MODIFYING SOYBEAN COMPOSITION BY PLANT BREEDING

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Plant breeding has played, and will continue to play, a significant role in the modification of soybean composition. In this discussion of plant breeding, I will use the more general definition which includes germplasm evaluation and genetic studies, as well as variety development and release. In this presentation, my comments about modifying soybean composition will be confined to examples of modifying the soybean seed.

Over the years plant breeders and geneticists have modified the composition of the soybean directly and indirectly. Many times plant breeders and geneticists have specifically attempted to change oil or protein levels or other specific components of the soybean seed. But they have also unknowingly altered composition in the process of varietal development or when concentrating on one specific component or character. It is probably safe to say that most soybean breeders have not emphasized composition at all, but other characteristics mainly having to do with productivity; the so-called agronomic characters, including yield, standability, maturity and resistance to hazards. Since soybeans are currently marketed by weight without regard to protein or oil content, farmers do not consider seed composition when selecting varieties. Consequently soybean breeders generally release varieties with high yield and good agronomic characters without giving major attention to oil or protein content or other components of composition. Composition is generally not evaluated until shortly before a new variety is released, and then only oil and protein levels are checked to make sure they have not been drastically altered.

In this paper, I will first discuss the general methods used by plant breeders as they attempt to alter soybean composition and then provide some specific examples of composition modification work that has been or is being done. Finally, I will attempt to provide some perspectives on the contributions plant breeding can make in further modifying soybean composition. I will restrict my discussion to the plant breeding considerations and not consider factors such as economic incentives, health issues, marketing strategies and other aspects which can play a significant role in determining what specific component is given attention.

Since plant breeding is a long-term undertaking (it takes 5-10 years to develop a variety plus perhaps several years of more basic genetic research), the alteration of composition by plant breeding must be approached with the expectation that the need for the "modified product" will be there when the breeding objectives have been achieved.

Actually, the breeding process is never finished since there is always the need for newer varieties that contain desired modifications along with improved agronomic performance.

The process of soybean composition modification by plant breeding begins with a decision that something in the soybean needs to be changed. The change may be a small or large percentage change or it may be the addition or elimination of a component. It may involve a major component like oil or protein or a specific enzyme or chemical substance like lipoxygenase-1 or linolenic acid. The decision on the need for change may be made by the scientist because of his/her particular interest, but often is made as a result of discussions with other scientists, processors, consumers and administrators. Once the decision on the desired modification is made, then detailed work can begin. For purposes of this discussion, I will assume that the analytical techniques for measuring the component have been developed so that the search for the desired level of the component can begin immediately.

The breeder frequently begins by screening the available germplasm for the presence of certain levels of the desired component. Named varieties, breeding lines, special genetic collections, the general soybean germplasm collection, and wild relatives of the soybean are available for screening. Each successive source listed above is a step further removed from an acceptable variety and generally represents additional time and effort needed in order to incorporate the modification into a variety that could be grown by soybean producers. If the desired level of the component was not encountered in the soybean and/or related species collections, then the breeder could consider using mutagens to induce changes or use transformation techniques to introduce a change from another species. Mutagenesis involves analyzing large numbers of seeds from treated plants attempting to find a seed with the desired modification. Transformation (which has not yet been successfully accomplished in soybeans) involves the transfer of foreign genetic material into the soybean, getting the trait expressed in the seed and passing it on to the next generation.

Once a desired modification is identified, it is necessary to ensure that the trait is heritable and to determine (at least in a general way) the type of inheritance involved. The type of inheritance will dictate the breeding procedure that can be used in variety development. Character or component traits are either qualitative or quantitative. Qualitative traits are controlled by a single gene, can be measured in distinct categories and are usually not influenced by the environment. Quantitative traits, on the other hand, are controlled by several genes, usually have a continuous distribution and can be greatly affected by the environment. An example of a strictly qualitative trait is the lack of the Kunitz trypsin inhibitor (Orf and Hymowitz, 1979b). This trait is controlled by one gene, has two distinct categories, has the presence or absence of the protein and is not affected by the environment. An example of a quantitative trait is protein level. It is controlled by many genes (the exact number is not known), has a continuous distribution (between 35% and 50%) and is affected by the production environment

(protein in a given variety can vary from 1 to 3% over years and locations).

Another consideration of qualitatively inherited traits is whether the gene is linked to other genes that may affect certain agronomic or compositional traits. Pleiotrophic effects, that is the effect a gene may have on other characters in the seed or plant, must also be considered. For example, metribuzin sensitivity (an undesirable character) is linked to phytophthora root rot resistance (a desirable character). Extra effort is required by the breeder to break the linkage before the desirable gene can be effectively used.

In the case of quantitative traits, breeders have to deal with two factors, heritability of the trait and correlation among traits (Brim and Cockerham, 1961). Heritability of a trait is usually defined as that proportion of the total variance that is due to genetic variation (the other part of the total is due to environmental variation). Hanson (1963) suggested that heritability estimates serve two purposes in plant breeding. First, such estimates measure the ease with which different traits can be selected in a population. For example, percent protein is more highly heritable than seed yield so it is easier to make progress in selecting for protein than selecting for yield. Heritability estimates are also used for predicting selection progress; that is, how much progress can be made toward achieving a particular goal under a given set of conditions. Knowledge of the relationship of one trait to another is important in plant breeding when selection is concentrating on one trait, because in selecting for one trait, other traits may be changed. Brim and Burton (1979) in a study to increase protein percentage were able to increase protein percentage from 42.8 to 46.1%, but the oil percentage decreased from 19.5 to 17.5%. This was due to a negative correlation between oil and protein.

Generally, modified composition is not found in highly productive, commercially acceptable varieties. So unless special incentives are offered, producers will not be interested in growing modified genotypes. Thus, the plant breeder needs to incorporate the specific modifications into an acceptable, productive genotype. The ease and length of time this takes depends on several factors including the type of inheritance and the genetic material that contains the modified trait. If the trait is controlled by a single gene (qualitative trait), the process is simple and quite rapid. Generally the backcross method is used to incorporate the gene into a productive variety. If winter nurseries and greenhouses are used, a modified variety might be available in about 5 years. An example of this was the incorporation of the genes for the lack of lipoxygenase-2 into the soybean variety 'Century' (Davies and Nielsen, 1987). If the modification desired is a quantitative trait, the development of a modified variety is much more difficult. Since several genes are involved, and all are not transferred from the original source into the desired genotype in a single cross but only during several cycles of crossing, the time to develop a variety can be quite long. The original source of the desired level of the modified component usually does not have high productivity and desirable agronomic characters due to linkages with detrimental characters,

negative correlations between the component and agronomic traits or the influence of the environment. Because of these difficulties, plant breeders generally expect to only partially adjust levels of a quantitative trait in an acceptable variety in a reasonable length of time (perhaps in the order of 10 to 15 years). Another approach that plant breeders have used to incorporate characters with multiple genes is a modified backcross procedure. In this procedure, some of the genes for modified composition are transferred from poor genetic backgrounds into higher yielding backgrounds (Wehrmann et al., 1987). This technique, however, requires the evaluation of large numbers of plants in each generation in order to recover a partially modified genotype and still takes 5 to 10 years.

Whenever a modification is incorporated into a good variety or genetic background, it is necessary to test for yield and other agronomic characteristics in order to make sure that the modification does not result in other undesirable changes. In the case of qualitative traits, relatively limited testing is required. However, in the case of quantitative traits, where the environment can exert a significant influence on the expression of a trait, more extensive testing is needed to adequately determine what affect the modification has had on other important agronomic traits such as yield, lodging, disease resistance, and oil and/or protein levels.

After this general account of what is involved in modifying composition by plant breeding, I would like to review some of the actual modification work that has been done by researchers. Since total protein and oil are the two components of soybean seed considered most valuable, considerable effort has been made to modify their levels.

Commercial varieties of soybeans contain about 40% protein and 20% oil. Lines in the germplasm collection, however, range in protein from about 30% to over 50% and from about 12% to almost 30% oil. If the related wild species are included the range is even greater. Although protein and oil content are quantitatively inherited (Brim, 1973), the heritability of each is moderate to high; thus, breeders have been able to modify protein and oil contents. Improved genotypes with protein contents as high as 50% have been reported (Brim and Burton, 1979; Miller and Fehr, 1979). Numerous researchers have been able to increase protein levels in many different genotypes. In some cases significantly higher protein levels have been incorporated into genotypes that are acceptable to growers (Wehrmann et al., 1987). Considerable improvements in oil content have also been achieved (Burton and Brim, 1981). Improved genetic material with 23% oil has been reported. There have been few attempts to breed for low levels of protein or oil but lower levels of oil have been observed in certain genotypes selected for high levels of protein because of a negative correlation between oil and protein level. Several studies (Brim and Burton, 1979; Shannon et al., 1972) have reported a negative correlation between yield and protein percentage, but there have been some exceptions. Such data indicates that high yielding, high protein soybean lines are possible. Low negative correlations between high oil and yield has generally been reported although some positive correlations have been noted (Brim,

1973); thus breeding high yielding, high oil lines should not be a major problem. Producing varieties with high protein, high oil and high yield would be very desirable but would be rather difficult from a breeding standpoint and may not be a realistic goal. Soybeans also contain carbohydrates, however, they have not received much attention by breeders. Genetic and breeding work on carbohydrates will be discussed later.

Protein, oil, and carbohydrates are general classifications of the primary constituents of soybean seed. Each of these primary constituents consists of many different compounds or components. First, I would like to consider some of the components of protein and research done to modifying these components.

Approximately 90% of the soybean seed protein is a globulin-type protein, which is composed of a number of fractions. These fractions have been identified by names and sedimentation densities. The identities suggested by Koshiyama (1983) are α-conglycinin (2S), a-conglycinin (7S), β-conglycinin (7S), Glycinin (11S) and poly-glycinin (15S). The 2S proteins contain several antinutritional factors, which will be discussed later. The 7S proteins have received increased attention recently and modification of the proteins has been suggested as a way to improve the quality of soybean protein (Wilson, 1987). Genetic variability exists for the 7S proteins (Beachy et al., 1983; Staswick and Nielsen, 1983), but plant breeders have not yet attempted to modify the 7S proteins. Modification of 11S proteins has also been suggested as a way to improve soybean protein quality (Koshiyama, 1983). Variability in 11S proteins has been reported in certain soybean varieties (Hughes and Murphy, 1983) and in certain lines of the wild relatives of soybean (Staswick et al., 1983). To my knowledge, soybean breeders have not attempted to modify the 11S proteins in improved cultivars. The 15S proteins have not yet been studied in detail.

There has been considerable research on the identification of specific 2S proteins and/or enzymes in soybeans as well as on the inheritance and incorporation of the variations into adapted varieties. Variant proteins or enzymes may have modified structures or activities or may be absent in certain lines. In almost all cases, the inheritance of the variants is relatively simple, usually of a qualitative nature. The next several examples show the progress and potential for modifying these components by plant breeding.

The Kunitz inhibitor is a major trypsin inhibitor found in soybeans. This protein is an antinutritional factor (Rackis, 1972). Four variants including a null type have been identified (Orf and Hymowitz, 1979a). Chick feeding studies with a genotype lacking the Kunitz protein have shown the effect of the lack of this protein (Bajaleih et al., 1978). Lack of the Kunitz trypsin inhibitor and the other variants of this protein have been incorporated into the variety 'Williams' (Bernard and Hymowitz, 1986a, 1986b). No adverse affects on yield or agronomic traits have been reported. Researchers have used these modified varieties to a limited extent, but they have not been grown commercially (R.L. Bernard, personal communication). Variants have also been

reported for the Bowman-Birk proteinase inhibitor (Stahlhat and Hymowitz, 1983), so incorporating modifications of this protein into productive varieties should be possible. Discussion of the Bowman-Birk inhibitor allows me to emphasize another point to consider when contemplating modification of soybean composition by plant breeding. The point is that in modifying one component in the soybean, the breeder may adversely affect other components. In the case of the Bowman-Birk inhibitor, Wilson (1987) suggests that although it may seem desirable from an antinutritional factor standpoint to develop genotypes devoid of the Bowman-Birk inhibitor, it must be remembered that this protein contains a relatively high level of cysteine, an essential amino acid present in relatively low concentrations in the soybean. Thus, the elimination of the Bowman-Birk protein by plant breeding becomes a value judgement of balancing the need for the sulfur amino acids in the protein against the antinutritional properties of the protein. Perhaps a better approach would be to search for a variant that lacks the antinutritional activity yet retains the essential molecular structure including the cysteines.

Another enzyme that has received considerable attention by geneticists and breeders is lipoxygenase. This enzyme has been implicated in flavor problems in soybean oil (Moll et al., 1979; Sessa, 1979). Three lipoxygenase isozymes (designated L-1, L-2, and L-3) occur in the seed of most soybean varieties (Yabuuchi et al., 1982). Genotypes which lack each of the isozymes have been identified (Hildebrand and Hymowitz, 1981; Kitamura et al., 1983; Davies and Nielsen, 1986). The lack of the individual isozymes and pairs of some of the isozymes, but not the lack of all three have been incorporated into the variety 'Century' (Davies and Nielsen, 1987). Recent research results indicate that the genetic removal of the L-2 isozyme only generally reduced off flavors associated with soybean products (American Soybean Association, 1987).

Variation in a number of isoenzymes and proteins in seed or germinating seed including urease, seed lectin, α amylase and β amylase have been reported (Hymowitz, 1983; Kiang and Gorman, 1983; Palmer and Kilen, 1987). In certain cases the variants have been incorporated into varieties (R.L. Bernard, personal communication). The commercial usefulness of these modified varieties depends on further research and demand for the modified varieties in the marketplace.

As we all know, the sulfur containing amino acids are present in limited amounts in soybeans. There has been screening of the cultivated as well as the wild soybean germplasm in search of variation in sulfur containing amino acids (Radford et al., 1977). The heritability of sulfur containing amino acids is moderate (Rosene, 1981) indicating progress can be made in modifying the levels. Because this approach deals with the sulfur containing amino acid deficiency from a quantitative genetics standpoint, some researchers suggest there might be easier ways to increase sulfur containing amino acids. Among the suggestions are to explore ways to increase the levels of specific protein components (such as the 11S protein or the Bowman-Birk protein) or to try to incorporate a gene from another species into soybean via transformation or some other molecular genetic manipulation.

Next, I would like to review some of the research conducted on modifying the components of soybean oil. Besides the considerable effort on modifying the total oil content of soybean, there has been a long-standing interest and effort in changing levels of the individual fatty acids. The fatty acid that has received the most attention is linolenic acid. The goal has been to develop a soybean with 3-4% linolenic acid. Screening of the germplasm did not reveal any genotypes with the desired level, but research to reduce the level of linolenic acid in soybean has been carried on for a number of years at Iowa State University (Hawkins et al., 1983) and North Carolina State University (Wilson et al., 1981) and more recently at Purdue University (Wilcox et al., 1984). Recurrent selection and/or mutagenesis has been used to develop lines with linolenic acid levels near 4%. Germplasm releases have been made from all three programs (Hammond and Fehr, 1983; Kuhr and Kinney, 1987; Wilcox and Cavins, 1986). The low linolenic acid levels have been incorporated into acceptable agronomic types and small scale industry tests have been conducted and acceptability of these genotypes for commercial production and from the industry for use in various products has been encouraging (W.R. Fehr, personal communication). Decisions are currently being made on the future use of low linolenic acid varieties and the use of the oil in industry. Preliminary results on research indicate that two genes may be involved in the genetic control of linolenic acid levels (Wilcox and Cavins, 1985; Kuhr and Kinney, 1987). The development of high yielding, high protein lines with lower levels of linolenic acid have been reported. North Carolina State Univ. researchers have attempted to breed for low linolenic acid by selecting for high oleic acid and have had some success using this indirect method (Wilson, 1987). Several commercial soybean breeders and public breeders are using low linolenic acid germplasm in their breeding programs.

Variation also exists for levels of palmitic, stearic, oleic, and linoleic acids (Wilson, 1987). Graef et al. (1985) reported producing three high stearic acid mutants by chemical mutagenesis. The high level of stearic acid was controlled by a single gene. In crosses of these mutants to high yielding genotypes, no detrimental associations of high stearic acid and agronomic characters were observed (Lundeen et al., 1987) although the level of stearic acid was not recovered in most improved lines. Earlier studies (Brossman and Wilcox, 1984) had shown no significant correlations between stearic acid content and agronomic characters. A low palmitic acid line has recently been developed at North Carolina (Wilson, 1987). Some recent work at the University of Illinois aimed at identifying plant enzymes that regulate protein and oil synthesis has indicated that a competitive interaction between enzymes is responsible for the relative amount of oil and protein in soybeans. The genes which control this enzyme activity have not been reported, but once they are, genetic control of oil and protein levels in soybean may be simplified.

Carbohydrates are generally not discussed much in soybeans, although the seed does contain starch and soluble sugars in addition to the structural carbohydrates. The oligiosaccharides, raffinose and stachyose are of most interest because they cause flatulence (Hymowitz

et al., 1972). There is genetic variability for total sugar content as well as oligiosaccharide content (Hymowitz et al., 1972; Openshaw and Hadley, 1981). Genetic studies have shown relatively high heritability for total sugars, but selection for total sugar content does not appear to affect oligiosaccharide content (Openshaw and Hadley, 1978). Genetic studies have shown total sugar content to be positively correlated with percent oil and negatively correlated with percent protein and yield (Hymowitz et al., 1972; Openshaw and Hadley, 1981). Few breeding programs, however, have attempted to modify total sugars or oligiosaccharides in soybean seed.

In this paper I have tried to demonstrate that significant modification of the soybean can be accomplished through plant breeding. Most plant breeders and geneticists would agree that if there is genetic variability for a character or trait, whether it be as general as total protein or as specific as activity of an enzyme, that trait can be modified by plant breeding. Although there are limitations to the amount of modification that can be done using naturally occurring variation in soybean or its relatives, additional useful changes can be produced using mutagenesis or possibly through biotechnology. In almost all cases, however, variations introduced by these latter methods will have to be manipulated to some extent by plant breeding in order to incorporate the desired modification into acceptable varieties which farmers will be willing to grow.

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