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Meta-analysis of environmental effects on soybean seed composition

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ABSTRACT

The value of commodity soybean depends on the concentration of protein and oil in the seeds. While seed composition is primarily genetically determined, environmental conditions during seed development also affect seed component accumulation, and can result in protein and/or oil deficits for processing. To understand the general environmental effects on soybean composition, we conducted a meta-analysis of published data quantifying the effect of water stress, temperature, and/or nitrogen supply on seed protein and oil accumulation and their final concentrations. The meta-analysis showed that water stress reduced the content (mg per seed) of protein, oil and residual seed fractions. Protein accumulation, however, was less affected than were oil and residual accumulation, resulting in an increase in final protein concentration (% dry weight). Growth at high temperature also increased protein concentration in a manner similar to that observed for water stress. But in neither case was the increase in protein concentration due to an increase in protein synthesis *per se*. Increasing nitrogen supply to seeds cultured *in vitro* and to plants grown hydroponically increased both final seed protein concentration and content. But the magnitude of seed component response to experimental manipulation under field conditions was far less than that observed in the Uniform Soybean Regional Field Tests. Greater knowledge of the physiological processes that regulate these responses is essential to predict when and where future protein deficits might occur. Limitations of the meta-analysis approach and implications for future research on soybean seed composition are discussed.

1. Introduction

The economic value of the soybean seed depends on its protein and oil concentration (Brumm and Hurburgh, 1990; Hurburgh, 1994). The final composition of the seed is known to vary by genotype and in response to environmental conditions during seed development (Brummer et al., 1997; Westgate et al., 1999; Vollmann et al., 2000; Yaklich et al., 2002; Fehr et al., 2003; Wilson, 2004; Nichols et al., 2006). Although genetics are generally considered the main determinant of composition, environmental variation in protein or oil concentration may determine years or locations where deficits in crude soybean oil or protein meal yield occur (Brumm and Hurburgh, 1990). A major challenge for the processing industry has been to develop a general model of environmental effects on seed composition to predict regions and/or environmental conditions that produce consistently high quality soybeans.

Soybean seed components are usually quantified in terms of their concentration ([mg component/mg dry weight] × 100). This has practical value for marketing purposes, but provides little insight into the genetic or physiological basis for regional or environmental variation. Concentration is a mathematical construct that relates the content of a particular component to the total weight of the seed (i.e. the sum of all components). Therefore, seed protein concentration depends not only on protein content (mg per seed) but also on the content of oil and the residual fractions (primarily cell walls, soluble carbohydrates, and minerals). As such, two varieties that produce seeds with the same protein content (mg per seed) could be categorized as high or low protein genotypes, depending on the content of other seed components. It is the accumulation rather than the final concentration of major seed components that directly reflects physiological processes active during seed development, such as the maternal supply of nutrients to the seed or metabolic capacity to synthesize protein. The final concentration of each component is simply a culmination of these processes. To predict how environmental conditions ultimately affect the final protein and oil concentrations, therefore, it is necessary first to understand how the accumulation of each individual seed component responds to environmental conditions during seed filling.

Numerous estimates of regional variation in soybean seed composition exist (Hurburgh et al., 1990; Hurburgh, 1994; Piper

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and Boote, 1999; Yaklich et al., 2002; Dardanelli et al., 2006; Brumm and Hurburgh, 2006). These estimations, however, confound both genotypic and environmental effects, which makes it impossible to separate the relative importance of these two factors. The magnitude of the environmental effects on soybean seed composition across years and locations has yet to be isolated in a systematic manner.

A classical approach to understand the effects of environmental factors on seed composition has been through manipulative experiments (e.g. Gibson and Mullen, 1996; Purcell et al., 2004; Pipolo et al., 2004). Westgate et al. (1999) presented a general qualitative review on environmental effects on soybean composition. But a comprehensive quantitative evaluation of environmental effects on soybean composition is lacking. Although a wealth of studies conducted under a range of conditions on diverse genetic backgrounds have been published, the data generated have not been synthesized into a general model of environmental effects on seed composition.

Meta-analyses are designed to integrate information from diverse studies and conditions to evaluate treatment effects on a set of independent variables (Curtis and Wang, 1998). This approach has been widely utilized in the medical and the social sciences and in some biological studies (Rosenberg et al., 2000), but has been used sparingly in crop physiology research (e.g. Ainsworth et al., 2002; Morgan et al., 2003). Meta-analyses can be particularly informative because they provide a quantitative estimate of effect sizes. The effect size is calculated as the response to a specific treatment (e.g. irrigation) relative to a control (e.g. no irrigation). Although this approach evaluates quantitative treatments as qualitative (i.e. control vs. stressed), appropriate data partitioning captures the quantitative nature of treatment effects and can account for differences in treatment timing and intensity. The effect sizes for the response to nitrogen fertilization, for example, can be partitioned into dosage ranges. In some cases, however, it is not possible to parse treatments because their magnitude and intensity are not documented in comparable units. Nonetheless, judicious data parsing prior to meta-analysis enables investigators to isolate potential experimental or biological influences on effect size. The main objective of this study was to use a meta-analytical approach to synthesize published data from studies addressing the effect of major environmental conditions on soybean seed composition. The meta-analysis incorporated studies focused on effects of water stress, temperature, and N supply on soybean seed component contents and concentrations.

2. Material and methods

2.1. Magnitude of environmental variation in seed composition in uniform field tests

The Uniform Soybean Test Northern Region (USDA-ARS) evaluates yield and composition of soybean varieties and germplasm with potential for commercial release. The data generated in these tests are particularly valuable for evaluating environmental effects because all locations use uniform management protocols and plots are replicated in standard statistical field designs. Seed harvested from the uniform tests are analyzed at the USDA-ARS National Center for Agricultural Utilization Research, Peoria, Illinois for protein and oil concentration and these data are reported on a moisture-free basis.

We re-analyzed 10 years (1989–1998) of composition data from the Uniform Soybean Test Northern Region (USDA-ARS) to assess the magnitude of environmentally induced variation in seed composition within the major soybean growing area in the U.S. Three varieties within maturity groups from 00 to IV having the greatest number of year-by-location replications were selected for analysis. Standard descriptive statistics were used to assess variation in composition across environments.

2.2. Database construction and evaluation

A database of published articles was constructed by searching the *ISI Web of Knowledge*® database that included the *Web of Science*® citation database (1945-present), the *Current Content Connect*® (1998-present), the *ISI Proceedings*® (1991-present) and the *Biosis previews*® (1980-present) (Appendix A). The search was intended to identify all published research articles that evaluated the effect of water deficit stress, temperature increase and nitrogen availability on seed component concentration (mg component per mg seed dry weight) and/or content (mg component per seed). Ideally, each study quantified both component concentration and component content, or provided seed weight from which we calculated component content. The majority of studies, however, evaluated treatment effects only on component concentration. If our database included only studies evaluating both variables, there would have been insufficient data to resolve treatment effects.

Studies that simply correlated rainfall or temperature during development with variation in seed composition were not included in the database. While this correlative approach might suggest a causal effect, it is often confounded by other factors. For example, a decrease in seed protein content associated with water deficit stress late in the season may have been caused by a concurrent increase in temperature or decrease in nitrogen supply. Therefore, only those studies in which the experimental design compared a manipulated treatment to a control treatment were included. Since meta-analysis considers each observation to be independent (Curtis and Wang, 1998), data for different years or experimental conditions (i.e. cultivars or other experimental factors) within each publication were treated as independent observations. This approach is consistent with other published meta-analyses (Curtis and Wang, 1998; Ainsworth et al., 2002; Jablonski et al., 2002; Morgan et al., 2003). Data were obtained directly from tables or by digitalizing data in figures utilizing DigitizeIt Software (Version 1.5.7, 2003 Bormann, Braunschweig, Germany). A description of the experimental details and treatments included in the meta-analysis is available online as supplemental information for this article.

Studies evaluating the effect of water stress on seed composition required a well watered control treatment and a water deficit treatment and were partitioned into pot or field experiments. Also, they were parsed by phenological stage when water stress was imposed: whole cycle (V1–R8), early reproductive period (R1–R5) or late reproductive period (R5–R8).

Studies that addressed the effect of temperature on soybean composition were included if they compared a control treatment to one at a higher temperature. Articles that evaluated temperature effects were classified further as *in vitro* or pot experiments. *In vitro* experiments cultured seeds or embryos in artificial nutritive solution to isolate them from the influence of maternal tissue (e.g., Obendorf et al., 1984). Pot experiments also were parsed by phenological stage when the temperature increase was imposed: whole cycle (V1–R8), whole reproductive period (R1–R8), early reproductive period (R1–R5) or late reproductive period (R5–R8). Piper and Boote (1999) observed that seed protein concentration responded differently to temperatures above or below $\sim$26 °C. Therefore, temperature studies were classified where possible according the range of temperatures explored: Low Range $<$26 °C and High Range $>$26 °C.

Studies evaluating the effect of nitrogen supply had a control treatment and a treatment with increased nitrogen availability.

These experiments were categorized as conducted *in vitro*, in hydroponics, or in the field. *In vitro* experiments included seed or embryo cultures at various levels of amino nitrogen supply. Hydroponics experiments involved intact plants cultured with nutrient solution in the absence of soil. Field experiments included various forms of fertilizer N and were parsed by phenological stage at which fertilizer N was increased: vegetative (planting-V6), early reproductive (R1–R5), and late reproductive (R5–R8). The effect size for response to fertilizer N also was analyzed according to N level applied with dosages ranging from <55 kg N ha^{-1} to >200 kg N ha^{-1}.

2.3. Statistical analysis

Meta-analysis evaluates treatment effects from different studies on a common scale of effect size. The natural log of the response ratio r (r = value in the treatment/value in the control) was utilized for this purpose (Hedges et al., 1999). This measure of effect size was selected because the transformation linearizes the response and generates a more normal distribution for r (Hedges et al., 1999). For clarity, however, data are presented graphically as relative responses ([(treatment − control)/control] × 100). MetaWin software was utilized to conduct the meta-analysis (Rosenberg et al., 2000).

If estimates of treatment variation (e.g. standard deviation) are available, it is possible to conduct a weighted meta-analysis in which effect sizes are weighted by the inverse of the sampling variance (Gurevitch and Hedges, 1999). This procedure increases the precision of the estimates and increases the resolving power for treatment effects since more weight is given to experiments with smaller errors (Gurevitch and Hedges, 1999). Since the majority of the articles dealing with soybean composition that could be included in the meta-analysis did not report a standard error or standard deviation for treatment means, effect sizes from all studies were given equal weight.

Confidence intervals for each effect size were calculated using the re-sampling (bootstrapping) technique developed by Adams et al. (1997) for meta-analyses. Briefly, this technique involves calculating the mean effect size for a randomly selected set of n studies. The process is iterated 4999 times and the output is ordered sequentially; the smallest and largest 2.5% values then are used as the lower and upper confidence intervals (Adams et al., 1997). An effect size was considered significant when the 95% confidence interval did not overlap with zero. Likewise, differences between treatment categories were considered significant if their 95% confidence intervals did not overlap (Gurevitch and Hedges, 1999).

3. Results

3.1. General environmental effect on soybean seed composition

To describe the environmentally induced variation in seed composition typically observed in the field, we analyzed 10 years of data from the Northern Region Uniform Soybean Tests. The subset of varieties with the greatest number of data years and locations in replicated trials were selected for evaluation. Fig. 1 shows that the variation in seed composition due to environmental factors was quite large. For example, the variety Lambert (MG0) varied from 33% to 40% protein across environments (Fig. 1a). Each of the data points in Fig. 1 corresponds to the mean of 3–4 replications, so the points outside the whiskers cannot be considered outliers. Averaged across all genotypes, the relative difference between maximum and minimum values was 18% for protein and 23% for oil.

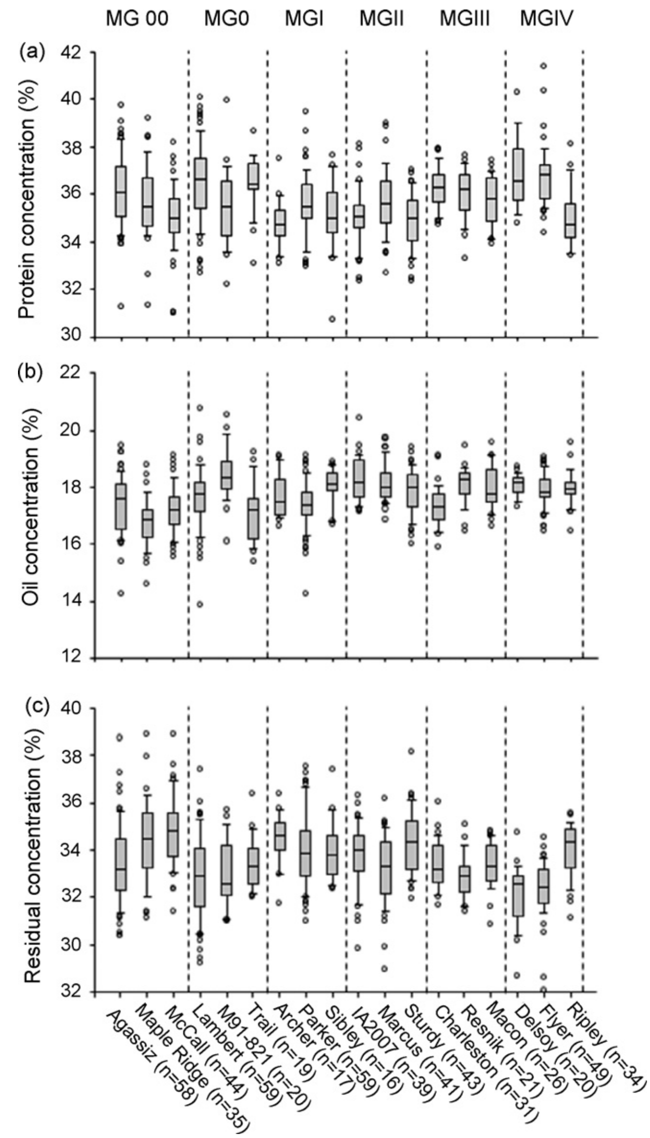

Fig. 1. Box-plot of environmentally induced variation on soybean seed protein (a), oil (b) and residual (c) concentration (%). Data are compiled from the Uniform Soybean Test-Northern Region, 1989–1998. The box indicates the upper quartile, the median and the lower quartile. The whiskers plus the box correspond to the 80% of data points. Points outside the whiskers have 20% chance of occurrence. N: number of replicated trials included for each variety. Bars shows 10th and 90th percentile.

3.2. The meta-analysis

Meta-analysis results are presented in two sections. First, we present the effect of water deficit stress, temperature increase, and nitrogen availability on seed component content (mg per seed). Then, we integrate the relative effect of environmental factors on each seed component contents to quantify its impact on final concentration (%). This approach clearly exposes the basis for variation in final concentration due to the impact water, temperature, and N supply on individual seed component accumulation.

3.2.1. Environmental effect on seed component content

3.2.1.1. Protein content (mg per seed). In general, water stress decreased seed protein content (Fig. 2a). Across all studies, the relative reduction was about 16% (overall category). In those few studies in which water stress was imposed early in reproductive

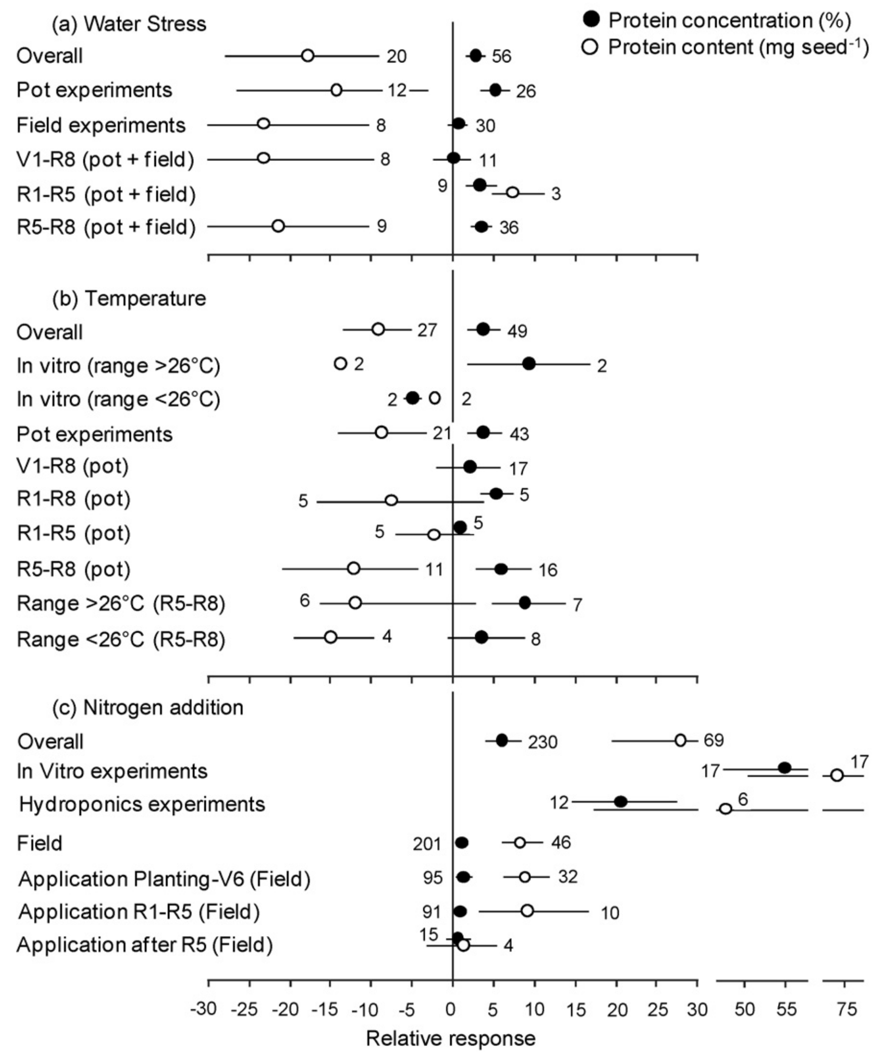

Fig. 2. Relative response [(treated − control)/control × 100] of soybean seed protein content (mg per seed) and concentration (%) to water stress (a), temperature increase (b), and nitrogen supply (c). Symbols and bars represent mean response and 95% confidence interval, respectively. The number of studies included in the meta-analysis are indicated in the figure.

development (R1–R5), however, seed protein content increased about 6%. This increase is likely a response to a decrease in seed number typically caused by water stress at this stage. Larger seeds develop on these plants which have a greater source to sink ratio during seed filling (Borrás et al., 2004).

An increase in temperature also reduced protein content (Fig. 2b). The relative reduction across all studies was ~9%. The effect size varied with the experimental approach and range of temperatures explored. Protein content *in vitro* was reduced by increasing temperatures much more in the High Range (14%) than in the Low Range (2%). In pot experiments, increasing temperature in the High Range during rapid grain filling (R5–R8) did not affect protein content significantly. But an increase in temperature in the Low Range reduced protein content by 15%. This difference in effect size likely reflects the lower protein content of seeds grown in the higher temperature range.

Providing supplemental nitrogen increased protein content about 27%, averaged across all studies (Fig. 2c). The magnitude of this effect size was determined primarily by the response of embryos grown *in vitro* (~60%). Whole plants grown in hydroponics also responded favorably to an increase in nitrogen supply, and to a much greater extent than did plants grown in the field. On average, seed protein content responded positively (~8%) to supplemental nitrogen fertilization across all field studies. A similar effect size was observed for supplemental N applied during vegetative growth (planting-V6) and early reproductive development (R1–R5). Nitrogen dose also had a significant impact on the effect size. During the vegetative stage, fertilizer N applied at <100 kg N ha^{-1} increased seed protein content about 2%, relative to the control, while application at >200 kg ha^{-1} increased seed protein about 14% on average (data not shown). Similar dosage-dependent responses were observed for supplemental nitrogen applications at early and late reproductive stages (data not shown).

3.2.1.2. Oil content (mg per seed). Water stress decreased oil content per seed dramatically (Fig. 3a). Across all studies, the reduction was ~25%, relative to the well-watered control treatments. The timing of stress also was very important. When water stress was imposed early in the reproductive period (R1–R5), the effect size was not different from zero. Water stress imposed during seed filling (R5–R8) decreased oil content about 35%.

Consistent with the decrease in seed protein content, increased temperature also reduced oil content 15% over all experiments (Fig. 3b). Timing again was important as there was no significant

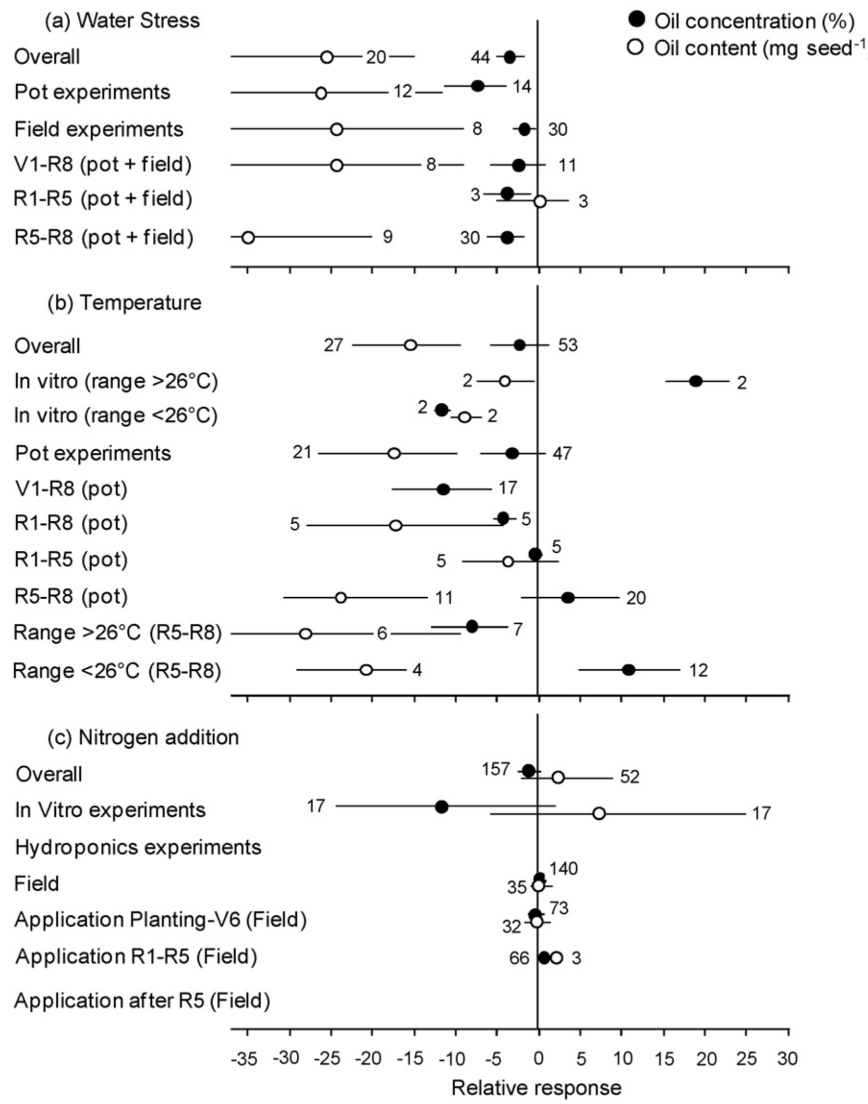

Fig. 3. Relative response [(treated − control)/control × 100] of soybean seed oil content (mg per seed) and concentration (%) to water stress (a), temperature increase (b), and nitrogen supply (c). Symbols and bars represent mean response and 95% confidence interval, respectively. The number of studies included in the meta-analysis are indicated in the figure.

response to increased temperature during R1–R5, while increased temperature at R5–R8 decreased oil content by ∼20%, relative to the control.

Increased nitrogen supply did not affect seed oil content significantly, averaged across all studies (Fig. 3c). An increase was observed in some *in vitro* studies, but the effect size was highly variable. Seed oil content did not respond to increased nitrogen supply in the field. Nor was there a significant response to nitrogen dosage (data not shown).

3.2.1.3. Residual content (mg per seed). Residual content (mg per seed of structural and non-structural carbohydrates) was reduced by water stress ∼20% across all experiments (Fig. 4a). The effect of developmental stage on effect size mirrored that observed for protein and oil content. Water stress imposed between flowering and beginning seed fill (R1–R5) increased residual content 4%, while stress during seed filling decreased residual content nearly 30% relative to the control.

Averaged across all studies, residual content was reduced 15% by an increase in temperature (Fig. 4b). *In vitro*, residual content decreased in response to a temperature increase in the High Range, but increased in the Low Range. Residual content in potted plant studies decreased significantly in response to higher temperature (∼15%); this response was similar in the high and low temperature ranges.

There was a small decrease in residual content in response to an increase in nitrogen supply, averaged across all studies (Fig. 4c). The lower residual value reflected the large decrease reported in the *in vitro* studies (∼11%). Increased nitrogen availability did not affect residual content in any of field studies, and there was no difference among nitrogen dosages (data not shown).

3.2.2. Combined effects of seed component content on final protein and oil concentration

The meta-analysis on seed component content revealed that the major seed components (protein, oil, residual) did not necessary respond to water supply, temperature, and nitrogen supply in the same direction or to the same extent. As such, the meta-analysis on seed component concentration (%) that follows is presented with particular emphasis on the relationships among individual component contents (*mg per seed*) as they determine final seed composition.

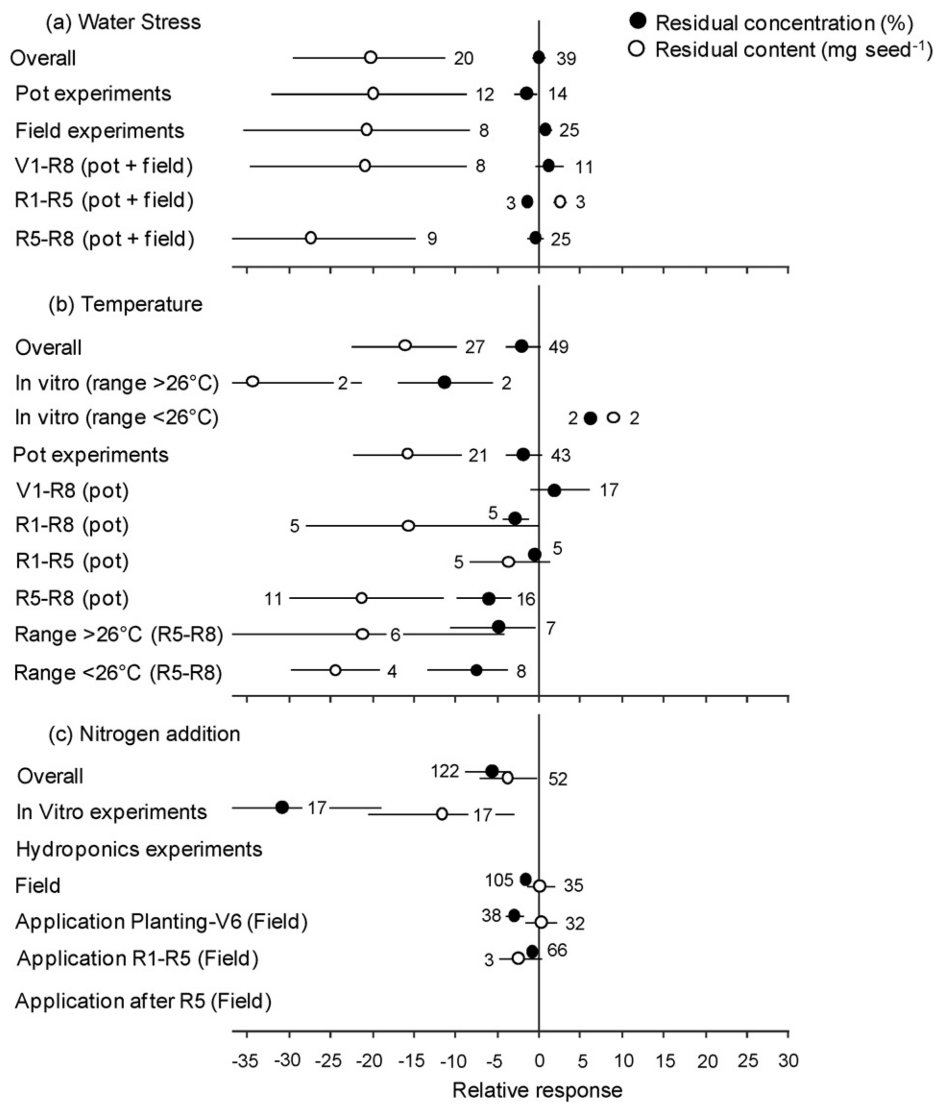

Fig. 4. Relative response [(treated − control)/control × 100] of soybean seed residual content (mg seed^{-1}) and concentration (%) to water stress (a), temperature increase (b), and nitrogen supply (c). Symbols and bars represent mean response and 95% confidence interval, respectively. The number of studies included in the meta-analysis are indicated in the figure.

3.2.2.1. Water stress. Water stress reduced seed protein, oil and residual content. On average, the negative impact was more pronounced on oil and residual than on protein content (Figs. 2a, 3a and 4a). Although the direction of response among the major seed components was similar, the difference in magnitude of response resulted in a significant increase in protein concentration (∼3%) and similar decrease in oil concentration (∼3%).

The effect size depended on the timing of the stress (Figs. 2a, 3a and 4a). Water stress imposed throughout the plant life cycle (V1–R8) reduced the content of all seed components to the same degree (∼25%). As a consequence, the final concentrations of protein and oil in the seed were not affected significantly. Water stress imposed during late reproductive development (R5–R8) decreased protein content less than oil and residual contents, on average. This difference in response resulted in a significant increase in final protein concentration in the stressed seeds (Fig. 2a). In contrast to this general pattern, stress imposed prior to rapid seed fill (R1–R5) resulted in an increase in protein content and concentration. This likely reflected an increase in source/sink ratio caused by a decrease in seed number per plant when stress is imposed at this stage of development (Borrás et al., 2004).

3.2.2.2. Temperature. Increasing temperature decreased seed protein, oil and residual contents in most cases, although the effect sizes generally was less than observed for water stress (Figs. 2b, 3b and 4b). On average, the decrease in content was less pronounced for protein (∼ 7%) than for oil or residual contents (∼15%), which resulted in a net increase in protein concentration in response to increased temperature.

The response of seed components to increasing temperature *in vitro*, i.e. in the absence of maternal tissues, depended on the temperature range explored (Figs. 2b, 3b, and 4b). At high temperatures (>26 °C) all seed component contents were reduced. Residual content, however, was affected to a greater extent, resulting in an increase in protein and oil concentration as temperature was increased. At lower temperatures (<26 °C) protein and oil contents decreased only slightly, but residual content increased causing a modest decrease in protein and oil concentration.

Increasing temperature around plants grown in pots decreased seed protein content about 9%, and decreased oil and residual contents 18–15%, on average (Figs. 2b, 3b, 4b). The smaller response of protein accumulation resulted in a significant increase in final protein concentration, with no changes in oil concentration and in residual concentration.

The range of temperatures also affected the direction and magnitude of the treatment effects. Treatments imposed in the high temperature range (>26 °C) has opposite effects on protein and oil concentrations (Figs. 2b and 3b). Changes in component contents were relatively large and in the same direction, but the decrease in protein content less pronounced in average (12%) than that of oil content (28%) and residual content (20%). The net result was a decrease in oil concentration and increase in protein concentration. An increase in temperature in the low temperature range (<26 °C) apparently had no significant effect on protein concentration and increased oil concentration (Figs. 2b and 3b). This result, however, is not consistent with the component content data showing that oil content decreased more than protein content. This should have led to an increase in protein concentration. The lack of consistency in this particular case is likely due to incomplete data on seed component content and concentration in studies conducted in the low temperature range.

3.2.2.3. Nitrogen. Averaged across all experiments, an increase in nitrogen supply increased seed protein content ~20%, with little change in oil content and a 5% reduction in residual content (Figs. 2c, 3c and 4c). The dramatic response of protein content, however, corresponded to only a modest (~5%) increase in protein concentration (Fig. 2c). The effect size for oil was not different from zero while residual concentration decreased more than 5% (Figs. 3c and 4c).

The magnitude of effect size on component concentration varied dramatically with experimental approach. Nitrogen treatments *in vitro* increased protein content ~60%, had only a small (non significant) impact on oil content of ~7%, and decreased residual content about 12% (Figs. 2c–4c). The net outcome of these component responses was an average increase in protein concentration of more than 50% relative to control treatments. In contrast, the response of seed protein concentration to increased nitrogen supply in the field was only about ~1% for all studies evaluated. There was, however, a tendency for an increase in response at greater nitrogen rates. In studies that applied nitrogen fertilizer nitrogen at >100 kg ha^{-1} between planting-V6, protein concentration increased ~2%. Similarly, for nitrogen applications >100 kg ha^{-1} at R1–R4, protein concentration responded ~3% (data not shown). Seed protein concentration did not respond to rates <100 kg N ha^{-1}; in no case were oil and residual concentrations affected by fertilizer N applications. When only those field experiments that reported both protein content and concentration were considered, the response of protein concentration to nitrogen fertilization was as great as 7% at rates >200 kg N ha^{-1} applied at planting (data not shown).

4. Discussion

To our knowledge, this meta-analysis is the first attempt to distinguish the responses of soybean seed components to specific environmental conditions across genetic backgrounds, experimental approaches, and stages of development. Analyzing the environmental effects on component content (mg per seed) as well as evaluating effects on component concentration (mg g^{-1}) revealed that an increase in concentration does not necessarily result from a stimulation of component synthesis. During water stress or high temperature synthesis of all the major seed components was inhibited. Differences in concentration result primarily because accumulation of individual components was not inhibited to the same extent.

4.1. Water stress

Water stress imposed during seed filling decreased oil and residual contents more than it did protein content. This generally resulted in an increase in final protein concentration. It is well established that water stress shortens the seed filling duration and apparently has little impact on rate of filling (Meckel et al., 1984; Westgate et al., 1989; Egli and Bruening, 2004). It is also well documented that water stress in soybean accelerates nitrogen remobilization from leaves (Brevedan and Egli, 2003; DeSouza et al., 1997). Since 50–100% of seed nitrogen is remobilized from leaves (e.g. Egli et al., 1983; Chapin et al., 1990; Turner et al., 2005), water stress during seed filling may increase the rate of N remobilization to the seed, resulting in a temporary increase in amino-N availability. While a significant amount of seed nitrogen is derived from soil uptake and N_2-fixation during the seed filling, remobilization of reduced nitrogen from the leaves will buffer a stress-induced shortage due to inhibition of uptake and fixation (Triboi and Triboi-Blondel, 2002). Synthesis of oil and carbohydrate by the seed, on the other hand, depends primarily on concurrent carbon fixation during seed filling (Yamagata et al., 1987). Therefore, a reduction in assimilate supply caused by water stress would likely have a more direct impact on the oil and residual component synthesis. Apparently, the increase in nitrogen remobilization from the leaves and pod walls is sufficient to compensate for the reduced rate of oil and residual synthesis resulting in a fairly constant seed growth rate, at least temporarily (Meckel et al., 1984; Westgate et al., 1989). Since water stress shortens the duration of seed filling, all component contents are reduced but protein synthesis is apparently less affected due to the increased amino-N remobilization, resulting in a net increase in protein concentration in the mature seed.

An alternative possibility is that the rates of protein, oil and residual accumulation are not affected by water stress, but durations of individual seed component accumulation are in fact affected differentially. That is, remobilization in water stressed plants may enable protein accumulation to continue longer than oil and residual accumulation. Our current studies are aimed at determining how environmental effects on the rate and duration of individual seed component accumulation affect their final concentration in the seed.

4.2. Temperature

According to our meta-analysis, accumulation of oil and residual are negatively affected by increased temperature in the high temperature range during seed filling, but protein accumulation was not significantly affected. This response to temperature also has been observed in rapeseed (*Brassica napus*) and sunflower (*Helianthus annuus*) (Triboi and Triboi-Blondel, 2002). Higher temperatures are known to increase seed growth rate and reduce seed filling duration (Egli and Wardlaw, 1980; Chimenti et al., 2001). Likewise, higher temperatures increase the rate of seed component accumulation up to an optimum while duration of accumulation is reduced (Bhullar and Jenner, 1985; Jenner et al., 1991). In soybean, an increase in the rate of nitrogen remobilization due to accelerated leaf senescence may support an increased rate of seed protein accumulation at higher temperature (Triboi and Triboi-Blondel, 2002; Egli and Wardlaw, 1980) even if total nitrogen uptake or fixation are reduced due to a reduced seed filling period or inhibited (Triboi and Triboi-Blondel, 2002). Apparently, the ability to maintain the rate of protein accumulation partially compensated for the shorter seed fill duration, resulting in a less deleterious effect on final protein content. Oil and residual contents, on the other hand, are more dependent on current photoassimilate production and are negatively affected by increased temperature, particularly in the high temperature range, primarily because of a shortened duration of seed filling (Triboi and Triboi-Blondel, 2002; Yamagata et al., 1987). In this case, there is no buffer (i.e. nitrogen in the leaves) to compensate for a shorter duration of filling; thus temperature stress only causes oil or residual concentrations to decrease.

Response of isolated embryos to increased temperature *in vitro* was markedly different from that observed in studies conducted on whole plants or in the field. The meta-analysis of *in vitro* experiments indicated that increased temperature in the High Range (>26 °C) markedly reduced the residual content of the seed, which increased both protein and oil concentrations. Embryos exposed to increased temperature in the Low Range (<26 °C) showed a modest increase in sucrose partitioning to oil (Iyer et al., 2008). These responses varied from those *in planta* in which elevated temperature caused an increase in final protein concentration and reductions in oil. The results indicate that temperature effects on seed component accumulation are moderated to a large extent by the response of maternal tissues.

4.3. Nitrogen

Seed protein content was much more responsive to an increase in nitrogen supply *in vitro* than in whole plant studies conducted in hydroponics or in the field. *In vitro* studies exposed embryos to increasing concentrations (and unlimited amounts) of amino nitrogen in the medium (Saravitz and Raper, 1995; Hayati et al., 1996; Pipolo et al., 2004). Since amino-N uptake is partially mediated by non-saturable facilitated diffusion (Zhang et al., 2007), amino acid uptake can continue at concentrations well above physiological levels likely to occur *in planta*. Consequently, very high protein contents and concentrations can be achieved by embryos grown *in vitro* under non-limiting conditions. These high values presumably reflect a genetic potential for protein accumulation that is not expressed *in planta*.

Whole plant studies confirm that fertilization with inorganic nitrogen inhibits nodule formation and nitrogenase activity (Streeter, 1988). In the presence of abundant soil NO_3^- or NH_4^+, soybean plants decrease amino-N transported to the shoot derived from atmospheric N-fixation. As such, application of fertilizer nitrogen to the soil may not lead to an increase in nitrogen availability to the seed. There are some evidence, however, that application of fertilizer nitrogen at very high rates (>200 kg ha^{-1}) early in development can result in measurable increases in protein concentration and content.

4.4. Field variation in seed composition vs. experimentally induced variation analyzed by meta-analysis

The relative variation (between percentile 90th and 10th) in seed protein and oil concentration for 10 years of the Northern Region Uniform Soybean Tests averaged across all varieties was 10% for protein and 11% for oil concentration (Fig. 1). If relative variation is estimated with average maximum and minimum it raises to 18% for protein and 23% to oil. Since each data point presented in the Fig. 1 corresponds to an entire experiment with 3–4 replications, none can be considered an outlier. Regardless of the type of estimation considered, this environmentally induced variation within the uniform field tests was, in general, greater than the variation observed for the field and greenhouse studies in which environmental variables were purposefully manipulated.

Why is the magnitude of seed component variation reported in experimental manipulation studies in the field less than that observed in the uniform field trials? It is possible that undocumented biotic stress from pests and diseases added to the variation among years and location in these trials. None of the field trails, however, reported infestations of pests or diseases. A more likely explanation is that the cultivars tested in field trials were exposed to multiple abiotic stresses simultaneously or sequentially during the season. Drought stress during seed filling is often associated with higher temperatures and low N availability (Sinclair et al., 1987). The occurrence of multiple stresses (water deficit, high temperature, low N availability) acting simultaneously has been shown to activate a suite of genes different from those induced by a single stress (Mittler, 2006). It is also possible that some of the uniform test locations encountered brief but extreme environmental conditions not recorded in the trial (e.g. Wardlaw et al., 2002; Rondanini et al., 2006). The occurrence of such multiple or repetitive stresses would not be accounted for in the manipulative experiments included in the meta-analysis.

4.5. Limitations and recommendations for future research on seed composition

Focusing the meta-analysis approach on seed component content along with concentration revealed several situations where an increase in seed component concentration was not associated with an increase in synthesis. This basic observation provides important insight for future experiments to understand the physiological factors regulating soybean seed composition. Final component concentrations (mg mg^{-1}) result from changes in the relative content of all seed components (mg per seed). These in turn are determined by the rate (mg seed^{-1} d^{-1}) and the duration of their accumulation (days). Resolving the complexity of seed composition into these simpler developmental components may provide a more rational approach for defining the genetic mechanisms that regulate seed metabolism and development.

That many studies have overlooked this complex interplay between seed metabolism and development is evident in their focus only on final component concentration. Studies lacking component content (or even seed mass) data limited the resolving power of the meta-analysis since it was necessary to utilize a combination of data sets to analyze the effect of each environmental factor on seed composition. The effect of N-supply on seed protein, for example, included 230 studies reporting seed protein concentration, only 69 of these reported seed protein content (Fig. 2c).

Considering quantitative treatments as qualitative also limits the resolving power of the meta-analysis. The intensity water stress, for example, was not always documented, or may have been reported in different units across studies. So it might not be possible to standardize the magnitude of the treatments in all cases. Relating effect sizes to treatment magnitude or intensity is possible only when treatments are explicitly quantitative (e.g. fertilizer rates). Nonetheless, qualitative treatment of environmental variables in the meta-analysis successfully depicted the general effects on soybean seed composition, and serves as a guide for future research.

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Appendix A

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A.1. Water stress

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Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fcr.2008.07.012.

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