

AGRONOMY & SOILS

Seed Size and Oil Content Are Key Determinants of Seedling Vigor in *Gossypium hirsutum*

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ABSTRACT

Despite the plethora of information on seed characteristics and seedling vigor in cotton, the near-continuous release of new cultivars coupled with limited information on seedling vigor for field conditions necessitates re-evaluation of seed characteristics important for vigorous seedling growth and plant establishment. Consequently, seedling vigor (fresh weight at the 2 to 3 leaf stage) and stand establishment were evaluated for 11 cotton cultivars across five locations in Georgia during the 2012 growing season. Seed size, seed oil content, and seed protein content were also evaluated for each cultivar. Cultivar differences in seedling vigor existed under field conditions for the genotypes evaluated. Genotypic correlations and regression analysis revealed a negative relationship between oil (%) and protein contents for quiescent seeds ($r^2 = 0.945$). Additionally, positive relationships were observed between seedling vigor and total seed oil content ($r^2 = 0.573$), and seedling vigor and seed size ($r^2 = 0.642$). Cultivar differences existed in germination responses to incubation time and temperature, but these responses were not necessarily predictive of seedling vigor. These data indicate that information on seed oil content and seed size could help identify cultivars or seed sources with potential for high seedling vigor for use in production scenarios where reduced seedling vigor may limit productivity.

Past research on cotton seedling vigor has shown that substantial variability can exist in seedling vigor due to genotype and prevailing environmental

conditions during seed development (Abdelmagid and Osman, 1975; Kerby et al., 1989; Peacock and Hawkins, 1970). Wanjura et al. (1969) utilized variation in cotton seed quality and planting depth to generate differences in time to seedling emergence. These researchers observed that time to emergence was strongly correlated with yield, whereas seed germination responses and planting depths were less predictive of yield, which highlighted the importance of vigorous seedling growth soon after planting (Wanjura et al., 1969). Subsequent research has been aimed at identifying key seed traits that contribute to enhanced seedling vigor (Ferguson and Turner, 1971; Krieg and Barte, 1975; Leffler and Williams, 1983).

When evaluated across broad taxonomic boundaries, it is generally accepted that large-seeded species produce more competitive seedlings that are larger, have deeper root systems, can more effectively utilize natural resources, and withstand environmental stresses better than smaller-seeded species (Coomes and Grubb, 2003; Muller-Landau, 2012). A number of authors have evaluated the possibility of using seed physical characteristics as indicators of potential seedling vigor (Barte and Krieg, 1974; Ferguson and Turner, 1971; Krieg and Barte, 1975; Leffler and Williams, 1983). Ferguson and Turner (1971) reported that cotton seedling vigor was more closely associated with degree of seed filling (determined using x-ray photography) than either seed weight or seed volume, where completely filled seeds (100%) exhibited greater emergence and seedling growth than partially-filled seed. A number of subsequent studies revealed that seed density could be predictive of seedling vigor in cotton, where larger, high-density seeds provided greater seedling vigor (Krieg and Barte, 1975; Leffler and Williams, 1983) than either small or low-density seeds. Barte and Krieg (1974) observed that increases in cottonseed density were also accompanied by an increase in the total nutrient content (lipids, carbohydrates, and nitrogen) of the quiescent seed.

For field-grown cotton, seed germination and early seedling development occurs in a few generalized stages: seed imbibition, radical protrusion and

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elongation coupled with seed reserve mobilization, and emergence, coupled with a transition to photosynthetic autotrophy (Bradow and Bauer, 2010; Turley and Chapman, 2010). Prior to the transition to photosynthetic autotrophy, the initial phases of seedling growth are fueled by the available nutritive reserves of the cotyledons. For cotton, these reserves are primarily lipids and proteins (Turley and Chapman, 2010). Because lipids represent the most energy-dense storage compound of the quiescent cotton seed that is utilized during post-germinative growth, it is suggested that higher seed oil content would enhance seedling vigor by providing more chemical energy to the growing seedling (Bartee and Krieg, 1974). However, positive correlations between seed protein content and seed germination have also been observed (Abdelmagid and Osman, 1975). For cotton, the quantity and composition of cottonseed macronutrients is strongly influenced by cultivar (Dowd et al., 2010; Pettigrew and Dowd, 2012), and cultivars with high crude oil concentrations also tend to have lower protein concentrations (Pettigrew and Dowd, 2012).

Unseasonably high temperatures and limited rainfall in southern Georgia during the 2011 growing season resulted in poor stand establishment, and a large number of Georgia cotton growers were forced to make the costly decision to replant at a later time during the growing season (Collins and Whitaker, 2012). Despite the plethora of information on seed characteristics and seedling vigor in cotton, the near-continuous release of new cotton cultivars by commercial breeding programs coupled with limited information on seedling vigor for field conditions necessitates a re-evaluation of seed characteristics critical for vigorous seedling growth and establishment. For the present study, the hypothesis is that cultivar differences in seedling vigor would be positively associated with seed size, oil content, and rapidity of germination responses. Consequently, the objectives of the current study are as follows: 1) to evaluate seedling vigor (seedling fresh weight) and stand establishment for 11 commercially-available cotton cultivars under field conditions at five locations in Georgia during the 2012 growing season, 2) to quantify seed size, seed oil content, and seed protein content for those same cultivars, 3) to quantify the relationship between seed characteristics and seedling vigor for all cultivars studied, 4) to evaluate seed germination responses to time and temperature for all cultivars studied.

MATERIALS AND METHODS

Plant Material and Study Sites. To evaluate genotypic differences in seed characteristics and seedling vigor in cotton (*Gossypium hirsutum* L.), seeds from 11 commercially-available cotton cultivars were utilized. From a single bag of seed for a given cultivar, seed were collected and utilized for analysis of seed size, seed oil and protein concentrations, and seed germination responses. The seeds remaining in each bag were utilized for planting large-scale on-farm trials to obtain stand establishment and seedling vigor data. The 11 cultivars utilized in the present study were as follows: Americot Inc., NG 1511 B2RF[®]; Bayer Crop Science, FM 1740 B2F[®], FM 1944 GLB2[®], ST 5458 B2RF[®]; Dow AgroSciences, PHY 375 WRF[®], PHY 499 WRF[®], PHY 565 WRF[®]; Monsanto Company, DP 1137 B2RF[®], DP 1050 B2RF[®], DP 1252 B2RF[®], and DP 0912 B2RF[®]. Because cultivar differences in seed physical and chemical properties can be influenced by genetics, environment during seed development, and environment during seed storage, each cultivar was assigned a letter (A-K) to prevent assumptions of strictly genotypic effects since only one seed lot was used for each cultivar to more accurately characterize the relationship between seedling vigor and seed characteristics. Cool and warm germination test results for each cultivar are presented in Table 1. Seed treatments were applied according to the best management protocols utilized by each seed company.

Table 1. Warm and cool germination (%) for 11 commercially-available cotton cultivars (represented by letters A-K). Seeds of each of these cultivars were subsequently used for evaluations of seed size, oil content, protein content, and field determinations of seedling vigor.

Cultivar	Warm Germination (%)	Cool Germination (%)
A	93	90
B	96	88
C	95	86
D	96	72
E	96	65
F	95	85
G	96	80
H	94	88
I	96	83
J	96	87
K	93	92

Seeds were planted at five on-farm locations, representing five cotton producing counties in southern Georgia (USA): Ben Hill, Berrien, Colquitt, Irwin, and Worth. The soils for these locations ranged from a loamy sand to a sandy loam soil type, and seeds were planted at a 0.91 m inter-row spacing in plots having a minimum plot width of four rows and minimum plot length of 274 m. At each location, there were three replicate plots per cultivar. Planting dates for the 2012 growing season in Ben Hill, Berrien, Colquitt, Irwin, and Worth counties were May 22, May 17, April 30, May 24, and May 11, respectively. Average soil temperatures from planting until five days after planting were obtained from the nearest weather station to each site associated with the Georgia Automated Environmental Monitoring Network (<http://www.georgia-weather.net/>), and are provided in Table 2. For all locations, soil temperatures were greater than the recommended minimum threshold (18.3°C). At each location, field preparation and crop management were conducted according to the typical production practices of each producer using University of Georgia extension recommendations (Collins et al., 2013).

Table 2. The average soil temperature (T_{soil}) at three depths (5, 10, and 20 cm) within the soil profile from planting until five days after planting.

County	T_{soil} 5 cm (°C)	T_{soil} 10 cm (°C)	T_{soil} 20 cm (°C)
Ben Hill	26.6	26.4	25.7
Berrien	23.6	23.3	23.0
Colquitt	21.3	21.1	21.2
Irwin	27.4	27.0	26.4
Worth	24.1	23.3	23.2

Field Measurements of Seedling Vigor and Stand Establishment. To avoid the potentially confounding effects of site-specific differences in soil physical characteristics, the cotton crop was monitored weekly at each location until plants were in the two to three leaf stage of development. At this time, plant stand establishment was determined by quantifying the number of plants in three separate 3.0 m sections of each plot (three replicate plots per cultivar and location). The number of plants m^{-1} row was averaged from the three sections, and plant population (plants ha^{-1}) was estimated for each plot. To quantify cultivar differences in seedling vigor at the two to three leaf stage of development, 20 seedlings from each plot ($n = 3$ for each cultivar at each location) were cut at the base of the plant at the soil surface. Immediately after destructive harvest, above-ground fresh weight (in g) was determined for all seedlings taken from a given

plot using a CS 2000 digital scale (Ohaus Corporation, Pine Brook, NJ) located in the field immediately adjacent to each plot. Average fresh weight per plant was then determined in g plant^{-1} .

Seed Oil and Protein Determination. Seed protein and oil content were quantified by non-invasive, time-domain proton-nuclear magnetic resonance ($^1\text{H-NMR}$) as described previously (Horn et al., 2011). Chemometric algorithms were developed for the simultaneous estimation of protein and oil content in seeds following pulsed-field $^1\text{H-NMR}$ analysis in a Bruker minispec 20 (Bruker Corporation, The Woodlands, TX). Three g samples of cottonseed were measured in quadruplicate ($n = 4$) and means and standard errors were reported for each genotype. Samples were equilibrated to 40°C for two h prior to acquisition of $^1\text{H-NMR}$ data. The Bruker minispec 20 model was updated with a PA-247 probe for rapid signal processing and improved signal-to-noise, especially for seed protein scans.

Seed Germination Responses. Seed germination responses *in vitro* were characterized for 11 commercially-available cotton cultivars using a thermogradient table described previously (Grey et al., 2011; Wallace et al., 2013). In the present study, two tables consisting of solid aluminum blocks measuring 2.4 m long by 0.9 m wide by 7.6 cm thick with a mass of 470 kg were utilized. To establish the thermal gradient, a one cm hole at each end of the tables allowed a chilled or heated mixture of ethylene glycol:water (1:10) to be pumped into the table at a rate of 3.8 L min^{-1} , thus establishing a temperature gradient along the length of the table and a stable temperature at any given position along the width of the table. Using thermocouples (Insulated PR-T-24 wire, Omega Engineering Inc, Stamford, CT) mounted to the undersurface of the aluminum table and a data logger (Graphtec Midi Logger, GRAPHTEC Corporation, 503-10 Shinano-cho, Totsuka-ku Yokohama, Japan), temperatures were continuously monitored and data was downloaded daily in 30 min intervals to ensure stable incubation temperatures throughout the duration of the experiment. To evaluate seed germination responses to time and temperature, incubation temperatures ($\pm 0.5^\circ\text{C}$) of 18, 25, 30, 35, 40, and 50°C were utilized.

Seeds of each cultivar (15 seeds per plate) were evenly spaced on filter paper in 100 x 15 mm Petri dishes and placed at positions on the table corresponding to the temperatures previously noted. Subsequently, five ml of distilled water was poured into each plate, the cover placed on each plate, and the filter paper kept moist for the duration of the experiment via supplemental additions when needed. Seeds were incubated

for five days at each temperature and germination was determined at 0.25, 0.50, 1, 2, 3, 4, and 5 d incubation intervals. Seeds were counted as germinated when the radicle visibly protruded beyond the seed coat, with data then converted to a percentage. The experiment was run in quadruplicate ($n = 4$), and the interactive effects of time and temperature on percent germination were evaluated using response surface analysis.

Statistical Analysis. To evaluate the effect of cultivar on seed size (mg), seed oil and seed protein concentrations (%), plant population (plants ha^{-1}) and seedling fresh weight (g), one-way analysis of variance (ANOVA) was conducted with means separation performed via conventional LSD *post hoc* analysis. Field data (plant population and seedling fresh weight at the two to three leaf stage) were combined across all five county trial locations. The relationships between all parameters were initially evaluated using correlation analysis. The dependence of seedling vigor on seed oil and seed size was quantified using linear regression.

The interactive effects of time and temperature on seed germination responses in cotton were evaluated using response surface analysis. The response surface model is a form of multiple, non-linear regression that uses a combination of linear and quadratic terms and cross-products of linear terms to describe the interactive effects of multiple independent variables (time and temperature) on a single independent variable (percent germination) (Freund et al., 2003). For each cultivar the response surface model was derived from 168 data points (six temperatures by seven sample times by four replicates).

Comparative analyses (ANOVA), correlations, and response surface analyses were conducted using JMP Pro 10 (SAS Institute, Cary, NC), and linear regression analysis was conducted using Sigma Plot 11.0 (Systat Software Inc., San Jose, CA).

RESULTS AND DISCUSSION

Seed size, seed oil, and seed protein were significantly affected by cultivar (Table 3; $P < 0.0001$). For example, F had the largest seeds (112 mg seed $^{-1}$), where seeds from this cultivar were statistically larger than the ten remaining cultivars. D seeds were the smallest (78 mg seed $^{-1}$) and were statistically smaller than the ten remaining cultivars. Seed oil concentration was highest for H (24.7%), where seeds of this cultivar had significantly higher oil concentrations than ten other cultivars. The lowest seed oil concentration was observed for D (17.7%) and was not differ-

ent than two other cultivars (C and B). Seed protein concentration was highest for C (30.1%), and was not different than the seed protein concentrations of two other cultivars (D and B). The lowest seed protein concentrations were observed for H (21.7%), where the seed protein concentrations of this cultivar were significantly less than ten other cultivars. These data are in agreement with a number of previous reports, where seed size, oil content, and protein content of the quiescent cottonseed are strongly influenced by cultivar (Kohel et al., 1980; Pettigrew and Dowd, 2012). It is also important to note that the amount of each of the aforementioned biochemical constituents of the cottonseed can also be impacted by the environment encountered during seed ontogeny or storage (Abdelmagid and Osman, 1975; Kerby et al., 1989; Peacock and Hawkins, 1970; Pettigrew and Dowd, 2012). As a result, cultivar variation in seed physical or chemical characteristics in the present study could be the result of both genetic and environmental factors.

Analysis of stand establishment (plant population) and seedling vigor (plant fresh weight at the two to three leaf stage) data revealed a significant effect of cultivar on both parameters ($P = 0.044$ for plant population and $P < 0.0001$ for plant fresh weight) (Table 3). When data were combined for analysis across the five field locations, H had the highest plant population (71,900 plants ha^{-1}), and was not different than five other cultivars (G, J, F, B, and A). The lowest plant population was observed for I (60,400 plants ha^{-1}), which was only significantly lower than H and G. Although a final plant population of 71,700 plants ha^{-1} is recommended by the University of Georgia Cooperative Extension Service, maximal lint yields have been obtained at plant populations as low as 43,000 plants ha^{-1} (Collins et al., 2013), suggesting that the plant populations of all cultivars in the present study would be suitable for obtaining maximum lint yields in cotton. Plant fresh weight at the two to three leaf stage was highest for H (2.66 g plant $^{-1}$), which produced statistically larger plants than eight other cultivars. The smallest plants were produced by C (1.93 g plant $^{-1}$) which was not different than two other cultivars (E and D). Importantly, the findings of the present study illustrated that cultivar differences existed in early seedling growth, which has been correlated with lint yield in previous studies (Wanjura et al., 1969). Consequently, if seedling vigor is a major limiting factor in a given cotton production system (i.e. high soil tension, drought, etc.), cultivars could be utilized that would exhibit more vigorous early season growth and potentially be able to withstand early season stresses.

Table 3. The effect of cultivar on seed size, % seed oil, % seed protein, plant population and seedling fresh weight at the 2-3 leaf growth stage. Values represent means \pm standard error. n = 15 for all parameters except for seed oil and seed protein (n = 4). Values not sharing a common letter within a given column are not significantly different (LSD; $P < 0.05$).

Cultivar	Seed Size (mg seed ⁻¹)	Seed Oil (%)	Seed Protein (%)	Plant Population (1000 plants ha ⁻¹)	Fresh Weight (g plant ⁻¹)
A	91 \pm 0.10 ^f	21.8 \pm 0.26 ^c	25.8 \pm 0.54 ^b	65.3 \pm 2.9 ^{abc}	2.24 \pm 0.12 ^{cd}
B	96 \pm 0.28 ^e	17.9 \pm 0.06 ^e	29.6 \pm 0.36 ^a	67.4 \pm 2.1 ^{abc}	2.50 \pm 0.11 ^{ab}
C	83 \pm 0.34 ^h	17.8 \pm 0.07 ^e	30.1 \pm 0.27 ^a	60.8 \pm 2.9 ^c	1.93 \pm 0.09 ^e
D	78 \pm 0.34 ⁱ	17.7 \pm 0.14 ^e	29.9 \pm 0.45 ^a	62.3 \pm 3.4 ^c	1.97 \pm 0.07 ^e
E	89 \pm 0.15 ^g	21.0 \pm 0.18 ^d	25.7 \pm 0.25 ^b	63.8 \pm 3.4 ^{bc}	2.06 \pm 0.08 ^{de}
F	112 \pm 0.29 ^a	22.3 \pm 0.07 ^b	24.0 \pm 0.05 ^c	65.3 \pm 1.6 ^{abc}	2.41 \pm 0.08 ^{abc}
G	103 \pm 0.18 ^c	22.3 \pm 0.05 ^b	23.1 \pm 0.30 ^c	70.0 \pm 2.3 ^{ab}	2.28 \pm 0.08 ^{bcd}
H	110 \pm 0.17 ^b	24.7 \pm 0.19 ^a	21.7 \pm 0.17 ^d	71.9 \pm 2.1 ^a	2.66 \pm 0.10 ^a
I	97 \pm 0.48 ^{de}	22.0 \pm 0.11 ^{bc}	25.6 \pm 0.45 ^b	60.4 \pm 1.8 ^c	2.23 \pm 0.08 ^{cd}
J	91 \pm 0.26 ^f	22.3 \pm 0.19 ^b	25.4 \pm 0.22 ^b	67.4 \pm 2.6 ^{abc}	2.37 \pm 0.10 ^{bc}
K	97 \pm 0.32 ^d	21.2 \pm 0.11 ^d	25.4 \pm 0.23 ^b	64.0 \pm 2.8 ^{bc}	2.39 \pm 0.08 ^{bc}

Table 4. Pearson product-moment correlation coefficients for relationships between seedling fresh weight, plant population, seed protein (% protein), seed oil (% oil), and seed size. Data were averaged across all replicates and locations for each of 11 varieties prior to correlation analysis.

	Fresh Weight	Plant Population	% Protein	% Oil	Seed Size
Fresh Weight	-	0.744	-0.601	0.607	0.802
Plant Population	-	-	-0.614	0.553	0.610
% Protein	-	-	-	-0.972	0.790
% Oil	-	-	-	-	0.727

A correlation matrix for seed and seedling parameters is provided in Table 4. A number of strong relationships were observed. For example, a positive relationship is reported between plant population and plant fresh weight ($r = 0.744$), where cultivars exhibiting the highest plant populations also produced larger seedlings at the two to three leaf stage of plant development. Because we chose seedling fresh weight as our indication of seedling vigor, positive relationships between seed-based parameters and seedling fresh weight indicate which key seed traits may be associated with seedling vigor. For example, positive relationships between seedling fresh weight by % oil ($r = 0.607$) and seedling fresh weight by seed size ($r = 0.802$) were noted, suggesting that these traits could be useful for potentially predicting seedling vigor in the field. In contrast, seed protein content was negatively correlated with seedling fresh weight ($r = -0.601$), and a strong, negative correlation ($r = -0.972$) was observed between protein and oil concentrations.

The relationship between seed oil and protein in the present study agrees closely with the findings of Pandey and Thejappa (1975) and Pettigrew and Dowd (2012) who reported negative relationships between seed oil and protein concentration. Overall, the results presented in Table 4 suggest that cultivars having higher oil concentration and larger seed size also tended to have lower seed protein concentrations and higher seedling fresh weight.

To evaluate dependence of some of the aforementioned parameters from Table 4 upon one another, linear regression analysis was conducted. Seed protein concentration (% protein) exhibited strong, negative linear dependency on seed oil concentration (% oil) ($r^2 = 0.945$; Fig. 1). Consequently, varieties having the highest oil concentration also had the lowest protein concentration. Seedling fresh weight at the two to three leaf stage was weakly dependent upon oil concentration (Fig. 2A; $r^2 = 0.369$); however, seedling fresh weight was strongly dependent upon seed size (Fig. 2B;

$r^2 = 0.642$). To evaluate the relationship between seedling fresh weight and total oil content available per seed, average % oil was multiplied by average seed size for each cultivar. Consequently, the oil content per seed was regressed against seedling fresh weight, and a much stronger linear relationship was observed between oil content and seedling fresh weight ($r^2 = 0.573$; Fig. 2C) than for % oil and seedling fresh weight (Fig. 2B). These findings support the hypothesis that differences in seedling vigor under field conditions, even for the large number of field locations utilized in the present study, are dependent upon seed size and total seed oil content per seed. A likely explanation for a stronger relationship between seedling vigor and total oil content compared to % oil is that total oil content per seed is a measure of the amount of stored chemical energy available for post-germinative growth prior to photosynthetic autotrophy of the developing seedling (Bartee and Krieg, 1974).

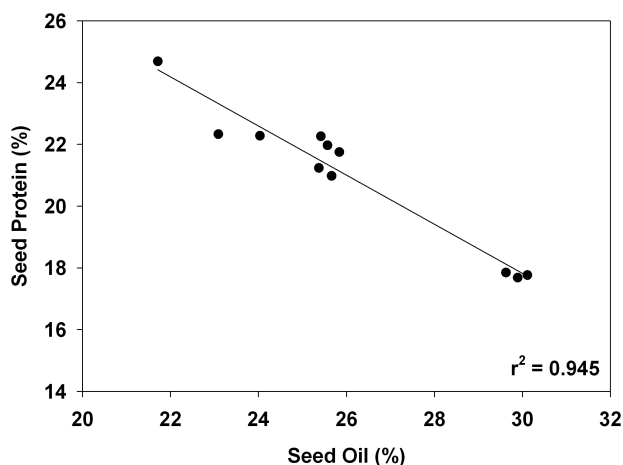


Fig. 1. Linear regression of seed protein (%) versus seed oil (%) for 11 commercially-available cotton cultivars. Each data point represents the mean of four replicate protein and oil measurements.

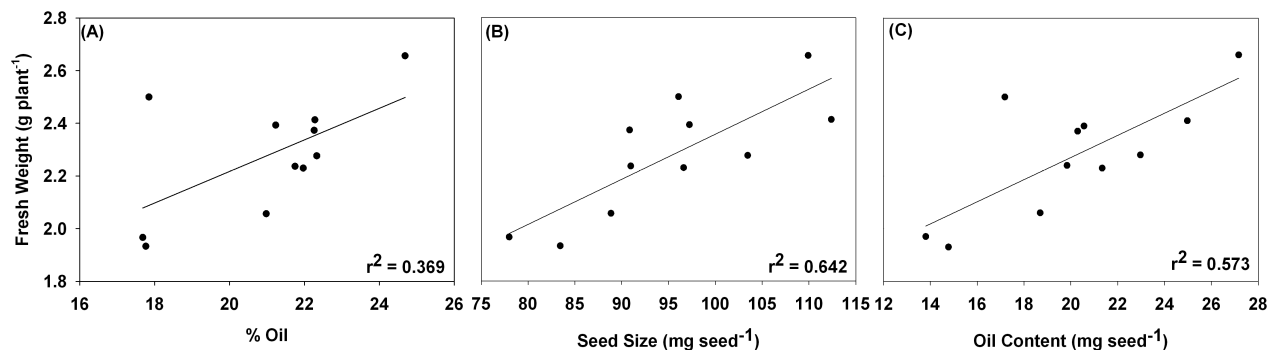


Fig. 2. Linear regression of seedling fresh weight at the 2-3 leaf stage versus % oil (A), seed size (B), and total seed oil content (C) for 11 commercially-available cotton cultivars. Fresh weight data were averaged from 5 locations, 4 replicate plots at each location, and 20 plants per plot (each data point represents the average weight of 400 seedlings).

The response of germination (%) to the interactive effects of temperature and incubation time for each cultivar is presented graphically in Fig. 3. When averaged across all varieties, the predicted time to reach maximum germination was 3.8 ± 0.24 d (mean \pm standard deviation) and the predicted temperature at which maximum germination could be expected was $27.8 \pm 4.5^\circ\text{C}$. There was substantial variation in germination responses to temperature, where the temperatures producing maximum germination ranged from 34°C for E to 18°C for K (Table 5). In Georgia, it is recommended that cotton be planted when soil temperatures are $\sim 18^\circ\text{C}$ for three days with a warming trend forecasted for the next five days (Collins et al., 2013). The genetic variability in germination responses to temperature reported in the present study suggest that cotton should be planted in both a variety and temperature-specific manner to provide optimal germination. Within the five-day incubation period used in the present study, values predicted by the response surface model for the maximum % germination were $> 80\%$ (from 81.9% for K to 103.4% for J) for all varieties except for F (28.8%) and G (64.6%) (Table 5). It is important to note that the low germination percentages *in vitro* reported for F and G are not necessarily indicative of poor stand establishment and seedling vigor under field conditions. For example, F had the lowest percent germination *in vitro*, yet plant population and seedling fresh weight was not statistically different than cultivars with the highest seedling vigor (Table 3). Other authors have reported disparity between field data and *in vitro* seed germination data (Ferguson and Turner, 1971); however, these authors primarily reported reduced performance in the field relative to the laboratory. A possible explanation for these seemingly disparate results is that the time to reach maximum germination for these two cultivars may actually be longer than the 5 d incubation time utilized in the present study.

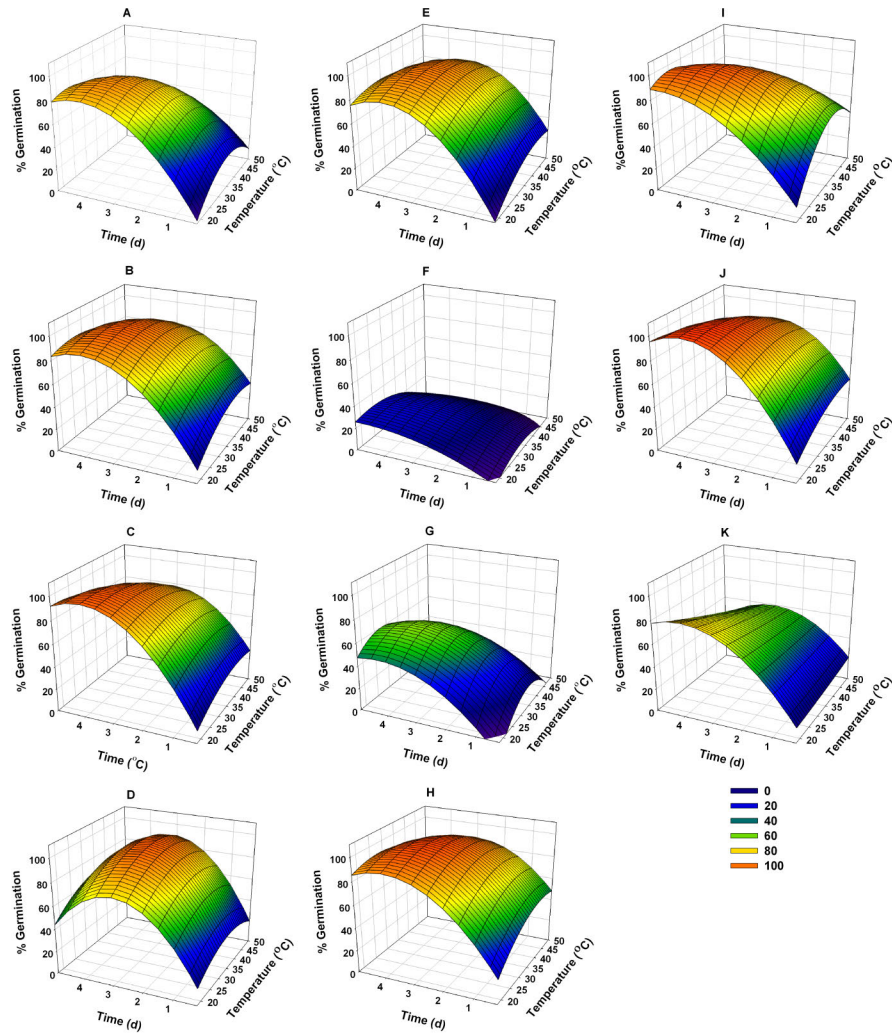


Fig. 3. A three-dimensional response-surface profile plot with % germination as the response variable and incubation temperature and time as the dependent variables for 11 different cotton cultivars. For each cultivar, the response surface model was derived from 168 data points (six temperatures \times seven sample times \times four replicates).

Table 5. Estimated time and temperatures at which maximal germination percentages were reached for 11 different cotton cultivars. The response surface function for each cultivar (graphically represented in Fig. 3) was used to estimate time, temperature, and maximum % germination during a five-day incubation period. For J and H, the response surface model over-predicted percent germination.

Cultivar	Time (d)	Temperature (°C)	Germination (%)	r ²	P value
A	3.8	27.1	89.9	0.688	< 0.0001
B	3.7	31.7	97.2	0.631	< 0.0001
C	3.9	24.4	98.4	0.682	< 0.0001
D	3.8	28.4	98.8	0.746	< 0.0001
E	3.6	34.0	93.5	0.648	< 0.0001
F	4.4	26.3	28.8	0.546	< 0.0001
G	3.8	29.6	64.6	0.664	< 0.0001
H	3.5	32.8	100.7	0.558	< 0.0001
I	3.7	28.8	99.8	0.609	< 0.0001
J	3.8	24.4	103.4	0.600	< 0.0001
K	4.1	18.0	81.9	0.499	< 0.0001

CONCLUSIONS

The major findings of the current study are: 1) that cultivar differences in seedling vigor exist under field conditions for the commercially available genotypes evaluated, 2) that the size and total oil content of the quiescent seed was predictive of seedling vigor under field conditions, and 3) that cultivar differences existed in *in vitro* germination responses to incubation time and temperature, but this was not necessarily predictive of seedling vigor under field conditions. A practical implication of the current study is that seed size and total oil content could potentially be used as screening parameters to estimate seedling vigor of a given genotype or seed lot. Such information would allow a producer to select a cultivar based upon potential lint yields and seedling vigor in production scenarios where seedling vigor may limit productivity. However, to more rigorously attribute differences in seedling vigor to variation in seed size and oil content it would be useful to produce all experimental seed in one location and subject them to uniform processing and seed treatments. Additionally, the genotypic mechanisms contributing to cultivar differences in germination response to temperature should be examined further, as early season soil temperatures (high or low) can strongly influence stand establishment and productivity.

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REFERENCES

- Abdelmagid, A.S., and A.M. Osman. 1975. Influence of storage period and temperature on viability and chemical composition of cotton seeds. *Ann. Bot.* 39:237-248.
- Bartee, S.N., and D.R. Krieg. 1974. Cottonseed density: associated physical and chemical properties of 10 cultivars. *Agron. J.* 66:433-435.
- Bradow, J.M., and P.J. Bauer. 2010. Germination and seedling development. p. 48-56. *In* J.M. Stewart, D.M. Oosterhuis, J.J. Heitholt, J.R. Mauney (eds.) *Physiology of Cotton*. Springer, New York, NY.
- Coomes, D.A., and P.J. Grubb. 2003. Colonization, tolerance, competition and seed-size variation within functional groups. 18:283-291.
- Collins, G.D., and J. Whitaker. 2012. The 2011 crop year in review. p. 3-4. *In* G.D. Collins, C. Li, D. Shurley (eds.) *Cotton Research-Extension Report—2011*. University of Georgia.
- Collins, G.D., J. Whitaker, S. Culpepper, G. Harris, B. Kemmerait, C. Perry, P. Roberts, D. Shurley, and A. Smith. 2013. 2013 Georgia cotton production guide. Publication No. CSS-13-01. University of Georgia Cooperative Extension Service, Tifton, GA.
- Dowd, M.K., D.L. Boykin, W.R. Meredith Jr., B.T. Campbell, F.M. Bourland, J.R. Gannaway, K.M. Glass, and J. Zhang. 2010. Fatty acid profiles of cottonseed genotypes from the national cotton variety trials. *J. Cotton Sci.* 14:64-73.
- Ferguson, D., and J.L. Turner. 1971. Influence of unfilled cotton seed upon emergence and vigor. *Crop Sci.* 11:713-715.
- Freund, R., R. Littell, and L. Creighton. 2003. Regression using JMP®. SAS Institute Inc., Cary, N.C.
- Grey, T.L., J.P. Beasley Jr., T.M. Webster, and C.Y. Chen. 2011. Peanut seed vigor evaluation using a thermal gradient. *Int. J. Agron.* doi:10.1155/2011/202341
- Horn, P.J., P. Neogi, X. Tombokan, S. Ghosh, B.T. Campbell, and K.D. Chapman. 2011. Simultaneous quantification of oil and protein in cottonseed by low-field time-domain nuclear magnetic resonance. *J. Am. Oil Chem. Soc.* 88:1521-1529.
- Kerby, T.A., M. Keeley, and S. Johnson. 1989. Weather and seed quality variables to predict cotton seedling emergence. *Agron. J.* 81:415-419.
- Kohel, R.J. 1980. Genetic studies of seed oil in cotton. *Crop Sci.* 20:784-787.
- Krieg, D.R., and S.N. Bartee. 1975. Cottonseed density: associated germination and seedling emergence properties. *Agron. J.* 67:343-347.
- Leffler, H.R., and R.D. Williams. 1983. Seed density classification influences germination and seedling growth of cotton. *Crop Sci.* 23:161-165.
- Muller-Landau, H.C. 2012. The tolerance-fecundity trade off and the maintenance of diversity in seed size. *Proc. Natl. Acad. Sci. U.S.A.* 107:4242-4247.
- Pandey, S.N., and N. Thejappa. 1975. Study on relationship between oil, protein, and gossypol in cottonseed kernels. *J. Am. Oil Chem. Soc.* 52:312-315.

- Peacock, H.A., and B.S. Hawkins. 1970. Effect of seed source on seedling vigor, yield, and lint characteristics of upland cotton, *Gossypium hirsutum* L. *Crop Sci.* 10:667-670.
- Pettigrew, W.T., and M.K. Dowd. 2012. Interactions between irrigation regimes and varieties result in altered cotton-seed composition. *J. Cotton Sci.* 16:42-52.
- Turley, R.B., and K.D. Chapman. 2010. Ontogeny of cotton seeds: gametogenesis, embryogenesis, germination, and seedling growth. p. 332-341. *In* J.M. Stewart, D.M. Oosterhuis, J.J. Heitholt, J.R. Mauney (eds.) *Physiology of Cotton*. Springer, New York, NY.
- Wallace, R.D., T.L. Grey, T.M. Webster, and W.K. Vencill. 2013. Increased purple nutsedge (*Cyperus rotundus*) tuber sprouting with diurnally fluctuating temperatures. *Weed Sci.* 61:126-130.
- Wanjura, D.F., E.B. Hudspeth Jr., and J.D. Bilbro Jr. 1969. Emergence time, seed quality, and planting depth effects on yield and survival of cotton (*Gossypium hirsutum* L.). *Agron. J.* 61:63-65.