Factors influencing the PROTEIN AND OIL CONTENT of soya beans

By Prof Rob Gous

Soya bean, *Glycine max* (L.) Merr., contains about 200g of oil and 400g protein/kg seed dry matter. It is the major oilseed and protein crop in many regions of the world, providing approximately 60% of the world supply of vegetable protein. For both animal feed and human food utilisation, a high and stable seed protein content is desirable.

In South Africa, the soya bean industry is growing rapidly, with many first-time producers realising the benefits of this crop. However, in order to ensure that the local crop is acceptable to animal feed producers, it is essential that the protein and oil contents of the seeds produced are close to the quantities expected. A number of factors are known to influence the content of protein and oil in soya bean seeds, and these are described below.

The factors can be divided into those associated with the plant, the soil, the climate/environment and with the rhizobia involved in nitrogen fixation.

Plant factors

There are no differences in protein content among seeds within the same pod, but seed protein content has been shown to increase linearly from the lowest to the highest fruiting node of both normal and high-protein strains of soya bean (Escalante and Wilcox, 1993). The increase is from 344 to 432g/kg for normal and 420 to 509g/kg for high-protein genotypes. This increase in protein content is observed in both determinate and indeterminate varieties. The greatest range in protein within plants has been reported to be from 349 to 510g/kg for indeterminate and from 340 to 487g/kg for determinate plants.

The oil content of soya beans is somewhat different. It also varies with pod position on the plant and on the raceme (flower-bearing branches), but in this case the position of the seed in the pod also causes variation in oil content. Seeds from the lower half of plants are on average 5g/kg higher in oil than those from the upper half; beans near the tip of long, terminal racemes have less oil than those further down; and seeds in the tip of the pod have the highest oil content (Collins and Carter, 1956).

If an accurate determination is to be made of the oil and protein content of individual plants, the analysis of seed samples, representative of the entire plant, is essential.

Genetic variation in seed protein content is considerable, thus plant geneticists have the potential to increase protein content by selection. However, there is an inverse relationship between seed yield and seed protein content, especially in indeterminate types. This is true even though the seed protein and oil contents from determinate and indeterminate plant types are similar. Determinate varieties, therefore, appear to have a better potential to combine high seed yield with high seed protein than indeterminate varieties (Wilcox and Guodong, 1997).

Soil/fertilisation factors

Soya beans obtain nitrogen (N) from the soil and from symbiotic fixation, when nodulated with effective strains of rhizobia. These two sources of N may need to be supplemented with fertiliser N for maximum seed yield.

In an analysis of 637 data sets published between 1966 and 2006, Salvagiotti *et al* (2008) demonstrated that, on average, 50 to 60% of the soya bean N demand was met by biological N_2 fixation. In most instances, the amount of fixed N was not sufficient to replace the N removed from the field in harvested seed. Soya bean yield was more likely to respond to N fertilisation in high-yield (>4,5tons/ha) environments.

A negative exponential relationship was observed between N fertiliser rate and N_2 fixation, when N was applied on the surface or incorporated in the topmost soil layers. Deep placement of slow-release fertiliser below the nodulation zone, or late N applications during reproductive stages, may be promising alternatives for achieving a yield response to N fertilisation in high-yielding environments.

Sugimoto *et al* (2001) reported a positive correlation between the contents of protein and oil in seeds from nodulated soya bean plants. Seeds from nodulated plants grown on urea-treated soil exhibited higher protein and lower oil contents than those from plants grown on soil treated with coated slow-release N fertiliser (LP-100). The contents of these compounds, in seeds from nodulated plants grown on LP-100 soil, were almost the same as those from non-nodulated plants on the same soil. These observations indicate that N economy in roots during seed maturation affects the contents of storage compounds.

Mineral N absorption

Yield has been shown to be directly related to mineral N absorption in the first stages of the reproductive growth period (R2) and to high N_2 fixation rates at stage R6, whereas seed protein content was related to N_2 fixation efficiency during the reproductive growth period until late stages (R2 – R6 + 10d). N is therefore utilised independently for yield and for seed protein content (Fabre and Planchon, 2001).

Ham *et al* (1975) measured the responses to three readily available fertilisers (ammonium nitrate, urea, and urea plus sulphur) and two slow-release fertilisers (S-coated urea and urea formaldehyde), which were mixed into the top 20cm of soil before planting. One non-nodulating and two nodulating soya bean lines were used.

The response to N fertiliser was greater with the non-nodulating line than with the other two lines. Nitrogen fertilisation increased seed yield, weight per seed, seed protein percentage and kilogram of protein per hectare. Plant height and lodging were either increased or unaffected, depending upon locations and/or year. Seed oil percentage decreased following N fertilisation. However, the total oil production usually increased due to larger yields.

All sources of fertiliser decreased N_2 fixation, plant nodule weight, nodule number and weight per nodule. Increases in seed yield and/or seed protein percentage, in the nodulating lines, suggest that N_2 fixation failed to supply the amounts of N essential for maximum seed yield and/or protein percentage.

The oil content of soya beans vary with pod position on the plant and on the flower-bearing branches. (Photograph: news.uga.edu)

Inoculated seed

In the experiment above, sulphur (S) fertiliser increased seed yield in one case and decreased it in two other cases, depending on location and year. The total S percentage and S-containing amino acids of the seed were not increased with S additions.

In a pot experiment and with seeds inoculated with *Bradyrhizobium* inoculum before sowing, Morshed *et al* (2008) found that N application progressively and significantly increased the yield of soya bean up to the N rate of 26,5kg/ha, where the highest seed yield of 6,85g per plant was obtained. Nutrient uptake and protein content in seeds also increased with increasing levels of N (up to the same rate of 26,5kg N/ha).

A trial conducted in Canada, in which the effects of N fertilisation on yield and protein content were measured, demonstrated that the benefits of N fertilisation depend critically on whether the soya bean seeds have been effectively inoculated prior to planting. The use of an inoculant promoted root nodule formation, enabling the soya bean crop to fix its own N throughout the growing season, leading to high yield and quality at harvest.

However, in non-inoculated treatments it was apparent that 112kg N/ha as fertiliser or manure was insufficient to meet soya bean needs, since crops ripened prematurely and produced lower yield and seed protein. Where seeds were inoculated,

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high soil N may have inhibited nodulation by the root, leaving the crop dependent on soil N supply to meet needs throughout the growing season.

These results suggest that high levels of soil N reduce soya bean yield and quality, and that high soil N from fertilisation tends to cause increased vegetative growth, increased lodging and lower yield and protein content. The effects are likely due to reduced root nodulation, which is required for full season N supply to the crop. This has implications for nutrient management, suggesting that the application of N fertiliser or manure – on soya bean fields where the seeds have been effectively inoculated – is an inefficient use of this fertiliser and may adversely affect the crop.

Nitrogen fixation

Nitrogen fixation by soya beans provides N nutrition in a highly sustainable and economically competitive way and, most importantly, increases the seeds' protein content. N fixation by soya beans may be limited by various environmental conditions. Thus, there exists the need to optimise symbiotic fixation through agronomic and plant-breeding research.

In two experiments conducted in Australia by Pritchard and Cesari (1996), N fertiliser had no significant effect on protein content at one of the sites, indicating that nodules alone were able to maintain both protein content and seed yield, at this site where the previous soya bean crop had been inoculated. At the second site, where soya beans had not been grown previously, mean protein content (39,0%) under flood irrigation was somewhat lower (41,7%) than at the other site, indicating a possible benefit of higher rhizobia populations in soils with a soya bean history.

Apparently poor nodulation is not uncommon on fields where soya beans have been planted for the first time. Some of the reasons for this include a lack of viable bacteria having been placed on the seed, possibly due to improper storage of the product; the inoculant might not have stuck to the seed or was added unevenly; or there was a problem with plant infection. Excess nitrate, present in the soil at planting time, will also inhibit infection. If there is excess N in the soil, the plant will first use this nitrogen before allowing proper nodules to form.

In fields with a history of soya beans, the nodules can form later, but in first-time fields the opportunity may be missed. The roots may have grown past the point where the inoculant was placed, or the bacteria may have died due to dry conditions. This may be the reason why old forage fields sometimes have nodulation failures when first seeded with beans.

Delayed root infection

The initial high N level in the field may cause delayed root infection. For fields with a history of soya beans, the bacteria will survive for many years in most soils, once introduced. However, poor nodulation can occur even in an established field if the soil has a low pH or is sandy, if there is excess nitrate in the soil or if it is very dry.

Rhizobial inoculants can only improve yields when the legume crops do not have enough N. Inoculants will not solve additional problems, such as a lack of other soil nutrients (see below). N already present in the soil or left over from earlier fertiliser applications may reduce the N fixation and inoculation benefit. When there are already many rhizobia present in the soil that can stimulate effective N fixation, inoculation may not provide much further benefit.

For proper nodulation to occur, a relatively high number of rhizobia must be present in the soil. Soya bean plants secrete chemical signals (flavonoids) into the soil from the roots. These signals are detected by the bacteria, which in turn send a chemical signal back to the root to then elicit nodulation in the plant.

Factors that influence nodulation, nodular growth and nitrogen fixation include excessive or insufficient moisture, soil temperature, soil pH, diseases, organic matter and soil nitrate availability, as well as the rhizobial quality and bacterial strain in the soil. N fixation in soya beans is highly susceptible to soil drying, being more susceptible to water-deficit stress than any other physiological processes in the plant. This may partially explain why soya beans do poorly in dry years, when compared to other crops.

Soya beans generally do not respond to pre-plant N fertilisation, but there are a few exceptions, including poorly drained soils, low organic matter, low residual N, acidic soils and dry conditions.

Soil trace minerals

The efficiency of the process of N fixation in soya beans can be limited by micronutrient deficiencies, especially of molybdenum (Mo). Soya beans generally respond positively to fertilisation with Mo in soils of low fertility and in fertile soils, depleted of Mo due to long-term cropping. Seeds enriched with Mo could be a viable alternative to exterior seed treatment, because certain forms of Mo have been shown to be toxic to rhizobium when applied to seed at the time of inoculation.

Severe manganese (Mn) deficiency (less than 15ppm Mn in the leaves) has been shown to increase seed protein percentage and decrease seed oil percentage. Interestingly enough, seed from plants with extremely low leaf Mn levels contains higher percentages of linoleic, palmitic, linolenic and stearic acids, and a lower percentage of oleic acid. The percentages of seed protein, seed oil, and fatty acids changed markedly at low leaf Mn levels, but remained relatively constant above leaf Mn concentrations of 15 to 20ppm. Amino acid content in seed protein is relatively unaffected by Mn.

Locality and climate

Vollmann *et al* (2000) reports that the protein content of soya beans grown in the northern regions of the world is reduced, owing to climatic conditions such as low temperatures and high volumes of precipitation. In the United States, seed protein content in the Western and Eastern Corn Belt has been lower than in southern production regions over a number of seasons.

Similarly, protein content is reported to be low in the northern locations of Northeast China and in the northern sites of Europe, where large seasonal variations are observed in protein content. Apart from other findings, it was recently discovered that low root-zone temperatures reduce nitrogen fixation, which might explain the low protein content commonly found in northern areas of soya bean cultivation.

In their investigation, seed protein content was the highest for soya bean crops grown under moderately dry conditions and a high temperature during the seed-filling period. In experiments on stress during the seed-filling period, both drought and high-air temperature conditions enhanced the protein content by approximately 30 to 50g/kg, whereas oil content and grain yield were reduced.

At constant water-supply levels, both protein and oil contents were increased at a high temperature and fatty acid composition was most affected by temperature, whereas amino acid composition was stable. In other investigations dealing with water stress and irrigation of early maturing soya beans, the importance of timing the irrigation was emphasised. The protein content was highest with irrigation after the flowering stage, whereas it was lower with continuous water supply. This might be due to higher yield levels or higher oil content at optimum levels of water supply, which would dilute a given amount of protein.

Environmental conditions

After observing the variation in seed protein content of early maturing soya beans grown in Central Europe, their conclusion was that environmental conditions, i.e. both seasonal and location effects, can modify seed protein content considerably. Low protein content may be due to insufficient nitrogen fixation in cool seasons or to high levels of precipitation and a low temperature during the seed-filling period.

Karr-Lilienthal *et al* (2004) obtained soya beans from five leading soya bean-producing countries (Argentina, Brazil, China, India and the United States), which had been imported to the United States and processed into soya bean meal (SBM) under uniform conditions. Soya beans from China had the highest crude protein content, while soya beans – and the resultant soya bean meal – from Argentina had the lowest.

In *Table 1* the chemical composition of the soya bean samples is given (two from India, a low- and a high-protein sample) and then again after processing under standard conditions. It is interesting to note the very low protein content of the Argentinian soya beans (32,6%).

Table 1: Chemical composition of an individual sample of soya beans from five geographic locations, and of soya bean meals prepared from those produced in different regions, but processed under uniform conditions in the United States (from Karr-Lilienthal et al, 2004).

	Soya bean source					
	Argentina	Brazil	China	India		USA
				Low	High	
Soya bean composition						
DM (%)	91,0	90,5	90,6	93,2	91,9	90,1
Crude protein*	32,6	39,3	44,9	37,5	39,6	37,1
Fat*	14,1	13,6	12,9	13,1	12,8	15,1
Soya bean oilcake composition						
Crude protein*	47,4	57,0	58,5	54,6	57,8	53,2
Fat*	4,4	4,4	4,6	5,6	2,9	4,1

* On a dry-matter basis.

Maestri *et al* (1999), while working in Argentina, identified a trend towards lower protein content as latitude increased and a negative correlation between latitude and oil content. Also, altitude above mean sea level correlated positively with protein and oil contents and oleic acid percentage, while a negative correlation between altitude and linoleic acid was found.

Fatty acid composition has been shown to be strongly affected by temperature. Linolenic and linoleic acids decreased markedly, whereas oleic acid increased as temperature increased; palmitic and stearic acids, however, remained unchanged (Wolf *et al,* 1982). Oil content was positively correlated with temperature and protein content increased at the highest temperature.

Piper and Boote (1999) summarised a large data set, in which the temperature effects on protein and oil content of soya beans were measured. Oil content increased along with increasing temperature, with an optimum at 25 to 28°C above which the oil concentration declined. The protein concentration was either constant or only slightly increased with decreasing mean temperature below 28°C.

At temperatures greater than 28°C, protein concentration increased linearly with temperature. In addition to temperature, shortening day length may enhance the protein concentration by increasing the rate of nitrogen translocation to the seed and seed growth rate.

Water as a contributing factor

Sionit and Kramer (1977) studied the effects of controlled water stress on yield, applied at various stages of development and on two varieties of soya bean. Plants stressed during flower induction and flowering produced fewer flowers, pods and seeds than controls, because of a shortened flowering period and abortion of some flowers.

Stress during early pod formation caused the greatest reduction in the number of pods and seeds at harvest. However, yield – as measured by seed weight – was reduced most by stress during early formation and pod filling. However, water stress did not materially affect the oil or protein content of the seeds at any stage of growth.

Dornbos and Mullen (1992) studied the effect of environmental stress during soya bean seed fill on the chemical composition of the seed, which is known to reduce yield, viability and vigour. Across experiments, severe drought increased the protein content by 4,4%, while the oil content decreased by 2,9%. As drought stress increased, the protein content increased and the oil content decreased linearly at each ambient temperature.

> **FATTY ACID COMPOSITION HAS BEEN SHOWN TO BE STRONGLY AFFECTED BY TEMPERATURE.**

Drought stress

Seeds from plants exposed to 35°C during seed fill, contained 4% more protein and 2,6% less oil than those exposed to 29°C, when averaged across drought stress levels. Drought had little effect on the fatty acid composition of the oil, but high ambient temperatures reduced the proportion of the polyunsaturated components.

A meta-analysis of published data reported by Rotundo and Westgate (2009) showed that water stress reduces the content (mg per seed) of protein, oil and residual seed fractions. Protein accumulation, however, was less affected than oil and residual accumulation, resulting in an increase in final protein concentration (percentage dry weight). Growth at a high temperature also increased protein concentration in a manner similar to that observed with water stress. However, in neither case was the increase in protein concentration due to an increase in protein synthesis.

It is important to note that although the seed protein concentration increased during drought, the actual yield of protein was less. It is of key importance to the industry to maximise the yield of soya bean oil and protein per hectare, not necessarily the percentage of these components in the seed.

Agronomic practices

Delaying the planting date: The protein content of soya bean seeds can be increased by delaying the planting date. Helms *et al* (1990) conducted a study to determine if the gross value per hectare was increased sufficiently – due to the increased protein content, resulting from a delay in sowing date – to offset the decreased oil content and seed yield economically. By delaying sowing by five weeks, the seed protein content increased from 34,1 to 34,9%, but the oil content and seed yield decreased from 18,9 to 17,9% and 2,95 to 2,17tons/ha, respectively. The increased value due to a higher protein content did not compensate economically for the decreased oil content and yield from late sowing.

Plant density: Boroomandan *et al* (2009) applied three levels of plant density (15, 30 and 45 plants/m^2). Yield increased significantly (with 528kg/ha) as density increased from 30 to 45 plants/m^2, but seed protein was unaffected.

Since factors such as longitude, altitude, environmental temperature (heat units) and rainfall play major roles in determining the quality of seeds produced, it is apparent that soya bean producers have only a limited capacity to influence the oil and protein contents of seeds. Nevertheless, seed inoculation prior to planting, the judicious application of N and micro-elements such as Mo and Mg, and the choice of variety and planting time are managed by the farmer. These factors can make a difference and contribute to the quality and yield of soya beans, as indicated above.

More information and references are available from Prof Gous at gous@ukzn.ac.za.