THE UNIVERSITY OF ZAMBIA

ANTHER CULTURE OF FINGER MILLET (ELEUSINE CORACANA)

BY CHILUBA BOWA

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SUPERVISOR: DR. K. MUNYINDA

Abstract

A study was conducted at the University of Zambia tissue culture laboratory, School of Agricultural Science (Crop Science Department) from March to September 2002. The objectives of the study was to establish which growth media was most suitable for anther culture of Nyika variety of finger millet. The other objective was to establish the optimal number of days of pre-shock treatment which gives the highest response. Anther response was the proportion of microspores that survived and developed from the inoculated anthers. These grew into muticellular filamentous structures with cells actively dividing with less the diploid number of chromosomes.

The importance of the study is that anther culture provides a method for the production of homozygous lines over the course of a few months, rather than the several generations required using conventional whole plant techniques .The resulting haploid plants are homozygous and breed true.

Three different media namely N6 ,NN and MS and pre shock treatment of duration 0, 4 ,8 and 12 days were evaluated for anther culture response. Anther response varied with pre shock treatment. The combination of N6 and 4 day pre shock treatment gave the highest response .

The results are indicative of the possibility of growing finger millet from pollen following further improvement in pollen culture conditions. The resultant homozygous plants with desirable traits could be selected as elite lines or used for further crop improvement to produce superior finger millet varieties.

Dedication

I dedicate this work to the memory of my late father ***Mr. Dancewell Bowa,*** my mother for her support, love and encouragement throughout my life, my brothers and sisters for being there for me. Friends and relatives who stood by me through the years and supported and believed in me. Lastly, but not the least, I dedicated this work to my lovely daughter, Mwape.

Acknowledgements

I would like to express my indebtedness to my supervisor Dr. Munyinda for his sound and useful advise and suggestions. I would also like to thank him for all his financial support through the course of the project. I would like to thank him for making this project materialize by keeping me on my toes with his meticulous attention to the slightest detail. He provided guidance, wisdom and vision to completion of the project.

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I express my gratitude to Mr. Chipampe, the Chief Technician; Miss Olga Kamanga and her Lab Assistant Mr. Japhet Phiri.

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Lastly, but certainly not the least, I thank God Almighty for his many blessings and guidance.

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Chapter 1

1. Introduction

Haploid plants can be produced through *in vitro* culture of male gametophytic Cells at the microspore or immature pollen developmental stage. These cells respond *in vitro* by undergoing embryo genesis or haploid callus proliferation.

Plants can be regenerated through shoot and root induction in haploid callus and through culture of haploid embryo's. It is possible to produce homozygous doubled haploids, pure breeding lines through chromosome doubling treatments applied to haploid cells (Barnubas et.a.l. 1991) or plants (Siebel and Pauls, 1989).

Guha and Musheshuari (1964) were the first to produce plants in Datura innoxia through the culture of anthers containing immature pollen. Since then, haploid plant production has been reported in more than 200 species (Dunwell 1986).

Microspore culture has a number of practical uses (Dunwell, 1985, Keller et.al 19870), Kasha et al. 1990). The main advantage is the reduction in time to develop new variety, whereas the use of microspore culture can reduce this by 3 – 4 years (Ulriech et.al. 1984).

Secondly, one can rapidly fix traits in the homozygous condition. Homozygous, doubled haploid lines can be produced in one generation, therefore eliminating 3 – 4 years of selfing or backcrossing to produce true breeding lines.

Thirdly, the efficiency at which selection can take place is also improved. The phenotype of the plant is not masked by dominance effects. Traits conditioned by recessive genes can be easily identified. Fourthly, a much smaller sample of doubled haploids is required when screening for desirable recombinants than would be the case for conventional diploid populations.

In this project, the assessment of response of finger millet (Eleusine Coracana) to Anther culture was carried out.

The main objectives were:-

(i) to identify which type of growth media gives the best response to Anther culture in finger millet.

(ii) To determine the optimum number of days of pre-shock treatment of the inoculating material.

Chapter 2

2. Literature Review

2.2.1 Origin and DIstribution

For many years it was considered that *E. Coracana* evolved from E. indica either in Africa or in India. Kennedy-O-Byrne (1957) shows that the Afro Asian types from Asia and lowland Africa with smaller florets, bear a closer resemblance to *E. indica* than the African highland types, which seem closer to *E. africana*. It is thus possible that the two types *E. africana* had separate origins, the former from *E. indica* and the latter from. Nevertheless, he considered it more likely that *E. africana* was derived from *E. indica* by chromosome doubling, followed by the selection of large grain mutants for use as food from *E. africana* in north eastern tropical Africa. Intermediate weedy contaminants occur in fields of finger millet in Uganda and appear to be hybrids between the two tetraploids, *E. africana* and *E. coracana*.

Finger millet is a plant whose contemporary distribution in the warmer regions is of world-wide origin. It is true origin is masked by it's wide dissemination in ancient times and the ease and rapidly with which it becomes established as a native plant. It certainly appears indigenous to East Africa most probably the highlands of Uganda and Ethiopia but elsewhere there is invariably considerable evidence indicating it's introduction though it achieved little

importance outside Africa and India. From examination of available literature, it can be concretely confirmed that finger millet is in fact indigenous to East Africa.

2.2.2. Uses

Finger millet is an important staple food in parts of East and Central Africa, and India particularly in Mysore, It is the principle cereal grain in northern and parts of Western Uganda and in North eastern Zambia. It is also used for malting and brewing. The grain is ground into flour and made into a stiff mush or porridge by adding to boiling water and stirring during heating until it's the right consistency. It is eaten with other foods when available, such as sweet potatoes, peas, beans, groundnuts and occasionally meat.

The great merit of finger millet is that it can be stored for long periods of up to ten years or more without deterioration or weevil damage. Consequently it is an important famine food and was stored for this purpose in parts of Uganda during the British administration.

The growing crop also suffers little from bird damage. The straw can be used as fodder and the fields are often grazed after the heads have been harvested.

2.2.3 *Ecology*

Finger millet is grown mainly in the tropics in Africa. It is usually grown at attitudes between 1000 – 2000 m. In Uganda, it is the staple crop in the drier areas between 1000 – 1500 m with an annual rainfall of 900 _1250 mm, in which there is a long hot dry season, which makes the area unsuited to permanent crops other than cassava. It requires a well distributed rainfall during the growing season with an absence of prolonged droughts. Dry weather is required for drying the grain at harvest in drier areas with unreliable rainfall. Sorghum and bulrush millet are better suited than finger millet. It will not tolerate such heavy rainfall as rice or maize. In Madras and Mysore in India finger millet is the staple crop in areas with an annual rainfall of 800 – 900 mm: with less rainfall irrigation is required. Thomas (1970) says that the crop grows best in Uganda where the average maximum temperature exceeds 27°C and the average minimum does not fall below 18°C.

Finger millet is an important cereal crop in the high rainfall areas of Zambia, requiring a moderate well distributed rainfall during the growing season with an absence of prolonged droughts. It is extensively grown in Northern, Luapula and North Western Provinces and to a lesser extent in the Copperbelt and Central Provinces that is agro-ecological zone III and II respectively.

The crop is grown on a variety of soils, but reasonably fertile, free-draining sandy loams are preferred. It cannot tolerate water logging. Sustri (1952) says that it thrives best in India and on red lutentic loams. In the low fertility soils of

Northeastern Zambia, it is grown in chitemene ash culture.Finger millet is acid tolerant .Most genotypes are very tolerant to acid soil conditions

Finger millet cannot tolerate competition in the early stages of growth and early and thorough weeding is essential.

Finger millet is a short day plant, a 12 – hour photoperiod being optimal. The time to maturity is influenced by temperature as well as photoperiod as is also the case with sorghum.

2.2.4 Structure

A robust free tillering, tufted annual grass, 40 – 100 cm, tall taking 2 ½ - 6 months to mature.

Roots: Finger millet has a shallow branched, fibrous adventitious root system from base of main stem and tillers.

Stems and Leaves: Lower nodes of the stem are usually semi-procumbent, the upper part is erect, the stool is compressed, smooth, 40 – 100 cm in height, 4 – 12 mm thick, bearing numerous leaves, the sheath is flattened, overlapping, split along entire length, glabrous except for occasional hairs on margins. The ligule is thin, short, fimbriate; the blade is linear often folded with strong midrib being glabrous or with few scattered hairs with acute tips.

Inflorescence:_Terminal digitate inflorescence of 3 – 9 (usually 4 – 6) dense sessile spikes, straight and spreading, or incurved and compact. Often with an additional 1 – 2 short spikes carried 2 – 4 cm below terminal whorl. About 60 – 80 spikelets per spike densely crowded, arranged alternately on rachis in two overlapping rows along outer sides of spike.

Spikelets are awnless, ovate-elliptic, flattened, with 6 – 12 florets arranged alternately in two parallel rows on a zig zag rachilla and with two basal glumes ovate, strongly keeled, nerves 3 – 5, margins hyaline.

Fruits

4 – 7 per spikelet, about 1 – 2 mm in diameter , globose smooth or rugose, varying in colour from orange red, reddish brown, dark brown to nearly black, a white seeded form is also known. The pericarp remains distinct during development and at maturity appears as a papery structure surrounding the seed. 400 – 500 seeds per gram.

2.2.5 Husbandry

In Madras and Mysore in India two crops per year are often taken, an early crop "Kar ragi" from May to August and the main season crop 'hain ragi' from July to November or early December. It is often grown in mixed cultivation with other crops such as peas. It usually receives a generous dressing of cattle or sheep manure, which is sometimes applied with the seed at sowing. In India the crops may be broadcast, and tilled about 2.5 cm deep in rows 7.5 – 25 cm

apart with a seed rate of 20 kg/ha or more. The seedlings may be raised in nurseries and transplanted when 3 – 4 weeks old. This is sometimes done for the rain fed crop and always where the crop is irrigated. The seedbed may be compacted after sowing by driving a flock of sheep on the field.

In Northern Uganda finger millet is usually planted in the second year of shifting cultivation following continued sesame. It may be interplanted with pigeon peas or sorghum, the proportion of the latter increasing in the drier areas . In Zambia,Finger millet is planted in the first year of shifting cultivation.

A firm seedbed is required. It is often planted before the rains break either before or after the cotton has been uprooted, or after a shallow ploughing, with a seed rate of 5 – 10 kg/ha. If a dry spell occurs after germination the growing plants may be killed off and resowing is necessary. In all countries the rain fed crop is grown on the flat. In western Uganda and Tanzania the grass roots, weeds and organic matter are piled in little heaps, which are burnt and the ash spread before broadcasting the seeds.

Great importance is attached to thinning and weeding when the finger millet is 2.5 – 5 cm high. The most serious weed in Africa is the wild *Eleusine africana*. It is particularly serious in broadcast crops and usually matures with the crop, shedding its seeds before the finger millet is harvested. Drilling is increasing in popularity, as this permits weeding in the interows and also makes thinning easier. The usual spacing is about 25 cm between rows, and the plants are

thinned to 10 – 12.5 cm apart. Striga Spp. Witchweed is seldom serious as the crop is well weeded.

2.2.6 *Insects*

Generally speaking, the need to control pests like the stem borers is less frequent on finger millet than other crops like sorghum. Stem borers are one of the major insect pests of finger millet especially when the crops is planted late and cause reduction in yield. Other insects include the phytophagous lady bird. *Epilachu Similis* the shoot fly (*Atherigonia* Spp) Stalk borers (*Sesamia* Sp) and Aphids .

The *Eleusines* are reputed to be relatively free of insect pests. Spraying the crop with insecticides as per manufacturer recommendations can easily control insects.

2.2.7 *Diseases*

Blust (*pyricularia* Sp) is a serious disease in finger millet throughout most of its growing region (MAFF. 1998) and literature suggests that Blust may occur in epidemic proportions and that has loss of grain yield may exceed 50% in some years. (McRae 1922) .

Several fungicides have been known to control the disease in other areas but are expensive for small scale farmers. Therefore control measures are very important in areas where it occurs.

2.2.8 *Plant breeding using anther culture*

Anther culture provides a method for the production of homozygous lines over the course of a few months, rather than the several generations required using conventional whole plant techniques. (Cho et.al 1985 Morrison and Evans 1988. Snape 1989) the doubled haploid plants resulting from Anther culture are homozygous and breed true. Also because they harbor no hidden traits, the use of doubled haploids for breeding also improves the efficiency with which superior genotypes can be identified. (Knapp 1991 Mitehell et al 1992 Bjorshet et al 1993)

Anthers contain pollen, and Anther culture involves the culture of these structures in vitro. The immature pollen or microspores contained within the anther either give rise directly to embryos, called androgenesis, or to callus tissues, which in turn is induced to regenerate plants under the influence of growth regulators added to the culture medium. Pollen is haploid and the cells produced from pollen or microspores culture are haploid as well when plants are regenerated from haploid cells, a haploid plant is produced. Haploid plants are sterile and can produce no seed. However, a spontaneous duplication of chromosomes often occurs within Anther culture-derived cullus cells, resulting in the production of fertile, doubled haploid plants. Because the two copies of genetic information within such plants are identical, the plants are fully homozygous and breed true.

2.2.9 Factors affecting success of anther culture

(i) ***Growth conditions of donor plants.***

The growth conditions of the donor plants used to provide the anthers for culture have a profound effect on the yield of microspore derivatives and reproducible results can therefore be achieved only by the use of environments with controlled temperatures, photoperiods and light intensities without such environments, massive fluctuations in response must be expected. The optimum requirements for each species are different and no general recommendations can be given.

(ii) ***Age of donor plants***

It is recommended that buds are harvested from plants at the beginning of the flowering period. If it is necessary to continue experiments over an extended period then unused buds must be removed from the plant and not allowed to mature.

(iii) ***Stage of pollen development***

The optimum stage of pollen development at the time of harvest of the buds is of vital importance but differs between species.

(iv) ***Sterilization methods***

Prior to the culture of isolated anthers,the investing floral tissue is removed and where appropriate leaf material after sterilization of the outer surface

with most species, sterilization is effected by a 10 minute treatment with hypochlorite solution (2% available chlorine) containing a wetting agent.

(v) Pre-treatment of buds or anthers

In many species, the best yields of microspore derivatives have been obtained from cultured anthers which have been previously subjected to a particular temperature pre-treatment prior to culture.

(vi) Dissection methods

Anthers of most species are of sufficient size to be removed from the flowers with relative ease. However , in some species such as tropical cereals of the genera panicum, pennisetum and setovia, dissection of the compound. Inflorescences in order to remove the minute anthers is tedious and time consuming and it is recommended that whole inflorescence be cultured. This can be best effected by slow shaking in liquid medium.

(vii) CULTURE MEDIUM

Although often considered of prime importance in culture protocols, the culture medium is subordinate to the prior variables of growth conditions and pollen stage.

Chapter 3

3. Materials and methods

3.1 Materials

The finger millet variety grown was that of Nyika variety. The seed was obtained from Misamfu Research Centre located in Kasama, in the Northern Province in region III.

The planting was done in the glass house situated at the University of Zambia, School of Agricultural Science and the experiment was conducted in the tissue culture laboratory.

The seed was sown on 15/11/01 in sterilized soil in buckets of 60 x 50cm. The time of sowing to harvest took about 3 to 4 months. The other materials used were a weighing balance, microscope, slides, one plate cooker, autoclave, laminar airflow cabinet, sterilizing oven, pH meter, 1 liter glass bottles, test tubes, sterile bottles containing petri dishes, tweezers, sharp blades, spirit lamp, timer, refrigerator, alluminium foil, macro stock solutions, distilled water, Murashige and Skoog media, Nitsh and Nitsh media and N_6 media (Chu 1978) Eriksson 1968).

The composition of the 3 different types of media are listed in table 1.

TABLE 1: Composition of Murashige and Skoog (MS) Nitsch and Nitsch (NN) and N6 (chu 1978), Eriksson (1965)

Component	N_6	NN	MS
MACRONUTRIENTS	**Mgl^{-1}**		
KNO_3	2830	950	1900
NH_4NO_3	-	720	1650
$MgSO_4$ $7H_2O$	185	185	370
KH_2PO_4	400	68	170
NaH_2PO_4O	-	-	-
$CACL_2 2H_2O_3$	166	166	440
$(NH_4)_2$ SO_4	463	-	-
Micronutrients			
H_3BO_3	1.6	10.	6.2
$MnSO_4.4H_2O$	3.3	19	22.3
$ZnSO_4$ $7H_2O$	1.5	10	8.6
NaM_oO_4 $2H_2O$	0.25	0.25	0.25
$CuSO_4$ $5H_2O$	0.025	0.025	0.025
$CoCL_2$ $6H_2O_3$	-	0.025	0.025
KL	0.8	-	-
$FeSO_4$ $_7H_2O$	27.8	27.8	37.3
Na.EDTA	37.3	37.3	37.3
Vitamins and organics			
MyO-Inositol	-	100	100
Nicotinic Acid	0.5	5	0.5
Pyridoxine Hcl	0.5	0.5	0.5
Thiamine Hcl	1.0	0.5	0.1
Glycine	4.0	5.0	2.0
Phytohormone	1.0		
		-	-
Sucrose	50g	20g	30g
PH	5.8	5.5	5.8

3.2 Methods

3.2.1 Determination of pollen development stage

The spikelets containing the anthers were severed from donor plants using sharp blades. The anthers were then removed and examined under the microscope from a squash prepared from the removed anthers. The uninucleate stage of pollen development was then noted and used as a basis for selection of the anthers.

The anthers containing pollen that was at the uninucleate to binucleate stage of development were those found at the lower end of the spikelets while those at the top had more mature pollen.

3.2.2. Culture media

The media used in the experiment was MS media (murashige and Skoog), modified N_6 media (Chu 1978) and NN media (Nitsch and Nitsch). The composition of these are shown in tables 1

The MS media was prepared by adding 30g of sucrose to a 1 liter capacity glass bottle. The sucrose was dissolved in distilled water after which 7g of agar, 100 ml of stock solution were also added to the glass bottle. Five ml of Iron solution and 1ml of micro nutrients (Table1) were also added to the bottle. The volume of the solution was brought to 1 litre with distilled water

and allowed to boil for slightly over an hour. The pH of the solution was adjusted by the use of Na0H and HCL and determined on a pH meter. The solution was then poured out into equal test tubes and autoclaved for 30 minutes.

The same procedure was followed in preparation of NN and N_6 media . The variations were that for NN media 30g of sucrose was added, and for N_6 ,50g of sucrose were added. The N_6 media was also modified in that a cytokinin (6-benzoaminopurine) was in the concentration of one ppm.

3.2.3 Shock treatment of anthers

The spikelets containing the anthers were removed from the donor plants, wrapped in aluminum foil and subjected to a pre shock treatment of 4^0C.The duration of the treatment varied from 0 days, 4 days, 8 days and 12 days.

3.2.4 Culture of anthers

The spikelets were surface sterilized by the immersion in 70% ethanol to generally remove dust particles and get rid of air bubbles. The spikelets were then placed in 2% sodium hypochlorate for 10 minutes and subsequently rinsed three times with sterile distilled water. The instruments were sterilized each time after handling tissue by dipping them in 95% ethanol as well. This was done to minimize contamination.

The anthers were removed from the spikelets using sterilized instruments. In instances were the husks were too small, the casing of the anthers were severed and the anthers exposed.

The cup of the culture test tube containing the media was removed, the inoculum transferred on the medium, the neck of the test tube flamed and cup replaced in quick succession.

These operations were carried out under aseptic conditions in a laminar-air flow chamber.

3.2.5 Incubation

After inoculation the test tubes containing the three different media, (MS, NN and N_6) were incubated at room temperature and uniform light intensities. The test tubes were frequently rotated in their racks to ensure even distribution of light.

3.6. Experimental design

The two factor completely randomized design was employed. The experiment factors where media and days of cold shock treatment. The parameters measured were anther response and duration of response from date of inoculation. Anther response was the proportion of microspores that survived and developed from the inoculated anthers. These grew into muticellular filamentous structures with cells actively dividing with less the diploid number of chromosomes.

3.7 Statistical analysis

This was analyzed by the use of MSTAT.C computer soft ware. The data was subjected to statistical analysis using the Analysis of Variance. (ANOVA 1). The means were then separated using the Duncans multiple range test .

Chapter 4

4.0 Results and discussion

The response varied with media and shock treatment. The combination that gave the highest response ($P \leq 0.05$) was N_6 media and 4 day pre shock treatment with a 46.4% response. This is illustrated in Figure 1, 2 and 3.The combination that yielded the lowest response was MS media and 8 day pre shock treatment.

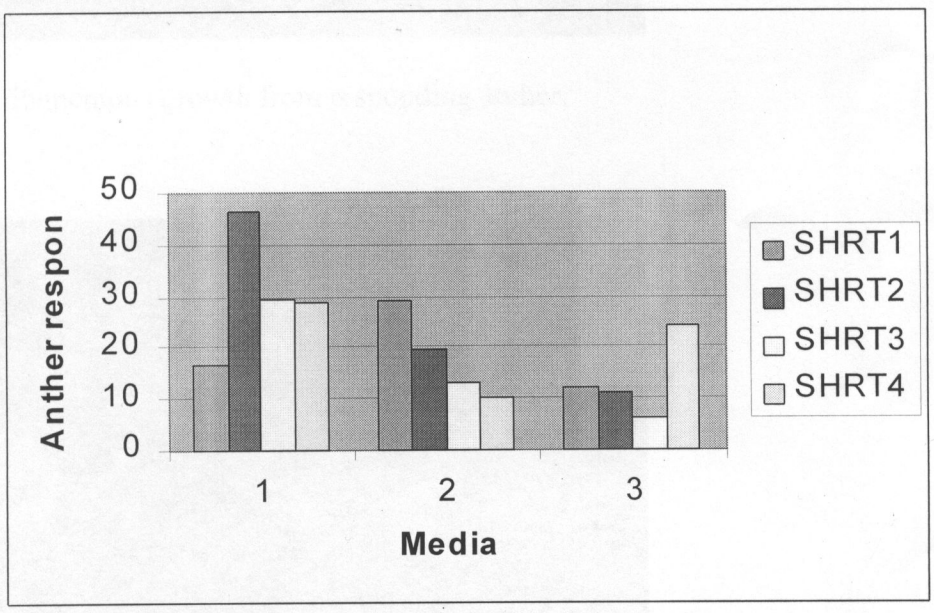

Figure 1. Effect of media and shock treatment on anther response

Key

Media 1 -	N6 (Chu, 1978), Eriksson, 1965)	ShockTRT 1 - O days
Media 2 -	NN (Nitsh and Nitsh)	ShockTRT 2 - 4 days
Media 3 -	MS (Murashige and Skoog)	ShockTRT 3 - 8 days

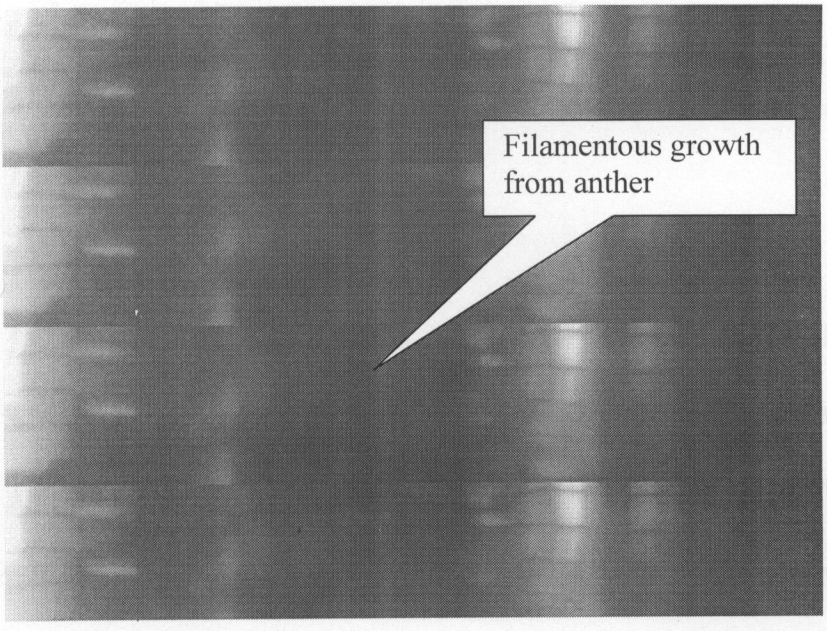

Figure 2. Filamentous growth from responding anther.

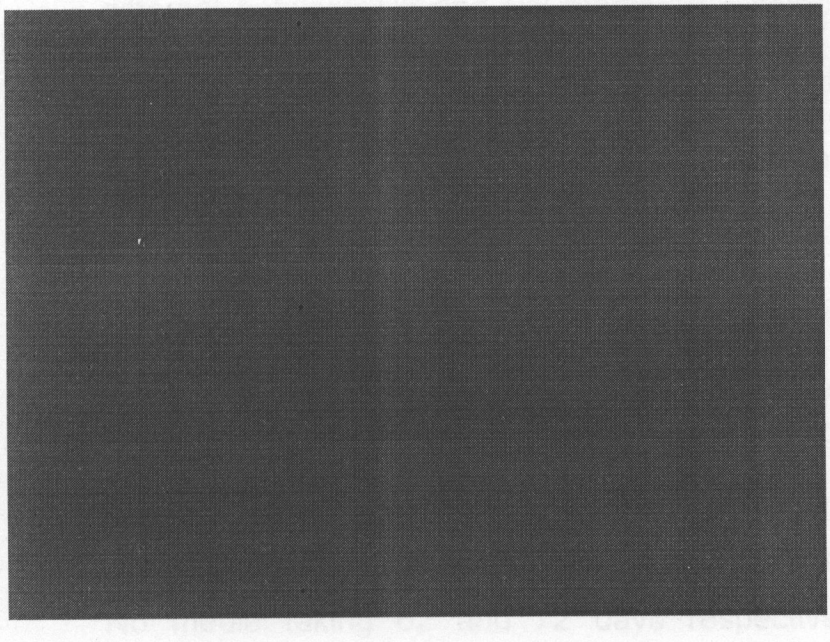

Figure 3. Close up view of filamentous growth.

With no shock treatment, anther response was non significant across the 3 different media.

The effect of 4 days pre shock treatment varied across the 3 media. The combination of N6 media and pre shock treatment 2 was significantly higher than the rest giving 46.4% response. Eight days pre shock treatment also varied across the 3 media. The combination of N6 media and pre shock treatment 3 was significantly higher than that of NN media and pre shock treatment 3 by 16.7%. Twelve days pre shock treatment was different between N6 and NN media. The combination of N6 media and pre shock treatment 4 was significantly higher by 18.7% than the combination of NN media and pre shock treatment 4.

Effect of media and shock treatment on duration of response

Days to response of anthers varied with media. The longest duration to response was in N6 Media with 4 days pre shock treatment taking 156 days.Days to response for shock treatment 4 was least across all media. The duration of response for shock treatment 1and 3 was minimal only in N6 media taking 62 and 72 days respectively, while that for shock treatment 2 was least in NN media. taking 62 days. This is illustrated in Figure 4.

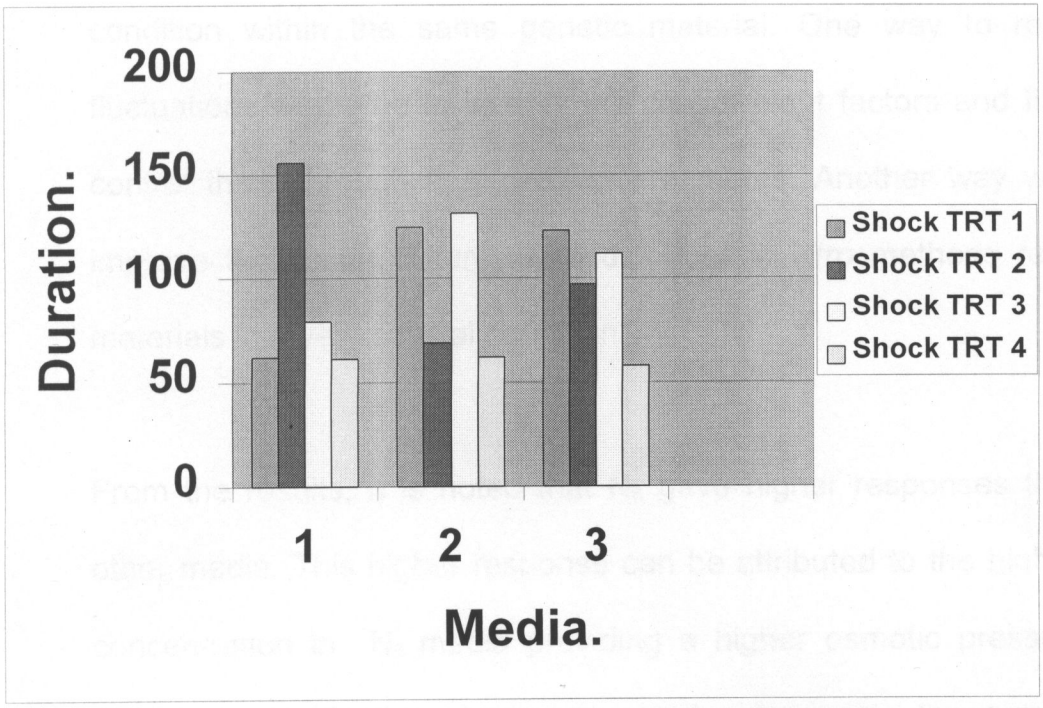

Figure 4. Effect of media and shock treatment on days to response (Duration) of anther culture.

4.2 Discussion

In anther culture each individual species requires it's own unique conditions while in certain species even different varieties of the same plant may require certain changes to be made to the same procedure to get positive responses. In this study, different media was inoculated with anthers subjected to different days of cold shock treatment.

The response to the shock treatment varied across all three media, the different response of anthers to media and pre shock treatment can be

attributed to the plant material being in a slightly different physiological condition within the same genetic material. One way to reduce such fluctuations would be to identify the crucial plant factors and find ways to control them through controlled environments. Another way would be to improve the tissue culture methods. Poor *in vitro* methods require plant materials in a very special condition.

From the results, it is noted that N_6 gave higher responses than did the other media. This higher response can be attributed to the higher sucrose concentration in N_6 media providing a higher osmotic pressure.Various empirical investigations have separated species into those requiring low sucrose concentrations and those whose anthers produce better results on higher concentrations of sucrose

This division of species seems to be related to known separation according to whether their mature pollen is bicellular (solanaceae, Liliaceae) or tricellullar (Gramineae, crucifereae). The former group require low osmotic, while the latter group require high osmotic pressure conditions. Therefore, the finger millet required a higher concentration of sucrose present in the N_6 media.

The addition of the hormone (6- Benzoaminopurine) to the media can also attribute to the better performance of this media. Finger millet has been

separated into a group of species requiring the addition of hormones to anther culture media.

N_6 media had a higher concentration of selected salts than the other media and it has been reported in some species that better response is observed on higher salt concentrations.

Chapter 5

5.1 Conclusion

The conclusion to be drawn from the study is that response of Finger millet anthers is dependent on media and pre shock treatment.

The media, pre shock treatment combination which gave the highest response was N_6 media with a pre shock treatment of 4 days.

5.2 Recommendations

Further studies should be carried out on the combination of N6 media and 12 day pre shock treatment as it gave the minimum days to Finger Millet anther response.

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Appendices 1

Table 2 ANOVA analysis of the factors media and shock treatment

Source	Degree of freedom	Sums of squares	Mean squares	F valve	Probability
Factor a	2	1106. 95	553.47	13.4	0.009
Factor b	3	266.15	88.71	2.15	0.1467
Ab	6	1400..96	233.49	5.66	0.0053
Error	12	494.42	41.201		
Total	**23**	**3268.47**			

C. V. 30.60%

Table 3 ANOVA analysis for duration

Source	Degree of freedom	Sums of squares	Mean squares	F valve	Probability
Factor a	2	302.571	151.286	2.23.86	0.1492
Factor b	3	9452.693	3150.898	46.62	0.00
Ab	6	15682.996	2613.833	38.676	0.00
Error	12	810.975	67.581		
Total	**23**	**26249.3**			

C. V. 8.69%

* NOTE in the anova tables under the source column:

Factor A represents Media

Factor B represents cold shock treatment

AB represents media and shock treatment interaction.

Appendices 2

Mean separation

Table 4 Effect of media and shock treatment on response

Media/shock treatment	Response (%)
12	46.4 A
13	29.7 B
21	29.0 B
14	28.77 B
34	24.07 BC
22	19.78 BCD
31	16.65 BCD
11	16.62 BCD
23	13.18 CD
32	11.19 CD
24	10.11 CD
33	6.267 D

Means followed by the same letter are not significantly different at the 5% level of probability = 0.05

TABLE 5; effect of media and shock treatment on duration of anther response

Medi/shock interaction	Response(%)
12	156 a
23	131 B
21	125 B
31	123 B
33	112 BC
32	97 C
13	79 D
22	69 DE
24	62 DE
11	62 DE
14	61 DE
34	58 E

Means followed by the same letter are not significantly different at 5% level of probability = 0.05

Note

In the mean separation columns, under the media/shock interactions, the first digit represents media and the second represents cold shock treatment.

KEY (F***IRST DIGIT)***

1 = N_6 Media (Chu 1978), Eriksson 1965)

2 = NN Media (Nitsch and Nitsch)

3 = MS Media (Murashigeand Skoog)

(SECOND DIGIT)

1 = 0 days to shock treatment

2 = 4 days to shock treatment

3 = 8 days to shock treatment

4 = 12 days to shock treatment