

Heat treatment and meristem culture as a means of freeing potato varieties from viruses X and S

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Zusammenfassung, Résumé p. 268

Summary

Potato varieties were freed from viruses X and S by culturing meristems isolated from sprouts of heat-treated tubers. *Duke of York* and *Royal Kidney* were freed from viruses X and S and *Doon Star Wilding* from virus X but not from virus S.

Of 137 meristems, 76 germinated, 12 developed into plants. Seven plants were free from viruses X and S, three were from virus X and two remained infected by both viruses.

Six plants from meristems treated for at least 23 days at 32–35 °C were free from viruses X and S, three of four meristems treated for 5–10 days were free from virus X while one plant free from virus X but not from virus S originated from an untreated sprout.

Varieties differed in tolerance to heat treatment – early varieties were more tolerant than late varieties. Varieties also differed in response to growth in culture – meristems of early varieties germinated more readily than those of a maincrop. Photoperiodism may influence the growth of varieties of different maturity in culture.

Introduction

Virus-free units of over a hundred varieties of potato are maintained by the Department of Agriculture and Fisheries for Scotland for supply to producers of virus-tested seed potatoes in Scotland. Although these units provide nuclear material for over 90% of the certified seed acreage, a few varieties are still grown of which all plants are virus-infected, principally with viruses X and S. These varieties are of commercial interest both in Scotland and overseas but constitute a serious health hazard as sources of infection in areas where seed potatoes are grown, in addition to practical difficulties involved in maintaining stocks of them to comply with certification standards.

This investigation was undertaken in an attempt to produce virus-free units of some of these varieties.

A demonstration of concentration gradients of tobacco mosaic virus in tobacco plants by Limasset et al. (1949), followed by the discovery that virus concentration is low in immature leaves and often absent from the growing point (Limasset & Cornuet, 1949), gave a lead to a method of producing virus-free units from infected plants. Meristem culture was employed by Morel & Martin (1952) to obtain virus-free dahlia

plantlets from infected shoots. The technique has since been successfully used by Morel & Martin (1955), Kassanis (1957), Quak (1961), Svobodova (1964) and Kassanis & Varma (1967), among others, to free potato varieties from viruses A, X, Y, M, S and leaf-roll.

Hot-water treatment has long been employed as a means of freeing canes and cuttings of various woody plants from certain viruses. Herbaceous plants are usually sensitive to temperatures required to eradicate most viruses. This difficulty has been overcome to some extent by employing lower temperatures for extended periods. Kassanis (1949) eradicated leaf-roll virus from infected potato tubers by incubating tubers in air at 37.5°C for at least 25 days while Quak (1961) freed carnation plants from virus by dissecting out tips from infected shoots incubated at 40°C for 6–8 weeks. More recently, Mellor & Stace-Smith (1967) eliminated virus X from the potato variety *Netted Gem* by incubating shoots at 33–37°C air temperature. One X-free cutting was obtained after 15 weeks and the proportion increased with further treatment. Later (1968), by combining heat treatment with axillary bud culture, 50% of the plants were free from virus X and 15% free from virus S after 8 weeks of treatment. After 18 weeks of treatment, 100% of the plants were free from virus X but no further increase in the proportion of plants free from virus S occurred.

Materials and methods

Heat treatment was applied to tubers of three varieties all infected with viruses X and S – *Duke of York* (infected with the virus B strain of virus X), *Doon Star Wilding* (a variant of *Doon Star*) and *Royal Kidney*. Twenty-five tubers of each variety were desprouted and incubated at high humidity in a Copenhagen seed-germinator at 32–35°C. Diffuse lighting was applied for 16 hours a day.

Sprouts were removed at intervals of 7–10 days, dipped in absolute alcohol and transferred to a 1:20 Deosan solution for 15 minutes. After washing in sterile water, the sprouts were dried between sheets of sterile filter paper preparatory to dissection. Meristems of 150–200 µm (occasionally including the first leaflet primordium) were placed on the surface of Morel & Muller (1964) medium contained in 2-oz (57-g), wide-mouth, screw-cap bottles (Fig. 1 and 2). A 16-hour photoperiod was employed. Plantlets were sub-cultured at intervals of three to four weeks.

The high-potassium medium described by Morel & Muller (1964) contains per litre: $(\text{NH}_4)_2\text{SO}_4$ (1.0 g), $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (0.5 g), KCl (1.0 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.25 g), KH_2PO_4 (0.125 g), sucrose (20 g), gibberellic acid (0.1 mg) and agar (5 g); 1 ml of Burkholder & Nickell (1949) solution was added, substituting Fe-EDTA at 7.2 g per litre as the iron source. All organic constituents were added to the autoclaved medium via the Seitz filter.

Cuttings 1–2 cm in length taken from plantlets growing on the culture medium were rooted in compost under small beakers. Tests for virus X were carried out serologically and by inoculation to tobacco; serological tests were made for virus S.

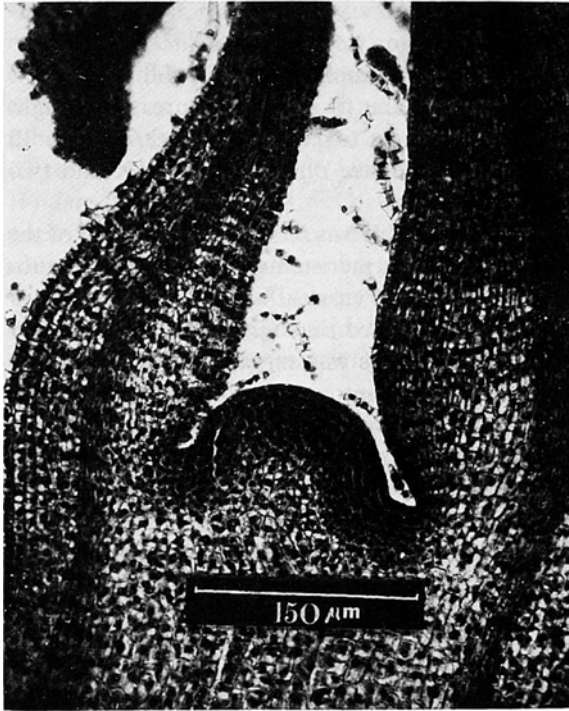


Fig. 1. Longitudinal section of the apex of a potato sprout showing the apical meristem enclosed by two leaflet primordia.

Abb. 1. Längsschnitt durch den Apex eines Kartoffelkeims, das apikale Meristem darstellend, eingeschlossen von zwei Primordialblättchen.

Fig. 1. Section longitudinale de l'apex d'un germe de pomme de terre montrant le méristème apical entouré de deux ébauches de folioles.

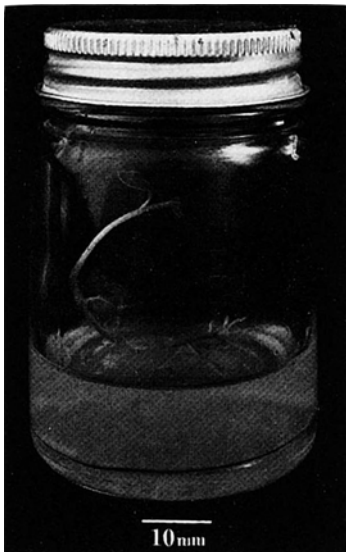


Fig. 2. Virus-free potato plantlet growing on Morel & Muller medium.

Abb. 2. Virusfreies Kartoffelpflänzchen auf Nährstoff nach Morel & Muller angezogen.

Fig. 2. Plantule de pomme de terre libre de virus poussant sur le milieu Morel & Muller.

Results and discussion

Of 137 meristems cultured on Morel & Muller Medium, 76 germinated within two weeks; 16 formed plantlets of which 4 were lost due to rooting failure. After eight weeks some were of sufficient size for cuttings to be taken. On testing, 7 of the 12 plants were free from viruses X and S, three were free only from virus X and two remained infected by both viruses.

The number of meristems free from viruses X and S was related to the length of the period of heat treatment. Plantlets originating from meristems which had been subjected to treatment for at least 23 days were free from viruses X and S. For periods of treatment of less than 23 days, there is some evidence that virus X is more readily eradicated by heat treatment than virus S (Table 1) as was suggested by Stace-Smith & Mellor (1968), though these authors found that the proportion of cultured axillary buds free from virus S was not increased by extending the period of heat treatment past a certain time. One plant free from virus X (but not from S) was obtained without prior heat treatment. A batch of meristems isolated after 16 days of heat treatment failed to produce plants.

A critical period for the elimination of viruses from the meristem occurred at or shortly after the tenth day of heat treatment. Of three meristems of *Duke of York* which developed into plants following 10 days of heat treatment, one was free from

Table 1. Tests for virus made on plants derived from meristems of sprouts incubated at 32–35°C.

Variety ¹	Days of Heat treatment ²	Results of virus tests ³	
		virus X	virus S
<i>Duke of York</i>	0	+	+
<i>Duke of York</i>	0	—	+
<i>Doon Star Wilding</i>	5	—	+
<i>Duke of York</i>	10	+	+
<i>Duke of York</i>	10	—	+
<i>Duke of York</i>	10	—	—
<i>Royal Kidney</i>	23	—	—
<i>Royal Kidney</i>	23	—	—
<i>Royal Kidney</i>	35	—	—
<i>Royal Kidney</i>	70	—	—
<i>Royal Kidney</i>	70	—	—
<i>Duke of York</i>	93	—	—

¹ Sorte – Variété

² Anzahl Tage Hitzebehandlung – Jours de traitement à la chaleur

³ Ergebnisse der Virusteste – Résultats de tests

Tabelle 1. Untersuchungen auf Virusbefall an Pflanzen aus Triebmeristemen (Inkubation bei 32–35°C).

Tableau 1. Tests virologiques sur plantes provenant de méristèmes de germes inoculés à 32–35°C.

viruses X and S, the second was free from virus X but not from S while the remaining one remained infected by both viruses.

Varietal differences in tolerance to heat treatment were observed – tubers of the early varieties *Duke of York* and *Royal Kidney* were tolerant in comparison with tubers of *Doon Star Wilding*, a maincrop. The early varieties continued to sprout for 12 to 14 weeks at 32–35°C whereas many of the sprouts on tubers of *Doon Star Wilding* died after 2 to 3 weeks and the tubers rotted. Hakkaart & Quak (1964) reported differences in response to heat treatment in varieties of chrysanthemum but conceded that this may have been related to the condition of the plants rather than to varietal characteristics. In later work, MacDonald (unpublished) noted that tubers of the early varieties *Eclipse* and *Ninetyfold* were more tolerant of heat treatment than were tubers of the late-maturing *Abundance* and *Arran Chief*. One meristem of *Doon Star Wilding* developed into a plantlet, most of the remainder formed single primordial leaflets and died. Failure of the growing point was attributed to the effects of heat treatment.

Varieties differed in their response to growth on Morel & Muller Medium. Such differences may be determined by the selective requirements of a variety from the medium or by the effects of various environmental factors such as duration and intensity of lighting and of heat treatment on the sprouts and meristems. Mellor & Stace-Smith (1967) noted that the growth of plants of *Netted Gem* (a maincrop) under heat treatment improved when the plants were removed from constant lighting to a photoperiod of 12 hours. *Duke of York* and *Royal Kidney* germinated rapidly and grew vigorously on the culture medium whereas *Doon Star Wilding* (and in the later experiment *Abundance* and *Arran Chief*) often failed to germinate and any plantlets that developed were weak and chlorotic.

Photoperiodism may be a vital factor in determining the response of varieties to growth on media. Early varieties are known to be adapted to long-day growing conditions whereas the reverse is true of late varieties – the 16-hour photoperiod employed in this investigation would tend to favour the growth of early varieties. The effects of photoperiodism on the tolerance of varieties to heat treatment and on the growth of varieties on culture media are being investigated.

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Zusammenfassung

Hitzebehandlung und Meristemkultur als Mittel zur Befreiung von Kartoffelsorten von den Viren X und S

In Schottland wurden durch Kultivierung von Meristemgewebe aus Keimen von Kartoffelknollen (Hitzebehandlung bei 32–35°C) virusfreie Pflanzen von vorher mit Virus X und S infizierten Sorten gewonnen.

137 Meristeme von 150–200 µm wurden auf die Oberfläche der Morel & Müller: – (1964) Nährlösung in 2 oz. (57g) – Musterflaschen mit grosser Oeffnung gebracht (Abb. 1 und 2). Eine Photoperiode von 16 Stunden wurde angewandt. 76 Meristeme keimten und 16 entwickelten sich zu Pflänzchen, von denen 4 keine Wurzeln bildeten. Nach 2–3 Monaten wurden den Pflänzchen auf der Nährlösung Stecklinge von 1–2 cm Länge entnommen und in Kompost bewurzelt. 7 Pflanzen waren frei von den Viren X und S, 3 waren frei von Virus X, und zwei blieben von beiden Viren befallen (Tabelle 1).

Alle 6 Pflanzen von Meristemen, die während mindestens 23 Tagen einer Hitzebehandlung unterworfen wurden, waren frei von Virus X und S. Bei einer Behandlungsdauer von weniger als 23 Tagen zeigten die Ergebnisse, dass das Virus X leichter zu eliminieren war als Virus S – 4 von 6 Pflanzen waren frei von Virus X bei einer

Hitzebehandlung von bis zu 10 Tagen Dauer. Eine virus-X-freie, aber nicht virus-S-freie Pflanze wurde erzielt ohne vorherige Hitzebehandlung (Tabelle 1).

Die Sorten unterschieden sich in ihrer Fähigkeit, der Hitzebehandlung zu widerstehen – frühe Sorten schienen toleranter zu sein als spät reifenden Sorten. *Duke of York*, *Royal Kidney* und andere früh reifende Sorten keimten nach 12–14 Wochen bei 32–35°C immer noch, während Knollen von *Doon Star Wilding* und andere mittelfrüh reifende Sorten nach 2–3 Wochen verfaulten.

Sortenbedingte Unterschiede in der Keimung und im Wachstum auf der Nährlösung wurden notiert. Auch hier keimten die Meristeme früher Sorten schneller, während jene später reifender Sorten sich oftmals nicht entwickelten. Es wird angenommen, dass dies eine photoperiodische Reaktion sein könnte, da man glaubt, dass frühe Sorten Langtag-Bedingungen, wie zum Beispiel der von uns angewendeten 16-Std.-Photoperiode, angepasst sind, während späte Sorten sich eher für Kurztag eignen. Die Untersuchung wird fortgesetzt.

Résumé

Traitement à la chaleur et culture de méristème comme moyens de débarasser les variétés de pomme de terre du virus X

On a obtenu en Ecosse des souches sans virus de variétés infectées de longue date par les virus X et S, grâce à la culture de tissu méristématique excisé de germes de tubercules de Pomme de terre incubés à 32–35°C.

137 méristèmes de 150–200 µm ont été déposés sur la surface d'un milieu de Morel & Muller (1964) contenu dans des flacons spéciaux, à large ouverture d'une contenance de deux onces (fig. 1 et 2). La photopériode consistait en 16 heures de lumière. 76 méristèmes ont germé et 16 se sont développés en plantules, parmi lesquelles 4 ne sont pas enracinées. Après 2 à 3

mois on a prélevé, sur les plantules des milieux de culture, des boutures de 1–2 cm de longueur, qui ont été mises à l'enracinement dans du compost. Sept plantes se sont révélées sans virus X et S, trois étaient libres de virus X et deux étaient encore infectées par les 2 virus (tableau 1). Toutes les six plantes provenant de méristèmes et qui subirent un traitement à la chaleur pendant un minimum de vingt-trois jours, furent trouvées sans virus X et S. Les résultats obtenus avec des traitements d'une durée inférieure à vingt-trois jours indiquent que le virus X est plus rapidement éliminé que le

virus S – quatre plantes sur six sont débarassées du virus X avec des traitements à la chaleur jusqu'à 10 jours. On a obtenu, sans traitement préalable à la chaleur, une plante libérée du virus X mais non du virus S (tableau I).

Les variétés diffèrent quant à leur capacité de résistance au traitement à la chaleur – les variétés précoces paraissent plus tolérantes que les tardives. *Duke of York*, *Royal Kidney*, ainsi que d'autres variétés hâtives continuent à germer après 12–14 semaines à 32–35°C, tandis que les tubercules de *Doon Star Wilding* et d'autres variétés de grande culture pourrissent

après 2–3 semaines.

On observe également des différences variétales dans la germination et la croissance sur le milieu de culture – de nouveau les méristèmes des variétés précoces germent rapidement alors que ceux des variétés tardives qui, souvent, ne se développent pas. Il est supposé que cette réaction est de nature photopériodique puisque les variétés précoces doivent être adaptées aux conditions de jours longs tels que ceux de la photopériode utilisée dans l'essai, tandis que les variétés tardives se plaisent mieux en jours plus courts. Les recherches continuent.

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