Final Technical Report

Project Title: Improved Soybean Oil for Biodiesel Fuel

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Project Locations: University of Nebraska-Lincoln, near mead. Illinois Crop Improvement Association, Juan Diaz, PR. Department of Agriculture Engineering, University of Idaho, Moscow, ID.

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Written by: Tom Clemente (University of Nebraska-Lincoln), Jon Van Gerpen (University of Idaho).

Subcontractors: Jon Van Gerpen (University of Idaho)

Cost-Sharing Partner: Nebraska Research Initiative

Project Contacts: Tom Clemente (tele: 402-472-1428/ e-mail: tclemente1@unl.edu). Mike Behne, Research Grants & Contracts (tele: 402-472-6323/ e-mail: mbehne1@unl.edu).

DOE Project Team: Golde Field Office, Golden, CO 80401; Fred Gerdeman (tele: 303-275-4928/ e-mail: fred.gerdeman@go.doe.gov) and Renae Binstock (tele: 303-275-4772/ e-mail: renae.binstock@go.doe.gov).

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Executive Summary

Over 70 million acres of soybean are planted in the US on a yearly basis, which translates to an annual supply of roughly 4,200 million gallons of vegetable oil for use in food, feed or industrial applications. This volume accounts for over 50% of the world's supply of vegetable oil. Less than 4% of this renewable oil supply is used for industrial applications, with the major industrial product from soybean oil being biodiesel. A number of positive attributes are realized with the use of biodiesel blends in engines, including increased biodegradation in spills, improved flashpoint of the fuel, reduced toxicity, lower emissions, with the exception of oxides of nitrogen (NOx), and improved lubricity.

While other feedstocks are available for biodiesel, expanding the use of a feedstock must be evaluated in terms of the environmental impact of the cropping system. Soybean as a feedstock for biodiesel has a significant benefit, being a legume it does not require nitrogen fertilization. This in turn drastically improves its net energy balance, with an estimate 93% more energy in the derived biodiesel than required for its production. Soybean is approximately 18% total oil, with a fatty acid profile primarily composed of 13% palmitic acid, 4% stearic acid, 18% oleic acid, 55% linoleic acid and 10% linolenic acid. This fatty acid profile makes soybean oil rather oxidative unstable, due to the high proportion of polyunsaturated fatty acids (linoleic and linolenic acids). With respect to biodiesel an oxidized fuel will compromise engine performance. Moreover, soybean oil saturated fatty acid percentage, palmitic and stearic acids, which compose approximately 17% of the profile, negatively affects cold flow properties of the derived biodiesel. For these reasons, researchers have been exploring avenues to develop vegetable oils high in the monounsaturated fatty acid, oleic acid, and low in saturated fatty acids, thereby creating a feedstock that will produce oil in which the derived biodiesel will possess enhanced oxidative stability and improved cold flow properties.

Conventional genetic approaches to increase the oleic acid content of soybean oil have led to some success. However, the elevated oleic acid (40%-70%) germplasm developed though these avenues have some significant drawbacks. First, the novel trait is impacted by environment, requiring growth in warmer climates for stability of the elevated oleic acid trait. Second, the elevated oleic acid trait developed through conventional means tends to be linked with reduced yield. In addition, multiple genetic loci are associated with the elevated oleic acid trait in soybean derived through conventional tools, which significantly complicates the breeding process. On the other hand, by implementing the tools of biotechnology it is feasible to perturbed fatty acid biosynthesis in soybean such that the fatty acid profile of seed oil is high in oleic acid (>85%) and low in saturated fatty acid (< 6%). In contrast to the conventional approach, a biotechnology strategy results in the novel oil trait being inherited as a single dominant trait, which facilitates breeding.

The goal of this program was to generate information on the utility of soybean germplasm that produces oil, high in oleic acid and low in saturated fatty acids, for its use as a biodiesel. Moreover, data was ascertained on the quality of the derived soybean

meal (protein component), and the agronomic performance of this novel soybean germplasm. Gathering data on these later two areas is critical, with respect to the first, soybean meal (protein) component is a major driver for commodity soybean, which is utilized as feed supplements in cattle, swine, poultry and more recently aquaculture production. Hence, it is imperative that the resultant modulation in the fatty acid profile of the oil does not compromise the quality of the derived meal, for if it does, the net value of the novel soybean will be drastically reduced. Similarly, if the improved oil trait negative impacts the agronomics (i.e. yield) of the soybean, this in turn will reduce the value of the trait.

Over the course of this program oil was extruded from approximately 350 bushels of soybean designated 335-13, which produces oil high in oleic acid (>85%) and low in saturated fatty acid (<6%). As predicted improvement in cold flow parameters were observed as compared to standard commodity soybean oil. Moreover, engine tests revealed that biodiesel derived from this novel oil mitigated NOx emissions. Seed quality of this soybean was not compromised with respect to total oil and protein, nor was the amino acid profile of the derived meal as compared to the respective control soybean cultivar with a conventional fatty acid profile. Importantly, the high oleic acid/low saturated fatty acids oil trait was not impacted by environment and yield was not compromised.

Improving the genetic potential of soybean by exploiting the tools of biotechnology to improve upon the lipid quality of the seed for use in industrial applications such as biodiesel will aid in expanding the market for the crop. This in turn, may lead to job creation in rural areas of the country and help stimulate the agricultural economy. Moreover, production of soybean with enhanced oil quality for biodiesel may increase the attractiveness of this renewable, environmentally friendly fuel.

Comparison of the actual accomplishments with the goals and objectives of the project

Objective 1: Evaluate engine performance with soydiesel derived from soybean germplasm high in oleic acid, low in saturated and polyunsaturated fatty acids

Accomplishments: Oil was extruded from approximately 350 bushels of soybean event designated 335-13, which is a genetically enhanced soybean derived from biotechnology that carries a genetic element designed to simultaneously down-regulate two genes involved in fatty acid biosynthesis, *FatB* a palmitoyl thioesterase and Fad2-1, a Δ^{12} desaturase, in a seed specific fashion. Down-regulation of the former leads to reduction in palmitic acid from about 13% to approximately 3%, while reduction in the latter leads to reduction in the polyunsaturated fatty acids, linoleic and linolenic acids, with a concomitant increase in oleic acid, from approximately 18% to over 85%.

Fuel characteristics were ascertained for neat methyl-esters and isopropyl-esters derived biodiesel. In addition, exhaust emissions were monitored across three engine setups with neat methyl-esters and one engine setup with neat isopropyl-esters.

Objective 2: Analyze the amino acid composition of the meal (protein component) of the novel soybean germplasm.

Accomplishments: As mentioned above, monitoring the amino acid composition is critical, for if the quality of the derived meal from the novel soybean is compromised the probability of such a product hitting the marketplace is significantly reduced.

Proximate analysis along with amino acid profile of ground samples from event 335-13 along with 79 samples from progeny populations derived from crosses of 335-13 with elite soybean germplasm along with the parental line, A3237, from which event 335-13 was developed. The data revealed no significant variation in either amino acid profiles or total oil and protein content.

Objective 3: Evaluate agronomic performance of the high oleic/low saturated and polyunsaturated fatty acid germplasm across multiple environments.

Accomplishments: In 2004 and 2005 event 335-13 was grown in one location in Juana Diaz, Puerto Rico and at two locations in Nebraska, Lincoln and near Mead. All field trials were conducted in accordance with USDA/APHIS guidelines governing the release and interstate movement of regulated transgenic plants.

The Puerto Rico and Nebraska sites allowed for monitoring of the affect of environment on the novel fatty acid profile. While the two Nebraska sites were utilized for a comprehensive study on the agronomic performance of event 335-13.

The 335-13 event was originally developed from Asgrow genotype, A3237, which was released as a cultivar in the early 1990s. Hence, the overall agronomics of event 335-13 was not expected to be superior to current soybean cultivars currently under production. For this reason crosses were initiated in order to introduce the novel fatty acid trait into more elite soybean germplasm. To this end, over the course of this program we monitored the progeny derived from a cross 335-13 X RMPLC1-311-128, the later soybean genotype was developed at the University of Nebraska's soybean breeding program. A total of 125 progeny lineages, designated with prefix Ux1625, were grown in Nebraska and Puerto Rico sites. Data was ascertained on stability of the novel oil trait, along with other agronomic parameters, including lodging, days to harvest, hilum color, and total protein/oil.

Summary of program activities

Objective 1: Evaluate engine performance with soydiesel derived from soybean germplasm high in oleic acid, low in saturated and polyunsaturated fatty acids.

In 2004 a bulk planting of event 335-13 covering approximately 15 acres was harvested to secure sufficient material to allow for testing of a biodiesel derived from the novel oil. The oil was extruded from two separate batches totaling approximately 350 bushels. Both methyl- and isopropyl- esters were prepared from the novel soybean oil. Studies were conducted with either a John Deere 4045T engine coupled to a General Electric DC dynamometer (TLC 2524), a 1994 Dodge Ram 2500 with a 5.9 liter Cummins diesel engine (B series) tested on a SF 602 Superflow Chassis Dyno or a John Deere 31350 tractor attached to a PTO dynamometer. Biodiesel prepared from canola oil and standard commodity soybean oil were used as controls, along with petroleum-based diesel fuel. Soybean and canola biodiesel fuels were purchased from Air Energy (Creston, WA) and Seattle Biodiesel (Seattle, WA), respectively. Figure 1 displays the fatty acid profiles of the respective methyl-ester biodiesel fuels used in the studies, and the corresponding fuel properties are listed in Table 1. Methyl-esters derived from the high oleic soybean had improved cold flow properties, with both cloud and pour points drastically reduced as compared to the corresponding methyl-esters derived from commodity soybean oil (Table 1). Cold flow parameters of the methyl-esters derived from canola oil were more closely aligned with the high oleic acid soybean biodiesel. These data reflect the impact of the fatty acid profile on cold flow, namely both the high oleic acid soybean oil and canola oil have reduced saturated fatty acid content as compared to commodity soybean oil (Fig 1).

Figure 1: Fatty acid profile of the respective biodiesel fuels evaluated in the program

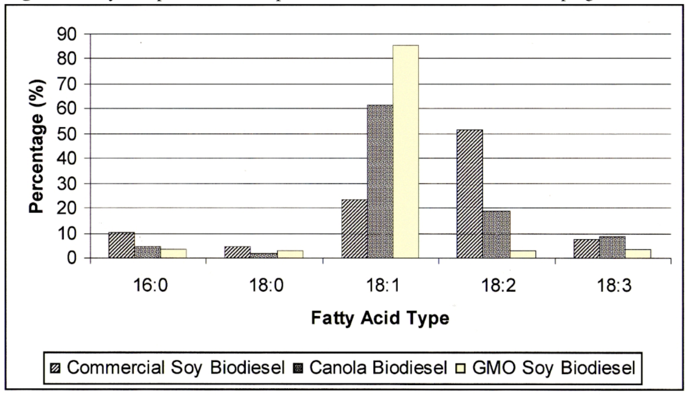


Table 1 Properties of the respective test fuels

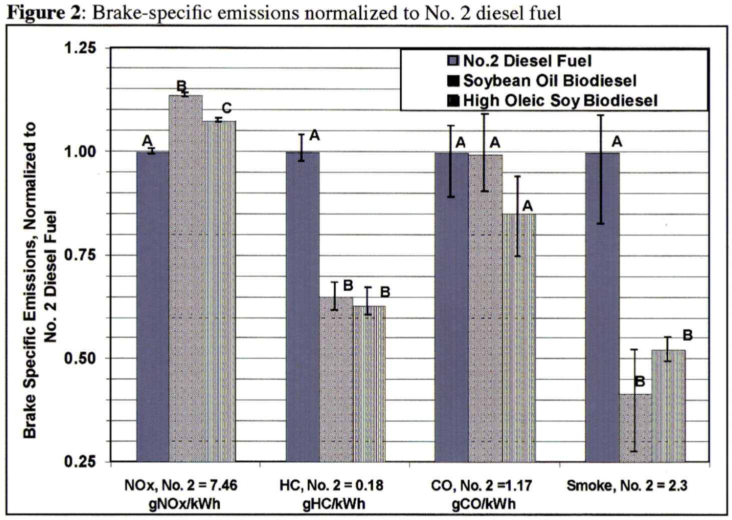
Property	Soybean Methyl Esters	HO Soybean Methyl Esters	Canola Methyl Esters	HO Soybean Propyl Esters	No. 2 Diesel
Lower Heating Value (Btu/lb)	16100	16208	16188	18267	18518
Carbon (%)	77.2	77.0	77.3	79.6	86.8
Hydrogen (%)	11.9	12.2	12.0	13.1	12.8
Oxygen (%)	11.3	11.1	11.1	7.3	N/A
Cetane No.	47.2	51.5	49.4	57.1	44.0
Specific Gravity	0.883	0.881	0.879	0.870	0.846
Cloud Point (°C)	-1	-5	-2	-10	-6
Pour Point (°C)	0	-9	-9	-18	-9
Total Glycerol (% wt.)	0.097	0.068	0.004	0.01	N/A
Kinematic Viscosity (cSt @ 40°C)	4.012	4.780	4.783	5.907	2.686

HO soybean columns refer to biodiesel derived from high oleic acid soybean oil from event 335-13

Exhaust emission tests were first conducted with a John Deere 4045T engine, coupled with a General Electric DC dynamomoter. The various brake emissions results are shown in Fig 2. Notably, the brake-specific NOx emissions of the high oleic acid soybean biodiesel and standard commodity soybean biodiesel were 7.5% and 13.5% higher than No. 2 diesel, respectively (Fig 2).

The start of injection, start of combustion, and ignition delay time are shown in Table 2. The ignition delay is the elapsed time between the start of fuel injection and the start of combustion as determined from the first rise in the heat release rate. The average ignition delay times of No. 2 diesel, soybean commodity biodiesel and high oleic acid biodiesel were 3.97, 3.26 and 3.14 crank degrees, respectively.

Figure 2: Brake-specific emissions normalized to No. 2 diesel fuel



Normalized brake-specific emissions are given with the No. 2 diesel fuel acting as the baseline. Actual No.2 diesel values are shown below the bars. Error lines are shown within the respective bars, letters above the bars represent statistical differences (p=0.05).

A second set of exhaust emission tests were run using Cummins engine B series. Included in this test was canola oil derived biodiesel. Under the conditions used in this test an interesting trend was observed, with the three biodiesel fuels derived from oils obtained from, standard soybean, high oleic acid soybean and canola, displayed lower brake-specific NOx at lower load conditions. At full load, both canola and soybean oils exhibited increased brake-specific NOx, while the 335-13 derived biodiesel remained below that of No. 2 diesel. At full load and low loads, brake-specific NOx increased with canola oil- and standard soybean oil- derived biodiesel, which correlated with the respective cetane values determined for the respective biofuels. However, the differences observed in NOx emission under in this test were not significantly different.

Table 2: Start of injection, start of combustion and ignition delay time of the test fuels

Fuel	Start of Injection	Start of Combustion	Ignition delay Time
No. 2 diesel	-7.09 (±0.08)	-3.11 (±0.07)	3.97 (±0.03)
Soy biodiesel	-7.72 (±0.06)	-4.46 (±0.10)	3.26 (±0.04)
335-13 biodiesel	-7.78 (±0.02)	-4.64 (±0.04)	3.14 (±0.02)

Start of injection and combustion values, crank angle degrees before top dead center, and ignition delay column values are crank angle degrees.

The third set of exhaust emission tests were conducted using a John Deere 3150 tractor connected to a PTO dynamometer, and tested at low and high load conditions with 335-13 and canola standard soybean biodiesel fuels along with No. 2 diesel. The increase in brake-specific NOx for the biodiesel fuels at low load increased by 23.5% and 27.5% for the 335-13 and canola biofuels, respectively, as compared to No. 2 diesel. While at high load test run with 335-13 and standard soybean biodiesel brake-specific NOx increased by 15.1% and 21.1%, respectively, as compared to No. 2 diesel.

Only with the John Derrre 4045T engine set-up were brake-specific NOx emissions significantly reduced. Nonetheless, a similar trend was observed using the other two engine set-ups, i.e. high oleic acid soybean oil derived biodiesel tends to mitigate NOx emission. These data, combined with the added benefits of increased oxidative stability due to the drastic reduction in polyunsaturated fatty acids, where it is well documented that methyl-linoleate and methyl-linoleneate oxidize 12 to 25 times faster than methyl-oleate, and improved cold flow properties, demonstrates that a soybean low in saturated fatty acids, and high in oleic acid is a superior feedstock than standard commodity soybean for biodiesel.

Objective 2: Analyze the amino acid composition of the meal (protein component) of the novel soybean germplasm.

In 2004 and 2005 yield trials were conducted under both irrigation and dryland conditions. In addition to the novel soybean event 335-13, included in these studies were the parental cultivar of 335-13, A3237, and elite soybean cultivars NE3001, NE2801, RMPLC1311-128 and U98-307-917. The latter four soybean genotypes were developed at the University of Nebraska's soybean breeding program.

Table 3 Affect of fatty acid profile on accuracy of NIR analysis

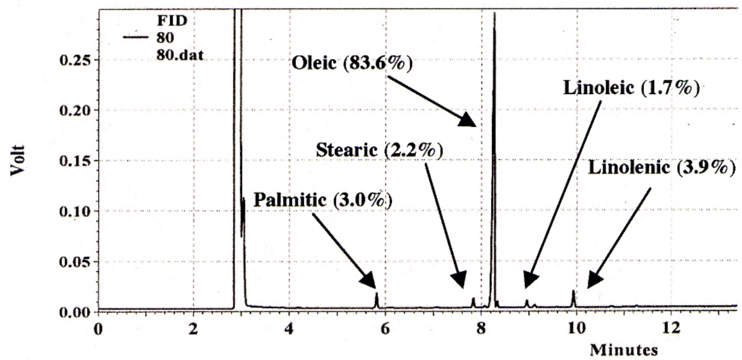
	Proximate		NIR	
Line	%Oil	%Protein	%Oil	%Protein
NE3001	19.6±0.6	37.7±0.7	21.8±0.3	39.6±0.4
U98-307917	18.9±0.6	38.4±0.4	20.7±0.1	40.4±0.3
RMPLPC1	21.1±0.3	35.4±0.3	23.8±0.2	37.4±0.3
Ux1625-12*	19.1±0.8	37.8±0.8	22.0±0.6	43.3±0.7
Ux1625-183*	19.1±0.6	38.8±0.6	21.7±0.5	44.4±0.7
335-13*	18.0±0.4	39.3±0.5	20.8±0.0	45.1±0.4

Line column indicates soybean genotypes. Those with * reflect soybean genotypes with high oleic acid oil.

Total oil and protein were determined by near infrared analysis (NIR) taken from random samples from the respective plots under both irrigation and dryland environments. The data tabulated from the NIR analysis revealed that while total oil levels were not significantly changed in the high oleic acid/low palmitic acid soybean event 335-13 under the environments grown in Nebraska (data not shown). However, the NIR data revealed that total protein levels were significant enhanced in event 335-13 as compared to the respective control variety A3237 (data not shown). This was an unexpected result given that oil and protein levels are typically inversely related in soybean. This led us to go back and randomly sample irrigated plots from 2004 and 2004, along with a subset of progeny derived from the cross designated Ux1625 (see above), and get proximate analysis to ascertain protein and oil content and compare the data with that obtained through NIR analysis. The data collected from this study comparing total oil/protein as determined by wet-bench proximate analysis verses the rapid NIR readings indicated NIR estimates of total oil in general mirrored that observed for the wet-bench approach, with NIR tending to over estimate oil levels at about 2% (Table 3). However, NIR tended to artificially elevate total protein estimates of the seed with the altered fatty acid ratio, with NIR estimates consistently measuring approximately 4% greater than the wet-bench assay (Table 3). Hence, the results obtained from the systematic scan with NIR to monitor total oil/protein from the test plots planted in 2004 and 2005, which indicated a significant rise in total protein in seed with a drastic shift in the fatty acid profile towards oleic acid, was not due to the perturbation of seed metabolism, but rather misread from a NIR apparatus calibrated to meet specifications for standard commodity soybeans.

We analyzed 79 samples from the 2005 progeny populations derived from the high oleic acid soybean event 335-13. Included in the analyses were samples of the parent soybean lines NE3001, RMPC1-311-128, U98-307-917 and A3237. As mentioned previously the four soybean lines, NE3001, RMPC1-311-128, A3237 and U98-307917 produce oil with conventional fatty acid profiles. Amino acid profile was ascertained from each sample. The data revealed no significant variation in the amino acid profiles (data not shown). These data, both amino acid profiles and total oil/protein analyses, suggests that seed protein quality and quantity, along with oil quantity are not compromised in event 335-13 or progeny derived from crosses with this event.

Figure 3: GC trace displaying fatty acid profile of soybean event 335-13



<u>Objective 3</u>: Evaluate agronomic performance of the high oleic/low saturated and polyunsaturated fatty acid germplasm across multiple environments.

In 2004 and 2005 event 333-13 was grown in one location in Juana Diaz, Puerto Rico and two locations in Nebraska. The Puerto Rico and Nebraska locations allowed for the monitoring of the affect of environment on the novel fatty acid profile. The two Nebraska sites were utilized for a comprehensive study on the agronomic performance of event 335-13.

Soybean plantings were harvested in March of 2004 and April 2005 from the Puerto Rico locations and during the second week of October for the Nebraska plantings in 2004 and 2005. Fatty acid analysis conducted on 219 randomly selected seeds from the Puerto Rico harvest in 2004, while the samplings from Nebraska sites in 2004 and Puerto Rico harvest in 2005 were conducted on 11 and 18 random bulk extracted samples, respectively. From the Nebraska harvest in 2005 fatty acid analysis was conducted on extruded oil derived from approximately 12 bushels of 335-13. The fatty acid profiles ascertained from the respective harvests are shown in Table 4. The dataset reveals the stability of the novel fatty acid profile is not impacted by harvest location. Percentage of

Table 4: Fatty acid profile observed from event 335-13 across two environments

Location	16:0	18:0	18:1	18:2	18:3
PR2004	4.1±0.5	2.3±0.2	86.4±2.5	2.7±1.7	3.5±0.4
NE2004	3.9±0.7	2.4±0.2	85.5±2.7	2.9±1.5	4.2±0.9
PR2005	3.8±0.3	1.7±0.1	87.9±1.5	2.6±1.3	2.8±0.2
NE2005*	4.3	2.9	85.2	3.2	3.1

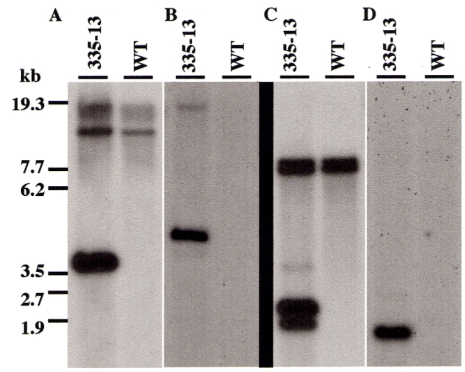
Location column refers to Puerto Rico 2004 (PR2004), Nebraska 2004 (NE2004), Puerto Rico 2005 (PR2005), and Nebraska 2005 (NE2005). * indicates fatty acid profile determined from bulk extruded oil. Columns 16:0- % palmitic acid, 18:0- % stearic acid, 18:1- % oleic acid, 18:2- % linoleic acid, and 18:3- % linoleic acid.

oleic acid averaged between 85.2% and 85.5% from the Nebraska harvests, and 86.4% and 87.9% from the Puerto Rico harvests (Table 4). An example of one of the gas chromatograph traces is shown in Figure 3.

The integration pattern of the foreign locus in event 335-13 was determined by Southern blot analysis. The results are shown in Figure 4. The data suggests the foreign allele in event 335-13 resides at a single locus with two to three copies.

Yield trials were conducted on four lineages, homozygous lines originally derived from two different T_1 individuals, of event 335-13 at the two Nebraska locations in 2004 and 2005, under both irrigated and dryland conditions. Included in the trials were five additional soybean lines, A3237, NE2801, NE3001, RMPLC1311-128, and U98-307-917. As mentioned above A3237 is the genetic background of event 335-13, while the later four genotypes were developed at the University of Nebraska's soybean breeding program. Data was ascertained on yield, lodging, date to maturity and height.

Figure 4: Southern blot analysis on event 335-13



4A: Total genomic DNA digested with *Sst* I, and probed with FAD2-1 ORF. **4B**: Panel A re-probed with *bar* ORF. **4C**: Total genomic DNA digested with *Hind* III and probed with Fad2-1. **4D**: Panel C re-probed with *bar* ORF.

Data collected on the agronomic parameters from the field studies in Nebraska in 2004 and 2005 are summarized in Tables 5 & 6. The data revealed that under both irrigated and dryland conditions no significant variation in yield was observed between the parental genotype A3237, and the transgenic lineages derived from soybean event 335-13. However, significant differences were observed between the groupings of A3237 with 335-13 lineages and the other genotypes. This is undoubtedly related to the improved genetic potential of these more advanced breeding genotypes.

Table 5: Agronomic performance of event 335-13, 2004

Line	Location	Yield	Lodging	MAT	Height
335-13-51-6	MD	53.5±3.0	1.8±0.5	36.0±0.0	31.5±1.7
335-13-51-9	MD	51.1±4.1	1.8±0.5	34.8±2.5	32.0±1.4
335-13-63-13	MD	52.4±1.0	1.8±0.5	36.0±0.0	31.8±1.9
335-13-63-8	MD	53.9±2.1	2.0±0.0	36.0±0.0	32.8±1.2
A3237	MD	52.1±5.0	1.3±0.5	34.5±1.7	36.8±1.0
NE2801	MD	56.6±9.3	1.0±0.0	33.5±3.6	29.0±1.6
NE3001	MD	64.0±7.2	1.0±0.0	32.3±2.5	29.3±2.6
RMLPC1	MD	54.3±3.9	1.8±0.5	34.3±2.9	32.8±2.6
U98-307	MD	62.3±0.3	1.0±0.0	31.0±0.0	33.0±0.6
335-13-51-6	SD	48.0±8.8	1.0±0.0	36.0±0.0	33.0±0.8
335-13-51-9	SD	44.6±11.5	1.3±0.5	36.0±0.0	32.8±3.0
335-13-63-13	SD	44.0±9.1	1.5±0.6	36.0±0.0	35.5±1.9
335-13-63-8	SD	40.5±1.8	1.0±0.0	36.0±0.0	31.3±0.6
A3237	SD	42.4±1.9	2.0±0.0	36.0±0.0	35.0±0.8
NE2801	SD	56.6±6.5	2.0±1.2	36.0±0.0	29.8±1.7
NE3001	SD	56.8±5.9	1.8±1.0	36.0±0.0	31.5±1.3
RMLPC1	SD	45.6±11.1	1.8±0.5	36.0±0.0	34.8±0.5
U98-307	SD	56.7±13.1	1.8±1.0	36.0±0.0	37.5±2.4
335-13-51-6	MI	63.9±0.8	1.0±0.0	44.0±0.0	34.8±1.1
335-13-51-9	MI	61.1±3.3	1.0±0.0	44.0±0.0	35.5±2.4
335-13-63-13	MI	65.6±5.7	1.5±0.6	42.0±4.0	33.8±1.3
335-13-63-8	MI	66.5±4.3	1.3±0.5	44.0±0.0	34.5±2.4
A3237	MI	58.3±3.0	1.0±0.0	46.3±1.3	36.8±1.0
NE2801	MI	66.0±4.6	1.0±0.0	36.0±0.0	30.5±1.3
NE3001	MI	78.6±3.6	1.0±0.0	36.0±0.0	30.0±2.2
RMLPC1	MI	59.8±4.7	1.0±0.0	39.0±3.5	33.5±1.3
U98-307	MI	79.6±4.0	1.3±0.6	38.0±0.0	38.8±1.5
335-13-51-6	SI	47.7±7.4	2.5±1.0	38.8±1.5	34.8±3.3
335-13-51-9	SI	50.7±5.6	2.8±1.0	38.8±1.5	35.5±3.3
335-13-63-13	SI	46.7±4.7	1.8±0.5	38.8±1.5	35.0±2.4
335-13-63-8	SI	46.1±7.4	1.8±0.5	38.8±1.5	34.0±0.8
A3237	SI	50.4±2.8	2.0±0.0	37.0±0.8	34.0±1.8
NE2801	SI	60.0±12.6	1.5±0.6	38.0±0.0	29.8±2.8
NE3001	SI	62.7±3.6	2.3±1.0	38.0±0.0	33.3±1.0
RMLPC1	SI	46.0±9.5	3.5±1.3	38.8±1.5	34.8±2.6
U98-307	SI	60.9±9.1	3.0±0.8	38.0±0.0	35.0±2.3

Location column reflects site planted, MD: Mead dryland; SD: Steven's Creek dryland; MI: Mead irrigated MD: Mead irrigated. Yield column refers to mean bushels/acre. Lodging column indicates mean lodging score (1= no lodging, 5= lodged). MAT refers to days to maturity. Height column, mean (inches).

Table 6: Agronomic performance of event 335-13, 2005

Line	Location	Yield	Lodging	MAT	Height
335-13-51-6	MD	51.5±3.4	1.0±0.0	27.0±1.2	35.3±1.5
335-13-51-9	MD	52.2±7.6	1.0±0.0	27.0±1.2	36.0±1.4
335-13-63-13	MD	48.5±6.8	1.0±0.0	28.0±0.0	36.8±2.2
335-13-63-8	MD	49.6±5.6	1.0±0.0	27.5±1.0	35.5±2.5
A3237	MD	50.9±1.3	1.0±0.0	26.5±1.0	35.5±2.5
NE2801	MD	42.7±4.3	1.0±0.0	18.0±2.3	28.3±1.7
NE3001	MD	50.5±2.7	1.0±0.0	21.5±1.7	25.0±1.6
RMLPC1	MD	40.5±7.3	1.0±0.0	23.0±0.0	28.0±2.7
U98-307	MD	56.4±5.3	1.0±0.0	26.0±0.0	33.5±1.7
335-13-51-6	SD	54.1±5.2	1.3±0.5	34.5±1.0	33.5±3.1
335-13-51-9	SD	51.5±10.5	1.5±0.6	33.0±2.0	33.0±6.0
335-13-63-13	SD	52.8±8.7	1.5±0.6	33.5±2.5	34.5±3.1
335-13-63-8	SD	53.3±6.0	1.3±0.5	35.0±1.2	33.5±1.0
A3237	SD	56.7±4.1	1.5±0.6	35.5±1.0	35.5±0.6
NE2801	SD	65.6±1.8	1.5±0.6	20.0±1.2	33.3±2.2
NE3001	SD	64.1±2.5	1.0±0.0	18.5±1.0	24.8±4.5
RMLPC1	SD	59.9±6.0	1.8±1.0	22.5±1.0	36.5±1.7
U98-307	SD	57.5±9.0	1.5±0.6	25.3±5.9	35.5±3.9
335-13-51-6	MI	47.0±3.8	1.0±0.0	26.3±3.0	34.8±1.7
335-13-51-9	MI	49.2±1.9	1.0±0.0	25.0±3.5	35.0±1.4
335-13-63-13	MI	47.3±2.0	1.0±0.0	27.0±1.0	34.3±1.5
335-13-63-8	MI	47.1±2.4	1.0±0.0	26.3±2.4	36.3±1.0
A3237	MI	46.3±0.8	1.0±0.0	26.5±1.0	34.0±0.8
NE2801	MI	45.0±4.1	1.0±0.0	25.5±3.3	27.3±2.2
NE3001	MI	48.5±4.3	1.0±0.0	25.5±2.9	24.3±1.7
RMLPC1	MI	46.4±3.3	1.0±0.0	22.3±2.9	31.3±1.9
U98-307	MI	53.2±4.4	1.0±0.0	20.3±5.1	32.0±1.8
335-13-51-6	SI	57.1±5.5	1.8±1.0	38.0±0.0	36.3±2.1
335-13-51-9	SI	60.0±3.5	1.8±0.5	38.0±0.0	37.3±2.2
335-13-63-13	SI	60.1±1.7	1.8±1.0	34.5±2.5	36.8±3.6
335-13-63-8	SI	58.1±6.3	1.3±0.5	37.0±1.2	37.0±2.2
A3237	SI	62.1±3.2	1.0±0.0	37.0±1.2	38.0±2.2
NE2801	SI	65.9±2.1	1.0±0.0	24.0±1.2	31.8±2.9
NE3001	SI	65.3±0.6	1.0±0.0	25.0±0.0	28.5±6.5
RMLPC1	SI	62.1±2.2	2.8±0.5	33.3±5.7	36.0±4.2
U98-307	SI	68.3±2.6	1.8±0.5	37.5±1.0	36.3±3.9

Location column reflects site planted, MD: Mead dryland; SD: Steven's Creek dryland; MI: Mead irrigated MD: Mead irrigated. Yield column refers to mean bushels/acre. Lodging column indicates mean lodging score (1= no lodging, 5= lodged). MAT refers to days to maturity. Height column, mean (inches).

Products developed under the award and technology transfer activities

Publications

Tat M. E., P. S. Wang, J. H. Van Gerpen, T. E. Clemente. 2007. Exhaust emissions from an engine fueled from high-oleic soybeans. J. Am. Oil. Chem Soc. 84: 865-869.

Graef G., B. J. LaVallee, P. Tenopir, M. Mustafa, B. J. Schweiger, A. J. Kinney, L. H. Van Gerpen, T. E. Clemente. 2008. A high oleic acid and low palmitic acid soybean: Agronomic performance and evaluation as a feedstock for biodiesel. In preparation

Abstracts

Wang, P. S., J. Van Gerpen, J. Thompson, T. Clemente. 2006. NOx emissions from the combustion of several biodiesel fuels. Western States Section Combustion Institute. Spring 2006 Meeting

Wang, P. S., J. Van Gerpen, J. Thompson, T. Clemente. 2006. Engine performance with biodiesel derived from high oleic soybean oil. ASABE International Meeting, July 2006.

T. Clemente. 2006. Transgenes in soybean. Second Annual Soybean Biotechnology Symposium. University of Missouri.

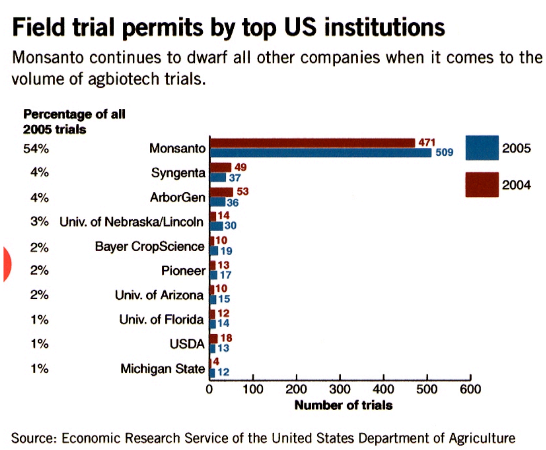
T. Clemente. 2006 A pipeline for evaluating novel soydiesel derived from biotechnology American Chemical Society, Atlanta, GA.

J. Van Gerpen. 2006 Engine emissions from high oleic acid soybean oil. Cellular and Molecular Biology of the Soybean Conference, Lincoln Nebraska.

T. Clemente. 2007. Genetic engineering of soybean: Trait evaluation in an academic environment. Plant & Animal Genome, San Diego CA.

Other products

Monies from this project help defray the cost of establishing a dedicated field and processing facility for testing of regulated soybeans. This unique infrastructure allows for the testing of agronomics along with down-stream evaluation of potential applications derived from processed seed, under strict identity preservation. The strength of this infrastructure was highlighted in a publication in the journal Nature Biotechnology, which compared the



number of release permits for planting of regulated transgenic plants requested by leading US institutions. As can be seen by the graph taken from this publication, the University of Nebraska ranked 4th in 2004 and 2005.

In addition the Van Gerpen group purchased a NOx analyzer that greatly facilitated the studies carried out over the course of this program.