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Effect of Potato Microtuber Size on the Growth and Yield Performance of Field Grown Plants

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Abstract: To examine the effect of the size of the potato microtuber (MT) produced *in vitro* on the posterior field performance, we examined the growth and yield of the late maturity cultivar Norin 1 using four sizes of MT: 0.3−0.5 g, 0.5−1 g, 1−3 g and 3−5 g, and conventional seed tubers (CT) (approximately 50 g). The experiment was conducted at Hokkaido University, Sapporo Japan in 1998 and 1999. The tubers were planted in May of each year, in a randomized block design with three replications. Plants from MT lighter than 0.5 g showed a slower initial leaf and tuber growth than heavier MT, but around the full flowering stage there was no significant difference with the MT size in leaf or tuber growth. CT plants showed higher initial leaf and tuber growth compared with MT plants, especially in 1999. No differences in growing period, number of tubers, and tuber fresh and dry yield were observed with the MT size. However, in 1999, the growing period was longer and tuber fresh and dry yields at harvest were higher in CT plants. MT of all sizes used in the study can be used for direct field planting, but more studies are needed to increase the yield stability of MT plants.

Key words: In vitro tubers, Leaf area index, Seed tuber, *Solanum tuberosum* L.

Healthy potato seed production in the field is a major problem in the potato production system in many countries, especially in those located at low latitudes (Maldonado et al., 1998; Hirpa et al., 2010). In some countries, approximately 50% of the expenditures on inputs for potato production are due to the potato seed (Horton, 1987).

There are alternatives for healthy potato seed production in countries located in low latitudes. One is the use of true potato seed (TPS), but it is not widely adopted due to the slow initial growth of plants, large genetic variation of the progeny and few available hybrids or materials developed for TPS production (Almekinders et al., 1996). The concomitant use of TPS with micropropagation techniques could be valuable for potato seed production (Carputo et al., 1994; Simmonds, 1997).

Most of the world potato producers use micropropagation techniques to achieve healthy tuber seed (Jones, 1988). The *in vitro* micropropagated potato plants can produce plantlets and microtubers (MT) (Goodwin and Adisarwanto, 1980; Uyen and Zaag, 1985). These materials can be planted in a greenhouse or can be directly planted in the field. Usually, the tubers harvested from these materials planted in pots are small and are called minitubers. Plantlets, minitubers and MT have advantages and disadvantages and although the production of MT has increased its utilization is still low.

At present, one of the reasons for the low utilization of MT as potato seed by the growers in field cultivation, is the high production cost of MT (Zaag, 1990). Donnelly et al. (2003) claimed that as the number of stem segments per culture flask increased, the total weight of the MT per flask increased, but the number of large MT decreased. Large MT also took more time to be produced, raising then the production costs.

Studies have been reported on the field performance of potato plants grown from minitubers of different sizes (Lommen, 1994; Lommen and Struik, 1994), plants from MT and conventional seed tubers (CT) (Haverkort et al., 1991), and plants from CT and field transplanted plantlets (Leclerc and Donnelly, 1990). However, there are few studies comparing the field performance of directly planted MT of different sizes.

Our hypothesis was that the differences in growth and yield between the plants were caused by the different size of MT planted and the aim of this study was to examine the shoot and tuber growth and yield of potato plants field-grown from MT of different sizes in comparison with those of plants from CT.

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Abbreviations: LAI, leaf area index; MT, microtuber; CT, conventional seed tuber; DAE, days after emergence; DW, dry weight; TPS, true potato seed.

Materials and Methods

The experiment was conducted on the Experimental Farm of the Field Science Center for Northern Biosphere, Sapporo, Japan (43º04′N) in 1998 and 1999. Conventional potato seed tubers provided by the Tokachi Experimental Station, Hokkaido, and MT provided by the Kirin Brewery Co. Ltd. (Japan), both of the late maturity cultivar Norin 1, were planted by hand on 7 May 1998 and 14 May 1999, in rows 0.75 m apart and distance between plants of 0.25 m at about 3 cm and 7 cm planting depths for MT and CT, respectively, in a randomized complete block design with three replications. Four MT sizes were used in 1998: 0.3–0.5 g (super small, SS); 0.5–1 g (small, S); 1–3 g (medium, M) and 3–5 g (large, L) and two MT sizes in 1999: SS and M.

Fertilizer was given at the time of planting at the rate of 70, 110, 90 and 30 kg ha^{-1} of N, P_2O_5, K_2O and MgO, respectively, in both years. Insects and diseases were controlled according to the standard practice of the Hokkaido University Farm, and plots were hand-weeded during the early growth stage and were not irrigated.

Plant emergence, flowering and physiological maturity were visually recorded at 2–3 day intervals and these stages were determined when at least 70% of the plants were emerged, flowered or the leaves of plants turned yellow, respectively. The rate of emergence (%) was calculated as the number of plants emerged/number of tubers planted×100.

Four plants in each plot were sampled in both years at four growth stages: tuber formation, start of flowering, full flowering and maximum shoot growth stages. Accordingly, observations were made at 22, 36, 58 and 77 days in 1998 and at 22, 33, 55 and 77 days after emergence (DAE) in 1999. In 1998, plants from MT of SS size were sampled only at 77 DAE and at harvest, and in 1999, plants from MT of SS size were not sampled at 55 DAE.

Except for the final harvest, at each sampling stage in both years, the number of tubers (>1 g), shoot and tuber dry weight (DW) were recorded after oven dried for 72 hr (70ºC and aeration) and leaf area index (LAI) was measured. To measure LAI, we stripped all leaves of each plant and measured the leaf area of randomly selected sub-samples of around 1000 cm^2 $plant^{-1}$ with an automatic area meter (AMM-9, Hayashi Denko, Japan). Total leaf area of the four sampled plants was calculated as follows: measured leaf area of sub-sample x total leaf DW/sub sample leaf DW. Leaf area index was then calculated by adjusting the total leaf area for the plant density in 1 m^{-2} (5.33 pl m^{-2}). In both years, after physiological maturity (leaf yellowing) 10 (1998) or 12 (1999) plants of each replication were sampled to calculate the number of tubers, tuber fresh and dry yield. Meteorological data for May to October, the potato growing season, were recorded at the agrometeorological station of the Experimental Farm, Hokkaido University.

Analysis of variance to test significance of differences between plants from MT of each size and CT was conducted and when the difference was significant between the treatments means were tested by Tukey test at the 5% level.

Results

1. Initial development and leaf area index

No significant difference in the time from planting to emergence was observed among the tuber size classes (Table 1). However, the plants from larger tubers flowered earlier than those from smaller tubers in both years, and percentage of emergence was smaller in the plants from SS MT than in those from L MT and CT in 1998.

Table 2 shows the LAI of plants from MT and CT at each growth stage. The lightest MT had the lowest LAI until the start of flowering (i.e., around 30 DAE). The smaller the MT size, the lower the LAI was. Thereafter, the difference in LAI between plants from MT and CT disappeared.

2. Tuber development and harvest

The plants from MT formed fewer tubers than the plants from CT at early growth stage (Table 3). However,

Table 1. Days from planting to emergence, from emergence to flowering and percentage of emergence in potato plants grown from microtubers of different size and conventional seed tubers of about 50 g.

Tuber size (g)	Days to Emergence		Days to Flowering		% of Emergence	
	1998	1999	1998	1999	1998	1999
0.3–0.5	19	18	43 a[1)]	44 a	82 b	99
0.5–1	19	–	43 ab	–	90 ab	–
1–3	19	18	40 b	41 b	91 ab	99
3–5	19	–	33 c	–	97 a	–
50	18	19	33 c	30 c	99 a	100
ANOVA	ns[2)]	ns	**	**	**	ns

[1] Numbers followed by the same letters are not statistically different by Tukey test (α=0.05)

[2] ns: not statistically different ($P \geq 0.05$), **: statistically different at 0.01.

Table 2. Leaf area index at different growth stages of potato plants grown from microtubers of different size and conventional seed tubers of about 50 g.

Tuber size (g)	Tuber Formation		Start of Flowering		Full Flowering		Maxium Shoot Growth	
	1998	1999	1998	1999	1998	1999	1998	1999
0.3 – 0.5	–	0.39 b[1)]	–	1.46	–	–	5.27	3.32
0.5 – 1	0.40 b	–	2.38 c	–	4.58	–	4.02	–
1 – 3	0.54 b	0.67 ab	2.73 b	2.20	4.70	3.06	5.24	3.19
3 – 5	0.60 b	–	3.18 a	–	4.64	–	4.70	–
50	0.97 a	1.05 a	2.58 bc	2.17	4.11	2.83	4.73	3.34
ANOVA	**[2)]	*	**	ns	ns	ns	ns	ns

[1] Numbers followed by the same letters are not statistically different by Tukey test (α=0.05)
[2] ns: not statistically different ($P \geq 0.05$), * and **: statistically different at $P < 0.05$ and 0.01, respectively.

Table 3. Number of tubers (m^{-2}) at different growth stages of potato plants grown from microtubers of different size and conventional seed tubers of about 50 g.

Tuber size (g)	Tuber Formation		Start of Flowering		Full Flowering		Maxium Shoot Growth	
	1998	1999	1998	1999	1998	1999	1998	1999
0.3 – 0.5	–	1.3 b[1)]	–	16 b	–	–	68 a	69
0.5 – 1	3.1 b	–	35	–	49	–	45 b	–
1 – 3	4.9 b	7.6 b	42	30 b	43	47	40 b	64
3 – 5	3.6 b	–	45	–	34	–	43 b	–
50	31.6 a	21.3 a	36	57 a	43	52	47 b	64
ANOVA	**[2)]	**	ns	**	ns	ns	**	ns

[1] Numbers followed by the same letters are not statistically different by Tukey test (α=0.05)
[2] ns: not statistically different ($P \geq 0.05$), **: statistically different at 0.01.

Table 4. Tuber dry weight (g m^{-2}) at different growth stages of potato plants grown from microtubers of different size and conventional seed tubers of about 50 g.

Tuber size (g)	Tuber Formation		Start of Flowering		Full Flowering		Maxium Shoot Growth	
	1998	1999	1998	1999	1998	1999	1998	1999
0.3 – 0.5	–	0.07 b[1)]	–	6.20 c	–	–	697 b	399
0.5 – 1	0.40 b	–	20.4 c	–	304 b	–	548 c	–
1 – 3	0.57 b	0.50 b	21.5 c	19.3 b	320 b	227 b	655 bc	496
3 – 5	0.84 b	–	65.2 b	–	296 b	–	614 bc	–
50	7.30 a	4.65 a	104 a	91.0 a	431 a	347 a	824 a	531
ANOVA	**[2)]	**	**	**	*	*	**	ns

[1] Numbers followed by the same letters are not statistically different by Tukey test (α=0.05)
[2] ns: not statistically different ($P \geq 0.05$), * and **: statistically different at $P < 0.05$ and 0.01, respectively.

after full flowering stage there was no difference in the number of tubers between MT and CT plants. In 1998, plants from SS MT had a higher number of tubers than the plants from the other seed tubers at the maximum shoot growth stage.

Tuber DW was heavier in the plants from CT than from MT at all growth stages except at the maximum shoot growth stage in 1999 (Table 4). The plants from a larger MT tended to have a heavier tuber than those from a smaller MT until the start of the flowering stage.

In 1998, no difference between the plants from CT and MT of any size was observed in any harvest parameters recorded (Table 5). However, in 1999, the time elapsed from emergence to plant maturity (growing period), was longer in the plants from CT than from MT, and fresh and dry yields of tubers were higher in plants from CT than MT. In this year, there was no difference in any parameter between the plants from SS MT and M MT. The number of tubers at harvest did not vary with the size of seed tubers in both years. However, since MT plants had one main

Table 5. Number of days from emergence to physiological maturity, number of tubers and fresh and dry tuber yields at harvest of potato plants grown from microtubers of different size and conventional seed tubers of about 50 g.

Tuber size (g)	Growing Period (days)		Number of Tuber (m^{-2})		Fresh Tuber Yield (kg m^{-2})		Dry Tuber Yield (kg m^{-2})	
	1998	1999	1998	1999	1998	1999	1998	1999
0.3 – 0.5	140	132 b[1)]	57	55	7.13	4.09 b	1.51	0.75 b
0.5 – 1	139	–	53	–	6.01	-	1.26	–
1 – 3	139	129 b	47	55	6.83	4.28 b	1.51	0.75 b
3 – 5	142	–	43	–	6.60	-	1.37	–
50	143	139 a	49	59	7.05	5.96 a	1.39	0.99 a
ANOVA	ns [2)]	**	ns	ns	ns	**	ns	*

[1] Numbers followed by the same letters are not statistically different by Tukey test (α=0.05)
[2] ns: not statistically different ($P \geq 0.05$), * and **: statistically different at $P < 0.05$ and 0.01, respectively.

Table 6. Average monthly temperature, total monthly radiation and precipitation in 1998 and 1999.

Month	Average Temperature (C°)			Accumulated Solar Radiation (MJ m^{-2})			Accumulated Precipitation (mm)		
	1998	1999	avg[1)]	1998	1999	avg	1998	1999	avg
May	13	12	12	550	445	529	56	73	50
June	15	17	16	499	534	531	80	31	46
July	20	21	20	468	436	488	73	137	77
August	21	24	21	373	472	474	90	90	123
September	19	19	17	362	346	359	210	102	150
October	13	11	11	283	245	262	74	72	98
Total				2535	2478	2643	582	504	543

[1] The average value of 30 years, calculated with the data from 1970 to 1999.

stem while CT plants had more than two main stems (data not shown), the number of tubers per main stem was higher in MT than in CT plants.

Discussion

In general, the average monthly temperature was lower in 1998 than in 1999 (Table 6). For instance, the average temperature in August of 1999 was more than 2°C higher than the average of last 30 years. The accumulated monthly radiation in the 1999 growing season was lower than the average of 30 years. The precipitation pattern showed large differences between the seasons. The accumulated precipitation in the 1998 season larger than the 30-year average while the growing season of 1999 had a low precipitation, principally in the last three months.

The similarity of the period from planting to emergence among plants from MT of different sizes and CT observed in this study (Table 1) was probably due to the shallower planting depth of the MT. Since we did not observe differences among the plants from MT of different sizes in the days to emergence, the depth of around 3 cm is considered to be suitable for planting MT in the field. The longer period from plant emergence to flowering observed in plants grown from the smallest MT could reflect their slower initial development; even MT of 0.3 g, could be used for field planting, although it tended to show a lower percentage of emergence. A lower percentage of emergence of MT compared with CT has also been reported by other workers (Haverkort et al., 1991; Lommen and Struik, 1994). The lower percentage of emergence of the smallest MT observed in 1998, could be due to the lower precipitation observed in this year at the time of the initial growth (Table 6). Therefore, we can speculate that in areas with poor or irregular precipitation at the time of MT planting, proper irrigation might be necessary to obtain a homogeneous canopy in the field.

A smaller LAI was observed in plants from small MT than from large MT and CT at the beginning of the growing season, and no difference was observed in LAI after the start of flowering about 30 DAE (Table 2), which is in agreement with our previous findings (Kawakami et al., 2003; 2006). The initial smaller LAI in the plants from small MT suggests that a narrow planting distance could decrease the initial difference in LAI among plants grown from seed tuber of small size.

MT size apparently did not affect the number of formed tubers, although it was lower than CT plants until the start of flowering stage (Table 3). It seems that only plants from

MT of SS size have different patterns of tuber growth, and it varies depending on the year. On the other hand, MT size affected the rate of tuber bulking (Table 4), that is, the MT size has a higher effect on tuber bulking than in tuber formation. This result corroborates our previous studies (Kawakami et al., 2003; 2004) that demonstrated that the difference in tuber initiation between plants from MT and CT was smaller than the difference in tuber bulking.

MT size seemed not to affect the length of the growing period, although in 1999 the growing period of plants from MT was shorter than that of plants from CT (Table 5). Lommen (1999) also did not find any difference in growing period between CT plants and *in vitro* plantlets. We can speculate that in the year with severe early drought or soil water stress as observed in June of 1999, plants from MT show a greater reduction in the growing period than plants from CT, because plants from MT have a higher shoot to root ratio at an early growth stage (Lommen and Struik, 1994). We also observed that MT size did not affect the growing period within the range of MT size used in this study.

The absence of a difference in the number of tubers between plants from MT and CT observed in this study, does not agree with the findings of Tsuda et al. (2010) who observed that MT plants of Norin 1 cultivars formed on average 9% less tubers than CT plants. This absence of difference in the number of tubers between MT and CT plants should be useful when using MT for potato seed production.

In this study, we did not observe any significant difference in tuber yield with the MT size, although in 1999 MT plants yielded less than CT plants (Table 5). Struik and Lommen (1990) stated that the use of MT as seed tubers would only be feasible when they were larger than 0.5 g. In our experiment, no significant difference in fresh or dry tuber yield was observed among the MT classes, suggesting that the use of the MT of even 0.3 g may be a feasible practice under the field condition. The tuber fresh yield of about 41 to 66 t ha^{-1} observed in the plants from MT demonstrates the high yield potential of MT as seed tubers in the field, although the yearly variation in tuber yield was higher in plants from MT than in plants from CT. Previously (Kawakami et al., 2006), we found that soil water stress affected tuber yield of plants from MT and CT in a similar way, so more studies about others factors effecting tuber yield of plants from MT may be necessary to assure the yield stability of plants grown from these materials.

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