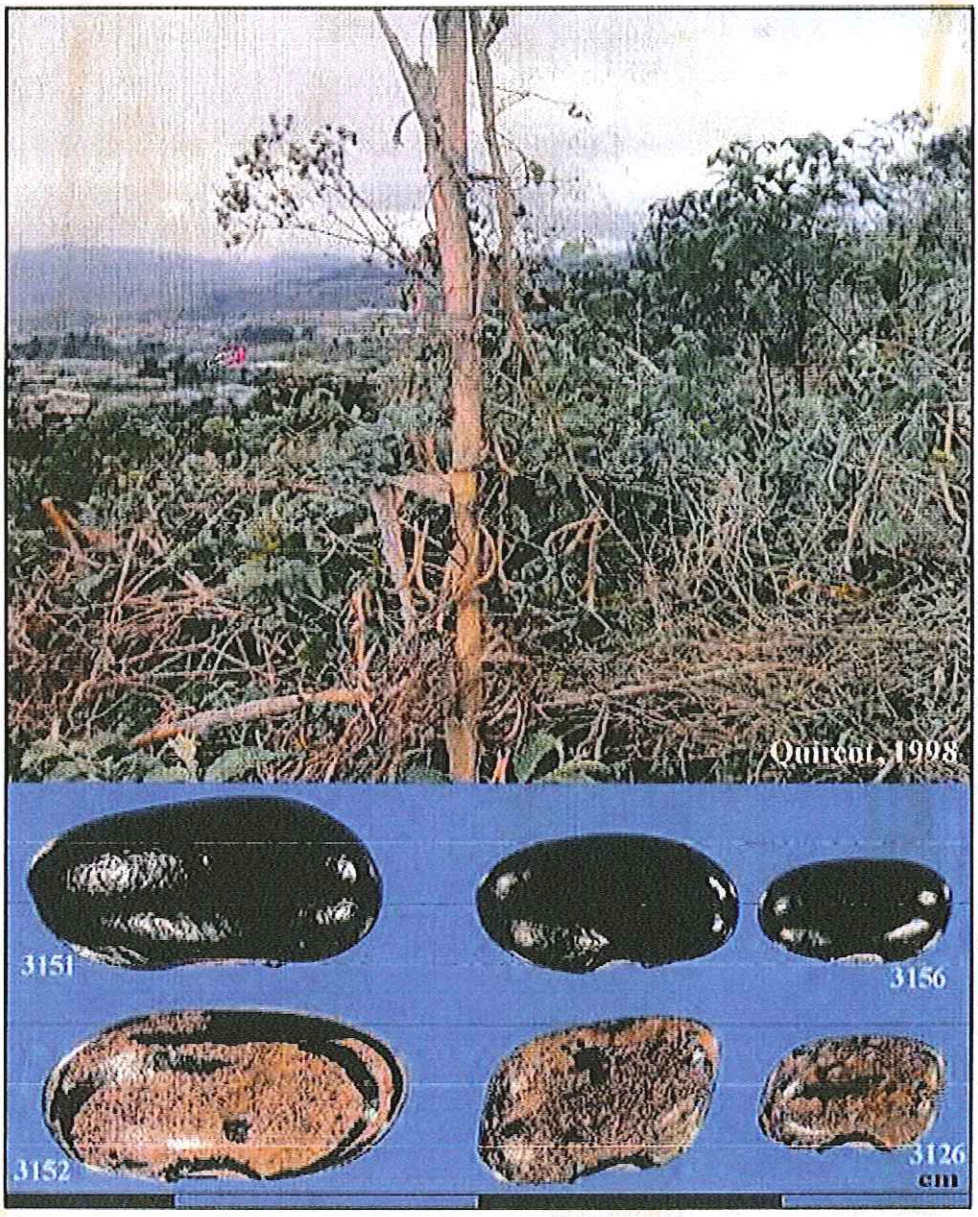

"GENE FLOW ANALYSIS FOR ASSESING THE SAFETY OF BIOENGINEERED CROPS IN THE TROPICS"

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Part 1 – Ecogeographic survey of the target species

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We present here evidence about the distribution and ecology of the target species in Costa Rica. A risk – real or potential – with transgenical crops is the movement of artificial transgenes to the natural flora through pollen, namely to genetically compatible species (Rissler & Mellon 1996). These are species belonging to the same biological entity or gene pool, or related species usually belonging to the same evolutionary phylum. An important step related to the introduction and management of transgenical crops is thus in the precise mapping of all populations of such species through ecogeographic surveys; the field work will also bring evidence about synchrony of flowering and vectors of pollen.

Our model crop – common bean, *Phaseolus vulgaris* L. – has long been known as an autogamous legume (Bliss, 1980), with almost no outcrossing. An ample variation in outcrossing has however been reported, from 0.5% to more than 65% (Brunner & Beaver 1989; Ferreira et al. 2000; Wells et al. 1988). In the context of safe introduction and management of transgenical crops, gaining insight on that variation and the factors causing it is relevant, particularly in the tropics where interruption in the biological cycles (because of the winter at extreme latitudes) is almost nil. Part 2 will deal with evidence of gene flow on experimental station through the measurement of outcrossing through seasons, and other factors affecting its variation.

The genetics of the common bean is such that in case of cross between cultivated and wild forms traits of wild forms will be dominant in the early generations (Bassett 1996; Leakey 1988). In order to establish the direction of the cross (important in the case of transgene from transgenical crops to wild relatives), we will thus need to study traits controlled by nuclear genes and polymorphisms of cpDNA. The hybrid swarms – sometimes called wild-weed-crop complexes (Beebe et al. 1997) - are also worth studying as they could behave as temporary reservoirs of transgenes or as bridges towards non transgenical landraces. Part 3 examines with help of molecular markers materials collected in the field and thought to result from past hybridizations. It aims at certifying cases of gene flow, its directions, and at providing some insight about the persistence through time.

Given our model crop, under our present knowledge (Freytag & Debouck 2002), we have to consider only the Phaseoli section of the genus. The four related species are thus: *P. albescens, P. costaricensis, P. dumosus*, and *P. vulgaris* (itself, as wild form and as landraces). In the context of Costa Rica, *P. albescens* is not to be considered, since this species is restricted to western Mexico (Ramírez Delgadillo & Delgado Salinas 1999). *P. dumosus* as cultivated species is grown as 'cuba' apparently in several places in Costa Rica, although poorly reported by agronomic authorities and agricultural statistics. As feral it is widely present in many mountainous ranges of the country. It has been shown to belong to the *P. vulgaris* phylum through cpDNA analysis (Schmit et al. 1993). However, it would with very great difficulties be crossed with the common bean as pollen receiver (Camarena & Baudoin 1987), since the hybrid embryos would survive and result into mature plants only through embryo rescue. *P. dumosus* would thus not be a species to consider in this part of the survey.

Although not much wide crossing has been done so far, *P. costaricensis* – shown to belong to the phylum of the common bean (Delgado Salinas et al. 1999; Schmit et al. 1993) – seems genetically compatible with the common bean (Singh et al. 1997). In contrast with *P. dumosus*, hybrids between *P. vulgaris* and *P. costaricensis* (as donor of pollen) have been easily obtained; to our knowledge the key cross with *P. costaricensis* as cytoplasm (pollen receiver) parent has not been attempted. It is thus good cautionary practice to include *P. costaricensis* in our ecogeographic survey; progress in the mapping of all populations has been significant (Araya Villalobos et al. 2001; Debouck et al. 1989) since the discovery of the species (Freytag & Debouck 1996). Similarly, for the wild common bean, the mapping of all populations has made progress (Araya Villalobos et al. 2001) since the first report of presence for this country (Debouck et al. 1989).

Results and discussion

Phaseolus costaricensis Freytag & Debouck

With the collections done this year (Table 1, 2), we know about 24 populations of this species, mainly distributed on the southern slope of the Volcanic Cordillera in the Central Valley, from N Zarcero (the Tapezco population # 3120) to Río Birrís (the easternmost population # 3128). It is also present on the northern slope of Cerros de Escazú (south of the Central Valley), from Bebedero (# 3138) to Hda. Tres Ríos (# 3144). This range includes three watersheds: Río Vírilla, Río Reventazón and Río San Carlos. *P. costaricensis* is also present in the extreme W of Cordillera de Talamanca, in the watersheds of Río Pirrís (# 2122, 2135), Río Naranjo-Savegre (# 2132), and Río Chirripó Pacífico (# 2126, 2128, 3111, 3112, 3113). Four populations have been added to the total reported in 2001 (Araya Villalobos et al. 2001). Our searches for it to the northwest of Costa Rica (e.g. Cordillera de Tilarán, Monteverde), and to the southeast (e.g. Fila Cotón, Sierra de Coto Brus) have failed; one should note that the vegetation type bmh-MB where it thrives is absent from these regions (Araya Villalobos et al. 2001).

This species is usually found in humid montane forests bmh-MB (Araya Villalobos et al. 2001); it is the vicariant species replacing wild *P. vulgaris* in such vegetations at a slightly higher altitude and rainfall, with a dry season of three months. We have found two places where the local populations of *P. costaricensis* would have hybridized, and tentatively with *P. dumosus*: one in Aserrí (# 2114) and three in Quircot (# 3149, 3157, 3158). Interestingly, these two places were the ones where *P. costaricensis* and wild *P. vulgaris* were found together (also in Bebedero and Zarcero). Pollination is by humming-birds (# 3120, 3127, 3144) and carpenter bees (# 2116, 2132, 3127), as indicated by the field notes.

Phaseolus vulgaris L.

With the collections done this year (Table 1, 3), we know of 18 populations of wild common bean distributed in four watersheds at intermediate altitude in Costa Rica. Ten populations have been found in the Central Valley or watershed of Río Vírilla (ending in Río Grande de Tarcoles), seven on the southern slope (#2097 Tarbaca, 2111 Aserrí, 3136 San Miguel Desamparados, 3137 Bebedero, 3140 Parque Iztarú, 3143 Hda. Tres Ríos, 3178 Guatuso), and three on the northern slope (#3106 Chagüite, 3132 Zarcero, 3133 Sabana Redonda). Six populations have been found in the upper valley of Río Grande de Candelaria: five on the northern slope (# 3131 Jérico, 3134 Tranquerillas, 3135 Chirogres, 3147 El Tigre, 3148 Manzano), and one on the southern slope (# 3184 Río Tarrazú). The mountainous range that separates these two watersheds – Cerros de Cedral o de Escazú – has thus the largest number of populations: twelve (7+5). One population has been found in the upper valley of Río Reventazón (# 3126 Quircot), which is the only one so far on the Atlantic slope of the continental divide. One population has been found in the upper valley of Río Pirrís (# 3168 Copey), the southernmost population to the southeast of the country. Our attempts to find wild common bean in other parts of Costa Rica, namely the upper Río Savegre, Río División and Río Chirripó Pacífico have failed so far. There might still be one population in the watershed of Río Pirrís (the slope north of San Marcos de Tarrazú, but heavily cleared for coffee plantations), and one on the slope of Fila Bustamante. All vegetations where it thrives reported on maps of life zones (Bolaños M. & Watson C. 1993; Gómez Pignataro 1986; Tosi, 1969) have been visited.

Wild common bean is usually found in subhumid montane forests (bmh-P, bh-MB and bmh-MB: (Araya Villalobos et al. 2001)) at intermediate altitude (Table 2), now largely cleared for coffee plantations and urban areas. In this habitat, the end of the rainy season coincides with the flowering period, and mists are not frequent; bean plants thus escape pressures from diseases such as anthracnose and root rots, as well as drought stresses, and seed dispersal will occur during the dry season (3-4 months) (Araya Villalobos et al. 2001). Germination of wild bean will occur from July onwards, with flowering in September-December anticipating thus synchronization of flowering with landraces during that period. Synchronization in the case

of bush varieties, usually planted in October-November, will take place only during a short period (late November- December). Carpenter bees, bumble bees and honey bees have been seen as active in the pollination at Quircot, Jérico, and Sabana Redonda, as indicated by the field notes.

On the other hand, the continuing contact between wild forms and cultivars of common bean is going down, because of the use of herbicides or the practice of weeding. In Quircot the use of atrazin in maize has virtually eliminated the wild bean from certain plots. In El Manzano and in Chirogres, weeding has seriously reduced the wild bean to just a very few plants. Coffee plantations and use of herbicides therein have eliminated the population # 3131.

For economic reasons (competitive prices of grain commodities), the traditional association of maize and beans ('fríjol tapado') is much lesser planted in the Central Valley of Costa Rica as compared to decades ago. As a matter of fact, the association has almost disappeared from the valley; the contact between the wild relative and the bean crop is nowadays scarce. During the course of the project and during previous years to it, we were able to identify in the field five places (Table 4) where hybrid swarms or wild-weed-crop complexes (Beebe et al. 1997) have existed. These were: Tarbaca (alt. 1750 masl), Aserrí (1560 masl), Quircot (1540 masl), Zarcero (1610 masl), and possibly Manzano (1370 masl). Out of these sites in 1998, the only one with sufficient planting of common bean and acreage was Quircot, and thus chosen for additional studies. In the period 1987-1998, the site of Tarbaca disappeared as original because of housing and road expansion, and that of Zarcero was converted into a quarry in 1998-2000. In Aserrí the planting of common bean has been greatly reduced, and so were the weedy forms. At Quircot and Manzano, planting of common bean has been switched to that of *P. dumosus* 'cuba'; some traditional landraces have been present at Quircot since 1998 (e.g. 'Higuerilla' # 3152, 'Vainica amarilla' # 3153), sometimes as escape from cultivation.

The fact that large planting of common bean has disappeared at the five sites is a problem inasmuch we cannot infer about the importance of variants and weedy forms that can be generated by recent/ continuing gene flow as seen elsewhere (Beebe et al. 1997). It is however useful as it would allow to see the permanence over time of effects of gene flow once demonstrated. In Table 4 about the others reported as "none", a survey is however being done every time intermediate types possibly resulting from gene flow have been observed. One can imagine that it is very difficult to certify that any of the wild common bean population has not been in contact with landraces in the past. Indeed before 1850 and the increase of coffee plantations in Central Costa Rica it is likely that the acreage of common bean landraces was much higher than at present. For instance, Puriscal is today well-known for its coffee, while 'purisco' means 'bean blossom' in rural language in that part of Costa Rica.

Literature Cited

Araya Villalobos, R., W.G. González Ugalde, F. Camacho Chacón, P. Sánchez Trejos & D.G. Debouck. 2001. Observations on the geographic distribution, ecology and conservation status of several *Phaseolus* bean species in Costa Rica. Genet. Resources & Crop Evol. **48:** 221-232.

Bassett, M.J. 1996. List of genes - *Phaseolus vulgaris* L. Annu. Rept. Bean Improvement Coop.(USA) **39:** 1-19.

Beebe, S., O. Toro Ch., A.V. González, M.I. Chácon & D.G. Debouck. 1997. Wild-weed-crop complexes of common bean (*Phaseolus vulgaris* L., Fabaceae) in the Andes of Peru and Colombia, and their implications for conservation and breeding. Genet. Resources & Crop Evol. **44:** 73-91.

Bliss, F.A. 1980. Common bean. *In:* W. R. Fehr and H. H. Hadley (eds.) Hybridization of crop plants. American Society of Agronomy, Crop Science Society of America, Madison, Wisconsin, USA. pp. 273-284.

Bolaños M., R.A., V. Watson C. 1993. Mapa ecológico de Costa Rica. Centro Científico Tropical. Scale 1:200.000. 9 sheets, San José, Costa Rica.

Brunner, B.R.& J.S. Beaver. 1989. Estimation of outcrossing of the common bean in Puerto Rico. HortSci. **24:** 669-671.

Camarena, F. & J.P. Baudoin. 1987. Obtention des premiers hybrides interspécifiques entre *Phaseolus vulgaris* et *Phaseolus polyanthus* avec le cytoplasme de cette dernière forme. Bull. Rech. Agron. Gembloux **22:** 43-55.

Debouck, D.G., R. Araya Villalobos, R.A. Ocampo Sánchez & W.G. Gonzalez Ugalde. 1989. Collecting *Phaseolus* in Costa Rica. FAO/IBPGR Plant Genet. Resources Newsl. **78/79:** 44-46.

Delgado Salinas, A., T. Turley, A. Richman & M. Lavin. 1999. Phylogenetic analysis of the cultivated and wild species of *Phaseolus* (Fabaceae). Syst. Bot. **24:** 438-460.

Ferreira, J.J., E. Alvarez, M.A. Fueyo, A. Roca & R. Giraldez. 2000. Determination of the outcrossing rate of *Phaseolus vulgaris* L. using seed protein markers. Euphytica **113:** 259-263.

Freytag, G.F. & D.G. Debouck. 1996. *Phaseolus costaricensis*, a new wild bean species (Phaseolinae, Leguminosae) from Costa Rica and Panama, Central America. NOVON **6:** 157-163.

Freytag, G.F. & D.G. Debouck. 2002. Taxonomy, distribution, and ecology of the genus *Phaseolus* (Leguminosae-Papilionoideae) in North America, Mexico and Central America. Botanical Research Institute of Texas. SIDA Botanical Miscellany 23, Fort Worth, Texas, USA, 300p.

Gómez Pignataro, L.D. 1986. Vegetación de Costa Rica - Apuntes para una biogeografía costarricense. Vol. 1. Editorial Universidad Estatal a Distancia, San José, Costa Rica, 327p.

Leakey, C.L.A. 1988. Genotypic and phenotypic markers in common bean. *In:* P. Gepts (ed.) Genetic resources of *Phaseolus* beans. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 245-327.

Ramírez Delgadillo, R. & A. Delgado Salinas. 1999. A new species of Phaseolus (Fabaceae) from west-central Mexico. SIDA **18:** 637-646.

Rissler, J. & M. Mellon. 1996. The ecological risks of engineered crops. Massachusetts Institute of Technology Press, Cambridge, Massachusetts, USA, 168p.

Schmit, V., P. du Jardin, J.P. Baudoin & D.G. Debouck. 1993. Use of chloroplast DNA polymorphisms for the phylogenetic study of seven *Phaseolus* taxa including *P. vulgaris* and *P. coccineus*. Theor. Appl. Genet. **87:** 506-516.

Singh, S.P., D.G. Debouck & W.M. Roca. 1997. Successful interspecific hybridization between *Phaseolus vulgaris* L. and *P. costaricensis* Freytag & Debouck. Annu. Rept. Bean Improvement Coop. (USA) **40:** 40-41.

Tosi, J.A. 1969. Mapa ecológico, República de Costa Rica, según la clasificación de zonas de vida del mundo de L.R. Holdridge. Centro Científico Tropical, San José, Costa Rica, 1 hoja.

Wells, W.C., W.H. Isom & J.G. Waines. 1988. Outcrossing rates of six common bean lines. Crop Sci. **28:** 177-178.

Table 1 – List of materials found in 2003-2004, sites and coordinates.

Collectors' Number	Species	Province, district, closest site	Longitude	Latitude	Altitude (masl)
3165	*leptostachyus*	Cartago, San Nicolás, Quircot	83°56'W	9°54'N	1540
3166	*oligospermus*	Cartago, Tobosí, Tablón	84°02'W	9°49'N	1470
3167	*leptostachyus*	Cartago, Tobosí, Tablón	84°02'W	9°49'N	1470
3168	*vulgaris* silv.	San José, Sta. María de Dota, Copey	83°57'W	9°39'N	1600
3169	*lunatus* silv.	San José, Sn Marcos Tarrazú, Sn Lorenzo	84°03'W	9°38'N	1420
3170	*tuerckheimii*	San José, Sta. María de Dota, Los Angeles	83°58'W	9°38'N	1860
3171	*tuerckheimii*	San José, Sta. María de Dota, Cima de Dota	83°55'W	9°40'N	1980
3172	sp.	San José, Sn Isidro, Sta. Eduviges	83°45'W	9°30'N	1520
3173	sp.	San José, Sn Isidro, Las Nubes	83°46'W	9°28'N	1570
3174	*Manihot brachyloba*	San José. San Pedro, Sta Teresa	83°34'W	9°21'N	1110
3175	*lunatus* silv.	San Jose, San Pedro, Sn Jerónimo	83°30'W	9°21'N	1190
3176	*xanthotrichus*	San José, Alajuelita, Llano de Alajuelita	84°07'W	9°52'N	1400
3177	*lunatus* silv.	San José, Alajuelita, Llano de Alajuelita	84°07'W	9°52'N	1470
3178	*vulgaris* silv.	San José, Desamparados, Guatuso	84°02'W	9°51'N	1380
3179	*oligospermus*	Cartago, Tobosí, Coris	84°00'W	9°52'N	1430
3180	*xanthotrichus*	Cartago, Tobosí, Coris	84°00'W	9°52'N	1430
3181	*lunatus* silv.	Cartago, Tobosí, Coris	84°00'W	9°52'N	1430
3182	*talamancensis*	San José, Sta María de Dota, *do.*	83°59'W	9°39'N	1490
3183	*tuerckheimii*	San José, San Pablo, *do.*	84°02'W	9°43'N	1720
3184	*vulgaris* silv.	San José, San Pablo, Sn Cristobal Sur	84°01'W	9°44'N	1450

Table 2 – List of populations of *P. costaricensis* found, sites, watershed, coordinates and year.

Collectors' Number	Province, district, closest site	Watershed	Longitude	Latitude	Altitude (masl)	Year found
1. 2093	Cartago, San Nicolás, Cta Chinchilla	Reventazón	83°53'W	9°54'N	1650	1987
2. 2095	San José, Aserrí	Vírilla sur	84°06'W	9°51'N	1470	1987
3. 2102	San José, Alajuelita, San Miguel	Vírilla sur	84°07'W	9°52'N	1620	1987
4. 2116	San José, Aserrí, Piedra	Vírilla sur	84°07'W	9°52'N	1590	1987
5. 2118	Cartago, La Unión, La Carpintera	Vírilla sur	83°58'W	9°53'N	1600	1987
6. 2119	Cartago, Tres Ríos, Dulce Nombre	Vírilla sur	83°57'W	9°57'N	1750	1987
7. 2122	San José, Sta. María de Dota, Copey	Pirrís	83°57'W	9°40'N	1660	1987
8. 2126	San José, Sn Isidro, Pueblo Nuevo	Chirripó P.	83°40'W	9°26'N	1550	1987
9. 2128	San José, Sn Isidro, Herradura	Chirripó P.	83°37'W	9°30'N	1690	1987
10. 2132	San José, Sn Isidro, Providencia	Naranjo	83°51'W	9°34'N	1990	1987
11. 2135	San José, Sn Isidro, Copey	Pirrís	83°55'W	9°37'N	2080	1987
12. 3111	San José, Herradura, Herradura	Chirripó P.	83°37'W	9°29'N	1550	1998
13. 3112	San José, Buena Vista, La Piedra	Chirripó P.	83°40'W	9°31'N	1500	1998
14. 3113	San José, Buena Vista, N La Piedra	Chirripó P.	83°41'W	9°31'N	1880	1998
15. 3115	Cartago, Sn Rafael, hacia Cot	Reventazón	83°54'W	9°53'N	1560	1998
16. 3118	Cartago, Pacayas, Cervantes	Reventazón	83°47'W	9°54'N	1570	1998
17. 3120	Alajuela, Zarcero, Tapezco	San Carlos	84°24'W	10°13'N	1710	1998
18. 3122	Alajuela, Zarcero, Río Seco	Vírilla norte	84°23'W	10°10'N	1610	1998
19. 3127	Cartago, Cartago, Quircot	Reventazón	83°56'W	9°54'N	1510	1998
20. 3128	Cartago, Pacayas, Río Birrís	Reventazón	83°47'W	9°55'N	1520	1998
21. 3138	San José, Escazú, Bebedero	Vírilla sur	84°10'W	9°54'N	1700	2002
22. 3139	San José, Sn Rafael, Vista de Mar	Vírilla norte	83°58'W	9°58'N	1790	2002
23. 3142	Cartago, San Nicolás, Río Tarras	Reventazón	83°55'W	9°55'N	2000	2002
24. 3144	Cartago, La Unión, Hda Tres Ríos	Reventazón	83°59'W	9°54'N	1630	2002

Table 3 – List of populations of wild common bean found, sites, watershed, coordinates and year.

Collectors' Number	Province, district, closest site	Watershed	Longitude	Latitude	Altitude (masl)	Year found
1. 2097	San José, Tarbaca	Vírilla sur	84°07'W	9°49'N	1750	1987
2. 2111	San José, Aserrí	Vírilla sur	84°07'W	9°52'N	1560	1987
3. 3106	Alajuela, Carrizal, Chagüite	Vírilla norte	84°10'W	10°06'N	1510	1998
4. 3126	Cartago, San Nicolás, Quircot	Reventazón	83°56'W	9°54'N	1540	1998
5. 3131	San José, Desamparados, Jérico	Candelaria n	84°03'W	9°49'N	1540	1998
6. 3132	Alajuela, Alfaro Ruíz, Zarcero	Vírilla norte	84°23'W	10°10'N	1610	2002
7. 3133	Alajuela, Poas, Sabana Redonda	Vírilla norte	84°14'W	10°07'N	1380	2002
8. 3134	San José, Aserrí, Tranquerillas	Candelaria n	84°07'W	9°48'N	1500	2002
9. 3135	San José, Aserrí, Chirogres	Candelaria n	84°06'W	9°48'N	1480	2002
10. 3136	San José, Desamparados, Sn Miguel	Vírilla sur	84°04'W	9°51'N	1370	2002
11. 3137	San José, Escazú, Bebedero	Vírilla sur	84°10'W	9°54'N	1600	2002
12. 3140	Cartago, La Unión, Pque Iztarú	Vírilla sur	83°58'W	9°54'N	1750	2002
13. 3143	Cartago, La Unión, Hda Tres Ríos	Vírilla sur	83°59'W	9°54'N	1500	2002
14. 3147	San José, Aserrí, El Tigre	Candelaria n	84°06'W	9°49'N	1450	2003
15. 3148	San José, Desamparados, Manzano	Candelaria n	84°05'W	9°49'N	1370	2003
16. 3168	San José, Sta. María de Dota, Copey	Pirrís	83°57'W	9°39'N	1600	2003
17. 3178	San José, Desamparados, Guatuso	Vírilla sur	84°02'W	9°51'N	1380	2003
18. 3184	San José, San Pablo, Sn Cristobal Sur	Candelaria s	84°01'W	9°44'N	1450	2003

Table 4 – List of populations of wild common bean found, possible weedy hybrid forms, sites, and year.

Collectors' Number for Wild forms	Collectors' Number for Weedy forms	Province, district, closest site	Year found
2097	2098	San José, Tarbaca	1987
2111	2115	San José, Aserrí	1987
3106	none	Alajuela, Carrizal, Chagüite	1998
3126	3151, 3155, 3158	Cartago, San Nicolás, Quircot	1998
3131	none	San José, Desamparados, Jérico	1998
3132	3121	Alajuela, Alfaro Ruíz, Zarcero	2002
3133	none	Alajuela, Poas, Sabana Redonda	2002
3134	none	San José, Aserrí, Tranquerillas	2002
3135	none	San José, Aserrí, Chirogres	2002
3136	none	San José, Desamparados, Sn Miguel	2002
3137	none	San José, Escazú, Bebedero	2002
3140	none	Cartago, La Unión, Pque Iztarú	2002
3143	none	Cartago, La Unión, Hda Tres Ríos	2002
3147	none	San José, Aserrí, El Tigre	2003
3148	possibly one	San José, Desamparados, Manzano	2003
3168	none	San José, Sta. María de Dota, Copey	2003
3178	none	San José, Desamparados, Guatuso	2003
3184	none	San José, San Pablo, Sn Cristobal Sur	2003