

IN VITRO CULTURE OF EXCISED ROOTS, SORGHUM

VULGARE VAR. SUDANESE

by

Susan H. Lee

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DEPARTMENT OF BOTANY

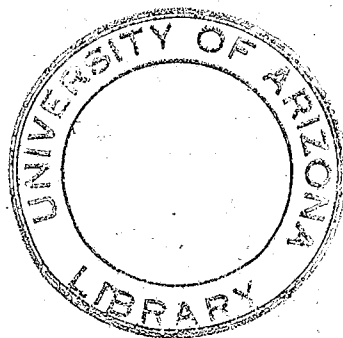
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This thesis has been approved on the date shown below:

Walter S. Phillips 8/12/55
WALTER S. PHILLIPS Date
Professor of Botany

REVIEW OF LITERATURE

The growing root tip has long been considered a favorable object for experiments on growth and differentiation. With the development of tissue culture many investigations have been made with excised root tips. The existence of many nutrient formulas for the successful establishment of dicotyledonous excised root cultures is the result of such efforts. The most widely used formulas are modifications of those of White (37), Gautheret (15), and Nobécourt as cited by Morel and Wetmore (19).

In 1951 White (38) outlined the studies to that date dealing with the general nutrition of plant tissue cultures. Following White, Gautheret (16) adequately summarized the literature on this subject to 1954, and later Nobécourt (20) presented a similar review although quite limited. Street (31) gives an excellent review of the advances made in excised root culture with the most emphasis placed on dicot roots, specifically those of the tomato.

A great deal of difficulty has been met by investigators attempting to establish root or even callus cultures of monocotyledons. As Almestrand (5) states, many results have not been published as they have been negative in nature. For this reason material concerning culture of excised roots of monocotyledons is very sparse as compared with the wealth of information available for dicotyledonous root culture.

Some of the earliest attempts to culture excised roots by

Robbins (22, 23), Kotte as cited by Roberts and Street (30), and White (35, 36) involved monocot roots. Robbins (22, 23) and Robbins and Maneval (25, 26) demonstrated the growth of excised maize roots was improved by the addition of autolyzed yeast or peptone to the culture medium although the growth rate declined with each subculture and eventually all growth ceased.

Galligar (11) using a modified Pfeffer's formula found 1.0 mm. root tips of dent corn had a tendency toward development of laterals and suppression of extensive elongation, but the growth behavior was erratic. Adding dextrose and peptone to modified Pfeffer's formula (14) gave similar results and this work indicated that corn root tips were less able to accumulate dry weight when cut at 1.0 mm. than when excised at 1.0 cm. In 1938, again working with corn (12), she found there was some correlation between growth behavior of excised root tips and the type of food stored in the seeds, root tips from grains high in starch reserves growing best. She varied the temperature of cultivation in another study (13), finding 20° C. to be optimum for corn. In no instance was Galligar able to culture corn roots extensively.

Robbins and White (27, 28) and Robbins et al (29) also conducted work aimed at continuous growth of corn root tips. They found substitution of fructose, xylose, or maltose for glucose to have no beneficial effect upon growth. Difco agar, water extracts of agar, autolyzed and dried yeast, and ash elements were unsuccessfully tried as additives. These workers concluded that a deficiency in solution existed.

Burström as abstracted (7, 8) studied the cell division and elongation of isolated wheat roots cultured on solutions of different glucose concentrations as well as the effect of fructose, sucrose, and maltose on such growth. He suggested fructose utilization only occurred after conversion to glucose. Indeterminate growth of excised wheat roots was not obtained.

Using media with the ability to support the continuous growth of excised roots of tomato or pea, Almestrand (1, 2, 3, 4), and Fiedler as cited by Roberts and Street (30) investigated the responses of several different excised cereal roots to changes in the medium composition, such changes involving alterations of the nature and concentration of the constituents and supplementation. The growth rate, after temporary enhancement, declined and finally ceased in all cases.

Success in culturing maize roots thru 88 subcultures was claimed by McClary (18). He reported all roots increased in length throughout a 115-day period at an average of approximately 8 mm./day. However, Bonner and Bonner (6) and Robbins (24) were unable to repeat this work, and it is suggested by Roberts and Street (30) that McClary's success was due either to the particular strain of maize used or to some unknown factor of his technique.

In 1951 Morel and Wetmore (19) with callus tissue were the first to succeed in the field of tissue culture of monocotyledonous plants. Using tuber tissue cubes of Amorphophallus (Hydrosme) rivieri Dur., they obtained, in modified Gautheret's medium, proliferation in vitro of tissue for potentially indefinite periods. Their studies indicated that these

tissues required growth substances found in the milk of immature coconuts.

The continuous cultivation of excised rye roots has been accomplished by Roberts and Street (30). They found the growth of excised Petkus II rye roots in modified White's medium to be stimulated by the addition of BDH-peptone or Difco yeast extract, the most marked stimulation being in lateral root growth. Eighty percent of the growth stimulation by the yeast extract was attributed to its L-tryptophane content.

In 1957 Robb (21) cultivated in vitro explants from the bulb scales of Lilium speciosum Thun. He found that these explants proliferated and differentiated to regenerate bulblets in 15-16 weeks using a modified White's medium.

Almestrand (5), working with 4 different kinds of excised cereal root tips, wheat, rye, oats, and barley, obtained a clone of rye, subcultured by excision of both main tips and laterals, having indeterminate growth. With wheat, oats, and barley, root growth for extended periods of time was not possible. Almestrand discusses the aspects of metabolism in their respect to the presence or absence of growth of the root tips involved in this study, linking disturbance of a metabolic phase with lack of growth.

The purpose of this investigation was to attempt establishment of two clones of excised roots having indeterminate growth, one of Sorghum vulgare var. sudanese type Redbine 60 and the other of Sorghum vulgare var. sudanese type Martin. The problem involved determination

of a medium suitable for excised root growth of these two types of Sudan grass.

MATERIALS AND METHODS

Grains of two types of Sudan grass, Sorghum vulgare var. sudanese type Martin and type Redbine 60, were obtained from the Agronomy Department, The University of Arizona at Tucson.

Immersion of the grains for 30 minutes in a Tide detergent solution, approximately 6 gms. of detergent powder in 400 ml. water, followed by a second 30 minute dip in a chlorox solution, 1 part chlorox to 2 parts water, gave sterilization found to be optimum for maximum germination and minimum contamination. A magnetic mixer was used to agitate the solutions, allowing for uniform seed coverage.

The sterile grains were then washed three times with sterile, de-ionized, triple distilled water and placed, evenly spaced to permit root development without excessive contacts, in steam sterilized Petri dishes containing three thicknesses of #1 Whatman filter paper thoroughly moistened with de-ionized, triple distilled water. The Petri dishes with the grains were placed in the dark in a culture chamber at 22° C. and allowed to germinate. After germination had proceeded for one week, the primary root having attained a length of approximately 7-10 cm., 1.0 cm. primary root tips were excised and transferred aseptically to the appropriate culture solutions.

All manipulations were performed in a sterile Fisher inoculation chamber equipped with gas, ultra-violet light, fluorescent light and positive pressure forcing all filtered air outward. Sterilization of such

chamber was effected by exposure to ultra-violet radiation for a thirty minute period. Instruments used for transferring and excision were sterilized in boiling 70% ethanal for 10 minutes. A 2% amphyll solution was used as a surface disinfectant. Sterile technique was checked out to insure minimum contamination due to human manipulation.

Because during the first phases of experimental work substantial growth of primary root tips was not obtained, it was deemed advisable to culture 1.0 cm. lateral root tips from one-week old seedlings. No difference in growth results was found.

Culture vessels were usually 125 ml. Erlenmeyer flasks of pyrex glass. In the first trials in this work, each flask was supplied with 50 ml. of solution and then charged with a single root tip. However, when it was found that root browning occurred within one week, the amount of solution per vessel was changed to 10 ml. As a check, tomato roots grown for a one week period in 10 ml. of solution excelled in this quantity of solution and appeared to not differ in growth from those cultured in 50 ml. Occasionally 5 cm. diameter pyrex Petri dishes were employed as culture vessels. It was found that excised tomato roots grew as well in these dishes as in the Erlenmeyer flasks.

For sterilization filtration pyrex #36060 funnels, Buchner type, with fritted discs of ultra-fine porosity were used. Before usage such filters were cleaned with a hot acid mixture of concentrated H_2SO_4 and concentrated HNO_3 , as recommended by the manufacturers. Following the acid treatment, the funnels were washed first with a 30 ml. aliquot of

distilled water followed by one of 30 ml. of de-ionized, triple distilled water. The cleaned funnels, fitted snugly through cork stoppers and covered with aluminum foil, were assembled with clean vacuum flasks and clean tygon tubing to form the complete apparatus to be used for sterilization filtration. All openings were covered by aluminum foil. After assemblage, each individual set up, wrapped in gauze, was thoroughly encompassed with brown wrapping paper and tied with string. The apparatus was sterilized in this condition by autoclaving at 15 lbs. pressure for 15 minutes and was then stored for later usage. When used, immediately after unwrapping, the open end of the tygon tubing was connected to the vacuum outlet, which surface had been wiped with the surface disinfectant. Upon completion of filtration, a clamp was applied near the end of the tubing connected to the outlet and the vacuum gradually decreased. Precautions were taken to guard against deterioration of heat-labile substances during filtration. Filtration apparatus was cooled by refrigeration before use, and ice baths were employed during the actual filtration process. A final step was filtration of the exterior of the set-up by ultra-violet radiation. If a solution sterilized in such a manner was not to be used immediately, it was placed under refrigeration and its container re-sterilized externally just preceding handling thereof. All solutions were used within 24 hours.

All water used for solutions, sterilization of grains, germination, and for the final washing of glassware was de-ionized, triple distilled, the second and third distillations carried out in pyrex glass.

Storage was also in pyrex. Chemicals used for solutions were of analytical grade.

All glassware was cleaned by an overnight immersion in potassium dichromate-sulfuric acid cleaning solution followed by three washings of tap water, three washings of distilled water and three final washings using de-ionized, triple distilled water.

To check culture technique using a plant whose reactions were known, a clone of excised roots of tomato, Lycopersicon esculentum Mill., was successfully established using White's solution (39). Within a week's time a 1.0 cm. root tip increased 10 or more times in length with the appearance of dozens of laterals. The formula for White's solution used is outlined below:

Mineral solution was prepared:

$\text{Ca}(\text{NO}_3)_2$	200.00	mg./l.
Na_2SO_4	200.00	mg./l.
KCl	80.00	mg./l.
NaH_2PO_4	8.25	mg./l.
MnSO_4	2.25	mg./l.
ZnSO_4	.75	mg./l.
H_3BO_3	.75	mg./l.
KI	.375	mg./l.
MgSO_4	720.00	mg./l.
(dissolved in water before added to the above)		

To the mineral solution sucrose and ferric sulfate, dissolved in water, were added:

sucrose 20.00 g./l.

$\text{Fe}_2(\text{SO}_4)_3$ 2.50 mg./l.

Finally, the following four organic compounds, dissolved in water, were added:

glycine 30.00 mg./l.

nicotinic
acid 5.00 mg./l.

thiamine 1.00 mg./l.

pyridoxine 1.00 mg./l.

RESULTS

Using White's solution as a standard medium for culture, various modifications in the form of additions and pH variations were tried. The results are given in the paragraphs following.

WHITE'S SOLUTION: Employing the same solution and identical technique, parallel trials were made for each type of Sudan grass. Each trial included between 16-20 excised 1.0 cm. root tips, one tip per flask. Browning occurred within one week, and at the end of three weeks the shriveled roots were completely brown with no appreciable elongation or lateral root formation. This experiment was repeated with similar results.

MODIFICATION OF WHITE'S SOLUTION: The same White's solution with the addition of 80 mg./l. KNO_3 and the reduction of KCl from 80 mg./l. to 65 mg./l., corrections for the nutrient formula made by P. R. White in a personal communication, was used for culture of the two types of Sudan grass. These root tips also became brown within a week, thoroughly brown by three weeks and lacked substantial elongation as well as lateral root formation.

BUFFERED SOLUTIONS: Stock solutions, 0.01 M phosphate buffered with a KH_2PO_4 - K_2HPO_4 system, were prepared using White's modified formula for the basic solution. These solutions were of the following pH's: 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5. A white precipitate occurred in slight amount at pH 6.5, moderate precipitation appeared at pH 7.0, and

at pH 7.5 this precipitate was heavy. After preparation 50 ml aliquots of these solutions were placed in 125 ml. Erlenmeyer flasks, stoppered in the usual fashion with non-absorbent cotton wrapped in gauze, and were autoclaved at 15 lbs. pressure for 15 minutes. The remaining reserve amounts of the solutions were distributed to 250 ml. and 500 ml. Erlenmeyer flasks, autoclaved, and stored in a chamber at 22° - 25° C.

Autoclaved Once: An initial pH run was conducted with the Erlenmeyer flasks containing 50 ml. aliquots of solution subjected to one autoclaving treatment. For each type of Sudan grass there were 3 flasks per pH. Growth was negative as is shown in Table 1.

Autoclaved Twice: A similar pH run was conducted using the reserve autoclaved pH stock solutions, the solutions thereby ultimately being twice autoclaved. As before, there were 3 flasks per pH per type Sudan grass employed. The results were negative as also may be seen in Table 1.

LACTOSE SUBSTITUTION FOR SUCROSE: Lactose, in identical quantity, ie., 20 g./l., was substituted for sucrose in White's modified formula, lactose being a β -glycoside on hydrolysis giving D-glucose and D-galactose, sucrose being an α -D-glucopyranosyl- β -D-fructofuranoside giving D-glucose and D-fructose upon hydrolysis. Solutions 0.01 M phosphate buffered, of six different pH's, the pH range being 5.0 to 7.5, were utilized, and for each pH there were four 125 ml. Erlenmeyer flasks containing 50 ml. of solution per each type of Sudan grass. (Table 2). A corresponding control run was made with 0.01 M phosphate buffered stock

<u>Sorghum vulgare</u> var. <u>sudanese</u> type Redbine 60						
solution	pH of solutions					
	5.0	5.5	6.0	6.5	7.0	7.5
autoclaved once	0 (0)	0 (0)	.1 (0)	0 (0)	0 (0)	0 (0)
	0 (3)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)
autoclaved twice	0 (0)	0 (0)	0 (0)	.2 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	.1 (0)	0 (0)	0 (1)
	.1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<u>Sorghum vulgare</u> var. <u>sudanese</u> type Martin						
solution	pH of solutions					
	5.0	5.5	6.0	6.5	7.0	7.5
autoclaved once	0 (0)	0 (0)	.4 (0)	0 (0)	.1 (0)	0 (0)
	0 (1)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (2)	0 (0)	0 (0)
autoclaved twice	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)
	0 (0)	0 (2)	0 (0)	0 (0)	0 (1)	0 (1)
	0 (0)	.3 (0)	0 (0)	0 (0)	.2 (0)	0 (0)

Table 1. Growth increments, expressed in cm., after 1 week of 1.0 cm. root tips in Whites modified solution, 0.01 M phosphate buffered and steam sterilized, 50 ml. of solution and 1 root tip per flask; number of lateral primordia indicated in parentheses.

<u>Sorghum vulgare</u> var. <u>sudanese</u> type Redbine 60.						
flask #	pH of solutions					
	5.0	5.5	6.0	6.5	7.0	7.5
1	.1 (0)	0 (0)	0 (0)	.1 (0)	0 (0)	0 (1)
2	0 (0)	.4 (1)	0 (0)	0 (0)	0 (0)	0 (0)
3	0 (0)	0 (0)	0 (0)	0 (0)	.2 (1)	0 (0)
4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<u>Sorghum vulgare</u> var. <u>sudanese</u> type Martin						
flask #	pH of solutions					
	5.0	5.5	6.0	6.5	7.0	7.5
1	0 (0)	0 (0)	0 (2)	.1 (0)	0 (0)	0 (0)
2	0 (0)	.1 (0)	.3 (0)	0 (0)	0 (0)	0 (0)
3	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	.2 (0)
4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 2. Growth increments, expressed in cm., within 1 week of 1.0 cm. root tips in White's modified solution, 0.01 M phosphate buffered, with the substitution of lactose, 20 g./l., for sucrose, 50 ml. of solution and 1 root tip per flask; number of lateral primordia indicated in parentheses.

solutions having sucrose as the carbohydrate source. No appreciable growth occurred in either case.

EPICOTYL EXTRACT: It was found that when the complete seedling was cultured, Sudan grass, both Redbine 60 and Martin, root growth excelled in White's modified buffered solution at pH 6.0; elongation occurred during one week cultivation to the order of several centimeters, and laterals as well as adventitious roots developed extensively.

Therefore, at this point a new variable, epicotyl extract, was introduced while the pH was maintained at 6.0. Since Robbins and White (28) had been unsuccessful in their attempt to grow excised corn roots by adding milk from corn grains to the medium, an extract of the grain was not tried.

The first prepared extracts were the products of epicotyls above the mesocotyls of seedlings germinated for the excision of root tips. Excised epicotyls were placed in 50 ml. buffered stock solution, pH 6.0, for extraction. Twenty-seven epicotyls were included in the 50 ml. Redbine 60 extract while 25 were in the extract of Martin. A Waring blender pulverized the excised epicotyls for 15 minutes. Repeated filtration through #1 Whatman filter paper to clear the solutions of debris preceded sterilization filtration. Using a steam sterilized syringe, 5 ml. and 3 ml. portions were added to 10 ml. aliquots of sterile, twice autoclaved pH 6.0 buffered stock nutrient solution. There were for each type of Sudan grass 5 flasks for each concentration

and 5 control flasks lacking the extract, a total of 15 flasks. Negative results were obtained (Table 3), and it was suspected that the extracts were of insufficient concentration.

Consequently, the second attempt at extraction, identical in actual extraction manipulations to that of the first attempt, involved epicotyls explicitly cultivated for this purpose. Grains of Redbine 60 and Martin were planted separately in previously moistened vermiculite to an approximate depth of 1.22 cm. in large pyrex trays. These growing trays were placed in a culture chamber at 22° C. under total darkness for 8 days. At the end of this time the epicotyls, as before including everything above the mesocotyl, were excised and placed in 50 ml. of pH 6.0 buffered stock solution. A total of 700 epicotyls were excised and included in the Redbine 60 extract while a sum of 900 composed that of Martin.

To wash each filtrate 25 ml. of pH 6.0 stock solution was added. The run with Redbine 60 epicotyl extract was comparable in design to that of Martin. Pyrex Petri dishes, 5.0 cm. in diameter, containing 10 ml. of twice autoclaved pH 6.0 stock nutrient solution, were charged by means of a sterile syringe with 3 ml. and 5 ml. portions of sterile extract, manipulations taking place within the sterile inoculation chamber. Five Petri dishes per treatment were involved, including a control. The results shown in Table 3 indicate that this effort also failed.

COCONUT MILK: Coconut milk, the fluid endosperm nourishing the developing Cocos nucifera L. embryo, has been recommended as an

<u>Sorghum vulgare</u> var. <u>sudanese</u> type Redbine 60			
solution	control	3 ml. of extract	5 ml. of extract
with first epicotyl extract (27 epicotyls in 50 ml.)	0 (0)	0 (1)	0 (0)
	0 (0)	0 (0)	0 (0)
	0 (1)	.1 (0)	0 (0)
	0 (0)	0 (0)	0 (0)
	fungal contam.	0 (0)	0 (0)
with second epicotyl extract (700 epicotyls in 50 ml.)	0 (0)	fungal contam.	0 (0)
	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	.1 (0)
	0 (0)	.2 (0)	0 (1)
	0 (0)	0 (0)	0 (0)
<u>Sorghum vulgare</u> var. <u>sudanese</u> type Martin			
solution	control	3 ml. of extract	5 ml. of extract
with first epicotyl extract (25 epicotyls in 50 ml.)	0 (0)	bacterial contam.	0 (0)
	0 (0)	bacterial contam.	0 (0)
	0 (0)	0 (0)	0 (0)
	.1 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (1)
with second epicotyl extract (900 epicotyls in 50 ml.)	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (1)
	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)

Table 3. Growth increments, expressed in cm., within 1 week of 1.0 cm. root tips in White's modified solution, 0.01 M phosphate buffered, with the addition of epicotyl extract, 13-15 ml. of solution and 1 root tip per culture vessel; number of lateral primordia indicated in parentheses.

excellent constituent of certain tissue culture media furnishing a necessary unknown growth factor or factors by van Overbeek et al (32, 33), van Overbeek et al (34), Caplin and Steward (9, 10), Morel and Wetmore (19), and Goris and Duhamet (17). In some instances autoclaved milk has been superior to un-autoclaved in stimulation.

To prepare coconut milk for use a mature coconut was broken with a hammer and the milk caught in aluminum foil. Debris of solution was eliminated by repeated filtration through #1 Whatman filter paper to produce a relatively clear solution. Transfer of the sterile milk to culture flasks containing the various pH stock buffered nutrient solutions was facilitated by means of a sterile syringe and was done in the sterile inoculation chamber.

Un-autoclaved Milk: Varying the pH, the effect upon root growth of filtration sterilized milk, 3% and 15% by volume, was observed. There were 9 flasks per pH per type grass, 3 for each treatment, a total of 108 flasks with each flask containing 10 ml. of solution.

Table 4 presents the results that were obtained. Although the positive growth increments are scattered and a \pm 1.0 mm. deviation must be considered in all measurements, growth appears to be stimulated at pH 5.5 with Redbine 60 roots. This is especially true when coconut milk is 15% by volume.

Autoclaved Milk: With the pH constant at 5.5, autoclaved coconut milk, 3% and 15% by volume, supplemented the nutrient solution.

<u>Sorghum vulgare</u> var. <u>sudanese</u> type Redbine 60						
solution	pH of solutions					
	5.0	5.5	6.0	6.5	7.0	7.5
control	0 (0)	0 (1)	0 (0)	0 (3)	0 (0)	0 (2)
	0 (0)	0 (0)	0 (2)	.5 (0)	0 (0)	0 (0)
	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
3% milk	0 (0)	.4 (0)	.2 (0)	.5 (0)	0 (0)	0 (0)
	0 (0)	.3 (0)	.1 (2)	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	.5 (0)	0 (0)
15% milk	.3 (0)	.2 (1)	0 (0)	0 (0)	0 (0)	0 (0)
	0 (0)	.2 (0)	0 (0)	0 (0)	0 (1)	0 (0)
	0 (0)	1.0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<u>Sorghum vulgare</u> var. <u>sudanese</u> type Martin						
solution	pH of solutions					
	5.0	5.5	6.0	6.5	7.0	7.5
control	0 (0)	0 (0)	0 (0)	.5 (0)	0 (2)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	1.0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
3% milk	0 (0)	0 (1)	0 (0)	1.0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (1)	0 (1)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
15% milk	.4 (0)	0 (0)	0 (0)	.4 (0)	0 (0)	0 (0)
	.2 (1)	.4 (0)	0 (0)	0 (0)	0 (1)	0 (1)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 4. Growth increments, expressed in cm., within 1 week of 1.0 cm. root tips in White's modified solution, 0.01 M phosphate buffered, with the addition of filtration sterilized coconut milk, 10 ml. of solution and 1 root tip per flask; number of lateral primordia indicated in parentheses.

This run consisted of 30 flasks with 10 ml. of solution apiece, 5 flasks per treatment per grass type. There was no significant growth (Table 5).

L-TRYPTOPHANE: L-tryptophane, concentration of 0.44 mg./l., according to Roberts and Street (30), was added to buffered pH stock nutrient solutions at the 6 different pH's in another study with the two types of Sudan grass. There were a total of 16 flasks per pH, 4 control Martin, 4 control Redbine 60, 4 Martin with tryptophane, and 4 Redbine 60 with tryptophane. Each flask was filled with 10 ml. of solution. All flasks were autoclaved, resulting in solutions with all constituents except L-tryptophane twice autoclaved.

Growth increments may be observed by examination of Table 6. Some growth did occur at pH 7.0 and pH 7.5 with Redbine 60 roots upon addition of tryptophane to the nutrient solution. Further work is necessary to indicate the significance of such growth as growth of the controls at these pH's also seemed to be stimulated. In addition, Martin root growth, pH 6.5 and pH 7.5, appeared to benefit from tryptophane addition.

INDOLEACETIC ACID: Indoleacetic acid was then tried at a concentration of 10^{-9} g./ml., according to Roberts and Street (30), and pH from 5.0 to 7.5 was also included as a variable. As with tryptophane, 16 flasks per pH were supplied with 10 ml. of pH buffered stock nutrient solution, 8 being control flasks and 8 with indoleacetic acid added, each 8 equally divided between Martin and Redbine 60. These solutions with IAA were likewise autoclaved (30), the indoleacetic acid in solution actually being subjected to one autoclaving whereas the

<u>Sorghum vulgare</u> var. <u>sudanese</u> type Redbine 60			
flask #	control	3% milk	15% milk
1	0 (0)	0 (0)	0 (0)
2	.5 (0)	0 (0)	0 (0)
3	0 (0)	0 (1)	fungal contam.
4	0 (0)	0 (0)	0 (0)
5	0 (1)	0 (0)	0 (0)
<u>Sorghum vulgare</u> var. <u>sudanese</u> type Martin			
flask #	control	3% milk	15% milk
1	0 (0)	0 (0)	0 (0)
2	0 (0)	.3 (0)	0 (0)
3	0 (0)	0 (0)	0 (0)
4	0 (2)	0 (0)	0 (0)
5	0 (0)	0 (0)	0 (0)

Table 5. Growth increments, expressed in cm. units, within 1 week of 1.0 cm. root tips in White's modified solution, 0.01 M phosphate buffered, with the addition of autoclaved coconut milk, 10 ml. of solution and 1 root tip per flask; number of lateral primordia indicated in parentheses.

<u>Sorghum vulgare</u> var. <u>sudanese</u> type Redbine 60						
solution	pH of solutions					
	5.0	5.5	6.0	6.5	7.0	7.5
control	0 (0)	0 (0)	0 (2)	0 (0)	.1 (1)	0 (0)
	0 (0)	.1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.3 (0)
	0 (1)	0 (0)	0 (0)	0 (0)	.5 (0)	0 (0)
L-trypto- phane added	0 (0)	0 (0)	0 (0)	fungal contam.	1.3 (0)	fungal contam.
	0 (1)	fungal contam.	0 (3)	fungal contam.	0 (0)	0 (0)
	0 (1)	0 (0)	0 (0)	0 (0)	1.0 (0)	0 (0)
	0 (0)	fungal contam.	0 (0)	0 (0)	0 (0)	1.0 (0)
<u>Sorghum vulgare</u> var. <u>sudanese</u> type Martin						
solution	pH of solutions					
	5.0	5.5	6.0	6.5	7.0	7.5
control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	0 (0)	.2 (1)	0 (0)	0 (0)	0 (0)	.2 (0)
	0 (0)	0 (0)	.1 (0)	0 (0)	0 (0)	0 (1)
L-trypto- phane added	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	.5 (0)
	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	.5 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 6. Growth increments, expressed in cm. units, within 1 week of 1.0 cm. root tips in White's modified solution, 0.01 M phosphate buffered, with the addition of 0.44 mg./l. L-tryptophane, 10 ml. of solution per culture vessel. (Lateral primordia indicated in parentheses).

rest of the components having undergone the treatment twice.

Resulting growth increments (Table 7) were not sufficient in extent to be recognized as significant for this work but are definitely worthy of further investigation. The almost uniform growth increase at pH 7.0 with Martin roots upon addition of indoleacetic acid to the nutrient solution seems promising but assuredly needs rechecking. When all individual increments are added, the total for Redbine 60 controls is 0.6 cm. as compared with 1.8 cm. when indoleacetic acid was added. For Martin the controls' total is 0.3 cm. as compared with 4.3 cm. upon addition of indoleacetic acid.

BACTO-PEPTONE, YEAST EXTRACT, AND CASEIN HYDROLYSATE: A final attempt at culture embraced the simultaneous addition of three substances (30), Bacto-peptone (Difco certified), yeast extract (Difco certified), and casein hydrolysate (Technical), each in concentration of 10 mg./l. Aliquots of 10 ml. of previously autoclaved pH buffered stock solutions with these three substances in appropriate amount were distributed to flasks. The entire pH range was employed, pH 5.0 to 7.5. After being plugged properly, the flasks were autoclaved in the usual fashion. Regarding number of flasks, the procedure was the same as that for the indoleacetic acid study.

The results (Table 8) for Martin were inconclusive. Redbine 60 roots with the addition of peptone, yeast extract, and casein hydrolysate increased in length in at least 50% of the cultures at pH's 6.0, 6.5, 7.0 and 7.5. Growth was obtained even though contamination occurred in 1/6 of the cultures.

<u>Sorghum vulgare</u> var. <u>sudanese</u> type Redbine 60						
solution	pH of solutions					
	5.0	5.5	6.0	6.5	7.0	7.5
control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	.1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	.1 (0)	0 (0)	0 (0)	0 (0)
	0 (0)	0 (1)	.2 (0)	.1 (0)	0 (0)	.1 (0)
IAA added	0 (0)	.5 (0)	0 (0)	.5 (0)	0 (0)	0 (0)
	0 (0)	.3 (2)	0 (1)	0 (0)	0 (0)	0 (0)
	.5 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	bacter. contam.	0 (0)
<u>Sorghum vulgare</u> var. <u>sudanese</u> type Martin						
solution	pH of solutions					
	5.0	5.5	6.0	6.5	7.0	7.5
control	0 (0)	.1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	.2 (0)	0 (0)	0 (2)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
IAA added	0 (0)	0 (0)	0 (0)	0 (0)	.3 (0)	0 (0)
	0 (0)	1.0 (0)	0 (0)	0 (0)	.3 (0)	0 (0)
	2.0 (0)	0 (0)	fungal contam.	.2 (0)	.3 (0)	0 (0)
	0 (0)	.2 (1)	0 (0)	0 (0)	0 (0)	0 (0)

Table 7. Growth increments, expressed in cm. units, within 1 week of 1.0 cm. root tips in White's modified solution, 0.01 M phosphate buffered, with the addition of 10^{-9} g./ml. IAA, 10 ml. of solution per culture vessel. (Lateral primordia indicated in parentheses).

<u>Sorghum vulgare</u> var. <u>sudanese</u> type Redbine 60						
solution	pH of solution					
	5.0	5.5	6.0	6.5	7.0	7.5
control	0 (0)	0 (0)	0 (3)	0 (0)	0 (0)	.7 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	.1 (0)	0 (0)	.3 (0)	0 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
peptone, yeast extract, & casein hydroly- sate added	0 (0)	0 (0)	0 (0)	.2 (0)	0 (0)	.5 (0)
	0 (0)	0 (1)	0 (0)	0 (0)	1.0 (0)	fungal contam.
	0 (0)	fungal contam.	.3 (0)	1.0 (0)	.2 (0)	.2 (1)
	0 (0)	fungal contam.	.2 (0)	fungal contam.	0 (0)	0 (0)
<u>Sorghum vulgare</u> var. <u>sudanese</u> type Martin						
solution	pH of solutions					
	5.0	5.5	6.0	6.5	7.0	7.5
control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (2)
	0 (0)	0 (1)	0 (0)	.3 (0)	0 (0)	0 (0)
	0 (0)	0 (1)	0 (0)	0 (0)	.4 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
peptone, yeast extract, & casein hydroly- sate added	0 (1)	0 (0)	0 (0)	.4 (0)	0 (0)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	0 (0)	.5 (0)	0 (0)	0 (1)	0 (1)	0 (0)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 8. Growth increments, expressed in cm., within 1 week of 1.0 cm. root tips in White's modified solution, 0.01 M phosphate buffered, with the addition of 10 mg./l. each of peptone, yeast extract, and casein hydrolysate, 10 ml. of solution and 1 root tip per flask; number of lateral primordia indicated in parentheses.

Root browning occurred within 1 week for each phase of work, including varied pH's, lactose substitution, addition of epicotyl extracts, coconut milk, L-tryptophane, indoleacetic acid, and Bacto-peptone, yeast extract, and casein hydrolysate in combination. The roots lacked substantial elongation and lateral root formation.

Because root tips universally turned brown within one week, browning root material was selected at random, smears made, and Gram's stain employed to determine the presence of internal fungal or bacterial contamination. In no case was such contamination found. The possibility of viral contamination does exist.

SUMMARY

Determination of a medium suitable for the indeterminate growth of excised Sudan grass roots, type Martin and type Redbine 60, was attempted, the root tips being cultured in darkness in a constant temperature chamber. Initially, White's standard medium for dicot root culture was used.

Alterations of this medium were made; the solution was phosphate buffered; lactose was substituted for sucrose; epicotyl extract, coconut milk, L-tryptophane, and indoleacetic acid were added separately, and peptone, yeast extract, and casein hydrolysate were added in combination. Clones of excised roots were not successfully established as growth ceased in all cases before initiation of the second passage.

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