VARIABILITY OF STORAGE ROOT-SPECIFIC GENE EXPRESSION IN TRANSGENIC
‘HIGH PROTEIN’ SWEETPOTATOES (IPOMOEA BATATAS L., PI 318846-3)
ENGINEERED WITH AN ARTIFICIAL STORAGE GENE (ASP-1).

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Variability of expression of native genes was analyzed in a number of ASP1-transgenic “high protein” sweetpotato lines. We have modified the nutritional quality of sweetpotato, a NASA candidate crop, genotype PI 3188463 by using a CaMV35S driven (292bp) synthetic gene (asp-1) coding for a storage protein extremely rich in essential amino acids. This change in nutritional value is associated with a two to threefold increase in total protein content and essential amino acid levels in transgenics especially line TA3. All transgenic plants expressed the ASP-1 protein detectable by immunoblot analysis; however, the increased protein content was primarily due to enhanced levels of native proteins such as sporamin and β-amylase the most abundant storage proteins in sweetpotato. To investigate the molecular basis (effect of asp-1 on) of the high accumulation of sporamin and β-amylase in the ASP1-plants, storage roots, at four developmental stages, were taken from the “high protein” sweetpotato lines, a GUS transgenic plant, and the parental control grown under hydroponic conditions. Considerable variation in the total protein in storage roots and specifically sporamin and β-amylase expressions was observed between individual transgenics per sequential harvest in SDS-PAGE and Western blot analyses. The yield of transgenics TA3 and TA2 plants was largely comparable to the parental control. The sporamin level was found to be higher at the very early stages of TA3 and TA5 compared to TA1, TA2 and TA4 but was comparable to controls. The relative levels of sporamin gradually (temporally) increased in the developing roots of all plants. However, the sporamin content was found to be 150% to 300% (TA3) higher at maturation in all transgenic plants compared with the parental control and the GUS plant in the third and fourth harvest of hydroponic and soil cultures. Lines TA1 and TA5 had the lowest protein expression when compared to the controls at 90 days post planting (DAP). However at 136 DAP these lines accumulated up to 12 and 16% protein on a dry weigh basis, respectively. Although protein content differed significantly in all transgenics and was higher than the controls, Northern data showed that roughly equal steady state level of ASP1-transcripts accumulated in all transgenics, inferring that chromosomal position effects of ASP1-transgene and physiological determination are suggested as triggers for the variations in total protein. Sporamin and β-amylase RNA levels were higher in the asp-1 transgenics compared to the controls and GUS transgenics. Sporamin transcripts were generally higher in TA3 than in TA2. The increase in gene expression appears to be a consequence of enhanced mRNA transcription of stability rather than gene amplification because sporamin genes occur in 10 copies, per haploid genome, in ASP1-transgenic as well as non-ASP1-transgenic (GUS) and control backgrounds. This indicates that asp-1 expression is central to the accumulation of these major native proteins in “high protein” sweetpotato revealing a temporal expression profile in all five ASP1-transgenic sweetpotato lines.

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