THE EFFECT OF PLANTING DENSITY ON POTATO (*SOLANUM TUBEROSUM* L.) MINITUBER NUMBER, WEIGHT AND MULTIPLICATION RATE

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Abstract
The study was aimed to investigate the effect of potato (*Solanum tuberosum* L.*) in vitro* plantlets planting density under greenhouse conditions on obtained minitubers number per unit area, multiplication rate and their weight distribution. Three cultivars of different maturity ('Monta' – early maturity, 'Prelma' – medium early and 'Mandaga' – medium late maturity) were used for the study. *In vitro* plantlets were planted in a greenhouse of State Priekuli Plant Breeding Institute, Latvia in 2014 at four planting densities (PDs) 63 plants per m^2, 95 plants per m^2, 142 plants per m^2 and 184 plants per m^2 respectively. Fertilized peat was used as a substratum. Significant effect of planting density ($p<0.001$) and cultivar ($p<0.01$) was found on analyzed yield parameters. Increased planting densities resulted in increased minitubers number per m^2 (from 272 minutubers m^{-2} at PD 63 plants m^{-2} to 414 minutubers m^{-2} at PD 184 plants m^{-2}), decreased multiplication rate (4.3 to 2.7 minitubers per planted plant) and mean fresh weight of minitubers (from 20.26. g to 12.11 g). The highest increase of minitubers number per m^2 was observed within size (weight) range 3 to 5 g. Minitubers number per m^2 increase within bigger size ranges (5 to 10 g, 10 to 20 g) was less pronounced. Slight insignificant ($p=0.330$) decrease of minitubers number >20 g was observed in relation of planting density increase (112 minitubers m^{-2} at PD 95 plants m^{-2} to 84 minitubers m^{-2} at PD 184 plants m^{-2}).
Key words: *Solanum tuberosum*, potato, minitubers, planting density, tuber size distribution.

Introduction

Initial potato seed stock material (known as breeder's seed in Latvia), which is free of tuber-borne pathogens, especially virus diseases, is crucial in any potato seed production system. Traditional systems involving clonal selection for obtaining of healthy seed stocks could take more than 10 years to obtain seed material at satisfactory amounts. Therefore, nowadays most of seed production systems worldwide involve healthy *in vitro* plants mass-propagation at initial stage of seed production with subsequent minitubers production, which is called rapid multiplication. This system was involved more than three decades ago both worldwide and in Latvia.

Rapid multiplication of seed stock material allows obtaining of healthy initial seed material at big amounts thus minimizing field generations, as well as fastening seed production of new cultivars.

Potato *in vitro* plants can be planted both in field (Tadesse et al., 2001; Särekanno et al., 2010) or in greenhouse conditions. Growing of minitubers in solid substrates in greenhouses is still the most common and robust minitubers growing method (Struik and Wiersema, 1999), although soil-less production systems are very popular (Lommen, 2007).

The aim of seed stock multiplication is to produce as many minitubers of adequate size as possible. It has been reported that one potato plant produces 2 to 5 minitubers on average (Struik, 2007); however, larger amount of minitubers from one plant can be also obtained (Roy et al. 1995).

As reviewed by I. Dimante and Z. Gaile (2014), several factors such as soil type, substratum layer, fertilizing protocols, extra lightening etc., and their combination can affect progeny minitubers yield parameters.

Manipulation with the *in vitro* plants planting density could be considered as one of the most popular ways to manage minitubers number per area unit (Roy et al., 1995; Lommen and Struik, 1992; Veeken and Lommen, 2009; Jin et al., 2013). Multiplication rate usually changes conversely if to compare with minitubers number per area unit change (Veeken and Lommen, 2009). The solution of this issue greatly depends on what is considered as the most efficient approach by a producer – increased multiplication rate or bigger numbers of minitubers per area unit.

Low mean fresh weights of minitubers can affect their field performance. Therefore, it should not be the main goal itself to obtain many minitubers at any size.

Some authors mention that too small minitubers have larger losses during the storage (Lommen, 1993) and smaller yield when planted in field (Lommen and Struik, 1995; Karafyllidis et al., 1997; Barry et al., 2001). As various authors use various minitubers sizes for their field performance experiments, it is not clearly stated which minitubers size (by dimensions or by weight) can be considered as big enough. Wiersema et.al., (1987) state that minitubers bigger than >5 g are sufficiently large for good field performance; nevertheless, experiments with smaller minitubers have shown adequate field performance as well (Lommen et al., 1995).

Assumptions based on our previous experience, allow us state that in this study we consider minitubers size (weight) of 3 g as a threshold which could be

appropriate and commercially applicable. However, all grown minitubers have been counted and weighted to see the proportion of acceptable fraction.

The purpose of this study was to investigate minitubers multiplication rate (number of minitubers per planted plantlet) and final number of minitubers per area unit in relation with potato *in vitro* plantlets planting density. Additionally, minitubers size distribution both across various size ranges and in cumulative stratum was in the scope of this study.

Materials and Methods

The experiment was carried out at State Priekuli Plant Breeding Institute (SPPBI, latitude 57°31′ N, longitude 25°34′ E), Latvia in 2014 with plantlets of cultivars (CVs) bred at SPPBI 'Monta' (early maturity), 'Prelma' (medium early) and 'Mandaga' (medium late maturity) at four planting densities (PDs).

In vitro propagation of plantlets

Only virus indexed plantlets with no virus diseases detected were subjected to further micropropagation. *In vitro* plants were propagated routinely at Potato tissue culture laboratory of State Priekuli Plant Breeding Institute. Single node cuttings were sectioned and placed in test tubes (1 cutting per tube) on fresh MS medium (Murashige and Skoog, 1962), supplemented with 30 g L^{-1} regular sugar from supermarket and 6 g L^{-1} food grade agar. Subculturing of microplants was performed once every 4 weeks. The temperature in growth room was 20–26 °C; photoperiod was 16/8 h day and night respectively.

Planting of in vitro plantlets and crop husbandry practises in a greenhouse

In vitro plantlets of three CVs were planted in a greenhouse at four PDs – 63 plants m^{-2}, 95 plants m^{-2}, 142 plants m^{-2} and 184 plants m^{-2} respectively.

Plastic boxes with permeable sides and bottom were used for planting of *in vitro* plantlets. Inner dimensions of the boxes were 0.55 m × 0.35 m × 0.20 m (length × width × height). Fertilized peat with pH adjusted to 5.3 was placed in boxes at 0.13 m height. Peat contained macronutrients at following rates kg per m^3: N 0.30; P 0.24, K 0.24; Ca 0.37; S 0.18 Mg 0.05 kg. Following micronutrients were added at such rates g per m^{-3} as follows: B 3.6; Mo 2.4; Mn 1.9; Cu 1.8; Fe 1.1 and Zn 0.48 g. The peat was entirely moistened with water before planting.

Desired planting densities were obtained by modifying the procedure, described by A. Veeken and W. Lommen, 2009. Thirty five holes per box were pressed in the peat in rectangular order (5 rows with 7 holes each). Each hole was 8 cm deep and 2 cm in diameter. *In vitro* plantlets of 10 cm length were planted into pressed holes, obtaining the maximum density by planting plantlets into each of 35 holes. The density of 142 plants m^{-2} was obtained by planting 27 plants per box (planting into the second and the fourth row was reduced to three holes at equal distance between plants). For the density of 95 plants m^{-2} the first, third and fifth row were planted with four plants, the second and the fourth row – with 3 plants. Eighteen plants per box were planted in this case. The planting density of 63 plants m^{-2} (12 plants per box) was obtained by planting plantlets only in the first, third and fifth rows and reducing planting to four holes per row. With decreasing of the PD, distance between plants at each separate row remained constant within the same PD. This approach contributed to uniform planting densities and uniform distances between planted plants over CVs and replications.

Plants were watered by hand five times per week during the first four weeks of the growth. Later watering was reduced to three times per week.

Foliar fertilizer applications were used three times per growing season starting at the sixth week after planting and following once every ten days. One litre of media used for applications contained 1.34 g KH_2PO_4, 1.34 g KNO_3, 1.34 g $Ca(NO_3)_2$, 0.7 g $MgSO_4$.

Planting was conducted on 23 April 2014, haulms were removed by hands and minitubers of CVs 'Monta' and 'Prelma' were harvested 78 to 79 days after planting (DAP) and 90 DAP for CV 'Mandaga'.

The environmental conditions in greenhouse were poorly controlled. Regardless of extensive ventilation, the air temperature reached more than 30 °C on some days.

Experimental design and statistical analysis

The split-plot design with 3 replications (blocks) was used in this experiment with cultivars assigned as main plots and planting density assigned as sub-plots. Cultivars were randomized within each block, planting densities were randomized within each main plot (cultivar).

Each sub-plot was surrounded by boxes with plants of the same cultivar and the same planting density in order to avoid side effects as well as competition between different CVs or PDs.

Data on minitubers number per planted plant (multiplication rate), minitubers number per m^2, mean weight of minitubers as well as minitubers size distribution were collected, calculated and subjected to analysis.

The obtained data was analyzed using the SPSS program, version 17.0. Significance level used for the separation of means was α=0.05. The analysis of variance was performed to evaluate the effects of treatments and Least Significant Difference test (LSD) was used to separate the significant treatment

means. Relationship between dependent variables was examined by Pearson's correlation coefficient, when applicable.

Results and Discussion

Number of minitubers

Planting density of *in vitro* plants and cultivar had significant effect on such minitubers yield parameters as multiplication rate (mean number of minitubers per planted plant), number of minitubers per m^2 and mean minitubers weight (Table 1). This finding confirms results obtained by A. Veeken and W. Lommen (2009). No significant interaction effect was found between CV and PD on these parameters. A. Veeken and W. Lommen (2009) figured out an interaction between CV and PD in case of such yield parameters as minitubers number per m^2 and mean minitubers fresh weight.

Partition sum of squares showed that main factors (together PD and CV) explained more than 50% of the variance in all analyzed yield parameters, PD being as dominant factor determining yield

Table 1

Number of minitubers per planted plant, per m^2 and mean fresh weight analysis at different planting densities of *in vitro* derived plants

Planting density, plants m^{-2}	Multiplication rate (mean number of minitubers per planted plant)				Mean number of minitubers per m^2				Mean weight of minitubers, g			
	>0 g	SE	>3 g	SE	>0 g	SE	>3 g	SE	>0 g	SE	>3 g	SE
means within cultivars												
'Monta'												
63	5.4^{b}	0.57	**4.7^{c}**	0.32	342^{a}	35.8	**293^{a}**	19.8	13.96	1.135	**15.88**	1.072
95	4.4^{b}	0.26	**3.9^{b}**	0.25	416^{a}	24.3	**368^{b}**	23.7	13.47	2.369	**14.81**	2.345
142	2.9^{a}	0.29	**2.5^{a}**	0.21	414^{a}	41.9	**354^{ab}**	30.4	14.21	0.963	**16.21**	0.784
184	3.2^{a}	0.15	**2.6^{a}**	0.08	591^{b}	28.1	**481^{c}**	15.3	10.94	1.606	**12.97**	1.629
$LSD_{0.05}$	1.2	×	**0.8**	×	108	×	**75**	×	NS	×	**NS**	×
'Prelma'												
63	4.1^{b}	0.12	**3.9^{c}**	0.13	256^{a}	7.6	**247^{a}**	8.0	21.31^{c}	1.039	**22.08**	1.133
95	3.3^{ab}	0.27	**3.0^{b}**	0.26	309^{ab}	25.3	**288^{ab}**	24.7	18.87^{bc}	1.359	**20.13**	1.643
142	2.9^{a}	0.56	**2.4^{ab}**	0.31	409^{bc}	79.3	**340^{ab}**	44.5	13.51^{ab}	2.965	**15.38**	3.002
184	2.5^{a}	0.11	**2.1^{a}**	0.14	451^{c}	19.8	**377^{b}**	26.2	12.62^{a}	1.427	**14.74**	1.361
$LSD_{0.05}$	1.0	×	**0.7**	×	140	×	**97**	×	6.05	×	**NS**	×
'Mandaga'												
63	3.5	0.84	**3.2**	0.70	218^{a}	53.1	**204^{a}**	44.3	25.51^{c}	4.18	**26.51^{c}**	3.543
95	3.2	0.23	**3.1**	0.32	305^{ab}	21.9	**290^{ab}**	29.9	21.15^{bc}	1.953	**22.32^{bc}**	2.425
142	2.6	0.17	**2.4**	0.16	363^{bc}	24.1	**335^{bc}**	22.4	14.28^{ab}	0.801	**15.30^{ab}**	0.890
184	2.4	0.03	**2.1**	0.02	432^{c}	6.1	**384^{c}**	3.0	12.77^{a}	0.553	**14.12^{a}**	0.686
$LSD_{0.05}$	NS	×	**NS**	×	102	×	**95**	×	7.69	×	**7.24**	×
means over cultivars												
63	4.3^{c}	0.42	**3.9^{c}**	0.30	272^{a}	26.2	**248^{a}**	19.2	20.26^{b}	2.122	**21.49^{b}**	1.904
95	3.6^{b}	0.23	**3.3^{b}**	0.20	343^{b}	21.7	**315^{b}**	18.7	17.83^{b}	1.495	**19.08^{b}**	1.550
142	2.8^{a}	0.20	**2.4^{a}**	0.12	395^{b}	28.0	**343^{b}**	17.1	14.00^{a}	0.938	**15.63^{a}**	0.943
184	2.7^{a}	0.15	**2.3^{a}**	0.10	491^{c}	27.1	**414^{c}**	18.9	12.11^{a}	0.705	**13.94^{a}**	0.695
$LSD_{0.05}$	0.6	×	**0.5**	×	61	×	**46**	×	3.31	×	**3.24**	×
P value for effects of factors												
PD	***	×	***	×	***	×	***	×	***	×	***	×
CV	**	×	**	×	**	×	**	×	**	×	**	×
PD × CV	NS	×	NS	×	NS	×	NS	×	NS	×	NS	×
partition of sum of squares (main factors), η^2, %												
PD	44	×	60	×	54	×	56	×	38	×	37	×
CV	21	×	12	×	19	×	16	×	18	×	16	×

>0 g=total number of minitubers, >3 g=minitubers bigger than 3 g, SE=standard error, PD=planting density, CV=cultivar, PD×CV=interaction planting density × cultivar

** – effect of the factor significant at $p<0.01$; *** – effect of the factor significant at $p<0.001$; NS – not significant ($p\geq0.05$).

Values labelled with a similar letter are not statistically significantly different ($p\geq0.05$)

parameters variance. PD determined even 60% of multiplication rate variance and 57% of mean minitubers number per m^2 variance within minitubers size (weight) range >3 g.

The effect of increased PD showed a significant increase of progeny minitubers number per m^2, but it led to reduced multiplication rates (number of minitubers per planted plant) simultaneously. Similar tendencies were obtained by W. Lommen and P. Struik (1992) and R. Roy et al. (1995).

Considering minitubers size >3 g, the highest increase of minitubers number per area was found between PD 63 and 95 plants m^{-2} (1.3 fold increase). PD change from 142 to 184 plants m^{-2} increased minitubers number significantly ($p<0.01$) as well. Regardless of 1.5 fold increase of planted plants between PD 95 and 142, obtained minitubers number per m^2 did not increase significantly ($p=0.203$). The results published by W. Lommen and P. Struik (1992) showed phenomena that gradual increase of PD did not necessarily mean simultaneous increase of minitubers at significant rates between any used PD, whereas the results obtained by A. Veeken and W. Lommen (2009) confirmed significant increase of minitubers number per m^2 coincidentally with gradual increase of PD.

Increase of minitubers number per m^2 was more evident (1.7 fold increase of tubers >3 g) than simultaneous multiplication rate decrease (1.4 fold decrease within tuber size range >3 g) between two marginal PDs. Furthermore, at two highest densities minitubers number per plant remained at the lowest level and did not change significantly. This finding could contribute to optimization of PD with respect to multiplication rate retain. Similar trend was observed by W. Lommen and P. Struik (1992) at the highest planting densities. In our study the change of multiplication rate and minitubers number per m^2 showed similar trends both in size ranges >0 g and >3 g. However, increase of minitubers number in size range >0 g was slightly bigger (2 fold increase) than was in size range >3 g (1.7 fold increase) which was mainly due to increase of <3 g minitubers number (Figure 1).

The effect of cultivar on all analyzed yield parameters was significant. As it can be figured out from the Table 1, cultivar 'Monta' had higher multiplication rate and mean minitubers number per m^2 than cultivars 'Prelma' and 'Mandaga' both in size range >0 g and >3 g.

However, the largest increase of minitubers number per m^2 was observed for cultivar 'Mandaga' (1.9 fold increase between PD 63 and 184 of minitubers >3 g), this effect was especially evident between PD 63 and 95, where minitubers number >3 g had 1.4 fold increase. At the same time multiplication rate showed an insignificant decrease ($p>0.05$).

Mean minitubers weight and minitubers size distribution

Higher PDs resulted in decreased mean minitubers fresh weight (Table 1) similarly to findings of other researchers (Roy et al., 1995; Veeken and Lommen 2009; Jin et al., 2013).

Higher PDs resulted in higher minitubers number per m^2 (Table 1). Based on this relation, minitubers number per m^2 and mean minitubers weight was subjected to correlation analysis.

Statistically significant negative correlation was found between mean minitubers number >0 g per m^2 and mean minitubers weight >0 g ($r = -0.767$, $p<0.001$) as well as between mean minitubers number >3 g per m^2 and mean minitubers weight >3 g ($r = -0.708$. $p<0.01$). This finding conforms to the study of Jin et al. (2013), which has stated that minitubers size distribution depends on the total number of minitubers produced per unit area and the mean minitubers weight.

Nevertheless, mean minitubers weight over cultivars did not reduce significantly between PD of 142 and 184 plants m^{-2} in both size ranges >0 g ($p=0.251$) and >3 g ($p=0.293$) (Table 1). Furthermore, only cultivar 'Mandaga' had significant ($p<0.01$) mean minitubers weight decrease within minitubers >3 g between the smallest and the largest PDs. Within size range >0 g, mean tuber weight decrease was observed for cultivars 'Prelma" and 'Mandaga'.

At the two lowest PDs minitubers of size range >20 g were produced at the largest amounts per m^2 if compared to other size ranges. However, the difference of tuber number of this particular size range between PDs was not significant ($p=0.330$). The largest total amount of minitubers at two highest PDs was obtained within the size range 10 to 20 g (Figure 1). The smallest total amount of tubers was obtained within the size ranges <3 g and 3-5 g at all PDs.

Significant ($p<0.01$) increase of minituber number within the size range <3 g, 3–5 g, 5–10 g, and 10–20 g was determined by the PD (Figure 1). Despite smaller absolute values, the largest proportional increase between PDs was observed within the size range 3–5 g, when a 4.5 fold increase was found between the smallest and the largest PD. The major proportion of minitubers number increase within this particular size range was found between PD 63 and 93 plants per m^2. Within next minitubers size ranges a tuber number increase in relation with PD was less pronounced, reaching two times increase in the range 5–10 g and 1.7 times increase in class 10–20 g between the smallest and the largest PD. In the size range >20 g minitubers number change was not significant ($p=0.330$) between PDs, even a slight decrease was observed. R. Roy et al. (1995) and A. Veeken and W.

Lommen (2009) observed a similar tendency when the minitubers number increase within size ranges was significantly determined by PD of up to particular minitubers weight.

Another important yield parameter is cumulative number of obtained minitubers, which is a total number of tubers over the certain size.

Significant ($p<0.001$) increase of minitubers >3 g and >5 g was determined by PD (Figure 2). Regardless of significant ($p<0.001$) minitubers number increase in the size range 10–20 g, no significant ($p=0.166$) changes were observed in cumulative stratum within minitubers bigger than >10 g. This phenomena can be explained by the fact that mentioned cumulative size range accumulated minitubers of >20 g weight which showed insignificant ($p=0.330$) changes with a tendency of minitubers number decrease with PD increase. Nevertheless, at the highest PD, tubers

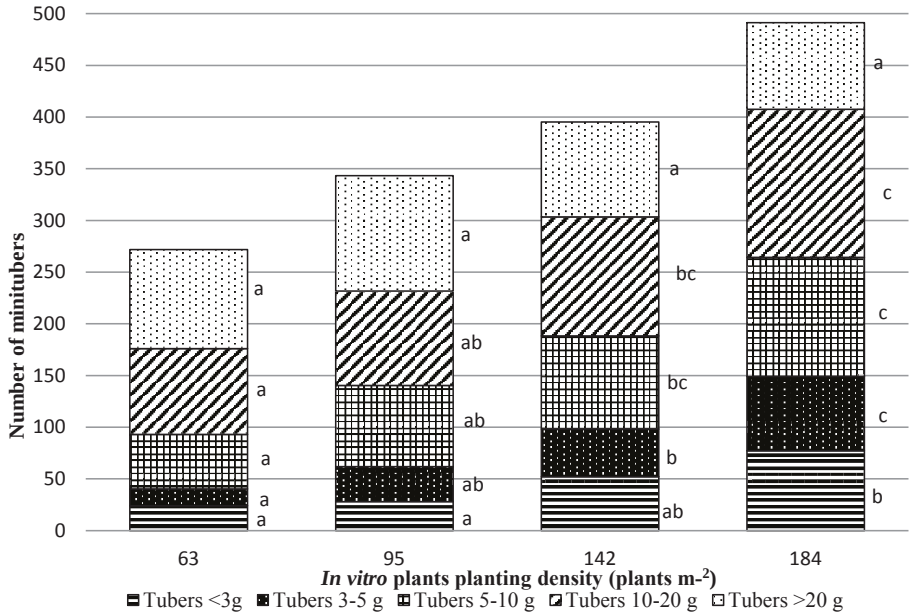

Figure 1. Minitubers weight distribution depending on *in vitro* plants planting density. Number of minitubers of the same size range with the same letter is not significantly different between planting densities ($p\geq0.05$).

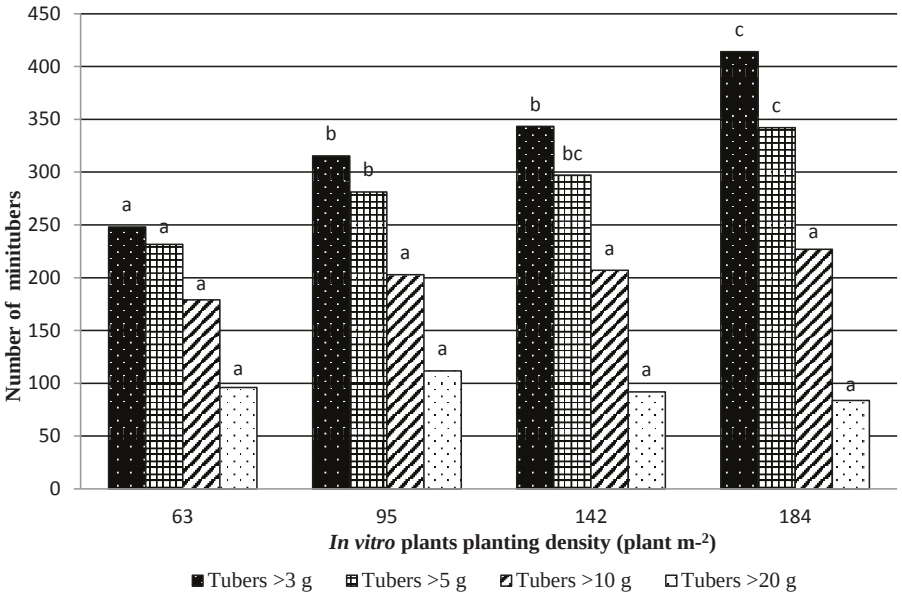

Figure 2. The cumulative number of minitubers depending on *in vitro* plants planting density. Number of minitubers of the same cumulative size range with the same letter is not significantly different between planting densities ($p\geq0.05$).

>10 g were still almost half of all produced tubers (46%).

Regarding the proportion change to total number of tubers per m^2, the total number of tuber >3 g decreased from 91% to 84% between the marginal PDs (data not shown). This tendency was caused by the respective increase in tuber number of size <3 g. The same trend (a slight decrease in percentage stratum) was observed in all cumulative size ranges.

Practical considerations

Minitubers number per area unit and the tuber number per planted plant of desirable size are two main yield parameters. It depends on a producer which of the two parameters is accepted as the major one. In case when a minitubers' grower is *in vitro* plants producer at the same time, minitubers number per area unit could become the most important parameter. The desirable minimum minitubers size depends mostly on quality of storage conditions and on minitubers field performance during subsequent field generation. Smaller minitubers size can require more careful field practices, which is not always affordable.

The number of planted plants per m^2 increased more considerably than minitubers number m^{-2} increase (2.9 fold and 1.7 fold (>3 g) to 1.8 (>0 g) fold increase between the lowest and the highest PD respectively; Table 1). Nevertheless, a decrease of multiplication rate was less evident. This could let us assume that increased PD can contribute to optimization of minitubers production at limited greenhouse space. Nevertheless, more data must be obtained during the repetitive experiments in order to see which PD could be considered as the optimal one.

Conclusions

1. The number of obtained minitubers per m^2 per planted plant (multiplication rate) and mean tuber weight was significantly determined by cultivar ($p<0.001$) and planting density ($p<0.001$).
2. Increased planting densities led to reduced multiplication rate, reduced mean tuber weight and increased minitubers number per m^2.
3. An increase of tuber number per m^2 was significantly ($p<0.01$) determined by planting density within size ranges <3 g, 3–5 g, 5–10 g, and 10–20 g of up to the size range >20 g where a slightly insignificant ($p=0.330$) decrease was observed. The same trend was observed in tuber number cumulative stratum up to the size range >10 g. However, even at the highest PD harvested minitubers of size >10 g were still almost half of all produced tubers (46%).

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