

Appendices

Enabling technologies and services provided in past 5 years

APPENDIX A. Stocks acquired, preserved, and distributed, with associated work “at a glance”

Current size of collection: Number of populations / clones maintained

Botanical seed populations

123 wild species 3,833

7 cultivated species 1,061

total 4,894

In vitro clones

Named commercial cultivars 265

Primitive Andean cultivars 47

Genetic stocks 285

Breeding stocks 186

total 783

Total 5,677

New acquisitions (including five collecting trips to southwest USA organized and led)

Foreign donated clones 92

USA wild species collections 56

Total 148

New taxonomic determinations = 431 (http://www.ars-grin.gov/nr6/potato_taxon_names.html)

Seed Increases (grow families of 20 parents in greenhouse, hand intermate 6-8 times, harvest berries, process and store seeds) = 879

Tissue culture maintenance transfers (take a nodal cutting from stock tube, transfer it to a tube with new media to revitalize) = 32,625

ID growouts (field plantings to confirm offspring are true to parental type) = 855

Disease tests (primarily for presence of systemic virus or viroid) = 3,900

Germination tests = 6,093 and seed viability (Tetrazolium) tests = 264

Ploidy determinations = 162

46 Germplasm distributions: Number of units and orders by state and region¹
 47 See also *SPECIFICS OF NRSP6 GERMLASM IMPACT ON SAES SCIENCE*, p. 38-41
 48 and *DISTRIBUTION DETAIL TABLES*, p. 42-48.

State	Region	Units	Orders	Regional summary	
Illinois	NC	92	6	14,229 units = 64%	298 orders = 54%
Indiana	NC	26	1		
Iowa	NC	17	5		
Kansas	NC	3	2		
Michigan	NC	468	22		
Minnesota	NC	1,064	36		
Missouri	NC	42	8		
North Dakota	NC	20	3		
Ohio	NC	68	13		
Wisconsin	NC	12,429	202		
Connecticut	NE	24	1		
Dist of Columbia	NE	61	1		
Maine	NE	222	12		
Maryland	NE	328	14		
Massachusetts	NE	280	4		
New York	NE	1,418	40		
Pennsylvania	NE	116	10		
Alabama	S	3	1	1,849 units = 8%	48 orders = 9%
Arkansas	S	169	6		
Florida	S	26	4		
Georgia	S	5	1		
Kentucky	S	18	5		
Mississippi	S	16	2		
North Carolina	S	78	5		
South Carolina	S	1	1		
Tennessee	S	15	3		
Texas	S	1,489	13		
Virginia	S	29	7		
Alaska	W	139	6	3,682 units = 17%	119 orders = 22%
Arizona	W	57	5		
California	W	488	27		
Colorado	W	54	6		
Hawaii	W	237	4		
Idaho	W	874	22		
Montana	W	10	2		
New Mexico	W	77	2		
Oregon	W	479	16		
Utah	W	6	2		
Washington	W	1,261	27		
US Total		22,209	547		

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50 ¹ Plus 29 foreign countries receiving a total of 6,832 units in 110 orders.

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52 **APPENDIX B.** Data and related service provided in past 5 years

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54 Evaluation records maintained = 57,167 total observation records.
55 Seed Increase records generated and maintained = 1,562 accession increase records.
56 Field plots documented = 2,404 field plots computerized
57 Characterization data generated = 9,552 data points gathered from published literature.
58 Provenance data records maintained = 4,952
59 Cooperator records in GRIN maintained and updated = 740 total cooperators, 375 “active”.
60 Records updated and contributed to Intergenebank Potato Database = 7,665 with 393 new.
61 Website updates = 25
62 Annual Technical Committee meetings organized = 5
63 Led *American Journal of Potato Research* as Editor in Chief
64 Led Potato Crop Germplasm Committee as Chairman
65 Foreign visitors hosted = 27
66 Domestic visitors hosted = many
67
68 Information dissemination = 96 publications. Scholarly publications below from NRSP6 staff
69 and Wisconsin associated scientists documented in Annual Reports 2004-08. An additional 553
70 publications by other users of NRSP6 stocks are documented at [http://www.ars-](http://www.ars-grin.gov/nr6/)
71 [grin.gov/nr6/](http://www.ars-grin.gov/nr6/)

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86.	Vega, S.E., A.H. del Rio, J.B. Bamberg, and J.P. Palta. 2004. Evidence for the up-regulation of stearoyl-ACP ($\Delta 9$) desaturase gene expression during cold acclimation. Am. J. Potato Res. 81:125-135.
87.	Vega, S.E., J.B. Bamberg and J.P. Palta. 2004. Characterization of gibberellin requirements for various diploid and tetraploid gibberellin deficient mutants. Presented at 88 th Annual Meeting of PAA, Scottsbluff, NE, Aug. 8-12, 2004. p. 60. (Abstract)
88.	Vega, S.E., J.B. Bamberg and J.P. Palta. 2005. Characterization of gibberellin requirements for various diploid and tetraploid gibberellin deficient mutants. Am. J. Potato Res. 82:94. (Abstract)
89.	Vega, S.E., J.P. Palta and J. Bamberg. 2006. Exploiting cultivated germplasm to breed for enhanced tuber quality. In A.J. Bussan and M. Drilias (eds.). Proceedings of the Wisconsin's Annual Potato Meeting. pp. 143-144. (Abstract)
90.	Vega, S.E., J.P. Palta and J.B. Bamberg. 2004. Evidence for the mitigation of gibberellin deficiency symptoms by root zone calcium in GA-deficient mutants of potato. Presented at 88 th Annual Meeting of PAA, Scottsbluff, NE, Aug. 8-12, 2004. p. 61. (Abstract)
91.	Vega, S.E., J.P. Palta and J.B. Bamberg. 2005. Evidence for the mitigation of gibberellin deficiency symptoms by root zone calcium in GA-deficient mutants of potato. Am. J. Potato Res. 82:94-95. (Abstract)
92.	Vega, S.E., M. Aziz, J. Bamberg, A. Verma, and J.P. Palta. 2006. Screening potato germplasm for carboxy-peptidase inhibitor and its potential anticancer property. In Potato Association of America/Solanaceae 2006 Annual Meeting. p. 160 (Abstract)
93.	Vega, Sandra E., Jiwan P. Palta and John B. Bamberg. 2006. Exploiting cultivated germplasm to breed for enhanced tuber calcium accumulation ability. Am J Potato Res 83:136. (Abstract)
94.	Vega, Sandra E., Jiwan P. Palta and John B. Bamberg. 2006. Root zone calcium can modulate GA induced tuberization signal. Am J Potato Res 83:135. (Abstract)
95.	Vega, Sandra E., John B. Bamberg and Jiwan P. Palta. 2006. Gibberellin-deficient dwarfs in potato vary in exogenous GA ₃ response when the <i>ga₁</i> allele is in different genetic backgrounds. Am J Potato Res 83:357-363.
96.	Villamon, F.G., D.M. Spooner, M. Orillo, E. Mihovilovich, W. Perez, and M. Bonierbale. 2005. Late blight resistance linkages in a novel cross of the wild potato species <i>Solanum paucissectum</i> (series <i>Piurana</i>). Theor. Appl. Genet. 111:1201-1214.

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APPENDIX C. R&D, techniques and tools that enable efficient germplasm collecting, preservation and evaluation (coded with numbered publication in Appendix B).

29	Is it necessary to create a balanced bulk of berries from seed increase parents to preserve genetic diversity? Conclusion: Little risk of genetic loss in an over-all seed bulk. Full paper accepted.
27	Is there an impact of high-use agrichemicals on native wild species populations growing close to cultivation in Peru? Conclusion: Screenhouse tests indicate that commonly-used chemicals have a marked impact on reproduction parameters, suggesting that populations in remote areas may be less impacted and have more diversity.
56	Is there a difference in efficiency of diversity capture by seeds versus tubers in two model species of the southwest USA? Conclusion: Diversity captured depends on breeding system. Full paper accepted.
	Does fertilization that increases seed yield also increase seed quality? Conclusion: Not consistently—better germination was not generally correlated with more seed yield.
	Can seed increased be performed in the field under floating row cover? Conclusion: Yes, high seed yield and germination resulted with no evidence of contaminating pollinations by bees. Abstract in press.
10	Can hidden recessives in disomic polyploids be revealed in outcross hybrids? Made 3rd of 4 generations to test this.
18,28	Do eco-geographic parameters predict genetic diversity? Conclusion: Yes, in some species, apparently based on breeding system.
12,36	Is more diversity captured at relatively inaccessible sites reached only by hiking and primitive camping, compared to easy drive-up sites? Conclusion: Yes, suggesting much more collecting is warranted. Full paper submitted.
9	Is diversity inadvertently lost by seedling selection when transplanting seed increase parents? Conclusion: No.
31	Are accessions in CIP and VIR genebanks really the same as their reputed duplicates at NRSP6? Conclusion: Mostly, with a few important exceptions.
22	Can re-collections of reputed nematode resistant stocks from Arizona provide additional resistance resources? Conclusion: Yes, suggesting re-collection is warranted.
43,57	Does propagule type and growing location change relative tuber antioxidant levels of species? Conclusion: Yes.
44	Does species' ploidy effect dispersion? Conclusion: Yes.
95	Do gibberellin mutants respond to GA differently in different genetic backgrounds? Conclusion: Yes, suggesting there are important modifiers of this locus.
15	Does cytoplasm contribute to the high frost resistance of <i>S. commersonii</i> ? Conclusion: No.
11	Do potato species vary in within-population heterogeneity, and does this influence estimates of relatedness? Conclusion: Yes.
45,46	Does taxonomy predict economic traits? Conclusion: Generally not!

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APPENDIX D. Custom materials developed that enable germplasm evaluation [coded to publications in Appendix B]

P-less mutant. Discovered a unique pigmentless mutant in *S. fendleri* that demonstrates the potential of hidden recessives in allopolyploids and a tool for study of species dispersion in Mexico. [19]

GA mutant. Discovered and described a gibberellin deficient mutant (*ga₁*) useful for study of the many economically-important physiological processes in potato that are influenced by this hormone. Created pure populations for study at both diploid and tetraploid levels, and identified a spontaneous reversion clone. [8]

Crazy sepal mutant. Discovered an absolute sterile (*cs₁*) that serves as a research tool for floral development, and would reliably prevent transgene escape if incorporated into cultivars. [20]

Inbred *S. chacoense* developed. Close relatives to cultivars are usually heterogeneous heterozygotes, so not convenient for genetic analysis. This novel inbreeding mutant was advanced to the 11th selfed generation and made available for distribution.

***S. jamesii* extreme tuber dormancy.** Ability to study and manipulate tuber dormancy would of enormous value for potato. We identified germplasm with tubers that remain firm for 8+ years. [13]

“Cultivarish” project. To incorporate wild diploid species into the cultivated genepool, breeders need a good cultivated diploid parent. We are developing a diploid *tuberosum* population recurrently selected for good flowering and fertility, and produces cultivar-like (i.e., “cultivarish”) tubers in the field.

Coldbreeding. Frost stress is a major worldwide problem of the potato crop. We have developed hybrids with extremely frost hardy wild species and organized their testing in the Andes. [63,85]

Microdontum Multiplex Project (MMP). Created tubers for screening 90+ families of *S. microdontum* for an array of useful traits (calcium, pH, tomatine, antioxidants, late blight, soft rot, protein), looking for correlations between traits, and comparing core collections based on these phenotypic traits versus one derived by DNA markers.

Tuber acidity. Did first broad survey of tuber pH. Identified low pH germplasm that may associate with disease resistance, processing quality, nutritional and other valuable traits. Created broadest segregating populations for study. [17]

Calcium. Identified germplasm with high tuber calcium, which mitigates many tuber defects related to stress and disease. Created broadest segregating populations for study. [6, 14]

125 **PI2 natural anti-appetite component in potato.** Organized survey of many named cultivars
126 and breeding stocks for higher levels of the active component of commercial diet aid “Slendesta”
127 by Kemin Co.

128
129 **Antioxidants.** Organized first broad screening of antioxidants in exotic potato, identifying
130 populations in breeding-friendly species with extremely high levels. [43,57,58]

131
132 **Nematodes.** Found new sources of resistance by comparing NRSP6 and VIR collections. [48]

133
134 **Tuber potassium.** Found large variation for K accumulation capacity of tubers among species.
135 [16]

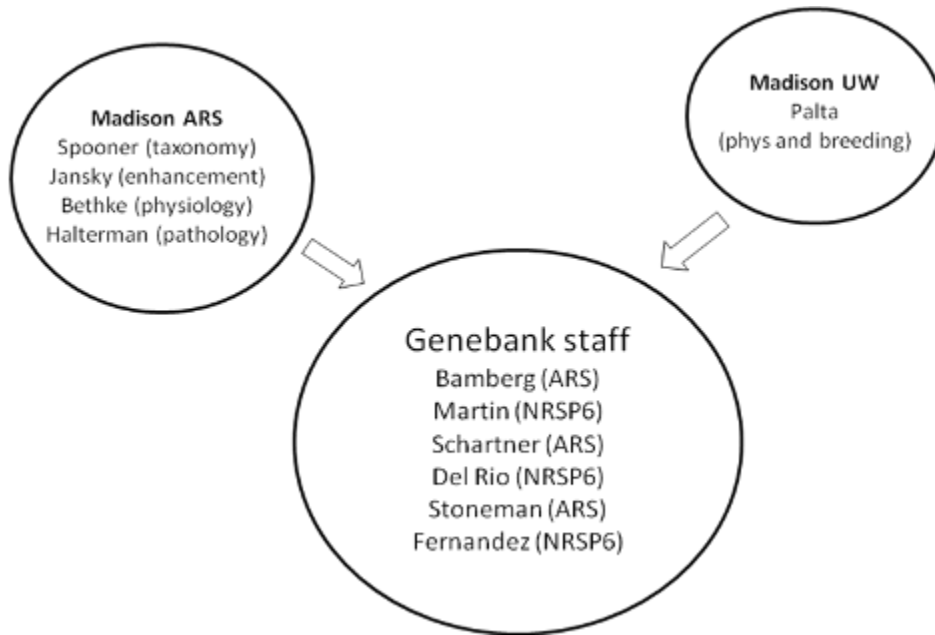
136
137 **Potato Carboxypeptidase Inhibitor.** Found wide species variation for this unique anti-cancer
138 protein. [85, 92]

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APPENDIX E.

A platform to leverage associated contributors from USDA/ARS and UW and Grant support



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Publications:

Appendix B lists publications by NRSP6 staff and associates in the past 5 years that demonstrate support for the NRSP6 collection by resources beyond the NRSP6 budget. These include those by:

- D. Spooner (ARS) with 35 publications using NRSP6 germplasm for taxonomic determinations and methods, origins of wild and cultivated potato, ploidy effects on speciation, predictivity of taxonomy (based on evaluation of germplasm for traits of early blight, Colorado potato beetle, white mold), with several of these involving international and/or intergenebank collaboration.
- S. Jansky and/or P. Simon (ARS) with 5 publications evaluating disease and pest resistance traits in NRSP6 stocks and their relationship with taxonomic predictivity.
- J. Palta (UW) with 24 publications on physiological studies related to use of NRSP6 germplasm for enhanced tuber calcium, characteristics of gibberellin mutants, frost tolerance, potassium accumulation, anti-cancer screening for potato carboxypeptidase inhibitor, and calcium fertilization in the Peruvian highlands.

172 **Grants:**

173

174 In addition to salary and base budget contributions from these associates, below are notable
175 extramural awards (total grant amounts summed by category), where PI or Co-PI are NRSP6
176 associated scientists pursuing characterization and evaluation of the collection (in \$K).

177

178 J. Palta et al.

179 Genetics and physiology of tuber calcium: 569

180

181 D. Spooner et al.

182 Taxonomic documentation, determination and predictivity : 1,035

183 Intergenebank collaboration with Vavilov Inst. genebank: 67

184 Intergenebank collaboration with International Potato Center (CIP, Lima, Peru): 400

185

186 S. Jansky et al.

187 Evaluation of germplasm for starch, PVY, *Verticillium*: 362

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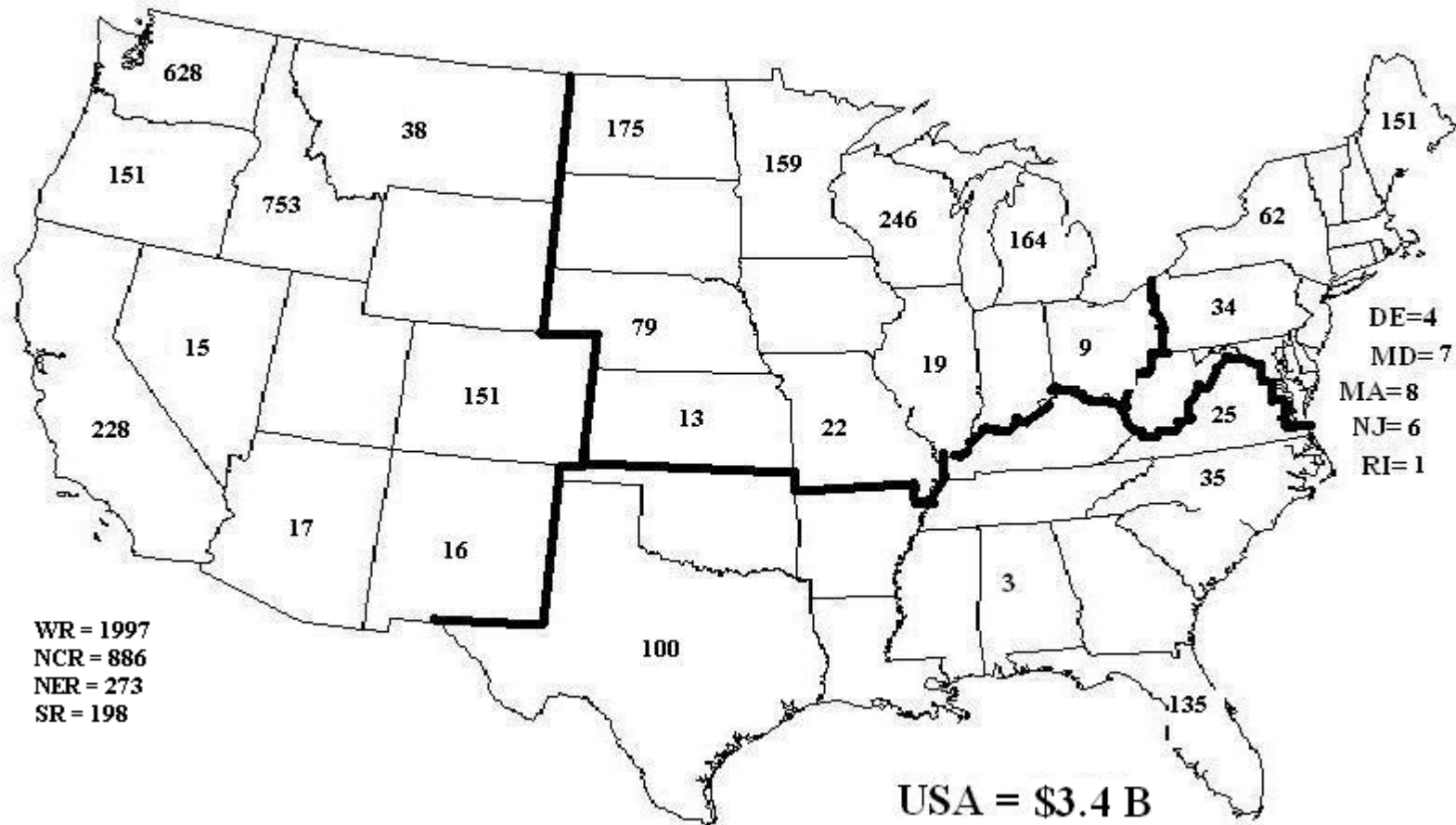
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190 **How does NRSP6 pertain as a national issue?**

191

192 **Appendix F. Potato production value in \$M by state and region, 2009 (updated from 2007 values)**

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195 **APPENDIX G.** Impact of breeding with NRSP6 stocks

196 **See also Revised General Impact Statement, page 36 and following use statistics tables**

197

198 **Past five years**

199

200 A total of 27 varieties were published in *American Journal of Potato Research* in the past 5
201 years, and all have NRSP6 exotic germplasm in their pedigrees. Notably:

202

203 *LRC 18-21* (Canada) advanced breeding line. Used *S. chacoense* from NRSP6 as a potent source
204 of resistance to *Verticillium*, the 2nd leading constraint to potato yield in North America.

205 *Defender* (Idaho et al.). Late blight resistance from NRSP6 germplasm originally obtained from
206 Poland that has wild resistant species from Mexico in its pedigree. Late blight is the
207 leading disease of potato with control costs of \$3B annually worldwide.

208 *Dakota Diamond* (North Dakota et al.). Great-grandmother is *S. chacoense* 472812, a wild
209 potato species from NRSP6 originally collected in Argentina.

210 *PA99N82-4* (Washington et al.). Advanced line (bred with the Mexican wild species *S.*
211 *bulbocastanum* from NRSP6), contributing high resistance to nematodes that can only
212 otherwise be controlled by fumigation with highly toxic chemicals at an estimated cost of
213 \$20M per year in the US.

214

215 **Other specific examples of NRSP6 germplasm success**

216

217 *Yukon Gold*, one of the most popular and name-recognized tablestock cultivars. Has *S. phureja*
218 195198, an exotic cultivated species from NRSP-6 as a grandparent, and was bred using the
219 Wisconsin-developed 2n gamete technique.

220 *Alaska Frostless* was bred with *S. acaule*, a potato species from NRSP6 with extreme frost
221 hardiness.

222 *Prince Hairy & King Hairy* were bred introgressing glandular hairs from the NRSP6 wild
223 species *S. tarijense* as a defense against insects.

224 *Atlantic* and its progeny are the backbone of chipping cultivars in the US, deriving these qualities
225 from *S. chacoense* from NRSP6.

226

227 **General**

228

229 About 50% of the four-fold advance in potato yields have been due to genetic improvement and
230 about 1% of annual value of all crops may be credited to exotic germplasm. Pro-rated, this is a
231 total of \$10-25 million benefit from germplasm per year for potatoes in the USA.

232

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234

Example voucher of NRSP6 impact on industry

235



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237

238 **Implementation / Plans / Participation**

239
240 **APPENDIX H. Administration, NRSP6 staffing and Associated contributors**
241 **and participation.**

242
243 **Administration and Technical (current configuration)**

244
245 *State Agricultural Experimental Stations*

246	Technical Representatives		
247			
248	Southern Region	Secretary (2010)	J. C. Miller, Jr.
249	Western Region	Chair (2010)	I. Vales
250	North Central Region	D. Douches	
251	Northeastern Region	Vice Chair (2010)	W. De Jong

252	Administrative Advisors		
253			
254	Southern Region		C. Nessler
255	Western Region		L. Curtis
256	North Central Region	Lead AA (2010)	M. Jahn
257	Northeastern Region		E. Ashworth

258
259 *United States Department of Agriculture*

260	Agricultural Research Service		
261			
262	Technical Representative		C. Brown
263	National Program Staff - Germplasm		P. Bretting
264	National Program Staff - Potato		G. Wisler
265	Midwest Area Director		L. Chandler
266	Vegetable Crops Research Unit Leader		P. Simon
267	Lead Scientist, NRSP-6 Project Leader & Curator		J. Bamberg

268			
269	National Institute of Food and Agriculture		A. M. Thro

270			
271	Animal and Plant Health Inspection Service		J. Abad

272			
273	<i>Agriculture and AgriFood Canada</i>		B. Bizimungu

274
275 **Full contact information at: <http://www.ars-grin.gov/nr6/techlst.html>**

276
277 **NRSP6 staff**

278 See Appendix I, budget proposal detail

279 **Associated contributors**

280 See Appendix E

281 **Participation**

282

283 The sense of “participation” as formatted in the NRSP Guidelines “Appendix E” is not a good fit
284 with how NRSP6 functions, and the current entries in NIMSS are not representative.

285

286 Administrative and technical participation in NRSP6 is configured as per the first section of this
287 appendix. Those individuals represent all of their respective SAES directors and germplasm
288 users, as well as USDA/APHIS, -ARS, -NIFA, and Canada. Although not official participants,
289 private industry is always represented at annual meetings and communications to the TAC. In
290 addition, Appendix E of this document details how local USDA/ARS and University of
291 Wisconsin staff play a special participatory role in enhancing NRSP6 service. Concerning
292 Intergenebank linkages, the project renewal text cites evidence of participation (in various
293 contexts like collecting; technical exchanges, training & research; data management) of other
294 potato genebank throughout the world. Finally, the multitude of germplasm users (represented in
295 the distributions and publications data presented in Appendix A & B) may be considered
296 participants since they use raw NRSP6 germplasm to create new breeding stocks and publish
297 results of studies, all which eventually cycle back through NRSP6 to enable and inform
298 germplasm use by future germplasm users.

299

300 **APPENDIX I. Revised 07 20 10 pursuant to RC question #2**

301 Budget Request with History and Status details

302

303 **a. History and status -- staff.**

304

305 It is difficult to objectively apportion contributions from various associated programs, so this
 306 section presents only resources under the direction of the Project Leader. The table below shows
 307 that over the past 15-20 years, the program has lost significant strength in terms of base human
 308 resources in the proposed FY11-15 budget (temporary labor is not included, as it is relatively
 309 difficult to track, but this has also surely declined).

310

311	historic	
312 Staff	FTE	FY11-15 plan FTE
313 =====		
314 Lead Scientist	1.00 F	1.00 F
315 Research support	1.00 F	0.50 M + 0.50 F*
316 Project Assistant	1.00 W&M	0.80 M
317 Seed tech	1.00 M	0.75 F
318 IT tech	1.00 M	1.00 F
319 Gardener	1.00 W&M	0.50 F*
320 Grad Student	0.50 M	0.00
321		
322 Subtotals	4.50 W&M	1.30 M
323	2.00 F	3.75 F
324		
325 Total	6.50	5.05
326 =====		

327 **NOTES**

328

- 329 1. Employer: F=Fed, M=MRF, W=UWisc, F*=UW staff paid with ARS funds.
- 330 2. In several pre-FY90 years, two Techs, two Grad Students, and Equipment were funded by
 331 NRSP6.
- 332 3. Since FY90, research support for Lead Scientist has not been provided by ARS as appointed
 333 TY, but paid by NRPS6 Grad student funds, grants, and ARS discretionary. In FY04,
 334 switched this research support position's employer with federal IT Tech for no net gain.
 335 ARS increased staff support represented in 0.75 Seed Tech = \$32K current. Proposed
 336 ARS FY11-15 support is for 0.50 Research and 0.50 Gardener.
- 337 4. In FY09, 1.2 FTE (0.40 Proj Asst + 0.80 Gardener) UWisc salary support lost.
- 338 5. Besides these FTE losses, funds for supplies, extra labor and evaluation have, of course,
 339 substantially eroded with NRSP6 flat budgets over past 20 years. ARS discretionary
 340 funding also was reducing with uptick expected in FY10 (discretionary totals for FY05
 341 through FY10 = \$94K, \$83K, \$88K, \$77K, \$71K, \$110K). These reductions have
 342 eliminated contracted cooperative evaluation studies except those supported by grants.

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b. History and status – resources.

Introduction. Given recent budget uncertainty (detailed below), reliably tabulating projections of total resources in section c. following (i.e., for up to 6 years into the future) is difficult, and it is even less clear precisely how the spending of those funds would be partitioned. Thus, we present each year as an equal average of expected spending assuming annual inflation equal to that of recent years (2.8%). At these funding levels, actual spending in the first years will be a little less than shown for salaries and a little more than shown for discretionary outlays (supplies, labor, travel), and vice versa in later years. As for the staff analysis above, budget request Table c. figures show only resources under the direction of the Project Leader.

MRF. The original FY06-10 project renewal proposed budget increases above the current \$162K to address inflation. Then a revision was requested for 5% progressive reductions per year. Then a phase-out revision was requested for years 1-5 at \$150K, \$110K, \$75K, \$50K, \$50K, respectively. We were on that course for the first two years, so lost \$40K in FY07. Dialog by NPGCC convinced the directors that a flat \$150K should be restored in FY08, but a mistake in the annual budget request process required an extraordinary vote to avert a loss of \$40K again that year. FY09 is at \$150K and the same is anticipated for FY10.

UW. During the current project term, UW reconsidered its 25+ year partnership with the genebank, and a phase-out of the 1.20 FTE support was decided, becoming complete at the start of FY09. UW continues to contribute substantial infrastructure and utilities (the latter at least \$40K annually) at the Peninsula Agricultural Research Station (PARS) farm where NRSP6 is located, with no formal direct charges. It is unclear how or if the state budget crisis and resulting mandate for spending reductions at UW Ag Research Stations will impact NRSP6 guest status at PARS.

USDA/ARS. ARS continues commitment to vigorous support of the genebank project.

It should be noted that USDA also devotes substantial resources through USDA/APHIS quarantine services for potato imports, and development and maintenance the GRIN national germplasm data computerization system. Both of these are critical to NRSP6 success.

**c. BUDGET REQUESTS SUMMARY
FY11-15**

**NRSP6 - the US Potato Genebank:
Acquisition, classification, preservation, evaluation and distribution
of potato (*Solanum*) germplasm**

See also Appendix I, Section b above for introductory comments

NRSP-6 US Potato Genebank Project FY11-15										
MRF (in \$K)										
MRF inputs	Proposed FY11 (year 1)		Proposed FY12 (year 2)		Proposed FY13 (year 3)		Proposed FY14 (year 4)		Proposed FY15 (year 5)	
	Dollars	FTE	Dollars	FTE	Dollars	FTE²	Dollars	FTE	Dollars	FTE
SALARIES & Sal Fringe	105.0	1.30	108.0	1.30	111.0	1.30	114.1	1.30	117.3	1.30
WAGES & WageFringe	25.5	0.80	26.3	0.80	27.0	0.80	27.8	0.80	28.5	0.80
TRAVEL	4.0		4.0		4.0		4.0		4.0	
SUPPLIES & Maintenance	15.4		11.8		8.0		4.1		0.2	
EQUIPMENT/ CAPITAL IMPROVEMENT										
TOTAL	150.0	2.10	150.0	2.10	150.0	2.10	150.0	2.10	150.0	2.10

Assuming 2.8% salary increases.

UW to continue contributions of facilities, utilities & related services estimated at not less than \$40K in FY10 dollars.

Direct salary support by UW discontinued at start of FY09.

NRSP-6 US Potato Genebank Project FY11-15

USDA/ARS (in \$K)

ARS inputs	Proposed FY11 (year 1)		Proposed FY12 (year 2)		Proposed FY13 (year 3)		Proposed FY14 (year 4)		Proposed FY15 (year 5)	
	Dollars	FTE	Dollars	FTE	Dollars	FTE ²	Dollars	FTE	Dollars	FTE
ARS employee SALARIES & Sal Fringe	364.4	4.05	371.7	4.05	379.1	4.05	386.1	4.05	394.4	4.05
Other SALARIES & Sal Fringe	0.0		0.0		0.0		0.0		0.0	
WAGES & WageFringe										
TRAVEL	8.0		8.0		8.0		8.0		8.0	
SUPPLIES & Maintenance	88.9		80.5		72.0		63.9		54.4	
EQUIPMENT/ CAPITAL IMPROVEMENT	0.0		0.0		0.0		0.0		0.0	
Indirect Research Costs	65.2		66.3		67.4		68.5		69.7	
TOTAL	526.5		526.5		526.5		526.5		526.5	

Assuming about 2.0% salary increases

Assessment

APPENDIX J. NIFA Review report

Suggested external reviewers:

USDA/ARS genebank leaders:

Candy Gardner -- Ames, IA (candice.gardner@ars.usda.gov, 515-294-3255)

Gary Pederson -- Griffin, GA (gary.pederson@ars.usda.gov, 770-228-7254)

Randy Nelson -- Urbana, IL (randall.nelson@ars.usda.gov, 217-244-4346)

Kim Hummer -- Corvallis, OR (kim.hummer@ars.usda.gov, 541-738-4201)

State cooperators

Richard Veilleux -- Blacksburg, VA (potato@vt.edu, 540-231-5584)

Craig Yenchow -- Raleigh, NC (craig_yencho@ncsu.edu, 919-513-7417)

Jiwan Palta -- Madison, WI (jppalta@wisc.edu, 608-262-5782)

Chrisian Thill -- St. Paul, MN (thill005@umn.edu, 612-624-9737)

USDA/ARS cooperators

Richard Novy -- Aberdeen, ID (rich.novy@ars.usda.gov, 208-397-4181)

Kathy Haynes -- Beltsville, MD (kathleen.haynes@ars.usda.gov, 301-504-7405)

Jeff Suttle -- Fargo, ND (jeff.suttle@ars.usda.gov, 701-239-1257)

Canadian

Ken Richards -- Ag Canada, Saskatoon, SK (ken.richards@agr.gc.ca, 306-956-7641)

Agnes Murphy -- Ag Canada, Fredericton, NB (agnes.murphy@agr.gc.ca, 506 459-5679)

Larry Kawchuk -- Ag Canada, Lethbridge, AB (kawchuk@agr.gc.ca, 403-317-2271)

Industry and individuals

Bob Hoopes -- Frito-Lay, Rhinelander, WI (robert.hoopes@fritolay.com, 715-365-1615)

Caius Rommens -- Simplot, Boise, ID (caius.rommens@simplot.com, 208-322-1540)

Dan Ronis -- Frito-Lay, Rhinelander, WI (daniel.ronis@fritolay.com, 715-365-1618)

Rick Machado -- Menifee, CA (farmrik@gmail.com, 909-672-3094)

Appendix J. Supplemental material added post-external review:
See final section (page 49) for NRSP-RC “5-question” letter brief responses.

MILESTONES for service to SAES scientists¹

Revised 07 20 10 pursuant to RC questions #1, #3 and #4

(see also Section C., Implementation, C.1.a.i., ‘Plan for future activities’, p. 8-9 of the proposal. Appendix B, Accomplishments also provides a reasonable quantitative measure of expectations for the next term).

Each year, FY11-15

- 1) Conduct a study to identify, acquire and advertise availability of new cultivars and wild relatives of potato that would be of most use to SAES customers.
- 2) Plan and conduct one collecting trip to the southwest USA.
- 3) Consult with the four Technical Representatives who will have surveyed SAES customers in all states in their respective regions, then pool, prioritize, and implement ideas for improving service and customer satisfaction.
- 4) Multiply at least 200 populations, 900 in vitro stocks and 70 tuber families; with associated 800 virus and 1000 germination tests in order to support rapid and complete SAES access to vigorous, disease-free samples of genebank holdings.
- 5) Process all orders within one week of receipt.
- 6) Update inventory and health status records of all germplasm on GRIN.
- 7) Update website and contact customers announcing germplasm and other news three times per year.

FY11

(this addresses NRSP-RC questions #3 and #4 regarding other sources of support)

- 8) During FY11, genebank staff will work with UWisc administration and the TAC to gather information pursuant to: a) a proposal for fees for services, and b) potential mechanisms for state, industry and private support of the genebank. These will be discussed and moved to action at the 2012 Technical Advisory Committee meeting [also addresses R. Cavalieri phone remarks 07 23 10].

¹ these yearly milestones mesh with and efficiently reinforce those of the corresponding USDA/ARS genebank project 3655-21000-051-00D “Conservation and Utilization of Potato Genetic Resources”

US Potato Genebank, NRSP6

Revised 07 20 10 pursuant to RC question #5

GENERAL IMPACT STATEMENT

Potato is the number one US and world vegetable in terms of production, value, and consumption. Considering its high satiety index and palatability, and its balanced protein, wide adaptability, and high productivity, it will play an increasingly important role in providing food security in developing countries and delivering new health-promoting nutrients to diets worldwide. Such food and health benefits carry with them a great economic impact, *even in areas where potatoes are not grown*. Annual healthcare cost of obesity is about \$147B. In 2009 we started working with Kemin company to improve the yield of PI2, a safe and effective appetite suppressant from potato. Cancer costs the nation about \$90B. With cooperators R. Navarre and C. Miller we made progress in identifying anti-cancer potato germplasm (*jamesii* antiproliferation and high tomatine *okadae*) for use in breeding. Stroke is the 3rd leading cause of death in the USA, the leading cause of disability, and costs \$43B annually. Hypertension promoted by sodium is a prominent risk factor. Estimates indicate that a high potassium diet would reduce hypertension and avert 100,000 deaths each year and \$12B in annual healthcare costs would be saved. In 2009 we prepared test samples and arranged funds and cooperators for screening for high potassium germplasm. The total US cost of just these three diseases each year is about 100 times that of the total annual farmgate value of the potato crop, so we conclude that the prospect of making a significant impact through nutrition compares favorably with using germplasm to increase yield or reduce production costs. With R. Navarre, we also identified a *phureja* clone with extremely high antioxidants, well-known for their health-promoting effects. With the high per capita consumption of potato, and a genebank with the world's most diverse and available source of new genes and germplasm information, NRSP6 is well positioned to support such contributions.

Beyond providing stocks, NRSP6 staff members are involved in discovering and developing associated germplasm tools and information. Among these are self compatibility, gibberellin, and 2n gamete mutants; cut-stem pollination, hormone pre-treatment of seeds for better germination, haploid-extracting pollinators, and 2n gamete breeding technique. Yukon Gold, one of the most popular and name-recognized tablestock cultivars, has *S. phureja* 195198, an exotic cultivated species from NRSP6 as a grandparent, and was bred using the 2n gamete technique.

Evaluation for a wide variety of useful traits has also been designed, contracted and documented by staff. Such work is the foundation for deploying exotic genes in new cultivars. One recent example is the release of cultivar PA99N82-4 bred with the Mexican wild species *S. bulbocastanum* from NRSP6. It has high resistance to nematodes that can only be controlled by fumigation at an estimated cost of \$20M per year, not counting the "cost" in risks to human and environmental health posed by use of toxic chemicals.

The genebank goal is maximum diversity. But because funds for collecting, preserving, distributing and evaluating are limited, reaching that goal depends on maximizing efficiency

through quality control and technology R&D. Thus, we collaborate with other world genebanks to study the partitioning and vulnerability of diversity in our collections. Examples of impact of this area are the intergenebank potato database, identification of more diversity-intense sites for future collecting, and confirming that the rare alleles within some populations within certain species are not explained by introgression of alleles common in another sympatric species.

One way the overall impact of these contributions can be measured is by the occurrence of NRSP6 germplasm in the pedigrees of new, improved potato cultivars. About 70% of all potatoes grown in the US have germplasm from the genebank in their pedigrees. Both cultivar releases published in the American Journal of Potato Research in 2008, ‘Premier Russet’ and ‘Dakota Diamond’, have exotic species from NRSP6 in their pedigrees. The great-grandmother of the latter is *S. chacoense* 472812, a wild potato species originally collected in Argentina.

Another gauge of impact is in the numerous publications in 2009 providing information that pushes potato science forward. In 2009, 51 papers, 18 abstracts, and 4 theses reporting the results of studies associated with NRSP6 *Solanum* stocks were recorded.

The impact of the genebank is expected to increase in the future for several reasons. 1) Mutants discovered and characterized by staff will be increasingly valuable as research models. 2) Intragenic transformation of potato has now been demonstrated and identified as a kind of GMO much more accepted by the consumer, so useful exotic potato genes will be increasingly valuable as the technology to easily insert them into existing cultivars improves. 3) Potato is rapidly expanding in large new growing regions, so the need for genetic resources for breeding in new environments and for new tastes will surge. 4) Loss of wild habitats and other limits on collecting will make it even more important to understand how to efficiently keep what we already have—thus, enhancing the importance of in-house R&D on the partitioning and vulnerability of diversity. 5) The revolution in electronic information exchange gives NRSP6 an opportunity to provide more complete and timely germplasm data, advice, and stocks, and detect and develop opportunities for new traits and germplasm applications. 6) Potato genetic resources will be increasingly mined for nutritional traits that reduce healthcare costs and suffering as evaluation and breeding technology advances.

SPECIFICS OF NRSP6 GERMPLASM IMPACT ON SAES SCIENCE on a REGIONAL BASIS

[The following section created in response to R. Cavalieri phone remarks of 07 23 10]

Below are highlights of regional narrative reports of NRSP6 germplasm use (from NRSP6 TAC meeting reports 2006-2009). This is followed by a table summarizing the number of peer reviewed publications recorded in Annual Reports 2006-2009 for selected state scientists by Region (full details available on genebank website).

These show germplasm research is promoting advances of knowledge and improved cultivars *which would not be possible if NRSP6 germplasm were not available to SAES scientists.*

WESTERN

Tristate program involves several OSU, UI, and WSU scientists and breeders who are working with ARS colleagues to use NRSP6 germplasm to improve many potato traits: corky ring spot, nematodes, antioxidants, black dot, iron content, tube worm, PVY, late blight.

Amyeric Goyer (OSU) testing NRSP6 stocks for Thiamine and Folate 2009 and 2010.

Isabel Vales (OSU) used genebank stocks for PVY, late blight resistance, value added potatoes (antioxidants, colorants, etc.). Used two sources of resistance to PVY (*stoloniferum*, and *andigena*) and MAS.

NORTH CENTRAL

James Bradeen (UM): Characterizing *verticillium* resistance in *polyadenium* potato somatic hybrids in the field and in the greenhouse. Resistance Gene Diversity Assessment: completed optimization of LR-PCR for recovery of RB (late blight resistance) alleles from genomic DNA of *bulbocastanum*. R gene genetics and comparative genomics, isolating more than 120 candidate resistance genes from *bulbocastanum*. Herbicide Tolerance: used ten primitive (1EBN) potato species to establish herbicide usage guidelines for field research. Using material from the NRSP-6 potato genebank to study avirulence proteins of late blight using *demissum* derivatives.

Christian Thill (Univ Minn): Genetic diversity for many traits having economic importance is being found. Resistance to late blight 13 Mexican and South American species was evaluated. Reported that male fertility and the production of 2n pollen was sufficient to facilitate introgression of resistance to cultivated potato. Manipulated ploidy (*pinnatisectum*) for hybrids to cultivated potato. Using South American germplasm, reported resistance to both tuber worm and blight, and proposed a breeding strategy to co-introgress both traits from the wild potato species. Also working on scab and virus resistance using NRSP6 germplasm.

David Douches (MSU) has a diploid breeding program for germplasm enhancement involving seven species from the genebank. For late blight, working with *microdontum* and *berthaultii*, *verticillium* resistance (*S. chacoense*), and Colorado potato beetle resistance. Michigan will soon release a cold chipper (*tarijense* and *phureja* are in its background). Evaluating a diploid population for Colorado Potato Beetle resistance. Also evaluating *microdontum* selections for tuber late blight resistance in cooperation with genebank staff, and have identified a potent R gene. Germplasm is being evaluated for ornamental potential. Looking for natural genetic variation for PVY resistance and the great potential for intragenic transformation developed by Simplot for using potato genes mined from the NRSP6 genebank stocks. Also using NRSP6 stocks for light chip color directly from field and after storage, dormancy, scab resistance, tuber moth. Douches and De Jong (Cornell) lead a SolCap grant that uses NRSP6 germplasm and involves many SAES scientists.

Jiwan Palta (UW) traits of interest include: cold chipping (*raphanifolium*), late blight (*bulbocastanum*), tuber calcium (*microdontum*, *kurtzianum*), pH involved with glycemc index, acrylamide formation, quality (25 species), vitamin content, cold tolerance (*acaule*, *commersonii*), anti-cancer (*okadae*), potassium (*phureja*), tuber dormancy (*jamesii*). The Wisconsin program is a closely integrated with the genebank's evaluation mission.

Susie Thompson (NDSU): Using NRSP6 stocks for breeding resistance for jelly end, ring rot, late blight, cold chipping—found that *verrucosum* has a gene complementary to the RB gene for late blight resistance. Used *demissum* and *chacoense* to hybridize with *tuberosum* to enhance disease, pest and stress resistance in breeding lines and potential releases, and also to improve quality traits, including processing qualities. Several hybrids are at various stages of early generation selection.

David Hannapel (Iowa State): Optimize stable, transgenic expression systems in select native Andean cultivars obtained from the genebank (*andigena*, *chaucha*, *stenotomum*) that eliminate unwanted marker DNA. Also working on genetics and physiology of tuberization.

NORTH EASTERN

The NE breeding effort has involved scientists from Penn State and Univ Maine cooperating with ARS Beltsville and the NC and NJ programs, studying many traits from NRSP6 germplasm (particularly *phureja* and *stenotomum*). New variety releases almost always have NRSP6 germplasm in their pedigrees.

B. de los Reyes (Univ Maine) used 15 wild species accessions screening for drought, salinity, and CPB resistance screening.

Walter DeJong (Cornell) uses germplasm for association analyses for shape, pigmentation, and carbohydrate metabolism.

SOUTHERN

J. C. Miller, Jr. (TAMU) uses genebank stocks for breeding and research. Found very high levels of antioxidants in *microdontum* and *pinnatisectum*, and showed importance of GxE. Working on use of exotics to combat Zebra Chip complex, and genebank-developed mutant to study genetic basis of sports of Russet Norkotah. Has found strong anti-prostate cancer properties in extracts of the USA species *jamesii* from the genebank.

Craig Yencho (NCSU) is breeding for resistance to internal heat necrosis with exotic potato germplasm (*phureja*). A wild species (*chacoense*) is being used for Colorado potato beetle resistance breeding. Also exploring the potential of NRSP6 germplasm as ornamentals.

Richard Veilleux (VPU) created doubled monoploids (*phureja*) from the genebank which are the basis of the potato genome sequencing project, and is using NRSP6 germplasm to examine the inheritance of glycoalkaloids.

Jeff Davis (LA State Univ). Used 25 genebank accessions for Electrical Penetration Graph studies to determine the nature of the aphid resistance; antixenosis or antibiosis.

Publications involving NRSP6 stocks, 2006-2009

Selected scientist / breeder authors

(as recorded in NRSP6 Annual Reports)

Scientist	region	Institution	number	
Bradeen	NC	Univ Minn	17	
Douches	NC	Mich State U	11	
Grafius	NC	Mich State U	3	
Gudmested	NC	ND State U	6	
Hannapel	NC	Iowa State U	3	
Jiang	NC	Univ Wisc	13	
Palta	NC	Univ Wisc	21	
Radcliffe	NC	Univ Minn	3	
Ragsdale	NC	Univ Minn	3	
Rouse	NC	Univ Wisc	2	
Secor	NC	ND State U	5	
Thill	NC	Univ Minn	2	
Thompson	NC	ND State U	7	96
Christ	NE	Penn State	14	
DeJong	NE	Cornell	8	
Ewing	NE	Cornell	2	
Fry	NE	Cornell	5	
Halseth	NE	Cornell	3	
Lambert	NE	Univ Maine	3	
Porter	NE	Univ Maine	5	40
Miller	S	TX A&M	13	
Sterret	S	Virginia Tech	4	
Veilleux	S	Virginia PolyTech	8	
Yencho	S	NC State Univ	2	27
Davidson	W	CO State	1	
Goyer	W	Oregon State U.	7	
Hamm	W	Oregon State U	2	
Hane	W	Oregon State U	10	
James	W	Oregon State U	12	
Knowles	W	Wash State U	8	
Love	W	Univ Idaho	14	
Mosley	W	Oregon State U	10	
Pavek	W	Univ Idaho	9	
Stark	W	Univ Idaho	4	
Vales	W	Oregon State U	9	86

NRSP6 Distribution Detail Tables 2000-2009

a. Summaries:

USA University recipients

REGION	ORDERS	UNITS	STA
NC	561	36634	9
NE	98	2451	9
S	38	2657	6
W	159	5578	10
	856	47320	34

USA Non-University recipients

REGION	STA	ORDERS	UNITS
NC	11	117	1524
NE	9	91	2140
S	12	82	770
W	10	168	4178
Total		458	8612

Foreign

COUNTRIES	ORDERS	UNITS
36	251	24577

TOTAL

COUNTRIES	ORDERS	UNITS
37	1565	80509

b. University recipient: Region detail **NCR**

ORG	ORDERS	UNITS	STA	WHO
University of Chicago	3	45	Illinois	J. Castillo
University of Illinois	1	53		K. Robertson
Iowa State University	8	53	Iowa	D. Hannapel, Y. Hou
Kemin Inc (coop with Univ Wisc)	7	861		J. Greaves
Michigan State University	32	1624	Michigan	M. Carvallo-P, D. Douches, W. Kirk
University of Minnesota	59	1861	Minnesota	J. Bradeen, J. Davis, I. Dinu, J. Flynn, L. Gao, R. Hayes, J. Jenkins, J. Lau, M. Meeks, D. Mollov, E. Quirin, M. Sanchez, R. Spangler, C. Thill, C. Tong, D. Zlesak
Saint Louis University	1	4	Missouri	J. Preiszner
University of Missouri	1	29		P. Kear
University of Nebraska	1	14	Nebraska	L. Sutton
North Dakota State University	11	483	North Dakota	B. Farnsworth, N. Gudmestad, A. Lafta, J. Lorenzen, S. Thompson
Ohio State University	7	57	Ohio	M. Kleinhenz, K. Perry, Y. Wang, S. Kamoun
University of Wisconsin	116	6117	Wisconsin	M. Martin, R. Aburomia, M. Bamberg, L. Boiteux, B. Bowen, J. Busse, A. Charkowski, Y-K Chen, Y-S Chung, R. Coltman, L. Colton, A. del Rio, D. Fajardo, I. Goldman, H. Groza, E. Haga, M. Iovene, J. Jiang, H-S Kim, S. Lara-C, A. Tek, L. McCann, R. Moreyra-C., M. Norby, J. Palta, L. Plhak, J. Pritchard, B. Pudota, F. Rodriguez, D. Rouse, E. Silva, J. Song, R. Stupar, S. Vega, A. Witherell
ARS (coop with Univ Wisc)	314	25433		D. Halterman, A. Hamernik, R. Hanneman, S. Jansky, H. Ruess, P. Simon, D. Spooner, S. Stevenson, J. Bamberg, P. Bethke, J. Busse, J. Schartner
	561	36634		

c. University recipient: Region detail **NER**

ORG	ORDERS	UNITS	STA	WHO
Yale University	2	27	Connecticut	S. Dinesh-K, J. Song
Delaware State University	1	18	Delaware	A. Tucker
Unity College	1	1	Maine	E. White
University of Maine	13	218		Z. Ganga, A. Reeves, A. Mukherjee, G. Porter, B. del los Reyes
University of Maryland	2	21	Maryland	Y-J Ahn
ARS coop with NE breeding	22	779		K. Deahl, K. Haynes, L. Wanner
Hampshire College	1	2	Massachusetts	J. Keach
Mount Holyoke College	1	248		A. Fray
University of Massachusetts	2	50		H-J Kim
Rutgers University	1	3	New Jersey	R. Di
Cornell University	43	1025	New York	W. DeJong, M. DiLeo, S. Doganlar, B. Fry, C-S Jung, L. Miller, K. Perry, R. Plaisted, C. Stuart, W. Tingey, J. Van Eck, Y-E Wang, L-X Yu,
Lehman College	1	12		V. Doyle
NY Bot. Garden/CUNY	1	14		V. Doyle
Cold Spring SUNY coop	3	26		Z. Lippman
Penn State University	2	4	Pennsylvania	J-K Na, Y-H Wang
Temple University	1	2		T. Messner
University of Vermont	1	1	Vermont	S. Lewins
	98	2451		

d. University recipient: Region detail **SR**

ORG	ORDERS	UNITS	STA	WHO
University of Central Florida	3	9	Florida	D. Henry, S. Kumar
University of Florida	1	2		D. Allen
University of Kentucky	1	3	Kentucky	M. Mahala
Louisiana State University	1	29	Louisiana	J. Davis
North Carolina State University	9	240	North Carolina	M. Clough, L. Gomez, C. Yencho
University of North Carolina	2	6		G. Copenhaver, S. Grant
Sul Ross State Univ (coop with U Wisc)	1	6	Texas	M. Powell
Texas A&M University	11	2098		J. Drawe, A. Hale, J. C. Miller, N. Nzaramba
Virginia Polytechnic Inst. & State Univ.	9	264	Virginia	J. Jelesko, F. Medina-B, R. Veilleux, J. Watkinson
	38	2657		

e. University recipient: Region detail **WR**

ORG	ORDERS	UNITS	STA	WHO
Northern Arizona University	1	34	Arizona	T. Ayers
University of Arizona	4	50		P. Jenkins, M. McCarthy,
NPS coop with Univ Arizona	1	11		M. Weesner
ARS coop with University of CA	1	8	California	B. Baker
University of California	25	444		M. Coffey, N. Dudek, M. Flanagan, B. Igic, C. Quiros, C. Rummold, S. Scheidt, N. Sinha, R. Voss, X. Wang, U. Wirtz, E. Albrecht
Adison University	6	275	Colorado	P. White
Colorado State University SLVRC	13	415		B. Deavours, H. Gruszewski, D. Holm, S. Jayanty, J. Vivanco, F. Goktepe, B. Spencer
Metropolitan State College of Denver	1	3		Z. Williamz
University of Colorado	1	6		T. Ranker
University of Hawaii	5	247	Hawaii	H. Keyser, D. Oka
University of Idaho	12	1279	Idaho	C. Bates, M. Dibble, A. Karasev, D. Khu, J. Lorenzen, S. Love
ARS (coop with tristate breeding)	22	207		D. Corsini, R. Novy, J. Whitworth
Montana State University	2	10	Montana	E. Nichols
University of New Mexico	1	69	New Mexico	T. Lowrey
Oregon State University	16	638	Oregon	B. Charlton, T. Chen, A. Goyer, R. Martin, A. Monteros, M. Townsend, S. Yilma, I. Vales
Brigham Young University	2	8	Utah	D. Atwood, S. Mogensen
BLM coop with Univ Wisc	1	2		T. Tolbert
Utah State Univeristy	1	3		S. Ripple
ARS (coop with tristate breeding)	38	1820	Washington	R. Navarre, C. Brown, R. Hannan
Washington State University	6	49		D. Culley, J. Keach, S. Salimath, C. Whitney
	159	5578		

f. Distribution summary: **USA non-University**

REGION	STA	ORDERS	UNITS
NC	Illinois	3	19
NC	Indiana	5	45
NC	Iowa	4	8
NC	Kansas	3	4
NC	Michigan	21	379
NC	Minnesota	8	94
NC	Missouri	17	113
NC	Nebraska	1	3
NC	North Dakota	2	9
NC	Ohio	19	252
NC	Wisconsin	34	598
		117	1524
NE	Delaware	3	10
NE	DC	7	681
NE	Maine	12	175
NE	Maryland	19	280
NE	Massachusettes	3	42
NE	New Jersey	1	3
NE	New York	29	758
NE	Pennsylvania	16	190
NE	Vermont	1	1
		91	2140
S	Alabama	7	117
S	Arkansas	9	236
S	Florida	14	67
S	Georgia	2	9
S	Kentucky	5	17
S	Mississippi	2	16
S	North Carolina	11	114
S	Oklahoma	2	2
S	South Carolina	3	4
S	Tennessee	3	15
S	Texas	15	123
S	Virginia	9	50
		82	770
W	Alaska	11	236
W	Arizona	2	36
W	California	73	2028
W	Colorado	5	23
W	Hawaii	1	75
W	Idaho	25	501
W	New Mexico	6	92
W	Orgegon	14	82
W	Utah	7	29
W	Washington	24	1076
		168	4178
Total		458	8612

g. Distribution summary: **Foreign**

COUNTRY	ORDERS	UNITS
Argentina	6	977
Belarus	10	649
Belgium	4	29
Brazil	1	25
Canada	85	3284
Chile	5	84
China	9	185
Colombia	4	46
Czech Republic	3	24
Egypt	1	4
Ethiopia	1	45
France	6	247
Germany	4	191
Guatemala	1	29
Hungary	4	118
Iceland	1	6
India	9	3198
Indonesia	1	42
Israel	1	1
Italy	1	1
Jamaica	1	21
Japan	12	825
Kuwait	3	155
Luxembourg	1	10
Mexico	14	8692
Netherlands	10	386
Peru	13	2406
Poland	6	72
Romania	3	78
Russian Federation	11	737
Slovakia	1	44
South Korea	8	1807
Spain	3	30
Switzerland	4	27
Turkey	1	8
United Kingdom	3	94
	36	251
		24577