Registration of N6002 Soybean Germplasm with Enhanced Yield Derived from Japanese Cultivars Fukuyutaka and Nakasennari and Elevated Seed Protein Content

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ABSTRACT

The release of N6002 (Reg. No. GP-397, PI 674171) soybean [Glycine max L. (Merr.)] is part of an effort to broaden the genetic base of North American soybean breeding programs. N6002 was developed and released by the USDA–ARS and the North Carolina Agricultural Research Service in September 2014 as a conventional maturity group (MG) VI soybean germplasm derived from hybridization of the cultivar Young and USDA germplasm line N6202. N6002 is an F₁-derived line that traces 25% of its pedigree to the Japanese cultivars Fukuyutaka and Nakasennari. The remaining pedigree (75%) is derived from ‘Young’. N6002 was tested in three sets of multistate yield trials in the southern United States: recombinant inbred line (RIL) yield trials, the United Soybean Board Southern Diversity Yield Trial Project, and the USDA Uniform Soybean Tests–Southern States. Over 39 environments, N6002 averaged 8.3% higher yield than parents Young or N6202, and 97% of the elite check ‘NC-Roy’. Although seed yield of N6002 was higher than Young, its seed protein content was comparable to that of Young and greater than that of NC-Roy. The superior yield of N6002 compared with Young demonstrates that yield-enhancing alleles were transferred to the Young background from Japanese cultivars. The elevated seed protein content of N6002 compared with NC-Roy and the parity of protein content with Young suggest that these yield-enhancing alleles may aid in mitigating the well-known negative correlation between seed yield and protein content.

IN THE DECADES since the development of modern agricultural research, development, and production techniques, the rise of soybean [Glycine max L. (Merr.)] from a regional crop in East Asia to a worldwide staple has been a major success story (Carter et al., 2004). Soybean oil and high protein meal now enjoy global demand. Soybean's long history of domestication and cultivation with concurrent selection by farmers to fit local conditions in Asia has led to a wealth of genetic diversity in the crop. However, the spread of this Asian genetic diversity was not concomitant with the global spread of soybean in the 20th century. The globalization of soybean was driven primarily by practical production needs and market demand rather than an interest in maintaining genetic diversity. Thus, the genetic base of most non-Asian soybean production and associated breeding programs is considerably smaller than for Asian programs (Carter et al., 2004; Hyten et al., 2006).

Over the past 100 yr, soybean breeders have been highly successful in adapting soybean to various growing environments in the United States and Canada (Orf et al., 2004). However, the narrow germplasm base on which North American soybean breeding rests may complicate the ability of breeders to deliver continued yield advances (Gizlice et al., 1994; Hyten et al., 2006). This narrow base has led to a current near consensus among US soybean breeders that an expanded germplasm pool is needed for applied breeding efforts (Carter et al., 2009b). The registration of N6002 (Reg. No. GP-397, PI 674171) is part of an ongoing USDA–ARS effort to mitigate the potential problems associated with this narrow breeding pool.

The yield superiority of the N6002 germplasm release over ‘Young’, the only adapted US parent used in its development, provides clear evidence that beneficial yield alleles were derived from exotic Asian germplasm and suggests that they are likely to be useful in applied US soybean breeding. The substantial exotic pedigree (25% based on coefficient of parentage) in this

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Abbreviations: CCRS, Central Crops Research Station; RIL, recombinant inbred line; SAVE, Soybean Asian Variety Evaluation; SCN, soybean cyst nematode; TARS, USDA–ARS Tropical Agricultural Research Station.
Materials and Methods

Antecedents of N6002

N602 (PI 658498) is a large-seeded, high-protein, MG VI germplasm released by the USDA–ARS (Carter et al., 2010). It was selected for large seed size, diverse pedigree, good yield, and high protein content. Across 43 regional test environments, N602 yielded 92% of the check cultivar NC-Roy (PI 617045; Burton et al., 2005). Young (PI 508266) is a MG VI cultivar released by the USDA–ARS in 1987 (Burton et al., 1987) and has become a landmark cultivar, being incorporated into the pedigrees of numerous cultivars (Boerma et al., 1992; Paris et al., 2006; Tyler and Young, 2004).

The Japanese ancestors of N6002, ‘Fukuyutaka’ (PI 506675) and ‘Nakasennari’ (PI 507079), were evaluated in the United States during the Soybean Asian Variety Evaluation (SAVE) trials in 1996 and 1997 (Manjarrez-Sandoval et al., 1997). Nakasennari, a Japanese MG V cultivar, yielded 86% of the appropriate US check cultivars across 10 environments, higher than any other Asian cultivar included in the MG IV-V SAVE trials. Fukuyutaka, a MG VII cultivar from Japan, yielded 73% of the appropriate US checks in the MG VI-VIII SAVE trials.

Pedigree

N6002 is derived from the cross of USDA–ARS germplasm release N602 × Young. N602 is derived from a cross of the USDA–ARS germplasm N6201 (PI 619615; Carter et al., 2003) × USDA–ARS breeding line N95-7390. N6201 is developed from a cross of Young by the Japanese cultivar Nakasennari. Nakasennari is descended from a cross of the Japanese landrace Houjaku and the Japanese line Nema Shirazu (PI 594231; Shoji Miyazaki, personal communication, 1996). Nema Shirazu is a selection from the landrace Geden Shirazu (PI 227557). N95-7390 is derived from a cross of Young × Fukuyutaka. Fukuyutaka is descended from the Japanese landraces Oka Daizu (PI 594241) and Shiro Daizu 3 (PI 417319). N6002 traces its pedigree to Young (75%), Nakasennari (12.5%), and Fukuyutaka (12.5%).

Breeding Line Development

The initial hybridization of N602 × Young was made at the Central Crops Research Station (CCRS) at Clayton, NC, during summer 2003. The F1 plants were grown in the winter nursery at the USDA–ARS Tropical Agricultural Research Station (TARS), Isabela, PR. The F1 plants were advanced using the single-seed descent procedure (Brim, 1966) at CCRS during the summer of 2004. The F2 plants were grown at the winter nursery and subjected to single-seed descent at TARS during the winter of 2004–2005. The F2 single plants were grown at the Sandhills Research Station, Jackson Springs, NC, in summer 2005. Two hundred fifty-seven full-sib F2 single plants were harvested, and the progeny were grown in individual rows at the Caswell Research Farm, Kinston, NC, during summer 2006.

One of these rows was selected and assigned the breeding line designation N06-10237, which was renamed N6002 on release.

Breeding Line Evaluation

N6002 was evaluated in three sets of yield trials across the southern United States. In cooperation with the University of Georgia and the University of Arkansas, N6002 was evaluated in yield trials from 2007 to 2010 (a total of nine environments) (recombinant inbred line yield trial), along with 33 of its F1-derived full-sib recombinant inbred lines (RILs), parents, grandparents, and the elite MG VI cultivar NC-Roy. At least three replications were used for each environment. N6002 was also evaluated for agronomic performance in the Southern Diversity Yield Trial Project sponsored by the United Soybean Board (Diversity Trial). In these cooperative yield trials, N6002 was compared to experimental MG VI lines from southern breeding programs, all of which had at least 12.5% exotic pedigree, plus elite check cultivars in 13 environments across the southeastern and Mid-South United States from 2009 to 2012. At least three replications were used in each trial. N6002 was also tested in the USDA Uniform Soybean Tests–Southern States, Preliminary Group VI (Uniform Tests) in replicated trials in 2010, 2011, and 2012 (a total of 17 environments in Alabama, Arkansas, Georgia, Mississippi, North Carolina, and South Carolina; Gillen and Shelton, 2011, 2012, 2013). Three replications were used in each trial.

Plot Technique

In both the RIL yield trials and the Diversity Trials, plot technique was as previously described (Carter et al., 2009a; Carter et al., 2010). Briefly, plots consisted of either three or four rows, with the outside two rows serving as buffer and the middle row(s) being harvested for yield measurement. Row spacing ranged from 76 to 96 cm and planted row length from 6 to 7 m. Plots were end trimmed at maturity to between 3.6 and 4.6 m long. Plots were seeded at a rate of about 344,000 seeds ha−1. In the Uniform Tests, plot technique was as previously described (Gillen and Shelton, 2011, 2012, 2013).

Traits Evaluated

Seed yield was recorded for all plots in all trials. Lodging was rated in one or two replications of each trial using the 1-to-5 visual scale described by Fehr (1987), in which a score of 1 indicates no lodging and 5 indicates that all plants are prostrate. In the Diversity Trials, height was measured on three randomly selected plants from two replications in each trial. Maturity, the first day on which at least 95% of pods were mature, was rated visually in single-row observation plots at 1 October = 1. Trait evaluation methods for the uniform tests have been reported elsewhere (Gillen and Shelton, 2011, 2012, 2013). In the RIL yield trials and Uniform Tests, protein and oil content were measured on a zero moisture basis using a near-infrared spectrometer at the USDA–ARS National Center for Agricultural Utilization Research, Peoria, IL (American Association of Cereal Chemists, 1999).

As part of the Diversity Trials, pod dehiscence (shattering) was estimated visually in single-row observation plots at Sandhills Research Station, Jackson Springs, NC, from 2009 to 2012. A scale of 0 to 10 was used, in which 0 indicates no...
Resistance to southern root-knot nematodes [Meloidogyne incognita (Kofoid and White) Chitwood] was evaluated in the greenhouse at the University of Georgia using the 1-to-5 scale described by Hussey and Boerma (1981), in which 1 indicates no galls and 5 indicates many galls. Resistance to Asian soybean rust (caused by Phakopsora pachyrhizi Syd.) was rated visually in the field at Attapulgus, GA, by the University of Georgia soybean breeding program, under the direction of R. Boerma and Z. Li, in 2009 and 2012. A scale of 1 to 5 was used in which 1 indicates no lesions and 5 indicates substantial lesions in the upper canopy, and both experimental and check lines were compared to the resistant check ‘Hyyuga’ (PI 506764; Monteros et al., 2007). Seed quality ratings were determined using the 1-to-5 scale of overall seed quality developed by Green et al. (1965), in which 1 indicates very good seed quality and 5 indicates very poor seed quality.

### Statistical Analysis

Analysis of the RIL yield trials was performed using PROC GLM in SAS (SAS Institute, Cary, NC). Initially, potential outliers for each trait were eliminated using residual analysis. Data points were removed if the residual was greater than the following arbitrary boundaries for each trait: lodging (1.5), seed protein content (16 g kg⁻¹), seed oil content (16 g kg⁻¹ seed weight), 100 seed weight (2.5 g 100 seed⁻¹), maturity (8 d), yield (672 kg ha⁻¹). Approximately 2% of the data points were removed as outliers using this approach. After removal of outliers, PROC GLM was used to calculate adjusted means and identify differences among genotypes for each trait of interest.

For analysis of the Diversity Trials, a two-stage analysis was performed in which the adjusted mean of each genotype within each environment was determined using the LSMeans statement in PROC MIXED in SAS. To avoid the challenges caused by the changing of test entries each year (Piepho and Mohring, 2006), analysis was performed only on a set of seven selected genotypes grown in all 4 yr in which N6002 was grown. In the second stage of the analysis, each environment was treated as a replication and the ANOVA procedure was used to assess differences among the genotypic adjusted means.

Analysis of the Uniform Tests was performed only on data from lines which were present in all years in which N6002 was tested (note: Young was coded ‘YoungBC4LX’). The published analysis (Gillen and Shelton, 2011, 2012, 2013) was used to obtain the environment’s coefficient of variation (CV) for yield. Individual trials that had a CV >15% for yield were omitted from further analysis. Analysis of variance was performed on the raw data of remaining environments to obtain adjusted means using the MIXED procedure of SAS Version 9.3_M1 for Windows (SAS Institute, 2010). A model with genotype as fixed effect and location, year, location × year, replication (location × year), location × genotype, year × genotype, and location × year × genotype as random effects was analyzed for yield, maturity, lodging, height, seed size and seed quality. Protein and oil data were never replicated in a trial; therefore these traits were analyzed using a model with genotype as a fixed effect and location, year, and location × year, location × genotype, and year × genotype as random effects. Fisher’s protected least significant difference (LSD) was calculated.

Statistical analysis was conducted using SAS as described above with results presented in Tables 1 to 3. For the in-text presentation of results obtained from consolidating the results from multiple sets of trials, that is, the RIL yield trial, Diversity Trial, and Uniform Tests, weighted means over environments are presented. The weighted means were calculated using a genotype’s mean trait value for an individual trial, which was weighted for the number of environments in which the trait was measured. Then the average of the weighted mean over all three sets of trials was calculated.

### Seed Purification and Increase

Seed increase and purification for N6002 was performed at Sandhills Research Station, Jackson Springs, NC, beginning in 2010, using seed from yield trials harvested in 2009 as a planting source. Before planting, seed were 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, seed were cleaned by hand to remove off-types for hilum color, seed coat color, and other visual qualities. Seed was planted in six-row increase blocks at a seeding rate of approximately 13 seed per meter of row. Plots were rogued both at flowering time and at maturity to remove off-types. The middle four rows of the six-row plot were harvested using a plot combine, while the outer two rows were left as buffer to reduce the likelihood of contamination from lodged plants or insect-_vectored outcrosses. To further

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**Table 1. Performance of soybean germplasm N6002 in recombinant inbred line yield tests. N6002 was grown in nine environments in Arkansas, Georgia, and North Carolina in 2007 to 2010. N6002 was compared with its parents Young and N6202, its grandparents N6201 and N95-7390, and the check line NC-Roy.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Yield kg ha⁻¹</th>
<th>Maturity d (1 Oct. = 1)</th>
<th>100-seed weight g</th>
<th>Lodging 1–5†</th>
<th>Seed protein content g kg⁻¹‡</th>
<th>Seed oil content %</th>
</tr>
</thead>
<tbody>
<tr>
<td>N6002</td>
<td>2430</td>
<td>31</td>
<td>16.3</td>
<td>2.2</td>
<td>425</td>
<td>200</td>
</tr>
<tr>
<td>Young</td>
<td>2133</td>
<td>33</td>
<td>15.3</td>
<td>2.5</td>
<td>424</td>
<td>205</td>
</tr>
<tr>
<td>N6202</td>
<td>2353</td>
<td>33</td>
<td>17.5</td>
<td>2.3</td>
<td>438</td>
<td>191</td>
</tr>
<tr>
<td>N6201</td>
<td>1997</td>
<td>34</td>
<td>20.6</td>
<td>2.3</td>
<td>419</td>
<td>198</td>
</tr>
<tr>
<td>N95-7390</td>
<td>2233</td>
<td>32</td>
<td>20.2</td>
<td>2.3</td>
<td>434</td>
<td>197</td>
</tr>
<tr>
<td>NC-Roy</td>
<td>2512</td>
<td>32</td>
<td>13.3</td>
<td>2.0</td>
<td>412</td>
<td>199</td>
</tr>
<tr>
<td>No. of environments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>202</td>
<td>2.5</td>
<td>0.9</td>
<td>0.3</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

† 1 = no lodging, 5 = complete lodging (Fehr, 1987).
‡ Protein and oil measured on a zero moisture basis.
reduce chances for cross-contamination, the first 3 kg of seed harvested from each increase block were discarded. The plot combine was cleaned between increase plots with a gas-powered leaf blower. After several years of observation, it was determined that the presence of buffer rows provided little-to-no benefit for reduction of cross-contamination between increase plots. Therefore, beginning in 2013, increase plot dimensions were changed to three rows wide, with all rows being harvested to allow more efficient use of available field space. All other production protocols remained constant.

### Plant Characteristics

#### Agronomic and Botanical Description

N6002 is a determinate, early MG VI soybean with gray pubescence and purple flowers. Over 30 environments in the Diversity Trials and the USDA Southern Uniform Trials, N6002 averaged 12 cm shorter than its parent Young and 2 cm shorter than its parent N6202 (Tables 2–3). N6002 also averaged 2 cm shorter than the conventional check NC-Roy and 3 cm shorter than Dillon (PI 592756; Shipe et al., 1997) over the 30 environments.

Maturity of N6002 averaged 2 to 3 d earlier than both Young and N6202 over 37 environments (Tables 1–3). On average, N6002 matured on the same day as the check cultivar Dillon (29 environments) and 4 d earlier than NC-Roy (37 environments).

N6002 showed no evidence of susceptibility to pod dehiscence, even when left in the field until December, with an average rating of zero over 4 yr of observations, as part of the Diversity Trials. In these same trials, Young, N6202, Dillon, and NC-Roy also exhibited little or no pod dehiscence (Table 2).

Over the three sets of trials, the mean lodging score of N6002 (2.2) was similar to that of parents Young (2.4) and N6202 (2.2) and the check line NC-Roy (2.3). Lodging scores for N6002 were also similar to the check Dillon (1.8) over the 29 environments in the Diversity Trials and Uniform Tests (Table 2–3), although in the subset of 12 environments represented by the Uniform Tests, the mean lodging score of N6002 was significantly greater \((p < 0.05)\) than that of Dillon (Table 3).

N6002 was susceptible to Asian soybean rust (rating = 3.75). Its rating was not significantly \((p < 0.05)\) different from those of Young (3.7), N6202 (2.75), Dillon (3.5), or NC-Roy (4.5) (Table 2), but all experimental and check lines had significantly
greater incidence of rust than resistant check Hyuuga (1.5). N6002 was also susceptible to the southern root-knot nematode, with a rating of 4.7, which was significantly (p < 0.05) greater than that of resistant cultivar Dillon (1.8) but not significantly different from that of susceptible Young (4.4), N6202 (4.6), or NC-Roy (5.0) (Table 2). In tests conducted in conjunction with the Uniform Tests, N6002 was susceptible to soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) Race 2 corresponding to HG Type 2.5.7, SCN Race 3 (HG Type 0), SCN Race 3 (HG Type 5.7), and Race 5 (HG Type 2.5.7), with mean ratings of 4.9, 4.9, 4.0, and 4.9, respectively (Gillen and Shelton, 2011, 2012, and 2013). N6002 was resistant to southern stem canker (*Diaphorina phaseolorum* (Cooke & Ellis) Sacc. var *mieridionalis* Fernández) (Gillen and Shelton 2011, 2012, 2013).

### Seed Yield Performance

Over all three sets of trials (39 environments), N6002 averaged 8.3% greater yield than its adapted parent Young with no loss of seed protein content (Table 1–3). N6002 yielded 8.3% greater than its other parent N6202 and only 2.8% lower than the elite MG VI line NC-Roy. N6002 also yielded on par with Dillon over 30 environments (Table 2, Table 3). In the RIL yield trials, N6002 yielded 2430 kg ha⁻¹, which was significantly (p < 0.05) greater than Young (2133 kg ha⁻¹) or N6201 (1997 kg ha⁻¹), and numerically greater than N6202 (2353 kg ha⁻¹) or N95-7390 (2233 kg ha⁻¹). N6002 yielded 96% of NC-Roy (2512 kg ha⁻¹, Table 1). In the diversity trial, the mean seed yield of N6002 was 3123 kg ha⁻¹ over 13 environments, which was significantly (p < 0.05) greater than either Young (2906 kg ha⁻¹) or N6202 (2911 kg ha⁻¹). Yield was comparable to the MG VI check Dillon (3097 kg ha⁻¹) and 99% of NC-Roy (3164 kg ha⁻¹) (Table 2). In the Uniform Tests, yield of N6002 (3498 kg ha⁻¹) was greater (p < 0.12) than that of Young (3272 kg ha⁻¹), and comparable to that of AGS606RR (3622 kg ha⁻¹), Dillon (3559 kg ha⁻¹), or NC-Roy (3633 kg ha⁻¹) (Table 3). The genotype × year effect was not significant (p = 0.05), while the genotype × location and genotype × location × year effects were significant (p < 0.05). However, all three genotype × environment effects were small relative to both the main effects and to the residual with F-values of 1.4, 2.67, and 1.65, respectively (data not shown).

### Seed Traits

Seed of N6002 has a yellow seed coat and imperfect black hilum. Over all three sets of trials (30 environments), the 100-seed weight of N6002 was 1.2 g greater than that of Young and 5.3 g less than N6202. The 100-seed weight was 2.8 g higher than NC-Roy over 30 environments and similar to Dillon over 22 environments (Table 1–3). The average seed quality score for N6002 was 2.0, which was not significantly different from any of the checks (Table 3).

Over 22 environments, seed protein content of N6002 was 242.8 g kg⁻¹, comparable to that of Young, even though seed yield was 8.3% greater (Table 1, Table 3). Seed protein content of N6002 was 14.9 g kg⁻¹ lower than that of N6202. Seed protein content of N6002 was 13.6 g kg⁻¹ greater than that of NC-Roy and 15 g kg⁻¹ greater than Dillon over 22 and 14 environments, respectively. Seed oil content of N6002 was 203 g kg⁻¹, which was 6.3 g kg⁻¹ less than Young and 5.8 g kg⁻¹ greater than N6202 over 22 environments (Table 1, Table 3). Seed oil content was comparable to NC-Roy and 9.0 g kg⁻¹ less than Dillon over 22 and 14 environments, respectively.

## Conclusions

The high yield of N6002, plus its acceptable lodging and height characteristics, demonstrates the potential to overcome the challenges of yield drag often associated with use of exotic genetic material (Sharma et al., 2013). By surpassing the yield of both the adapted cultivar parent Young and germplasm parent N6202 (the first US release derived from Nakasennari and Fukuyutaka), the performance of N6002 provides a clear example of the presence of beneficial yield alleles derived from Asian cultivars. Although the ultimate utility of this release in applied commercial breeding is yet to be determined, the fact that its only US adapted parent is Young, a landmark cultivar, suggests that N6002 carries yield alleles that are not common in—and thus, potentially valuable to—the southern US germplasm breeding pool. Additionally, the improved yield of N6002 without a decline in seed protein content in comparison to the North American parent Young suggests that application of underutilized exotic genetic resources may help US soybean breeders to mitigate the negative genetic correlation between seed protein content and seed yield (Wilson, 2004). Germplasm N6002 was higher in seed protein than elite cultivar NC-Roy and yielded only slightly less.

## Availability

Small quantities of N6002 seed are available from the corresponding author for research and breeding purposes for a minimum of five years after the date of this publication. It is requested that appropriate recognition be made if this germplasm release leads to new cultivars, germplasm releases, and/or scientific discoveries. Seed of N6002 has been deposited in the National Center for Genetic Resources Preservation (NCGRP) and will be made available five years after publication of this article.

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