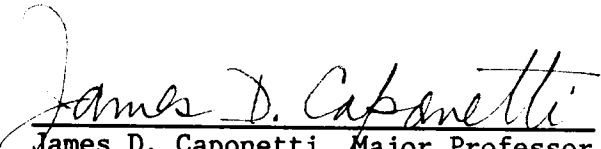
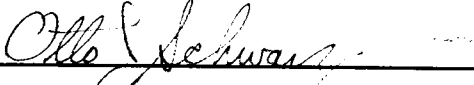
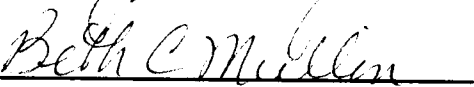


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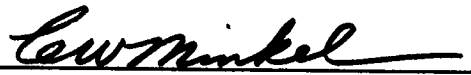
I am submitting herewith a thesis written by Wei Shao entitled "Callus Production and Organogenesis in Sweet Pepper (Capsicum Annuum L.)." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Botany.


James D. Caponetti, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:


Vice Provost
and Dean of The Graduate School

CALLUS PRODUCTION AND ORGANOGENESIS IN SWEET
PEPPER (CAPSICUM ANNUUM L.)

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Wei Shao
December 1988

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ABSTRACT

Cotyledon and hypocotyl explants of tissue cultured seedlings of three varieties of pepper, Capsicum annuum L., were aseptically cultured on MS medium supplemented with the auxins 2,4-D, NAA, and IAA separately in a range of concentrations from 0 to 5×10^{-5} M. Callus formation occurred on media with all three auxins in four weeks. Medium with 10^{-6} to 10^{-5} M 2,4-D produced the greatest fresh and dry weights of callus on the explants of all three varieties. In many instances, roots formed on callus on media with NAA and IAA, but not with 2,4-D. Organogenesis of shoot buds was achieved best on cotyledon explants on medium with a combination of the auxin IAA and the cytokinin BA.

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I. INTRODUCTION

Interest in this reseach problem was generated by a visit to Dr. Caponetti from Mr. James H. Sterling, Field Manager of Moody Dunbar, Inc., a capsicum pepper grower and canner with offices and cannery in Limestone, Tennessee. According to Mr. Sterling, the company suffers large annual losses of pepper due mainly to two destructive pepper diseases, bacterial spot disease caused by the bacterium Xanthomonas vesicatoria and southern stem blight caused by the fungus Sclerotium rolfsii.

Bacterial spot disease first appears as small yellowish spots on the underside of young leaves. On older leaves, the spots turn dark green and enlarge to 1 1/4 inches in diameter. Severely spotted leaves turn yellow and abscise. Plants severely affected lose all of their leaves except the few young ones at the top. The fruit develop blister-like spots about 1/4 inch in diameter. These spots turn brown and crack open. In damp weather, various decay-producing organisms can enter through these spots and cause the fruit to rot. Southern stem blight fungus attacks the stem of the pepper plant near the ground level during rainy weather. The fungus girdles the stem causing all leaves to wilt, yellow, and die.

Control of both diseases has been obtained by selected pesticide sprays and by crop rotation. Attempts to eliminate the problem by developing disease-resistant varieties through traditional methods of plant breeding have not been successful. While reading several agricultural magazines and trade journals, Mr. Sterling had learned that new varieties of agronomic plants could be developed by methods of plant

tissue culture. This led to the consultation with Dr. Caponetti about the idea of developing disease-resistant pepper plants. Producing such new plants is a long term project because it involves many complicated molecular techniques. At the moment, it would be more feasible to begin the process by employing some of the more simple techniques of plant tissue culture. A logical starting point would be the production of callus tissues from an amenable organ of the plant and then induction of organogenesis in the callus tissues.

Callus is mainly an unorganized proliferating mass of parenchyma cells (Constable, 1984). Callus can occur naturally in some plants as the result of injury (Sinnott, 1960), microorganism invasion (Braun, 1954), or even by insect feeding (Pelet, et al., 1960). Since callus cultures of carrot and tobacco were made possible by the tissue culture techniques of Gautheret, Nobecourt and White respectively in 1939 (Dodds and Roberts, 1985), much research has been done on the initiation and growth of callus. The callus itself could be employed in biochemical and growth studies (Gamborg, et al., 1976). Many efforts have been focused on economic plants. For example, in carrot (Caplin and Steward, 1948; Caplin, 1963; Steward and Rao, 1970; Tripathi and Ram, 1982; Bender and Neumann, 1978); in tobacco (Das, et al., 1956; Keitt and Skoog, 1959; Murashige and Skoog, 1962; Fox, 1964; Linsmaier and Skoog, 1965; De Fossard, 1974; Rogers, et al., 1974; Einset, 1977; Chang and Hsing, 1978; Collins, et al., 1978; Zatzkyo, et al., 1982); in potato (Steward and Caplin, 1951; Lingappa, 1957); and in tomato (Coleman and Greyson, 1977; Chin, et al., 1981), and also in other dicotyledonous and monocotyledonous plants (Schenk and Hildebrandt, 1972), conifers

(Beatty, 1987), and ferns (Byrne, 1984). Several reviews have described the initiation and maintenance of callus (Yeoman, 1970; Schenk and Hildebrandt, 1972; Yeoman and Macoleod, 1977; Gamborg, et al. 1976; Fosket and Tepfer, 1978; Yeoman and Forche, 1980; Constable, 1984; Dodds and Roberts, 1985).

Sweet pepper (Capsicum annuum L.) is another important economic plant. Some work has been done on sweet pepper. Rogers, et al (1974) were the first to induce callus formation in C. annuum by using thin stem cross-sections as explants. Callus and roots were obtained from the sections. Gunay and Rao (1978) obtained callus from cotyledon and hypocotyl explants. Fári and Czáká (1981) induced callus from the basal part of hypocotyl explants of C. annuum.

As for organogenesis, Gunay and Rao (1978) were the first to obtain shoots and roots separately from the cotyledon and the hypocotyl explants of C. annuum. Fári and Czáká (1981) studied the relationship between the position of the hypocotyl explant and the morphogenetic response of shoot and root formation of C. annuum var. T. Hatvani. They found that the apical section of the hypocotyl produced only buds, the middle portion developed roots mainly, and the basal sections produced only abundant callus.

Most of the work which has been done so far on C. annuum is anther culture and androgenesis. Kuo, et al. (1973) cultured the anthers of C. annuum on anther media. Callus and embryoids were induced from the anthers and plantlets developed later. Wang, et al. (1973) obtained callus and haploid plantlets from the anthers of a variety of pepper named Yeo Hsien Redpepper. In the same year, George and Narayanaswamy

(1973) studied androgenesis from the anther of the pepper variety Grossum Sendt. However, less than 0.1% of the cultured anthers produced viable plantlets. MacDonald and Grant (1973) tested the effects of the ethylene-releasing chemical Ethrel on mitotic division in the pollen of pepper cultivar 'Hybrid Early Bountiful.' The results implied that Ethrel had no effect in inducing mitotic divisions in the pollen. Novak (1974) worked on haploid callus induction from anther cultures of C. annuum. The work showed that IAA or NAA alone induced no callus, and that 2,4-D alone induced some cell proliferations. Kinetin or BA improved the callus growth. Sibi, et al. (1979) obtained haploid plants through anther culture of C. annuum. Most of the embryoids that were produced developed into plantlets. Recently, Vagera and Havranek (1985) noted that the addition of activated charcoal to medium had a positive effect on androgenesis in capsicum pepper. However, there was a negative effect of the charcoal on the further development of the plantlets.

Protoplast culture has also been studied in C. annuum. Mishra and Arora (1978) were able to induce the protoplasts of C. annuum to develop new walls. The work was improved on by Saxena, et al. (1981). They obtained flowering plants of California Wonder variety of pepper through protoplast regeneration, callus induction, and callus differentiation into whole plants.

The research presented here tested callus production of hypocotyl and cotyledon explants of three domestic varieties of Capsicum annuum on media with three different auxins, and described organogenesis of shoot buds and roots induced from the cotyledons of the same varieties by adding different combinations of IAA and BA to media.

II. MATERIALS AND METHODS

The plants used in this research were three varieties of sweet capsicum pepper, Capsicum annuum L. var. Yolo Wonder B, var. Yolo Wonder L, and var. Dunbar Pimiento. The seeds were donated by Mr. James H. Sterling, Field Manager of Moody Dunbar, Inc., Limestone, Tennessee. The seeds were stored in a refrigerator at 5° C until used.

Seedling Generation

The seeds of each variety were surface sterilized in a 20% v/v Clorox solution in small Griffin beakers for 20 minutes under sterile conditions in a laminar flow hood. Then, the seeds were rinsed twice with sterile, distilled water. After rinsing, the seeds were placed into sterile petri dishes. The dishes provided the seeds with a temporary, sterile, storage place until they could be transferred to sterile culture medium.

The medium used to germinate the seeds was MS medium (Murashige and Skoog, 1962) containing only the inorganic salts at half the original strength, and 1% Difco Bacto-Agar. The pH of the medium was adjusted to 5.8 with a 1 N KOH solution before adding the agar. The medium was then distributed to 25 mm x 150 mm culture tubes, 20 ml per tube, and autoclaved at 250° F and 15 lbs/in.² pressure for 20 minutes. The seeds were then transferred to the surface of the medium in the culture tubes, one seed per tube. The cultures were placed in a dark area of a culture room in which the temperature was set at 25° C.

After one week in darkness, the seeds of variety Yolo Wonder B and Yolo Wonder L had germinated to the point of producing primary roots 1

cm long. The seeds of variety Dunbar Pimiento had slight root development, and needed a second week for 1 cm primary root development. At this point, shoot development had not begun, and all seedlings were etiolated. All cultures were then brought out into the light in the same culture room where the illumination was 2500 lux at the level of the cultures. Illumination was provided by a combination of fluorescent tubes and incandescent bulbs in a 16 hour light and an 8 hour dark photoperiod. After a second week for seeds of varieties Yolo Wonder B and L and a third week for Dunbar Pimiento out in the light, the seedlings had turned green and achieved a hypocotyl length of about 3 cm, and each had two well-developed cotyledons.

Callus Production from Seedling Explants

For callus production, whole cotyledons and 1 cm long sections of hypocotyl were used as explants. Under sterile conditions, the cotyledons and hypocotyl explants were placed on MS medium (Murashige and Skoog, 1962) as modified by Linsmaier and Skoog (Linsmaier and Skoog, 1965), and supplemented with the auxins 2,4-D, NAA, and IAA respectively at concentrations of 0, 10^{-7} M, 5×10^{-7} M, 10^{-6} M, 5×10^{-6} M, 10^{-5} M, and 5×10^{-5} M. The media were gelled with 1% Difco Bacto-Agar after adjusting the pH to 5.8 and distributed to 25 mm culture tubes, 20 ml per tube. All the media were autoclaved at 250° F and 15 lbs/in.² for 20 minutes. Cotyledons were placed randomly on media with adaxial or abaxial side down on the surface of the medium. Hypocotyl sections were placed in a horizontal position on the surface of the medium.

All cultures were maintained in the same culture room and under

the same temperature and light conditions previously described. After 4 weeks in culture, calluses were individually removed from culture, and the fresh weights of the callus were determined using aluminum weighing cups. Dry weights were determined after the fresh callus had been placed in an oven set at 60° C for 12 hours. For each treatment, 10 to 14 replicates of cultures were measured, and the experiment was repeated. Means, standard deviation, and Duncan's Multiple Range Test were calculated for the replications of each treatment according to the methods of Joyner (Joyner, 1985).

Shoot Organogenesis from Seedling Explants and Callus

For shoot organogenesis, only the blades of the cotyledons were used as explants. The blades were placed with abaxial side down on the MS medium used for callus production but supplemented with the auxin IAA and the cytokinin BA. The IAA concentrations were 0, 10^{-7} M, 5×10^{-7} M, 10^{-6} M, 5×10^{-6} M, 10^{-5} M, and 5×10^{-5} M. The BA concentrations were 5×10^{-6} M, 10^{-5} M, and 5×10^{-5} M. Each concentration of IAA was combined with each concentration of BA so that a total 21 combinations were tested with the three varieties of pepper.

The cultures were maintained in the same culture room under the same conditions of light and temperature. The percentage of the explants which had formed shoot buds was determined after two weeks of culture. For each treatment, 10 to 14 replicates of cultures were measured and the experiment was repeated.

III. RESULTS

Seed Germination

In general, seeds of angiosperms contain sufficient mineral nutrients to produce healthy seedlings. Therefore, pepper seeds were, at first, placed in culture on a water-1% agar medium at pH 5.8. The seeds germinated but development was poor. Seedlings exhibited the usual symptoms of lack of mineral nutrients. In later germination trials, seeds were placed on MS medium containing only the inorganic salts at full strength and at half the full strength each with 1% agar and the pH adjusted to 5.8. Growth measurements were not taken, but seedlings on MS with salts at half strength were larger and healthier looking than those on MS medium with salts at full strength. At the same time, it was noticed that seedling development was better if seed cultures were placed in the dark during initial root development (about one week) with subsequent shoot development and further root development in the light (additional one or two weeks).

Callus Production from Seedling Explants

Initial Callus Formation on Explants

Data from the literature and from preliminary experiments demonstrated that (1) the cotyledons and hypocotyl parts of the seedling were capable of producing callus, (2) that the auxins 2,4-D, NAA, and IAA were the most effective for callus initiation, and (3) that auxin concentrations in the vicinity of $5 \times 10^{-6} \text{M}$ induced the largest mass of callus.

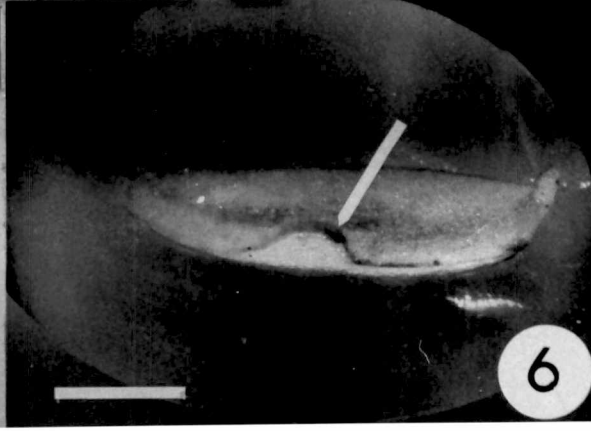
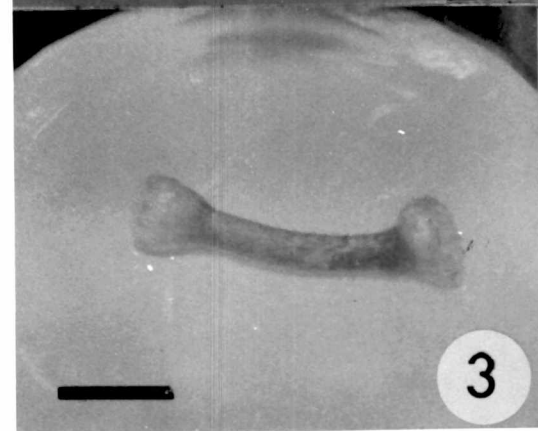
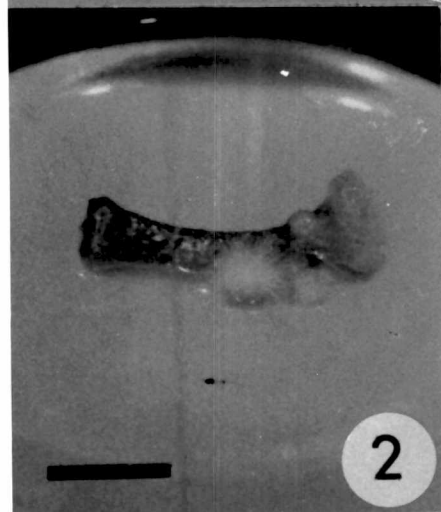
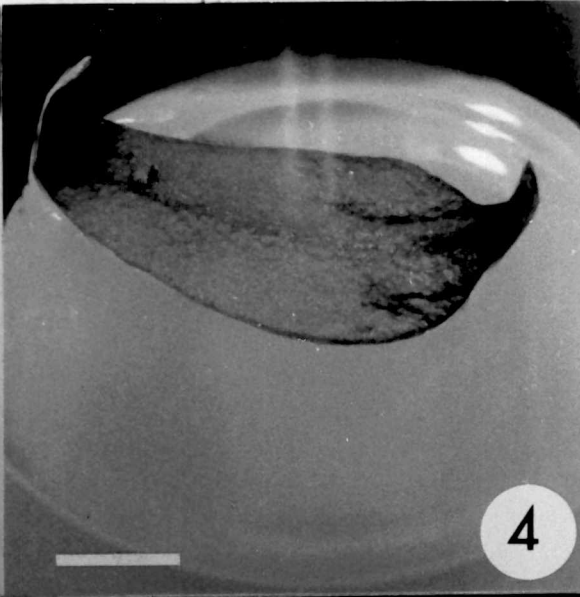
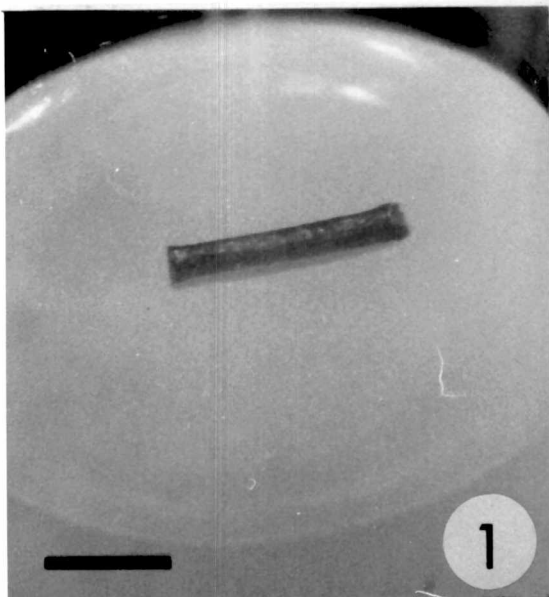
Initial callus formation differed between the two seedling explants. After being placed on appropriate medium (plate 1, figure 1), hypocotyls swelled slightly after several days in culture. Callus formation always began at the cut ends of the explant, but there was random variation of callus initiation. With approximately equal frequency, callus developed at one end first (plate 1, figure 2), and then a few days later the other end (plate 1, figure 3), or at both ends simultaneously (plate 1, figure 3), or just at one end with the other end turning brown. It seemed that polarity did not play a role in callus initiation. In any case, callus growth spread toward the middle of the hypocotyl explant, and, under appropriate conditions, completely engulfed the explant.

When cotyledon explants were placed on appropriate medium (plate 1, figure 4), callus initiation began at the cut, basal end of the blade after several days in culture. While callus developed at the cut end of the blade, an area of leaf tissue near the middle of the blade died (turned brown) beginning at the edge of the leaf and progressing to the midvein (plate 1, figure 5). A half moon shaped dead area of leaf tissue was produced, and callus formed from living leaf tissue in a semi circle around the dead tissue. Thus, a callus growth spread in two directions (plate 1, figure 6) and eventually engulfed the cotyledon explant.

The Effect of 2,4-D on Callus Growth

The auxin 2,4-D was the most effective in inducing callus formation on both cotyledon and hypocotyl explants of the three varieties of pepper. The color and texture of the callus varied with the concentration of 2,4-D in media. During the early stages of development,

- Plate 1. Early stages of callus formation on cotyledon and hypocotyl explants.
- Figure 1. Hypocotyl explant of Dunbar Pimiento on medium with $5 \times 10^{-6} \text{M}$ IAA for 1 day. The bar represents 5 mm.
- Figure 2. Hypocotyl explant of Dunbar Pimiento on medium with $5 \times 10^{-6} \text{M}$ IAA for 10 days. One end formed callus. The bar represents 5 mm.
- Figure 3. Hypocotyl explant of Yolo Wonder B on medium with 10^{-6}M 2,4-D for 10 days. Both ends formed callus. The bar represents 5 mm.
- Figure 4. Cotyledon explant of Dunbar Pimiento on medium with $5 \times 10^{-6} \text{M}$ IAA for 1 day. The bar represents 5 mm.
- Figure 5. Cotyledon explant of Dunbar Pimiento on medium with $5 \times 10^{-6} \text{M}$ IAA for 10 days. Necrotic tissue formed in the middle of the blade. The bar represents 5 mm.
- Figure 6. Cotyledon explant of Yolo Wonder B on medium with 10^{-5}M NAA for 10 days. Callus formed at the cut end and surrounding the necrotic tissue (arrow). The bar represents 5 mm.



the callus on all the explants was green and firm. After four weeks, explants on media with no 2,4-D (controls) had very little callus development (plate 2, figures 7, 8). The callus of the explants which were on media with low concentrations of 2,4-D (10^{-7} , 5×10^{-7} M) remained green and firm (plate 2, figures 9, 10). Callus on media with 10^{-5} and 5×10^{-5} M 2,4-D became brown or dark brown and soft (plate 2, figures 11, 12). However, callus from the explants on media with 10^{-6} and 5×10^{-6} M were white and soft (plate 3, figures 13, 14).

The amount of callus produced also varied with the concentration of 2,4-D in media. This can be observed in plates 2 and 3, figures 7 to 14. Tables 1, 2 and 3 show fresh and dry weights of callus produced on cotyledon and hypocotyl explants of the three pepper varieties on media containing a range of 2,4-D concentrations. Data are shown for one experimental test. A duplicate experiment yielded similar results. The data indicate that both the fresh and the dry weights of callus increased for both explants and all varieties as the concentration of 2,4-D in the medium increased up to 10^{-5} M 2,4-D. Maximum weights for Yolo Wonder B explant callus were obtained on media containing 5×10^{-6} and 10^{-5} M 2,4-D. For Yolo Wonder L, it was 5×10^{-6} and 10^{-5} M 2,4-D. For Dunbar Pimiento, it was 10^{-6} M, 5×10^{-6} M, and 10^{-5} M 2,4-D. For callus on media with 5×10^{-5} M 2,4-D, the weights showed a decrease.

The Effect of IAA on Callus Growth

The auxin IAA was not so effective as 2,4-D in callus production. Furthermore, the color and texture of the callus did not vary so much as the callus on media with 2,4-D. In most cases, the calluses formed on media with IAA were green and firm during the first and second weeks of

- Plate 2. Callus formation on cotyledon and hypocotyl explants on MS basal medium (control) and on media with different concentrations of 2,4-D. The bar represents 5 mm.
- Figure 7. Hypocotyl explant of Yolo Wonder B in control experiment for 4 weeks. Roots and small amount of callus formed. The bar represents 5 mm.
- Figure 8. Cotyledon explant of Yolo Wonder B in control experiment for 4 weeks. Small amount of callus formed at the cut end. The bar represents 5 mm.
- Figure 9. Hypocotyl explant of Yolo Wonder B on medium with $10^{-7}M$ 2,4-D for 4 weeks. Green and firm callus formed. The bar represents 5 mm.
- Figure 10. Cotyledon explant of Yolo Wonder B on medium with $10^{-7}M$ 2,4-D for 4 weeks. Green and firm callus formed. The bar represents 5 mm.
- Figure 11. Hypocotyl explant of Yolo Wonder B on medium with $5 \times 10^{-5}M$ 2,4-D for 4 weeks. Brown and soft callus formed. The bar represents 5 mm.
- Figure 12. Cotyledon explant of Yolo Wonder B on medium with $5 \times 10^{-5}M$ 2,4-D for 4 weeks. Brown and soft callus formed. The bar represents 5 mm.

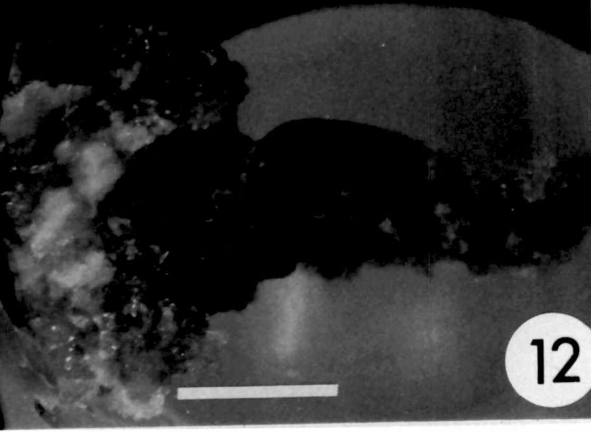
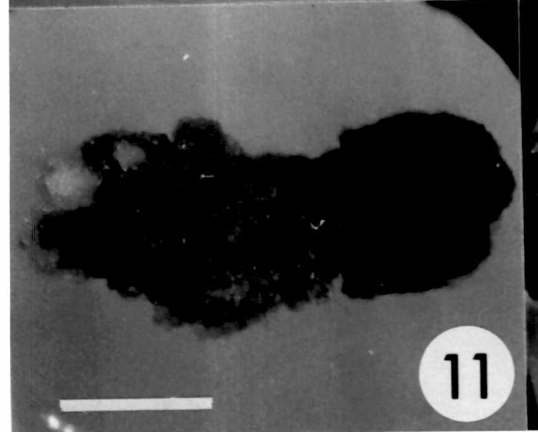
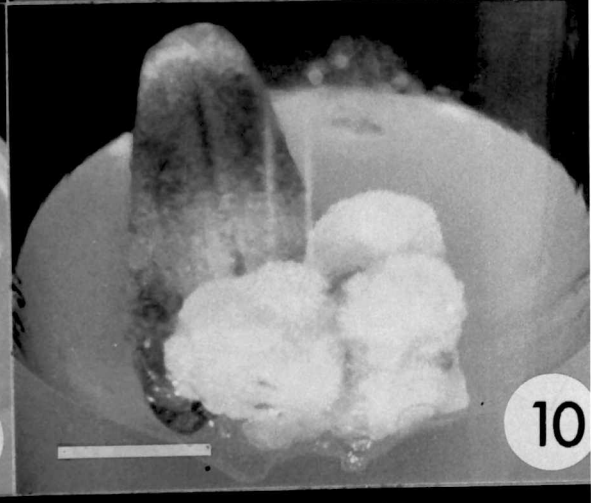
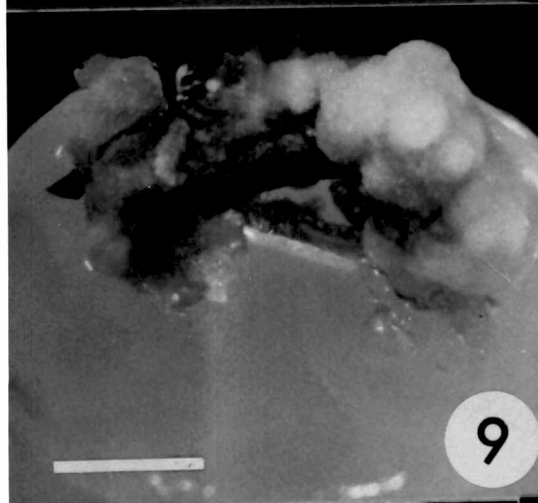
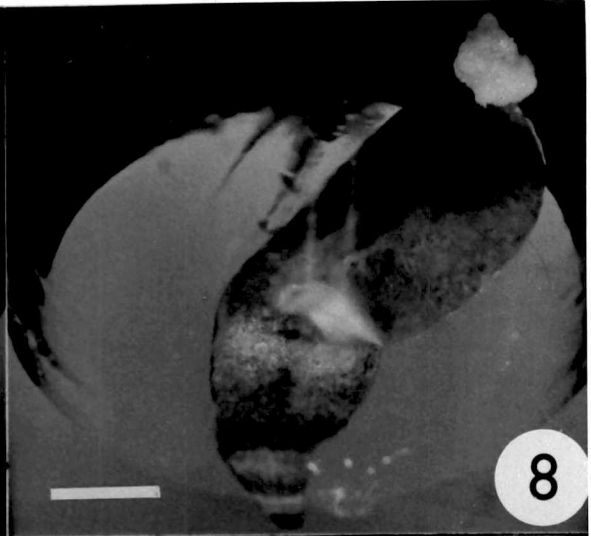
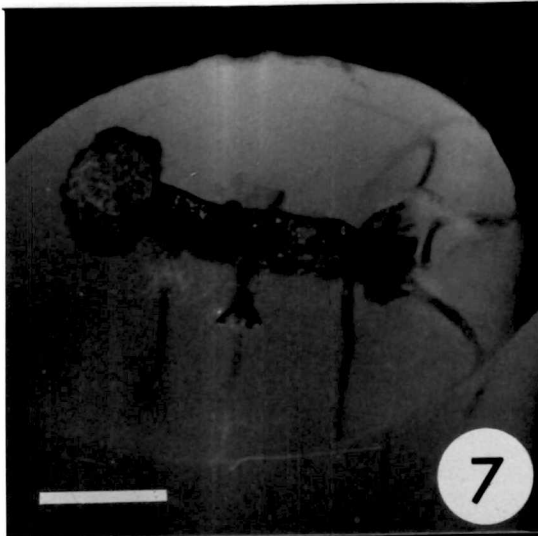


Plate 3. Callus formation on cotyledon and hypocotyl explants on medium with $5 \times 10^{-6} \text{M}$ 2,4-D.

Figure 13. Cotyledon explant of Yolo Wonder B on medium with $5 \times 10^{-6} \text{M}$ 2,4-D for 4 weeks. White and soft callus formed. The bar represents 5 mm.

Figure 14. Hypocotyl explant of Yolo Wonder B on medium with $5 \times 10^{-6} \text{M}$ 2,4-D for 4 weeks. White and soft callus formed. The bar represents 5 mm.

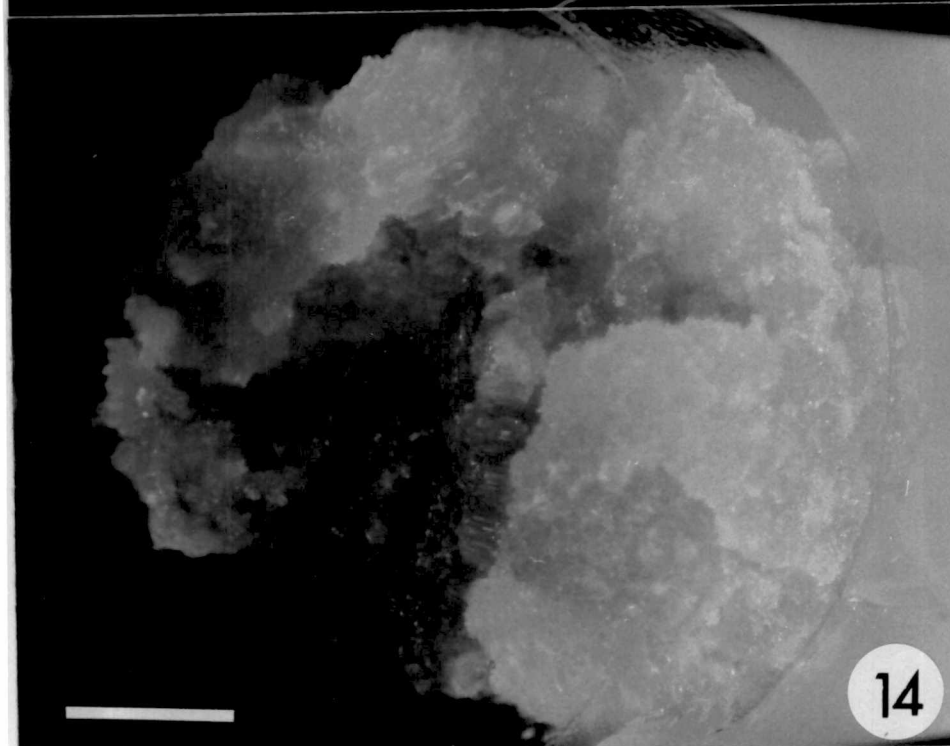
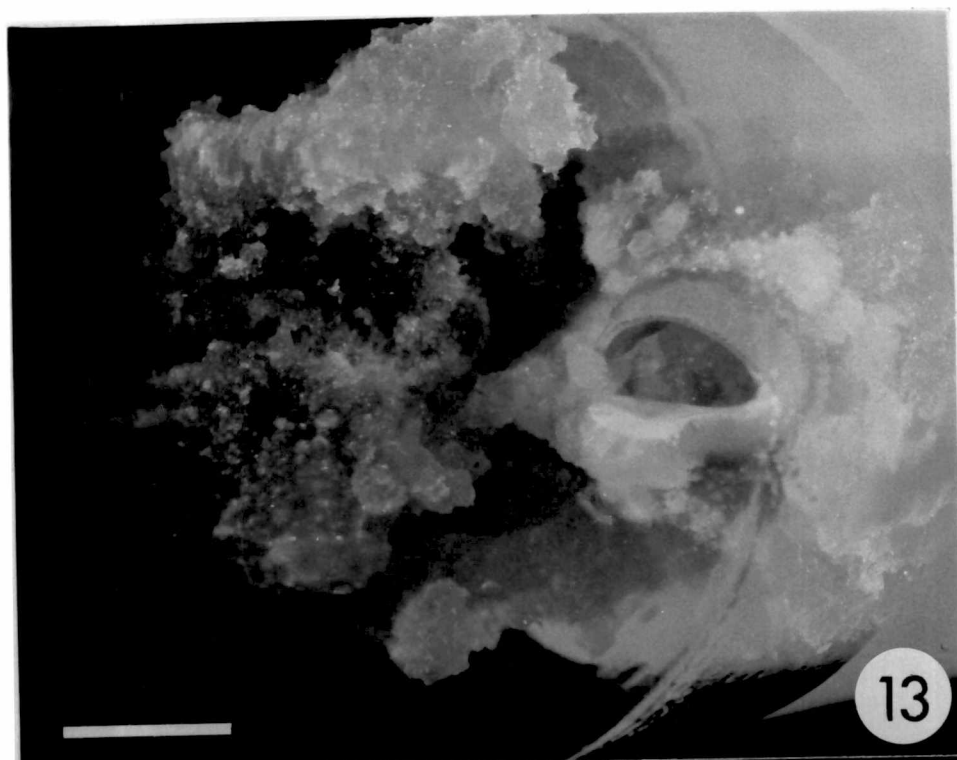


Table 1. Fresh and Dry Weights in Grams of Callus Produced on Cotyledon and Hypocotyl Explants of Yolo Wonder B on Media with 2,4-D.

2,4-D (M)	Cotyledon		Hypocotyl	
	FW ¹	DW ²	FW	DW
0	0.056±0.012 C ³	0.008±0.001 C	0.038±0.006 C	0.004±0.001 D
10 ⁻⁷	0.138±0.024 BC	0.020±0.006 CD	0.135±0.025 BC	0.011±0.003 CD
5x10 ⁻⁷	0.251±0.029 BC	0.024±0.003 CD	0.231±0.065 BC	0.023±0.005 BC
10 ⁻⁶	0.415±0.059 BC	0.036±0.005 BC	0.602±0.083 B	0.039±0.003 B
5x10 ⁻⁶	1.424±0.257 A	0.068±0.011 A	1.592±0.196 A	0.069±0.007 A
10 ⁻⁵	1.458±0.224 A	0.072±0.010 A	1.623±0.330 A	0.064±0.011 A
5x10 ⁻⁵	0.597±0.156 B	0.052±0.009 AB	0.309±0.085 BC	0.023±0.005 BC

¹ FW = fresh weight

² DW = dry weight

³ Means with the same letter are not significantly different by Duncan's Multiple Range Test (P = 0.05).

Table 2. Fresh and Dry Weights in Grams of Callus Produced on Cotyledon and Hypocotyl Explants of Yolo Wonder L on Media with 2,4-D.

2,4-D (M)	Cotyledon		Hypocotyl	
	FW ¹	DW ²	FW	DW
0	0.084±0.009 B ³	0.015±0.001 D	0.057±0.012 C	0.008±0.001 C
10 ⁻⁷	0.150±0.017 B	0.021±0.002 CD	0.118±0.025 D	0.011±0.003 C
5x10 ⁻⁷	0.388±0.043 B	0.041±0.003 BC	0.179±0.037 D	0.016±0.003 C
10 ⁻⁶	0.359±0.107 B	0.030±0.008 BCD	0.847±0.120 C	0.054±0.007 B
5x10 ⁻⁶	1.996±0.443 A	0.094±0.014 A	2.752±0.426 A	0.080±0.011 A
10 ⁻⁵	1.887±0.371 A	0.080±0.011 A	1.836±0.253 B	0.077±0.011 A
5x10 ⁻⁵	0.728±0.145 B	0.050±0.007 B	1.177±0.299 C	0.054±0.012 B

¹ FW = fresh weight

² DW = dry weight

³ Means with the same letter are not significantly different by Duncan's Multiple Range Test (P = 0.05).

Table 3. Fresh and Dry Weights in Grams of Callus Produced on Cotyledon and Hypocotyl Explants of Dunbar Pimiento on Media with 2,4-D.

2,4-D (M)	Cotyledon		Hypocotyl	
	FW ¹	DW ²	FW	DW
0	0.058±0.007 B ³	0.012±0.001 C	0.030±0.004 C	0.004±0.001 C
10 ⁻⁷	0.110±0.021 B	0.016±0.002 BC	0.174±0.038 BC	0.020±0.004 C
5x10 ⁻⁷	0.072±0.014 B	0.013±0.004 C	0.726±0.137 B	0.050±0.008 B
10 ⁻⁶	0.955±0.139 A	0.069±0.008 A	1.743±0.281 A	0.091±0.011 A
5x10 ⁻⁶	1.241±0.266 A	0.069±0.010 A	2.280±0.364 A	0.088±0.010 A
10 ⁻⁵	1.087±0.090 A	0.061±0.003 A	1.674±0.333 A	0.075±0.012 A
5x10 ⁻⁵	0.437±0.069 B	0.031±0.004 B	0.155±0.027 BC	0.013±0.005 C

¹ FW = fresh weight

² DW = dry weight

³ Means with the same letter are not significantly different by Duncan's Multiple Range Test (P = 0.05).

culture, and became brown and firm during the last two weeks of culture (plate 4, figure 15, 16). The production of white soft callus was uncommon and random. The more interesting result was that numerous roots formed in many cultures of both cotyledon and hypocotyl explants (plate 4, figure 16, 17, 18). Many cultures of both cotyledon and hypocotyl explants randomly produced roots directly without forming callus. Many explant cultures produced both callus and roots randomly. In those cases, roots originated on the explant tissue and not on callus tissue (plate 4, figure 17). A few cultures produced callus only (plate 4, figure 19). Before evaluating cultures for callus production, root development on explants was noted, and the percentages of cotyledon and hypocotyl explants which produced roots were recorded. Roots were then removed and discarded from those explant cultures containing them before callus weights were determined.

The amount of callus produced varied with the concentration of IAA in media, but not so widely as callus on media with 2,4-D. Tables 4, 5, and 6 show fresh and dry weights of callus produced on cotyledon and hypocotyl explants of the three pepper varieties on media containing a range of IAA concentrations. Data are shown for the second experimental test. The data for first experiment included root weights with callus weights and thus discounted. It was observed, however, that the extent of callus development in both experimental tests was very similar. The data indicate that both the fresh and the dry weights of callus increased only slightly for both explants and all varieties as the concentration of IAA in the medium increased. It is difficult to match a maximum callus weight with a particular IAA concentration because

Plate 4. Cotyledon and hypocotyl explants on media with different concentrations of IAA.

Figure 15. Hypocotyl explant of Yolo Wonder B on medium with 10^{-7} M IAA for 4 weeks. Small amount of callus formed and roots started to emerge (arrow). The bar represents 5 mm.

Figure 16. Cotyledon explant of Yolo Wonder B on medium with 10^{-7} M IAA for 4 weeks. Roots and callus formed. The bar represents 5 mm.

Figure 17. Hypocotyl explant of Yolo Wonder B on medium with 5×10^{-6} M IAA for 4 weeks. Roots and callus formed. The bar represents 5 mm.

Figure 18. Cotyledon explant of Yolo Wonder B on medium with 5×10^{-6} M IAA for 4 weeks. Roots and callus formed. The bar represents 5 mm.

Figure 19. Hypocotyl explant of Yolo Wonder B on medium with 5×10^{-5} M IAA for 4 weeks. Callus formed. The bar represents 5 mm.

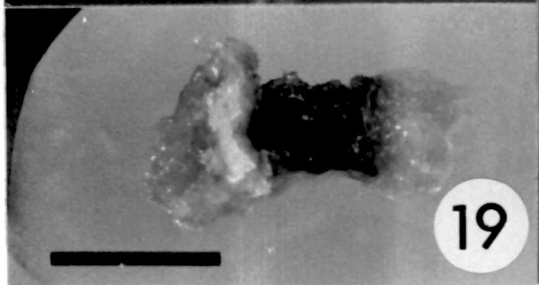
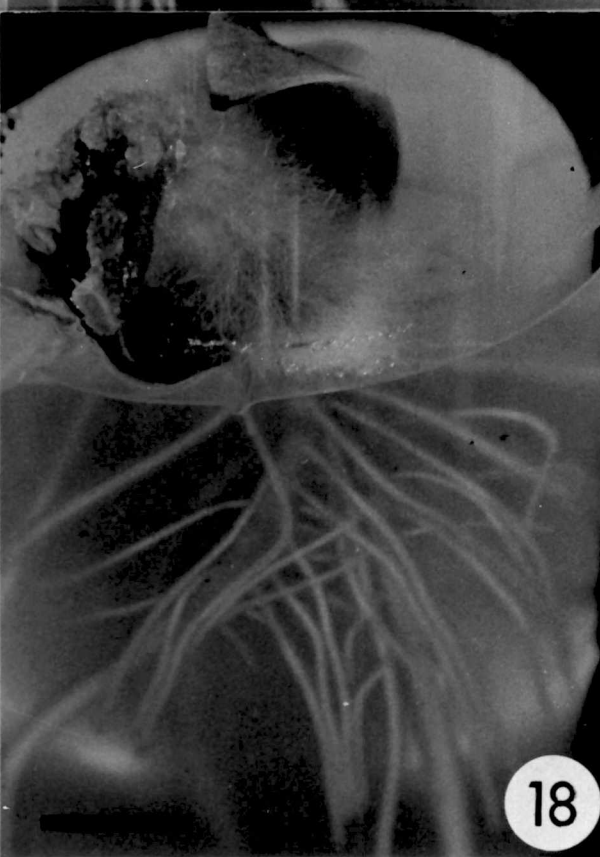
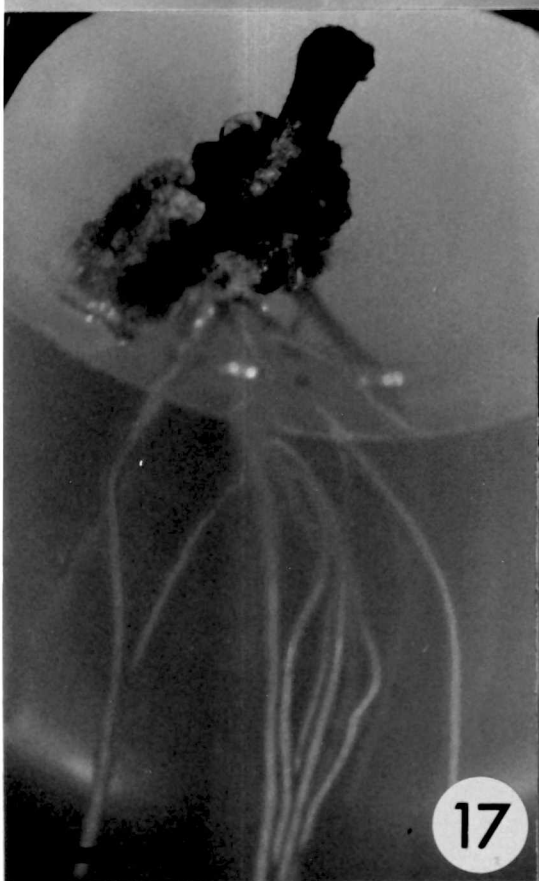
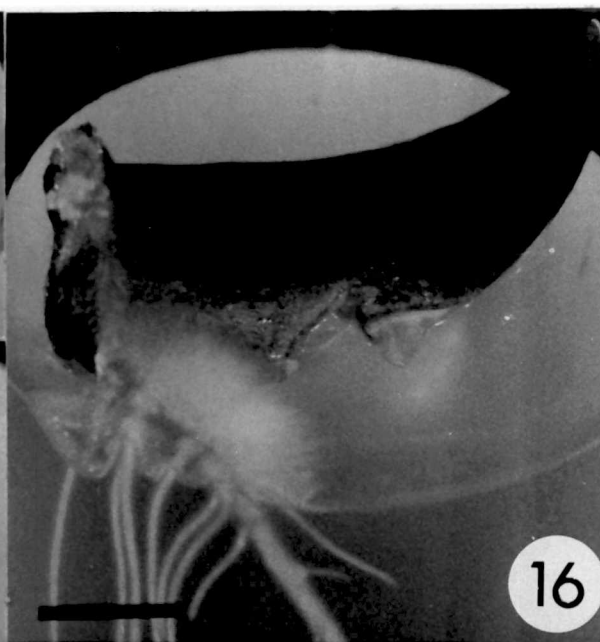
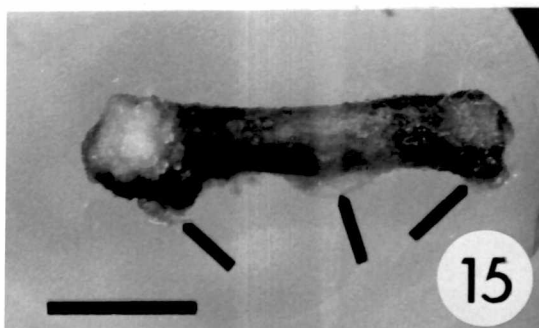


Table 4. Fresh and Dry Weights in Grams of Callus Produced on Cotyledon and Hypocotyl Explants of Yolo Wonder B on Media with IAA.

IAA (M)	Cotyledon		Hypocotyl	
	FW ¹	DW ²	FW	DW
0	0.056±0.012 C ³	0.008±0.001 C	0.038±0.006 C	0.004±0.001 D
10 ⁻⁷	0.090±0.020 BC	0.013±0.001 BC	0.037±0.009 C	0.010±0.002 BCD
5x10 ⁻⁷	0.078±0.013 C	0.013±0.001 BC	0.067±0.010 BC	0.009±0.001 DC
10 ⁻⁶	0.126±0.026 ABC	0.019±0.003 AB	0.144±0.028 AB	0.014±0.002 ABC
5x10 ⁻⁶	0.175±0.031 A	0.018±0.003 AB	0.131±0.026 AB	0.012±0.003 ABC
10 ⁻⁵	0.168±0.031 AB	0.019±0.003 AB	0.172±0.023 A	0.018±0.003 AB
5x10 ⁻⁵	0.209±0.042 A	0.023±0.005 A	0.206±0.048 A	0.019±0.004 A

¹ FW = fresh weight

² DW = dry weight

³ Means with the same letter are not significantly different by Duncan's Multiple Range Test (P = 0.05).

Table 5. Fresh and Dry Weights in Grams of Callus Produced on Cotyledon and Hypocotyl Explants of Yolo Wonder L on Media with IAA.

IAA (M)	Cotyledon		Hypocotyl	
	FW ¹	DW ²	FW	DW
0	0.056±0.012 B ³	0.015±0.001 B	0.057±0.012 C	0.008±0.001 B
10 ⁻⁷	0.079±0.012 B	0.016±0.003 B	0.040±0.011 C	0.008±0.001 B
5x10 ⁻⁷	0.078±0.006 B	0.014±0.001 B	0.053±0.010 C	0.009±0.001 B
10 ⁻⁶	0.106±0.012 B	0.015±0.002 B	0.052±0.007 C	0.008±0.001 B
5x10 ⁻⁶	0.070±0.008 B	0.015±0.002 B	0.103±0.009 BC	0.008±0.001 B
10 ⁻⁵	0.163±0.032 A	0.027±0.007 A	0.153±0.031 B	0.017±0.003 A
5x10 ⁻⁵	0.096±0.013 B	0.013±0.002 B	0.242±0.046 A	0.016±0.003 A

¹ FW = fresh weight

² DW = dry weight

³ Means with the same letter are not significantly different by Duncan's Multiple Test (P = 0.05).

Table 6. Fresh and Dry Weights in Grams of Callus Produced on Cotyledon and Hypocotyl Explants of Dunbar Pimiento on Media with IAA.

IAA (M)	Cotyledon		Hypocotyl	
	FW ¹	DW ²	FW	DW
0	0.058±0.007 B ³	0.012±0.001 A	0.030±0.004 B	0.004±0.001 B
10 ⁻⁷	0.111±0.023 A	0.018±0.003 A	0.080±0.010 A	0.010±0.002 AB
5x10 ⁻⁷	0.075±0.011 AB	0.013±0.002 A	0.065±0.008 AB	0.007±0.001 B
10 ⁻⁶	0.119±0.017 A	0.018±0.002 A	0.081±0.017 A	0.011±0.002 A
5x10 ⁻⁶	0.060±0.008 B	0.012±0.002 A	0.074±0.012 A	0.011±0.002 A
10 ⁻⁵	0.092±0.014 AB	0.018±0.002 A	0.100±0.015 A	0.011±0.002 A
5x10 ⁻⁵	0.078±0.010 AB	0.014±0.001 A	0.063±0.010 AB	0.010±0.002 A

¹ FW = fresh weight

² DW = dry weight

³ Means with the same letter are not significantly different by Duncan's Multiple Range Test (P = 0.05).

maximum callus production varied along the concentration gradients of IAA. This is especially true for callus production in variety Dunbar Pimiento.

The Effect of NAA on Callus Growth

The auxin NAA was also not so effective as 2,4-D in callus production. Moreover, the color and texture of the callus also did not vary so much as the callus on media with 2,4-D. Calluses formed on media with NAA concentrations of 10^{-6} M and less were green and firm. At media concentrations of 5×10^{-6} and above, callus tended to be white and soft. As in those cultures on media with IAA (plate 4, figures 14 to 19, page 23), roots formed in many cultures of both cotyledon and hypocotyl explants, and in the same patterns. Before evaluating cultures for callus production, root development on explants was noted, and the percentage of cotyledon and hypocotyl explants which produced roots were recorded. Roots were then removed and discarded from those explant cultures containing them before callus weights were determined.

The amount of callus produced varied with the concentration of NAA in media, but not so widely as callus on media with 2,4-D. Tables 7, 8, and 9 show fresh and dry weights of callus produced on cotyledon and hypocotyl explants of the three pepper varieties on media containing a range of NAA concentrations. Data are shown for the second experimental test. The data for first experiment included root weights with callus weights and thus discounted. It was observed, however, that the extent of callus development in both experimental tests was very similar. The data show that both the fresh and the dry weights of callus increased for both of the explants and for all varieties as the concentration of

Table 7. Fresh and Dry Weights in Grams of Callus Produced on Cotyledon and Hypocotyl Explants of Yolo Wonder B on Media with NAA.

NAA (M)	Cotyledon		Hypocotyl	
	FW ¹	DW ²	FW	DW
0	0.056±0.012 D ³	0.008±0.001 E	0.038±0.006 C	0.004±0.001 C
10 ⁻⁷	0.224±0.051 D	0.030±0.007 ED	0.207±0.051 BC	0.020±0.007 BC
5x10 ⁻⁷	0.270±0.032 CD	0.033±0.003 CD	0.348±0.045 BC	0.030±0.004 B
10 ⁻⁶	0.599±0.168 B	0.056±0.014 AB	0.291±0.072 BC	0.028±0.006 BC
5x10 ⁻⁶	0.518±0.081 B	0.046±0.007 BC	0.817±0.148 A	0.062±0.009 A
10 ⁻⁵	0.466±0.061 BC	0.039±0.005 BCD	0.858±0.129 A	0.059±0.009 A
5x10 ⁻⁵	0.817±0.052 A	0.065±0.019 A	0.542±0.105 AB	0.031±0.007 B

¹ FW = fresh weight

² DW = dry weight

³ Means with the same letter are not significantly different by Duncan's Multiple Range Test (P = 0.05).

Table 8. Fresh and Dry Weights in Grams of Callus Produced on Cotyledon and Hypocotyl Explants of Yolo Wonder L on Media with NAA.

NAA (M)	Cotyledon		Hypocotyl	
	FW ¹	DW ²	FW	DW
0	0.084±0.009 D ³	0.015±0.001 B	0.057±0.012 B	0.008±0.001 D
10 ⁻⁷	0.141±0.024 CD	0.026±0.001 AB	0.083±0.017 B	0.012±0.001 CD
5x10 ⁻⁷	0.290±0.030 BC	0.038±0.004 A	0.332±0.042 AB	0.032±0.005 A
10 ⁻⁶	0.342±0.068 BC	0.035±0.007 A	0.353±0.051 A	0.036±0.025 AB
5x10 ⁻⁶	0.466±0.078 AB	0.038±0.006 A	0.442±0.087 A	0.033±0.007 ABC
10 ⁻⁵	0.256±0.045 CD	0.026±0.004 AB	0.499±0.107 A	0.037±0.007 AB
5x10 ⁻⁵	0.555±0.062 A	0.036±0.005 A	0.288±0.035 AB	0.018±0.002 ABD

¹ FW = fresh weight

² DW = dry weight

³ Means with the same letter are not significantly different by Duncan's Multiple Range Test (P = 0.05).

Table 9. Fresh and Dry Weights in Grams of Callus Produced on Cotyledon and Hypocotyl Explants of Dunbar Pimiento on Media with NAA.

NAA (M)	Cotyledon		Hypocotyl	
	FW ¹	DW ²	FW	DW
0	0.058±0.007 D ³	0.012±0.001 C	0.030±0.004 D	0.004±0.001 C
10 ⁻⁷	0.091±0.014 D	0.015±0.002 C	0.055±0.006 D	0.007±0.001 C
5x10 ⁻⁷	0.115±0.024 D	0.015±0.003 C	0.034±0.001 D	0.004±0.001 C
10 ⁻⁶	0.450±0.100 BC	0.042±0.008 AB	0.486±0.084 BC	0.040±0.008 B
5x10 ⁻⁶	0.551±0.065 AB	0.047±0.007 A	0.863±0.174 B	0.040±0.006 B
10 ⁻⁵	0.569±0.081 A	0.052±0.006 A	1.324±0.237 A	0.073±0.012 A
5x10 ⁻⁵	0.357±0.017 C	0.027±0.003 BC	0.223±0.006 CD	0.013±0.001 C

¹ FW = fresh weight

² DW = dry weight

³ Means with the same letter are not significantly different by Duncan's Multiple Range Test (P = 0.05).

NAA in the medium increased. However, maximum weights of callus varied among the explants and the varieties. Like the experiments with IAA, it is difficult to match a maximum callus weight with a particular NAA concentration because maximum callus production varied along the concentration gradients of NAA for all three varieties.

Organogenesis from Seedling Explants

Root Induction on Seedling Explants

It was reported in the callus production section of RESULTS that numerous roots formed in cultures of both cotyledon and hypocotyl explants (plate 4, figures 16, 17, 18, page 23) on media with IAA and with NAA. Even a few cultures of the controls produced roots (plate 2, figure 7, page 14). Explants on media with 2,4-D did not produce roots. While the callus cultures were being evaluated for callus production, the developmental morphology of the roots from cotyledon and hypocotyl explants was noted, and the percentages of cotyledon and hypocotyl explants which produced roots were recorded.

The roots from the cotyledon explants usually originated from the abaxial surface of the blade (plate 4, figures 16, 18, page 23) and tended to develop near the leaf base or in the middle of the blade near the midvein. The roots from the hypocotyl explants usually originated from the two cut ends of the cylinder-shaped explant and later more roots developed on the rest of the explant sequentially from the ends toward the middle. In many instances, more roots were produced from the callus of both types of explants after callus had developed on them. In all of the explant cultures on media with IAA and with NAA, roots

developed within two weeks after the explants were placed in culture. Within four weeks after culture, many roots were estimated to be about 10 cm in length. In general, roots formed earlier on explants which were on media with IAA than those on media with NAA. Also, the roots growing from the explants on media with IAA appeared to be longer and thinner than those roots growing on explants on media with NAA. The percentages of cotyledon and hypocotyl explants which produced roots on media with IAA are shown in Table 10. The percentages for roots on media with NAA are shown in Table 11. In both situations, the percentages of roots fluctuated widely between hormonal concentrations, explants, and varieties. It is difficult to match a maximum percentage with a particular hormone concentration because maximum percentages of roots varied widely along the concentration gradients of IAA and of NAA for both explants and for all three varieties. All that can be said is that most cotyledon and hypocotyl explant cultures of the three pepper varieties produced roots under the influence of IAA or NAA at concentrations in media varying from $10^{-7}M$ to $5 \times 10^{-5}M$.

Shoot Bud Formation on Seedling Explants and Callus

Preliminary experiments with hormone combinations demonstrated that media with BA and IAA induced the greatest percentages of shoot buds on cotyledon and hypocotyl explants of the three varieties than did other combinations. Media with BA and 2,4-D, did not induce any shoot bud formation at all on any of the explants. Explants on media with BA and NAA only produced callus which was green and firm. Explants on media with BA and 2,4-D only produced callus which was white and soft.

Preliminary experiments also showed that cotyledon explants produced

Table 10. The Percentages of Cotyledon and Hypocotyl Explants Forming Roots on Medium with IAA for All Three Varieties of Pepper.

IAA (M)	Yolo B		Yolo L		Pimiento	
	C ¹	H ²	C	H	C	H
10 ⁻⁷	15.4	30	7	73	0	60
5x10 ⁻⁷	13.3	42.9	23	58.8	8	72.7
10 ⁻⁶	14.3	69.2	41.5	45.5	8	30.8
5x10 ⁻⁶	50	81.8	15.4	36.4	9	87.5
10 ⁻⁵	58.3	59.3	40	91.7	30.8	66.7
5x10 ⁻⁵	50	70	30.8	50	50	71.4

C¹ = cotyledon

H² = hypocotyl

Table 11. The Percentages of Cotyledon and Hypocotyl Explants Forming Roots on Medium with NAA for all three Varieties of Pepper.

NAA (M)	Yolo B		Yolo L		Pimiento	
	C ¹	H ¹	C	H	C	H
10 ⁻⁷	30.3	41.6	25	37.5	14.3	61.5
5x10 ⁻⁷	63	33.3	60	39	50	42.9
10 ⁻⁶	85.7	55.6	50	41.7	58.3	69.2
5x10 ⁻⁶	100	100	50	54.5	92.3	81.8
10 ⁻⁵	66.7	54.5	16.7	42.9	83.3	58.3
5x10 ⁻⁵	30	28.6	40	10	66.7	70

C¹ = cotyledon

H² = hypocotyl

shoot buds much more frequently than hypocotyl explants.

A detailed experiment based on preliminary experiments was designed in order to study the effect of different combinations of BA and IAA containing media on shoot bud formation on cotyledon explants. Cotyledon explants were placed on the different media abaxial side down on the medium.

In contrast to callus and root development on cotyledon explants placed on media with IAA alone (plate 5, figure 20), cotyledon explants placed on media with BA and IAA produced shoot buds (plate 5, figures 21, 22, 23, and 24). The first stage was callus development on the cotyledon explant within two weeks after initial culture of the explant in a manner similar to that described previously in the section on callus production (plate 5, figure 21). Immediately after callus formation, numerous, tiny, immature, shoot buds appeared on the callus at the junction of blade tissue and callus, and then extending all around the callus (plate 5, figures 22 and 23). This pattern of shoot bud development occurred mostly on the abaxial side of the blade. In a very few instances, shoot buds developed on the callus of the adaxial side of the cotyledon explant. After three weeks, shoot buds became larger and more distinct (plate 5, figure 24). After four to five weeks, the shoot buds in cultures on medium with $5 \times 10^{-5} \text{M}$ BA and any other concentration of IAA produced enhanced leaf blade growth and no further growth of the callus (plate 6, figure 25). By contrast, the shoot buds in cultures on medium with $5 \times 10^{-6} \text{M}$ or 10^{-5}M BA with any other concentration of IAA produced more green and firm callus and no further shoot bud development (plate 6, figure 26). In fact, the additional

Plate 5. Cotyledon explants on medium with 5×10^{-5} M IAA and on medium with different combinations of BA and IAA.

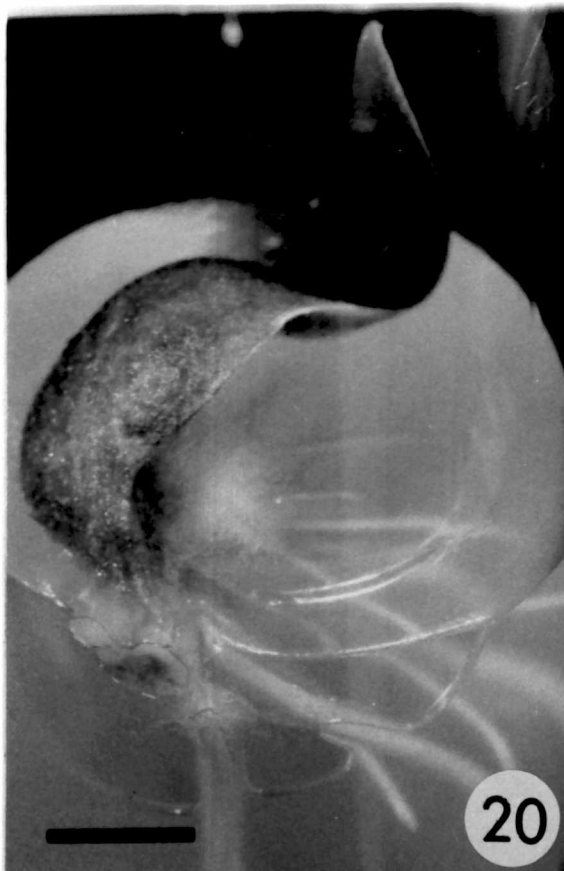
Figure 20. Cotyledon explant of Yolo Wonder B on medium with 5×10^{-5} M IAA. Many roots formed on the abaxial side. The bar represents 5 mm.

Figure 21. Cotyledon explant of Dunbar Pimiento on medium with 5×10^{-7} M IAA and 5×10^{-6} M for 13 days. Callus formed at the cut end and surrounding the necrotic tissue.

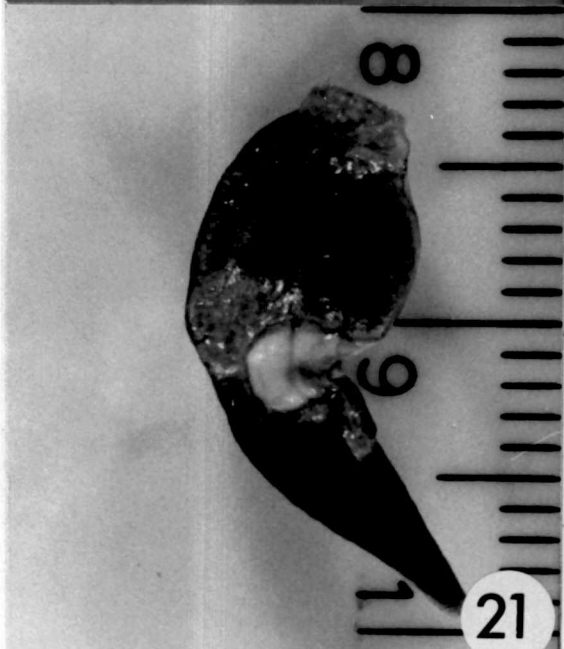
Figure 22. Cotyledon explant of Yolo Wonder B on medium with 10^{-5} M IAA and 5×10^{-5} M BA for 13 days. Green, tiny, immature shoot buds formed on callus (arrows).

Figure 23. Cotyledon explant of Yolo Wonder B on medium with 5×10^{-7} M IAA and 5×10^{-5} M BA for 2 weeks, showing enlarged green buds.

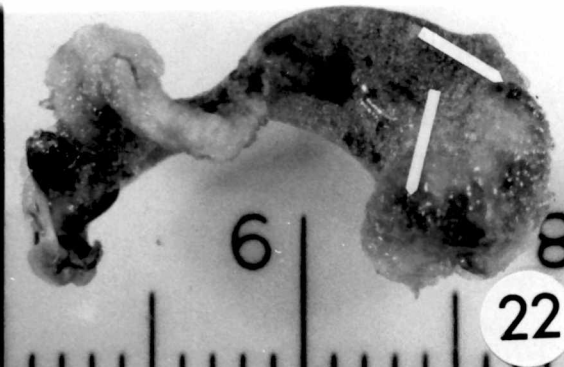
Figure 24. Cotyledon explant of Yolo Wonder B on medium with 10^{-5} M IAA and 5×10^{-5} M BA for 3 weeks. Shoot buds distinct.



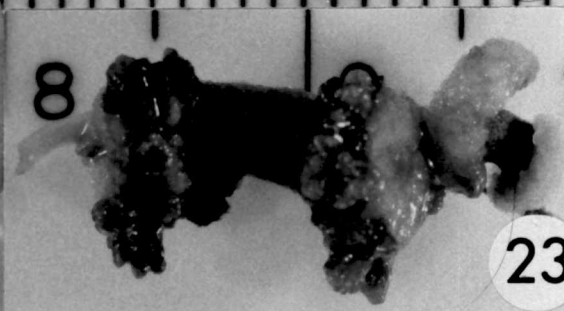
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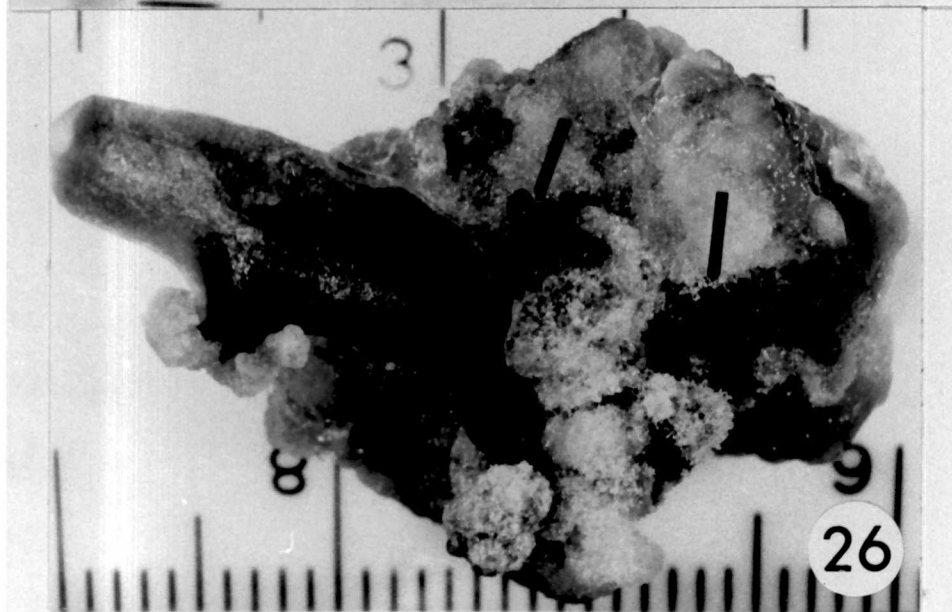


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Plate 6. Late stages of shoot bud organogenesis.

Figure 25. Cotyledon explant of Yolo Wonder B on medium with 10^{-5} M IAA and 5×10^{-5} M BA for 5 weeks, showing buds with enlarged leaves.

Figure 26. Cotyledon explant of Yolo Wonder B on medium with 10^{-7} M IAA and 10^{-5} M BA for 4 weeks. Callus shown engulfing shoot buds.



callus growth engulfed some shoot buds and buried some out of sight.

At the end of the two weeks of culture time, the percentages of cotyledon explant cultures which produced shoot buds were determined. The data are presented in Table 12. The table shows that the percentages of shoot buds fluctuate between hormonal combinations in all three varieties. It is difficult to match a maximum percentage with a particular hormone combination because maximum percentages of shoot buds varied along the concentration gradients of any BA concentration with one IAA concentration, and any IAA concentration with one BA concentration for all three varieties. All that could be said is that all cotyledon explant cultures of all three pepper varieties produced shoot buds under the influence of the combination concentrations of BA and IAA tested in these experiments.

Attempts were made to obtain large shoots from shoot buds. Selected shoot bud cultures were transferred to MS basal medium plus 1% charcoal and to MS basal medium without charcoal. After four weeks of culture, shoot buds showed some growth in cultures with and without charcoal. Cultures are still in progress. In addition, attempts were made to obtain whole plants. Selected shoot bud cultures were placed on MS medium with 5×10^{-6} M IAA, and medium with 5×10^{-6} M NAA with the hope of inducing root development. After two weeks of culture, roots did not appear. Cultures are still in progress.

Table 12. The Percentages of Cotyledon Explants Forming Shoot Buds on Media with Combinations of BA and IAA for All Three Varieties of Pepper in Two Weeks.

		Yolo B			Yolo L			Pimiento		
BA (M)		5×10^{-6}	10^{-5}	5×10^{-5}	5×10^{-6}	10^{-5}	5×10^{-5}	5×10^{-6}	10^{-5}	5×10^{-5}
IAA (M)										
0		53.9	46.2	30.8	33.3	38.5	69.2	57.1	64.3	76.9
10^{-7}		90.9	72.7	38.5	40.0	23.1	92.3	57.1	54.5	83.3
5×10^{-7}		66.7	85.7	100	72.7	61.5	91.7	61.5	92.9	100
10^{-6}		71.4	92.3	80.0	61.5	83.3	81.3	85.7	92.3	92.3
5×10^{-6}		71.4	85.7	92.3	66.7	92.3	93.3	83.3	92.3	100
10^{-5}		50	85.7	100	92.6	92.9	100	60	91.7	100
5×10^{-5}		19.2	78.6	50	21.4	77.8	38.5	21.4	53.8	85.7

IV. DISCUSSION

This research on three selected, domestic varieties of sweet pepper has demonstrated that cotyledon and hypocotyl explants of cultured seedlings are capable of producing (1) callus on media supplemented with the auxins 2,4-D, IAA, and NAA, (2) roots on media with IAA and NAA, and (3) shoot buds on media with IAA plus the cytokinin BA. Gunay and Rao (1978) reported the induction of callus, roots, and shoot buds on cotyledon and hypocotyl explants of seedlings of three varieties of sweet pepper that differed from those utilized in this research. They obtained results similar to those presented here. However, since they cultured explants on media with only one concentration of hormone, their results are inconclusive. The present research involves the testing of a series of increasing concentrations of hormones in media in order to determine maximum efficacy for callus production and organogenesis.

The data for callus production show that the auxin 2,4-D was more effective than IAA and NAA in inducing callus formation on both cotyledon and hypocotyl explants of all three varieties of pepper tested. Moreover, auxin concentrations in the vicinity of $5 \times 10^{-6} \text{M}$ 2,4-D induced larger masses of callus than did IAA and NAA. These findings have not been reported previously for capsicum pepper, but have been observed in callus research in other plants (Yeoman and Macleod, 1977). Gunay and Rao (1978) simply induced callus formation on cotyledon and hypocotyl explants of pepper seedlings on MS medium with 1 mg/l of 2,4-D, or with IAA, or with NAA. For 2,4-D, 1 mg/l is about $5 \times 10^{-6} \text{M}$. For IAA, it is about $6 \times 10^{-6} \text{M}$, and for NAA, it is about $6 \times 10^{-6} \text{M}$. It is

interesting to note that in the work of Gunay and Rao, medium with 1 mg/l of 2,4-D was the most effective concentration for callus production. That fact matches the work reported here. Rogers et al (1974) reported callus formation on stem segments excised from young nodes of sweet pepper plants on MS medium with 1 mg/l of NAA, 1 mg/l of kinetin, and 5 mg/l of 2,4-D. Fári and Czaká (1981) showed that hypocotyl segments of seedlings of sweet pepper variety T. Hatvani responded differently. On MS medium with 1 mg/l of IAA and 2 mg/l of BA, apical segment of hypocotyl produced shoot buds, middle segments produced roots, and basal segments produced callus. This finding was not observed in the research reported here.

Another important finding which has not been reported previously for sweet pepper is that callus color and texture varied as the concentration of hormones in the media varied, and the time of culture varied. In all cases, callus was green and firm during the first two weeks of culture. During the last two weeks of culture, callus on media with 10^{-7} M and 5×10^{-7} M 2,4-D remained green and firm. Callus on media with 10^{-6} M and 5×10^{-6} M 2,4-D was white and soft. Callus on media with 10^{-5} M and 5×10^{-5} M 2,4-D was brown and soft. During the last two weeks of culture, callus on media with all concentrations of IAA were brown and firm. And during the last two weeks of culture, callus on media with NAA concentrations of 10^{-6} M and less were green and firm, and those on media with 5×10^{-6} M and higher were white and soft. Such findings are new for sweet pepper callus, but have been reported previously for callus of other plants (Aitchison et al., 1977). The important point is that one can obtain the desired color and texture of sweet pepper callus for a

particular research requirement by altering the auxin concentration in the medium. Firm callus would be more suitable for callus studies per se, whereas soft callus would be more suitable for the preparation of cell suspension cultures.

As previously stated, the present research has shown that cotyledon and hypocotyl explants of seedlings are able to produce roots on media with IAA and NAA, but not with 2,4-D. This finding is not new. This is a well known fact as reported by Reinert, et al. (1977), by Gunay and Rao (1978) for their varieties of sweet pepper, and for explants of other plant tissues in many publications which have been well reviewed by Yeoman and Forche (1980). Moreover, Yeoman and Forche (1980) indicated that although 2,4-D induced cell division very well, it tended to suppress organogenesis. They suggest that NAA or IAA alone or in combination with BA, Zeatin, or 2iP may be more effective. In addition, the present research has shown that large percentages of explants on media containing a rather broad range of IAA or NAA concentration can produce large numbers of roots.

It was interesting to observe that explants on media with IAA or with NAA produced roots with no accompanying shoots for the formation of whole plants. This situation appears to be a general phenomenon in plant tissues as reported by Pierik (1987). This aspect requires more future research, and would make a valuable contribution to the area of organogenesis.

As mentioned earlier, the present research has shown that large percentages of cotyledon explants can produce shoot buds from produced callus on a rather broad range of concentrations of a combination of BA

and IAA. Gunay and Rao (1978) obtained similar results with all of their pepper varieties, but only on media containing 2 mg/l of BA plus 1 mg/l of IAA. Fári and Czáká (1981) obtained the same results with apical segments of hypocotyl explants of their pepper variety. It is interesting that shoot buds could be obtained easily on explants exposed to media containing a combination of BA and IAA, but very difficult to obtain large plants from the shoot buds. Repeated attempts on different media have thus far failed with the pepper varieties in this research. Rogers, et al. (1974) also reported the failure to obtaining large plants. Further research is needed on this aspect. By contrast, this is a common occurrence in many other species of plants as reported in reviews by Reinert, et al (1977), and Bhojwani and Razdan (1983). Gunay and Rao (1978) and Fári and Czáká (1981) obtained large plants from shoot bud cultures of their varieties. Beginning with the work of Skoog and Miller (1957), it has become a well known fact that explant cultures on media with a high concentration of a cytokinin in combination with a low concentration of an auxin favor shoot induction (Bhojwani and Razdan, 1983). It is interesting that almost all the shoot buds were induced from the abaxial side of cotyledon explants. To obtain a high frequency of shoot bud formation, it seems important to place the cotyledon explant with abaxial side down. The importance of correct placing of the explant was indicated by Pierik (1987).

This reseach is a necessary first step toward solving disease problems in sweet pepper by tissue culture methods. A more extensive knowledge of callus production and organogenesis has been obtained. This knowledge can now be applied to further reseach in sophisticated

techniques of molecular botany in an effort to obtain disease resistant plants by tissue culture techniques. At the same time, further research should explore the possibility of obtaining cell suspension cultures from soft pepper callus, and protoplast cultures from soft callus, shoot buds, and cotyledons of cultured seedlings. Molecular techniques on these tissue cultures could also yield disease-resistant pepper plants in the field.

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