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RESEARCH ARTICLE

Characterization of butterfly pea (*Clitoria ternatea* L.) accessions for morphology, phenology, reproduction and potential nutraceutical, pharmaceutical trait utilization

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Abstract Butterfly pea, *Clitoria ternatea* is used in Africa as a companion crop and in the United States as an ornamental. The USDA, ARS, PGRCU curates 28 butterfly pea accessions. Butterfly pea accessions were transplanted from about 30-day-old seedlings to the field in Griffin, GA around 01 June 1999, 2003, 2006–2007 or directly sown in 2001. At 50% maturity, 19 accessions were characterized for morphology, phenology, and evaluated for regeneration. High quality plants regenerated from all accessions produced 6 to more than 3,400 total seeds. Butterfly pea can be successfully grown and regenerated in Griffin, GA. Coefficients of variation and principal component analysis revealed considerable variability among accessions for morphological and reproductive traits. Butterfly pea has potential to be used as a nutraceutical, pharmaceutical, or food. The flavonoid quercetin has been shown to reduce upper respiratory infections in humans while delphinidin and malvidin identified in butterfly pea flowers may inhibit various forms of cancer.

Keywords Butterfly pea · *Clitoria ternatea* · Genetic resources · Nutraceutical · Phytopharmaceutical · Principal component analysis

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Introduction

Butterfly pea, *Clitoria ternatea* L. is a member of the Leguminosae (Fabaceae) family, Phaseoleae tribe, and Clitoriinae subtribe, found in many countries worldwide (NPGS 2008). Butterfly pea has a diploid chromosome number of $2n = 16$ (Joson and Ramirez 1991). The plant is a tall, slender, climbing herbaceous vine with five leaflets, white to purple flowers, and has deep roots. Butterfly pea is self-pollinated, however segregating genotypes have been identified, indicating partial outcrossing probably exists (Cook et al. 2005).

Butterfly pea grows well in a range of soil types (pH 5.5–8.9) including calcareous soils and tolerates excess rainfall and drought. The primary method of propagation is by seed, and the plant can be grown with or without support such as trellises for efficient pod harvesting. Butterfly pea plants produce large quantities of seed and re-seed due to seed shattering at maturity. The plants compete well with weeds once established, however some weed control is recommended after mowing the crop. This allows subsequent regrowth of butterfly pea to gradually dominate the weeds. If a pure stand is needed, weed control can be accomplished by cultivation during early growth. Generally it is recommended to cut the butterfly pea plants at 10 cm above ground level for use as hay forage, however if it is used for pasture, 25 days of regrowth is required. Butterfly pea plants contain protein ranging from 14% to 20% while seeds contain 38% protein, 5% total sugars, and 10% oil. Butterfly pea is tolerant to most pests and diseases. In Sudan, butterfly pea is grown for dense cover when grown with support crops such as sudan grass, sweet sorghum, and sunn hemp (ECHO 2006). In Barbados, butterfly pea has been established with elephant grass, guinea grass, coastal bermuda grass, Rhodes grass, and *Leucaena* (ECHO 2006). Butterfly pea is also used in the USA as an ornamental. Even though various fungi and nematodes have been found to infect butterfly pea plants, the economic threshold is usually not reached to warrant control (ECHO 2006).

The objectives of this study were to (1) characterize variability among regenerated butterfly pea accessions for branching, foliage, plant size, flower color, days to initiation of the first flower, days to 50% maturity, seed number, seed weight and percent germination in Griffin, GA, USA during 1999, 2001, 2003, 2006, and 2007 and (2) to discuss several underutilized traits.

Materials and methods

All butterfly pea accessions studied are conserved and curated at the USDA, ARS, Plant Genetic Resource Conservation Unit located in Griffin, GA, USA. Seed accessions studied were collections from 12 diverse countries (Table 1). Approximately 50 butterfly pea seeds from each of 18 accessions were planted in 6.4 cm × 7.0 cm jiffy pots (Hummert International, Earth City, MO) containing Metro Mix 200 potting soil (Scotts Sierra Horticultural Products Company, Marysville, Ohio) each year (1999, 2003, 2006, 2007) during April through June in a greenhouse at a range of 21–26°C or directly sown in the field by hand during June, 2001. After approximately 30 days, butterfly pea plants were transplanted (1999, 2003, 2006, 2007) or directly sown by hand during 2001 to field regeneration plots in clayey, kaolinitic, thermic typic kanhapludults soil series at Griffin, GA. About 25–50 plants representing each accession per plot were regenerated in one 6 m row plot with 3–6 m between rows. A typical regeneration cycle includes butterfly pea plots separated by at least two different legume species to decrease outcrossing as much as possible. Plots were irrigated using sprinklers as necessary. Data were recorded based on a rating scale from 1 to 9 for amount of branching and foliage where 1 = 91–100%; 2 = 81–90%; 3 = 71–80%; 4 = 61–70%; 5 = 51–60%; 6 = 41–50%; 7 = 31–40%; 8 = 21–30%; 9 = 1–20% of the plants are producing branches and foliage based on visual observations. Mean plant size was calculated by multiplying length by average width of plants growing in each row (plot) at 50% flowering. The number of days from planting to initiation of first flower was recorded along with a visual observation of the number of days from planting to 50% maturity of plants within each plot (row). Mature seeds were harvested from each butterfly pea accession 3–6 months after transplanting or direct seeding. Pods were hand harvested as they matured, dried at 21°C, 25% RH for 2–4 weeks, and threshed. Seeds were then counted and weighed.

A principal component analysis was used to characterize phenotypic variability among these butterfly pea accessions regenerated in one environment, to determine correlations, as well as establishing relationships among butterfly pea accessions analyzed for morphological and reproductive components. Standard errors (SE) and coefficients of variation (CV) were also determined to confirm variability using principal component analysis (2002–2003 SAS software program, SAS Institute, Cary, NC, USA).

Results and discussion

Quality plant regeneration occurred for most of the butterfly pea accessions tested. Morphological, phenological, and reproductive characteristics observed among accessions are in Table 1. In general, considerable variability in morphological phenology and reproductive traits among accessions was reflected by wide ranges for these characteristics (Table 2). Phenotypic variation for the PGRCU butterfly pea collection can be explained on the basis of the diverse geographic origins of these accessions as well as plant selection leading to the possible development of ecotypes and/or cultivated varieties. The amount of branching and foliage ranged on a scale of 1–8. Most of the accessions produced high branching and foliage indexes averaging 1 (91–100%). However, the accessions PI 164250, PI 258379, and PI 641948 produced the fewest branches (averaging five) and foliage while PI 322364 from Brazil produced the least amount of

Table 1 Morphological, phenological and reproductive traits of *Clitoria ternatea* evaluated from 1999 to 2007

Acc. no.	Origin	Branching	Foliage	Mean plant size (cm^2)	Flower		Seed			
					Color	Days to start of	Days to 50% maturity	No.	Wt. (g)	% germ.
1999										
209315	Virgin Islands	1	1	2,100	Blue	65	–	1,194	60	73
538311	Dominican Rep.	1	1	6,500	Blue	71	–	410	21	100
2001										
164250	Brazil	1	–	3,200	Dark blue	53	–	353	17	30
451721	Mexico	1	1	2,000	Blue	47	–	2,998	156	83
2003										
641948	Virgin Islands	5	5	–	Dark blue	93	153	216	11	70
164250	Brazil	5	8	3,200	Dark blue	107	194	290	–	–
209592	Cuba	1	1	9,000	Blue	127	170	459	21	94
213499	India	1	1	2,100	Light blue	107	178	305	–	–
226265	Kenya	2	3	1,800	Light blue	90	177	96	3	80
258379	Taiwan	6	6	2,000	Blue and purple	65	150	158	6	–
283231	Sudan	3	3	2,400	Light and dark blue	76	149	1,040	50	84
295357	Taiwan	1	1	2,800	White light and dark blue	65	149	1,383	76	87
311506	Brazil	1	1	2,400	Dark blue	76	159	1,200	53	93
316204	Australia	1	1	6,600	Dark blue	76	142	1,010	52	79
319465	Tanzania	1	1	2,700	Light blue	87	194	860	28	74
320977	Sierra Leone	3	1	2,450	Dark blue	65	149	800	42	82
322364	Brazil	3	5	2,450	Dark blue	83	140	572	27	82
2006										
209591	Cuba	1	1	4,950	White	93	192	190	8	–
226265	Cuba	3	3	4,950	Light blue	89	176	3,185	99	73
258379	Cuba	2	2	4,500	Purple	131	168	6	–	–
283232	Sierra Leone	1	1	5,040	Dark blue and purple	88	193	702	30	83
538311	Sierra Leone	1	1	5,850	–	–	191	1,196	48	80
2007										
209591	Sierra Leone	1	1	6,960	White and blue	120	197	2,722	131	–
258379	Sierra Leone	1	1	3,685	Dark blue	108	120	3,404	167	–
SE		0.30	0.41	416		4.71	5.03	208	10.48	3.62
C.V. (%)		77	92	51		26	13	99	91	19

Table 2 Phenotypic traits in butterfly pea based on data from 1999, 2001, 2003, 2006, and 2007 field regenerations

Variable	Maximum	Minimum	Range	Mean	SD
Branching rating	6.00	1.00	5.00	1.96	1.51
Foliage rating	8.00	1.00	7.00	2.17	1.99
Mean plant size (cm^2)	9,000.00	1,800.00	7,200.00	3,897.00	1,995.00
No. of days to start of flowering	131.00	47.00	84.00	86.17	22.62
No. of days to 50% maturity	197.00	120.00	77.00	167.10	22.51
Seed number	3,404.00	6.00	3,398.00	1,031.00	1,020.00
Seed weight (g)	167.00	3.00	164.00	52.67	48.07
% Germination	100.00	30.00	70.00	79.23	15.00

foliage. The accession PI 538311 from the Dominican Republic produced good branching and foliage in 1999 and 2006. The accession PI 209591 from Cuba produced good branching and foliage in 2006 and 2007 as well. The coefficients of variation for branching and foliage was 77% and 92%, respectively (Table 1). These values indicate greater variability for foliage than for branching between all accessions tested. Mean plant size based on growing area within rows (plots) ranged from 1,800 to 9,000 cm^2 among accessions over all years (Table 2). The accession PI 538311 from the Dominican Republic produced the largest plants (6,500 cm^2) in 1999 as well as in 2006 (5,850 cm^2). The accession PI 164250 produced similar plant size as PI 538311 during 2001 and 2003 (3,200 cm^2). The accession PI 209592 from Cuba produced the largest plants (9,000 cm^2) during 2003 followed by PI 316204 from Australia (6,600 cm^2), and PI 283232 from Sierra Leone (5,040 cm^2) during 2006. During 2007, the accession, PI 209591 from Cuba produced the largest plants (6,960 cm^2) per plot. The coefficient of variation for mean plant size per plot was 51% indicating low variation between accessions (Table 1). Flower colors ranged from white to purple with the majority (seven) of the butterfly pea accessions producing dark blue flowers. Seven accessions produced either blue or light blue flowers while four accessions produced segregating colors including mixtures of white, light blue, blue, dark blue, and purple flower colors within the same accession (Table 1). See Fig. 1 for some variations for butterfly pea flower colors.

The earliest butterfly pea flower occurred in the accessions, PI 451721 from Mexico and PI 164250 from Brazil, with first flowers forming 47 and 53 days after planting, respectively during 2001 (Table 1). The accessions, PI 164250 (during 2003), PI 209591, PI 209592, PI 213499, and PI 258379 averaged first flower 117 days after planting. All other accessions produced the first flower on average of 79 days after planting. The earliest maturing (120 days) butterfly pea accession was PI 258379 from Taiwan in 2007. The accessions PI 283231, PI 295357, PI 316204, PI 320977, and PI 322364 matured an average of 146 days after planting. All other accessions matured on average 178 days after planting. The coefficients of variation for days to first flower and days to 50% maturity were 26% and 13%. These values indicate low variability for these phenological traits (Table 1).

Variable seed numbers and weights were observed among butterfly pea accessions (Table 1). Butterfly pea seed numbers ranged from 6 to 3,404 with seed weights per accession ranging from 3 to 167 g for all 5 years (Table 2). The accession, PI 209315 from the Virgin Islands produced 1,194 seeds weighing 60 g in 1999. The accession, PI 451721 from Mexico produced 2,998 seeds weighing 156 g in 2001. From 2003 to 2007, PI 283231, PI 295357, PI 311506, PI 316204, PI 226265, PI 538311, PI 209591, and PI 258379 produced the most seeds with an average of 1,893 weighing an average of 85 g. Ten accessions produced an average of 497 seeds weighing an average of 28 g. The lowest seed production was observed in PI 226265, PI 258379, and PI 209591 averaging 113 seeds weighing an average of 6 g.

Since branching, foliage production, plant size, seed number, seed weight, and percent germination are quantitative traits, the extensive variability between accessions is probably attributed to the environment in which they were regenerated. However, the coefficient of variation (Table 1) for seed

Fig. 1 *Clitoria ternatea* flower colors. From left to right, PI 538311, PI 226265, and PI 226265

number (99%) and seed weight (91%) were all high indicating high variability between the accessions tested. Within accession variation was determined in several butterfly pea accessions including PI 164250, PI 209591, PI 226265, PI 258379, and PI 538311 with two reps consisting of 25–50 plants each. Coefficients of variation within these accessions were 123% for seed number and 111% for seed weight. These values indicate considerable variation within these five butterfly pea accessions.

In general, high variation in morphological, phenological, and reproductive traits among butterfly pea populations was reflected by wide ranges for these characteristics (Table 2). Phenotypic variation for the PGRCU butterfly pea collection can be explained on the basis of the diverse geographic origins of these accessions. The goal of plant genetic resources conservation efforts is to conserve crop species and their wild relatives so that sufficient genetic and phenotypic diversity of important characteristics support crop improvement basic research.

Principal component analysis

The first four principal components had Eigen-values (Table 3) greater than 1.0, and together they explained 91% of the total variation for this group of phenotypic, phenological, and reproductive traits. The first principal component had an Eigen-value of 2.8013, explaining 35% of the entire variation. Branching and days to flower traits contributed greatly to variation for this principal component, with Eigen-vectors equal or greater than 0.40 (Table 4). The second principal component's Eigen-value was 1.8622 and it explained 23% of the total variation. Reproductive traits including seed number and seed weight contributed greatly to variation for this principal component, with Eigen-vectors above 0.50. The third principal component's value was 1.4441 and it explained 18% of the total variation. Phenological traits including days to first flower and 50% maturity with Eigen-vectors above 0.45 contributed greatly to its variation. The fourth principal component had 1.1778 as its Eigen-value and it explained 15% of the total variation. Plant size and days to 50% flowering contributed the greatest amount to its variation. Branching was significantly correlated with foliage ($r^2 = 0.9053$) and seed number was significantly correlated with seed weight ($r^2 = 0.9654$).

Table 3 Eigen-values and the proportion of total variability among butterfly pea accessions (1999–2007) as explained by the principal components

Principal component	Eigen-value	% Variability	% Cumulative
1	2.8013	35.02	35.02
2	1.8622	23.28	58.29
3	1.4441	18.05	76.35
4	1.1778	14.72	91.07
5	0.4355	5.44	96.51
6	0.2324	2.91	99.42
7	0.0386	0.48	99.90
8	0.0077	0.10	100.00

Potential nutraceutical traits

Butterfly pea contains numerous phytochemicals for possible use in the nutraceutical area. Several flavonoids including quercetin, robinin, and ternatin are found in butterfly pea flowers (ILDIS 1994). Blue flowers of butterfly pea contains the flavonoid quercetin (ILDIS 1994). A recent clinical trial

Table 4 Eigen-vectors, principal components for eight morphological and seed traits in butterfly pea accessions (1999–2007)

Trait	Principal components							
	1	2	3	4	5	6	7	8
Branching	0.45	−0.23	0.25	0.28	0.04	0.76	0.07	0.05
Foliage	0.32	−0.37	0.37	0.34	0.16	−0.60	0.32	0.0042
Mean plant size	−0.34	0.36	0.14	0.47	−0.49	0.07	0.48	−0.09
Days to flower	−0.40	0.04	0.47	0.39	0.18	−0.01	−0.61	0.22
Days to 50% maturity	−0.25	0.17	0.51	−0.48	0.43	0.14	0.43	0.08
Seed number	0.34	0.56	0.13	0.10	0.24	−0.05	−0.17	−0.65
Seed weight	0.35	0.55	−0.15	0.12	0.14	−0.09	0.05	0.69
Percent germination	−0.30	−0.08	−0.49	0.40	0.64	0.11	0.22	−0.10

concluded that quercetin significantly reduced upper respiratory tract infection incidence among cyclists during a 2 week period of intensified exercise (Nieman et al. 2007). Quercetin was also clinically proven to reduce blood pressure in hypertensive people (Edwards et al. 2007). Ternatin found in the blue petals of butterfly pea has been shown to be anti-inflammatory in animal studies (Rao et al. 2003). Blue flowers of butterfly pea also contain robinin with potential as an antioxidant (Lau et al. 2005). Butterfly pea flowers are edible for humans and are used in the cuisine at the Ritz Carlton, South Beach, Florida where the fitness-minded Chef utilizes the flowers in salads (edible flowers). White and dark blue flowers produced anthocyanin indexes ranging from 1.0 to 26.8, respectively while the flavonoid kaempferol content in butterfly pea flowers was 658.6 ng/μl (Morris and Wang 2007). Other flavonoids are found in butterfly pea leaves including nicotiflorin (ILDIS 1994). Nicotiflorin has been shown to inactivate botulinum neurotoxins which cause botulism in mammals (Sawamura et al. 2002). Chinese scientists have proven in an animal model that nicotiflorin has protective effects on reducing memory dysfunction, energy metabolism failure and oxidative stress in dementia (Huang et al. 2007).

Potential pharmaceutical traits

Some of these flavonoids have potential as pharmaceutical agents as well including kaempferol and quercetin. A recent clinical trial revealed that both kaempferol and quercetin provided evidence of pancreatic cancer prevention for current smokers (Nothlings et al. 2007). Isoquercitrin found in blue butterfly pea flowers has been clinically proven to improve symptoms in patients with chronic venous insufficiency when used as a constituent with other flavonols (Schaefer et al. 2003). In addition both quercetin and isoquercitrin are effective eosinophilic inflammation suppressors with potential for treating allergies (Rogerio et al. 2007). The flavonoid delphinidin found in butterfly pea flowers may have breast cancer chemopreventive potential (Singletary et al. 2007). Both delphinidin and malvidin (found in butterfly pea flowers) may result in apoptosis (Srivastava et al. 2007). Delphinidin may also prevent and successfully treat additional forms of cancer (Lamy et al. 2006). Delphinidin and petunidin (found in butterfly pea flowers) inhibited breast cancer cell growth by 66% and 53%, respectively (Zhang et al. 2005). Another flavonoid, ternatin found in butterfly pea blue petals (ILDIS 1994) has reportedly been discovered to suppress body weight and fat in mice (Shimokawa et al. 2007). The flavonoid, astragalin found in both leaves and flowers of butterfly pea has been suggested to be a potent inhibitor of cellular inflammatory responses induced by a periodontal pathogen and may be useful for the prevention of periodontitis (Kou et al. 2008). The steroid, β-sitosterol found in butterfly pea flowers (ILDIS 1994) has reportedly been found to inactivate HIV, influenza, herpes virus and poxviruses (Kotwal 2007). Root extracts of butterfly pea had significant antibacterial activity against both gram-positive and gram-negative bacteria (Malabadi et al. 2005). So far, none of these phytochemicals are extracted from butterfly pea for use in the nutraceutical or pharmaceutical markets.

Conclusions

Butterfly pea genetic resources contain numerous traits which have the potential to be valuable in the nutraceutical and pharmaceutical industries. These butterfly pea resources could be used in many ways. Flowers could be harvested and used in salads, and vital phytochemicals could be extracted and marketed as nutraceuticals. Flavonoids could be extracted from leaves of butterfly pea plants growing in the field. Since butterfly pea plants produce beautiful flowers, plants and seeds could be used or marketed as ornamentals. The remaining butterfly pea plants could be left in the field as pasture, cut for hay or allowed to continue growing in the field for cover cropping purposes which incorporates valuable nutrients back into the soil. This is the first report on characterization of butterfly pea genetic resources regenerated in Georgia, USA.

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