Selection of low phytate soybeans and QTL mapping of phytate genes in soybean with a simple, rapid, and quantitative procedure

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Phytic acid, *myo*-inositol hexa*kis*phosphate ($InsP_6$) is the major storage form of P in seeds. Lacking sufficient phytase, non-ruminant animals cannot digest phytate in soybean (*Glycine max* (L.) Merr.) meal and excrete undigested phytate in animal waste. The low P bioavailability of animal-feeds and land application of the animal manure cause excess accumulation of P in soil and water. Breeding soybeans with low phytate and correspondingly high inorganic P content provides a practical strategy to avoid these problems.

In this study, we have developed a reliable, convenient phytate assay in selecting low phytate soybean breeding lines and QTL mapping of the genes conditioning the low phytate trait. The colorimetric procedure (Vaintraub and Lapteva, 1988), based on the reaction between ferric chloride and sulfosalicylic acid, was modified and standardized to measure crude soybean phytate extracts. The important changes include overnight extraction for consistency and convenience, and the addition of neutral salts to remove protein interference, followed by centrifugation. The modifications allow the use of an ordinary low-speed centrifuge and have increased the applicability of the original method.

We have generated 122 $F_{2:4}$ breeding lines from a cross of CX1834-1-6 (a low phytate line; Wilcox *et al*., 2000) with V99-3337 (high phytate line). Association between phytate data obtained by our method and the segregation of SSR (simple sequence repeat) marker alleles in the 122 $F_{2:4}$ families agrees with the map location of phytate-controlling QTLs reported by Walker *et al*. (2002). A breeding line selected for low phytate using QTL markers proved to have the lowest phytate content with our phytate assay.

Phytate content assessed by our procedure was significantly correlated ($r = 0.96$) with those obtained by the HPLC method (Kwanyuen and Burton, 2005). In comparison to the HPLC method, our protocol is simple, inexpensive, and high throughput. It provides a suitable method for efficient screening of low phytate breeding lines from large populations and for developing molecular markers closely linked to low phytate genes for marker-assisted selection.

References

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