VOL. 8, NO. 4, APRIL 2013 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

CONSERVATION OF BANANA cv. AMBON KUNING ON VARIOUS MEDIA IN VITRO FORMULA

Sunyoto Makful dan Nofiarli
Indonesian Tropical Fruit Research Institute, Jl.Raya Solok, Aripan Solok, West Sumatera, Indonesia
E-Mail: ali_swarna@yahoo.co.id

ABSTRACT

Genetic resources should be sustained to support the genetic improvement in the future. One of the efficient techniques to conserve the banana germplasm collection is the *in vitro* culture using the modification of MS media (Murashige and Skoog). Formulas created by modifying the concentration of salt and sucrose (GS) in the MS media. The research objective is to obtain storage media of banana's explants on the in vitro. The Research has done in tissue culture, breeding and germplasm laboratory Indonesian Tropical Fruits Research Institute, from July 2004 to March 2005. Research was used completely randomized design with 7 media storage treatments such as GS0 = MS + sucrose 3% (control medium) that always used for the *in vitro* of banana multiplication and GS1 = ½ MS + sucrose 3%, GS2 = ½ MS + sucrose 6%, GS3 = ½ MS + sucrose 9%, GS4= ¼ MS + sucrose 3%, GS5 = ¼ MS + sucrose 6%, GS6 = ¼ MS + sucrose 9%. The experiment results indicated that modification of salt and sucrose in the MS media were can inhibit the vegetative plantlet growth, so it possible to used for conservation of banana genetic resources. GS2 that contains ½ MS of salt + 6% sucrose was potential for storage of the ambon bananas clones on the *in vitro*. Planlets that come from GS2 storage media were having the smallest inhibition during acclimatization (in vivo).

Keywords: banana, *in vitro*, conservation, breeding, germplasm.

INTRODUCTION

Banana production has many losses caused by various plant diseases, for example, *Fusarium* wilt and bacterial wilt. Bacterial wilt disease was caused by *Pseudomonas celebensis*; it was first reported 85 years ago. It was found in Selayar Islands, Sulawesi. Similar diseases was caused by *P. solanacearum* that widespread in Java and some other areas that can reduce banana yield between 27-36% and in 1998 the greater decrease in yield between 67.5 to 85.5% (Hermanto *et al*., 1998). The rate of fusarium wilt attack (*Fusarium oxysporum f.* sp. *Cubense*) was larger than bacterial wilt. According to the Plant Quarantine Centre (1983) Fusarium wilt has invaded banana plants in almost every province in Indonesia, except for East Nusa Tenggara and Papua.

In order to save the existence of national commercial banana clones from extinction, so the conservation action needs to be done. Generally, banana clones conservation was performed in vitro under optimum environmental conditions. Plants are grouped based on genetic similarity, and then cultivate in intensive culture by applying standard techniques. The main obstacle in the banana clones conservation on the in vivo is need vast land and very expensive maintenance operational costs (Grout W.V., 1991).

Alternative ways for banana clones conservation are more secure and efficient need to be investigated. Since most commercial banana plants propagated in vegetative method, then the in vitro conservation can be recommended the application by using selective media (Bonnier *et al*., 1992). The in vitro conservation proved to be very efficient to use for maintenance the genetic resources to various high value of fruit crops, because it does not require a large place (Bonnier and van Tuyl, 1996). Meanwhile, in vitro conservation can reduce the risk damage by pests and diseases and facilitate the monitoring and management (Bonnier *et al*., 1996).

The basic principle on the *in vitro* maintenance of genetic resources is to slow the plantlets growth. This can be done by storing the in vitro culture of banana clones under low temperature (Grout, 1991). Storage techniques culture under low temperatures has been successfully used for the conservation of various types of commercial crops, including bananas (Bouman and de Klerk, 1990). Cryopreservation techniques have been applied to conservation the genetic plant resources by various research institutions and private companies in abroad. To apply the cryopreservation techniques required the cold storage temperature facilities that are modern and expensive. That facility is certainly not owned by the each laboratory in Indonesia. On the other hand the using low temperature is not always beneficial, because it can damage the tissue plantlets (chilling injury), especially on the kind of vulnerable fruit crops. This is made clear by Matsumoto *et al*. (1995) that plants response to low temperature is determined by genetic factors. Some genotypes fruit crops there is one has a wide tolerance range to low temperatures, while other fruit crops genotypes require environmental temperature with a very narrow range. For research institutions that do not have sophisticated cold storage facilities, it is necessary to attempt to apply other in vitro method conservation that accordance with their facilities.

The growth inhibition in the in vitro media can do include by providing osmotic stress and suboptimal nutrient at plantlets (Grout, 1991; Engelman, 1991). It has been successfully applied to the In vitro storage of lily germplasm. The combination of ¼ MS concentration and 9% sucrose real inhibit shoot growth of *Lilium longiflorum* and *L. henyi* without pressing the plantlets viability when

acclimatization stage. *L. plantlets longiflorum* and *L. henryi* are able to survive in that media during 28 months (Bonnier and van Tuyl, 1997).

The research objective is to obtain storage media of banana's explants on the in vitro by modifying the salt concentration and sucrose in MS medium.

MATERIALS AND METHODS

The research was conducted in tissue cultures laboratory, Breeding and Germplasm Indonesian Tropical Fruit Research Institute from July 2004 until March 2005.

This research used completely randomized design with 7 media storage treatments, each treatment includes 3 replicates and each replicate includes 25 bottles (1 bottle consist of 5 plantlets). The treatments are:

(1) GS0 = MS + 30 g sukrosa/l
(2) GS1 = ½ MS + 30 g sukrosa/l
(3) GS2 =½ MS + 60 g sukrosa/l
(4) GS3 = ½ MS + 90 g sukrosa/l
(5) GS4 = ¼ MS + 30 g sukrosa/l
(6) GS5 = ¼ MS + 60 g sukrosa/l
(7) GS6 = ¼ MS + 90 g sukrosa/l

Explants materials are buds of banana cv. Ambon Kuning from the in vitro culture with medium base MS + 30 g sucrose/l. MS medium was prepared by mixing macro elements, micro, and vitamins are adjusted with specified treatment. On MS media solution was given the sucrose that adjusted for treatment has been determined. Agar (merck) as much as 7 g / l and IAA 0.1% and BAP 0.2% was added to media before the entire mix media sterilized in autoclave. Banana shoots from the in vitro culture were separated one by one and flat cut approximately 1 cm in length. Five shoots were cultured in bottle that containing the medium in vitro that had been treated. In this research provided two bottles per replication (10 plantlets) for destructive observation at 1 time a month.

Explants observations conducted at 1 times a month i.e., at the explants age 1, 2, 3, 4, and 5 months after storage. Incubation temperature is 16 - 18°C.

RESULTS AND DISCUSSIONS

Bud initiation

Bud initiation of Ambon Kuning clones within the *in vitro* media occurs at the age of 15-21 days depends on the type of culture media (Table-1).

Based on the average value of GS2 and GS3 appear more quickly when compared with shoots in other media, including controls. Meanwhile, shoot initiation in GS2 occurs at the same time as the shoots initiation in control media. The differences in sucrose content caused the differences in the initiation of shoot speed between plantlets in GS2 and GS3 with controls media. GS2 and GS3 contain more 2-3 times sucrose than the media control thus providing greater source energy to support the shoots morphogenesis per plantlet. According to Sachs (1965) a lot of energy need when corpus cells inside of the meristem divide and enlarged to prepare the new tissue as the initial formation of leaf primordia. Beside energy, shoot formation requires the supply of macro nutrients, micro and vitamin. Availability of the three elements in the optimal amount can accelerate shoots initiation (Stange, 1965). This is evidenced from the shoots appearance was earlier in the GS2 and GS3 when compared with shoots appearing in GS1 and GS4. The content of macro elements, micro, and vitamins in GS2 and GS3 are 2 times larger than GS4, GS5 and GS6.

The research results indicate that the modification of salt and sucrose in MS medium significantly affect to shoots initiation. Increasing concentrations of sucrose can accelerate shoots initiation, whereas the decreasing concentrations of nutrients and vitamins in MS medium can be slowing the shoots appearance.

Table-1. When did the emerging shoots and banana's shoots number on the media initiation after one month cultured.

Initiation medium	Buds appear (day)	Buds number
GSO	15,50^{ns}	1,50^{ns}
GS1	18,75	1,00
GS2	15,00	1,25
GS3	15,25	1,50
GS4	21,00	1,00
GS5	20,50	1,00
GS6	19,25	1,00

ns: Numbers in the same column are not significantly different at 5% level

Plantlets growth

Based on the statistical analysis showed that plant height, leaves number, leaves length, root length, and roots number at each observation time was significantly influenced by the type of culture medium in vitro.

The plantlets growth at 4 weeks after the culture was slow. This is caused by limitations number of the availability of vegetative organs at each plantlet, so plantlets unable to use the available nutrients in the media at its optimum. The Most nutrients obtained through the cations diffusion and anions to the surface of the stem plantlets that touch media. With the growing number and root length and leaves number and leaves size, the plantlets growth more rapid in the period 5-10 weeks after culture. The increasing plant height, leaf number, and plantlets root length on control are highest compared to increasing variable observation in other media. Meanwhile, an increasing leaf length and roots number were highest in plantlets which cultured in the GS2 in observation period of 9-12 weeks after culture, the growth of plant height and root length that the slowest found in the plantlets in GS6 (Figure-1). Results analysis to

significance average of the treatment value on each variable within the observation period 2-5 months after culture are presented in Table-2 through Table-5.

Three months after culture are starting to look inhibition of plantlets growth that caused by the using of storage media. This is evident from the significant differences of height and leaves number of plantlets among all storage media and control media. The average of roots length on the storage media is also markedly lower than the control. Nevertheless the roots plantlet number at GS2 and GS3 on statistically analysis was not significantly different compared to control media. The shortest roots found in plantlets in GS6. Storage media was not produce a significant effect on the roots number. There is a tendency that the using of GS2 storage media stimulates the roots appearance that is more productive than the control. Nevertheless the number of roots plantlet at GS2 and GS3 on statistical analysis was not significant different compared to control. Four months after culture, the size of plantlet leaves in GS3 and GS6 were not increased. The leaves seem remain small and dark green. This is thought to be caused by the osmotic stress influence that caused by the sucrose using with the higher concentrations than GS0. According to Levitt (1972), plant visual symptoms that it was experiencing osmotic stress such as inhibition of leaf size growth. And next Levitt (1972), stated that in the plants leaf tissue that has the osmotic stress experiencing there is carbohydrates accumulation which cause dark green leaves. The plantlets leaf number on GS3 and GS6 tend to shrink. This is caused by the decay of bottom leaves after senescence.

Table-2. Plant height, leaves number, leaves length, root length, and root number of banana plantlets for 2 months after cultured in various storage media.

Conservation media	Plant height (cm)	Leaves number	Leaves length (cm)	Root length (cm)	Root number
GSO	2.70b	2.80b	2,30ab	2, 40b	3, 60b
GS1	2, 30a	2.00a	2,40ab	2, 00a	2, 80b
GS2	2, 50b	2.60b	2,70b	2, 50b	3, 80b
GS3	2, 00a	2.33a	2,40ab	2, 00a	2, 3a
GS4	2, 40b	2.20a	2,10a	2, 00a	2, 00a
GS5	2, 30a	2.30a	2,10a	2, 10a	2, 70b
GS6	2, 20a	2.00a	2,00a	2, 00a	2, 10a

* Means in the same column followed by the same letter are not significant different at 5% HSD test

Table-3. Plant height, leaves number, leaves length, root length, and root number of banana plantlets for 3 months after cultured in various storage media.

Conservation media	Plant height (cm)	Leaves number	Leaves length (cm)	Root length (cm)	Root number
GSO	6, 00b	6.00b	4, 60a	5, 62b	7, 40a
GS1	5, 30a	5.00a	4.40a	5, 42b	6, 80a
GS2	5, 40a	6.10b	4, 50a	5, 55b	7, 70a
GS3	5, 10a	5.20a	4, 00a	4, 40a	7, 70a
GS4	5, 00a	5.00a	4, 10a	7, 00c	7, 40a
GS5	5, 00a	5.00a	4, 20a	8, 60c	7, 60a
GS6	5, 10a	5, 00a	4, 10a	4, 10a	7, 50a

* Means in the same column followed by the same letter are not significant different at 5% HSD test

Among the seven *in vitro* media that used, GS6 has greatest influence on the plant inhibition (Figure-3). In the time period 4-5 months after planting still looks plantlets growth. The plantlets growth rate in a period of time varies with the type of media in vitro. Plantlets height in GS2 was rise faster than plantlets height in control or any other medium. But if done comparisons between plantlets at the observation time 5 months after culture, it appears that the plantlets in control media was relatively higher than the plantlets in GS2. Plantlets height in GS3 is not increase, whereas GS1 and GS2 increased respectively to 5.40 cm and 5.86 cm. The leaves number generally

increased, except in the plantlets in GS6 tend to decreased. Decreasing the leaves number in the GS6 due to the senescence was occurring faster than the leaf senescence in other media. The largest increasing in the leaves number found in the control media that followed by an increase in the leaves number in the GS2. The plantlets roots number generally fixed while the roots length generally increased until the 5th months observation period after cultured. The plantlets roots length in GS0 grow most rapidly, although in the end of the average value of the root length in GS0 still lower when compared with roots length in the GS2. Mean of plantlets leaf length at the observation time 5 months after culture was lower than observations at 3 months after culture. This happens because the leaves on the observation time were a new leaf that has a smaller size than the existing leaves in previous observation. Meanwhile, the leaves that have been old generally die, so it cannot be observed.

Table-4. Plant height, leaves number, leaves length, root length, and root number of banana plantlets for 4 months after cultured in various storage media.

Conservation media	Plant height (cm)	Leaves number	Leaves length (cm)	Root length (cm)	Root number
GSO	6.10b	7.10b	4, 90b	9, 92b	8, 40bc
GS1	5.30a	6, 00ab	4.50ab	8, 42ab	7, 80b
GS2	5.86b	6.70b	5, 20b	10, 55b	10, 0c
GS3	5.10a	5.40a	4, 10a	7, 10a	7, 70b
GS4	5.14a	5.20a	4, 70ab	9, 60b	8, 40bc
GS5	5.22a	5.20a	4, 40ab	9, 70b	8, 60bc
GS6	5, 20a	5.33a	4, 10a	7, 00a	6, 10a

* Means in the same column followed by the same letter are not significant different at 5% HSD test

Table-5. Plant height, leaves number, leaves length, root length, and root number of banana plantlets for 5 months after cultured in various storage media.

Conservation media	Plant height (cm)	Leaves number	Leaves length (cm)	Root length (cm)	Root number
GSO	6, 30b	7.40b	4, 90b	14, 30c	8, 40bc
GS1	5.40a	6, 10ab	4.50ab	10, 56b	7, 80b
GS2	5.9ab	6.94b	5, 20b	11, 86b	10, 0c
GS3	5.10a	5.10ab	4, 10a	9, 28b	7, 70b
GS4	5.34a	5.10ab	4, 70ab	11.21b	8, 40bc
GS5	5.81ab	5.10ab	4, 40ab	10, 88b	8, 60bc
GS6	5, 60a	4.13a	4, 10a	7, 87a	6, 10a

* Means in the same column followed by the same letter are not significant different at 5% HSD test

Based on the appearance of vegetative plantlets can be stated that the storage of in vitro banana clones that using GS2 better than the in vitro storage on other media (Figure-4). The plantlets growth in GS2 inhibited, but did not find symptoms of serious morphological transformations on vegetative organs, such as occurs in plantlets in GS3 and GS6 are characterized by shrinking the size of leaf area, branching appearance and hardening stem. The plantlets growth in ¼ MS media apparently decreased during the 3 months period after culture, probably caused by deficiency of the plantlets element (macro, micro, and vitamins) that were necessary for metabolic activity. Salisbury and Ross (1985), states that nutrient supplies under optimal can disrupt metabolic processes in plants that eventually suppress the plantlets growth. Disturbance metabolism may be decreased protein synthesis, inhibition of enzymatic activity due to the scarcity of the cofactors number, decreased synthesis of phosphorus compounds that has rich energy (ATP), and the suppression of hormonal activity in the body plantlets (Alexander and Mc Anulty, 1982). Inhibition of physiological reactions by reduction effect in salt concentration to be a ¼ MS that proved in lily plantlets that ¼ MS treatment stimulates root formation. This indicates that these treatments alter the physiological

reactions that lead to the dormancy induction (Bonnier and van Tuyl, 1997).

Increasing sucrose concentrations up to 9% also suppress the plantlets growth. This is caused by increased osmotic pressure media which is the source of environmental stress for plantlets. The abundance of sucrose content in the medium led to increase in excessive sucrose absorption by plant. Gradually the osmotic potential in the cell fluid becomes negative that disrupts the metabolic processes (Hanson and Wiliam, 1982). According to Salisbury and Ross (1985), that is the stress caused by changes in osmotic pressure will stimulate the ABA accumulation (abscisic acid) in the plant tissue, which in turn inhibits the plant growth in the in vitro media. Besides ABA accumulation, there is also inhibition of the cytokines synthesis that effect amplify the inhibition growth that caused by the ABA effect (Morgan 1984) (Figure-5).

The plantlets growth in acclimatization stage

After 5 months of storage period, each plantlet acclimatized in a greenhouse on rice husk, sand and manure. Three weeks after removal from the culture bottle, plant height to all the seedlings that came from plantlets observed.

The measurement results showed that the plants from the GS0 grow faster than plants from other media. Among the plants from storage media there are differences in the growth speed. The highest plants are plantlets from the GS2. This relates to the state of the current crop removal from the culture bottles. At the time, the plantlets from the culture bottles GS2 was highest compared with plantlets from another culture bottles. However, if observed the growth acceleration, it appears that the plants from GS2 were growing fastest and more resilient in the acclimatization media. Meanwhile, plant that comes from GS5 and GS6 grow slowly (Figure-6). This is presumably because these plants are still adapted to stressful conditions during the storage period. Stress on media GS5 and GS6, it can be restored in stages, as evidenced by the plantlets growth after transfer to greenhouse.

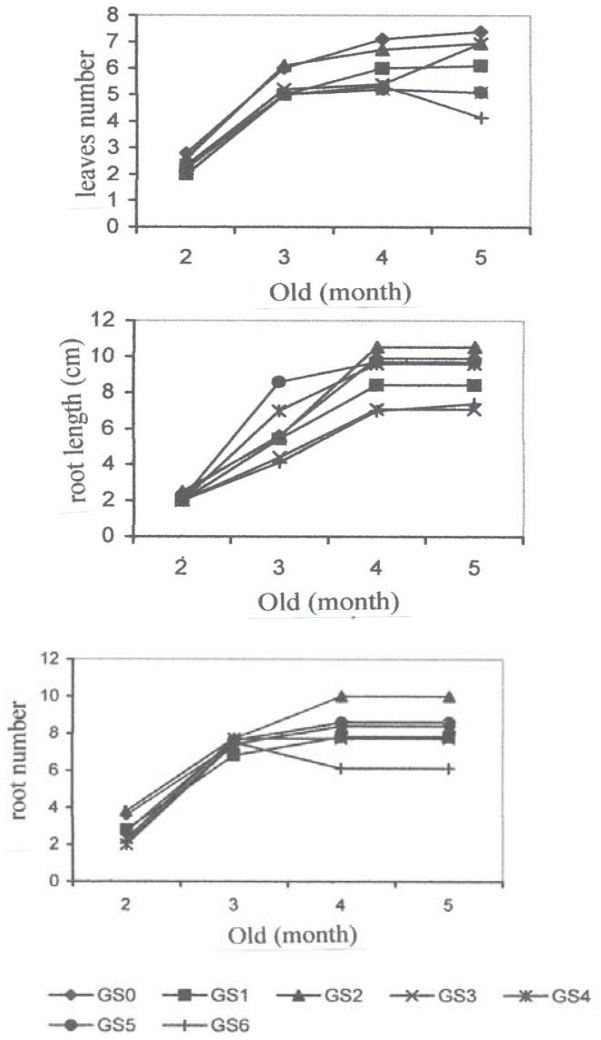

Figure-1. Plant height, leaves number, leaves length, root length, and root number of banana plantlets in various storage media formula.

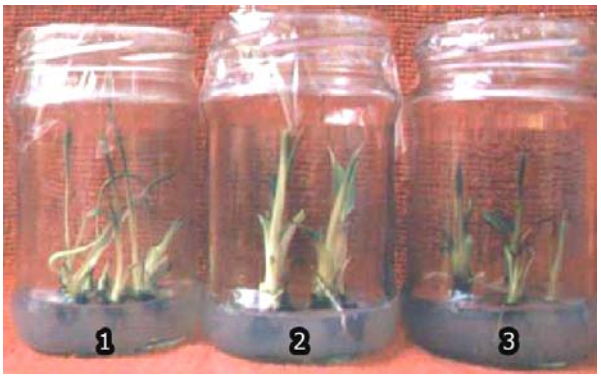

Figure-2. Increased plant height, leaves number, and root length of planlet in GS0 control media (1) better than GS2 (2) and GS1 (3), 2 months after culture.

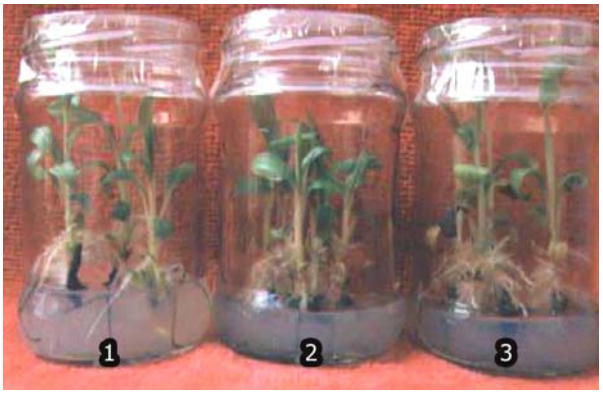

Figure-3. Growth Inhibition on GS6 (2) compare with GS2 (3) or GS0 as control media (1), 4 months after culture.

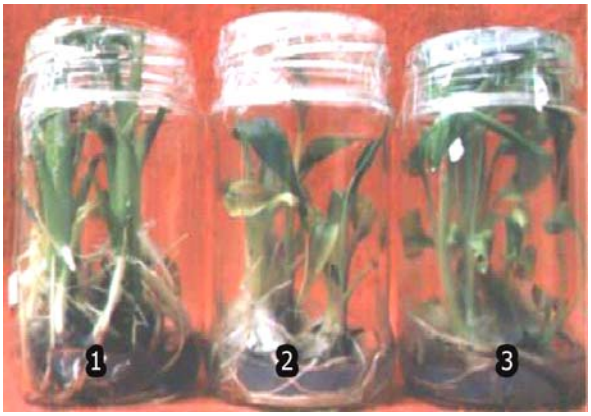

Figure-4. Planlets from GS2 (3) are better than GS1 (2) or GS0 as control media (1), 5 months after culture).

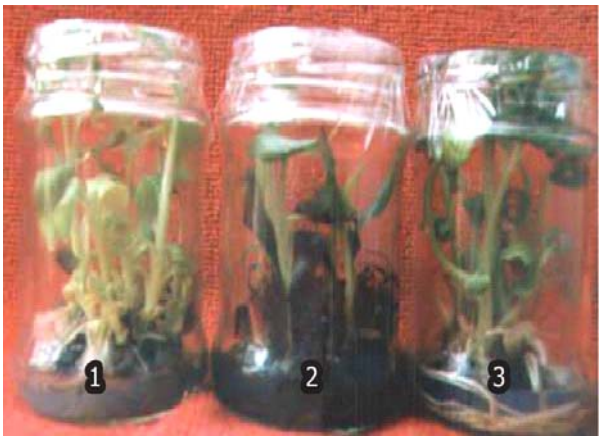

Figure-5. Planlets from GS0 (1) and GS6 (2) has senescence compared with GS2 treatment media (3), 5 months after culture.

Figure-6. Planlet from GS2 treatment (3), the most rapidly growing than GS5 (2) and GS6 (1).

CONCLUSIONS

Based on the research results, it can be concluded that the salt modification and sucrose on MS medium in vitro can slow the explants growth, so it was appropriate for the purposes of banana germplasm storage.

The formula of GS2 = ½ MS + 6% sucrose is the better formula storage medium for banana explants in vitro than the most other storage media formula.

Plantlets from the treatment of GS2 formula = ½ MS + 6% sucrose is the lowest plantlets that had growth inhibition when compared to other treatments acclimatized (*in vivo*).

ADVICE

Assessment of further research needs to be done to get the maximum storage time that did not affect the growth of acclimatization time (*in vivo*).

REFERENCES

Alexander G.V. and L.T. Mc. Nulty. 1993. Multielement analysis of plant-related tissue and fluids by optical emission spectrometry. J. of plant Nutrition. 3: 51-59.

Bonnier F.J.M, R. Jansen and J.M. van Tuyl. 1996. Long term lily scale bullet storage: Effects of temperature and storage in polyethylene bags. Ann. App. Biol. 129: 161-169.

Bouman H. and G.J de Klerk. 1990. Cryopreservation of lily meristems. Acta Hort. 266: 331-337.

Bouner F.J.M. and J.M. van Tuyl 1997. Long term in vitro storage of lily: Effects of temperature and concentration of nutrients and sucrose. Plant Cell, Tissue and Organ Culture. 49: 81-87.

Bounnier F.J.M. and J.M. van Tuyl. 1996. Freezing of vegetative germplasm of lily for 0 to 4 years. Acta Horticulture. 325: 643-658.

Engelman F. 1991. In vitro conservation of tropical plant germplasm-a review. Euphytica. 57: 227-243.

Grout W.V. 1991. Conservation in vitro. Acta Horticulture. 189: 171-178.

Hanson A.D. and D.H. William. 1982. Metabolic responses of mesophytes to plant water deficits. Annual Review of Plant Physiology. 33: 163-203.

Hermanto C., T. Setyawati dan and P.J. Santoso. 1998. Konfirmasi: Daerah endemik baru penyakit layu bakteri pisang di Sumatera Barat. Disampaikan pada Seminar Sehari PFI Komca Sumbar, Riau dan Jambi, Padang, 4 November, p. 11.

Levitt J. 1972. Responses of plants to environmental stresses. Academic Press, New York, USA. pp. 101-120.

Matsumo T., A. Sakai and K. Yamada. 1995. Cryopreservation of in vitro-grown apical meristems of lily by vitro-grown apical meristems of lily by vitrification. Plant Cell, Tissue and Organ Culture. 41: 237-241.

Morgan J.M. 1984. Osmoregulation and water stress in higher plant. Ann. Rev. Plant Physiol. 35: 299-348.

Rao D.G. 1981. Short note on bunchy top disease of Banana Indian Inst. of Hort. Res. Bangalore, India. pp. 142-143.

Sachs R.M. 1965. Stem elongation. Ann. Rev. Plant Physiol. 16: 53-72.

Salisbury F.B. and C.W. Ross. 1985. Plant physiology. Wadsworth Publishing Company. Belmont, California. A Division of Wadsworth, Inc. p. 540.

Stange L. 1965. Plant cell differentiation. Ann. Rev. Plant Physiol. 16: 119-140.