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Anther and Stem Tissue Callus from Healthy and Potato Virus X Infected Potatoes

Kallus fra støvknapper og stængelvæv af sunde og kartoffelvirus X inficerede kartoffelsorter

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Potatoe tissue has been cultured in vitro for different purposes. During the last decade the meristem technique has been successfully used to eliminate viruses from potatoes (Kassanis 1957, Morel et al. 1968, Stace-Smith & Mellor 1968, Svobodova 1964, Christensen 1968). Plant and callus cultures were studied by Kumar (1963). The aim of this project was to study callus formation, growth and differentiation from different types of tissue from healthy and virus infected varieties.

Material and methods

Stem tips, sections of petioles and stems, true seed and to a limited extent tuber-phloem were used in attempts to induce callus from 15 varieties. Anthers were isolated from three of these. The plant materials were surface sterilized with 1 % NaClO solution for 10-25 minutes, followed by 5 rinses of sterile water. The basic media used were W (White's), T (tobacco), C and D (Hildebrandt 1962) and MS (Murashige & Skoog 1962, modification: Vasil & Hildebrandt 1966). W-, T- and MS-medium are synthetic media containing only defined compounds. T-medium supplemented with coconutmilk and NAA (naphtalene acetic acid) is the C-medium, and further addition of 2,4-D makes the D-medium.

35 ml of media adjusted to pH 5,9 were pipetted into 150 ml prescription bottles and autoclaved 15 minutes at 15 lb/ sq inch. Solid media contained 0,7 % agar. The cultures were incubated in the dark or in 16 or 24 hr light at 26-28°C. Light was from Sylvania Grow Lux tubes and incandescent bulbs. Liquid cultures were incubated on rotary or reciprocating shakers at 60 cycles/ min. Cultures were maintained by monthly transfers. Growth and differentiation of single cells were studied in microcultures (Jones et al. 1960) and nurse cultures (Muir et al. 1954).

Results

Continous light was superior to dark, and solid media to liquid for raising callus. Callus became established within 1-3 months. Stem tips grew directly into callus, while petioles and stem tissue usually required 1-2 transfers before doing so, or they failed to become established on agar despite good callus development on the original explants. Tissue from tubers were unsatisfactory for this purpose, mainly because of contermination.

Callus initiation was best on D-medium. MS-medium with 0.04, 0.5 or 5 mg/1 kinetin did not support callus initiation and addition of 0.1 mg/1 NAA only kept the explanted tissue alive longer. On MS-medium with 0.5 mg/ kinetin and 0.6 mg/1 2,4-D some slow callus initiation was seen. T, W and C media failed to support callus induction.

Callus was obtained from Bx (Russet Burbank), N (Norland), R (Red la Soda), RW (Red Warba), S (Superior), Sc (Saco), US (US seedling 41956) and T (Tawa).

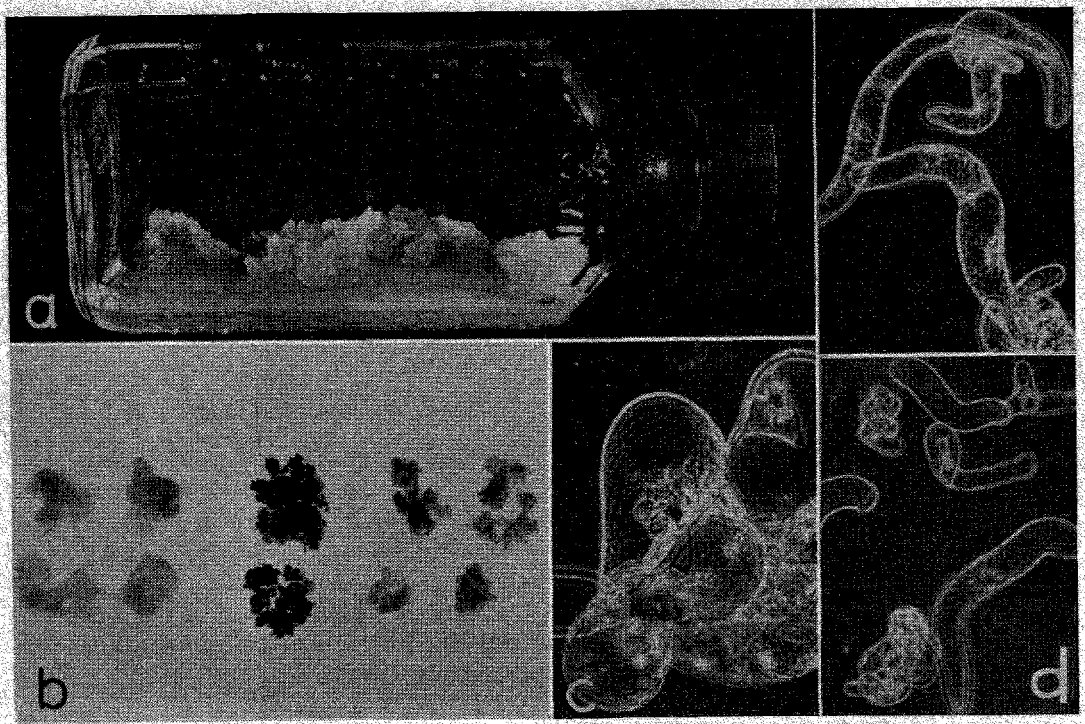

Fig 1. a. Anther callus of variety Tawa after 3 weeks on D-medium in 16 hr light (x 0.7). b. Callus after 4 weeks on D-medium, from left: US seedling 41956 (anther callus) and Red la Soda in 16 hr light and Red la Soda in dark. Notice difference in antocyanin concentration in Red la Soda (x 0.3). c. Cells from liquid Tawa anther callus culture, chloroplasts around nucleus (x 105) d. Cell types from liquid Russet Burbank callus (x 7). e. as fig. d, but Tawa anther callus.

Fig. 1. a. Støvdragerkallus af sorten Tawa efter 3 uger på D-substrat, 16 timers lys (x 0.7). b. Kallus efter 4 uger på D-substrat, fra venstre: US seedling 41956 (støvknapkallus) og Red la Soda i 16 timers lys og Red la Soda i mørke. Bemærk forskellen i antocyankoncentration hos Red la Soda (x 0.3). c. Celler fra rystekultur af Tawa støvknapkallus, kloroplaster omkring kærnen (x 105). d. Celletyper fra rystekultur af Russet Burbank- kallus (x 7). e. som fig. d, men Tawa støvknapkallus.

Anther Callus was established from varieties T, Sc and US, designated Ta, Sca and USa (fig. 1 a & b). Appearence and growth patterns were similar to those for the stem callus cultures. Anthers were selected from flowers prior to flowering. The ability to form callus rapidly declined along with the yellowing of the anthers, which takes place soon after the petals become coloured.

Cultures were usually transferred to fresh medium every 1-2 months, but USa-callus remained active as long as 4 months without transfers, and it grew better in dark than most other varieties. In light it formed chlorophyl as most other varieties did. The red skinned varieties N, R and RW produced abundantly antocyanin in light and to a minor extent in the dark.

True seed formed callus on D-medium and plants on T-medium, and transferred to D-medium these plants also formed callus. Seeds from selfed Merrimack and some seeds from Red Pontiac x Kathadin readily produced callus and plants, while seeds from selfed Ka-

thadin grew poorly. In 24 hr light, soft, whitish callus developed in 4-6 weeks. The growth pattern was similar to that of stem callus, but it matured earlier.

Callus growth was compared on D-, W- and MS-media with different concentrations of kinetin, NAA and 2,4-D. C- and T-media were unsatisfactory for this purpose.

On D-medium supplemented with 0.2 mg/1 kinetin and 0.5 instead of 0.1 mg/1 NAA callus production was equal to or better than on the basic D-medium. When further modified to 2.5 mg/1 NAA and 1 instead of 5 mg/1 2.4-D callus growth decreased, but chlorophyl production increased.

All isolates failed to grow on basic W-medium, but when supplemented with 1 mg/l 2,4-D, 0.2 mg/l kinetin and 0.5 mg/l NAA callus of varieties N, RW and USa made some growth, which slightly improved, when the NAA concentration was raised to 2,5 mg/1. All the modifications of W-medium were quite inferior to D-medium.

In order to work with a defined medium the MS-medium as a base was supplemented with different concentrations of kinetin, NAA and 2,4-D. IAA was omitted. Callus grew poorly without kinetin, even if NAA and 2,4-D were added. Among kinetin supplements 0.04, 0.2, 0.5, 2.0, 5.0 and 10.0 mg/1 the callus showed optimum growth at 0.2–0.5 mg/1. Above 2 mg/1 growth was poor or failed. NAA was nescessary for growth and was tested at 0.1, 0.5, 1.0 and 2.5 mg/1. The optimum was 0.5 mg/1 NAA at the 0.2-0.5 mg/1 kinetin level. With 2 mg/1 kinetin, 1.0 mg/1 NAA was nescessary to obtain good callus growth.

At 0.5 mg/1 or more of kinetin callus became light greyish and softer, whether or not it normally contained chlorophyl and antocyanin. The varieties differed in callus-growth on MS-media, and most of them grew better here than on D-medium. An exception was the USa-callus, which always grew best on D-medium, even if MS-media were supplemented with coconut milk.

2.4-D tested in the MS-medium up to 5 mg/1 along with 0.1 mg/1 NAA and 0.5 mg/l kinetin gave maximal callus yield at 3 mg/1. Perhaps 2,4-D and NAA have an additive effect in promoting callus growth, while only NAA in proper concentration promotes differentiation. 1 g initially of USa-callus on D-medium yielded 15-20 g in 6 weeks, while 5-15 g was normal for other varieties.

In liquid MS-medium cultures of stem callus as well as anther callus of most cultivars formed dense cell suspensions with responses similar to the compounds and concentrations used with agar media. Best callus growth was with 0.2 mg/l kinetin, 0.5 mg/l NAA and 1 mg/l 2,4-D. After 4-5 weeks in light the 35 ml liquid was converted into a semisolid mass of cells from the original transfer of 0.5 g of tissue. With 2 mg/l kinetin similar good growth was obtained, if NAA was increased to 1 mg/l.

Generally the optimal concentrations of these compounds were somewhat lower in liquid than in agar media, perhaps because the more intimate contact between medium and the tissue. For liquid cultures the MS-media were superior to the other media, even for isolates which otherwise grew best on solid D-medium.

Differentiation of embryoids from callus was seen in some cases, especially in liquid cultures in 16 hr light. MS-medium with 0.04 mg/l kinetin and 0.1 mg/l NAA was best, but some differentiation still appeared, if each of these concentrations were raised to 0,5 mg/l. Firm green callus pieces contained vascular tissue, and later formed roots in cultures of Sca, Ta, S and RW.

Morphology. Tracheids of various shapes were found, singly or organized in strands, in callus from agar media, most with 0.1 mg/l or less kinetin. The roots formed were of normal structure. The undifferentiated cells were of varying size and shape (fig. 1 c, d & e). In liquid media they were mostly elongated, and were found singly or in colonies. The maximum cell lenght was 300 μ. Chains of cells were also observed. Fresh liquid suspensions of callus from agar contained a few single cells, and the spherical type was the most common. Large

numbers of mitochondria and spherosomes were seen in the vigorously streaming cytoplasm. Chloroplasts were commonly seen and also starch granules, which later aggregated and obscured the nucleus.

Microcultures. In microcultures single cells usually remained active 1-3 weeks, but divisions were never observed. Colonies of 25-50 cells remained active as long as 2 months, and increased in size 10-20 times.

Single cells were also not observed to divide in nurse cultures.

Potato virus X in callus. Callus cultures from PVX-infected Russet Burbank were assayed for the virus on Gomphrena globosa. During the first 2-3 transfers PVX was always found in the callus, but in subsequent transfers the number of PVX-positive cultures decreased, and none were positive after 7 transfers. This were encouraging in terms of beeing able to differentiate virus-free plants from such callus. Agar as well as liquid callus cultures, including old mature cultures, were indexed.

Quite a number of the original Bx-isolates grew poorly from the start and soon declined, uncertain what role the virus played in that.

Discussion and conclusions

Potato varieties differ in their capacity to form callus. Because saprophytic bacteria often spread from tubers to stems the upper part of rapidly growing stems are best for isolation. The contermination rate was much lower in anther isolations. Anthers were best, when picked at the green or green-yellow stage. True seed was convenient as a source of callus, because contermination easily was controlled, but the unknown genetic combination may offer disadvantages, if plants are differentiated from such callus. No chlorophyl or antocyanin were formed in this callus.

Among the media tested only the D-medium was of practical value for callus initiation. The coconut milk was an important or nescessary factor during the callus initiation phase, while subsequently most established callus isolates grew well without.

Among the defined media only the MS-medium was of practical interest for callus growth. A supplement of NAA was nescessary, optimum 0.5 mg/l. Higher concentrations of NAA increased the chlorophyll concentration only. The optimum kinetin concentration was 0.2-0.5 mg/l. With higher concentrations of kinetin the chlorophyll concentration declined and above 2 mg/l callus growth was retarded. Though not nescessary for continued, unlimited callus growth, 2,4-D much increased it, and callus initiation was not possible without 2,4-D.

Among the liquid media the MS-medium was superior, supporting a very intensive growth. 2,4-D was of much less importance here, and some callus isolates grew equally well without it.

Differentiation was seen, mostly in liquid cultures, as firm green callus pieces with vascular tissue, and later normal roots developed. Depending on the medium, ordinary soft callus from agar media contained tracheids, singly or in strands. A low kinetin koncentration (0.04 mg/l) favoured differentiation. Also 0.1-0.5 mg/l NAA was nescessary for differentiation, while 2,4-D was omitted. A 16 hr day favoured differentiation, but not continous light.

Numerous attempts to raise callus from cultured single cells, with the aim later to induce plants from it, were unsuccessfull. The efforts included modifications of media and light, isolation at different age of cultures, and isolation in conditioned or fresh medium. The microcultures however were useful in studying cell details and activity, even though the cells never divided and died after 1-2 weeks. Colonies with 25 or more cells however did grow in the drop of medium in the microcultures for 2 months, increasing in colony-size 10-20 times.

Callus from PVX-infected plants first contained PVX, but after 7 transfers no cultures were PVX-positive. Several isolates survived a few transfers only, perhaps because of the precense of virus.

Along with the meristem technique, which has proved to be an effective method to elimi-

nate several viruses, the present results with seed, stem and anther callus suggest additional tools for producing virus-free and more productive potatoes.

Summary

Callus was obtained from healthy and PVX-infected potato varieties isolated from stems, stem tips, anthers and true seed. Differentiation of callus into embryoids with roots was obtained. PVX was originally present in certain of the established callus cultures, but was lost after 6-7 transfers. Combined with induced differentiation this provides a method for obtaining virus-free plants.

Sammendrag

Kallus isoleredes fra skudspidser, stængelstykker og støvknapper fra sunde og kartoffel virus X-inficerede kartoffelsorter, samt fra frø. Embryoider med rødder uddifferentieredes i nogle tilfælde fra callus. Kartoffel virus X var oprindeligt tilstede i callus fra inficerede planter, men kunne ikke påvises efter 6-7 overpodninger. Kombineret med induceret differentiering frembyder dette en metode til eliminering af virus i inficeret plantemateriale.

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