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GERMPLASM

Registration of USDA-N6003LP Soybean Germplasm with Low Seed Phytate

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Abstract

Soybean [*Glycine max* (L.) Merr.] meal is the main source of protein in poultry and swine rations worldwide. Phytate, the main storage form of phosphorous in soybean meal, is largely indigestible by monogastric animals and, thus, a major concern both for nutrition and for environmental pollution. USDA-N6003LP (Reg. no. GP-435, PI 689999) is a low-phytate (LP) determinate, lodging-resistant early maturity group (MG) VI soybean germplasm developed and released jointly by the USDA-ARS and the North Carolina Agricultural Research Service. USDA-N6003LP is derived from a backcross (BC1) between recurrent parent 'NC-Roy' and LP donor line USDA CX1834. NC-Roy is a high-yielding MG VI cultivar adapted to the southern United States. USDA-N6003LP has 60% lower phytate and 4.8 times higher inorganic phosphorus (Pi) contents in its seed than the seed of NC-Roy. It matures approximately 5 d earlier and has larger seed size and better lodging resistance ($P < 0.05$) compared with NC-Roy. Across 17 environments in the USDA Uniform Soybean Tests, Southern States and over four local yield trials in North Carolina, USDA-N6003LP yielded 91 and 97% of NC-Roy, respectively. Field emergences of this line in four tests in NC were 79 to 80% compared with 89 to 90% for NC-Roy. USDA-N6003LP is the first early MG VI LP germplasm release with good agronomic performance and relatively normal field emergence. It will be useful as parental stock for soybean breeders interested in developing LP soybean cultivars.

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Soybean [*Glycine max* (L.) Merr.] meal is the main source of protein in poultry and livestock rations worldwide (Wilson, 2004). Nearly 81% of soybean meal is fed to poultry and swine, the two main monogastric consumers of soybean meal (Soystats, 2016). Soybean meal is also on the rise as a protein source in aquaculture (Asche et al., 2013). The main storage form of phosphorous (P) in soybean meal is phytate, which is largely indigestible by monogastric animals and is a major concern for both animal nutrition and environmental pollution. Undigested phytate in the meal is excreted in waste, is digested microbially, and becomes a major source of P contamination in water bodies surrounding poultry and livestock farms. Excess P in streams and rivers can promote environmental havoc via algal blooms, hypoxia, and damage to aquatic fauna and flora (Schindler et al., 2008; Sinkko et al., 2013). Nutritionally, negatively charged phytate strongly binds to metallic cations, such as Ca, Fe, K, Mg, Mn, and Zn, making them insoluble and reducing the efficacy of these essential nutrients in animal rations (Pallauf et al., 1998; Plumstead et al., 2007; Leytem et al., 2008). In contrast, inorganic phosphate (Pi) is readily digestible (Wilcox et al., 2000; Bilyeu et al., 2008).

To alleviate the problem of poor phytate digestibility by monogastric animals, synthetic phytase is often applied commercially to soy-based feeds. However, the additive is expensive and less efficient than natural phytase activity, in that the efficacy of added phytase is affected by factors such as temperature, pH, and mineral concentration of the meal (Brejnholt et al., 2011; Hassaan et al., 2013).

To overcome the challenges posed by high-phytate soybean meal, researchers have identified genetic resources with low phytate (LP) via mutagenesis (Wilcox et al., 2000; Hitz et al., 2002; Yuan et al., 2007). The LP soybean line CX1834 showed 40% reduction in phytate and a 1.4-fold increase in Pi compared with

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Abbreviations: CCRS, Central Crops Research Station; KASP, Kompetitive Allele Specific polymerase chain reaction; LP, low phytate; MG, maturity group; PCR, polymerase chain reaction; Pi, inorganic phosphorus; TARS, Tropical Agricultural Research Station.

its normal parent, CX1514-4 (Yuan et al., 2007). The LP trait in CX1834 is controlled by two recessive alleles (Oltmans et al., 2004), and the two loci associated with the trait were mapped on chromosomes (Chrs) 3 (linkage group N) and 19 (linkage group L) (Walker et al., 2006; Gillman et al., 2009). However, LP soybean seed generally has lower field emergence compared with seed that has normal phytate (Spear and Fehr, 2007; Maupin et al., 2011).

Methods

Parental Lines and Their Pedigrees

USDA-N6003LP (Reg. no. GP-435, PI 689999) is derived from the backcross of the LP trait from donor line CX1834 to recurrent parent NC-Roy (Burton et al., 2005). NC-Roy is a high-yielding, non-genetically modified, late-maturity group (MG) VI cultivar developed and released jointly by USDA-ARS and the North Carolina Agricultural Research Service. CX1834 was derived from a cross between 'Athow' (Wilcox and Abney, 1997) and M153-1-4-6-14. Athow is a cultivar with normal phytate content, and M153-1-4–6-14 is a LP mutant developed from a breeding line CX1515-4 via ethyl methanesulfonate treatment at USDA-ARS/Purdue University (Wilcox et al., 2000; Maroof et al., 2009).

CX1515-4 is derived from the cross CRS3-998-24-1 × C1813. C1813 is a derived from breeding line C1655 × 'Pella 86' (Fehr et al., 1987). CRS3-998-24-1 is from a recurrent selection population developed to improve seed protein content. Breeding line C1655 is derived from 'Nebsoy' (Williams et al., 1980) × A75-305022. A75-305022 is derived from 'Wye' × ('Amsoy' × `Wayne') (Bernard, 1966; Weber, 1966).

Breeding Line Development

NC-Roy was hybridized with breeding line CX1834-1-6 at the Central Crops Research Station (CCRS), Clayton, NC, in 2000. The CCRS and all other North Carolina experiment stations referenced in this registration are part of the North Carolina Department of Agriculture and North Carolina State University. The F_1 plants were grown at the USDA-ARS Tropical Agricultural Research Station (TARS), Isabela, PR, during winter 2000–2001. The F_2 plants were grown at CCRS in summer 2001 along with NC-Roy. Random pollen was collected from the F_2 plants and backcrossed to NC-Roy. The backcross-derived (BC_1–F_1) plants were grown at TARS in winter 2001–2002. The BC_1–F_2 plants were increased subsequently at CCRS and at TARS (2002 and 2002–2003, respectively). In 2003, approximately 400 F_4 plants were harvested individually at CCRS. Seed of the F_4 plants was screened for the presence of elevated Pi in 2004 according to a previously published protocol from our Research Unit (Israel et al., 2006), and 14 plants tested positive. The 14 progeny rows were grown at TARS in winter 2004–2005. In 2007 and 2008, 13 of the 14 $BC_1F_{4:5}$ and $BC_1F_{4:6}$ breeding lines, respectively, were evaluated for yield at CCRS and the Tidewater Research Station near Plymouth, NC, in preliminary yield trials (data not shown). One breeding line was selected for further evaluation. During the inbreeding process and development of this breeding line, no direct selection was made for field emergence.

Breeding Line Evaluation

USDA-N6003LP (previously designated as N04-05-N41) was evaluated for seed yield and other agronomic traits from 2012 to 2015 in regional and in-house trials. USDA-N6003LP and NC-Roy were evaluated in 19 environments of the USDA Southern Regional Preliminary (MG-VI) Trials (Gillen and Shelton, 2013, 2014, 2015). The plot techniques for USDA Southern Regional Soybean Trials are described in Gillen and Shelton (2013, 2014, 2015). USDA-N6003LP was also evaluated in a total of four additional North Carolina environments in replicated trials during 2014 and 2015. In the North Carolina trials, each yield plot consisted of three 6.7-m-long rows with a row spacing of 96.5 cm. The plots were end trimmed to 4.57 m before harvesting at or near maturity. The seeding rate was approximately 30 seed m^{-1} of row. The middle row of each plot was harvested with a plot combine for assessment of yield and other seed traits. Maturity, plant height, and lodging data were collected before harvest. Maturity was defined as the first day on which >95% of pods were mature. Plant height was measured as the mean height of the main stem for three plants in the middle row of a plot at maturity. Lodging was rated on a scale of 1 to 5 (Fehr, 1987), where 1 = almost all plants erect, 2 = either all plants leaning slightly or few plants down, 3 = either all plants leaning moderately or 25 to 50% of the plants down, 4 = either all plants leaning heavily or 50 to 80% of the plants down, and 5 = all plants down. Seed quality was rated using a 1 to 5 scale, where 1 indicates very good seed quality and 5 indicates very poor seed quality (Green et al., 1965). Protein and oil contents were measured on a zero moisture basis with a Foss Infratec 1241 Grain Analyzer at the USDA-ARS National Center for Agricultural Utilization Research, Bio-Oils Research Unit, Peoria, IL.

Field Emergence

Soybean seed with LP has been reported to have lower field emergence than seed with normal phytate (Oltmans et al., 2005; Spear and Fehr, 2007; Maupin et al., 2011). Therefore, field emergence of USDA-N6003LP was evaluated in two separate trials in 2017 in North Carolina. The seed from the 2016 breeder seed increase plot was used for both trials. The first trial included NC-Roy, USDA-N6003LP and three additional check cultivars—'N8101' (Carter et al., 2009), 'N6202' (Carter et al., 2010), and 'NC-Raleigh' (Burton et al., 2006). The trial was grown at two locations—CCRS and at Caswell Research Farm near Kinston, NC—with six replications at each location arranged in a randomized block design. One hundred seeds of each line was planted in a 3.65-m-long row with an approximate planting density of 27 seed m^{-1}. Two weeks after planting, the normal looking seedlings in each row were manually counted and the number was recorded. A seedling with open unifoliate leaves, open or unopen trifoliate leaves, and apical meristem was considered normal. The second trial was also grown at two North Carolina locations (Caswell Research Farm and Tidewater Research Station) each with four replicates and just two genotypes—NC-Roy and USDA-N6003LP. Two hundred seeds of each genotype were planted in a 6.7-m-long row with an approximate planting density of 30 seed m^{-1}. Two weeks after

planting, the seedlings in each row were counted in the same manner as described above and the number was recorded.

Phytate Assay

The phytate content of each sample was determined using an indirect Fe colorimetric method as reported in previous studies (Burleson et al., 2012; Averitt et al., 2017). Briefly, 0.5 g of soybean powder was extracted with 10 mL of 0.5 M HCl. Starch and protein were removed with 0.9 mL of 20% (w/v) NaCl in ddH_2O before being treated with a ferric iron solution for 2 h followed by a color reagent. For each sample, 380 μL of the solution was then analyzed through 510-nm wavelength absorption on a FLUOstar Omega microplate reader (BMG Labtech), and phytate concentration was determined from a standard curve taken from seven known concentration standards.

Phosphate Assay

A few grams of seed were ground in a Retsch ZM100 grinder using a 1-mm filter. The ground samples were dried for 3 d at 65°C to a constant weight. Ten milligrams of dried meal was put in a Supelco vial (Sigma-Aldrich) and 10 mL of 80% (v/v) ethanol was added to the vial, after which the magnetic screw cap was securely tightened to close the vial. The vials were rotated in a hybridization oven at 65°C for 3 h and then centrifuged in a Sorvall HS-4 rotor (Thermo Fisher Scientific) at 2500 rpm for 5 min. Two hundred microliters of the supernatant solution was pipetted into a 96-well plate in triplicate and allowed to dry overnight in a hood. The dried material was solubilized for 1 h at room temperature in 200 μL of distilled water. Samples were pipetted up and down to ensure all phosphate in the well was solubilized. Phosphate was measured on a 100 μL aliquot of the sample using a PerkinElmer Victor 3 and the Abcam ab65622 Phosphate Assay Kit following the manufacturer's instructions.

KASP Assay

DNA was isolated from 20 mg of seed tissue using the Qiagen DNeasy Plant Mini Kit (Germantown, MD 20874). A pre-amplification step (Gillman et al., 2009) was used to enrich for amplicons containing the targeted SNP in the low phytate alleles, *lpa*1 and *lpa*2. Kompetitive Allele Specific polymerase chain reaction (KASP) assays (LGC Genomics) were used to identify the *lpa*1 and *lpa*2 alleles (shown in Table 1). Separate 10 μL pre-amplification was performed for *lpa*1 and *lpa*2 alleles using ~10 ng of DNA, 5 pM of the forward and reverse primers, 10× *Taq* polymerase buffer, 15 mM $MgCl_2$, 3.12 mM dNTP, and 1 unit of Thermo Fisher Scientific *Taq* polymerase. The DNA was denatured for 5 min at 95°C and the pre-amplification was performed for 45 cycles of 95°C for 30 s, 52°C for 30 s, 72°C for 30 s, with a final extension at 72°C for 5 min. Two microliters of the pre-amplification polymerase chain reaction (PCR) product was used in separate gene-specific KASP assays for *lpa*1 and *lpa*2. The components for PCR reactions include premade 2 μL of 2× KASP master mix (contains KASP 2× mix, 50 mM $MgCl_2$, and DMSO) (KBS-1016–002) and KASP assay primer mix (0.4 μM for the common reverse primer and 0.165 μM for the allele-specific forward primers). The KASP assay was performed with the PCR conditions of 94°C for 15 min for hot-start activation, 9 cycles of 94°C for 20 s and 65°C for 60 s (dropping 0.8°C per cycle), followed by 38 cycles of 94°C for 20 s and 57°C for 60 s. Endpoint genotyping was conducted following the manufacturer's instructions, and the values are the FAM and HEX channels of the BioRad Real-time CFX96 thermocycler (Bio-Rad).

Statistical Analysis

Performance results were accessed for USDA-N6003LP from the USDA Uniform Soybean Tests, Southern States for the Preliminary Group VI trials during 2012 to 2014 (Gillen and Shelton, 2013, 2014, 2015). Inspection of these data from each of the 19 environments, in which USDA-N6003LP was tested, revealed that two individual trials (Tallassee, AL, in 2013 and 2014) had coefficients of variation for seed yield greater than 15%, and these data were omitted from further consideration. Four genotypes in addition to USDA-N6003LP were common to all MG VI trials during 2012 to 2014, and these were subjected to analysis of variance for all traits using the procedure PROC GLM in SAS 9.4 (SAS Institute, 2013). Analysis of variance was performed on genotypic means from each environment. Genotypic mean squares from the analyses were tested for significance using the respective genotype × environment mean squares for each trait. Genotype was treated as a fixed effect and environment as a random effect. Least squares means were obtained via the PROC MIXED procedure in SAS 9.4. Fisher's protected least significant difference (LSD) was used for comparison of genotypic means. The data for field emergence, phytic acid, and inorganic phosphate were also analyzed using the same GLM procedure of SAS as above. Again, the genotypes were considered fixed and environments as random effects.

Table 1. DNA pre-amplification and KASP assay primer sequences of *lpa*1 and *lpa*2 alleles.

Purpose	Primer	Direction	Sequence (5′ to 3′)
Pre-amplification	*lpa*1_F	Forward	CCTACTCACTCCTCTGAAGAG
	*lpa*1_R	Reverse	TATGCTGCTGCCATGTATGAAAG
	*lpa*2_F	Forward	CTTAATCCTGGAGGATCAAGTTATGT
	*lpa*2_R	Reverse	AAAAGAAGCACAAATGTGAAGCTG
KASP assay	*lpa*1_A1_wt	FAM	GAAGGTGACCAAGTTCATGCTAGAAGAAGAAGAAAGCAAAACGATCGA
	*lpa*1_A2_mut	HEX	GAAGGTCGGAGTCAACGGATTAGAAGAAGAAGAAAGCAAAACGATCGT
	*lpa*1_C2b	Common reverse	CTCCTCTTCCTGAACAAGCTGTTTCTTT
	*lpa*2_A1_wt	FAM	GAAGGTGACCAAGTTCATGCTAGGAATTTGGCTGTACTGATAAATTC
	*lpa*2_A2_mut	HEX	GAAGGTCGGAGTCAACGGATTACTAGGAATTTGGCTGTACTGATAAATTT
	*lpa*2_C1	Common reverse	GTCTATCACGGTGGATACTCAGCTT

Characteristics

Agronomic and Botanical Description

USDA-N6003LP is a determinate, lodging-resistant, short-statured, early MG VI soybean with white flowers and gray pubescence. Averaged across 17 environments of USDA Regional Trials, USDA-N6003LP matured 5 d earlier than recurrent parent NC-Roy and 3 d earlier than check cultivar Dillon (Shipe et al., 1997) (Table 2). NC-Roy and Dillon are early and late MG VI cultivars, respectively. The 100-seed weight of USDA-N6003LP was significantly ($P < 0.05$) larger than that of NC-Roy and Dillon in the USDA Regional Trials (15.1 vs. 12.9 and 14.4 g, respectively) but smaller than that of NC-Dilday (17.0 g). NC-Dilday is a high-yielding cultivar released in 2016 by North Carolina State University (Loren Fisher, personal communication, 2019). Plant height of USDA-N6003LP was significantly ($P < 0.05$) shorter than that of NC-Roy or Dillon (80 vs. 90 and 95 cm, respectively) and on par with NC-Dilday (79 cm). Lodging of USDA-N6003LP was significantly less than NC-Roy and NC-Dilday and similar to that of Dillon (1.8 vs. 2.7, 2.2, and 2.0, respectively). Separate tests in North Carolina produced similar comparisons between NC-Roy and USDA-N6003LP for maturity, lodging, and 100 seed weight (Table 3). Seed protein contents of USDA-N6003LP and NC-Roy were nearly identical in both the USDA Regional Trials and in-house state trials in North Carolina (Tables 2 and 3). Its oil content was slightly lower than NC-Roy in the USDA Regional Trials but did not differ in the in-house trials (Tables 2 and 3). However, USDA-N6003LP was significantly ($P < 0.05$) higher in seed protein and lower in seed oil content than NC-Dilday (422 vs. 393 and 206 vs. 233 g kg^{-1}) (Table 2).

Averaged over 17 environments in the USDA Southern Regional Trials, the seed yield of USDA-N6003LP (3328 kg ha^{-1}) was 91, 92, and 83% of the yield of recurrent parent NC-Roy, Dillon, and NC-Dilday, respectively (Table 2). In our in-house trials over four North Carolina environments during 2014 and 2015, USDA-N6003LP yielded 97% of NC-Roy and matured 7 d earlier than NC-Roy (Table 3).

Field Emergence

In the first trial across two environments, USDA-N6003LP exhibited 80% field emergence that was not significantly ($P < 0.05$) lower than that (89%) of NC-Roy (Table 4). Emergence of three other check cultivars ranged from 88– 94% in the tests. The genotype x environment interaction term was significant ($P < 0.01$), reflecting the result that USDA-N6003LP had much lower (74%) germination at CCRS than it had at Caswell Research Farm (86%). By contrast, NC-Roy exhibited 89% field emergence in both of these environments. Thus, USDA-N6003LP had significantly lower ($P < 0.05$) field emergence

Table 2. Performance of USDA-N6003LP soybean germplasm in 17 environments of the USDA Southern Regional Preliminary Trials, 2012–2014.

Genotype	Yield	Maturity	Height	100-seed weight	Lodging	Seed quality	Protein	Oil
	kg ha^{-1}	d (1 Oct. = 1)	cm	g	1–5†	1–5‡	g kg^{-1}§	
USDA-N6003LP	3328	8	80	15.1	1.8	1.5	422	206
NC-Roy¶	3672	13	90	12.9	2.7	1.6	422	210
NC-Dilday¶	3995	13	79	17.0	2.2	1.7	393	233
Dillon¶	3623	11	95	14.4	2.0	1.6	422	219
LLL05-14	3290	11	78	13.5	2.0	1.6	429	207
No. of environments	17	17	17	15	17	10	15	15
Mean	3540	11	85	14.6	2.1	1.6	418	215
LSD (0.05)	291	2	5	0.7	0.3	0.2	8	4

† 1 = almost all plants erect; 2 = either all plants leaning slightly or few plants down; 3 = either all plants leaning moderately or 25 to 50% of the plants down; 4 = either all plants leaning heavily or 50 to 80% of the plants down; and 5 = all plants down.

‡ 1 = very good, 5 = very poor (Green et al., 1965)

§ Protein and oil measured on a zero moisture basis.

¶ NC-Roy is the recurrent parent of USDA-N6003LP. Dillon, NC-Dilday, and NC-Roy were all maturity group VI check cultivars for this trial. LLL05-14 is a high stearic breeding line from USDA-ARS Soybean and Nitrogen Fixation Unit in Raleigh, NC.

Table 3. Performance of USDA-N6003LP soybean germplasm and check cultivars over four North Carolina environments, 2014- 2015.

Genotype	Yield†	Maturity	100-seed weight	Lodging	Protein	Oil
	kg ha^{-1}	d (1 Oct. = 1)	g	1–5‡	g kg^{-1}§	
USDA-N6003LP	4270	18	17.8	1.8	422	210
NC-Roy¶	4371	25	13.2	2.3	418	207
NC-Raleigh¶	4539	29	13.2	2.6	385	240
Osage¶	4613	16	13.8	1.6	444	213
LSD (0.05)	499	3	0.9	0.6	19	7
No. of environments	4	4	4	4	2	2

† Yield trials were grown at Plymouth and Caswell, NC, in 2014 and 2015 using four replications per environment.

‡ 1 = almost all plants erect; 2 = either all plants leaning slightly or few plants down; 3 = either all plants leaning moderately or 25 to 50% of the plants down; 4 = either all plants leaning heavily or 50 to 80% of the plants down; and 5 = all plants down.

§ Protein and oil are on a zero moisture basis, which were measured only in 2015.

¶ NC-Roy is the recurrent parent of USDA-N6003LP. NC-Raleigh is a maturity group VII cultivar and Osage (Chen et al., 2007) is a maturity group V cultivar used as checks for this trial.

Table 4. Field emergence of soybean USDA-N6003LP, NC-Roy, and check cultivars in two environments (Caswell and Clayton, NC) in 2017.

Genotype	Field emergence		
	Caswell	Clayton	Mean
	%		
USDA-N6003LP	86	74	80
NC-Roy	89	89	89
N8101	89	92	91
N6202	89	87	88
NC-Raleigh	94	94	94
LSD (0.05)	6.7	4.5	9.3

than NC-Roy in Clayton but the difference was not significant in Caswell (Table 4). In a second trial, USDA-N6003LP had significantly ($P < 0.05$) lower (79%) emergence compared to (90%) of that of NC-Raleigh. In this test, there was no genotype x environment interaction. USDA-N6003LP and NC-Roy were the only two genotypes compared in this trial (data not shown).

Phytate and Inorganic Phosphate

Based on six environments (18 replicates) across 3 yr (2014–2016), the phytate content (1.06 mg g^{-1}) of USDA-N6003LP was only 39% of the phytate content (2.72 mg g^{-1}) of NC-Roy (Table 5). Inversely, the Pi content (38.34 nmole mg^{-1}) of USDA-N6003LP was 4.8 times greater than that (7.98 nmole mg^{-1}) of NC-Roy (Table 5). The low phytate as well as the high Pi contents in the seed of USDA-N6003LP should be very useful for better nutrition of the monogastric consumers of soybean meal and to reduce P pollution in the environment.

KASP Assay

The KASP assay confirmed that USDA-N6003LP has the homozygous recessive mutant alleles and NC-Roy has the two wild-type alleles at the two loci on Gm 3 and 19, as expected.

Seed Purification and Increase

Seed purification and increase for USDA-N6003LP was conducted at the Sandhills Research Station, Jackson Springs, NC, beginning in 2012, using seed from yield trials harvested in 2011 as a planting source. Before planting each year, seed was cleaned on a Clipper Eclipse 324 (A.T. Ferrell Company Inc.) to remove trash and eliminate both the smallest and largest 5% of seed to reduce off-types for seed size. After mechanical cleaning, the seed was cleaned further by hand to remove off-types for hilum color and seed coat color. Purification increase blocks were planted each year in three, four or six rows of approximately 100-m row length at a seeding rate of approximately 13 seed m^{-1} of row. Plots were rogued both at flowering time and maturity to remove off-types. Before harvest, the plot combine was thoroughly cleaned with a gas powered leaf blower and then inspected to remove residual seed. To further reduce chances for cross-contamination, the first 2 kg of seed harvested from each increase block was discarded. In 2016, 50 random plants were harvested individually from the purification block and grown as progeny rows at CCRS in North Carolina in 2017. Forty of the agronomically uniform progeny rows with the expected flower, pubescence, and pod colors were harvested and subjected to phytate and Pi assays as well as DNA marker screening as described previously. Of the 40 progeny rows, 35 had the expected low phytate and high Pi values and carried the homozygous recessive mutant alleles at the two loci on Gm 3 and 19. The seed of these 35 ($BC_1F_{4:10}$) rows were bulked to form the 2017 breeder seed source for USDA-N6003LP.

Table 5. Average phytate and inorganic phosphate (Pi) contents of soybean USDA-N6003LP and NC-Roy based on six environments of local tests, 2014- 2016.

Genotype	Phytate	Pi
	mg g^{-1}	nmole mg^{-1}
NC-Roy	2.72	7.98
USDA-N6003LP	1.06	38.34
LSD (0.05)	0.15	4.05
No. of environments†	6	6

† The tests were grown at Plymouth and Caswell, NC, during 2014, 2015, and 2016 using three replicates per environment.

Availability

Small quantities of USDA-N6003LP are available from the corresponding author for research and breeding purposes. Seed of USDA-N6003LP has also been deposited in the USDA-ARS National Laboratory for Genetic Resources Preservation, where it will be available for research purposes immediately upon publication. It is requested that appropriate recognition be made if this germplasm leads to new cultivar(s), germplasm release(s), and/or scientific discovery(s).

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