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Vol. 14(16), pp. 1373-1378, 22 April, 2015
DOI: 10.5897/AJB2014.14216
Article Number: 416430C52402
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Selection of *Gossypium hirsutum* genotypes for interspecific introgression from *G. arboreum* using ovule culture

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Received 29 September, 2014; Accepted 7 April, 2015

Ovule culture is one of the techniques currently used to introgress desirable traits from *Gossypium arboreum* germplasm into *G. hirsutum* cultivars. Twenty-six (26) *G. hirsutum* breeding lines were used as female parents in crosses with five *G. arboreum* accessions to determine if the *G. hirsutum* parent influenced the germination and recovery of plants from ovule culture. Variation in boll weight and the number of ovules per boll was observed for crosses with the *G. hirsutum* lines, but heavier bolls and a greater number of ovules per boll were not associated with a higher germination rate. Ovules derived from crosses with 16 *G. hirsutum* lines showed germination. Plants were recovered for seven of these lines (Acala GLS, DES 56, DES 119, Deltapine 50, Stoneville 132, Stoneville 506 and Stoneville 825) with vigorous growing plants derived from four crosses (DES 119 x PI 408763, Stoneville 506 x PI 408763, Acala GLS x PI 529779, and DES 119 x PI 615699). The breeding line DES 119 showed a better success rate and typically produced smaller bolls with fewer ovules. However, results would suggest the *G. arboreum* accessions had a greater influence on the success rate compared to the *G. hirsutum* lines.

Key words: Cotton, germplasm, immature embryo, tissue culture, wide-hybridization.

INTRODUCTION

Tetraploid upland cotton, *Gossypium hirsutum* L., is comprised of over 90% of global cotton production (Zhao et al., 2015). Cultivated *G. hirsutum* genotypes are considered to have a narrow genetic base, due in part to a monophyletic origin and the use of few genotypes in the breeding of new cultivars (Grover et al., 2012; Zhao et al., 2015). In contrast, the diploid species *G. arboreum* L. comprises less than 1% of global cotton production, but is considered an important source of genetic diversity for several traits (Liu et al., 2006). *Gossypium arboreum* is commonly grown on marginal lands with low inputs and is a source of drought tolerance (Basu, 1996; Maqbool et al., 2007) and pest resistance (Yik and Birchfield, 1984; Thengane et al., 1986; Miyazaki et al., 2012). The ability to transfer genes from *G. arboreum* to *G. hirsutum* is hindered by incompatibility barriers resulting in abortion of developing bolls (Mehetre and Aher, 2004). Techniques such as exogenous hormone application

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(Altman, 1988), hexaploid bridging lines (Sacks and Robinson, 2009), *in vitro* interspecific fertilization (Liu et al., 1992), protoplast fusion (Sun et al., 2006), and ovule culture/embryo rescue (Stewart and Hsu, 1978; Gill and Bajaj, 1987) have been used for interspecific hybrid development in cotton. Ovule culture has frequently been used for hybrid development between incompatible diploid species and for crosses between diploid and tetraploid cotton species (Mehetre and Aher, 2004). Several factors can influence the success of ovule culture such as the species being crossed, age of the excised ovules, cultural media, and environmental conditions for culturing and plant growth (Stewart and Hsu, 1978; Mehetre and Aher, 2004). Research has mainly focused on improving the culture media to increase the success rate of germination and plant recovery (Stewart and Hsu, 1978; Thengane et al., 1986; Gill and Bajaj, 1987; Sacks, 2008). Information is lacking to determine if the *G. hirsutum* parent used for interspecific introgression has an influence on the success rate. Thus, the focus of this study was to evaluate 26 *G. hirsutum* breeding lines to determine if the tetraploid parent used in crosses with *G. arboreum* genotypes influenced the growth and germination of immature ovules and the recovery of plants from tissue culture.

MATERIALS AND METHODS

Twenty-six (26) diverse *G. hirsutum* breeding lines (Table 1) and five *G. arboreum* accessions (PI 152088, PI 408763, PI 529724, PI 529779, and PI 615699) were selected from the germplasm collection maintained at the United States Department of Agriculture (USDA) Agricultural Research Service (ARS), Crop Genetics Research Unit in Stoneville, Mississippi. These germplasm lines were planted in the field at the USDA ARS in Stoneville during the 2013 growing season. Each line was planted in a single 3 m row and rows were spaced 0.9 m apart. Standard management practices were conducted for cotton production in Mississippi. The *G. hirsutum* lines were used as the female parent with the *G. arboreum* accessions used as the pollen parent. Flowers of the *G. hirsutum* lines were emasculated by splitting the staminal column with the fingernail and removing the corolla and androecium (Doak, 1934) between 06:30 to 07:30 a.m. Following emasculation, flowers were misted with a single application of a 100 mg L^{-1} solution of gibberellic acid to prolong boll retention (Miravalle, 1964). Pollinations were conducted the same morning between 10:00 to 11:00 a.m. Each emasculated flower was pollinated using a single *G. arboreum* flower. Seven days after pollination, the developing bolls were removed for ovule culture. Bolls were weighed, surface sterilized in an aqueous 0.2 M sodium hypochlorite solution for 15 min., transferred to a 95% ethanol solution for another 15 min., and then air-dried in a biological safety cabinet prior to excision of ovules. The number of immature ovules from each boll was counted. The ovules were cultured on MS basal media (Murashige and Skoog, 1962) with B5 vitamins (Gamborg et al., 1968) and supplemented with 20 g L^{-1} sucrose, 1.9 g L^{-1} potassium nitrate, and 5 g L^{-1} gelrite as a solidifying agent (Sacks, 2008). All ovules from a single boll were cultured in the same 100 × 15 mm sterile plastic Petri plate. Ovules were cultured at 30°C in a growth chamber with a 16 h photoperiod. Ovules were transferred to fresh media every 21 days. Seedlings that developed from the ovules were transferred to magenta plant culture boxes containing the same media used for ovule culture and grown under the same conditions. Seedlings that developed roots and shoots were planted in pots containing potting media (Metro-Mix 360, Sun Gro Horticulture, Agawam, MA) and covered with a beaker for seven days to prevent desiccation of the developing seedlings. Pots were placed in a growth room at 27°C with a 16 h photoperiod. At flowering, plants were transferred to a greenhouse for further evaluation.

RESULTS AND DISCUSSION

Boll weight and the number of ovules per boll are presented in Table 1. More variation in mean boll weight and mean number of ovules per boll was observed across the *G. hirsutum* breeding lines as compared to the *G. arboreum* accessions. For the *G. hirsutum* breeding lines, the mean number of immature ovules cultured across the five *G. arboreum* accessions ranged from 30 to 42. The breeding line Sure-Grow 747 produced the most ovules across the *G. arboreum* accessions. Mean boll weight across the *G. arboreum* accessions was also greater for Sure-Grow 747 with the highest mean boll weight recorded for Acala GLS. However, the greater number of ovules per boll and higher boll weights did not result in the production of more plants from culture. The breeding line DES 119 showed the lowest mean number of ovules per boll and low mean boll weight, but produced more plants than breeding lines with a greater mean number of ovules per boll and higher boll weights. Across the *G. arboreum* accessions no noticeable trend was apparent. A mean of 37 immature ovules were cultured from the crosses with the *G. arboreum* accessions. Crosses involving accession PI 408763 produced a greater mean number of ovules and heavy bolls across the 26 *G. hirsutum* breeding lines with ovules derived from crosses with PI 408763 and PI 615699 showing a higher frequency of germination (Table 2). However, accession PI 529724, which had the lowest mean boll weight across the 26 breeding lines, produced a greater number of plants. Altman (1988) showed that exogenous hormone application increased boll weight and suggested that heavy bolls may correspond to better quality seed being produced; however, production of plants from *G. hirsutum* x *G. arboreum* crosses was unsuccessful. Flowers for the 26 *G. hirsutum* breeding lines pollinated with *G. arboreum* pollen and not sprayed with gibberellic acid aborted bolls within five days after pollination (data not shown). No comparisons were conducted between bolls treated with gibberellic acid and non-treated bolls to determine if the treatment increased boll weight. Rapid abortion of non-treated bolls was observed for crosses with several *G. hirsutum* lines and the ovules from these bolls failed to grown in culture. The number of ovules per boll was within the range observed for the treated bolls (data not shown); however, no additional data were collected due to poor boll development and ovule growth in culture.

All ovules from the crosses treated with gibberellic acid

Table 1. Boll weight (g) at 7 days after pollination and number of ovules per boll from crosses between 26 *Gossypium hirsutum* breeding lines used as the female parent and five *G. arboreum* accessions (PI 152088, PI 408763, PI 529724, PI 529779, and PI 615699) used as the pollen parent to evaluate the germination and recovery of plants from ovule culture.

Female	PI 152088		PI 408763		PI 529724		PI 529779		PI 615699		Boll weight		Ovules	
	Weight	Ovule	Weight	Ovule	Weight	Ovule	Weight	Ovule	Weight	Ovule	Mean	SD	Mean	SD
Acala GLS	no boll		5.21	40	5.12	40	4.74	37	5.49	39	5.14	0.31	39	1.4
Auburn 82 RNR	4.37	40	4.78	37	3.76	35	4.64	39	3.19	35	4.15	0.66	37	2.3
Auburn 56	4.09	34	2.07	33	1.72	34	1.90	34	4.23	41	2.80	1.24	35	3.3
Bayou 7769	3.52	36	3.39	42	3.05	35	2.61	28	3.92	35	3.30	0.49	35	5.0
Coker 100	3.56	35	0.90	42	1.52	33	2.58	39	2.18	35	2.15	1.02	37	3.6
Coker 312	3.81	38	3.07	46	3.31	36	2.95	35	2.22	45	3.07	0.58	40	5.1
Delcott 277	3.07	36	3.30	35	2.08	31	2.85	39	2.75	39	2.81	0.46	36	3.3
DES 24	3.04	41	3.91	44	3.56	36	3.20	41	4.09	36	3.56	0.45	40	3.5
DES 56	2.93	37	3.32	42	2.18	33	2.49	34	2.78	38	2.74	0.43	37	3.6
DES 119	1.13	27	3.74	34	2.83	35	1.12	20	3.17	35	2.40	1.20	30	6.6
Deltapine 15	2.97	34	4.80	45	3.84	39	3.93	43	4.42	45	3.99	0.69	41	4.7
Deltapine 16	3.57	45	4.24	44	3.06	39	2.62	40	3.94	36	3.48	0.66	41	3.7
Deltapine 50	2.98	32	2.81	37	1.91	36	2.89	34	2.04	31	2.52	0.51	34	2.5
Deltapine 90	2.79	43	3.04	35	2.05	35	2.44	36	3.08	46	2.68	0.43	39	5.1
Empire	5.54	39	6.29	45	3.36	41	3.85	44	4.22	37	4.65	1.22	41	3.3
LA 887	2.54	37	4.26	42	3.67	34	2.85	35	3.27	44	3.32	0.68	38	4.4
MD51ne	3.69	43	3.19	35	3.60	43	2.88	35	2.41	35	3.15	0.53	38	4.4
Sealand 1	3.85	43	2.65	41	2.45	36	5.05	36	4.76	35	3.75	1.19	38	3.6
Stoneville 2	2.40	35	2.06	37	2.40	36	1.50	37	2.14	32	2.10	0.37	35	2.1
Stoneville 132	1.12	25	3.61	38	1.74	30	2.24	39	1.78	38	2.10	0.93	34	6.2
Stoneville 506	2.10	27	3.27	36	3.23	37	3.27	33	3.31	32	3.04	0.52	33	3.9
Stoneville 825	3.21	35	2.84	35	3.46	42	2.76	32	3.57	39	3.17	0.36	37	3.9
Sure-Grow 125	6.17	39	4.53	43	3.82	33	3.70	36	4.21	44	4.48	1.00	39	4.6
Sure-Grow 501	2.48	31	3.85	37	2.62	34	2.91	32	3.13	35	3.00	0.54	34	2.4
Sure-Grow 747	5.04	37	6.03	45	4.58	41	4.89	44	5.02	44	5.11	0.55	42	3.3
STV7A	2.52	32	3.73	45	1.99	40	3.00	34	1.61	14	2.57	0.84	33	11.8
Mean	3.30	36	3.65	40	2.96	36	3.07	36	3.34	37				
SD[1]	1.18	5.2	1.19	4.2	0.92	3.4	0.99	5.0	1.04	6.4				

[1]SD = standard deviation.

showed growth in culture. Variation in ovule growth within and between crosses was observed. Some ovules showed more than a 10-fold increase in size. These ovules that showed greater growth in culture typically did not germinate and produce seedlings. Germination

Table 2. Number of ovules showing germination in tissue culture and the number of plants recovered from crosses between 26 *Gossypium hirsutum* breeding lines used as the female parent and five *G*. *arboreum* accessions (PI 152088, PI 408763, PI 529724, PI 529779, and PI 615699) used as the pollen parent. All plants derived from crosses with *G*. *arboreum* accession PI 529724 died at the flowering stage.

Female	PI 152088		PI 408763		PI 529724		PI 529779		PI 615699	
	Germination	Plant	Germination	Plant	Germination	Plant	Germination	Plant	Germination	Plant
Acala GLS	no boll	no boll	0	0	0	0	1	1	0	0
Auburn 82 RNR	0	0	0	0	0	0	1	0	2	0
Auburn 56	0	0	0	0	0	0	1	0	4	0
Bayou 7769	0	0	0	0	0	0	0	0	0	0
Coker 100	0	0	0	0	0	0	0	0	0	0
Coker 312	0	0	0	0	0	0	0	0	0	0
Delcott 277	0	0	0	0	0	0	0	0	1	0
DES 24	0	0	1	0	0	0	0	0	1	0
DES 56	0	0	0	0	1	1	0	0	0	0
DES 119	0	0	3	1	0	0	0	0	1	1
Deltapine 15	0	0	5	0	0	0	0	0	1	0
Deltapine 16	0	0	0	0	0	0	0	0	0	0
Deltapine 50	0	0	0	0	1	1	0	0	0	0
Deltapine 90	0	0	0	0	0	0	0	0	0	0
Empire	0	0	0	0	0	0	0	0	0	0
LA 887	0	0	1	0	0	0	0	0	1	0
MD51ne	0	0	0	0	0	0	0	0	1	0
Sealand 1	0	0	0	0	0	0	0	0	0	0
Stoneville 2	0	0	0	0	0	0	0	0	0	0
Stoneville 132	0	0	0	0	1	1	0	0	0	0
Stoneville 506	0	0	2	1	0	0	0	0	0	0
Stoneville 825	0	0	0	0	1	1	0	0	1	0
Sure-Grow 125	0	0	2	0	0	0	0	0	0	0
Sure-Grow 501	0	0	0	0	2	0	0	0	1	0
Sure-Grow 747	0	0	0	0	0	0	0	0	0	0
STV7A	0	0	0	0	0	0	0	0	0	0

varied among the ovules derived from the crosses (Table 2). Germination was low compared to results reported by Stewart and Hsu (1978), Gill and Bajaj (1987), and Sacks (2008), but similar to the results reported by Altman et al. (1987) and Altman (1988). Germination was not observed for ovules derived from 10 (Bayou 7769, Coker 100, Coker 312, Deltapine 16, Deltapine 90, Empire, Sealand 1, Stoneville 2, STV7A, and Sure-Grow 747) of the 26 breeding lines. Ovule germination was not observed from *G*. *hirsutum* crosses with more than two *G*. *arboretum* accessions. Variation

in ovule germination was also observed across the *G*. *arboreum* accessions. Ovules derived from *G*. *hirsutum* crosses with accessions PI 408763 and PI 615699 showed better germination and among a greater number of *G*. *hirsutum* lines. Germination was observed from crosses with *G*. *arboreum* accession PI 615699 and 10 of the breeding lines (Auburn 82 RNR, Auburn 56, Deltapine 15, Delcott 277, DES 24, DES 119, MD51ne, LA 887, Stoneville 825, and Sure-Grow 501). In comparison, germination was not observed for ovules derived from any of the crosses with PI 152088.

Many of the developing seedlings showed little or no additional growth when transferred to media in magenta boxes. Other seedlings showed abnormal growth and failed to survive when transplanted into potting media. Seedlings that germinated from ovules derived from crosses between accession PI 529724 and the breeding lines Deltapine 50, DES 56, Stoneville 132, and Stoneville 825, successfully developed into plants; however, when these plants produced flowers they started to wither and die. These plants failed to develop a vigorous root system to support the growth of the plants. Other researchers have reported abnormal seedling growth and poor plant survival (Thengane et al., 1986; Gill and Bajaj, 1987). In crosses involving three of five *G*. *arboreum* accessions (PI 408763, PI 615699, and PI 529779), healthy and vigorous plants have been recovered (Table 2). Plants have been recovered from crosses DES 119 x PI 408763, Stoneville 506 x PI 408763, Acala GLS x PI 529779, and DES 119 x PI 615699.

Germination was observed for cultured ovules derived from crosses with 16 *G*. *hirsutum* breeding lines and plants were obtained for seven of these lines (Acala GLS, DES 56, DES 119, Deltapine 50, Stoneville 132, Stoneville 506, and Stoneville 825). Ovules derived from crosses with DES 119 produced more plants hence this line would be a desirable selection. Other *G*. *hirsutum* lines to be considered include Acala GLS, Auburn 56, DES 24, DES 56, Deltapine 15, Deltapine 50, Sure-Grow 501, Stoneville 132, Stoneville 506, and Stoneville 825. No cultured ovules showed germination from crosses with Deltapine 90; however, four additional crosses between Deltapine 90, and accessions PI 408763 and PI 529724, did result in the recovery of a single plant for each accession. The plant recovered from the Deltapine 90 x PI 529724 crosses failed to survive as was observed for the other plants derived from crosses with this accession. Multiple interspecific crosses were also conducted with Sure-Grow 747, but no plants were recovered from these additional crosses. However, cultured ovules derived from crosses with Sure-Grow 747 and other *G*. *arboreum* accessions have resulted in the production of plants at a low frequency with the recovery of one plant from crosses with 24 accessions. Sure-Grow 747 is a source of superior fiber quality traits, but the unsuccessful recovery of plants for the majority of the interspecific crosses would suggest other *G*. *hirsutum* lines evaluated in this study would be more desirable for ovule culture. These results would indicate that evaluation of advance breeding lines or recently release *G*. *hirsutum* cultivars would be useful for the identification of superior lines for ovule culture. The use of improved cultivars would reduce the time required to recover desirable yield and fiber quality traits. However, the *G*. *arboreum* accession used in the cross may have a greater influence on the recovery rate and survival of plants compare to the *G*. *hirsutum* parent. Multiple factors such as tissue culture media, parental genotypes, age of immature ovules cultured, and environment conditions (Mehetre and Aher, 2004) can influence the germination for cultured ovules and the recovery of plants. In this study, germination rates were low and modification to the media could increase the recovery of plants. Several media formulations have been published, but similar results across laboratories have not been achieved (Altman, 1988; Sacks, 2008). Selection of a subset of *G*. *hirsutum* breeding lines and multiples crosses to the lines would increase the likelihood of generating plants from ovule culture. For some *G*. *arboreum* accessions, such as PI 529724, other approaches may be required to recover plants. Crosses with this accession and several breeding lines resulted in plants, but all plants failed to survive. Whereas, the success rate for other *G*. *arboreum* accessions, such as PI 615699, was greater with ovule germination observed from crosses with numerous *G*. *hirsutum* breeding lines. The successful recovery of plants from three *G*. *arboreum* accessions used in interspecific crosses provides breeding lines for further evaluation in the introgression of desirable traits for cotton improvement.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENT

This research was funded by the United States Department of Agriculture, Agricultural Research Service project 6402-22000-051-00D.

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