

## **The elimination of viruses from garlic (*Allium sativum* L.) plants by thermotherapy and meristem tip culture**

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### **A b s t r a c t**

The production of virus free garlic plants from totally cvs. Jana, Mera and ecotype Zamojski was attempted by means of thermotherapy and meristem tip culture. The cloves and the aerial bulbils after hot air treatment in a growth chamber at 36°C for 30-35 days or at 26-28°C for 3-4 months in greenhouse were used to meristem tip culture on M. S. medium. In the 26-28°C treatment 19.5 % of meristems produced plants and 22.5 % of these were virus free. In the 36°C treatment 14.5 % of the meristems developed into plantlets and 34.6 % of them were virus free. The plantlets were indexed by "sap-dip" electron microscopy methods.

### **INTRODUCTION**

Cultivars of garlic (*Allium sativum* L.) world-wide, commonly have shown symptoms of leaf streaking and mild or severe mosaics. These symptoms occurred on all commercial cultivars as well as on garlic ecotypes. In most instances a complex of filamentous viruses, including carla- and potyviruses, has been isolated from affected plants. The identity of viruses contained in each complex is variable and some viruses have not yet been precisely identified (D e l e c o l l e and L o t, 1981; B o s, 1982; S w a r d, 1990; C o n c i et al., 1992). The following viruses have been identified by the immunoelectron microscopy decoration technique (IEM-D) in garlics in Poland: garlic latent virus (GLV), garlic mosaic virus (GMV), onion yellow dwarf virus (OYDV), shallot latent virus (SLV) and two unidentified viruses (a poty- and

a carlavirus). Multiple virus infections have been detected in all examined garlic plants (Szyndel and Lipiński, 1994).

Garlics are propagated by vegetative means through cloves and air bulbils (Machalowski, 1977) and this facilitates virus transmission. Garlic viruses are also very easily transmitted by sap, aphids and mites (van Dijk et al., 1991; Yamashita et al., 1991).

Investigations in other countries have been undertaken to obtain virus-free garlic plants using thermotherapy followed by meristem tip culture. It was reported that bulb yield and clove size were substantially increased in virus-free garlic cultivar stocks (Havranek, 1975; Messiaen et al., 1981; Walkey and Antill, 1989; Rabiti and Marani, 1992).

The objective of this research was to obtain virus-free garlic plants in Poland.

## MATERIALS AND METHODS

Garlic cloves from totally infected cv. Mera and ecotype Zamojski as well as cloves and bulbils from cv. Jana were kindly provided by Dr. Z. Lipiński, Dept. Vegetable Corps, Warsaw Agric. Univ.

General thermotherapy and meristem tip culture methods described by reviews of Hollings (1965) and ten Houten et al. (1968) were used in these investigations. In meristem tip culture experiments explants (with and without primordial leaves) were excised from cloves and bulbils. Plant fragments were surface sterilized with 1 % NaOCl for 2 min., 70 % ethanol for 2.5 min. and rinsed 4 times with distilled water. The medium contained M/S basic salts (Murashige and Skoog, 1962) complemented with (mg/l): inositol – 100.0, thiamine HCl – 0.4, glycine – 2.0, pyridoxine HCl – 0.5, nicotinic acid – 0.1, adenine sulfate – 80.0, IAA – 0.1, sucrose – 30 000 and Bactoagar – 6 g/l. Cultures were maintained at 25°C under a 5 000 Lux fluorescent light for 16 h.

Two different groups of plants were used for elimination of garlic viruses by meristem culture: plants after thermotherapy and plants kept in greenhouse.

Hot air treatments were carried out in a growth chamber at 36°C for 30-35 days. Cloves and bulbils were dipped in a mixture of fungicides (Topsin M, Sadoplon, Apron 1:1:1 – 3 g/l) and sown in seedbeds with a mixture of equal volumes of peat moss, soil and perlite.

In the greenhouse experiment: cloves and bulbils were treated with fungicide mixture described above and sown in pots containing peat moss. They were maintained at 26-28°C for 3-4 months under 4 000-6 000 Lux sodium lights for 18 h.

Plantlets which developed from meristems were indexed by "sap-dip" electron microscopy techniques according to the standard procedure described by Szyndel (1992), and Szyndel and Lipiński (1994). The electron microscopy preparations were examined in JEM 100 C electron microscope (JEOL) in the Laboratory in Electron Microscopy of the Warsaw Agriculture University.

## RESULTS AND DISCUSSION

Virus-free garlic plants were obtained by meristem tip culture with and without heat treatment of plants (Table 1). Although the survival of explants was low (16.5 %), a relatively high percentage (29.2 %) of virus free plants seems to be satisfactory. C o n c i and N o m e (1991) received from 14 % to 67 % of virus-free plants in an analogous study.

Although all explants were surface sterilized, most of the successful surviving meristems in culture were destroyed by bacteria and fungi. High mortality of explants of *Allium* species was usually associated with microbiological contaminations of cultures, as well as damage to the meristems during extraction (B r e m e r, 1990). After thermotherapy 14.6 % meristems developed into plants, however more meristems (19.5 %) survived and developed into plants without thermotherapy (Tables 2 and 3). High temperature sometimes has a negative influence on the physiological condition of plants, although larger meristem tips with primordial leaves can be excised after thermotherapy and they will survive much better (H o l l i n g s, 1965; t e n H o u t e n et al., 1968). Garlic plants of cv. Mera were the most sensitive to thermotherapy and were most strongly affected by microbiological contaminants. Thermotherapy treatments reduced the percentage of successful explant development of this cultivar to only 5.5 %.

The efficiency of eliminating viruses from garlic plants after thermotherapy was 34.6 % (Table 3) and 22.5 % virus-free plantlets developed from meristems not treated with hot air (Table 2).

Meristems were cut from cloves and air bulbils to eliminate viruses from plants cv. Jana. Although thermotherapy reduced the number of bulbil meristems developed in culture, the number of virus-free garlic plants increased (Tables 2 and 3).

Presented results were consistent with those reported earlier, that virus-free garlic plants can be obtained by meristem tip culture without thermotherapy (H a w r a n e k, 1972; P e ñ a - I g l e s i a s and A y u s o, 1974; B h o j w a n i, 1980). Large numbers of meristems should be cut to produce virus-free plants by meristems tip culture alone (C o n c i and N o m e, 1991).

All plants which development from meristems were indexed for presence of virus particles by EM. Plantlets were transferred from media to pots containing peat moss and were examined when leaf growth was clearly noticed. In "sap-dip" preparation from plants which harboured viruses, many flexuous, filamentous particles were always observed (Fig. 1). Concentration of both poty- and carlavirus-like particles were very high. No virus particles were detected in preparations from sap of "virus-free" plants. According to the literature viruses can not always be eliminated from garlic plants by using the methods presented here e.g. garlic yellow virus – GYSV (C o n c i and N o m e, 1991). This virus has not yet been detected in Polish garlic ecotypes, and "virus-free" plants received in Poland confirm that GYSV probably does not occur there (S z y n d e l and L i p i ń s k i, 1994).

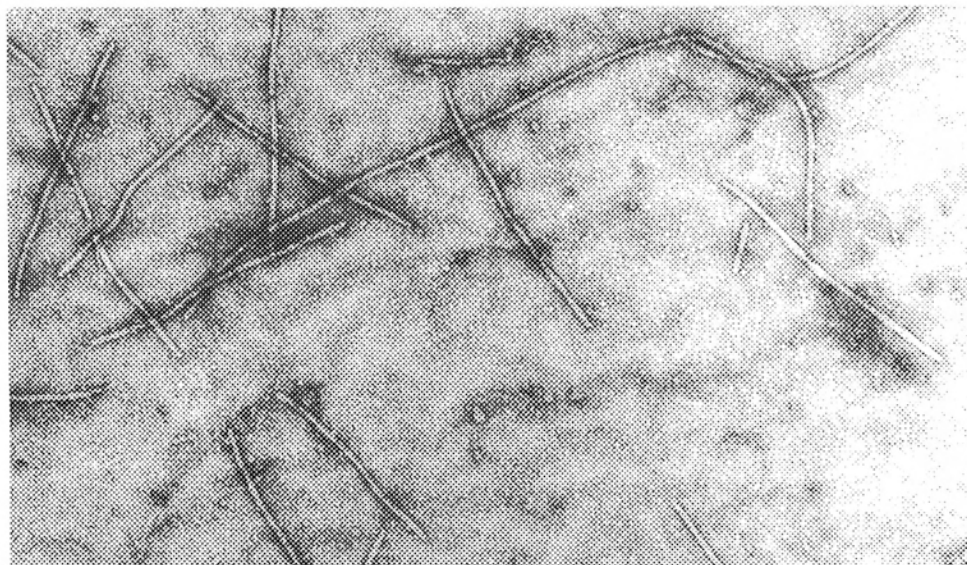


Fig. 1. Filamentous virus particles similar to poty- and carlaviruses in the sap from garlic plants cv. Mera (Magn. x 66 000)

Virus-free garlic plants, especially new Polish cultivars Jana and Mera, should be used as basic propagation materials to establish commercial production. The rapid multiplications of virus-free clones could be done by tissue culture methods (Peña-Iglesias and Ayuso, 1974; Bhojwani, 1980; Matsubara et al., 1990). The next steps should be the field acclimatization of *in vitro* plants (Osa and Sugawara, 1980; Matsubara and Chen, 1989) and agronomic evaluation of virus-free garlic crops (Marani et al., 1988; Walkey and Antil, 1989).

Table 1

The efficiency of virus eliminating from garlic plants by meristem tip culture after heat treatment or without heat treatment

Method used for virus eliminating	The number of cut meristems	The number and percentage of obtained plants	The number and percentage of virus-free plants
Heat treatment + meristem tip culture	335	49 (14.6 %)	17 (34.6 %)
Meristem tip culture alone	205	40 (19.5 %)	9 (22.5 %)
Total	540	89 (16.5 %)	26 (29.2 %)

Table 2

The efficiency of virus eliminating from garlic plants grown in a greenhouse (26-28°C) by meristem tip culture depending on the plant organ which the meristems were cut from

Garlic cultivar (ecotype)	Plant material which meristems were cut from	The number of cut meristems	The number of obtained plants	The number of virus-free plants
Jana	bulbils	38	15	1
Jana	cloves	37	7	4
Mera	cloves	68	9	0
Zamojski	cloves	62	9	4
Total		205	40 (19.5 %)	9 (22.5 %)

Table 3

The efficiency of virus eliminating from garlic plants by heat-therapy (36°C) and meristem tip culture

Garlic cultivar (ecotype)	Plant material which meristems were cut from	The number of cut meristems	The number of obtained plants	The number of virus-free plants
Jana	bulbils	51	7	4
Jana	cloves	43	7	6
Mera	cloves	55	3	2
Zamojski	cloves	168	32	5
Total		335	49 (14.6 %)	17 (34.6 %)

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## Odwirusowanie roślin czosnku (*Allium sativum* L.) metodami termoterapii i hodowli stożków wzrostu

### Streszczenie

W celu uzyskania wolnych od wirusów roślin czosnku odmian Jana, Mera i ekotypu Zamojski zastosowano metodę hodowli stożków wzrostu połączoną z zabiegiem termoterapii roślin albo bez traktowania roślin wysoką temperaturą. Wsadzone w substrat torfowy ząbki i cebulki powietrzne przetrzymywano w termostacie w temperaturze 26-28°C przez 30-35 dni (termoterapia) lub w szklarni w temperaturze 26-28°C przez 3-4 miesiące (doświadczenie szklarniowe). Z roślin, które wyrosły w tych warunkach, wycinano merystemy do hodowli na pożywkach M.S. Z merystemów wyciętych z roślin rosnących w szklarni 19,5 % rozwinęło się w rośliny, z których 22,5 % było wolnych od wirusów. Po termoterapii 14,6 % merystemów rozwinęło się w rośliny, a z nich 34,6 % było wolnych od wirusów. Rośliny były testowane elektronmikroskopową techniką „sap-dip”.