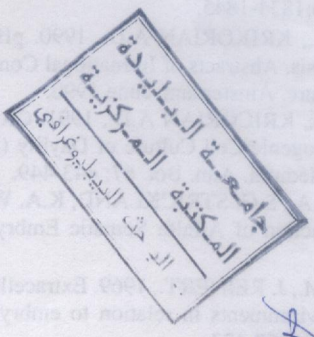

character of the vascular reorganization is concurrent with the concept of discontinuous circumferential changes in shoot apex responsible for qualitative changes in phyllotaxis (Zagórska-Marek 1987, 1994, Meicenheimer, Zagórska-Marek 1989). The sectorial character of morphogenetic changes in *Anagallis* was already reported by the author of this work for the circumferential spacing of leaf primordia in shoot apices with changing phyllotaxis (Kwiatkowska 1993). The vascular pattern change in the transitions from spiral Fibonacci to trimerous phyllotaxis, and from the trimerous to spiral Lucas, is more difficult to interpret, because in shoots with these patterns the mode of connection between the stem and leaf vasculature is not uniform.

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The author wishes to thank Prof. B. Zagórska-Marek for all the discussions and the help in correcting the original manuscript. The final stages of this research have been supported by a grant (No 6 P204 046 06) from KBN (State Commitee for Scientific Research).

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ZMIANY ROZWOJOWE PIERWOTNEGO SYSTEMU WASKULARNEGO PĘDÓW *ANAGALLIS ARVENSIS* L.

STRESZCZENIE

Zmiany rozwojowe pierwotnego systemu waskularnego pędów *Anagallis* są ściśle związane ze zmianami wzoru filotaksji. W ontogenezie tego gatunku naprzemiennie występują spiralne i okółkowe wzory ulistnienia. Porządek ich występowania jest następujący: Początkowy wzór, naprzeciwległy, zmienia się we wzór skrętoległy Fibonacciego, a następnie dalej we wzór okółkowy z trzema liśćmi w okółku. Ten z kolei przechodzi we wzór skrętoległy Lucasa. Sporadycznie występuje także bezpośrednia transformacja wzoru okółkowego naprzeciwległego we wzór okółkowy z trzema liśćmi w okółku. System waskularny *Anagallis* jest zawsze zamknięty, mimo że występują zarówno okółkowe jak i spiralne wzory ulistnienia. Liczba śladów odchodzących do każdego liścia jest również stała. W trakcie wszystkich transformacji filotaksji wzrasta liczba sympodiów waskularnych i liczba śladów liściowych przebiegających w cylindrze waskularnym. Wzrost ten jest najczęściej stopniowy: podczas transformacji liczba sympodiów wzrasta jednostkowo, a liczba śladów o jeden lub dwa. Jednorazowe dodanie dwóch sympodiów występuje wyłącznie w trakcie najrzadziej spotykanej transformacji pomiędzy wzorami okółkowymi z dwoma i trzema liśćmi w okółku. Najczęściej wzrost liczby sympodiów jest równoczesny z transformacją, jednak w przypadku zmiany wzoru okółkowego z trzema liśćmi w okółku we wzór skrętoległy Lucasa, wzrost ten jest opóźniony czasami aż o dziesięć plastochronów. Mimo zmieniającej się filotaksji pędów *Anagallis*, w obrębie cylindra waskularnego można wyznaczyć sektor, w którym ułożenie sympodiów waskularnych jest takie, jak gdyby utrzymywany był poprzedni wzór ulistnienia.

SŁOWA KLUCZOWE: *Anagallis arvensis*, pierwotny system waskularny pędu, transformacje filotaksji.

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FACTORS INFLUENCING CUCUMBER (*CUCUMIS SATIVUS* L.) SOMATIC EMBRYOGENESIS. I. THE CRUCIAL ROLE OF pH AND NITROGEN IN SUSPENSION CULTURE

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ABSTRACT

A method of obtaining and the characteristics of an embryogenic stabilised cucumber (*Cucumis sativus* L.) suspension culture which has many similarities to the carrot model are presented. The Specific Type I cells and proembryogenic mass were present in such a suspension. The maintenance of the proembryogenic stage took place in medium containing 2,4-D as the sole growth regulator, subsequent stages of embryogenesis occurred in hormone-free medium. Embryonic structures were also observed in medium with auxin in the late stages of growth, probably due to the depletion of 2,4-D in the medium during subculture. The choice of the proper inorganic nitrogen sources and the maintenance of correct proportions between them had a significant effect on the formation of these structures. We have shown that the pH of the medium with an embryogenic culture became stabilized regardless of the initial pH value and depended on the medium composition. The inoculum used for the initiation of subsequent subcultures of the stable suspension culture was 1 part tissue to 300 parts medium and was small in comparison to the systems described for the cucumber so far. From 1 ml of basic suspension 7 embryos were obtained on medium without growth regulators 10 days after inoculation, and this amount increased to 21 after 3 weeks. From 3.2% of the somatic embryos it was posible to regenerate plants. The high yield and synchronisation of the process and the development of embryos without passing through callus tissue create the possibility of using this system for molecular investigations and in the technology of somatic seed production.

KEY WORDS: cucumber, 2,4-D, long-term cell suspension, nitrogen source, somatic embryogenesis, pH changes.

INTRODUCTION

Cucumber is a vegetable of economic importance, which warrants the use of modern technologies of vegetative multiplication. This includes artificial seed technology, which requires detailed information about the conditions essential for somatic embryogenesis. Malepszy (1988) has rewived various types of cucumber tissue cultures and the explants used for their initiation. Somatic embryogenesis in the cucumber is a fairly common phenomenon, nevertheless the variability of embryo formation in *in vitro* culture is so large that it is difficult to consider cucumber a model plant for the study of this process. The formation of somatic embryos in *in vitro* cultures does not always result in their conversion into normal plants. Embryogenic cucumber callus can be obtained from leaf explants via a characteristic phase designated gel-like callus (Malepszy and Nadolska-Orczyk 1983). Somatic embryogenesis in cucumber suspension culture initiated from a gel-like callus has been reported by Malepszy and Solarek (1986). Embryogenic callus cultures were also used for initiation of embryogenic cell suspensions by Chée and Tricoli (1988), Callebaut et al. (1987), Callebaut and Motte (1988), Bergervoet et al. (1989) and Rossi et al. (1990). As reported for carrot (Halperin, 1966) it is also possible to synchronize the embryogenic process in cucumber and obtain somatic embryos without callus formation (Wróblewski and Malepszy, 1992).

Early reports (Halperin and Wetherel, 1965; Halperin, 1966), in which somatic embryos were shown to develop from proembryogenic aggregates (PEM), indicated that the nitrogen source in the medium would play a significant role in this process. In embryogenic suspension cultures of alfalfa Stuart et al. (1987) and McDonald and Jackman (1989) observed pH changes as a result of selective uptake of ammonium ions by the growing tissue. Martin et al. (1987) and Rose and Martin (1975) studied on *Ipomoea* sp. suspension cultures the uptake of inorganic nitrogen from the medium. Some differences in inorganic nitrogen uptake in various cucumber lines were observed by Wróblewski et al. (1994). To date, however, the influence of the form of nitrogen on embryogenesis in cucumber suspension cultures has not been relationed.

MATERIALS AND METHODS

1. Preparation of plant material

Sterile seedlings of a highly inbred cucumber (*Cucumis sativus* L.) line (line B obtained from the Borszczagowski variety after 15 self-pollinations) were prepared as described previously (Malepszy, 1988), except that the temperature during seedling growth was maintained at 24°C, and the light intensity was 4 kLx (LF, Philips TLD 36W/33). Liquid cultures were initiated directly from primary explants by isolating vegetative shoot apices about 1,5 mm long.

2. Initiation of the suspension culture

Each 10 shoot apices obtained from sterile seedlings were placed in 100 ml of liquid medium in a 350 ml Erlenmeyer flask. The modified liquid Murashige and Skoog (1962) medium was used in which macroelements and iron were added at half the concentration, and microelements and vitamins at full concentration. The medium was also supplemented with 250 mgl^{-1} edamine, 40 gl^{-1} sucrose, 5 gl^{-1} glucose. 2,4-D was added at a concentration of 1mgl^{-1} as sole source of growth regulators. The pH of this medium was adjusted to 5.6 before autoclaving (17 min, 121°C). The nitrogen content of this medium is similar to E and F medium (see also Table 1) and [N-NH_4^+]:[N-NO_3^-] was approximately 10:19.5, as compared to 10:20 in E and F medium. The explants developing in liquid medium were passaged onto fresh medium every 2-3 weeks, each time taking about 300 mg of tissue as the inoculum per 100 ml medium. The cell suspension was formed by separation of cells from the explant and their division. An 8-10 month stable suspension (counting from the moment of initiation) was used for experiments and is called basal suspension. The growth curve was based upon the mass of cell aggregates and the number of single living cells per 1 ml. The mass of aggregates (0.15 mm to 2 mm in size) was measured by weighing them on sterile Petri dishes after collection from a sterile sieve. After this measurement the aggregates continued to proliferate in the same medium from which the culture was initiated. A Fuchs-Rosenthal chamber was used for density measurements. For pH and cell density measurements 0.5 ml in volume samples were taken from the culture. Starch present in the aggregates was detected with the use of an iodine in potassium iodide solution. Liquid-gas chromatography was used for measurement of 2,4-dichlorophenoxyacetic acid concentration using the siliceous column (HP-1, 10 m x 0.53 mm). Before measurement 2,4-D was estrified to the 2-chloroethanol ester. For 2,4-D measurement samples 2 ml in volume were collected and centrifuged. Only the supernatant was used for the assays.

3. Inoculation into hormone-free medium

The suspension was ready for inoculation into hormone-free medium 8-12 days after initiation of a fresh culture. 80 ml of the culture were filtered through a nylon sieve with mesh diameter 150 µm, and then centrifuged for 5 min at 100 x g. The pellet was resuspended in medium of the same composition as used for culture initiation, but without 2,4-D. Cells were washed in this manner three times. After resuspension in liquid medium (at a density of 1 x 10^5 cells per ml) cells and cell aggregates were placed in a Petri dish (10 cm in diameter, 4 ml liquid culture per each). The culture was placed in a growth chamber with light intensity of about 200 Lx (LF, Philips TLD 36W/33), temperature 26°C and a 16 hour photoperiod. The number of formed embryos was determined using black paper with a scale on it.

4. Influence of medium composition on culture behaviour

Two weeks after the last passage the basal suspension (see above) was sieved through a 150 µm mesh. The remaining aggregates were used as an inoculum to initiate new subcultures. Tissue from one flask was used to initiate all the performed tests, using 350 mg of tissue as an inoculum per one flask. The 350 ml Erlenmeyer flasks contained each 100 ml of liquid medium with various content of total inorganic nitrogen and different [NH_4^+]: [NO_3^-] ratio. The pH of the medium was adjusted after autoclaving (121°C, 17') by the addition of sterile KOH or HCl to levels 4.2 or 5.7 (see also Table 1). The parameters characterizing the growth dynamics of individual cultures were determined similarly as described above. Inorganic nitrogen content was modified for these tests by adding various amounts of potassium nitrate and ammonium nitrate to liquid medium composed of $CaCl_2$, $MgSO_4$, KH_2PO_4, Fe-EDTA at half the concentrations described by Murashige and Skoog (1962). Microelements and vitamins were present in full concentration of MS medium, sucrose in concentration 40 gl^{-1}, glucose 5 gl^{-1}, edamine 100 mgl^{-1} and 2,4-D – 1 mgl^{-1}.

TABLE 1. Proportions of inorganic nitrogen salts added into the medium.

Symbol of medium	Concentration of nitrogen salts [mM]		Inorganic nitrogen ion concentration [mM]		Total inorganic nitrogen ion concentration [mM]	pH adjusted after autoclaving
	NH_4NO_3	KNO_3	NH_4^+	NO_3^-		
A	2	0	2	2	4	5.7
B	2	0	2	2	4	4.2
C	2	18	2	20	22	5.7
D	2	18	2	20	22	4.2
E	10	10	10	20	30	5.7
F	10	10	10	20	30	4.2

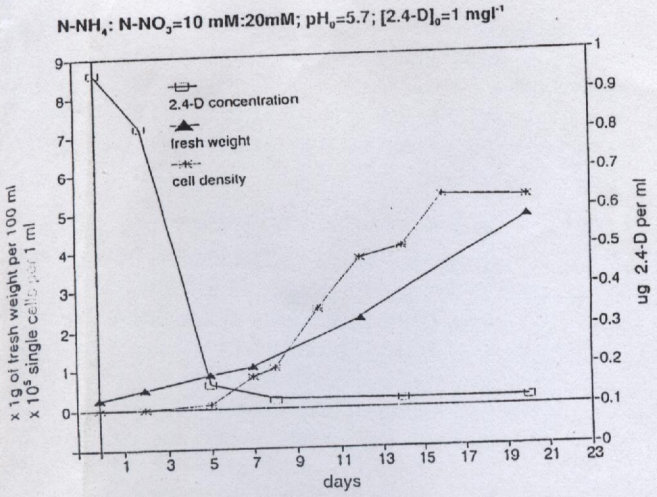

Fig. 1. Growth curves of cucumber established embryogenic cell suspension. The highest cell density was 5-6 x 10^5 per ml.

Inorganic nitrogen salts were added in proportions presented in Table 1. The presented media differ in $[NH_4^+]$:$[NO_3^-]$ proportions and in total nitrogen concentration.

The culture was performed in the growth chamber in an elliptical shaker (80 rpm, amp. 7) at 26°C with light intensity of about 200 Lx (LF, Philips TLD 36W/33) and a 16h photoperiod.

5. Plant regeneration

Embryos obtained after inoculating hormone-free medium were removed and placed on the same medium used for inoculation, but without glucose and supplemented with 7 gl^{-1} Difco agar and 25 gl^{-1} sucrose. The percentage of regenerated plants represents the ratio of seedlings to the number of embryos obtained from liquid culture. All embryos which appeared on hormone-free medium were subjected to the regeneration test. Regeneration occurred 2-4 weeks after placing the embryos on solid medium. The intensity of light was about 4kLx (LF, Philips TLD 36W/33) with the temperature and photoperiod similar to that described above. Embryo-like structures obtained in experiments analysing nitrogen effects were not tested for regeneration.

RESULTS AND DISCUSSION

1. Characteristics of culture growth and 2,4-D induced changes.

The growth curve of the cucumber long term suspension (Fig. 1) was similar to those described previously for other plants (Engvild, 1974). Seven days after initiation the culture shifted from lag phase to exponential growth. During the first 16 days of growth the mass of aggregates in suspension increased 15-fold. The plateau for the increase of single cells number per ml occurred at the 15th-17th day of culture growth. In contrast to previously reported inoculum ratios of 1:4, 1:10 (Bergervoet et al., 1989), 1:12.5 (Chée and Tricoli, 1988) or 1:25 (Rossi et al., 1990), we report here that the ratio of 1:300 (tissue to the medium) was sufficient to initiate suspension culture. The suspension proliferated in a medium in which 2,4-D was the sole hormone source. Such a system has previously not been described for cucumber. During culture a decrease in 2,4-D content in the medium was observed (Fig. 1) from 1 mgl^{-1} added to the medium to the detection limit (0.12 mgl^{-1}). This was probably due to the binding of 2,4-D by tissue as reported in carrot suspension cultures (Michalczuk et al., 1992) or to its enzymatic degradation. Microscopic analysis of the suspension has shown the presence of aggregates of proembryogenic mass (PEM – Fig. 2) described by Halperin (1966) in carrot cell culture. Some of such aggregates contained starch, which is in agreement with data presented for carrot (McWilliam et al., 1972). PEM aggregates did not display morphological differentiation, and single cells could not be seen in unstained, crushed preparations. In a stable cucumber suspension culture we also observed small cells with dense cytoplasm. These resembled Specific Type 1 cells described by Nomura and Kommamine (1985) in carrot suspensions. Small PEM aggregates 60-100 µm in diameter were probably formed by divisions of these cells and also by fragmentation of larger aggregates.

2. Embryo production

Plating of a cell suspension on medium without 2,4-D led to the formation of somatic embryos (Fig. 3). A seeding su-

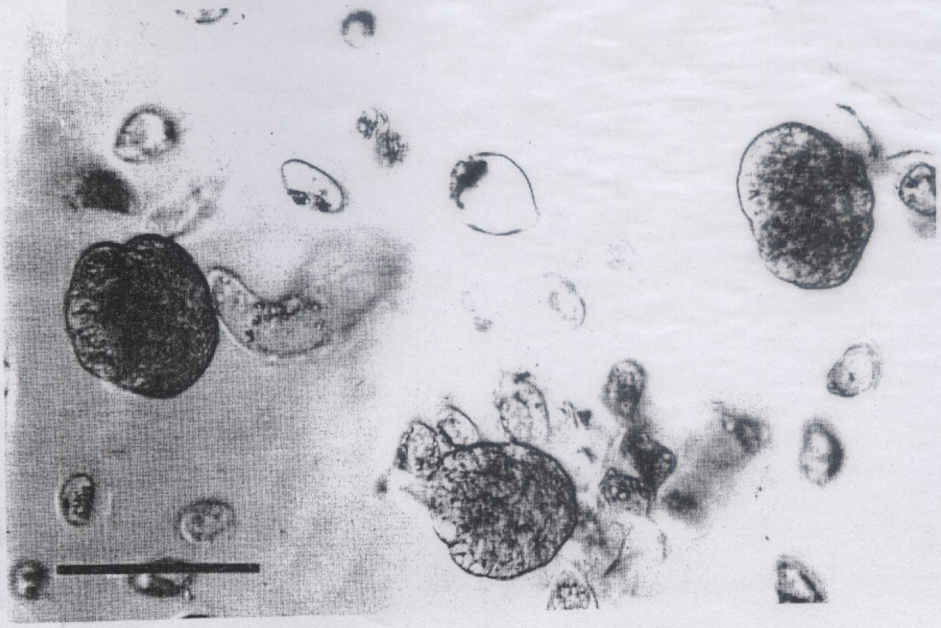

Fig. 2. Proembryogenic mass (PEM – described by Halperin, 1966) derived of Specific Type 1 Cells and probably during fragmentation of bigger aggregates, (bar = 100 µm).

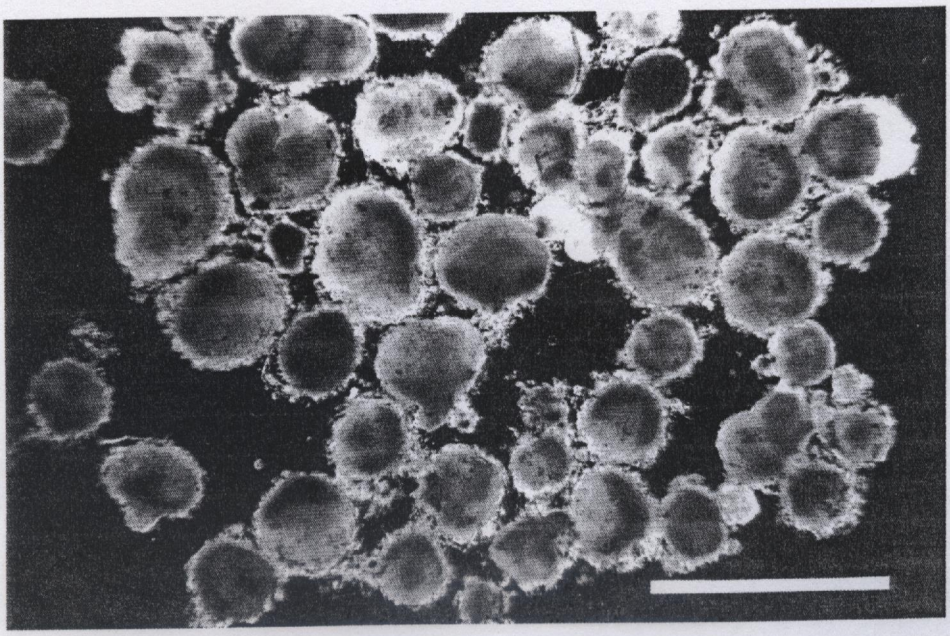

Fig. 3. Many globular and heart embryos developing 9 days after plating of fraction < 150 μm into 2,4-D-free medium, (bar = 1 mm).

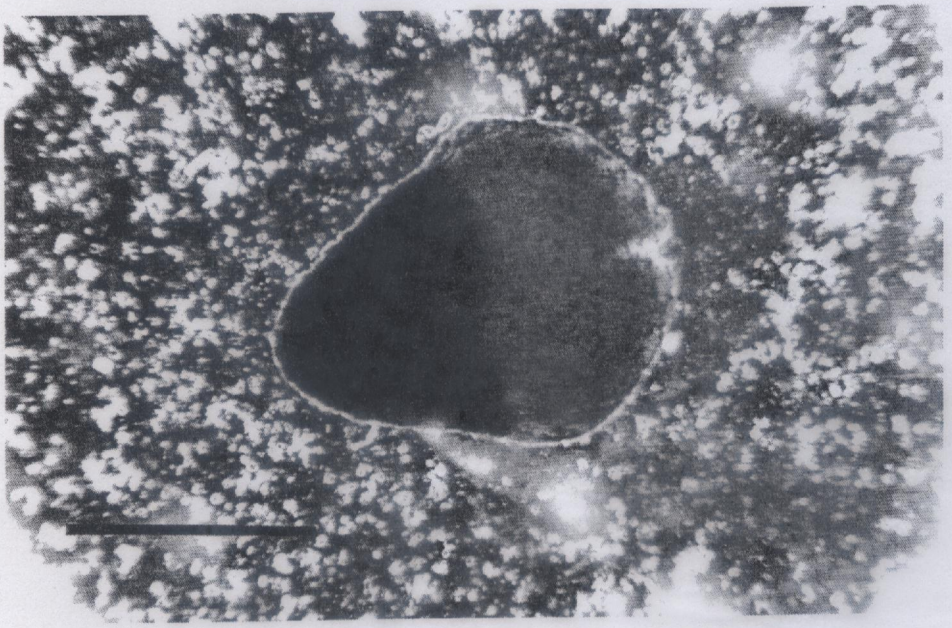

Fig. 4a. Abnormalities of embryo developing on hormone-free medium: overgrowing plumular part, (bar = 1 mm).

Fig. 4b. Abnormalities of embryo developing on hormone-free medium: three cotyledons, (bar = 1 mm).

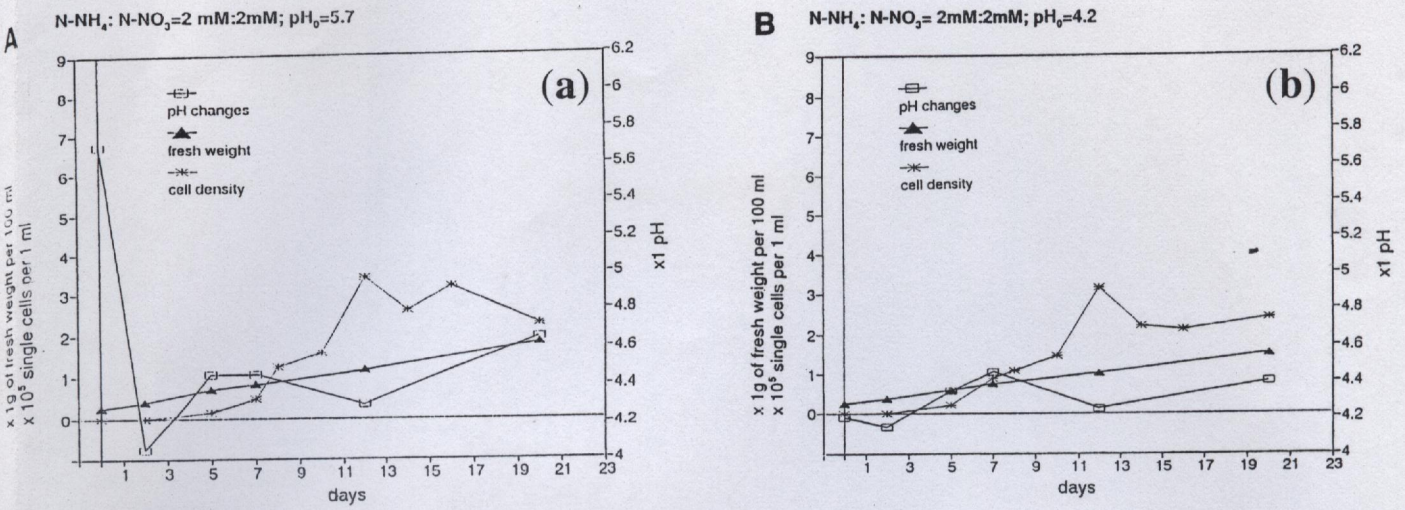

Fig. 5. Proportion of 2 mM : 2 mM of the NH_4^+ : NO_3^- ion and (**a**) pH_0 = 5.7, i.e. medium A, give a lower fresh weight and pH decrease to ~ 4.0 after 2 days and (**b**) pH_0 = 4.2, i.e. medium B, give no pH decrease.

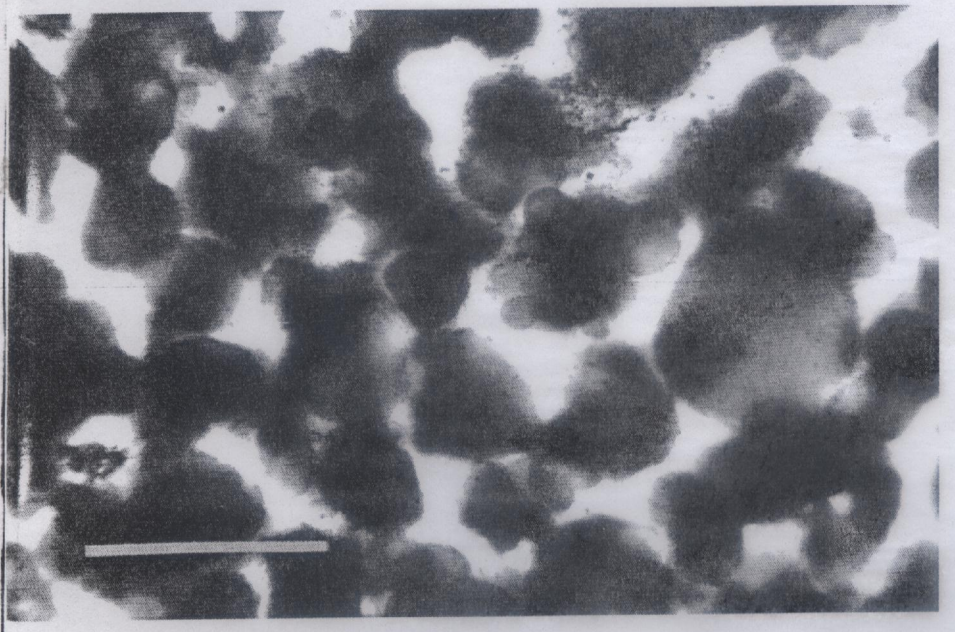

Fig. 6. Globular embryos after 20 days on the medium A and/or B were composed of meristematic tissue and overgrowth could be connected with plumular part.

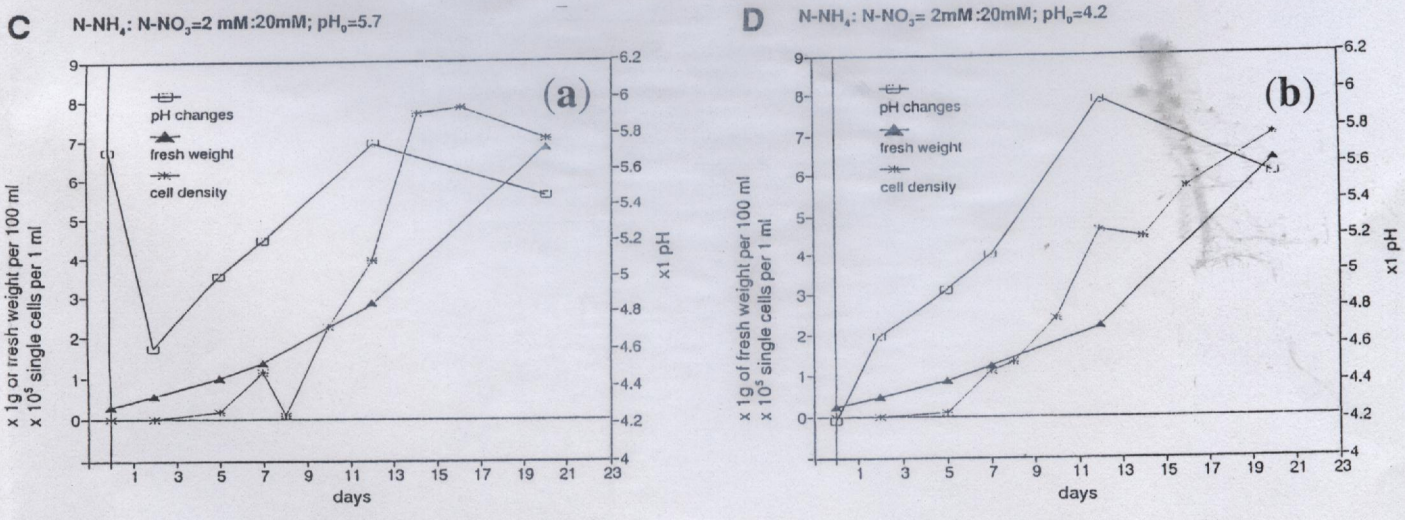

Fig. 7. Proportion of 2 mM : 20 mM of NH_4^+ : NO_3^- ion with (**a**) normal, initial pH_0 = 5.7, i.e. medium C, give after 2 days not such a drastic pH decrease as in case of the medium A and (**b**) lower initial pH_0 = 4.2, i.e. medium D, give a rapid increase of pH.

spension was composed of 95% single cells and 5% cell aggregates. Eight days after inoculation, globular and bipolar embryos reached the size of 200-400 µm in diameter. One ml of basal suspension (with 2,4-D) used for inoculation produced 7.2 embryos after 8 days. After additional two weeks more embryos appeared, thus the final yield obtained from 1 ml of basal suspension was about 21 embryos. Calculating this in respect to the mass of tissue used for initiating the subculture on medium supplemented with 2,4-D (1 part of tissue to 300 parts of medium; see above) about 1800 embryos could be obtained after 3-4 weeks starting with 300 mg. Mass of obtained aggregates larger then 150 µm was ~ 5 g and it could be sufficient for inoculation of 1.5 dm^3 of a new culture.

Three embryo-differentiation pathways were observed depending on the embryo origin: 1) one or more PEM cells rich in starch gave rise to an embryo; 2) the whole aggregate developed into embryo – in this case cells building PEM in general did not contain starch, 3) a single cell divided and formed a small aggregate which subsequently became an embryo. Most embryos which took longer (about 2 weeks) to appear on the plates were formed via a third pathway. Many abnormalities were observed during subsequent development of the embryos on the plates. For example, an overgrowth of the apical part often took place (Fig. 4a) and some embryos were found to have more than two cotyledons (Fig. 4b). For plant regeneration, embryos were removed from liquid medium 3 weeks after plating and 3.2 % of the embryos developed into plants.

3. Effect of the inorganic nitrogen source on the growth of the basal suspension (with 2,4-D).

3.1. In our experiments, the pH of individual cultures was stabilised, and subsequently did not undergo any dramatic changes. The pH of the culture became stabilised at various levels depending on medium composition. If nitrate ions were at a relatively higher concentration $[NH_4^+]$: $[NO_3^-]$= 2 : 20; media C and D according to Table 1, the pH was stabilised at a level of approximately 5.6-5.8 (Figs. 7a and 7b) whereas with the proportion of higher ammonium content ($[NH_4^+]$: $[NO_3^-]$= 10 : 20; media E and F) the pH was slightly lower at the level 5.0 (Figs. 9a and 9b). The presented diagrams (see for comparison Figs. 5a and 5b, Figs. 7a and 7b, Figs. 9a and 9b) show, that the stable pH of the culture did not depend on the initial pH level but only on the form and content of inorganic nitrogen in the medium. Other parameters such as tissue growth rate and changes in the single cell density per ml did not depend on the initial pH either. Figures 5a and 5b show culture growth parameters at 2 mM ammonium ion concentration and 2 mM nitrate. With an initial pH of 4.2 or 5.7 the culture medium reached a stable pH value after three days at about pH = 4.5. With an initial pH of 5.7 the pH value quickly dropped to 4.5 and with an initial pH of 4.0 the pH value rose only slightly. The presented data show that the growth of the cucumber embryogenic tissue depends mainly on the content of inorganic nitrogen salts in the medium. In alfalfa suspension cultures (McDonald and Jackman, 1989) pH changes in subsequent subcultures were caused by selective ammonium ion uptake. It is likely that this is also true for cucumber suspension cultures.

In contrast to previously reported result (Mc Donald and Jackman, 1989), we did not observe two distinct exponential phases during culture growth. Earlier work did not precisely define the tissue used for the experiments (Mc Donald and Jackman, 1989). We have used fairly homogeneous tissue (in so far as it is possible to define morphological or competence homogeneity in *in vitro* plant tissue cultures). Cultures consisted of variously sized aggregates composed of cells which contain dense cytoplasm and probably also a lot of storage substances. The larger aggregates appeared to undergo embryogenesis in hormone-free medium. In such situations clumps of somatic embryos were formed with the apical part directed towards the centre.

The medium which stimulated the most intense cell dispersion had ammonium and nitrate ion concentrations of 2 mM and 20 mM respectively (Figs. 7a and 7b). These conditions (media C and D) produced the maximum concentration of single cells per ml. Halperin (1966) has pointed out that NO_3^- ions derived from potassium nitrate stimulate dispersion in the Lin and Staba (1961) medium containing 55 mM KNO_3. In the light of the well-known interactions between potassium and calcium in stabilising cell wall biopolymers a larger role in dispersion should be probably assigned to K^+ ions.

3.2. Relations between embryo abnormalities and nitrogen source. Embryo or embryo-like structures were observed in

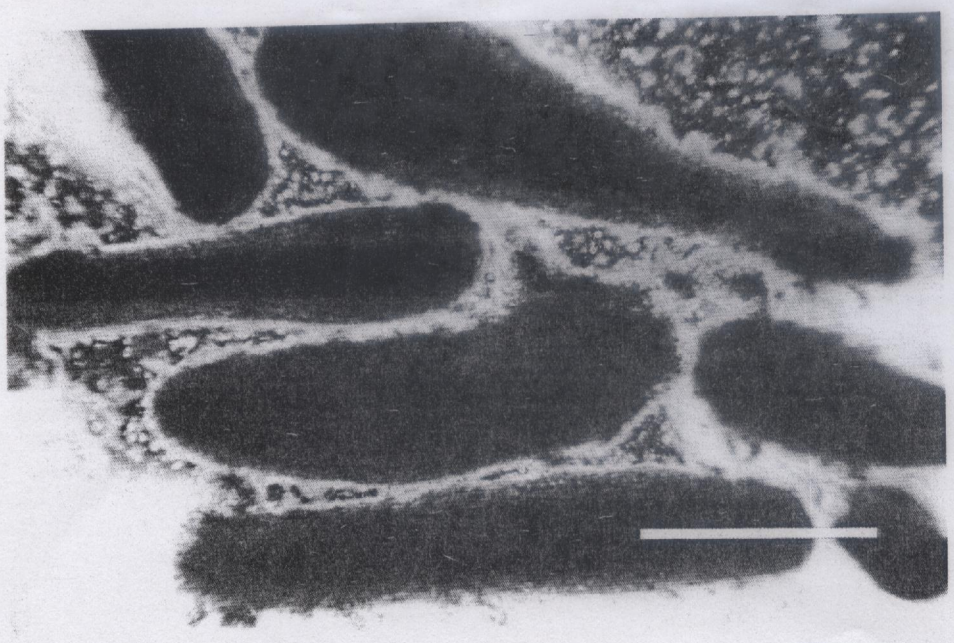

Fig. 8. Embryos with overgrowing of radicular part observed after 20 days of culture on C and/or D medium (bar = 1 mm).

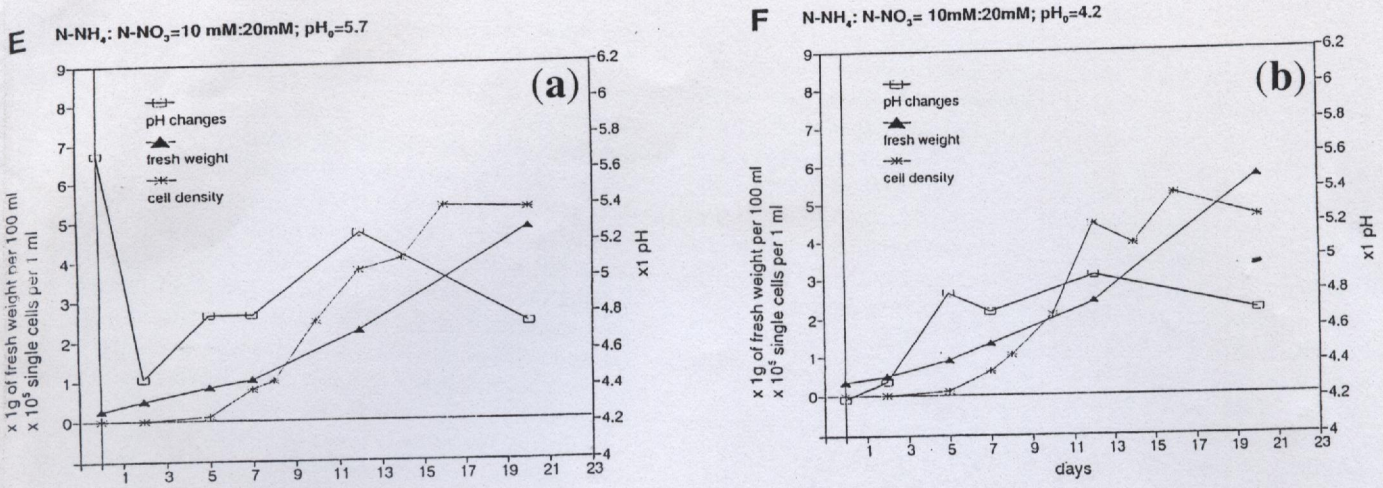

Fig. 9. Media with about the half NH_4^+ : NO_3^- of Murashige and Skoog (1962) proportion (media E, and F) and the initial pH_0 = 5.7 (**a**) or pH_0 = 4.2 (**b**) give at the start and in the following phases intermediate pH values.

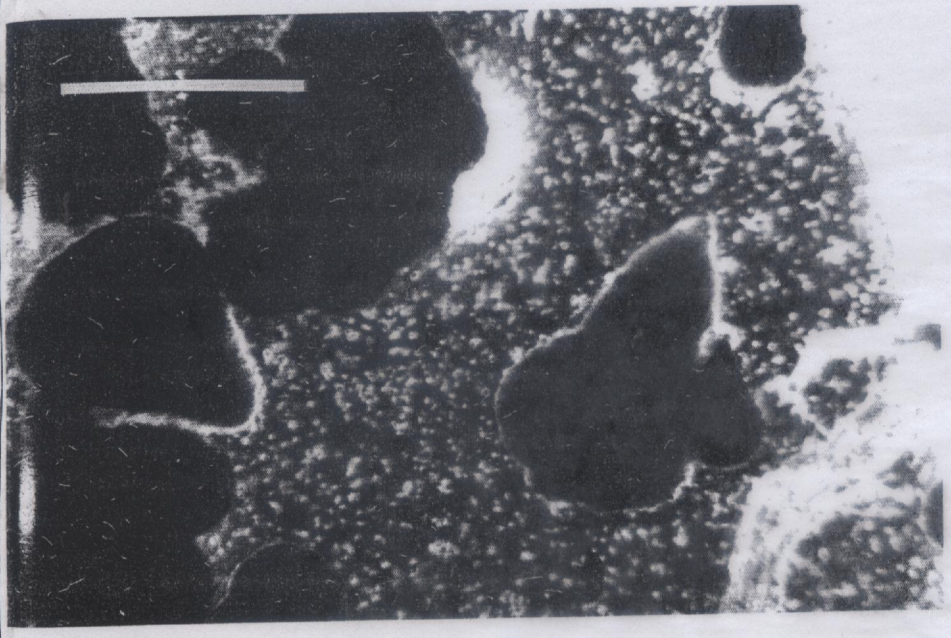

Fig. 10. Embryos originating on medium E and F had the some proportions as those developing after plating on the hormone-free medium. Arrows show places of cell detachment, (bar = 1 mm).

two cases; after the inoculation of a culture into hormone-free medium (Figs 3, 4a and 4b) and also in the presence of 2,4-D late in stationary phase of suspension culture. In the later case, embryogenesis probably took place due to depletion of 2,4-D from the culture (Fig. 1). Fig. 10 presents embryo-like structures (growing in media E and F; $[NH_4^+]$: $[NO_3^-]$=10 mM : 20 mM) with developed plumular part from which single cells detached similarly as from the calypter of plants developing *in vivo*. Some of these structures have abnormaly overgrown apical part. The structures (Fig. 8) grown in media C and D ($[NH_4^+]$: $[NO_3^-]$= 2 mM : 20 mM) are built to a large extent from larger, vacuolised cells. In this case only the radicular part of these structures has developed. Reaction in medium with a relatively high content of nitrate ions corresponds to observations made on *Ipomoea* sp. (Martin et al., 1977). In the carrot system however, only rhizogenesis occurred when nitrate was a sole nitrogen source, regardless of whether the suspension was proliferated on a medium with a reduced form of nitrogen or else without it after removal of 2,4-D (Kamada and Harada, 1979). The structures presented in Fig. 8 may either be considered as roots or as somatic embryos with an overgrown radicular part. Fig. 6 presents the appearance of structures grown in a medium with a nitrogen composition $[NH_4^+]$:$[NO_3^-]$ = 2 mM : 2mM (media A and B). Here the equilibrium of embryo-structure development is shifted in the direction of the apical part, what results in large globular structures composed of meristemic tissue. The preferential proliferation of apical meristemic tissue has also been reported in soybean tissue cultures (Finer, 1988). In carrot suspension cultures two types of aggregates were observed: PEM, which is competent for embryogenesis and aggregates forming roots only on the hormone-free medium (Halperin, 1964, Halperin, 1966). In the experiments reported here, the alternative morphogenetic events (embryogenesis and rhizogenesis) were stimulated using different nitrogen compositions. Some similarities exist between structures described here and morphological mutants in carrot cultures (Schnall et al., 1988). Structure resembling carrot mutant's RF-1 and GB-5 resulted when cucumber suspension cultures were grown under conditions described in Fig. 8 ($[NH_4^+]$: $[NO_3^-]$ = 2 mM :

20 mM; media C and D) and Fig. 6 ($[NH_4^+]$: $[NO_3^-]$ = 2 mM : 2 mM; media A and B) respectively. Similarly, the effects of pH on the formation of somatic embryos and the model of embryogenesis elaborated by Smith and Krikorian for the carrot (1989, 1990) and the daylily (1991) may be analogous to our observations of cucumber suspension cultures grown at low pH. Given the high yields, high degree synchronization and circumvention of callus tissue prior to somatic embryo formation, the system reported here should be useful in cucumber molecular biology and in somatic seed production technology

CONCLUSIONS

1. As with the carrot model, cucumber embryogenic suspension cultures can be maintained in a proembryogenic phase in medium in which 2,4-D is the sole hormone source; withdrawal of the hormone results in embryogenesis.
2. Estabilished cucumber suspension cultures have characteristic growth parameters. Specific Type 1 cells and the proembryogenic mass, both of which have been observed in carrot cultures, are present.
3. The yield of embryos formed on hormone-free medium (using fractions below 150 µm for inoculation) is up to 21 embryos per ml of basal suspension.
4. The inoculum used for the initiation of subsequent subcultures was very small in comparison with previously described for cucumber systems.
5. By choosing appropriate nitrogen proportion and content the development of somatic embryos can be stimulated to resembling typical development.

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CZYNNIKI WPŁYWAJĄCE NA EMBRIOGENEZĘ SOMATYCZNĄ OGÓRKA (*CUCUMIS SATIVUS* L.) I. DECYDUJĄCA ROLA pH I AZOTU W KULTURZE ZAWIESINOWEJ

STRESZCZENIE

Opisana została metoda otrzymywania ustalonej embriogennej kultury zawiesinowej ogórka (Cucumis sativus L.). Scharakteryzowano parametry wzrostu takiej zawiesiny i wykazano wiele podobieństw do modelu opracowanego przez Halperina (1966) dla marchwi. Podobnie jak u marchwi w zawiesinie takiej występowały proembriogenne Komórki Specyficznego Typu I oraz agregaty masy proembriogenicznej (PEM). Zawiesina proliferowała w pożywce uzupełnionej 2,4-D jako jedynym regulatorem wzrostu, a zarodki somatyczne rozwijały się na podłożu wolnym od hormonów. Struktury embrioidalne obserwowano także na pożywce z auksyną w końcowej fazie wzrostu zawiesiny, co było prawdopodobnie związane z ubytkiem 2,4-D z pożywki w wyniku akumulacji w tkance lub enzymatycznej degradacji w czasie trwania kultury. Wykazano, że różne zwartości oraz proporcje form nieorganicznego azotu miały wpływ na morfologię powstających struktur zarodkowych. Wartość pH pożywki z embriogenną kulturą zawiesinową nie zależała od pH pożywki zadanego na początku kultury. Zaobserwowano natomiast zależność odczynu pożywki od zawartości jonów amonowych i azotanowych.

Inokulum używane do zainicjowania kolejnych subkultur zawiesiny ustalonej było bardzo małe w porównaniu z danymi dotychczas publikowanymi i wynosiło 1 część tkanki w stosunku do 300 części pożywki. Z jednego ml zawiesiny, której kultura przebiegała na pożywce uzupełnionej 2,4-D, otrzymano 7,2 zarodków po 10 dniach od momentu posiewu na podłoże wolne od hormonów. Po 21 dniach liczba ta wzrosła do 21. Spośród tak otrzymanych zarodków 3,2 % rozwijało się w rośliny. Wysoki stopień synchronizacji rozwoju zarodków somatycznych, a także rozwój zarodków bez udziału tkanki kalusowej stwarzają możliwość wykorzystania systemu do badań molekularnych oraz w technologii produkcji somatycznych nasion.

SŁOWA KLUCZOWE: ogórek, 2,4-D, kultura zawiesinowa, źródło azotu, embriogeneza somatyczna, zmiany pH.