Two Soybean Genotypes Lacking Lipoxygenase-1

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ABSTRACT

The U.S. Department of Agriculture soybean germplasm collection (6,499 accessions) was screened for genotypes with greatly reduced or missing lipoxygenase-1 (L-1) [linolate: O2 oxidoreductase, EC 1.13.11.12] and lipoxygenase-2 and L-3 (L-2 and L-3) activity. The L-1 assay used linoleic acid dispersed in Tween-20 at pH 9.0 as the substrate (acid assay) and the L-2 and L-3 assay used lino-leic acid methyl ester dispersed in ethanol at pH 7.0 as the substrate (ester assay). The spectrophotometric assay based on conjugated diene formation at 234 nm was used in the qualitative screening procedure. Two plant introductions (PI), 133226 from Indonesia and PI 408251 from Korea lacked L-1 activity. Oxygen uptake, electrophoresis and isoelectric focusing confirm the lack of detectable L-1 activity in the seed of these two genotypes. Radial diffusion against soybean seed lipoxygenase antiserum showed that the two genotypes are missing a precipitin band that normal soybean genotypes and purified lipoxygenase from soybean seed exhibit. Neither the L-1 variants nor any other accessions tested had greatly reduced activity with the ester assay.

INTRODUCTION

Lipoxygenase [linolate: O2 oxidoreductase, EC 1.13.11.12] has been implicated as the principal cause of the undesirable flavors of soybean products (1,2) especially soy milk (3). Additionally, the lipid hydroperoxides resulting from lipoxygenase action can lead to loss of nutritive value by the destruction of certain vitamins and protein (4). Also, lipid hydroperoxides and their breakdown products may have toxic effects (4).

In commercial soybean crushing operations, there is usually from 15 to 20 min between flaking of the seeds and extraction of the oil. This lipoxygenase has ample time to initiate the oxidation of the linoleic and linolenic acids and their esters in the oil (5). Heat treatment of soybeans prior to oil extraction increases the stability of the oil, presumably due to the inactivation of lipid oxidizing enzymes such as lipoxygenase, but has the undesirable consequence of reducing the solubility of the protein (5-7).

Soybean seeds contain at least three lipoxygenase isozymes, all having molecular weights (MW) of about 100,000. With linoleic acid as the substrate, lipoxygenase-1 (L-1) has a pH optimum at 9.5, lipoxygenase-2 (L-2) has a pH optimum at 6.5 and lipoxygenase-3 (L-3) has a broad pH optimum from 4.5 to 9.0. The isoelectric points of the three isoenzymes are different: L-1 is the most acidic (8,9), L-1 is the most reactive with free linoleic acid, whereas L-2 and L-3 are most reactive with methyl linoleate or trilinolein (10). On an equal protein basis, L-1 is 2.5 times as active as L-2 at its pH optimum, and L-2 is 2.5 times as active as L-3 or L-3b (11). L-1 is at least 36 times more stable than L-2 at 69 C (12).

Hammond et al. (13) and Chapman et al. (14) investigated the genetic and environmental influences on a number of chemical components of soybean seed that
affect soybean oil quality. They found that the level of unsaturated fatty acids was strongly influenced by the environment, but lipoxygenase activity was predominantly under genetic control. They therefore suggested that low lipoxygenase activity be used as a criterion for selection in soybean breeding programs.

Hymowitz has taken a different approach for improving the nutritional quality of soybean. He theorized that, unless a chemical component of soybean seeds is absolutely necessary for the survival of the domesticate, genotypes should exist in nature which do not have the chemical component. Hymowitz and coworkers have found soybean genotypes in which the nutritional quality of soybean is absolutely necessary for the survival of the domesticate, genotypes groups O0 to IV maintained at the germplasm lab, USDA.

The objective of this study was to screen the U.S. Department of Agriculture soybean germplasm collection for genotypes with greatly reduced or missing L-1, L-2, or L-3 activity in mature seeds.

**MATERIALS AND METHODS**

**Germplasm**

The 5904 soybean [Glycine max (L.) Merr.] accessions and 595 wild soybean (G. soja Sieb. & Zucc.) accessions evaluated for L-1, L-2 and L-3 activity were obtained from Drs. R.L. Bernard, Urbana, IL, and E.E. Hartwig, Stoneville, MS, curators of the northern and southern U.S. Department of Agriculture soybean germplasm collections, respectively (Table 1).

**Lipoxygenase Extraction**

Lipoxygenase was extracted from each accession by grinding one or more whole seeds (totaling more than 100 mg) in 3 ml buffer (0.06 M Tris, 0.015 M CaCl₂, 13% sucrose, pH 8.2) at 4 C for 5 sec using a Brinkmann Polytron (Model PT 20) at maximal setting. The extracts were centrifuged at 3,000 x g at 4 C for 15 min. The supernatant was saved and used immediately for all analyses except for the qualitative screening procedure in which extracts that were frozen were analyzed. Extracts from seeds of the cv. Williams were used as standards in all assays and Williams seed extracts which had been boiled for 1 hr were used as controls.

**L-1 Substrate**

An aqueous linoleate stock solution containing Tween-20 as the dispersant was used as described by Yoon and Klein (18) except that it contained 0.02% citric acid to chelate possible prooxidant contaminants, such as copper and iron, and 5 x 10⁻³ M NaCN to inhibit heme oxidases (19). The stock solution was diluted with 4 vol of 0.2 M borate buffer, pH 9.0, for all assays giving a final concentration of 2.57 mM linoleic acid. The substrate always was prepared immediately prior to use from linoleic acid stored frozen under nitrogen.

**L-2 and L-3 Substrate**

For the stock solution, 0.2 ml methyl linoleate was dissolved in 50 ml 95% ethanol containing 0.02% citric acid and 5 x 10⁻³ M NaCN. This was diluted with 4 vol of 0.1 M phosphate buffer, pH 7.0 giving a final concentration of 2.41 mM methyl linoleate. The substrate was prepared immediately prior to use.

**Qualitative Screening Procedure**

In screening for variants for L-1 activity, 10 µl of the seed extracts were mixed with 2 ml aerated L-1 substrate and the change in absorbance at 224 nM (conjugated diene formation) at 22 C relative to the control was monitored with a Beckman UV recording spectrophotometer (20). The same procedure was used in screening for L-2 and L-3 variants except that the L-2 and L-3 substrate was used.

**Quantitative Assays**

The same procedures were used as in the qualitative screening procedure except that extracts from 6 seeds of each accession were analyzed using the substrates as above and with a 1:10 and 1:100 dilution of the L-1 substrate in pH 9.0 buffer. Ten µl of the undiluted seed extracts was used for the ester assay and 10 µl of a 1:100 dilution of the seed extracts was used for the acid assay. One unit of activity was defined as an absorbance increase of 0.01 (min)⁻¹ at 234 nm. The protein content of the extracts was determined by the Lowry method (21).

**Electrophoresis**

Electrophoresis of the seed extracts on 10% polyacrylamide was performed according to the method of Davis but at pH 8.3 (22). The current was 1 ma/gel for 5 min and 3.5 ma/gel for 50 min. The buffer was at 4 C initially and electrophoresis was performed at room temperature. A volume of seed extract containing about 6 mg of protein was applied to each gel. Lipoxygenase activity in the gels was detected according to the starch-iodine method of Guss et al. (19) and using a specific lipoxygenase staining technique involving o-dianisidine (23,24) at pH 9.0.