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Assessment of potato microtuber and *in vitro* plantlet seed multiplication in field conditions – Growth, development and yield

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ABSTRACT

Microtubers and *in vitro* plantlets are the basic materials of potato seed production. Minitubers are yield of tubers derived from the first generation of *in vitro* plantlets or from microtubers. At present the production of minitubers usually takes place under covers – in glasshouse, plastic tunnels and screenhouses. The aim of this research was to assess the possibility of carrying out such multiplications in field conditions. The growth, development and harvesting was estimated in *in vitro* plantlets, microtubers, minitubers and traditional seed potatoes. Additionally, three terms of *in vitro* plantlets and microtubers planting in the field were assessed (1st term – second week of April, 2nd term – fourth week of April, 3rd term – last week of June/first week of July). The weather conditions in 2006–2012 impacted greatly on the survival of *in vitro* plantlets and microtubers planted in a field, especially in the 1st and 3rd terms. In spite of clear differences in the size of planted seed material, the highest reproduction coefficient per one plant was recorded for traditional seed potatoes and microtubers, and the lowest for minitubers. While at the 1st and 2nd terms of planting the average number of tubers from one plant ranged between 6.7 and 8.8, at the 3rd term this value oscillated between 11.2 and 12.4. Analysis of the productivity of particular seed material clearly indicates a much higher share of potentially useful tubers derived from microtubers and *in vitro* plantlets than of traditional seed potatoes or minitubers. Microtubers and *in vitro* plantlets planted earlier (1st term of planting) or significantly later (3rd term) produced the greatest minituber yield. Because of a high risk connected with the dying out of plants and maintaining adequate coverage at its earlier planting, the 3rd term of planting of this material was much more favorable. At the same extreme weather conditions (drought and lack of rainfall at the beginning of after planting), which occurred in 2010 contributed to a very strong reduction plants in the field.

1. Introduction

Microtubers and *in vitro* plantlets are nowadays used as the base material in seed potato production in many countries. Primarily *in vitro* plantlets are produced from isolated meristems. However, their mass production is based on the multi-division of the stem plant and on stimulating these fragments to grow on special nutrients under control (Struik and Wiersema, 1999). Microtubers are also produced in laboratory conditions. These are miniature potato tubers of 4–12 mm (Ranalli, 2007), which produce a similar amount of fresh plant mass as *in vitro* plantlets but produce fewer mini-tubers (Wiersema et al., 1987). *In vitro* material has a particular significance in places where climate and epidemiological conditions do not allow for the use of traditional methods of healthy seed potato production at the highest level of qualification. Their fast and effective reproduction in laboratory conditions allows one to shorten the process of production of seed potatoes of a new cultivar to achieve a trading volume by up to 3–4 years. Using a traditional approach takes at least 7 years (Donnely et al., 2003). Moreover, the quality of produced seed potatoes on the basis of *in vitro* plantlets is incomparably better – it involves fewer viral, bacterial and fungal infections. For the production of minitubers, both microtubers and *in vitro* plantlets are used. Ranalli (1997) claims that due to the fact that the process of minituber production out of microtubers is 1–2 months longer, it is *in vitro* plantlets that are certainly more favorable.

Yet microtubers were favored due to their easy of storage and transport. The technologies of microtuber and minituber production are constantly being improved. Donnely et al. (2003) claim that a technology which could change the size of produced microtubers

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would totally eliminate the role of minitubers. The production of minitubers usually takes place in glasshouses, in plastic tunnels or in screenhouses. Production possibilities are still limited by the surface area occupied by the owned facilities. In a situation in which there is an increased demand for minitubers, the production of new ones is often uneconomical. Nowadays the new hydroponic systems significantly lower the costs of minituber production in comparison with a conventional production system. This is why hydroponics is particularly important in regions where, because of climate, the glasshouse exploitation costs are very high, due to *e.g.* extra heating and lighting.

Planting of microtubers and *in vitro* plantlets directly in the field is another solution which eliminates the necessity of owning the above mentioned facilities. Yet because of the delicacy of *in vitro* materials there is a risk involving their survival, especially during the initial growth period. There are many publications that are concerned with the productivity of *in vitro* plantlets, microtubers and minitubers and traditional seed potatoes in field conditions, but none of them compares the effectiveness of all these materials (Wattimena et al., 1983; McCown and Wattimena, 1987; Haverkort and van der Zaag, 1989; Leclerc and Donnelly, 1990; Ranalli et al., 1994a; Struik and Lommen, 1999; Kawakami et al., 2003; Pruski et al., 2003; Kawakami et al., 2006; Öztürk and Yildirim, 2010; Kawakami and Iwama, 2012). Moreover, most of their results are the consequence of only 1–2 years of research which in the case of field experiments is too short a period to observe certain permanent dependencies. There is often a difference of opinion regarding the practicality of direct planting of microtubers in the field, at the very least due to harvesting (Özkaynak and Samanci, 2005). Certainly it is easy to mechanize such planting, however, it was observed that plants grown out of microtubers have a very unstable level of harvesting in particular years in comparison with traditional seed potatoes (Leclerc and Donnelly, 1990; Kawakami et al., 2003), and it is to a large extent dependent on the size of planted microtubers (Kawakami and Iwama, 2012).

Moreover, direct planting in the field causes slow growth and development of plants. When bigger microtubers (over 0.5 g) were planted initially in a glasshouse and only later transferred into the field, the obtained harvest/yield was comparable with that obtained from traditional seed potatoes, but mainly from late maturing of cultivars (Haverkort et al., 1991). Also the physiological age and size of planted microtubers are relevant. Microtubers which were physiologically older were more prolific than the younger ones; this was particularly noticeable for later cultivars (Ranalli et al., 1994a). Ranalli et al., 1994b found that small microtubers in spite of a high breeding coefficient produced much weaker yields than minitubers and traditional seed potatoes. Molet (1997) observed that microtubers which were stored longer (7 months) produced much better yields than those stored shorter (3 months). Similar results were also obtained by Désiré et al. (1995), who recorded more number of tubers per plant from one plant in the case of microtubers which were physiologically older. Hassanpanah and Khodadadi (2009) observed that the older the planted *in vitro* plantlets are, the lower their breeding coefficient (number of tubers from one plant).

The aim of the research was to compare the productivity in field conditions of all the seed material (*in vitro* plantlets, microtubers, minitubers and traditional seed potatoes) in the context of seed production, and to assess the impact of different terms of planting of *in vitro* plantlets and microtubers on yield and its size distribution.

2. Material and methods

Field experiments were carried out between 2006 and 2012 in the north of Poland (Plant Breeding and Acclimatization Institute – National Research Institute, Department of Potato Protection and Seed Science in Bonin near Koszalin, 54°09′ N, 16°15′ E). We compared the growth and development of plants, their yield and its size distribution of material grown out of *in vitro* plantlets, microtubers and minitubers as well as traditional seed potatoes of three potato cultivars. The choice of varieties was related to their susceptibility to viruses (cv. Adam and cv. Quiny – susceptible, cv. Tajfun – mid-resistance). The assessment of progeny tubers infection with viruses (PVY, PVM and PLRV), out of the same experiment was described extensively in an earlier paper (Wróbel, 2014). In 2010–2012 we additionally assessed the impact of diversified terms of planting *in vitro* plantlets and microtubers in the field conditions on the above-mentioned features.

Seed material (*in vitro* plantlets, microtubers and minitubers) were supplied in each year of research from the Bank of Gene Material of the Plant Breeding and Acclimatization Institute – National Research Institute, Department of Potato Protection and Seed Science in Bonin. Seed potatoes were annually purchased directly from the breeder of a given cultivar. To make sure that they were not infected with viruses, prior to planting they were assessed in terms of their health and DAS ELISA in an eye test. No viruses were found in the research material.

2.1. Preparation of material and planting

The whole experiment comprised 3 replication because of the large number of microtubers and *in vitro* plantlets required, as well as difficulties in obtaining them. One small plot of land comprised 4 ridges (spacing 75 cm × 35 cm), each of which was randomly planted with seed material of different origin totaling 50 items: traditional seed potatoes, minitubers, microtubers and *in vitro* plantlets. Additionally, traditional seed potatoes were planted around the plots (with a single ridge on each side). In total, for each cultivar, 6 plots were created, out of which half were additionally protected with Sunspray 850 EC mineral oil in intensity 2 or 4% (2006 and 2007), usually in 7-day-long intervals subsequent to 90% of emergences appearing. The plots were additionally distributed at random within a larger plot of land, both protected and unprotected with mineral oil.

In the experiment referred to as 2nd term (Table 1), the major period of planting in the field was fourth week of April. During this period only traditional seed potatoes (35–45 mm diameter) and minitubers (15–30 mm diameter) were planted by hand, though in the case of minitubers because of their small size these were placed in the previously profiled ridges. Because of *in vitro* plantlets' delicacy and sensitivity as well as microtubers' sensitivity to early spring weather conditions (low temperatures), they were not planted directly in the field but seed material was first prepared in a glasshouse. For this purpose, approximately 7–10 days after the seed potatoes and minitubers had been planted in the field, microtubers (5–10 mm diameter) were prepared in a glasshouse, planting into pots of 8 cm diameter filled with peat substrate. Seven days later the *in vitro* plantlets were planted using the same methodology. When full emergence was reached on all of the plots planted with traditional seed potatoes and minitubers (depending on the year and cultivar it was approximately 23–39 days since planting), microtubers and *in vitro* plantlets which were well developed (average height about 10–15 cm) and well rooted, were hand planted in the field. They were planted deep enough not to stick out above the top of the ridge by more than 3–7 cm. The aim of delaying the planting of *in vitro* plantlets and microtubers was to ensure that the size of all the seed material growing in the field was equal at the time of the first virus infection threat.

In 2010–2012 we assessed the impact of an earlier planting (1st term – second week of April) and a much later planting (3rd term – last week of June/first week of July) of microtubers and *in vitro*