tion characteristics. However, 80-31-l (PPR 1) resulted from a cross-pollination of 'Grand Slam' (PPR 4) and 'Virginia' (PPR 3); this is not surprising in view of the heterozygosity of 'Virginia'. Progeny from self-pollinations of 80-31-l did not segregate into low and high PPR (Table 2). Since *P.* × *domesticum* may be a polyploid, a larger population of self-pollinated progeny would be needed to detect all possible segregates.

Postproduction quality can be used as a selection criterion in a breeding program since *P.* × *domesticum* segregates for petal retention. Few progeny with improved PPR were produced from either self- or cross-pollinations involving parents with low PPR. Four of the five breeding lines with consistently high postproduction ratings were self-pollinations of a parent with a PPR of 3; 'Virginia' was a parent of three of these breeding lines as well as ≈ 33% of all the breeding lines evaluated in this study. Another superior breeding line is 81-35-1, a hybrid that had one parent with a PPR of 3. The best approach to breeding *P.* × *domesticum* with improved postproduction characteristics is to use gentoypes with the highest petal retention.

Literature Cited

Armitage, A.M., R. Heins, S. Dean, and W. Carlson. 1980. Factors influencing flower petal abscission in the seed-propagated geranium. J. Amer. Soc. Hort. Sci. 105:562-564.

Benschop, M. and A.A. DeHertogh. 1969. An analysis of the post-harvest characteristics of cut tulips. Flor. Rev. 145 (3758):24-26, 62-65.

Conover, C.A., R.D. Heins, and G. Staby. 1981. Postproduction handling of container-grown plants. Flor. Rev. 168 (4364):20, 22.

Craig, R. 1982. Chromosomes, genes, and cultivar improvement. p. 380-410. In: J.W. Mastalerz and E.J. Holcomb (eds.). Geraniums. Pennsylvania Flower Growers, Univ. Park, Pa.

de Jong, J. 1979. Selection for keeping quality in gerbera, p. 263-267. In: L. Quagliotti and A. Baldi (eds.). Proc. Eucarpia Meeting Gen. and Breeding of Carnation and Gerbera, 24-28 Apr. 1978. presso la Minerva s.n.c. di Bollito, Torino, Italy.

Deneke, C.F. 1988. The post-harvest quality of *Pelargonium* × *domesticum* L.H. Bailey. PhD Diss., The Pennsylvania State Univ., Univ. Park.

Hanniford, G.C. and E.J. Holcomb. 1982. Regal geraniums, p. 161-169. In: J.W. Mastalerz and E.J. Holcomb (eds.). Geraniums. Pennsylvania Flower Growers, Univ. Park, Pa.

Harbaugh, B.K. and W.E. Waters, 1979. Evaluation of flowering potted plants under simulated home conditions. HortScience 14:743-745.

Sparnaaij, L.D. 1979. Current research on carnation with special reference to breeding, p. 47-55. In: L. Quagliotti and A. Baldi (eds.). Proc. Eucarpia Meeting Gen. and Breeding of Carnation and Gerbera, 24-28 Apr. 1978. presso la Minerva s.n.c. di Bollito, Torino, Italy.

van Eijk, J.P. and W. Eikelboom. 1976. Possibilities of selection for keeping quality in tulip breeding, Euphytica 25:353-359.

van Eijk, J.P. and W. Eikelboom. 1986. Aspects of breeding for keeping quality in *Tulipa*. Acta Hort. 181:237-243.

HortScience 27(1):57–58. 1992.

In Vitro Storage of Pineapple *(Ananas* spp.) Germplasm

Francis T. Zee[1] and Mark Munekata[2]

National Clonal Germplasm Repository, U. S. Department of Agriculture, Agricultural Research Service, P.O. Box 4487, Hilo, HI 96720

Additional index words. tissue culture, germplasm, medium-term storage

Abstract. Sterile distilled water was found to be an acceptable medium for the maintenance of tissue-cultured plantlets of three *Ananas* accessions. Eighty-one percent of the plantlets survived 12 months at 25C in 1 ml of sterile distilled water. Plantlets stored in water for 12 months were observed to be more vigorous than those that were cultured for 12 months in full-strength Murashige and Skoog (MS) medium. However, medium containing l/4-strength MS salts and full-strength organics, 3% sucrose, and agar gave the best plant survival and vigor. None of the plantlets in this study produced callus. Only a single instance of axillary budbreak was observed in explants stored on the l/4-strength MS medium.

Pineapple *[Ananas comosus* (L.) Merr.] is one of the 14 designated tropical fruit and nut crops maintained in the field gene bank at the National Clonal Germplasm Repository, Hilo (NCGR-Hilo), Hawaii, by the U.S. Dept. of Agriculture, Agricultural Research Service. In addition to field and greenhouse plantings, the 162 accessions of *Ananas* are maintained and distributed in vitro.

Frequent subculture of the in vitro collection onto new medium is laborious, costly, and uses valuable laboratory space. Recent studies on pineapple tissue culture have concentrated mainly on the development of rapid propagation methods (DeWald et al., 1988; Mathews and Rangan, 1979,1981; Mathews et al., 1976; Zepeda and Sagawa, 1981), and no efforts have been made to define a medium-term, low-maintenance in vitro storage protocol. Our goal was to develop a medium-term (minimum 12 months), low-input in vitro maintenance system through the manipulation of medium composition and culture environment.

This study was conducted using the *Ananas* accessions *A. comosus* (L.) Merr. cv. Ananas De Vaupes (HANA 51, P.I. 162769), *A. bracteatus* (Lindley) var. *rudis* (Bertoni) (HANA 73, P.I. 536877), and *A. comosus* cv. Philippine Green (HANA 129, P.I. 536949).

Axillary bud pieces (1 cm^3) were excised from mature greenhouse-grown plants and disinfected in 15% Clorox solution (5.25% sodium hypochlorite) and Tween-20 (two drops/100 ml) for 15 min followed by 10% Clorox + Tween 20 for 10 min. Pieces were then trimmed to 5 mm^3 in 5% Clorox and Tween and soaked for 1 h in 1% Clorox solution. Buds were rinsed in 1 ml of sterile water for 10 min and cultured on initiation medium (IM) containing full-strength Murashige and Skoog (MS) salts (Murashige and Skoog, 1962) plus (per liter) 100 mg *myo*-inositol, 2 mg glycine, 0.5 mg nicotinic acid, 0.5 mg pyridoxine·HCI, 0.4 mg thiamine·HCl, 2 mg 6-benzylaminopurine (BA), 2 mg naphthaleneacetic acid (NAA), 30 g sucrose, and 9 g Bacto-agar at pH 5.7 (DeWald et al., 1988; Zepeda and Sagawa, 1981).

Three weeks after culture initiation, buds that were green and free from microbial contamination were transferred to maintenance medium (MM) similar in composition to IM medium, but containing less salts (l/4 MS) and no growth regulators. Plantlets were grown on MM until the basal leaves were 4 cm long and then subcultured to liquid multiplication medium (MX). MX medium contained l/4 MS salts, full-strength organics, and 2 mg BA/liter. Lower leaves were removed from each plantlet to enhance budbreak in this medium. Liquid cultures were maintained in 14 x 150-mm test tubes on a rotary drum (30 cm in diameter) at 10 rpm.

Plantlets (1 cm long) from the multiplication medium were transferred to MM and allowed to grow until their basal leaves reached 7 to 9 cm. These plantlets were then harvested for the storage study.

Five storage treatments were compared using 14 x 150-mm disposable glass tubes as containers. Treatments included: plantlets stored in A) empty, sterilized tubes; B) 1 ml of sterile distilled water; C) 3.5 ml of a solid medium containing 1/2 MS salts, full-strength organics, 9 g agar/liter, and no sucrose; D) 3.5 ml of a solid medium containing full-strength MS salts, full-strength organics, 9 g agar/liter, and no sucrose; and E) 3.5 ml of a solid medium containing 1/4 MS, full-strength organics, 30 g sucrose/liter, and 9 g agar/liter. Treatment E is the same as MM currently used at the repository for the propagation and maintenance of 162 accessions of pineapple. Hana 73 was not included in Treatment E due to an insufficient number of explants at the initiation of the experi-

Received for publication 19 Apr. 1990. Accepted for publication 6 Aug. 1991. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Curator and Horticulturist.

[2]Research Assistant.

Table 1. Frequency distributions in plant vigor ratings of three *Ananas* accessions after 12 months in five storage treatments at 25C.

Accession	Rating of plants (no.)[z] Vigor	Moderate	Poor	Dead
Treatment A: empty sterile tube				
HANA 51	1	2	5	22
HANA 73	3	3	2	22
HANA 129	0	2	1	27
Treatment B: sterile distilled water				
HANA 51	12	2	12	4
HANA 73	8	8	6	8
HANA 129	10	9	6	5
Treatment C: 1/2 MS, organics				
HANA 51	14	3	10	3
HANA 73	28	2	0	0
HANA 129	15	5	7	3
Treatment D: MS, organics				
HANA 51	4	4	12	10
HANA 73	11	6	8	5
HANA 129	4	8	14	4
Treatment E: 1/4 MS, organics, 3% sucrose[y]				
HANA 51	23	7	0	0
HANA 129	26	4	0	0

[z]Total plants each line: 30.
[y]HANA 73 not tested.

Table 2. Mean vigor rating values for three *Ananas* accessions after 12 months of storage in five storage treatments at 25C.

Treatment	Accession HANA 51	HANA 73	HANA 129	Mean
	Rating			
A	1.4[z]	1.6	1.2	1.4 e[y]
B	2.7	2.5	2.8	2.7 c
C	2.9	3.9	3.1	3.3 b
D	2.1	2.8	2.4	2.4 d
E	3.8	---	3.9	3.9 a
Mean	2.5	2.7	2.7	

[z]Vigor ratings: 1 = dead, 2 = poor, 3 = moderate, 4 = vigorous.
[y]Mean separation within columns by Duncan's multiple range test, $P = 0.05$.

ment. Each treatment was replicated five times using six plantlets per replication. Culture tubes were arranged in a randomized complete block design. Cultures were maintained at 25C with 16 h light and 8 h darkness under white fluorescent lights. All plantlets were maintained in the same tubes for 12 months without subculture. Visual observations were made every 3 months. A point system was used to rate plant vigor after 12 months: 1 = dead, 2 = poor, 3 = moderate, and 4 = vigorous. The ratings were analyzed using the Statistical Analysis System (SAS).

No significant differences were observed between the two accessions of *A. comosus* (HANA 51, 129) in their growth responses within each storage treatment. All plantlets stored in empty sterile glass tubes (Treatment A) remained viable for at least 60 days and then gradually dehydrated, with lower leaves becoming chlorotic. An average of eight plantlets (27%) from each accession survived for 9 months, and a total of 11 plantlets (18%) from the two *A. comosus* accessions survived 12 months (Table 1), with low average vigor ratings. Hana 73 *(A. bracteatus)* appeared to survive desiccation better than pineapple. Eight plantlets (27%) survived 12 months but had low vigor (Table 2). After 12 months, most of the leaves on all the surviving plantlets were dried and grayish-brown with only a whorl of three to four small green leaves remaining in the center. Attempts to rejuvenate these plantlets using MX medium were moderately successful, resulting in ≈ 50% recovery.

The addition of 1 ml of sterile distilled water to each tube (Treatment B) prolonged the storage life of pineapple plantlets to 9 months with only 7% mortality. At the end of 12 months, 51 of the 60 plantlets (85%) from the two pineapple accessions and 22 of the 30 (73%) HANA 73 *(A. bracteatus)* plantlets survived. The vigor rating of HANA 73 was slightly lower than that of the pineapples (Table 2).

All plantlets from HANA 51 and HANA 129 stored exceptionally well on Treatment E (Table 1). Although no HANA 73 plantlets received Treatment E, plantlets of this accession maintained on MM were as vigorous as the two pineapple accessions. One HANA 51 plantlet in this medium developed a single side shoot. Plantlets of *A. bracteatus* cultured on Treatment C were vigorous and appeared to grow more rapidly than the two pineapple accessions on this medium.

The high salt concentration in Treatment D was detrimental for the medium-term, low-maintenance culture of pineapples. Although the survival rate of plantlets was similar to those in sterile water after 1 year, the mean vigor rating was significantly lower than for Treatments C and E (Table 2). Plantlets of *A. bracteutus* had slightly higher survival rates and vigor ratings than the two *A. comosus* accessions (Tables 1 and 2).

Available moisture was a critical factor for maintenance of pineapple in vitro. The addition of sterile distilled water satisfied this criterion as a short-term (6 months) storage medium. However, for medium-term storage, there was a significant loss of plant vigor. Also, sterile water was effective only if plantlets were >7 cm long. Plantlet survival rate and vigor were poor when smaller plantlets (2 to 4 cm long) were used (unpublished observations). Treatment E (l/4-strength salts, sucrose, and organics) appeared to be more effective than Treatments C and D for medium-term, low-maintenance storage of pineapple. In addition to the higher survival rate and vigor rating of plantlets, Treatment C also supported the growth of smaller plantlets (24 cm) (unpublished data).

As a result of this study, a revised maintenance and distribution protocol for tissue-cultured pineapple was adopted at the National Clonal Germplasm Repository in Hilo. Axillary buds from each accession are now cultured and grown on the IM medium. After 4 weeks, six healthy plantlets are transferred into 3.5 ml of l/4-strength MS (Treatment E) maintenance medium in glass tubes and stored without further subculture for 12 months. Three additional plantlets are cultured and maintained in the same conditions, but are used only for multiplication and distribution. To ensure the stability of the germplasm, the distribution stocks will be renewed yearly from greenhouse plants or from slow-growth materials in storage. In terms of savings in labor and storage space, this new procedure is an improvement over our original protocol of keeping three 250-ml flasks (75 ml of medium, five plantlets per flask) per accession for all functions (storage, multiplication, and distribution).

Tissue-cultured pineapple plantlets (4 to 9 cm long) are being distributed in empty sterile disposable glass tubes after they have been rinsed in sterile water. This method reduces the shipping weight as well as the incidence of en route contamination. Plantlets can be planted immediately after arrival at their destination without further treatment. This new protocol produced significant savings in space and labor at the NCGR-Hilo operation.

Literature Cited

DeWald, M.G., G.A. Moore, W.B. Sherman, and M.H. Evans. 1988. Production of pineapple plants in vitro. Plant Cell Rpt. 7:535-537.

Mathews, V.H., T.S. Rangan, and S. Narayanaswamy. 1976. Micro-propagation of *Ananas sativus* in vitro. Z. Pflanzenphysiol. Bd. 79. S. 450-454.

Mathews, V.H. and T.S. Rangan. 1979. Multiple plantlets in lateral bud and leaf explant in vitro cultures of pineapple. Scientia Hort. 11:319-328.

Mathews, V.H. and T.S. Rangan. 1981. Growth and regeneration of plantlets in callus culture of pineapple. Scientia Hort. 14:227-234.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Zepeda, C. and Y. Sagawa. 1981. In vitro propagation of pineapple. HortScience 16:495.