UNDERSTANDING VARIATION IN COTTONSEED OIL PERCENTAGES

An Undergraduate Research Scholars Thesis

by

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This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

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ABSTRACT

Understanding Variation in Cottonseed Oil Percentages

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Cottonseed grown in the US is most commonly either crushed as an oil seed or used by dairies in feed rations. While more than 85% of a cotton crop's value is from the lint, the seed represents an important economic component of the cotton industry. Over the past few decades, cottonseed size has been trending towards a smaller seed, as plant breeders have been selecting for seeds with a higher ratio of lint to seed weight to increase lint yield. Consequently, the value of the cottonseed has declined. To ultimately regain value within the cotton industry, cotton producers need to grow a crop with a large seed size and high oil content while maintaining high lint yields and fiber quality. The objective is to determine the current relationship between seed size, oil content, and lint yield among current cotton varieties grown by US cotton producers. Replicated field trials were grown at six locations in Texas with 25-40 entries of commercial cotton varieties. Measurements included lint yield, lint percentage (ratio of lint to seed weight), HVI™ fiber qualities, seed index (weight in grams of 100 seeds) and oil content. The oil content was measured by time domain nuclear magnetic resonance (TD-NMR) as described by AOCS. Data was analyzed in an ANOVA using SAS version 9.4 with PROC GLM. Because cotton

variety entries varied by location, each location was analyzed separately. Lint yield, lint percent, all fiber traits, seed index, and oil content were different among cotton varieties at all locations. There was an inverse relationship between seed size and lint percent, but there were several cotton varieties in which lint percent and seed size were both relatively high. This suggests that the traits may not be inextricably linked, and plant breeders can develop germplasm with both improved traits. In addition, there did not appear to be a relationship between seed quality (size and oil content) and fiber quality, which suggests those are independent traits.

DEDICATION

This thesis is dedicated to my lovely parents: Sridhar & Sailaja, whose guidance, love, and strength inspire me daily.

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1. INTRODUCTION

1.1 Significance of Biodiesel

Petrodiesel substitutes are heightening in demand as the production and sourcing of diesel from fossil fuels has adverse environmental and health effects (Unosson et al, 2021). Biodiesel offers a potential substitute to petrodiesels as they are eco-friendly due to their biodegradable, renewable, and non-toxic features. In addition, cottonseed oil produces low levels of smoke, emissions of particulate matter, and hydrocarbons compared to soybean or palm oil (Eloka-Eboka & Maroa, 2021). The fuel properties of cottonseed oil demonstrate the ability to be a formidable green alternative fuel in internal combustion engines with electrical generators (Eevera & Pazhanichamy, 2013).

1.1.1 Ease of Biodiesel Production

Essentially, biodiesels are fatty acid alkyl esters that can undergo a variety of processes with the triglycerides from raw materials into the desired product. Directly using highly viscous vegetable oils into engines is often associated with high engine deposits and excess thickening of lubricants. In order to solve such problems, solutions such as dilution, micro emulsification, pyrolysis, and transesterification have been developed (Schwab et al., 1987). In commercial biodiesel production transesterification process is widely used. The transesterification process involves a reaction of the triglyceride sourced from the animal fat/vegetable oil with an alcohol to form esters and glycerol (Knothe,2005).

1.1.2 The Transesterification Process

The transesterification process is dependent on catalyst concentration, catalyst type, temperature, methanol to oil molar ratio and agitation intensity (Rashid et al., 2009). *In the*

Evaluation of the Biodiesel obtained from Cottonseed Oil, the highest yield of cottonseed oil methyl ester, 96.9%, was found with the conditions of the optimum catalyst type of $NaOCH_3$, catalyst concentration 0.75%, temperature 65 ˚C, methanol to oil molar ratio 6:1, and agitation intensity 600 rpm. The study drew the major conclusion that the production of cottonseed oil methyl esters can reduce dependence on imported fossil fuels and diminish the competition between fueling and feeding the world that often riddles the rapeseed, soybean, and palm crop sources (Rashid et al., 2009). In addition, the lipase enzyme that is used in the commercial enzyme production is associated with a high cost. As a result, measures to reduce lipase production costs have been developed such as recombinant gene technology and microorganism manipulation (Tamalampudi & Fukuda, 2011). Such information demonstrates the ease of producing biodiesels from cottonseed oil and the validity in acquiring cottonseed oil.

1.2 Cottonseed Oil Nutritional Advantages

Cotton, often referred to as the “Golden Goose” in India, is a lucrative commercial crop as it produces food, feed, and fiber. In comparison, to other polyunsaturated oils, less hydrogenation is required for the cooking process due to the high levels of oleic, palmitic, and stearic acids present in cottonseed oil. Cottonseed oil is said to have 27% saturated fats which can be slightly disadvantageous nutritionally (List, 2016). Nonetheless, cottonseed oil requires a relatively small amount of hydrogenation which has added nutritional value as it is cholesterol free, is composed mostly of unsaturated fatty acids, and has a minimal amount of trans fatty acids when being processed for food (Agarwal, 2003). Furthermore, soybean oil and corn oil, which have been traditional sources of vegetable fat in the US food and feed supply chain, are becoming more expensive and less available have made industrial users of vegetable fats look for alternative sources of feedstock. The poultry production and food science industries rely heavily on vegetable fat sources for nutritional feed enhancement and other cooking purposes (Yang et al., 2019).

1.3 Food Industry Applications of Cotton Seed Oil

Cottonseed oil is pertinent to the food industry as a frying oil and that is partly due to high tocopherol presence. Tocopherols are antioxidants that can lengthen fry life (List, 2016). Using cottonseed oil also involves a desirable nutty flavor development (Ghazani & Marangoni, 2016). Cottonseed is also able to form the beta prime crystal giving it abilities related to plasticity and aeration (Ghotra et al., 2002). This allows it to be primarily useful in margarines, icings, and whipped toppings (Agarwal & Gopalakrishnan, 2007). Cottonseed oil is also able to undergo winterization making it suitable for salad dressings (Gibon, 2006). Even though cottonseed oil has proven to have certain benefits, it has some shortcomings.

1.4 Gossypol Predicament within Cottonseed Oil

Gossypol, a defensive mechanism against predators, is produced in the pigment glands of cotton stems, leaves, seeds, and flower buds (Gadelha et al., 2014). Gossypol is known to have adverse biological effects on animals who consume it as part of their diet. According to *Effects of Dietary Cottonseed Oil and Cottonseed Meal Supplementation on Liver Lipid Content, Fatty Acid Profile and Hepatic Function in Laying Hens*, it was found that due to the cottonseed oil in the hen's diets an interference with their fatty acid metabolism occurred (Yang et al., 2021). In addition, gossypol is known to have certain elements of toxicity causing such side effects: respiratory distress, impaired body weight gain, anorexia, weakness, apathy, female, and male reproduction and even death if not controlled (Gadelha et al., 2014). However, the toxicity of gossypol can prove to have advantageous applications as it can provoke infertility and be used as a contraceptive for men. There is a chance of irreversibility in infertility, thus should be taken if an individual decides to pursue permanent infertility (Coutinho 2002). However, this does not take over the need for gossypol limitation as it is still integral for animal feed usage. Thankfully, there are methods being developed to this effort, making cottonseed oil even more effective in nutritional feed enhancement, and delivering the potential for cottonseed oil to be expanded to even more animals.

1.4.1 Limiting Gossypol Production in Cottonseed Oil

Efforts to completely remove the gossypol from cottonseed products have been made but host-plant resistance is then compromised. Plant breeders have successfully developed cotton varieties that produce ultra-low gossypol cottonseed that is considered safe as food or as feed. The focus in this breeding effort involved silencing the σ-cadinene synthase gene. This gene reduced the levels in the seed by 97% without affecting the terpenoids that are essential for defense against insects and diseases (Rathore et al., 2020). Rathore et al. (2020) introduced breeding efforts concentrated on removing gossypol from the cottonseed, however not the plant. This decreases the toxicity effects of gossypol in feed consumption whilst protecting the plant from insects.

A widely used method to separate gossypol from oil is through solvent extraction, as gossypol is soluble in polar solvents. Applicable polar solvents include aqueous acetone, acetone & hexane mixtures, sequential extraction with hexane, aqueous acetone, and anhydrous acetone. Iron salt additions that bind to the gossypol have also been reported to render gossypol biologically inactive in animals (Hernandez, 2016).

1.5 Factors Associated with Cottonseed Oil Content

Through either indirect and/or direct pathways, genetics and the environment influence cottonseed oil percentage. In one study, seed was collected from a total of nine environments.

Based on a random effects model, indications were made that the environment and genetics influenced the relative level of fatty acids within the cottonseed oil (Dowd,2010). With variations in nutrient uptake and mineral needs, different characteristics develop in seed cultivar profiles thus, creating associations with different seed attributes. In another 2-year experiment, five sets of near-isogenic mutant cotton were investigated in relation to the seed fuzz phenotype. It was found that the fuzz less sets had higher concentrations of Ca and C and higher seed oil content compared to the fuzzy sets. Furthermore, the research stipulates that fiber maturity is associated with mobility of nutrients, most likely due to fiber development's involvement of carbon and nitrogen metabolism (Bellaloui et al., 2015). In three upland cotton cultivars, positive correlations between oil content and fiber maturity and negative correlations between oil content and percent immature seed were observed (Turner & Worley, 1976).

As stated, mineral and non-mineral nutrients have certain effects on cottonseed oil content as they can create different physical characteristics, and by relation differing oil and protein properties. The okra leaf type and long fruiting branches are related to higher amounts of cottonseed oil (Liu et al.,1994). Biosynthetic pathways involving triacylglycerol expression can be related to increased lipid biosynthesis in the seed. There are many different organelles and enzymes involved in acyl group related pathways, such as phosphatidylcholine (Bates et al., 2013). Some researchers from Huazhong Agricultural University observed an increased expression of triacylglycerol biosynthesis-related genes with the decrease of GhPEPC1 expression (Xu et al., 2016).

At Gorgon University of Agricultural Sciences an experiment was conducted where a strong linear relationship between seed weight and oil content was found (Pahlavani et al., 2009).

Eleven different cultivars were planted across five different fields in Georgia and differences in cultivars were observed. For cotton, lipids and proteins are the primary available nutrients for chemical energy during post-germinative growth. This leads to the stipulation that seed vigor is aided by cottonseed oil reserves. Consequently, a positive correlation was observed between seedling vigor, seed size, and seed oil content. However, a negative correlation was found between oil percentage and the protein contents of quiescent seeds (Snider et al., 2014). A two-year field experiment using two curly leaf scenarios was conducted in Stoneville, Mississippi which supports the inverse relationship of protein content and oil content. Lower levels of protein were observed in the 'Uzbek CRL' and 'DP 5690' wild types which were found to have higher levels of oil (Bellaloui et al., 2021). Other variables that negatively correlate with cottonseed oil content are the percentage of immature seeds and palmitic, stearic, and oleic acids (Sharif et al., 2019).

Presumably, genetics plays a role in the composition of lipid biosynthesis. Overexpression of the cotton gene GhDGAT1 via a seed specific promoter in cottonseed increases total oil content from 4.7% to 13.9% in a variety of different transgenic lines and generations. It was also found that there was a change in oil composition from mainly linoleic acid to unsaturated oleic acid (Wu et al., 2021). From a nutritional and technological perspective, higher oleic and lower linoleic acid contents are favorable (Hernandez et al., 2021). Through transcriptome analysis, decreased expression of genes related to GhPEPC1 were found to lead to the increased expression of triacylglycerol biosynthesis related genes (related to lipid biosynthesis) (Xu et al., 2016).

In a study conducted by researchers at Nanjing Agricultural University, it was found that total unsaturated fatty acids (UFA) and total essential amino acids (EAA) concentrations decreased notably under severe drought conditions. In addition, decreases in polyunsaturated to saturated fatty acids ratio (PUFA/SFA), health-promoting index (HPI), unsaturation index (UI), and the increased atherogenicity index (AI) were observed. The decrease in unsaturated fatty acids indicate that the oil concentration is unhealthy when subject to drought stresses (Li et al., 2022). Currently, there is a decrease in unsaturated fatty acids due to plant breeders desire for a decrease in cottonseed size.

1.6 The Current Trends Following Cottonseed Oil

Over the past few decades, the size of commercial cottonseed has been decreasing. Most experts in the cotton industry believe that plant breeders have favored increases in lint yield percentages, which have inadvertently caused seed size to shrink. Although an increase in lint yield is highly beneficial economically, smaller seed size has introduced a new set of problems (Personal Communication, Hague, 2022).

1.6.1 Disadvantages of Smaller Seed Size

Seed size can affect the seed’s ability to germinate and produce a healthy seedling. Small will struggle much than larger seed when germination conditions in field are not optimal due to cold temperatures, crusting soil, or other factors that impact stand establishment (S. Hague, personal communication 2022). The inability of cotton farmers to achieve a full stand of cotton can substantially impact yield and ultimately whole-farm profitability. Larger seed varieties are those that are ranging from 3,000 to 4,000 seeds per round. Medium sized seeds are 4,000 to 5,000 seeds per round. Smaller seed varieties are greater than 5,000 seeds per pound. Smaller seeds require more replanting than larger seed varieties when planting in adverse conditions (Edmisten, 2020). With larger seeds there is a greater chance of emergence and reserves for oil and protein content. Measuring such oil reserves is possible via oil content.

In the past decade, it is becoming an increasing practice of farmers to plant fewer seeds in an effort to reduce input costs. Many have switched from a goal of 40,000 plants per acre to 20-25,000 plants per acre. It is believed that lower plant populations become more water use efficient than higher populations as well as more nitrogen use efficient because an individual plant has more soil to exploit with less competition from adjacent cotton plants.

Most US cottonseed companies are actually encouraging farmers to plant fewer seeds per acre. Seed companies charge a GMO technology fee on a per acre basis, so they do not price their product on a per seed basis. In fact, it is usually to their advantage to sell less seed per acre so they do not have to spend as much on seed production. Moreover, this strategy allows seed companies place new varieties that usually have a limited amount of seed on more production acres in the first year of a product launch.

1.7 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy is a common tool used in analytical chemistry. NMR Spectroscopy uses the chemical environment of the nuclei of atoms to determine its molecular structure. Essentially, atomic nuclei possess the ability to spin and have a proton fueled electric charge. The rotation of this charged nucleus creates a measurable magnetic field Nuclear Magnetic Resonance-history physical principles, nuclear spin magnetic moment magnetic torque on a nuclear orientation energy. It is with this basis, that NMR spectroscopy determines phase changes, conformation and configurational alterations, solubility, and diffusion potential (M Singh & A. Singh, 2022). NMR Spectroscopy can be split into two different categories: Fourier Transform (FT-NMR) and Continuous Wave (CW) NMR Spectroscopy.

1.7.1 Fourier Transform NMR Spectroscopy (FT-NMR)

Fourier Transform NMR spectroscopy employs short duration pulses of radiofrequency radiation. The pulse duration is sufficient to excite all the NMR active nuclei present. The rest period between each pulse session provides the nuclei time to get back into their ground states. Fourier transform NMR can take multiple scans, which it then averages to increase the signal-to-noise ratio (Nuclear Magnetic Resonance).

1.7.2 Continuous NMR Spectroscopy (CW)

Continuous wave NMR spectrometers have the sample irradiated by a continuous radio frequency within a fixed magnetic field. Continuous wave spectrophotometers vary the current in a frequency coil to achieve resonance absorption signals. Often this is seen in university laboratories as it requires low maintenance and operational costs (Nuclear Magnetic Resonance).

1.7.3 NMR Spectroscopy uses in the Industry

Nuclear Magnetic Spectroscopy is sought after for a variety of applications by industries as an analytical method. In the early years of NMR, the technology was mostly used in pharmaceutical applications. The value of NMR for the pharmaceutical industry lies in the ability to determine the compounds structurally and general drug classification. For it to be used biologically, technological improvements related to line narrowing, high-field magnets, and spin coupling connectivity had to be developed. Prior to using NMR, structures of complex organic molecules were formed via tedious double resonance methods. For the determination of proteins, solvent signal suppression methods had been developed allowing peptide NH resonances to be included. Inevitably, aiding the determination of the complete 3D structures of small proteins (Becker 1993). This technology can be extended to biofluids. Biofluids are a determinator of the biochemical status of a living organism. The specific characteristic physiochemical properties of all biological fluids allow them to be differentiated by NMR technology (Lindon et al., 1999). An advancement in resolution called high-resolution magic angle spinning (HRMAS) enables NMR spectroscopy to semi-solid, gel-like samples. HRMAS uses edited pulse sequences making it suitable for food products and biological tissue analysis. In terms of agricultural applications: agricultural chemistry, characterization and dynamics of soil components, plant tissues, and in vivo organisms, HRMAS NMR is particularly useful (Mazzei & Piccolo, 2017).

Time-domain NMR (TD-NMR) a derivative of classical NMR is commonly used in structural analysis. TD-NMR uses infinite electric waves when interacting with the sample. The transition of the nucleus from the high energy level to the lowest energy level generates the NMR signal. TD-NMR takes advantage of the differences in molecular mobility between various items (Tang et al., 2019). In agriculture, TD-NMR is often used in food quality control and determination of oil content in intact oilseeds (Colnago et al., 2021). The determination of oil content for many different types of oilseeds such as cotton, sunflower, soybean, rape, palm, and coconut seeds makes the TD-NMR technology an important analytical tool for researchers, growers, crushers, processors, and traders (Sherazi & Mahesar, 2015). Essentially, anyone involved in any transactional, harvesting, or processing relationships with the crop of the oilseed. Furthermore, the use of toxic solvents or destruction of sample is completely avoided as this method is nondestructive to the sample.

2. METHODS

Fuzzy cottonseed was obtained from the ‘Commercial Variety Trials’ conducted by the Cotton Improvement Lab at Texas A&M University at College Station, Texas. Cotton Varieties were grown in randomized complete block designs with four replications per variety. Plots were approximately 11 meters in length and on 2-rows that were approximately 1 meter wide. Final plant stands were 10-12 plants per meter of row. Trials were conducted at Weslaco, Corpus Christi, College Station (irrigated and dryland), Chillicothe, and Commerce, Texas.

A 30-boll sample was hand harvested from 2 of the four replications to determine fiber quality, lint percent, and to obtain seed samples. Seed cotton samples were ginned on 10-saw laboratory gins. Seed samples were acclimated to a standard humidity. Seed indexes were calculated based upon the weight in grams of 100 fuzzy seeds. Prior to oil analysis, seed was dried in oven at 37.8 ˚C and then placed in desecrator to prevent additional moisture absorption. Oil content in seeds were measured with an NMR Spectrophotometer located at the USDA-ARS station in College Station. Results from the NMR were reported as a percentage of oil of the total seed weight. To determine mean values, coefficient of variation, and means separation among cotton varities for lint yield, lint percent, seed index, and seed oil content, data was analyzed with the PROC GLM function in SAS (SAS,2022). While many of the same cotton varieties were tested across all locations, each location differed from each other. Therefore, we calculated the analysis of variance separately for each location for all traits of interest. Simple linear regression was used to determine relationship within the dataset between the four variables of interest: lint yield, lint percent, seed index, and oil content. Our first approach was to pool all data and use the regression functions in Microsoft Excel (Microsoft 2022). Our next step was to calculate the covariance for a population sample using the formula:

Where:

N= Number of data values

=mean of y

=mean of x

=data value of y

=data value of x

The covariance of the population sample was calculated by location and across locations.

3. RESULTS & DISCUSSION

At all testing locations, we found highly significant differences among cotton varieties for seed index and oil content (Table 3.1). This was an expected finding and concurs with other researchers' findings (Campbell et al., Zeng et al., 2015). Some of the locations, such as the dryland (non-irrigated) trial at College Station and the trial at Commerce provided substantial stress on the plants but was not severe enough to negate genotypic influences over seed size or oil content. It was also noted that within a trial location, the amount of variation between replications for a cotton variety were low and the amount experimental error as reflected in the residual term in the ANOVA was relatively low. This suggests that evaluation of seed index and seed oil content does not require a high number of replications per site for a close estimation of cotton seed quality at a particular location.

Table 3.1. Analysis of variance for seed index (seed weight in grams per 100 seed) and seed oil content (percent of total seed constituents) from cotton varieties grown at multiple locations in Texas in 2021.

Location: Corpus Christi

Source	df	Seed Index MSE	Oil Content MSE
Variety	29	1.95**	18.61**
Replication	1	0.17	1.72
Experimental Error	29	0.37	1.68

** highly significant at a probability of > 0.01

Location: Weslaco

Source	df	Seed Index MSE	Oil Content MSE
Variety	34	1.82**	16.11**
Replication	1	2.06	<0.00
Experimental Error	34	0.12	0.47

** highly significant at a probability of > 0.01.

Location: College Station (dryland)

Source	df	Seed Index MSE	Oil Content MSE
Variety	25	2.07**	15.98**
Replication	1	3.09	0.01
Experimental Error	25	0.25	0.28

** highly significant at a probability of > 0.01.

Location: College Station (irrigated)

Source	df	Seed Index MSE	Oil Content MSE
Variety	35	1.93**	13.14**
Replication	1	1.43	0.03
Experimental Error	34	0.51	1.04

** highly significant at a probability of > 0.01.

Location: Commerce

Source	df	Seed Index MSE	Oil Content MSE
Variety	34	1.82**	11.46**
Replication	1	0.08	0.34
Experimental Error	34	0.74	2.10

** highly significant at a probability of > 0.01.

Because seed composition typically does not affect fiber quality, but will influence lint yield and lint percent (Campbell et al., 2016), we only reported lint yield, lint percent, seed index, and oil content. Because cotton varieties varied by location, we could not pool the data for means analysis, therefore we have reported each location separately (Tables 3.2-3.6).

Cotton trials grown at Weslaco usually allow researchers to evaluate the full genetic potential of cotton varieties because of the excellent growing conditions. Trials at Weslaco are typically planted in late February or early March because of the sub-tropical environment. In addition, the soil quality and availability of irrigation further enhance the productivity of the crop. However, the spring season at Weslaco was unusually cool and delayed the crop by about three weeks. Nevertheless, on average the lint yield was 1192 A^{-1} and the lint percent was 41.1, which is exceptionally high (Table 3.2). The seed index was 8.9 and seed oil content averaged 26.7%. Typically, seed index and oil content are positively correlated (Zeng et al., 2015). One particular variety, ‘DG 3520 B3XF’ had extremely high oil content at 32.5% and a slightly above average seed index of 9.6. This suggests that the seed had a high ratio of oil to other internal seed components such as proteins and simple carbohydrates.

Table 3.2. Lint yield, lint percent, seed index, and seed oil content from cotton varieties grown at Weslaco, TX in 2021.

Variety	Lint	Lint %	Seed Index	Oil
	lbs. A^{-1}	%	g c^{-1}	%
PHY 443 W3FE	1601	42.7	9.7	26.4
DP 1646 B2XF	1509	44.4	7.3	23.8
DG H959 B3XF	1506	42.8	9.0	31.1
FM 2398 GLTP	1488	43.3	9.2	23.8
ST 4550 GLTP	1467	43.9	8.0	22.8
DP 2038 B3XF	1466	47.1	6.9	25.0
PHY 390 W3FE	1449	42.0	8.6	28.8
PHY 332 W3FE	1402	41.8	9.2	27.1
ST 4990 B3XF	1392	40.8	8.1	22.0
DP 2012 B3XF	1385	40.5	8.2	26.2
SSG UA222	1365	39.7	9.1	29.6
DP 1948 B3XF	1345	41.7	7.7	25.1
ST 4993 B3XF	1338	45.2	8.0	23.9
DG 3520 B3XF	1327	40.0	9.6	32.5
ST 5091 B3XF	1291	45.0	8.3	24.1
TAM 16 SHS_05	1263	42.6	8.4	29.8
PHY 400 W3FE	1251	43.1	8.7	30.0
SSG UA107	1245	39.5	10.0	28.6
NG 4190 B3XF	1204	43.3	8.1	23.0
FM 1730 GLTP	1192	39.9	8.5	24.3
NG 4936 B3XF	1178	40.3	8.4	22.2
TAM 16 SHS_32	1129	40.5	8.9	28.8
ExCeed 6494	1110	38.1	9.7	27.0
TAM 16 SHU_05	1030	39.2	9.6	29.4
Exp. Line	1021	37.6	9.3	24.8
NG 5150 B3XF	1005	42.2	8.0	24.8
NG 3195 B3XF	1004	41.7	8.2	25.9
ExCeed 4344	996	39.0	9.7	26.3
TAM 16 WSO_08	974	36.1	10.8	30.4
FM 1830 GLT	914	43.6	8.1	23.9
Exp. Line	909	39.4	9.3	24.9
TAM 16 WSR_25	907	36.7	10.8	29.0
TAM 16 WSQ_70	832	37.5	10.3	29.5
PHY764 WRF	813	38.8	9.5	28.9
Mean	1192	41.1	8.9	26.7
CV, %	12.8	2.4	3.9	2.6
LSD $_{(0.05)}$	229	1.9	0.6	1.2

The trial conducted at Corpus Christi, TX, experiences a substantial amount of rainfall through most of the growing season. Consequently, productivity was higher than normal even with supplemental irrigation. Results in terms of lint yield, lint percent, seed index, and seed oil content were similar to those observed at the Weslaco location (Table 3.2 and 3.3). Seed index was 9.9 grams and there was a wider array of difference among varieties at Corpus Christi (Table 3.3) in comparison to Weslaco (Table 3.2). Again, DG 3520 B3XF was among the highest varieties for oil content at 30.9%.

Table 3.3. Lint yield, lint percent, seed index, and seed oil content from cotton varieties grown at Corpus Christi, TX in 2021.

Variety	Lint	Lint %	Seed Index	Oil
	lbs. a^{-1}	%	g c^{-1}	%
PHY 390 W3FE	1,463	43.9	8.6	30.7
NG 3195 B3XF	1,431	42.4	9.6	24.6
DP 1646 B2XF	1,390	43.1	7.9	22.5
PHY 332 W3FE	1,383	41.1	9.9	26.2
TAM 16 SHS_05	1,346	42.2	9.2	28.5
PHY 400 W3FE	1,338	41.9	9.5	29.8
ST 4550 GLTP	1,292	43.0	9.3	21.5
DP 1948 B3XF	1,280	41.6	8.7	21.3
DP 2012 B3XF	1,279	41.8	8.8	24.1
DG 3402 B3XF	1,278	41.7	9.9	27.9
FM 1830 GLT	1,277	41.4	8.9	23.4
PHY 443 W3FE	1,265	41.9	10.1	24.2
ExCeed 6494	1,225	39.1	10.2	23.4
Exp. Line	1,183	42.8	9.5	23.3
NG 4936 B3XF	1,137	40.6	9.0	21.6
TAM 16 SHS_32	1,115	40.5	9.8	27.7
Exp. Line	1,112	39.0	10.4	24.9
NG 4190 B3XF	1,112	43.5	9.3	21.2
SSG UA107	1,107	40.9	10.1	27.3
Exp. Line	1,094	38.6	10.7	24.8
TAM 16 WSO_08	1,070	36.3	12.0	28.7
NG 5150 B3XF	1,043	42.1	9.0	22.7
ExCeed 4344	1,012	37.8	9.9	23.9
DG 3520 B3XF	890	38.5	11.4	30.9
TAM 16 WSR_25	871	38.7	12.3	29.9
TAM 16 SHU_05	824	40.8	10.0	27.4
SSG UA222	816	39.1	10.6	28.5
TAM 16 WSQ_70	650	39.7	10.5	29.6
PHY 764 WRF	555	37.4	10.5	28.2
Mean	1111	40.7	9.9	25.8
CV, %	10.3	2.6	6.1	5.0
LSD **(0.05)**	152	2.1	1.2	2.5

There were two trials conducted at College Station in 2021. One was designed as a dryland or non-irrigated trial and the other was designed to be fully irrigated. Although the mean yields reflect differences in irrigation affects, 503 lbs A^{-1} for the dryland trial and 1,563 lbs A^{-1} for the irrigated trial, the actual differences were due to soil type and drainage. The 2021 growing season at College Station was unusually wet with May, June and July receiving unprecedented volumes and frequencies of precipitation. Even though the trials were conducted less than a kilometer away from each other, the internal drainage was much better for the 'irrigated' trial, which actually never received an irrigation treatment because soil moisture deficits that would trigger an irrigation were never reached. The 'dryland trial' suffered from waterlogging, which can have severe negative effects upon the productivity of cotton (Bange et al, 2004).

The lint yield in the College Station dryland trial was only 503 lbs A^{-1}, which would be expected from highly stressed cotton plants, but surprisingly the lint percent, 39.8%, seed index, 10.1 g, and oil content, 24.7%, were fairly normal (Table 3.4). Because most of the water logging stress occurred during the early part of the reproductive period (May – July) the plants were able to recover and produce normal cotton bolls, albeit fewer of them, during the latter stages of the crop's development. Once again, DG3520 B3XF had exceptionally high oil content.

Table 3.4. Lint yield, lint percent, seed index, and seed oil content from cotton varieties grown at College Station (dryland trial), TX in 2021.

Variety	Lint lbs. A^{-1}	Lint %	Seed Index g c^{-1}	Oil %
PHY 400 W3FE	728	41.0	10.4	27.8
NG 4936 B3XF	640	39.4	9.3	20.0
FM 1830 GLT	636	41.7	9.9	21.7
DP 2012 B3XF	578	40.0	8.0	22.1
DP 1646 B2XF	564	40.4	8.8	22.7
PHY 443 W3FE	564	43.0	10.4	23.4
PHY 390 W3FE	561	40.9	10.5	27.1
NG 4190 B3XF	530	42.6	9.0	21.7
ExCeed 4344	528	38.6	10.7	23.7
SSG UA 222	528	38.2	11.9	28.6
ST 4550 GLTP	516	42.9	9.4	20.6
DG 3520 B3XF	508	38.9	11.4	30.6
NG 5150 B3XF	503	41.8	8.3	23.5
PHY 764 WRF	497	37.4	11.2	25.0
DP 1948 B3XF	488	40.5	8.9	20.8
TAM 16 SHS_05	482	40.1	9.1	27.0
Exp. Line	465	38.5	10.0	22.1
Exp. Line	460	39.5	9.7	23.0
NG 3195 B3XF	451	40.9	8.8	24.1
TAM 16 SHU_05	437	38.4	10.5	25.7
TAM 16 WSQ_70	427	38.5	10.2	28.9
TAM 16 SHS_32	426	39.1	9.3	263
PHY 332 W3FE	421	41.3	9.7	23.7
ExCeed 6494	404	38.5	10.2	26.0
TAM 16 WSO_08	394	37.0	11.1	26.0
TAM 16 WSR_25	392	37.1	13.1	27.7
Mean	503	39.8	10.1	24.7
CV, %	23.8	2.4	4.9	2.2
LSD (0.05)	249	2	1.0	1.0

Results from the College Station irrigated trial were not slightly unanticipated (Table 3.5). While lint yield was high, 1,563 lbs. A^{-1}, as was lint percent, 40.5%; seed index and oil content were lower than expected. Perhaps the excessive rainfall exerted subclinical damage to bolls to the point where they were not shed but failed to provide adequate constituents for growth

(Jackson and Gerik 1990). While DG 3520B3XF only had an oil content of 25.4 percent, it was still among the highest in the trial. This suggests that differences among cotton varieties for seed qualities remain relative across these trials. However, Campbell et al. (2016) reported that from his observations in South Carolina, there could be significant genotype X environmental interactions.

Table 3.5. Lint yield, lint percent, seed index, and seed oil content from cotton varieties grown at College Station (irrigated trial), TX in 2021.

Variety	Lint lbs. A^{-1}	Lint %	Seed Index	Oil
DP 1646 B2XF	1,937	42.3	7.9	17.6
DP 2038 B3XF	1,848	45.0	8.2	15.9
PHY 443 W3FE	1,845	40.8	10.2	18.5
Exp. Line	1,839	42.5	8.7	17.6
DP 2012 B3XF	1,788	40.6	8.9	19.7
ST 4993 B3XF	1,775	44.8	9.4	18.2
PHY 400 W3FE	1,760	42.6	8.9	22.8
Exp. Line	1,750	42.8	8.1	16.0
NG 4190 B3XF	1,671	42.2	8.5	17.1
Exp. Line	1,668	42.2	10.3	17.8
ST 5091B3XF	1,665	42.9	8.8	18.8
ST 4550 GLTP	1,660	44.3	9.2	16.6
PHY 332 W3FE	1,655	39.2	10.2	20.0
ST 4990 B3XF	1,639	38.7	9.5	16.4
Exp. Line	1,626	42.5	8.8	17.4
SSG UA 222	1,619	38.3	9.6	21.1
PHY 390 W3FE	1,603	41.6	8.6	21.4
NG 4936 B3XF	1,563	39.7	9.0	16.5
DP 1948 B3XF	1,549	40.9	8.8	17.1
Exp. Line	1,548	38.6	10.1	18.2
DG 3520 B3XF	1,518	38.4	12.1	25.4
ExCeed 4344	1,512	38.5	12.2	20.1
TAM 16 SHS_05	1,504	42.1	9.3	22.7
FM 2398 GLTP	1,493	42.4	11.0	18.0
NG 5150 B3XF	1,465	40.4	8.9	16.9
ExCeed 6494	1,462	41.9	10.2	20.5
Exp. Line	1,442	38.8	10.1	17.9
TAM 16 SHS_32	1,437	39.5	9.7	21.5
NG 3195 B3XF	1,401	42.3	8.9	20.1
FM 1730 GLTP	1,349	39.3	10.1	17.3
TAM 16 SHU_05	1,345	38.3	9.5	22.1
FM 1830 GLT	1,340	39.2	9.2	16.8
TAM 16 WSO_08	1,310	37.1	10.7	22.4
TAM 16 WSQ_70	1,294	38.0	10.5	23.2
TAM 16WSR_25	1,289	35.4	10.3	23.0
PHY 764 WRF	924	36.9	10.5	21.0
Mean	1,563	40.5	9.6	19.3
CV, %	10.2	2.0	7.4	5.3
LSD (0.05)	222	1.5	1.6	2.0

Commerce is located in northeast Texas and has the shortest growing season among the locations in this study. It is also a non-irrigated trial and encountered more drought stress in 2021 than any of the other locations. Lint yield averaged 538 lbs. A^{-1} across all varieties (Table 3.6). Seed index was 8.7 g and oil content was only 16.6%, which was the lowest of any location. Kohel and Cherry (1983) reported that because the cotton fruiting period occurs over several weeks, environmental conditions can affect seed quality. We surmise that the short growing season, coupled with drought stress, negatively affected the average seed quality at the Commerce location. Typically, crop management affects seed composition less than the influence of cotton varieties (Bednarz et al., 2006). DG 3520 B3XF was yet again in the top tier for seed oil content at Commerce.

Table 3.6. Lint yield, lint percent, seed index, and seed oil content from cotton varieties grown at Commerce, TX in 2021.

Variety	Lint lbs. A^{-1}	Lint % %	Seed Index	Oil
ST 4550GLTP	763	44.0	9.2	15.4
PHY 400W3FE	763	43.4	9.7	20.1
PHY 443W3FE	756	43.2	9.2	17.2
ST 5091B3XF	754	41.4	9.2	17.1
ST 4993B3XF	706	44.8	9.4	15.8
PHY 390W3FE	695	43.2	7.9	20.0
DP 2012B3XF	687	38.3	8.1	17.1
DG 3520B3XF	650	38.0	9.4	21.9
FM 1830GLT	628	41.6	8.4	14.4
DP 1646B2XF	626	41.9	7.7	15.0
NG 4936B3XF	606	39.9	7.7	13.8
DP 1948B3XF	561	40.9	8.1	15.9
Exp. Line	558	42.8	8.9	15.0
Exp. Line	545	43.2	7.8	15.5
PHY 332W3FE	542	41.1	9.3	18.6
NG 5150B3XF	539	41.8	7.6	13.3
Exp. Line	538	38.5	9.2	15.3
NG 3195B3XF	528	41.7	9.0	17.6
FM 1730GLTP	525	38.5	9.7	16.0
TAM 16WSO_08	523	35.1	11.3	22.0
ST 4990B3XF	519	39.0	10.6	15.5
ExCeed6494	498	37.6	7.6	14.9
TAM 16SHS_05	477	40.1	7.1	15.4
PHY 764WRFE	464	38.5	9.7	20.2
SSG UA222	459	37.6	8.9	19.6
Exp. Line	456	41.1	7.4	14.1
Exp. Line	445	36.0	9.4	15.9
NG 4190B3XF	399	39.3	8.6	15.8
ExCeed4344	397	37.5	9.3	17.9
FM 2398GLTP	397	40.4	6.5	11.8
TAM 16SHU_05	383	36.1	8.9	18.9
Exp. Line	380	37.6	7.8	15.5
TAM 16WSQ_70	364	35.0	8.3	17.3
TAM 16SHS_32	361	41.3	7.8	13.8
TAM 16WSR_25	354	35.6	12.9	22.4
Mean	538	39.9	8.7	16.6
CV, %	9.7	3.3	9.9	8.7
LSD $_{(0.05)}$	66	2.5	2.1	2.9

It is important to examine the relationship among traits for cotton breeding programs. These programs attempt to combine positive traits into a single line, which in turn meets the needs of the cotton grower and the downstream cotton industry. During our analysis, we compared four important cotton traits in all combinations: lint yield, lint percent, seed index, and seed oil content. Initially we pooled the data across varieties and locations.

The positive relationship between lint yield and lint percent has been well established (Wells and Meredith, 1984). Therefore, we expected to observe a positive correlation between these traits (Figure 3.1). However, because the growing conditions across our locations were so different, the degree of influence of lint percent as a mechanism of improving lint yield was less discernable in the pooled data.

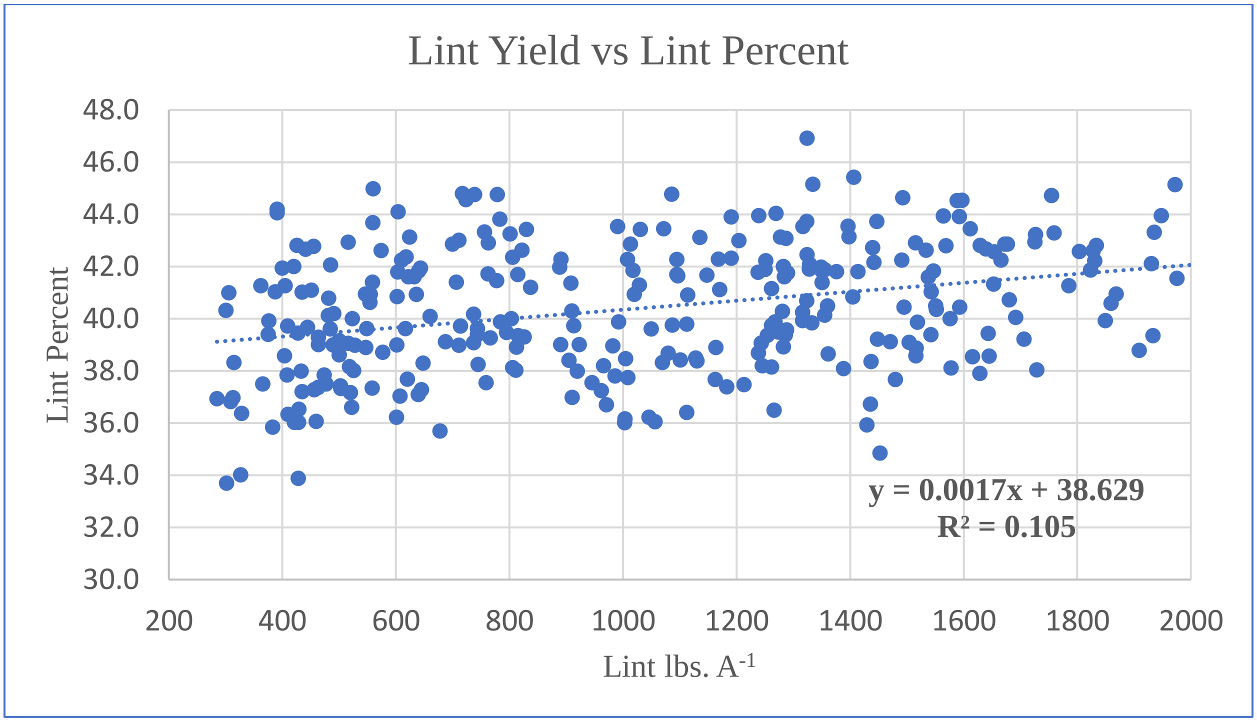

Figure 3.1. Relationship between lint yield and lint percent across all locations and cotton varieties in 2021.

The correlation between lint yield and seed index was slightly negative and almost flat (Figure 3.2). Other researchers have reported a high negative correlation between these traits (Zeng et al., 2015). Again, the large pool of data from diverse locations likely precluded us from discerning a stronger linkage between these traits.

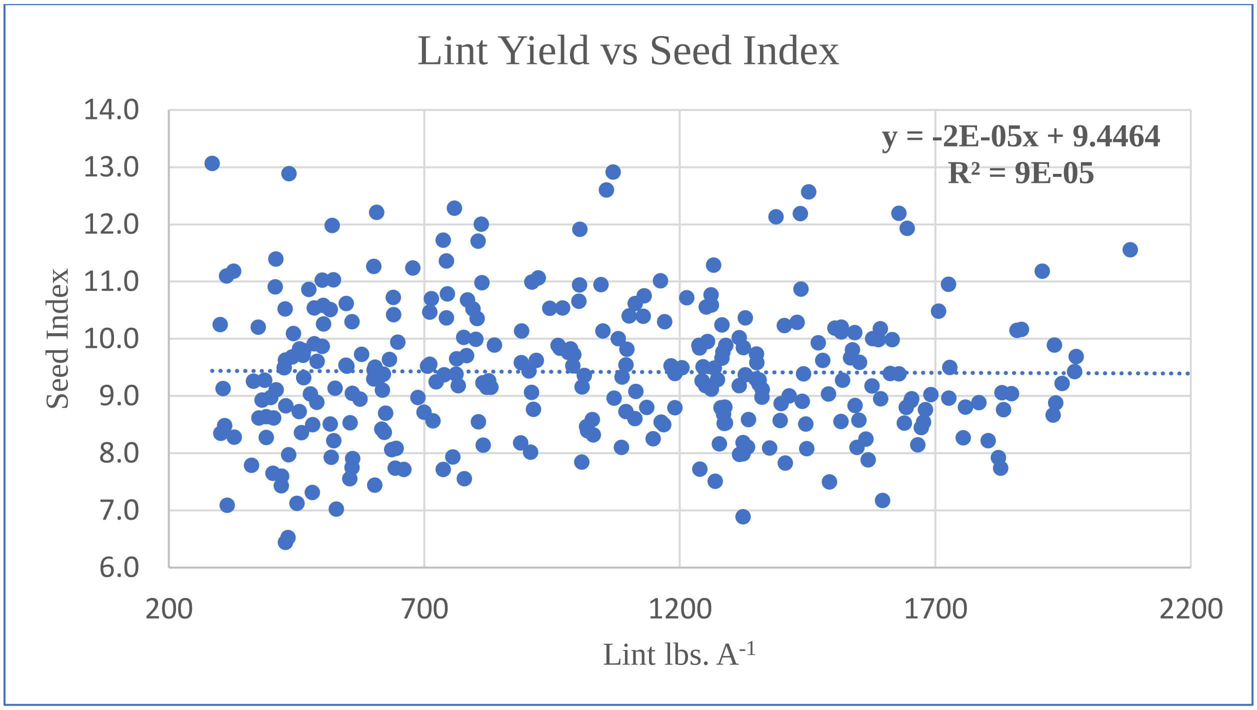

Figure 3.2. Relationship between lint yield and seed index across all locations and cotton varieties in 2021.

The linear regression between lint yield and oil content indicated a slight positive correlation between the traits (Figure 3.3). Campbell et al. (2016) and Kothari et al. (2016) both suggested that there is a strong negative relationship between lint yield and oil content. Because of the curve observed in the data set (Figure 3.3), we chose to conduct a polynomial regression analysis of the data (Figure 3.4). In doing so, we improved the R-square value from 0.0037 to 0.1802. While the later does not indicate a good fit, it does demonstrate that we could improve our prediction performance by looking at the data on a curve. In other words, the lower yielding cotton seemed to produce higher oil content as yields went up, whereas at the higher yielding locations, we observed the more commonly expected situation in which higher yields and oil content were inversely related. There may be a logical explanation to this phenomenon. Research has shown that larger seed produces better seedling vigor (Snider et al., 2014). Since the lower yielding environments would have favored varieties with higher oil content, it seems reasonable to assume those varieties with a genetic predisposition for high oil content would perform better than varieties with low oil content. And since it has also been reported that seed oil content has a higher heritability than even lint percent (Badigannavar and Myers, 2015), this could serve as an important breeding objective in low-yielding environments.

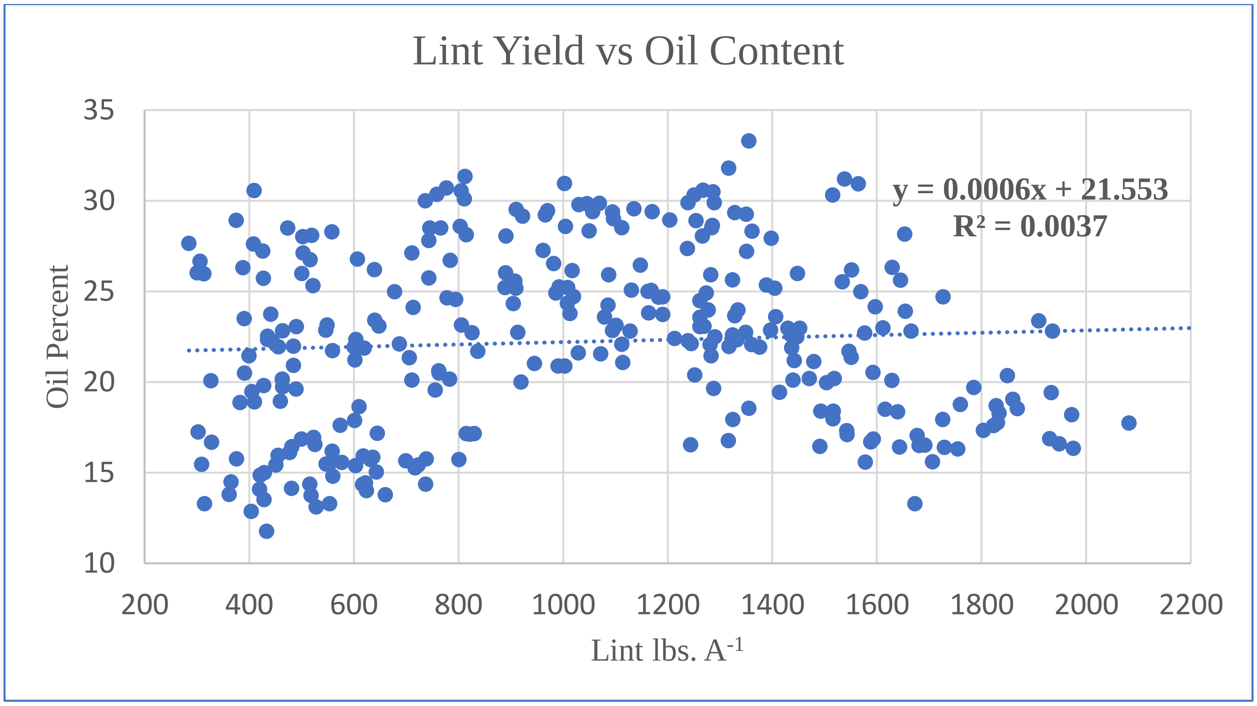

Figure 3.3 Relationship between lint yield and oil content across all locations and cotton varieties in 2021.

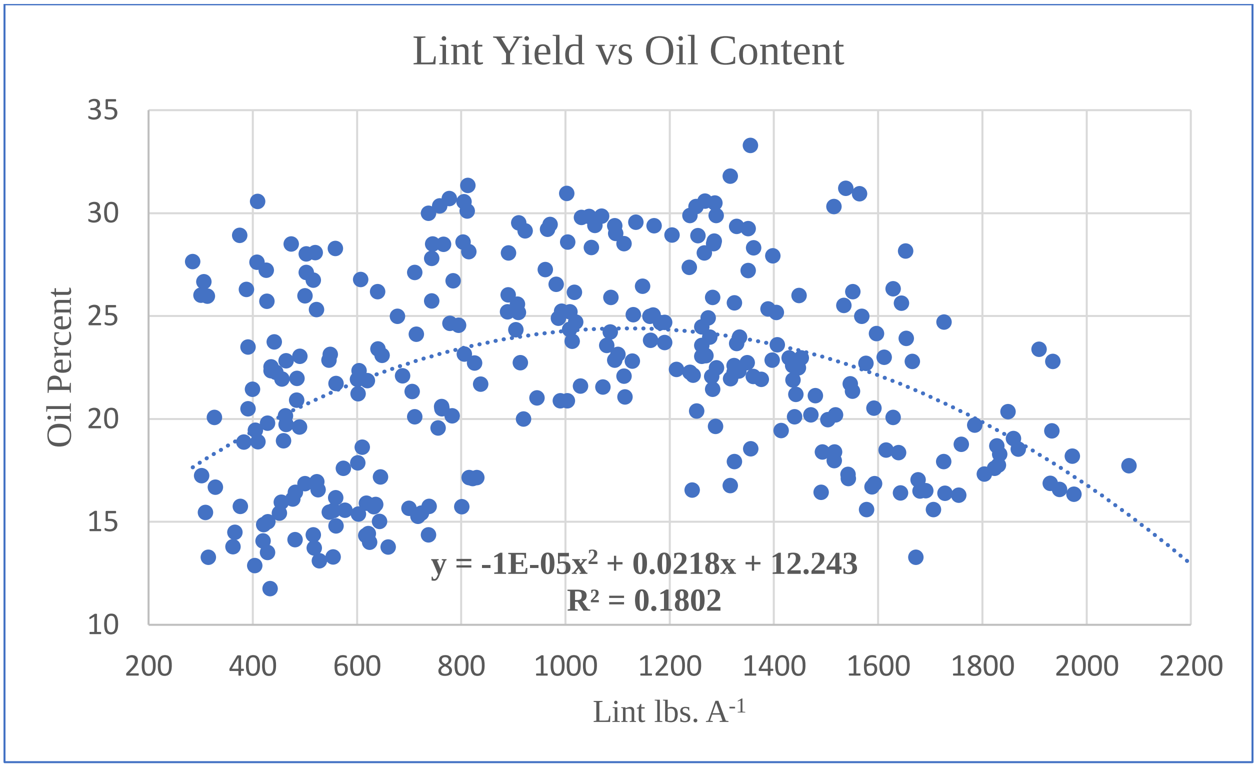

Figure 3.4. Polynomial regression between lint yield and oil content across all locations and cotton varieties in 2021.

Results from this study confirm what other researchers have reported (Campbell et al., 2011), that lint percent and seed index are negatively correlated (Figure 3.5). This can be particularly problematic in cotton breeding programs that use lint percent as an initial yield component when eliminating early-generation lines from their program without also accounting for seed index. The result can be an over-selection of lines with small seeds.

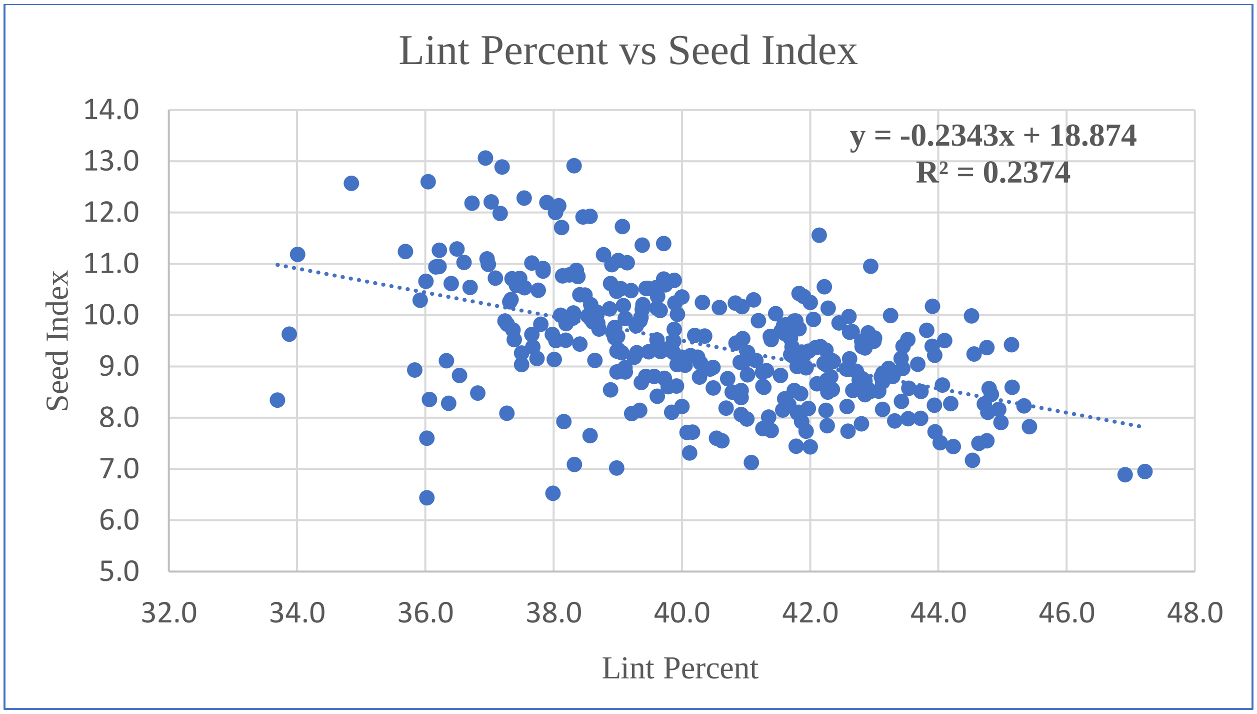

Figure 3.5. Relationship between lint percent and seed index across all locations and cotton varieties in 2021.

Most cotton studies that report relationships between lint percent and oil content, have indicated a strong negative correlation between lint percent and oil content (Zeng et al., 2015; Kothari et al., 2016). While our correlation was negative (-0.161x), the results were likely obnubilated by the wide array of growing conditions (Figure 3.6).

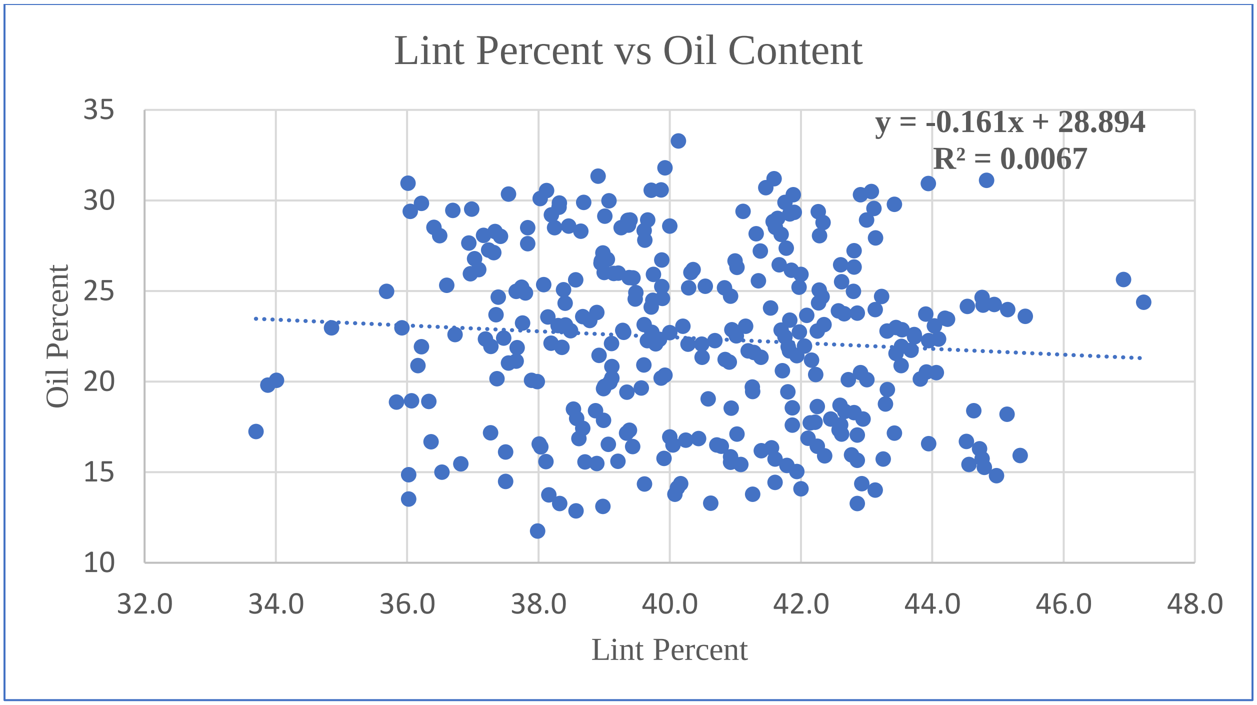

Figure 3.6. Relationship between lint percent and oil content across all locations and cotton varieties in 2021.

As seed size went up in our data set, so did oil percent (Figure 7). These findings are consistent with those from previous studies (Hinze et al., 2015; Zeng et al., 2015). This relationship offers cotton plant breeders a short cut to improving oil content in their germplasm if they will emphasize seed size as a selection criteria.

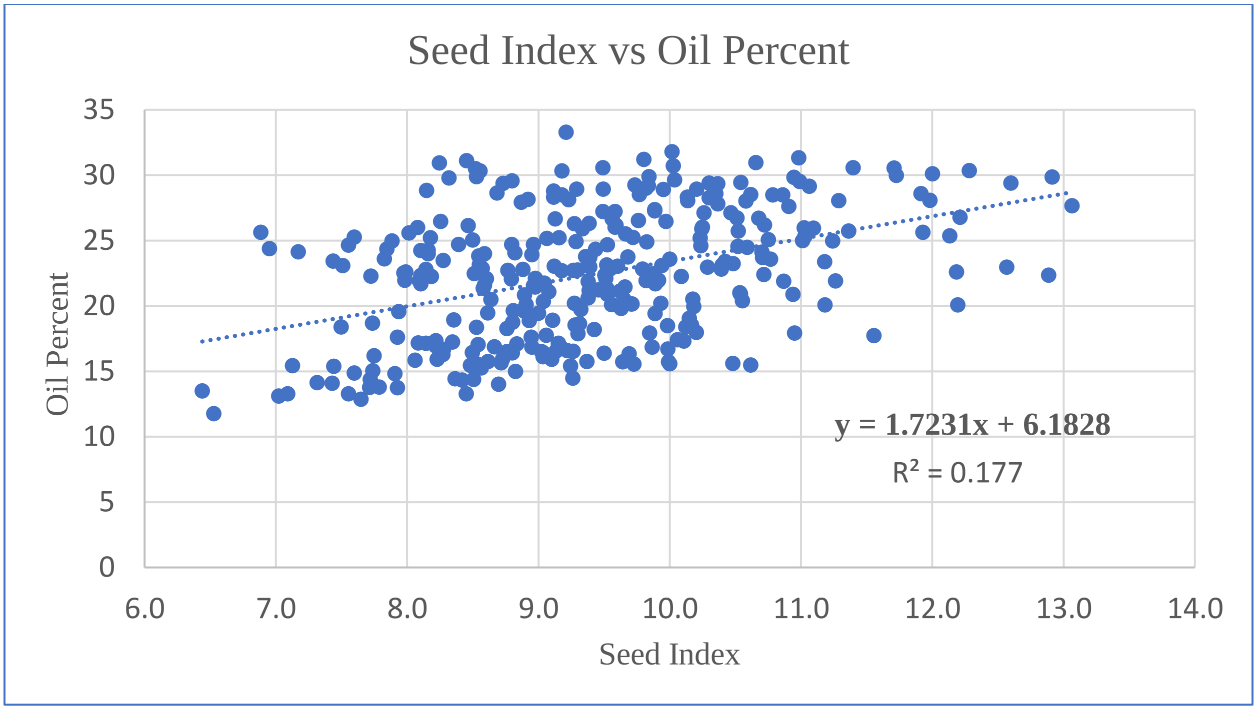

Figure 3.7 Relationship between lint percent and oil content across all locations and cotton varieties in 2021.

Because much of the data was confounded by the different growing conditions, covariances among traits were calculated for each location as well as a combined location covariance (Table 3.7). Our analysis indicted a positive relationship in four out of the five locations for lint yield and lint percent as well as across locations. The only exception was for the College Station dryland trial. The covariances for lint yield and seed size were negative except at College Station dryland and Commerce. This strongly suggests that stressful environments are poor locations in which to select for the simultaneous improvement of lint yield and seed size. Similarly, lint yield and oil content were also negatively associated in four out of five locations with the sole positive location being Commerce. A surprising cautionary finding was the positive covariance calculated across locations. Campbell et al., (2016) reported similar results in their study. Lint percent and seed size, as well as lint percent oil content were inversely related at all locations, which suggests that selection for one trait will frequently result in a decline in value for the other trait. Lastly, seed index and oil content had a positive covariance at all locations, which would be expected.

Table 3.7 Covariance analysis of sample populations for lint yield, lint percent, seed index, and seed oil content by location and combined locations for cotton trials in Texas in 2021.

Traits	Weslaco	Corpus Christi	College Station dryland	College Station irrigated	Commerce	Combined locations
Yield / Lint %	255	270	-44	309	256	372
Yield / Seed Index	-62	-137	17	-70	20	5
Yield / Oil %	-76	-245	-37	-248	40	140
Lint % / Seed Index	-1.98	-1.61	-1.58	-1.73	-0.33	-1.45
Lint % / Oil %	-2.94	-1.52	-2.46	-2.75	-1.42	-1.00
Seed Index / Oil %	1.28	1.27	2.16	1.37	2.04	2.47

4. CONCLUSION

Our study revealed several important findings. First, plant breeders need to evaluate their germplasm lines, especially early-generation material that does not yet have a lengthy performance record, in high-yielding growing environments. Selections and advancements in stressful conditions may introduce unintended interactions with the traits of interest. Even though selections need to be made in good production situations, it was demonstrated by DG 3520 B3XF that high oil content can be expressed in both high-yielding and low-yielding environments.

Secondly, our results suggest that large seeds are important in stressful growing conditions because they can likely provide additional energy support to newly emerged seedlings. This helps the plant to establish a strong root system. However, in low-stress conditions in which seed size for seedling vigor is not important, large seeds tend to have low lint percentages, which in turn can result in lower lint yields. Therefore, cotton breeders should probably set breeding goals for a moderate seed size with a seed index between 8.5 and 9.5 grams and a lint percent of at least 40%.

Thirdly, we demonstrated the relationship between seed index and oil content on a consistent basis. Plant breeders do not necessarily have to measure oil content in all of their breeding lines in order to make improvements in oil content. Seed index appears to be an excellent predictor of seed oil content.

All of these strategy suggestions could have a meaningful impact upon the cotton industry, the end-users of cotton seed because of the magnitude of the cotton seed grown in the

US and abroad. Implementing these approaches could have a substantial effect upon the availability and cost of our domestic and global food supply.

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