Rhynchosia minima (L.) DC. – regeneration, characterization and potential uses for natural products and flavonoids

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Summary

Rhynchosia minima (L.) DC. – regeneration, characterization and potential uses for natural products and flavonoids

Least snout bean, *Rhynchosia minima*, has been used very little worldwide, including the USA. Wild species of least snout bean genotypes are known to be native to the south-eastern USA, but none have been collected and deposited in the USDA, ARS, PGRCU collection. The PGRCU maintains 26 least snout bean accessions from Mexico, Central America, Caribbean Islands, and South America. Twenty-one accessions of least snout bean were transplanted as approximately 44-day-old seedlings to the field in Griffin, GA, USA, around 01 June 1998, 2001, 2002 and 2003. After 3 to 4 months, the accessions were characterized for morphological traits and evaluated for seed regeneration. High quality plants regenerated from all accessions produced from 3 to more than 23 000 total seeds per plot. Least snout bean can be successfully grown and regenerated in Griffin. Least snout bean has potential to be used in numerous ways, including for pharmaceuticals and other agricultural products. Natural products such as gallic acid identified in least snout bean pods and seeds may fight colon cancer, while the flavonoid, isovitexin, may reduce inflammation, as well as carcinogenesis.

Key words: Least snout bean, characterization, regeneration, pharmaceutical, flavonoid

Résumé

Rhynchosia minima (L.) DC. – régénération, caractérisation et utilisations potentielles pour les produits naturels et les flavonoïdes

Le pois colibri, *Rhynchosia minima*, a été peu utilisé dans le monde, y compris aux États-Unis. Les espèces sauvages des génotypes de *R. minima* sont indigènes dans le sud-est des États-Unis, mais aucune n'a été collectée ni déposée dans la collection PGRCU, ARS de USDA. La PGRCU maintient 26 accessions de *R. minima* provenant du Mexique, d'Amérique centrale, des Antilles et d'Amérique du sud. 21 accessions de *R. minima* ont été transplantées en champ sous forme de plantules âgées d'approximativement 44 jours à Griffin, Géorgie (États-Unis), les 01 juin 1998, 2001, 2002 et 2003. Après 3 à 4 mois, les caractères morphologiques des accessions ont été déterminés et la régénération à partir des graines a été évaluée. D'excellentes plantes ont été régénérées pour toutes les accessions avec une production de 3 à plus de 23 000 graines au total par parcelle. *R. minima* a pu être cultivé et régénéré avec succès à Griffin. Ses utilisations potentielles sont nombreuses, y compris pour des produits pharmaceutiques et agricoles. Des substances naturelles telles que l'acide gallique ont été identifiées dans les gousses. Les graines de *R. minima* peuvent être utilisées contre le cancer du côlon et les flavonoïdes, tels que l'isovitexine, réduisent l'inflammation et la cancérogenèse.

Resumen

Regeneración, caracterización y usos potenciales de _Rhynchosia minima_ para obtener flavonoides y productos naturales

En todo el mundo se ha utilizado muy poco el frijolillo (*Rhynchosia minima*). Se sabe que hay especies silvestres de genotipos de frijolillo nativos de la zona sudoriental de los EE.UU. que no se han recogido ni depositado en la colección del PGRCU, ARS, USDA (Unidad de Conservación de Recursos Fitogenéticos, Servicio de Investigación Agrícola, Departamento de Agricultura de EE.UU., siglas en inglés). El PGRCU conserva 26 accesiones de frijolillo de México, América Central, Islas del Caribe y América del Sur. Alrededor del 1º de junio de 1998, 2001, 2002 y 2003 se trasplantaron plantines de unos 44 días de edad de veintiun accesiones de frijolillo en los campos de Griffin, Georgia, EE.UU. Después de 3 a 4 meses se caracterizaron los rasgos morfológicos de las accesiones y se evaluó la regeneración de las semillas. Plantas de gran calidad regeneradas a partir de todas las accesiones produjeron de 3000 a más de 23 000 semillas por lote. El frijolillo puede crecer y regenerarse óptimamente en Griffin. Tiene muchas aplicaciones potenciales en productos farmacéuticos y otros productos agrícolas. Algunos productos naturales como el ácido gálico identificado en las vainas y semillas de frijolillo pueden servir contra el cáncer de colon, y el flavonoide isovitexin puede reducir la inflamación y la carcinogénesis.

Introduction

Least snout bean (*Rhynchosia minima* (L.) DC.) is a wild relative of pigeon pea and a member of the Leguminosae (Fabaceae) family, Phaseoleae tribe, Cajaninae subtribe, widespread in the tropics (ILDIS/CHCD 1994). Least snout bean is found on every continent and associated islands worldwide (FAO 2006). It grows wild in the states of Texas, Louisiana, Arkansas, Mississippi, Alabama, Georgia and Florida, USA (NRCS 2006). The prefix '*Rhync*' refers to its nose- or snout-shaped flower, and the suffix '*minim*' derives from its very small flower, seed and pod. Least snout bean is a diploid with a chromosome number 2n = 22 (Dundas 1990). PGRCU, Griffin curates a global collection of 26 least snout bean accessions.

The plant is a glabrous to pubescent and vine producing sub-erect legume. Stems are slender, numerous, and 80 to 120 cm long. Stipules are lanceolate and reach 2 to 3 mm in length. The leaves are trifoliolate, while the leaflets are rhomboid, ovate or sub-orbicular to broad-acute, apex acute or rounded, 0.5 to 3 cm long × 0.5 to 3 cm wide, and glabrescent to velvet texture. The inflorescence consists of a 6- to 12-flowered raceme 5 to 10 cm long. The calyx is 3 to 4 mm long, with five acuminate lobes. The 1-cm long corolla is yellow, whereas the standards are 5 to 7 mm long. The keel is as long as the standard, but the wings are shorter. Pods are 1 to 1.5 cm long × 0.4 to 0.6 cm wide, black when mature, two seeded, oblong, finely pubescent, with slight separation between seeds, and has short beaks. The seeds range from grey to tan, brown or black, with similar colours arranged on the seeds as spots (Morris unpublished) and are about 3 mm long, with a short hilum (FAO 2006).

The objectives of this study were, first, to regenerate and characterize least snout bean genetic resources for flower colour, days to first flower (DAP), and seed regeneration in Griffin, GA, USA, in 1998, 2001, 2002 and 2003, and, second, to discuss potential use of this plant for pharmaceutical products and forage.

Materials and methods

Twenty-five to fifty least snout bean seeds from each of 21 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, OH) each year (1998, 2001, 2002 and 2003) during February to May in a greenhouse with a temperature range of 21 to 26°C. Least snout bean seeds were directly seeded to the field on 20 June 2001 only. After approximately 44 days, previously germinated least snout bean plants were transplanted to field plots in clayey, kaolinitic, thermic typic kanhapludults soil series at Griffin (latitude 33°13′ N; longitude 84°16′ W). Plant characteristics of all accessions were recorded each year for 4 years over a 5-year period (1998, 2001, 2002 and 2003; Table 1). Seed regeneration and characterization can be accomplished without replications for the conservation of least snout bean germplasm, based on recommendations of the USDA, ARS, NPGS, Special-purpose Legume Crop Germplasm Committee (SPLCGC). Twenty-five to fifty plants of each accession per plot were transplanted in one 6-m row plot, with 3 to 6 m between rows. Plots were irrigated using overhead sprinklers as necessary. Flower colour and days to first flower were recorded when plants reached the initial inflorescence stage per plot. Mature pods were hand harvested from each accession 3 to 4 months after transplanting. Pods were dried at 21°C and 25% RH for 2 to 4 weeks, and threshed. Total seeds were counted, weighed and mass determined by dividing total seed weight by seed number.

Results and discussion

Successful plant regeneration occurred for all accessions tested, with variability in plant characteristics among accessions (Table 1). Variable days to first flower, seed number, weight and mass were observed. Fourteen accessions produced yellow flower colours, while 15 accessions produced yellow flowers with a red pigment mark on the petals. Accession PI 322621 did not flower in 2002. The number of days after planting (DAP) to first flower for all four years ranged from 32 to 153 days, with an average of 82 days. The coefficient of variation for days to first flower was 44.04%, indicating a fairly high level of variation between accessions (Table 1). Least snout bea seed numbers ranged from 0 to 23 579, with seed weight p accession ranging from 0 to 259.4 g over four years. Seed ma is a good indicator of relative seed size. In these accessior seed mass ranged from 0.008 to 0.030 g per seed. During 19 accession PI 322540 produced the earliest first flower (62 DA while all other accessions had an average first flower date 114 DAP. Accession PI 322614 produced 121 seed, weighi 1.10 g with a mass of 0.009 g per seed. Next in order of se production were PI 322623 (49 seed weighing 0.54 g) a PI 639275 (44 seed weighing 0.40 g). The accessions PI 3225 PI 286294, PI 385384, and PI 365004 produced an average 12 seed. These four accessions had a seed weight averagi 0.16 g and with an average mass of 0.015 g per seed. Duri the 2001 regeneration cycle, PI 292354, PI 304151, PI 3121 PI 316627 and PI 322619 produced the first flower at 46 D and averaged 2 812 seeds, weighing 29 g, with an avera mass of 0.010 g per seed. PI 319487 produced the latest fi flower (110 DAP), as well as 850 seed weighing 7.10 g a with a mass of 0.008 g per seed. During 2002, PI 322620 fro Brazil flowered the earliest (32 DAP), produced the most se (23 579), weighed (259.4 g) more than any other accession, a had a mass of 0.010 g per seed. Next in order of flower and se production were both PI 322623 and PI 322624 where the fi flower date occurred at 39 DAP for both accessions. PI 3226 produced 13 555 seeds weighing 149.1 g and a mass of 0.01 per seed, while PI 322624 produced 20 642 seeds weighi 206.4 g with a mass of 0.010 g per seed. Considerable se production occurred from PI 322540 (4 891 seed), PI 6392 (1 203 seed) and PI 322621 (931 seed). These three accessio weighed an average of 26 g, and had a mass of 0.010 g p seed. Accessions PI 538315, PI 322615, PI 322622, PI 5383 and PI 322614 produced the lowest average seed number (11 weighing an average of 1.02 g per accession in 2002. PI 3226 produced the most seed (9 176) weighing 88.1 g with a mass 0.010 g per seed in 2003, while PI 322618 produced 8 500 se weighing 72.3 g with a mass of 0.009 g per seed. The low seed production occurred in PI 538316, PI 322614 and PI 3226 with an average seed number of 1 536, weighing an average 15.2 g. Least snout bean accessions requiring regenerati are those that are low in viability, vigour and seed numb and aging. Low seed quality based on low vigour was w several accessions regenerated fewer seeds than in succeedi years. For example, PI 322623 produced more seed in 2 (13 555) than in 1998 (49) because fresh seed regenerated 1998 was used to increase the seed in 2002. Fresh seed enhanc germination as well as vigour among the regenerating plan Thus greater seed numbers were regenerated from these fres seeds. Variation within accessions was observed for seve accessions (see Table 1), however, this variation may be mask due to the initial low seed quality in some accessions tested

Segregation for DAP, total seed number and flower colo occurred in PI 322623 in 1998 and 2002, and in PI 538316 2002 and 2003, and for total seed number and flower colo in PI 322614 in 1998 and 2003. Since seed number, se weight, seed mass and DAP are quantitative characters, t variability between least snout bean accessions is probal

Table 1. Flower and seed characteristics for *Rhynchosia minima* accessions regenerated in the field at Griffin, GA, USA.

Accession (PI No.)†	Origin	Days after planting to first flower (DAP)	Flower colour	Seed		
				Total no.	Weight (g)	Mass (g/seed)
1998						
286294	Cote D'Ivoire (donated)	143	Yellow, red mark on petal	18	0.20	0.010
322540	Brazil (donated)	62	Yellow	20	0.24	0.010
322614	Brazil (donated)	103	Yellow	121	1.10	0.009
322623	Brazil (donated)	103	Yellow	49	0.54	0.010
365004	S. Africa (collected)	103	Yellow, red mark on petal	3	0.03	0.010
385384	Turkey (collected)	116	Yellow	6	0.18	0.030
639275	Virgin Islands (collected)	-	Yellow, red mark on petal	44	0.40	0.009
2001						
292354	Colombia (collected)	46	Yellow	2 487	22.60	0.009
304151	Mexico (collected)	46	Yellow	3 403	34.00	0.010
312193	Mexico (collected)	46	Yellow	2 356	33.00	0.014
316627	Venezuela (donated)	46	Yellow	2 562	25.40	0.010
319487	Tanzania (donated)	110	Yellow	850	7.10	0.008
322619	Brazil (donated)	46	Yellow, red mark on petal	3 256	30.00	0.009
2002						
286294	Cote D'Ivoire (donated)	114	Yellow, red mark on petal	21	0.23	0.011
322540	Brazil (donated)	69	Yellow	4 891	58.70	0.012
322614	Brazil (donated)	126	Yellow	0	-	-
322615	Brazil (donated)	153	Yellow, red mark on petal	158	1.60	0.010
322620	Brazil (donated)	32	Yellow	23 579	259.40	0.010
322621	Brazil (donated)	-	-	931	9.30	0.010
322622	Brazil (donated)	114	Yellow, red mark on petal	70	0.70	0.010
322623	Brazil (donated)	39	Yellow, red mark on petal	13 555	149.10	0.011
322624	Brazil (donated)	39	Yellow, red mark on petal	20 642	206.40	0.010
538315	Dominican Republic (collected)	114	Yellow, red mark on petal	338	2.70	0.008
538316	Dominican Republic (collected)	126	Yellow	9	0.08	0.009
639275	Virgin Islands (collected)	32	Yellow, red mark on petal	1 203	10.80	0.009
2003						
322614	Brazil (donated)	-	Yellow, red mark on petal	1 456	13.50	0.009
322618	Brazil (donated)	146	Yellow	8 500	72.30	0.009
322621	Brazil (donated)	132	Yellow, red mark on petal	1 000	10.00	0.010
322622	Brazil (donated)	119	Yellow, red mark on petal	9 176	88.10	0.010
538316	Dominican Republic (collected)	146	Yellow, red mark on petal	2 153	22.2	0.010
Std. error		8.06		1 134	12.3	0.003
CV		44.04%		176.6%	176.7%	126%

Note: † = Accession number in the USDA National Plant Germplasm System (NPGS), USA. CV = Coefficient of Variation.

due to segregating accessions, as well as the environment in which they were regenerated. However, the coefficients of variation were extremely high for all seed characteristics evaluated (Table 1). Both seed number and weight coefficients of variation of 176.6% and 176.7%, respectively. These very high coefficients of variation indicate considerable variability for these traits between accessions. The coefficient of variation was 126% for seed mass, indicating a large amount of between-accession variation as well.

This is the first report of characterization and successful regeneration of least snout bean genetic resources at Griffin, GA, USA.

Potential pharmaceutical uses

Many plant species provide the pharmaceutical industry with new sources of health enhancing products. Least snout bean has great potential as a new source of useful nutraceuticals, phytopharmaceuticals and agricultural products.

Several phytochemicals with the potential to alleviate several human health ailments have been identified in least snout bean. The following is a review of these potential new medicines, summarized in Table 2.

The aromatic natural product known as gallic acid exists in least snout bean pods and seeds (ILDIS/CHCD 1994). Gallic acid exhibited a 60% inhibition toward UVB-induced oxidation in erythrocytes and low density lipoprotein (Hsieh et al. 2005). Gallic acid has also been found to exert strong antiproliferative activity on human colon cancer cells (Lee et al. 2005). The natural product known as hydroquinone, found in least snout bean pods and seeds (ILDIS/CHCD 1994), has been shown to induce apoptosis in a human promyelocytic leukaemia cell line (Terasaka et al. 2005), and could be anticancerous to breast, lung and gastric cancer (Stagos et al. 2005). Protocatechuic acid could be used for the prevention of sepsis (Yan et al. 2004), as well as inhibiting the progression of oral carcinogenesis (Suzuki et al. 2003).

Several flavonoids in least snout bean leaves show health enhancing potential, including isovitexin, orientin, schaftoside, vicenin and vitexin (ILDIS/CHCD 1994). Isovitexin has shown benefit by reducing inflammation and carcinogenesis in mice cells (Huang et al. 2005). In mice, both orientin and vicenin protect against bone marrow damage from irradiation, suggesting protection of normal tissues in radiotherapy (Nayak and Devi 2005). They have shown weak antiviral activity against parainfluenza type 3 (Li et al. 2002), and significantly protected human lymphocytes against radiation for possible cancer therapy (Vrinda and Uma 2001). In addition, orientin and vicenin protect against irradiation-induced genomic damage and instability, which reduces delayed chromosomal abnormalities and tumorigenesis in adult mice (Uma and Satyamitra 2004). Orientin showed antifungal activity as high as the standard drug ketoconazole (De Campos et al. 2005). The flavonoid vitexin has antibacterial activity against Gram-negative bacteria (Basile et al. 1999). Both vitexin and schaftoside inhibited lung neutrophil influxes in mice, suggesting possible airway anti-inflammation caused by influenza and pneumonia (De Melo et al. 2005). Currently none of these natural products and flavonoids are extracted from least snout bean nor used as phytomedicine for humans. However, great potential exists.

This is the first reported discussion of potential uses of least snout bean in the pharmaceutical industry within the USA, as well as throughout the world.

Agricultural uses

In 1998, the author recorded various traits of interest from least snout bean regenerated in the field at Griffin. These traits included uniformity, vigour, pest damage and other useful qualities. The following is a brief description of qualities observed from least snout bean growing in the field.

The most vigorous accession, based on morphological appearance, was PI 322540 from Brazil, followed by PI 32261[illegible] PI 322623, PI 365004 and PI 639275. These same accessions were noted as being very uniform. Normally, the progression of senescence is fairly rapid in most legumes and the plants show curling and drying near the end of the growing season in the regeneration fields at Griffin. However, senescence appeared to be delayed somewhat, based on the observation of very few curling and drying leaves on accessions PI 28629[illegible] PI 322540, PI 322614, PI 322623, PI 365004 and PI 63927[illegible] indicating potential abiotic or biotic resistance or tolerance. Based on these observations, it is possible that flavonoids such as orientin may be responsible for the low abiotic and biotic stresses. In addition, PI 322540, PI 322614, and PI 6392[illegible] were noted as candidates for use as weed controlling living mulches, because few weeds were observed in these plots. The weed control may be caused by least snout bean smothering weeds or weed preventive phytochemicals may be released from the plants into the soil, thus preventing weed growth by acting as allelopaths. Interestingly, only PI 385384 from Turkey was observed to have extremely dark green leaves indicating potentially high protein levels for use as forage. Protein levels in least snout bean leaves need to be assessed further. Least snout bean shoots are high in calcium (19[illegible]

Table 2. Some useful phytochemicals identified in *Rhynchosia minima*.

Phytochemical	Plant part	Potential use
Aromatic natural products		
Gallic acid	Seed, pod	Anti-cytotoxic, antioxidant
Hydroquinone	Seed, pod	Anticancer
Protocatechuic acid	Seed, pod	Anticancer, antioxidant
Flavonoid		
Isovitexin	Leaf	Antioxidant
Orientin	-	Antiviral, anticancer, anti-fungal
Schaftoside	Leaf	Airway anti-inflammation
Vicenin	Leaf	Anticancer
Vitexin	-	Antiviral, airway anti-inflammation

ppm), carbohydrates (508 000 ppm), fibre (236 600 ppm) and phosphorous (2 400 ppm). Protein has also been found to be high in least snout bean seeds (236 000 ppm) and shoots (136 000 ppm) (Duke 2006). Least snout bean is capable of regenerating from rootstock in eroded soils in India (Dagar et al. 1978) and tolerated drought as well as waterlogged soil in Australia (Keating and Mott 1987). Least snout bean was shown to be palatable in rams and ewes, with 0.32 kg per day of weight gain (Shukla et al. 1970). Salt tolerance has been observed from least snout bean plants growing in soils with a salt concentration of 1.6 meq/100 g in Barbados. This salt concentration prevented the growth of 30 other plant species (Eavis et al. 1974).

Conclusion

High coefficients of variability were detected, indicating considerable variability for seed number, seed weight, seed mass and DAP between least snout bean accessions. Least snout bean genetic resources contain numerous traits that have potential for use in the development of natural products, or for extraction of flavonoids for use as pharmaceuticals. These least snout bean resources could be produced in the southern USA for multiple uses. For example, mature least snout bean pods and seeds could be harvested, dried and aromatic natural products extracted from pods or seeds. These products could be marketed as new ingredients in skin care products, nutraceuticals or phytopharmaceuticals. Live plants in the field could be harvested and processed by extracting specific flavonoid compounds from least snout bean leaves. The remaining least snout bean plants in the field could be allowed to continue growing for cover cropping or as forage for grazing animals.

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