TISSUE CULTURE AND DROUGHT RESISTANCE: GROWTH OF CACTUS (*ECHINOPSIS TURBINATA* L.) AND WHEAT (*TRITICUM AESTIVUM* L. EM. THELL. 'PONCA' AND 'KANKING')

By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF SYMBOLS

Cactus Experiments

The following symbols were used in the cactus results:

C - Callus

R - Root

Rs - Roots

S - Shoot

NC - New Callus

LNC - Little New Callus

SNC - Some New Callus

GNC - Good New Callus

VGNC - Very Good New Callus

W - White New Callus

T - Transparent New Callus

G - New Callus with Some Green Color

Re - Red Spots on New Callus

NoC - No Callus

Co - Contaminated

Da - Dark Callus

D - Dead

Wheat Experiments

The following symbols were used in the wheat results:

C - Callus

R - Root

Rs - Roots

CACE - Callus Along Cut End

CAX - Callus Along Explant

GCAX - Good Callus Along Explant

G - Green Spots Along Explant

Ctr - Center of Explant

Ch - Presence of Chlorophyll

NC - New Callus

NoG - No Growth

Br - Brown

D - Dead

Co - Contaminated

w/ - With

ND - No Data

CHAPTER I

INTRODUCTION

Wheat ranks first among world food crops, measured either by planted area or by size of harvest (Centro Internacional de Mejoramiento de Maiz y Trigo, 1976). The United States is the largest and most reliable exporter of grain. All countries except Canada, Australia, New Zealand, Argentina, and the Republic of South Africa have annual deficits and must import grain. The size of the granary in the United States is small compared to the projected increases in population (one billion more people by 1986). At the current level of production, the United States can feed only 50 million more people than live in the United States (Johnson, 1977). Clearly, production of wheat must increase to meet world food demands.

The southern Great Plains in the United States produces more hard red winter wheat than any other region in the United States. Despite the importance of this area for world wheat supplies, wheat land is being abandoned. For example, in 1976, 19.2 percent of the wheat land in Oklahoma was abandoned (Statistical Reporting Service, 1976). Two reasons account for this abandonment:

1. In the western part of the southern Great Plains where wheat is irrigated, the water table is falling due to irrigation, and irrigation water is increasing in cost, forcing many farmers to sell their farms (Mapp and Eidman, 1976).

2. Where farmers depend on natural rainfall to grow wheat, rains are unpredictable and suboptimum, causing a hazardous environment for growth. The primary reason for the high rate of wheat-land abandonment is drought. More than 95 percent of the wheat in Oklahoma is produced under dryland conditions.

Standard screening procedures for selecting drought-resistant plants entail prolonged, expensive operations in the growth chamber, greenhouse, and field (Hurd, 1976). Drought-screening techniques are urgently needed (Boyer and McPherson,1975; Hare, 1976). According to a report by the National Academy of Sciences (1976), resources for research on crop genetics and physiology in relation to adverse weather conditions are not adequate to insure a dependable food supply. Breakthroughs in wheat productivity are needed. Innovative approaches in the study of genetic variability must be undertaken now (Johnson, 1977). These new approaches, to be used in association with conventional breeding practices, must include techniques for differentiation of callus to produce complete plants.

Plant scientists around the world are making use of somatic cell culture techniques, with which they can evaluate large numbers of cells, each a potentially complete reproductive unit, with a small amount of cost, time, and labor compared to that required to evaluate full plants. Many crop species are being examined. But no one apparently has reported working with cultivars of wheat differing in drought resistance. It is important to study wheat since it is the main producer of both calories and protein for people of the world (Johnson, 1977). A panel, convened by the National Science Foundation, emphasized the need for tissue-culture work with wheat (Ozbun, 1976). Performance information

concerning drought resistance would greatly enhance the value of the world wheat collection which now contains 30,000 to 40,000 entries (Heyne and Campbell, 1976). This thesis reports tissue culture of drought-sensitive and drought-resistant cultivars of wheat.

Tissue culture of the cactus Echinopsis turbinata L. is also reported. Cacti, the majority of which are succulent, arid-land plants of varied habit, have thickened stems, which, besides functioning as water-storage organs, serve the plants also as photosynthetic organs, replacing the leaves, which are mostly either minute or early deciduous (Bailey, 1941). This type of structure lends itself to Crassulacean Acid Metabolism which enables the cactus to withstand long periods of drought. This characteristic of drought resistance is a desirable quality which is sought after in wheat. The general technique for doing tissue culture of cacti was known, so this was used to serve as a basis for developing tissue culture for wheat.

CHAPTER II

LITERATURE REVIEW

Plant tissue culture techniques have been developed during the last 40 to 50 years (White, 1932; Skoog, 1944; Skoog and Miller, 1957; Steward, Mapes, and Mears, 1958; Butenko, 1968; Kehr, 1975; Murashige, 1977). Recently, these techniques have been applied commercially and experimentally to grow plants.

For commercial purposes, in which rapid propagation is desired, tissue culture techniques to grow ornamental plants are being used (e.g., orchids, lilies, geraniums, gesneriads, gloxinia, gerbera, carnations) (Stone, 1963; Bertsch, 1967; Pillai and Hildebrandt, 1969; Chen and Holden, 1972, 1975; Murashige, Serpa, and Jones, 1974; Harmann and Kester, 1975; Kehr, 1975; Meyer, 1976; Davis et al., 1977; Stenberg, Chen, and Ross, 1977; Johnson, B. B., 1978a, 1978b). The development of whole crop plants from tissue culture is only at an experimental, rather than a commercial, stage. Many crop plants are being studied, including the following:

Dicotyledons

Alfalfa (Medicago sativa L.) (Saunders and Bingham, 1972; Walker, Yu, Sato, and Jaworski, 1978)

Bindweed (Convolvulus arvensis L.) (Ruesink, 1978)

Birdsfoot trefoil (Lotus corniculatus L.) (Saunders and Bingham, 1972)

Broad bean (Vicia faba L.) (Saunders and Bingham, 1972)

Carrot (Daucus carota L.) (Saunders and Bingham, 1972; Verma and Dougal, 1977)

Citrus (Citrus sinensis (L.) Osbeck) (Giladi, Altman, and Goren, 1977)

Clover (Trifolium sp.) (Saunders and Bingham, 1972)

Common Bean (Phaseolus vulgaris L.) (Saunders and Bingham, 1972)

Lupine (Lupinus sp.) (Saunders and Bingham, 1972)

Pea (Pisum sativum L.) (Saunders and Bingham, 1972)

Potato (Solanum tuberosum L.) (Murashige, 1977)

Soybean (Glycine max Merr.) (Saunders and Bingham, 1972; Hermina and Reporter, 1977)

Tobacco (Nicotiana tabacum L.) (Saunders and Bingham, 1972; Einset, 1977; Weatherhead, Burdon, and Henshaw, 1978)

Tomato (Lycopersicon esculentum Mill) (Meredith, 1978; Tal, Heikin, and Dehan, 1978)

White sweet clover (Trifolium repens L.) (Saunders and Bingham, 1972)

Monocotyledons

Asparagus (Asparagus sp.) (Saunders and Bingham, 1972)

Banana (Musa paradisiaca var. sapientum Kuntze) (Murashige, 1977)

Barley (Hordeum vulgare L.) (Yamada, 1977; Kartel and Maneshina, 1978; Shamina, Scheunert, and Koblitz, 1978)

Big bluestem (Andropogon gerardii Vitman) (Chen, Stenberg, and Ross, 1977)

Bromegrass (Bromus inermis Leyss.) (Yamada, 1977)

Flax (Linum usitatissimum L.) (Gamborg and Skyluk, 1976)

Maize (Zea mays L.) (Green and Phillips, 1975; Oswald, Nicholson, and Bauman, 1977; Yamada, 1977; Butenko, Kuznetsov, Skripka, and Zyat'kova, 1978)

Millet (Panicum miliaceum L.) (Yamada, 1977)

Oats (Avena sativa L.) (Cummings, Green, and Stuthman, 1976; Lörz, Harms, and Potrykus, 1976; Yamada, 1977)

Orchard grass (Dactylis glomerata L.) (Conger, Carabia, and Lowe, 1978)

Pineapple (Ananas comosus Merr.) (Murashige, 1977)

Rice (Oryza sativa L.) (Yamada, 1977)

Rye (Secale cereale L.) (Yamada, 1977)

Ryegrass (Lolium multiflorum Lam.) (Anderson and Stone, 1978; Conger et al., 1978)

Ryegrass (Lolium perenne L.) (Yamada, 1977)

Sorghum (Sorghum vulgare Pers.) (Yamada, 1977)

Sugarcane (Saccharum officinarum L.) (Koga and Kudo, 1977)

Tall fescue (Festuca arundinaceae Schreb.) (Conger et al., 1978)

Triticale (cross between wheat and rye) (Taira and Larter, 1978)

Wheat (Triticum aestivum L. em. Thell; T. dicoccum Schubl.; T. durum Desf.; T. monococcum L.) (Nakai and Shimada, 1975; Farmer and Lee, 1977; Yamada, 1977; Bennici and D'Amato, 1978; Collins, Vian, Phillips, 1978; Donovan and Lee, 1978)

Wheat-rye enbryos (Taira and Larter, 1978)

In general, the literature dealing with monocotyledons, especially the cereals, is scant compared to that concerning dicotyledons (Schenk and Heldebrandt, 1972; Sunderland, 1977; Yamada, 1977; King, Potrykus, and Thomas, 1978).

The experimental objectives with the crop plants are (Saunders and Bingham, 1972):

1. To produce plants which are genetically the same.
2. To produce plants which are genetically different.
3. To produce plants that are polyploid.
4. To produce plants that are haploid.

The production of anther-derived haploids in crop plants is receiving much study now, especially in the cereals (Clapham, 1977; Collins, 1977; King et al., 1978), including barley (Foroughi-Wehr, Mix, Gaul, and Wilson, 1976; Gonzalez-Medina and Bouharmont, 1978), triticale (Wang, Sun, Wang, and Chien, 1973; Sun, Wang, and Chu, 1974), and wheat (Ouyang, Hu, Chuang, and Tseng, 1973; Wang, Chu, Sun, Wu, Yin, and Hšu, 1973; Craig, 1975; Shimada and Makino, 1975; Picard and de Buyser, 1975, 1977; Rives and Picard, 1977; Schaeffer, Baenziger, and Worley, 1979).

Different species of wheat have different growth requirements in tissue culture. Durum wheats (Triticum durum Desf.) appear relatively easy to culture. Whole plants developing from durum-wheat callus have been reported several times (Mascarenhas, Pathak, Hendre, and Jagannathan, 1975; Mascarenhas, Pathak, Hendre, Ghugale, and Jagannathan, 1975; Bennici and D'Amoto, 1978). The basic propensity of common wheats (Triticum aestivum L. em. Thell.) is to form roots, and rarely, shoots (Gamborg and Eveleigh, 1968; Trione, Jones and Metzger, 1968; Shimada, Saskuma, and Tsunewaki, 1969; Prokhorov, Charnova, and Filin-Koldakov, 1974; Bhojwani and Hayward, 1977; Chin and Scott, 1977; Cure and Mott, 1978; O'Hara and Street, 1978; Pental and Gunckel, 1978; Shimada, 1978; Sánchez de Jiménez and Murillo, 1979). The growth of whole plants (shoots and roots) from

common wheat has been sporadic and results have not been reproducible (Bhojwani and Hayward, 1977; King et al., 1978).

Alfalfa, soybean, tobacco, carrot, sorghum, sweet clover, cabbage (Brassica oleracea var. capitata L.) and tomato are being grown, using tissue culture techniques, to study salt-tolerance and aluminum-tolerance (Mercado and Gollek, 1970; Nabors, 1976a, 1976b; Venne, 1976; Meridith, 1978; Croughan, Stavarek, and Rains, 1978; Herth and Meyer, 1978; Tal et al., 1978). Work on wheat has not looked at the difference in callus formation among cultivars varying in tolerance to salt, metals, or drought. King et al. (1978) suggested that drought resistance might be studied in vitro. The objective of the present study was to determine the difference in callus formation by a drought-resistant and a drought-sensitive cultivar of wheat.

Wheat was not used in the first experiments because the literature indicates difficulty in getting growth. To establish tissue culture techniques, a cactus was used. It was chosen as it is a drought-resistant plant. Cacti callus can withstand a remarkable degree of desiccation (Mauseth, 1977). Methods for tissue culture of cacti have been developed (King, 1957; Sachar and Iyer, 1959; Steinhart, 1962; Colomas, 1971; Minocha and Mehra, 1974; Mauseth and Halperin, 1975; Johnson, Koenigsberg, and Langhans, 1976; Mauseth, 1976; Johnson and Emino, 1977a, 1977b, 1979; Mauseth, 1977; Johnson, J. L., 1978). Fourteen experiments were done: nine with cactus and five with wheat. Tissue culture work with the cactus used in the experiments (Echinopsis turbinata L.) have not been reported. Two

cultivars of wheat (Triticum aestivum L. em. Thell.) were used: "Ponca," a drought-sensitive cultivar, and "KanKing," a drought-resistant cultivar (Todd and Webster, 1965).

CHAPTER III

MATERIALS AND METHODS

Cactus Experiments

Echinopsis turbinata L. (Cactaceae) is a low, growing cactus that originates from Argentina (Bailey and Bailey, 1942). It has 13 to 14 ribs, running longitudinally, with clusters of spines along the ribs. The spines are about 0.5 cm long. Two basal media were used to culture the cactus. These media are presented in Table I. These basal media were supplemented with different amounts of plant growth regulators in each experiment. The growth regulators used, their abbreviations, their molecular weights, reference for their structure, and the method of mixing stock solutions are as follows:

Auxins:

2,4-dichlorophenoxyacetic acid, 2,4-D, molecular weight 221 (Thimann, 1969); dissolved 50 mg 2,4-D with 10 to 15 ml 95% ETOH, then diluted to 25 ml with water.

indole-3-acetic acid, IAA, molecular weight 175 (Thimann, 1969); dissolved 50 mg IAA with 5 to 10 ml 0.5N KOH, then diluted to 25 ml with water.

indole-3-butyric acid, IBA, molecular weight 189 (Thimann, 1969); dissolved 50 mg IBA with 10 to 15 ml 95% ETOH, then diluted to 25 ml with water.

naphthalene-1-acetic acid, NAA, molecular weight 186 (Thimann,

TABLE I

MEDIA AND CONTENTS

Ingredients	Murashige and Skoog (MS) Medium[1] 1962 (mg/l)	Gamborg's B5 (B5) Medium[2] 1975 (mg/l)
$NaH_2PO_4.H_2O$		150
KNO_3	1900	2500
$(NH_4)_2SO_4$		134
$MgSO_4.7H_2O$	370	250
NH_4NO_3	1650	
KH_2PO_4	170	
Na_2EDTA	37.30	37.30
$FeSO_4.7H_2O$	27.80	27.80
$CaCl_2.2H_2O$	435	150
H_3BO_3	6.20	33
$MnSO_4.4H_2O$	22.30	
$ZnSO_4.7H_2O$	8.60	2
$Na_2MoO_4.2H_2O$	0.25	0.25
$CuSO_4.5H_2O$	0.025	0.025
$CoCl_2.6H_2O$	0.025	0.025
$MnSO_4.H_2O$		10
KI	0.75	0.75
Nicotinic acid	1	1
Thiamine.HCl	10	10
Pyridoxine.HCL	1	1
Myo-Inositol	100	100
Agar	5,500	5,500
Sucrose	30,000	20,000

Adjust final pH to 5.8 for MS media and 5.5 for B5 media with 0.2 N KOH or 0.2 N HCl.

Deionized water was used to mix all stock solutions and media.

[1]T. Murashige and F. Skoog. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15 (1962).

[2]O. L. Gamborg, Callus and cell culture, Plant tissue culture methods (1975).

1969); dissolved 50 mg NAA with 10 to 15 ml 95% ETOH, then diluted to 25 ml with water.

p-chlorophenoxyacetic acid, pCPA, molecular weight 187 (Thimann, 1969); dissolved 50 mg pCPA with 10 to 15 ml 95% ETOH, then diluted to 25 ml with water.

Cytokinin:

6-furfurylaminopurine, kinetin, molecular weight 215 (Fox, 1969); dissolved 50 mg kinetin with 5 to 10 ml 0.5N HCl, then diluted to 25 ml with water.

Gibberellin:

gibberellic acid, GA_3, molecular weight 346 (Cleland, 1969); dissolved 50 ml GA_3 to 25 ml with water.

To obtain explants from the cactus, the spines were trimmed. The whole plant then was soaked in saturated solution of benomyl, a fungicide, and water for four hours. It then was rinsed with a solution of Tween 20, Clorox, and water for 15 minutes. Then it was rinsed several times with sterile water under the hood (Catalog No. 11,000, Labconco Corporation, Kansas City, Missouri). The plant was sliced between the ridges. Each axil, with a cluster of clipped spines (an explant), was removed from the wedged slices and placed on the media in glass test tubes (15 cm long; 2.5 cm diameter) with plastic covers. Each explant was about 0.5 cm long x 0.5 cm wide.

Two media were used: one low in salts and one high in salts. The medium developed at the University of Wisconsin, Madison, Wisconsin, by Murashige and Skoog (1962) for tobacco-tissue culture has a high content of nitrate, potassium, and ammonium (Gamborg, 1975). The B5 medium, developed at the Prairie Regional Laboratory, National Research Council

of Canada, Saskatoon, Saskatchewan, Canada, by Gamborg (1975) for growing soybean tissue has a general concentration of inorganic nutrients which is lower than in the Murashige and Skoog medium.

The test tubes were placed in a growth chamber (Model E.54B, Percival, Boone, Iowa). The light intensity, provided by a combination of cool-white fluorescent and incandescent lamps, was 100 μE m^{-2} sec^{-1} at the top of the test tubes for 12 hours. The temperature in the growth chamber was 24°C and 19°C for day and night, respectively, each being 12 hour periods. The relative humidity varied between 36 to 42 percent for day and 40 to 52 percent for night.

After the explants were placed in tubes they were observed and recorded in days from culture time. The number of days and former media for explants will be shown in the following tables.

Data for Each Experiment

Cactus Experiment 1 (Table II)

Explants from E. turbinata L. were excised January 22, 1979, and placed onto B5 medium with the growth regulators, kinetin, ranging from 0 to 6450 μg/l, with an NAA constant of 180 μg/l, NAA ranging from 0 to to 1400 μg/l with a kinetin constant of 215 μg/l and 2,4-D; half of each treatment had 1100 μg/l 2,4-D added to it and half contained none. Due to an error in calculation of concentration, the amounts are small, so they will be shown in μg/l instead of mg/l, in Table II. The observations days are also shown in Table II.

Cactus Experiment 2 (Table III)

Explants from the cactus E. turbinata L. were excised February 28,

TABLE II

OBSERVATION DAYS FOR CACTUS EXPERIMENT 1

µg/l 2,4-D	Rep. No.	Kinetin (180 µg/l NAA Constant) µg/l 0	430	860	1290	1720	2150	3230	4300	5380	6450
1100	1	10		4	4	4	4	18	58	23	4
1100	2	23		35 200	35	10	51		10	4	4
0	1	23		4	18	10	120 133	35 44	10	10	4
0	2			10	18	4	23	10	23	23	4

µg/l 2,4-D		NAA (215 µg/l Kinetin Constant) µg/l 0	90	190	280	370	470	700	930	1170	1400
1100	1	4		4	10	23	23	23	23	4	10
1100	2			4	4	18	51	28	35	10	10
0	1		35	18		10	23	10	23	4	18
0	2		10	18	35 88	23	4	44	23	10	18

TABLE III

OBSERVATION DAYS FOR CACTUS EXPERIMENT 2

µg/l 2,4-D	Rep. No.	Kinetin (182 µg/l NAA Constant) 0	436	872	1310	1740	µg/l 2180	3270	4360	5540	6540
1100	1	14	7	14	14	14	21	7	7 239	7	7
1100	2	14	7	14	28	21	14	7	14	14	14
0	1		21 44 139	28	7	7 37 239	21	14	21	75	28
0	2	14	103	51	51	7	7	51	28 68	7	28 239

µg/l 2,4-D	Rep. No.	NAA (215 µg/l Kinetin Constant) 0	90	185	278	370	µg/l 460	695	930	1160	1390
1100	1	7	7	14	7	7	7	14	7	14 103 125	7
1100	2	7	14	14	14	14	7	7	7	7 68	21 37 68
0	1	239	7	7	7	7 68	7 37	7	7	21	14
0	2	7	7	14	14	7	7 75	7	14	21	7

1979, and placed onto MS medium with the growth regulators kinetin, ranging from 0 to 6540 µg/l with an NAA constant of 182 µg/l, NAA, ranging from 0 to 1390 µg/l with a kinetin constant of 215 µg/l, and 2,4-D; half of each treatment had 1100 µg/l 2,4-D added to it and half contained none. Because the cactus did not grow well in Gamborg's (1975) B5 medium (see Table X), Murashige and Skoog's (1962) basal medium was used. As stated before, it has a higher concentration of inorganic salts than does Gamborg's B5 medium (Gamborg, 1975). Due to an error in calculation of concentration, the amounts are small, so they will be shown in µg/l instead of mg/l in Table III. The observation days are shown in Table III also.

Cactus Experiment 3 (Table IV)

New callus from the cactus callus explant was transferred June 6, 1979, onto MS medium with the growth regulators kinetin ranging from 5 to 30 mg/l, NAA ranging from 2 to 15 mg/l, and IAA ranging from 2 to 30 mg/l. The explant came from Cactus Experiment 2. The days of the observations for Table IV and the concentrations of the kinetin in the media from which the explant was transferred are shown in Table IV. The callus of the original explants all grew with 1100 µg/l 2,4-D and 182 µg/l NAA constant (see Experiment 2).

Cactus Experiment 4 (Table V)

New callus from the cactus callus explant was transferred August 7, 1979, onto MS medium with the growth regulators, kinetin ranging from 5 to 20 mg/l, IBA ranging from 0 to 45 mg/l, GA_3 ranging from 0 to 45 mg/l, and pCPA ranging from 0 to 45 mg/l. The callus came from Cactus

TABLE IV

OBSERVATION DAYS AND PREVIOUS KINETIN CONCENTRATIONS FOR CACTUS EXPERIMENT 3

mg/l Kinetin	NAA (mg/l) 2	3	5	6	7	8	9	10	12	15
	13	75	75	19	75	13	13	77	13	13
5	3.270	3.270	3.270	3.270	3.270	4.360	4.360	4.360	4.360	4.360
10	75	13	13	75	75	75	13	27	75	13
	3.270	3.270	3.270	3.270	3.270	4.360	4.360	4.360	4.360	4.360
20	13	13	75	75	13	75	75	75	19	13
	3.270	3.270	3.270	3.270	3.270	4.360	4.360	4.360	4.360	4.360
30	13	13	75	75	75	75	75	13	75	75
	3.270	3.270	3.270	3.270	3.270	4.360	4.360	4.360	4.360	4.360
mg/l Kinetin	IAA (mg/l) 2	3	5	7.5	10	12.5	15	20	25	30
5	13	75	75	13	13	13	13	13	13 41	13
	0.436	0.436	0.436	0.436	0.436	1.310	1.310	1.310	2.180	2.180
10	13	13	19	13	13	13	13	13	19	13
	0.436	0.436	0.436	0.436	0.436	1.310	1.310	1.310	2.180	2.180
20	13	13	13	13	13	19	13	13	19	13
	0.436	0.436	0.436	0.436	0.436	1.130	1.310	2.180	2.180	2.180
30	13	19	13	13	13	19	13	13	13	13
	0.436	0.436	0.436	0.436	0.436	1.130	1.310	2.180	2.180	2.180

TABLE V

OBSERVATION DAYS AND PREVIOUS KINETIN/NAA CONCENTRATIONS
FOR CACTUS EXPERIMENT 4

mg/l Kinetin	IBA (mg/l) 0	5	10	15	20	25	30	35	40	45
5	57 1740-182	57 1740-182	41 1740-182	41 1740-182	57 1740-182	41 1740-182	41 1740-182	41 1740-182	41 6540-182	41 6540-182
10	57 1740-182	41 1740-182	41 1740-182	57 1740-182	57 1740-182	41 1740-182	57 1740-182	41 1740-182	41 6540-182	41 6540-182
15	57 1740-182	41 1740-182	57 1740-182	41 1740-182	41 1740-182	41 1740-182	57 1740-182	57 1740-182	57 6540-182	41 6540-182
20	57 1740-182	57 1740-182	41 1740-182	41 1740-182	41 1740-182	57 1740-182	41 1740-182	41 6540-182	41 6540-182	41 6540-182

mg/l Kinetin	GA_3 (mg/l) 0	5	10	15	20	25	30	35	40	45
5	41 6540-182	41 6540-182	57 6540-182	57 6540-182	57 6540-182	41 6540-182	41 1740-182	41 1740-182	41 1740-182	41 1740-182
10	41 6540-182	57 6540-182	41 6540-182	57 6540-182	41 6540-182	41 6540-182	41 1740-182	41 1740-182	57 1740-182	41 1740-182
15	41 6540-182	41 6540-182	41 6540-182	41 6540-182	41 6540-182	41 6540-182	41 1740-182	41 1740-182	41 1740-182	41 1740-182

TABLE V (Continued)

mg/l Kinetin	GA (mg/l) 0	5	10	15	20	25	30	35	40	45
20	41 6540-182	41 6540-182	41 6540-182	41 6540-182	41 6540-182	41 6540-182	41 1740-182	57 1740-182	57 1740-182	41 1740-182
mg/l Kinetin	pCPA (mg/l) 0	5	10	15	20	25	30	35	40	45
5	41 1310-182	41 1310-182	41 1310-182	41 215-930	41 215-930	41 215-930	41 215-930	41 215-930	41 6540-182	41 6540-182
10	41 1310-182	41 1310-182	41 1310-182	41 215-930	41 215-930	41 215-930	41 215-930	41 215-930	41 6540-182	41 6540-182
15	41 1310-182	41 1310-182	41 1310-182	41 215-930	41 215-930	41 215-930	41 215-930	41 215-930	41 6540-182	41 6540-182
20	41 1310-182	41 1310-182	41 1310-182	41 215-930	41 215-930	41 215-930	41 215-930	41 215-930	41 6540-182	41 6540-182

Experiment 2. The days of the observations and the concentration of kinetin-NAA, respectively (shown in µg/l), used to grow the original callus are shown in Table V. All original callus grew with 1100 µg/l 2,4-D (see Experiment 2). No auxin was used in this experiment.

Cactus Experiment 5 (Table XIV)

New callus from the cactus callus explant was transferred September 7, 1979, onto MS medium with the growth regulators, kinetin ranging from 5 to 30 mg/l and IAA ranging from 10 to 40 mg/l. New callus was rated on a scale of 1 to 10 with 1 being equal to 10 percent coverage and 10 being equal to 100 percent coverage of the explants. The explant came from Cactus Experiment 2. The days of the observations are noted at the top of each column. The concentration of kinetin, NAA, and 2,4-D used to grow the original callus was not noted. This may affect the results slightly. The results will be shown in means of four replications.

Cactus Experiment 6 (Table XV)

Because the high concentration of kinetin inhibited callus formation in Cactus Experiment 5 (Table XIV), the kinetin concentration was lowered in Cactus Experiment 6 to 1 through 4 mg/l; the IAA concentration stayed the same. New callus from the cactus callus explant was transferred October 30, 1979, onto MS medium with the growth regulators, kinetin and IAA. The explant came from Cactus Experiment 5 which had been transferred from Cactus Experiment 2. The new callus was rated according to color in this experiment, looking for a green color, showing the presence of chlorophyll. The days of the observations are noted

at the top of each column. The concentration of the growth regulators used in the former media of the explant was not noted. This may affect the results slightly.

Cactus Experiment 7 (Table VI)

New callus from the cactus callus explant was transferred November 28, 1979, onto MS media with the growth regulators kinetin ranging from 20 to 60 mg/l and pCPA at 5 and 10 mg/l. The explant came from the kinetin-pCPA treated media of Cactus Experiment 4. The days of the observations and the concentration of kinetin and pCPA, shown in mg/l, used to grow the original callus are shown in Table VI.

Cactus Experiment 8 (Table VII)

New callus from the cactus callus explant was transferred December 11, 1979, onto MS medium with the growth regulators, kinetin ranging from 30 to 70 mg/l, IAA, ranging from 2 to 8 mg/l, and NAA ranging from 4 to 8 mg/l. The callus explant came from Cactus Experiment 5. The days of observation are noted at the top of each column. The concentration of IAA/kinetin, respectively (in mg/l), used to grow the original callus is shown in Table VII.

A mistake in pipetting was made in the second half of this experiment, in which NAA and kinetin were used. Consequently, data are missing in the second part of Tables VII and XVII.

Cactus Experiment 9 (Table VIII)

Six new plants with roots and shoots, which developed from explants of Cactus Experiment 2, were transplanted on February 4, 1980, from the

TABLE VI

OBSERVATION DAYS AND PREVIOUS GROWTH REGULATOR CONCENTRATIONS FOR CACTUS EXPERIMENT 7

mg/l Kinetin	pCPA (mg/l) 5		10	
	14	41	14	41
20	5-15	5-15	5-20	5-20
20	5-15	5-15	5-20	5-20
20	5-15	5-15	5-20	5-20
20	5-15	5-15	5-20	5-20
30	5-25	5-25	5-30	5-30
30	5-25	5-25	5-30	5-30
30	5-25	5-25	5-30	5-30
30	5-25	5-25	5-30	5-30
40	10-5	10-5	10-10	10-10
40	10-5	10-5	10-10	10-10
40	10-5	10-5	10-10	10-10
40	10-5	10-5	10-10	10-10
50	15-5	15-5	15-25	15-25
50	15-5	15-5	15-25	15-25
50	15-5	15-5	15-25	15-25
50	15-5	15-5	15-25	15-25
60	20-5	20-5	20-20	20-20
60	20-5	20-5	20-20	20-20
60	20-5	20-5	20-20	20-20
60	20-5	20-5	20-20	20-20

TABLE VII

OBSERVATION DAYS AND PREVIOUS IAA/KINETIN CONCENTRATIONS
FOR CACTUS EXPERIMENT 8

mg/l Kinetin	IAA (mg/l) 2		4		2		4		6		8		6		8	
	29	64	29	64	29	64	29	64	29	64	29	64	29	64	29	64
30	10-20		10-20		10-20		10-20		10-20		10-20		10-20		10-20	
40	10-20		10-20		10-20		10-30		10-20		10-20		10-30		10-30	
50	10-30		10-30		10-30		10-30		10-30		10-30		20-30		10-10	
60	10-10		10-10		20-20		20-20		20-20		20-20		20-20		20-20	
70	10-10		10-10		20-20		20-20		20-20		20-20		20-20		20-20	
mg/l Kinetin	NAA (mg/l) 0		4		0		4		6		8		6		8	
30	. . .		30-30		. . .		30-30		30-30		30-30		30-30		30-30	
40	. . .		30-30		. . .		30-30		30-30		30-30		30-30		30-30	
50	. . .		30-30		. . .		10-5		10-5		10-5		10-5		10-5	
60	. . .		20-10		. . .		30-20		20-10		20-10		30-20		30-20	
70	. . .		30-20		. . .		30-20		30-20		30-20		30-20		30-20	

original culture tubes, into a soil-less medium, consisting of one part sand and two parts vermiculite (volume basis). The medium was placed in a "6-pak," a plastic container 13 cm long, 13 cm wide, and 6 cm tall which had six separate sections, one for each plant. Plants were removed from test tubes and the excess agar-medium was washed off with sterile water. The plants were put in the soil-less media which has been wet thoroughly and drained. After transplanting, the medium was treated with a dilute solution of benomyl and deionized water plus one drop of Tween 20 to act as a wetting agent. The 6-pak with plants was put on a tray and covered with a clear plastic bag and placed near a west-facing window in the laboratory (Room 303, Agriculture Hall, Oklahoma State University). The covering was removed for short periods of time daily, starting two weeks after the plants were planted. The time was gradually increased each day until the covering could be left off completely, which was about 10 to 12 days. The plants were fertilized with a dilute solution of Peter's 15-30-15 each watering.

Table XVIII (p. 38) shows the size of the cacti at the time of transplanting, February 4, 1980, and two months later. The concentrations of the growth regulators used to grow the plants are shown in Table VIII.

Summarization of Treatments

Table IX summarizes the growth regulator treatments from the first eight cactus experiments.

TABLE VIII

PREVIOUS GROWTH REGULATOR CONCENTRATIONS OF PLANTS IN EXPERIMENT 9

Plant No.	µg/l 2,4-D	NAA	Kinetin
1	0	182	436
2	0	182	1740
3	0	182	6540
4	0	370	215
5	1100	116	215
6	1100	137	215

TABLE IX

SUMMARY CHART OF GROWTH REGULATORS USED IN EXPERIMENTS 1 THROUGH 8

Exp. No.	Table No.	Basal Medium	2,4-D	IAA	NAA	pCPA	IBA	GA	Kinetin
			mg/l						
1	II	B5	0-1.1	---	0-1.40	---	---	---	0-6.45
2	III	MS	0-1.1	---	0-1.39	---	---	---	0-6.54
3	IV	MS	---	2-30	2-15	---	---	---	5-30
4	V	MS	---	---	---	0-45	0-45	0-45	5-20
5	XIV	MS	---	10-40	---	---	---	---	5-30
6	XV	MS	---	10-40	---	---	---	---	1-4
7	VI	MS	---	---	---	5-10	---	---	20-60
8	VII	MS	---	2-8	---	---	---	---	30-70
8	VII	MS	---	4-8	---	---	---	---	30-70

CHAPTER IV

RESULTS OF CACTUS EXPERIMENTS

Observations and previous concentrations are shown in Chapter III. Symbols for the results are shown in the List of Symbols.

Cactus Experiment 1 (Table X)

Many explants died or became contaminated. Four explants produced callus when the concentration of kinetin was varied and four explants produced callus when the concentration of NAA was varied. The occurrence of callus seemed random and not related to concentrations of kinetin, 2,4-D, or NAA. When roots and shoots developed, they appeared to come from the explant, not the callus tissue.

Cactus Experiment 2 (Table XI)

Callus formed from many explants with the Murashige and Skoog medium (Table XI). When grown with varying concentrations of kinetin and NAA, 24 and 31 explants formed callus, respectively. There were more dead explants when no 2,4-D was used than when 1100 ug/l 2,4-D was incorporated into the medium. But callus did form without 2,4-D. At 0 ug/l NAA, no callus formed, or explants died, except for one explant, with 0 ug/l 2,4-D. No auxin seemed to inhibit callus development. The roots and shoots that developed appeared to come from the explant and not from callus. Roots and shoots were formed both at low and high

TABLE X

CACTUS EXPERIMENT 1

		Kinetin (180 µg/l NAA constant) µg/l									
µg/l 2,4-D	Repli-cation #	0	430	860	1290	1720	2150	3230	4300	5380	6450
1100	1	CO	...	Co	Co	Co	Co	Co	C	D	Co
1100	2	D	...	C R,S	C	Co	D	...	Co	D,Co	Co
0	1	D	...	D	Co	Co	1 R 2 R	C R,S	Co	Co	Co
0	2	...	...	Co	Co	Co	Co	Co	D	D	D

		NAA (215 µg/l kinetin constant) µg/l									
ug/l 2,4-D	Repli-cation #	0	90	190	280	370	470	700	930	1170	1400
1100	1	D	...	D,Co	Co	D	D	Co	D,Co	Co	Co
1100	2	...	...	D,Co	D,Co	Co	D	C	C	Co	Co
0	1	...	C	Co	...	Co	D	Co	D	Co	D,Co
0	2	...	Co	D	C 1,R	D	D,Co	Co	D	Co	Co

TABLE XI

CACTUS EXPERIMENT 2

		Kinetin (182 µg/l NAA constant) µg/l									
µg/l 2,4-D	Replication #	0	436	872	1310	1740	2180	3270	4360	5540	6540
1100	1	C	C	C	C	C	C	C	C R,S	D	C
1100	2	C	C	C	C	C	C	C	C	D	C
0	1	...	C S R,S	Co	C	C S R	Co	Co	C	D	C
0	2	C	D	D	D	D	D	D	S R	D	S R

		NAA (215 µg/l kinetin constant) µg/l									
ug/l 2,4-D	Replication #	0	90	185	278	370	460	695	930	1160	1390
1100	1	D	C	C	C	C	C	C	C	C S R	C
1100	2	D	C	C	C	C	C	C	C	C S	C S R
0	1	NoC	Co	C	C	C S,R	C S	D	D	C	Co
0	2	C	D	C	C	D	C R	C	C	C	C

concentrations of kinetin. Under varying NAA concentrations, roots and shoots formed only when concentration of NAA was 370 µg/l.

Cactus Experiment 3 (Table XII)

More new callus formed when the original callus was transferred to media with varying concentrations of IAA than with varying concentrations of NAA. Kinetin concentration did affect callus formation. More new callus formed when the concentration of kinetin was low (5 mg/l) in the media + NAA, however, on the media with IAA the lowest concentration of kinetin (5 mg/l) inhibited callus growth. Roots developed from the new callus in only one case (25 mg/l IAA; 5 mg/l kinetin). No shoots developed from the new callus.

Cactus Experiment 4 (Table XIII)

No callus formed when either IBA or GA was used. New callus formed when pCPA was used. The kinetin concentration had little effect on new callus production. More new callus was formed when the kinetin concentration was 5 mg/l (lowest concentration in the experiment) and when it was 20 mg/l (highest concentration in the experiment). The low and high concentrations of pCPA inhibited the growth of new callus. No callus formed when the pCPA was 0 mg/l (lowest concentration). Callus did not form when the concentration of pCPA was 40 or 45 mg/l (highest concentrations); however, this may be the effect of the high kinetin: low NAA (6.54 mg/l:0.182 mg/l) concentration from the former media. Out of the 14 new callus-producing explants formed with pCPA, 10 of the original explants had grown with 0.93 mg/l NAA and four had grown with 0.185 mg/l NAA. Since more new callus was formed with the higher

TABLE XII

CACTUS EXPERIMENT 3

	NAA mg/l									
mg/l Kinetin	2	3	5	6	7	8	9	10	12	15
5	NC	Noc	NoC	NC	NoC	NC	NC	NoC	NC	NC
10	NoC	NC	NC	NoC	NoC	NoC	NC	Co	NoC	NC
20	Co	Co	NoC	NoC	NC	NoC	NoC	NoC	NC	NC
30	NC	NC	NoC	NoC	NoC	NoC	NoC	NC	NoC	NoC

	IAA mg/l									
mg/l Kinetin	2	3	5	7.5	10	12.5	15	20	25	30
5	NC	NoC	NoC	NC	NC	NC	NC	NC	NC R	NC
10	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
20	NC	NC	NC	NC	Co	NC	NC	NC	NC	NC
30	NC	NC	NC	NC	Co	NC	NC	NC	NC	NC

TABLE XIII

CACTUS EXPERIMENT 4

	IBA mg/l									
mg/l Kinetin	0	5	10	15	20	25	30	35	40	45
5	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC
10	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC
15	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC
20	NoC	NoC	Noc	NoC	NoC	NoC	NoC	NoC	NoC	NoC

	GA mg/l									
mg/l Kinetin	0	5	10	15	20	25	30	35	40	45
5	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC
10	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC
15	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC
20	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC	NoC

	pCPA mg/l									
mg/l Kinetin	0	5	10	15	20	25	30	35	40	45
5	NoC	NoC	NoC	NC	NC	NC	NC	NoC	NoC	NoC
10	NoC	NC	NC	NoC	NC	NoC	NoC	NoC	NoC	NoC
15	NoC	NC	NoC	NoC	NoC	NC	NoC	NC	NoC	NoC
20	NoC	NC	NoC	NoC	NC	NC	NC	NoC	NoC	NoC

concentration of NAA, this suggested that the NAA in the original tissue might have aided new callus formation.

Cactus Experiment 5 (Table XIV)

The highest concentration of kinetin used in this experiment (30 mg/l) inhibited new callus formation. The best growth of new callus occurred when the kinetin and IAA concentrations were 5 mg/l and 20 mg/l, respectively. On October 4, 1979, within one month after transfer of the original callus (on September 7), all four replications in this treatment, except one (90% coverage), had 100% coverage of the old callus with new callus.

Cactus Experiment 6 (Table XV)

New callus formed at all concentrations of kinetin (1-4 mg/l) and at all concentrations of IAA (10-40 mg/l). New callus tended to be more transparent when the kinetin and IAA concentrations were low (1 and 10 mg/l, respectively). Forty-three days after culturing, it was observed that at 1 mg/l kinetin, two explants produced a green callus, but at 4 mg/l kinetin, 10 green masses were produced.

Cactus Experiment 7 (Table XVI)

The best new callus grew with the highest kinetin concentration (60 mg/l) and the lower pCPA concentration (5 mg/l). This contradicted results from Cactus Experiment 5 (Table VI) in which the best callus growth occurred with a low concentration of kinetin (5 mg/l). But in Cactus Experiment 5, IAA was the auxin and in this experiment, pCPA was the auxin. Low concentrations of kinetin with IAA, therefore, appear

TABLE XIV

PERCENT NEW CALLUS COVERAGE IN CACTUS EXPERIMENT 5

mg/l Kinetin	IAA mg/l 10			20			30			40		
	Days After Culturing											
	12	27	33	12	27	33	12	27	33	12	27	33
5	3.25	7.25	8.5	7.25	9.75	10	6.25	9	10	8	9.5	9.75
10	6	9	9.25	1.75	5	6.75	3.75	6.5	8.5	4	7	8
20	3.75	5.5	6.25	1.5	3.5	3.75	2	3.25	4.5	3.25	6	6.25
30	.05	1.5	3	.25	1.75	2.75	.05	3.75	6.5	1.25	3.5	4.25

TABLE XV

CACTUS EXPERIMENT 6

mg/l Kinetin	IAA mg/l 10			20			30			40		
	10	43	91	10	43	91	10	43	91	10	43	91
1	NC	W	TG	NC	W	TG	NC	W	...[z]	NC	W	...
1	NC	W	TG	NC	W	T	Co	...	...	NC	W	...
1	NC	W	TG	NC	G	TG	NC	T	...	NC	G	...
1	NC	W	TG	NC	W	W	NC	W	...	NC	W	...
2	NC	W	TG	NC	W	W	NC	W	...	NC	W	...
2	NC	G	TG	NC	W	G	NC	G	...	NC	W	...
2	NC	W	TG	NC	W	T	NC	G	...	NC	T	...
2	NC	W	W	NC	W	T	NC	T	...	NC	T	...
3	NC	W	TG	NC	W	G	NC	W	...	NC	W	...
3	NC	W	G	NC	W	W	NC	W	...	NC	W	...
3	NC	W	TG	NC	W	TG	NC	T	...	NC	G	...
3	NC	W	G	NC	W	G	NC	W	...	NC	W	...
4	NC	G	G	NC	W	T	NC	W	...	NC	W	...
4	NC	W	G	NC	W	T	NC	G	...	NC	G	...
4	NC	G	G	NC	G	TG	NC	T	...	NC	G	...
4	NC	G	W	NC	G	W	NC	G	...	NC	G	...

[z] No data.

TABLE XVI

CACTUS EXPERIMENT 7

mg/l Kinetin	pCPA mg/l 5		10	
	14	41	14	41
20	LNC	SNC	SNC	GNC,Re
20	GNC	VGNC	SNC	GNC
20	NC	Co	SNC	GNC,G
20	LNC	GNC	SNC	GNC
30	GNC	VGNC,G	SNC	GNC
30	SNC	GNC	SNC	GNC
30	SNC	GNC	SNC	GNC
30	GNC	GNC	GNC	GNC
40	LNC	SNC	LNC	SNC
40	SNC	SNC	LNC	SNC
40	SNC	SNC	SNC	SNC
40	GNC	SNC	SNC	SNC
50	LNC	SNC	GNC	GNC,Re
50	GNC	GNC	GNC	GNC,Re
50	GNC	GNC,G	GNC	GNC,Re
50	LNC	SNC	GNC	GNC
60	VGNC,G	VGNC,G	VGNC	VGNC,G
60	VGNC,G	GNC,G	GNC,Re	GNC,Re
60	VGNC,G	VGNC,G	GNC,Re	GNC,Re
60	VGNC	VGNC,G	GNC	GNC,Re

to promote callus formation with this cactus. Under the optimum treatment in this experiment (60 mg/l kinetin; 5 mg/l pCPA), all new callus masses, except one, had green color. The high concentration of kinetin apparently enhanced chlorophyll formation. Red spots, which indicate anthocyanin formation, were especially prevalent at the highest kinetin concentration (60 mg/l) and the higher pCPA concentration (10 mg/l). No red spots appeared at the lower pCPA concentration (5 mg/l).

Cactus Experiment 8 (Table XVII)

The poor growth of the new callus with the high concentrations of kinetin, at different IAA concentrations, corroborates the observations made in the previous experiment (Cactus Experiment 5). Low concentrations of kinetin, with varying concentrations of IAA, appeared to promote callus formation with this cactus. No green color formed when callus growth was poor (Da, D, LNC, Table XVII). Callus growth had to be substantial (SNC, GNC, Table XVII) before tissue turned green. Green color appeared only at the higher IAA concentrations (6 and 8 mg/l), which suggested that IAA and kinetin were interacting to promote chlorophyll development.

Growth of new callus was poor with the concentrations of NAA and kinetin used. Good growth occurred only in two instances, both at the lower kinetin concentrations (30 and 40 mg/l) and the highest NAA concentration (8 mg/l). NAA appeared to act like IAA in that both auxins promoted the best callus growth when kinetin concentrations were low.

Cactus Experiment 9 (Table XVIII)

Two of the six new plants developed at relatively high kinetin

TABLE XVII

CACTUS EXPERIMENT 8

mg/l Kinetin	IAA (mg/l) 2		4		2		4		6		8		6		8	
	29	64	29	64	29	64	29	64	29	64	29	64	29	64	29	64
30	Da	D	Da	D	SNC	LNC	Da	LNC	SNC	GNC	SNC	SNC	NC	SNC	SNC	GNC
40	Da	D	SNC	SNC	SNC	LNC	SNC	SNC	Da	LNC	Da	LNC	GNC	SNC	GNC G	GNC
50	LNC	LNC	SNC	SNC	Da	LNC	LNC	LNC	SNC	SNC, G	NC, G	GNC, G	LNC	SNC	Da	D
60	Da	D	LNC	LNC	Da	LNC	Da	LNC	Da	LNC	NC	GNC	NC	GNC, G	NC	SNC, G
70	Da	LNC	NC	SNC	Da	D	Da	LNC	Da	D	NC	SNC	Da	D	Da	LNC

mg/l Kinetin	NAA (mg/l) 0		4		0		4		6		8		6		8	
30	...	...	Da	LNC	...	...	Da	D	LNC	SNC	NC	GNC	Da	LNC	Da	LNC
40	...	...	Da	NC	...	...	Da	D	Da	D	Da	LNC	Da	LNC	GNC	GNC
50	...	...	Da	D	...	...	Da	D	Da	LNC	Da	LNC	Da	D	Da	LNC
60	...	...	Da	Da	...	...	Da	D	Da	D	LNC	LNC	LNC	LNC	Da	LNC
70	...	...	Da	D	...	...	Da	LNC	Da	LNC	Da	LNC	Da	LNC	Da	D

concentrations (1.74 and 6.54 mg/l). In the other four cases, the kinetin concentration was less than 1 mg/l. A low auxin concentration, with the high kinetin concentration, appeared to promote the plant development from the explant. At higher auxin concentrations (.37 to 1.39 mg/l NAA with or without 1.1 mg/l 2,4-D), plants developed when only 0.215 mg/l kinetin was present.

After approximately two months in the soilless media, the plants grew an average of 50% of their original height.

TABLE XVIII

CACTUS EXPERIMENT 9

Plant Number	Size (cm) (Height; dia at widest part)	
	2/4/80	4/9/80
1	3.0; 1.0	5.0; 1.2
2	2.5; 1.0	3.0; 1.5
3	2.0; 0.8	3.2; 1.2
4	2.0; 0.8	3.2; 1.0
5	4.0; 1.4	4.2; 1.4
6	0.9; 0.5	1.8; 0.9

CHAPTER V

DISCUSSION OF CACTUS EXPERIMENTS

More growth occurred using Murashiga and Skoog's (1962) medium rather than Gamborg's (1975) medium. Callus growth was initiated over a range of concentrations of the auxins IAA (2-4 mg/l), NAA (0.09-15 mg/l), 2,4-D (0-1.1 mg/l), pCPA (5-35 mg/l), all in association with kinetin ranging in concentrations from 0-70 mg/l. Both high and low concentrations of auxins, and high and low concentrations of kinetin, resulted in callus formation. New callus tended to be more transparent where the kinetin and IAA concentrations were low (1 mg/l and 10 mg/l, respectively; see Table XV). In tobacco-callus cultures, low cytokinin concentrations produced watery tissue (Skoog and Schmitz, 1972). More green callus was produced at 4 mg/l kinetin level than 1 mg/l. Cytokinens and light interact in chloroplast formation. In tobacco callus without added cytokinin, plastids form in the light, but remain undifferentiated; callus cultured in the dark in the presence of cytokinin produces protoplastids, but no grana develop. Only in the presence of both light and cytokinin do normal chloroplasts appear (Skoog and Schmitz, 1972). The formation of chlorophyll in the absence of added cytokinin, when starved tissue is transferred to sucrose medium, is ascribed to the accumulation of natural cytokinins during the prior starvation period (Skoog and Schmitz, 1972).

Callus grown on media with higher concentrations of kinetin (60 mg/l) along with high pCPA concentrations (10 mg/l) in the media did show red spots indicating the presence of anthocyanin formation (see Table XVI). Others have noted red color, too, with cactus (Steinhart, 1962; Minocha and Mehra, 1974).

Curious interactions occur between pigmentation and auxin (Thimann, 1977). It is well known that auxin encourages root formation in many plant species (Thimann and Behnke-Rogers, 1950). Non-anthocyanin-forming varieties of Hibuscus, maple, and eucalyptus root poorly, while the anthocyanin-forming varieties will root well, in response to auxin (Thimann, 1977). In this tissue-culture experiment with cactus, the higher pCPA concentration seemed to stimulate anthocyanin formation.

More explants died when there was no 2,4-D incorporated in the media than when there was (1.1 mg/l; see Table XI). Minocha and Mehra (1974) found that callus of Neomammillaria, another cactus, would not grow unless 2,4-D was present in the media.

Roots and shoots were initiated from the explants when kinetin ranged in concentration from 0.436-6.54 mg/l (with 0.182 mg/l NAA constant, both with and without 1.1 mg/l 2,4-D) and when NAA concentration ranged from 0.37 to 1.4 mg/l (with 0.215 mg/l kinetin constant), both with and without 1.1 mg/l 2,4-D (Cactus Experiment 2, Table XI).

Because seed germination is slow (Hartmann and Kester, 1975, p. 630), cacti like Echinopsis turbinata L. are normally propagated by division of accessory buds (offsets) borne at the axillary buds of the plant. Propagation by this method takes time because only a few offsets are produced at one time from one mother-plant. Each axil with a cluster of spines is capable of producing a new plant. There are 13 to 14

ribs per plant and several clusters of spines per rib. The older the plant gets, the more clusters of spines per rib, because the rib elongates (see Graff, 1973, p. 620). If there are an average of five clusters with spines per rib on a plant with 13 ribs, this means each mother-plant can produce 65 explants for tissue culture. Therefore, propagation is more prolific with tissue culture than with the normal method of propagation by division.

Plants from desert regions are being screened for production of oil and fuel which can be put directly into tanks of diesel-powered cars (Nielsen et al., 1977); Adams et al., 1978; Maugh, 1979). "Petrochemical plantations" are envisioned where desert species would be grown on a large-scale basis (Nielsen et al., 1977). This research involves plants that grow in the arid western parts of the United States and includes Asclepidaceae (Asclepias), Buxaceae (jojoba), Euphorbiaceae (Euphorbia, Hevea, Jatropha, Pedilanthys), Myrtaceae (Eucalyptus). The cactus family (Cactaceae) has not been investigated for possible biomass production (i.e., burning cacti for fuel, as wood is burned) or for petrochemical production (i.e., extracting chemical substances from the cacti which can be injected into vehicles for fuel). Alkaloid synthesis in the cactus family has been studied (Steinhart, 1962). The cactus family includes about 10,000 species, virtually all of them originally from America, mainly the desert regions of Arizona and Mexico and the Andes Mountains in Bolivia and Peru (Nicolaisen, 1970, p. 194). The Western Hemisphere has an extensive resource (cacti) which might be tapped for energy. With tissue culture, it would be possible to propagate large numbers of cacti. They could be planted in plantations in

the now unproductive desert regions of the United States and might help provide energy for the country.

CHAPTER VI

MATERIALS AND METHODS

Wheat Experiments

To obtain explants from wheat, seeds were sterilized and placed in petri dishes as described by Gamborg (1975). Two changes were made after some experimentation:

1. Due to the high rate of contamination, it was found necessary to, first, rinse seeds with low concentration of mercuric chloride ($HgCl_2$) for one minute or less, then rinse with sterile water several times, followed by Gamborg's procedure. The germination rate was slightly affected, so more seeds were used.

2. It was found more convenient to mix deionized water with agar at 5.5 mg/l and pipette 15 ml into each petri dish. The dishes were then covered and sterilized before use.

After germination, when the shoots were approximately 1 cm long and roots usually longer, each shoot was excised, as close to the seed as possible, and placed in prepared tubes of media. Each root tip, approximately 1 cm long, was also excised and placed in prepared tubes of media. The tubes were then placed in the growth chamber under the same conditions described for the cacti.

Observations were made at different intervals of the growing period and recorded, in days, counting from the day the explant was put in the tube.

Data for Wheat Experiments

Wheat Experiment 1 (Table XIX)

Roots and shoots were excised from the germinated wheat seed of the cultivar "Ponca," May 29, 1979, and placed on B5 medium with the growth regulators IAA, ranging from 0 to 20 mg/l, and 2,4-D, ranging from 0 to 4.54 mg/l. The days of observations after culturing are shown in Table XIX.

TABLE XIX

OBSERVATION DAYS AFTER CULTURING FOR WHEAT EXPERIMENT 1

mg/l 2,4-D	IAA mg/l 0	3	5	7.5	10.0	12.5	15	20
				Shoots				
0	83	13 49	6	127	8 73	8	13	35
1.5	83	13	35	83	21	13	83	83
3.03	8	6	27	83	83	8	83	8
4.54	8	83	83	8	83	8 21 73	8	27
				Roots				
0	83	83	8	83	8	83	83	27
1.5	83	83	83	83	83	83	8 73	8
3.03	83	8 73	83	83	13	8	83	83
4.54	8	80	8	83	13 73	83	83	8

Wheat Experiment 2 (Table XX)

Callus from the roots and shoots from Wheat Experiment 1 was transferred to new B5 medium with the growth regulators kinetin, ranging from 3 to 15 mg/l, and IAA, at 10 and 15 mg/l. Two observations were made. The first was 26 days after the explants were transferred and the second was 48 days after transfer. The concentrations of the IAA and 2,4-D in the medium from which the callus was transferred are shown in Table XX. This will be shown only for the shoot callus transfer. The previous concentrations of the growth regulators were not recorded for the root callus transfer.

Wheat Experiment 3 (Table XXI)

Roots and shoots were excised from the germinated wheat seed of the cultivar "KanKing," October 4, 1979, and placed on B5 medium with the growth regulators NAA, ranging from 3 to 20 mg/l, IAA, ranging from 3 to 20 mg/l, and 2,4-D, ranging from 0 to 2 mg/l. The days of observations will be shown in Table XXI.

Wheat Experiment 4 (Table XXII)

Callus was transferred from Experiment 2, after 150 days of culture, onto B5 medium. Similar concentrations of kinetin (2 mg/l) and IAA (10 and 20 mg/l) were used in the culture of the second transfer (Table XXII), as were used in the culture of the first transfer (Table XX). The concentration of kinetin and IAA used to grow the callus explants was not noted.

TABLE XX

PREVIOUS CONCENTRATIONS OF GROWTH REGULATORS OF SHOOT CALLUS EXPLANTS FOR WHEAT EXPERIMENT 2

mg/l Kinetin	IAA mg/l 10		15	
	26 Days	48 Days	26 Days	48 Days
3	12.5-4.54		12.5-4.54	
3	12.5-4.54		12.5-4.54	
5	12.5-4.54		12.5-4.54	
5	12.5-4.54		12.5-4.54	
7.5	12.5-4.54		10-1.5	
7.5	10-1.5		10-0	
10	10-0		10-0	
10	10-0		7.5-4.54	
15	7.5-4.54		3-0	
15	7.5-4.54		3-0	

TABLE XXI

OBSERVATION DAYS AFTER CULTURING FOR WHEAT EXPERIMENT 3

mg/l 2,4-D	NAA (mg/l) 3	5	10	15	20
		Shoots			
0	21	15	95	77	15
1	95	77	21	21	77
1.5	77	21	21	77	77
		95			
2	21	77	77	77	21
	116				77
		Roots			
0	95	21	21	95	77
	116	95			
1	21	21	95	6	21
	77				
1.5	21	77	77	77	6
2	21	21	77	95	95
	IAA (mg/l)				
	3	5	10	15	20
		Shoots			
0	77	95	77	77	77
1	77	77	77	77	77
1.5	95	77	77	77	77
2	77	77	...	77	77
		Roots			
0	21	116	95	21	21
	77				
1	77	77	21	147	147
	95		95		
1.5	77	21	21	147	95
	95				
2	21	21	21	6	21
			77		77

TABLE XXII

OBSERVATION DAYS AFTER CULTURING FOR WHEAT EXPERIMENT 4

mg/l Kinetin	IAA mg/l 10	20
	Shoot Callus Transfer	
2	5	9
2	5	32
2	32	32
2	55	32
2	32	32
	Root Callus Transfer	
2	32	65
2	55	55
2	55	102
2	5	32
2	32	32 55

Wheat Experiment 5 (Table XXVIII)

Roots and shoots were excised from the germinated wheat seed of the cultivar "Ponca," December 20, 1979, and placed on B5 medium with the growth regulators NAA, ranging from 0 to 20 mg/l, and 2,4-D, ranging from 0.5 to 2.0 mg/l. All observations were made seven days after the explants were placed on the media.

Summarization of Treatments

Table XXIII summarizes the Wheat Experiments, the ranges of growth regulators used in each experiment and the cultivar which was treated.

TABLE XXIII

SUMMARY OF WHEAT EXPERIMENTS

Experiment Number	Table Number	Basal Medium	Cultivar	Tissue	2,4-D	IAA	NAA	Kinetin
					mg/l (range used in experiments)			
1	XIX	B5	Ponca	Root; shoot	0-4.5	0-20		
2	XX	B5	Ponca	Callus		10-15		3-15
3	XXI	B5	KanKing	Root; shoot	0-2		3-20	
3	XXI	B5	KanKing	Root; shoot	0-2	3-20		
4	XXII	B5	Ponca	Callus		10-20		2
5	XXVIII	B5	Ponca	Root; shoot	0-5.2		0-20	

CHAPTER VII

RESULTS OF WHEAT EXPERIMENTS

Wheat Experiment 1 (Table XXIV)

Shoots of the drought-sensitive cultivar of wheat, Ponca, formed callus at the cut end, near the seed. No callus from shoots was formed when the IAA was low (o mg/l) or high (20 mg/l). Callus formed at all concentrations Of 2,4-D, but roots formed on the callus at the highest 2,4-D concentration (4.54 mg/l).

No callus formed from roots when the 2,4-D concentration was zero. Callus formed on roots at low (0 or 3 mg/l), medium (10 mg/l), and high (15 mg/l) concentrations of IAA used in the experiment. The callus always started to grow from the center of the root explant. Callus production was poor for both shoots and roots. Out of 32 cultures of shoots, only eight formed callus. For the 32 cultures of roots, five formed callus.

Wheat Experiment 2 (Table XXV)

Roots formed on callus from shoots of Ponca (transferred from Wheat Experiment 1 above), but only at the low concentrations of kinetin (3, 5, or 7.5 mg/l) used in the experiment. New callus, some with roots, formed on callus from roots of Ponca (transferred from Wheat Experiment 1 above) at all kinetin concentrations used (3-15 mg/l), except for one (7.5 mg/l).

TABLE XXIV

WHEAT EXPERIMENT 1

mg/l 2,4-D	IAA mg/l 0	3	5	7.5	10.0	12.5	15	20
				Shoots				
0	D	CACE R on C	Co	CACE	CACE G	Co	Co	Co
1.5	D	Co	Co	D	CACE	Co	ND	ND
3.03	Co	Co	Co	D	D	CACE	ND	Co
4.54	Co	D	D	CACE	D	C w/R C on R G	CACE	Co
				Roots				
0	D	D	Co	D	Co	D	D	Co
1.5	D	D	D	D	D	D	C,Ctr G	D
3.03	D	C,Ctr G	D	D	C,Ctr	Co	D	D
4.54	C,Ctr	Co	Co	D	C,Ctr G	D	D	Co

TABLE XXV

WHEAT EXPERIMENT 2

mg/l Kinetin	IAA (mg/l) 10		15	
	26 Days	48 Days	26 Days	48 Days
	Shoot Callus Transfer			
3	Many R,CH	Many R,Ch	Many R,Ch	Many R,Ch
3	D	D	C,G	G now Br
5	Some R,G	Some R,G	Some R,Ch	Some R,Ch
5	Some R,Ch	Some R,Ch	Some R,Ch	Some R,Ch
7.5	1 R,Ch	1 R,CH	D	
7.5	1R,Ch	1 R,Ch	D	
10	D		D	
10	D		D	
15	D		D	
15	D		D	
	Root Callus Transfer			
3	NC,1R,Ch	NC,1R,Ch	NC,2R,Ch	More R
5	NC	Some Ch	D	
7.5	D		D	
10	Ch	Many R	Ch	Ch
15	NC,Ch	NC,CH	2R,Ch	Many R

Wheat Experiment 3 (Table XXVI)

In the first part of the experiment (with NAA), both roots and shoots of the drought-resistant cultivar of wheat, KanKing, formed callus at all concentrations of 2,4-D (0-2 mg/l) and NAA (3-20 mg/l) used in the experiment. Roots formed in shoot cultures at the lowest 2,4-D and NAA concentrations (0 mg/l 2,4-D; 3 mg/l NAA) and highest 2,4-D and NAA concentrations (2 mg/l 2,4-D: 20 mg/l NAA) used in the experiment. Callus, formed on root tissue, appeared from the center of the explant. Several roots had chlorophyll.

In the second part of the experiment (with IAA), shoots formed callus at the three lowest 2,4-D concentrations (0, 1, and 1.5 mg/l) and all but the highest IAA concentration (20 mg/l). Roots formed callus at the three highest 2,4-D concentrations (1, 1.5, or 2 mg/l) and at two concentrations of IAA (3 and 10 mg/l).

In both parts of the experiment, callus production was poor. Of 40 cultures of shoots, only 14 explants formed callus. Most of the callus was formed from the cut end of the shoot, near the embryonic part of the plant (seed). Of 40 cultures of roots, 14 formed callus. The use of NAA and 2,4-D resulted in more callus formation than the use of IAA and 2,4-D. In Wheat Experiment 3, ten shoots produced callus with NAA and four shoots produced callus with IAA. Seven roots produced callus with NAA and seven roots produced callus with IAA.

Wheat Experiment 4 (Table XXVII)

No new callus formed from callus of Ponca when it was transferred a second time.

TABLE XXVI

WHEAT EXPERIMENT 3

mg/l 2,4-D	NAA (mg/l) 3	5	10	15	20
		Shoots			
0	CACE, Rs	CO	D	CACE	Co
1	C	D	CACE	C	D
1.5	D	CACE G,Ctr	C	D	D
2	CACE(Good) G spots	D	CACE	D	C w/Rs Ch
		Roots			
0	Alive D	C(Ctr),Ch 1R from mid-explant	Ch	D	C(Ctr)
1	C(Ctr) R(Elongated)	C(Ctr)	Alive	Co	Ch
1.5	Ch	R(G)	C(Ctr)	C(Ctr)	Co
2	Ch	Ch	C(Ctr)	Ch	Alive
	IAA (mg/l) 3	5	10	15	20
		Shoots			
0	D	D	CACE	D	D
1	CACE	D	D	D	D
1.5	D	CACE	D	C,G	D
2	D	D	D	D	D
		Roots			
0	Ch C(tip)	D	Ch	Ch	Ch
1	Alive Ch	Ch	C(Ctr) Ch	D	D
1.5	C(Ctr) Ch	Ch	C(Ctr)	D	Ch
2	C(Ctr) and tip	Ch	Ch C	Co	Ch C

TABLE XXVII

WHEAT EXPERIMENT 4

mg/l Kinetin	IAA mg/l 10	20
	Shoot Callus Transfer	
2	Co	Co
2	Co	D
2	D	New R
2	D	D
2	D	D
	Root Callus Transfer	
2	D	D
2	D	D
2	D	D
2	Co	D
2	D	New Rs New Rs show Ch

Wheat Experiment 5 (Table XXVIII)

Shoots of the drought-sensitive cultivar of wheat, Ponca, formed callus, at the cut end, at all concentrations of 2,4-D (0.5-2.0 mg/l) and at all concentrations of NAA (0-20 mg/l), except for the 0 mg/l NAA concentration. Roots formed in the shoot culture at a low concentration of NAA (0.5 mg/l).

Callus formed on roots at all concentrations of 2,4-D (0.5-2.0 mg/l) and at all but the two lowest concentrations of NAA (0 and 0.5 mg/l). The higher concentrations of NAA (7.5-20 mg/l) resulted in good callus growth.

TABLE XXVIII

WHEAT EXPERIMENT 5

					NAA mg/l					
mg/l 2,4-D	0	0.5	1.0	3.0	5.0	7.5	10	12.5	15	20
					Shoots					
0.5	Co	Co	Co	Co	CACE	CACE	CACE	CACE	CACE	CACE
1.0	Co	CACE	CACE	Co	CACE	CACE	CACE	CACE	CACE	Co
1.5	Co	Co	Co	CACE	CACE	CACE	CACE	CACE	CACE	Co
2.0	Co	CACE w/Rs	CACE	Co	CACE	CACE	CACE	NoG	CACE	Co
					Roots					
0.5	Co	Co	CAX	CAX	CAX	GCAX	GCAX	GCAX	GCAX	GCAX
1.0	Co	Co	CAX	CAX	CAX	GCAX	GCAX	GCAX	GCAX	GCAX
1.5	Co	Co	CAX	CAX	CAX	GCAX	GCAX	GCAX	GCAX	GCAX
2.0	Co	Co	CAX	CAX	CAX	GCAX	GCAX	GCAX	CAX	CAX

CHAPTER VIII

DISCUSSION OF WHEAT EXPERIMENTS

Of the 72 shoots and 72 roots produced from Ponca seed, 33 and 37, respectively, formed callus (Tables XXIV and XXVIII). Of 40 shoots and 40 roots produced from KanKing seed, 14 and 14, respectively, formed callus (Table XXVI). The percentage of callus formation for Ponca was as follows: shoots, 45% and roots, 51%; for KanKing, it was shoots, 35% and roots, 35%.

Roots developed from shoot explants (Tables XXIV, XXVI, XXVIII) and from callus cultures from shoots (Tables XXV and XXVII). Roots also developed from root explants (Table XXVI) and from callus cultures from roots (Tables XXV, XXVII). In Wheat Experiment 2, roots formed on callus from shoots of Ponca (transferred from Wheat Experiment 1), but only at low concentrations of kinetin (3, 5, and 7.5 mg/l) used in the experiment. Dudits et al. (1975) noted root formation in wheat-callus tissues was increased by the presence of cytokinins. No new shoots formed. This agrees with previous observations cited in the Literature Review, which show that roots, but not shoots, form with ease from tissue cultures of wheat. The new callus that formed from shoots occured at the cut end, near the seed. This also agrees with previous observations which report that callus production is better if embryonic tissue is included in the culture (Shimada, 1975; Chin and Scott, 1977; Cure and Mott, 1978; O'Hara and Street, 1978). In Wheat Experiment 3,

the use of NAA and 2,4-D resulted in more callus formation than the use of IAA and 2,4-D. Sheridan (1973) found no callus formation with IAA at 10 or 50 mg/l. In Wheat Experiment 5, the higher concentrations of NAA (7.5-20 mg/l) resulted in good callus growth. This contrasts with work by O'Hara and Street (1978) who found that NAA did not enhance callus production. Chin and Scott (1977) found that, of all the auxins examined, NAA gave consistently good root induction and at the concentration of 1.0 mg/l, was adopted as standard root induction medium.

In Wheat Experiment 4, no new callus formed from the callus of Ponca when it was transferred a second time. The ability to form new callus is age-dependent (Trione et al., 1968). Others have noted that subculturing results in poor callus formation (Prokhorov et al., 1974; Dudits et al., 1975; Chin and Scott, 1977; O'Hara and Street, 1978).

Wheat Experiments 3 and 5 can be compared because in each experiment shoots and roots from seed grew with the same growth regulators. Out of 15 shoot and 15 root explants of Ponca, callus was produced on eight shoots and 15 roots (Table XXIV). Out of 15 shoot and 15 root explants of KanKing, callus was produced on eight shoots and five roots (Table XXVI). The percentage of callus formation for Ponca was as follows: shoot, 53% and roots, 100%; for KanKing it was shoots, 53% and roots, 33%. The numbers indicate that callus formation was similar for the drought-sensitive cultivar, Ponca, and the drought-resistant cultivar, KanKing, from shoots. Visual observation, however, indicated that callus formed with greater ease, and cell proliferation was more vigorous, with KanKing than with Ponca. Although this visual result is not quantified, the author suggests that growth of wheat from tissue culture might be more successful if drought-resistant cultivars were used rather

than drought-sensitive cultivars. Alternatively, cultivars could be screened for drought-resistance using tissue culture. Cultivars that produced callus readily on the tissue-culture medium might be more drought-resistant than those that grew slowly.

CHAPTER IX

SUMMARY

The cactus, Echinopsis turbinata L., grew best on a basal medium developed by Murashige and Skoog. Callus growth was initiated over a range of concentrations of the auxins, IAA (0-40 mg/l) NAA (0.09-15 mg/l) 2,4-D (0-1.1 mg/l), pCPA (5-35 mg/l), all in association with kinetin ranging in concentration from 0 to 70 mg/l. Both high and low concentrations of auxins, and high and low concentrations of kinetin, resulted in callus formation. Roots and shoots were initiated from the explants when kinetin ranged in concentration from 0.44 to 6.54 mg/l (with 0.18 mg/l NAA constant, both with and without 1.1 mg/l 2,4-D) and when NAA concentrations ranged from 0.37 to 1.4 mg/l (with 0.215 mg/l kinetin constant, both with and without 1.1 mg/l 2,4-D).

More research should be done comparing varying concentrations of auxins with varying concentrations of cytokinins (preferably in a Latin square with at least four replications): a) to test for the best combination of growth regulators to produce roots and shoots from each explant and b) to test for the best combination of growth regulators to produce callus, then the roots and shoots from the callus.

The wheat, (Triticum aestivum L. em. Thell.) "Ponca," being drought sensitive and "KanKing," being drought resistant grew well on a basal medium developed by Gamborg. Ponca and KanKing produced callus with the same growth regulators, (IAA, 0-20 mg/l; NAA, 0-20 mg/l; 2,4-D, 0-4.54

mg/l). Even though the numbers of callus produced from explants was similar with Ponca and KanKing, callus formed with greater ease, and cell proliferation was more vigorous with KanKing than with Ponca. This result suggested that growth of wheat from tissue culture might be more successful if drought-resistant cultivars were used rather than dought-sensitive cultivars. Alternatively, cultivars could be screened for drought resistance using tissue culture. Cultivars that produced callus readily on the tissue culture medium might be more drought resistant than those that grew slowly.

More research should be done comparing varying concentrations of auxins with varying concentrations of cytokinins (preferably in a Latin square with at least four replications) to test for the best combination of growth regulators to produce callus, then roots and shoots from callus. After a good combination of growth regulators is found to produce callus, some work should be done with varying concentrations of NaCl in the medium to test for callus formation under stress and to test callus transfers for the ability to overcome or survive stress. If drought resistant or tolerant cultivars survive or grow more vigorously, this might be used as a method of screening for drought resistance.

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VITA 2

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