Hydroponic Rescue and Regeneration of *Aeschynomene*, *Corchorus* species, and *Lablab purpureus* (L.) Sweet Genetic Resources

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Abstract

***Aeschynomene*, *Corchorus* species, and *Lablab purpureus* (L. Sweet) have uses ranging from forage, vegetables, nutraceutical, and medicinal. Many of these will not flower nor produce seed when grown under normal field conditions in Griffin or Byron, GA because of juvenility, photoperiod and freeze-sensitivities. Therefore alternative regeneration methods such as hydroponic techniques are required to increase high quality seed for these species. 40 accessions of *Aeshynomene*, *Corchorus* species, and *L. purpureus* were planted in the field at Griffin or Byron, GA from 2011-2013. 4 stem cuttings per accession with 3 true leaves were removed from plants and placed in a hydroponic cloner system inside the greenhouse during the Fall all three years. Most of the stem cuttings developed healthy root systems. After 1-2 wks., 2-4 well developed stem cuttings from each *Aeschynomene* accession with healthy root systems were placed in an aeroponic system while both the *Corchorus* and *L. purpureus* accessions were transplanted to plastic pots containing potting soil and maintained in the greenhouse. 14 seedlings from a low seed producing *L. purpureus* accession, Grif 16516 were placed in a nutrient film technique (NFT). Seed numbers ranged from 20-18,000 per accession for all species and seed weights ranged from 0.258-305.8 g per accession for the *Aeschynomene* species and *L. purpureus*. Seed weights were not determined for the *Corchorus* species. A fairly low amount of variability for seed numbers and weights occurred for both *Aeschynomene* and *L. purpureus* accessions based on coefficient's of variation (ranging from 51-64%, however the *Corchorus* accessions revealed greater variability for seed numbers based on there coefficient of variation (91%). The *L. purpureus* accession, Grif 16516 produced 1,629 seeds. These are very useful techniques to rescue juvenile, photoperiod, freeze-sensitive and low seed producing accessions for quality seed regeneration and should be useful for additional species as well.**

Materials and Methods

Aeschynomene

11 photoperiod-freeze-sensitive greenhouse-grown *Aeschynomene* accessions including 6 *A. americana*, 4 *A. americana* var. *americana*, and 1 *A. villosa* var. *villosa* were transplanted to the field at Griffin, GA (June 1, 2012-2013). 4 vegetative stem cuttings (15-20 cm long) with at least 3 true leaves per cutting were removed per accession from all accessions between November 7-18 each year. Each cutting was placed inside a hydroponic cloner (Fig. 1) on the same date inside the greenhouse maintained from 21-26° C. Each *Aeschynomene* cutting received tap water which was continuously sprayed onto each basal stem within the hydroponic cloner. 2 weeks later, stem cuttings with well developed root systems (Fig. 2) were placed in an aeroponic system (Fig. 3) in a randomized complete block design with 4 replications both years. Equal proportions (1:1:1) of a 2:1:6 and 0.5% Mn growth solution; a 0:5:4, 1.5% Mg, and 1% S bloom solution; and a 5:0:1 with 5% Ca, 0.0005% Co, 0.1% Fe, 0.05% Mn, and 0.0008% Mo micronutrient solution were sprayed on the root systems of each plant. pH and electrical conductivities were maintained at 6.0 and between 750-1500 µ S/cm, respectively. Fig. 4 shows pods.

Corchorus

4 photoperiod-freeze-sensitive *Corchorus* accessions including 2 each of *C. hirtus* and *C. olitorius* were transplanted to the field at Griffin, GA by the first week of June, 2011. 4 vegetative stem cuttings (15-20 cm long) with at least 3 true leaves per cutting were removed per accession from all accessions on 27 October, 2011. Each cutting was placed inside a hydroponic cloner (Fig. 1) and received tap water which was continuously sprayed onto each basal stem within the hydroponic cloner on the same date inside the greenhouse maintained from 21-26° C. About 2 weeks later, stem cuttings with well developed root systems (Fig. 5) were transplanted to plastic pots (Fig. 6) containing potting soil.

Lablab purpureus

25 juvenile-freeze-sensitive hyacinth bean accessions were directly seeded to field plots in Byron, GA on 26 April, 2011. Fig. 7 shows plants on 2 Sept., 2011. 4 woody and mature vegetative stem cuttings (15-20 cm in length) per accession with 3 true leaves per cutting were removed from hyacinth bean plants on 25 October, 2011. Each cutting was placed in hydroponic cloners (Fig. 1) and received tap water which was continuously sprayed onto each basal stem within the hydroponic clone machine inside a greenhouse maintained from 21-26° C on 25 October, 2011 in Griffin, GA. After 2 weeks, 2 to 4 well developed stem cuttings per accession with healthy root systems (Fig. 8) were transplanted to potting soil in plastic pots and placed in the same greenhouse. Plants were watered and fertilized as needed (Fig. 9). Seeds from hyacinth bean plants were harvested by hand from 24 Jan, 2012 to 6 May, 2012.

Grif 16516 which produced only 35 seeds in the field during 2009 was evaluated for its ability to regenerate more seed when grown in a high tunnel enclosed nutrient film technique system (NFT, Fig. 10) during the 2011 summer growing season at Griffin, GA. A submersible pump was used inside a 40 gal tank to pump equal proportions (1:1:1) of a 2:1:6 and 0.5% Mn growth solution; a 0:5:4, 1.5% Mg, and 1% S bloom solution; and a 5:0:1 with 5% Ca, 0.0005% Co, 0.1% Fe, 0.05% Mn, and 0.0008% Mo micronutrient solution in tap water from the higher end to the lower end of each tray by gravity flow before being collected and re-circulated. The pH was maintained at 6.0. Fourteen 30-day old seedlings from Grif 16516 growing in 6.4 cm x 7.0 cm jiffy pots containing potting soil per seedling were placed in this nutrient film technique system inside a high tunnel during the first week of May, 2011.

Fig. 1. Cuttings from all species were placed in these types of hydroponic cloners in the greenhouse.

Fig. 2. Rooted cuttings of *A. americana*, PI 544161 from the hydroponic cloner after 2-4 wks in the greenhouse.

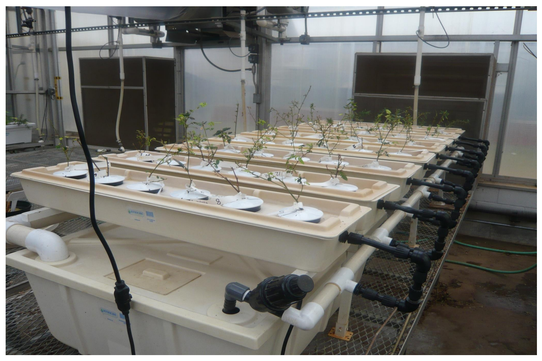

Fig 3. High quality *Aeschynomene* accessions producing pods in the aeroponic system inside the greenhouse on 29 January, 2013.

Fig. 4. *Aeschynomene* accessions in the aeroponic system inside the greenhouse after 10-14 days of growth in 2012.

Fig. 5. Rooted cuttings of *C. hirtus*, PI 478608 from the hydroponic cloner after 2-4 wks in the greenhouse.

Fig. 6. *Corchorus* species growing well in plastic pots containing potting soil in the greenhouse.

Fig. 7. Example of *L. purpureus*, Grif 16547 in the field, Byron, GA without flowers on 2 Sept., 2011.

Fig. 8. Example of rooted cutting from *L. purpureus*, Grif 16533 from the hydroponic cloner in the greenhouse after 2-4 wks.

Fig. 9. *L. purpureus* accessions rescued and regenerating in the greenhouse (12 Jan., 2012).

Fig. 10. *L. purpureus*, Grif 16516 producing mature pods in the nutrient film technique (NFT) hydroponic system inside the coldframe.

Table 1. Seed numbers and weight recorded from all hydroponically grown accessions.

Acc. (PI or Grif)	Species	Origin	Seed no.	Seed wt. (g.)
***Aeschynomene* (2012-2013 based on means, 0 seeds field produced)**				
544161	*A. americana*	Dominican Republic	308	1.208
420303	*A. villosa* var. *villosa*	Australia	287	0.517
544176	*A. americana*	Mexico	243	0.545
544115	*A. americana*	Venezuela	239	0.900
544105	*A. americana*	Panama	195	0.565
544118	*A. americana*	Venezuela	187	0.742
544319	*A. americana* var. *americana*	Mexico	176	0.721
544157	*A. americana* var. *americana*	Mexico	96	0.300
544216	*A. americana* var. *americana*	Mexico	93	0.372
544323	*A. americana* var. *americana*	Mexico	81	0.258
544080	*A. americana*	Mexico	73	0.406
Standard error			152	0.57
Coefficient of variation (%)			55	64
***Corchorus* (2011 based on total seeds, 0 seeds field produced)**				
478608	*C. hirtus*	Guinea	4,866	NA
560027	*C. hirtus*	Nigeria	18,000	NA
560040	*C. olitorius*	Nigeria	3,557	NA
560042	*C. olitorius*	Nigeria	3,938	NA
Standard error			3480	NA
Coefficient of variation (%)			91	NA
***Lablab purpureus* (2012 based on total seeds, 0 seeds field produced)**				
16511	All are *L. purpureus*	Senegal	484	126.8
16512		Myanmar	20	4.3
16517		Myanmar	53	19.2
16521		Myanmar	329	147.9
16528		Tanzania	219	52.4
16530		Indonesia	539	102.2
16534		Sudan	788	161.5
16541		Malawi	315	106.4
16542		S. Africa	396	107.8
16543		S. Africa	401	125.2
16545		S. Africa	217	113.9
16547		S. Africa	667	168.2
16549		S. Africa	738	194.9
16550		Indonesia	633	163.9
337535		Argentina	336	116.5
346440		U.S.	1127	305.8
347628		Ethiopia	701	162.8
364255		India	101	34.9
388005		Australia	263	88.2
388015		S. America	553	149.9
401553		Australia	228	49.9
451722		Mexico	738	161.2
532164		Oman	319	136.2
532166		Oman	449	146.0
543231		Bolivia	431	123.2
Standard error			52	12
Coefficient of variation (%)			59	51