeds. Notably, the endosperm tissue of cereal seeds occupies more than 80% of the total seed weight and represents a stable storage organ. Many seed-specific promoters have been isolated from several crops and used for the expression of recombinant proteins in transgenic plants. In particular, the promoters of genes encoding major rice storage protein glutelins, such as GluA2(gt1), GluB1 and GluB4, along with the strong bean phaseolin 7S and soybean glycinin 11S promoters, have been utilized for the production of several recombinant proteins, such as pharmaceuticals, in the endosperm and embryos of monocot and dicot seeds, respectively. Seed specific expression of seed storage proteins (SSPs) is determined by the combinatory interaction between several cis-regulatory elements, and transcription factors recognizing the individual cognate cis-element have been also characterized.

The accumulation levels of foreign recombinant proteins fundamentally depends on the yield potentiality of the target tissues (cells) of the host crop. Furthermore, the yield of recombinant protein products is determined at both the transcriptional and post-transcriptional level. The stability of mRNA, translational efficiency and codon-optimisation of recombinant genes based on codon bias in expressed host tissue have to be taken...
into consideration when designing a seed-based protein production platform.13-15 Furthermore, the accumulation levels of recombinant protein are determined by several steps or processes leading to the final destination site of the recombinant protein after translation. Deposition in intracellular regions has a critical effect on the accumulation of recombinant SSPs that are translated in the rough ER and stably stocked in the specialized storage organelle referred to as the protein body (PB). To increase the accumulation level of a recombinant protein, a suitable intracellular compartment must be chosen as the deposition site. In many seeds, PBs are the main deposition sites of SSs, which serve as nitrogen reserves for the germinating embryo. There are two types of protein bodies in rice endosperm cells, i.e., ER-derived PBs and protein storage vacuoles (PSVs).16-18 Most dicot seed proteins are deposited into PSVs, whereas seed proteins of monocot plants are sequestered into ER-derived PBs and PSVs. ER-derived PBs contain prolamins, while 7S and 11S globulins and rice glutein are deposited into PSVs. Although both PSV proteins and PB proteins are synthesized as secretory proteins on the rough ER, PSV proteins are transported to the PSV via Golgi apparatus or deposited directly as PAC vesicles, whereas PB proteins aggregate to form PBs in the ER lumen.19

The reduction of SSs is compensated for by the increased production of other SSs irrespective of any types of SSs.20 In particular, when seeds are deficient in PSV proteins such as glutein and globulin, the production of sulfur-poor 13 kD prolamins is preferentially increased in rice.21 Such preferential compensation may depend on the sulfur levels of the seed proteins.22 Therefore, when the production of some SSs is depressed using a knock-down approach (RNA interference and anti-sense RNA) or by mutation, the production of other types of endogenous seed proteins increases due to the compensatory or rebalancing mechanisms inherent in seeds, which maintain constant levels of total seed protein. This finding indicates that seed proteins may be replaced by foreign recombinant proteins, thereby maintaining proper nitrogen levels in the seed. That is, rebalancing the proteome occurs via the regulatory mechanism required for maintaining the total seed protein content. In the seeds of low glutelin rice mutants such as LGC-1 or the a123 three glutelins-less mutant, the prolamin levels significantly increase in a compensatory manner.23-25 The suppression of 7β-oxoglucanase production in soybean seeds results in an increase in 11S glycinin content via a redistribution of seed protein content.26 Furthermore, in transgenic rice, the suppression of genes encoding individual SSs or a combination of several SSs, such as glutelins, prolamins and globulin significantly affects the expression of other seed protein genes, as well as PB formation, as rebalancing the proteome occurs to maintain the protein content of rice seeds.27 Such a compensatory effect is regulated at both the transcriptional and translational levels. A similar situation was also reported in maize grains. The reduced production of both the 19 and 22 kD major α/zeins results in higher non-zein protein content.28 These studies suggest that the production yield of foreign proteins may be effectively increased by redirecting intrinsic seed protein production to foreign protein production. The production yields of foreign recombinant proteins are greatly increased by the simultaneous reduction of the expression of endogenous seed protein genes by RNA interference (anti-sense technology) or mutation. These techniques have enabled greater amounts of Psoralens glucaric acid arcein (arc) 5-1, Green Fluorescence Protein (GFP) and human growth hormone to be expressed as secretory proteins by ligating the signal peptide and the KDEL ER retention signal to the N and C termini of the desired recombinant protein, respectively, to improve their accumulation levels. These strategies result in higher yields of recombinant protein production than can be achieved by targeting the protein to the cytoplasm without the

©2013 Landes Bioscience. Do not distribute
Taken together, these studies suggest that the selection of a suitable intracellular compartment is a critical step for increasing the yield of recombinant proteins in seeds, since trafficking to the final deposition site requires the proper folding, assembly and post-translation modification of the protein and is related to the accumulation capacity of the seed. The specific suppression of endogenous seed proteins at the same deposition site as the desired recombinant protein provides a new strategy for increasing the accumulation levels of recombinant proteins in seeds. Further work will be required to determine whether this strategy will be applicable to other intracellular compartments.

Disclosure of Potential Conflicts of Interest
No potential conflict of interest was disclosed.

Acknowledgments
The author was supported by an “Agri-Health Translational Research Project” grant from the Ministry of Agriculture, Forestry and Fisheries of Japan.

Figure 1: Increasing cytokine production by suppressing prolamin protein deposition in ER-derived PBs. (A) Expression constructs used for the production of cytokines, human IL-10 (H-IL-10), mouse IL-6 (M-IL-6) and mouse interferon-γ (M-IFN-γ), in transgenic rice seeds. Rice 16 kD, 13 kD and 10 kD prolams were simultaneously suppressed by RNA interference using RSIS linked to the 5’ NTR of 10 kD and 16 kD prolams and the 3’ NTR of 13 kD prolamin. (B–D) The protein accumulation levels were examined in more than six independent transgenic rice lines per construct. A minimum of four positive seeds from each line were analyzed by western blotting using commercially purchased human IL-10 or His-tagged as controls.
