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Effects of Medium Surface Level Control on the Mass Propagation of Potato Tubers Using a Jar Fermentor Culture Technique

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Potato (*Solanum tuberosum* L.) tubers were propagated in a jar fermentor using a 2 step culture method which consisted of the shoot multiplication step (step 1) and the tuber induction and development step (step 2). Shoots which were multiplied in the aerial phase of the jar fermentor were more valuable for the mass propagation of tubers than the shoots cultured under continuously submerged conditions in the liquid medium. The development of tubers was strongly suppressed under the submerged conditions in step 2, whereas the development of tubers was stimulated only at the surface area of the medium, and this suppression might cause significant variation in tuber weight. Tubers contained about 20% (w/w) dry matter, irrespective of size and localization in the jar fermentor.

Introduction

Techniques for the *in vitro* propagation of potato (*Solanum tuberosum* L.) tubers are valuable because disease-free seed potatoes can be mass propagated and small tubers are easily stocked, transferred and distributed. Such *in vitro* propagated tubers are known as microtubers and they have sufficient potential for direct application in field cultivation despite their small size [1-4]. Numerous researchers have been studying the factors affecting *in vitro* tuberization[1,5-9].

Labor cost, which is the major obstacle for the industrial production of disease-free plants using *in vitro* culture techniques, can be reduced by a large scale culture system[10]. With regard to the rapid propagation of potato microtubers, Estrada *et al.* (1986) first reported a culture method using a liquid shake culture[11]. They used a two-step culture method; shoots were previously multiplied (step 1) and then tubers were induced on the shoots by changing the culture conditions (step 2). Their report strongly suggested the possibility of large scale culture. Subsequently, Akita and Takayama (1988) reported for the first time the mass propagation of potato microtubers in a jar fermentor using a two-step culture method similar to that of Estrada *et al.*[12]. The culture conditions should be changed in each step for the mass propagation of tubers because direct differentiation of tubers from undifferentiated tissues does not occur. On the other hand, potato shoots can be easily propagated and multiplied from single nodal segments and such multiple shoots

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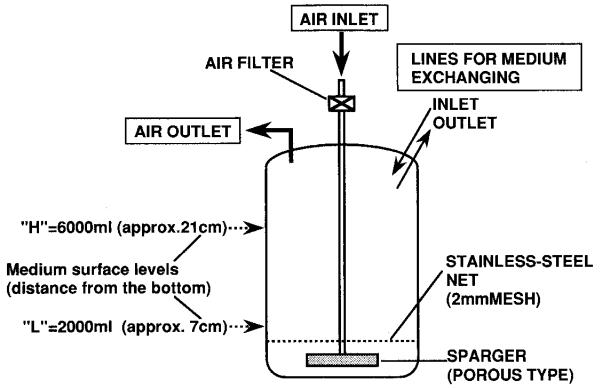

Fig. 1 Diagram of a jar fermentor used for mass propagation of potato tubers.

have numerous buds on which tubers can be induced. Thus one of the key factors in using a two step culture method is the number of buds with high tuberization potential produced.

In the present study, we investigated the conditions for efficient tuberization in potato using jar fermentors and the characteristics of the *in vitro* propagated tubers.

Material and Methods

1. *Plant material*

In vitro stock plants of potato (*Solanum tuberosum* L. cv. Yukishiro) were established from an apical meristem and maintained by serial subculture on MS solid medium.

2. *Culture medium*

Culture medium consisted of MS mineral salts plus organic constituents including thiamine-HCl (0.4 mgl^{-1}), myo-inositol (100 mgl^{-1}), pyridoxine-HCl (0.5 mgl^{-1}), nicotinic acid (0.5 mgl^{-1}), glycine (2.0 mgl^{-1}) and sucrose. Liquid medium containing 30 gl^{-1} sucrose or 90 gl^{-1} was used for shoot multiplication or induction and development of tubers, respectively. The solid medium contained 30 gl^{-1} sucrose and gelrite (2 gl^{-1}). pH was adjusted to 6.2 with 1 N NaOH before autoclaving (20 min, 121°C).

3. *Culture conditions*

3.1. *Stock plant*

Culture vessels for stock plants were 25×125 mm test tubes containing 10 ml of the solid medium. Test tubes were capped with silicon spongy plug (T-24, Shin-etu Silicon Co. Ltd., Tokyo). A single nodal segment was transplanted into a test tube and incubated at 25 °C under continuous irradiance of 2.5 Wm^{-2} from fluorescent lamps (Toshiba FL40 SW (100 V, 40 W)) for 4 weeks. Nodal explants for jar fermentor culture were taken from stock plants having approximately 10 nodes.

3.2. *Jar fermentor culture*

A jar fermentor (airlift type, approx. 20(d)×35(h) cm, vessel volume was 8000 ml) was used (**Fig. 1**). The culture method is illustrated in **Fig. 2**[12].

In step 1, 60 single nodal segments were transplanted into a jar fermentor containing 2000 ml (1-L) or 6000 ml (1-H) of MS medium which contained 30 gl^{-1} sucrose. After 4 weeks culture under continuous light (2.5 Wm^{-2}), numerous elongated shoots were formed. The entire medium was then exchanged with 2000 ml (2-L) or 6000 ml (2-H) of the MS medium containing 90 g l^{-1} sucrose (step 2). The cultures were incubated under continuous darkness at 25 °C for 5 weeks in step 2. Each jar fermentor was aerated 200 ml min^{-1}. for 2000 ml medium or 600 ml min^{-1}. for 6000 ml medium with sterilized air during culture.

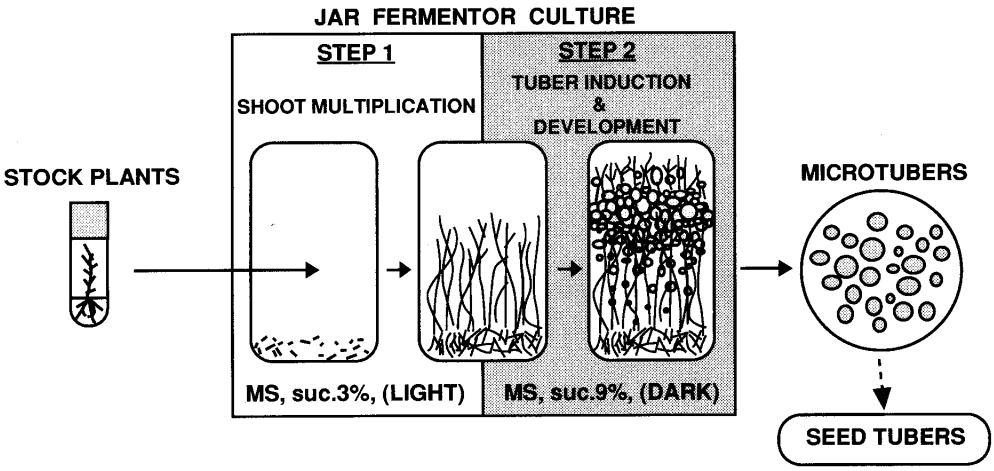

Fig. 2 Diagram of the culture scheme for the mass propagation of potato tubers in a jar fermentor.

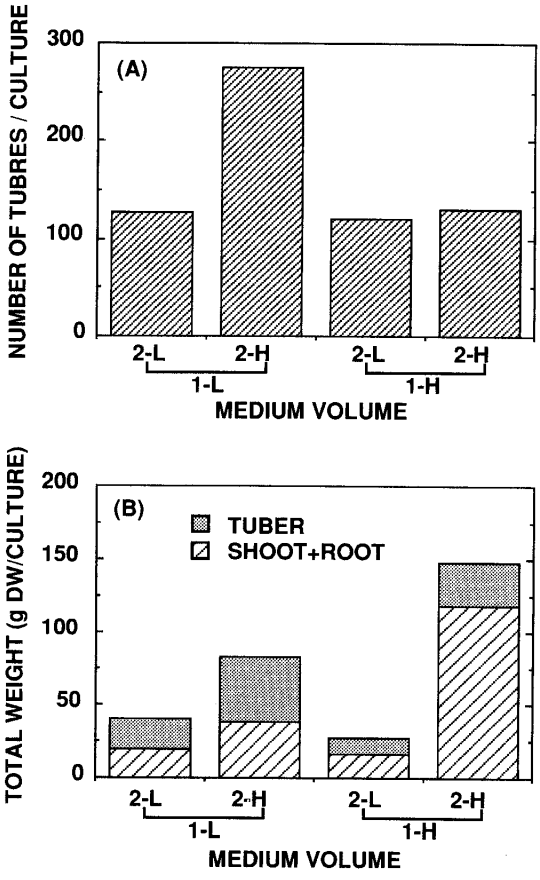

Fig. 3 Effects of medium surface level control on the number and total weight of tubers. Shoots were multiplied in 2000 m*l* (1-L) or 6000 m*l* (1-H) of medium in the step 1, and then tubers were induced in 2000 m*l* (2-L) or 6000 m*l* (2-H) of freshly changed medium in the step 2. Bars indicate means; (n=2).

Results and Discussion

1. *Growth of shoots during the step 1*

Most of the shoots grew in the aerial phase in the jar fermentor when 2000 m*l* of the medium was used (1-L). In contrast, almost all of the shoots were submerged in liquid medium when 6000 m*l* of the medium was used (1-H). The maximum height of the shoots was higher in 1-H than 1-L. Shoots multiplied in the liquid phase were thicker than those in the aerial phase but stems and leaves were slightly swollen in the liquid phase. No tubers were observed at the end of step 1.

2. *Tuber formation during the step 2*

Tubers were induced in step 2 by changing the culture condition. Tuberization was observed in the early period, within the first week of step 2, as we reported using liquid shake culture[13].

Figs. 3-A, 3-B show the number and total weight of tubers which were formed in the jar fermentor. The culture conditions of step 1 significantly affected tuberization in step 2. The number and total weight of tubers decreased when multiplied shoots were continuously submerged in the medium in step 1 (1-H). However, total tuber weight increased in the larger volume of medium in step 2 (2-H). These results suggest that culture conditions for the multiplication of shoots, such as submergence of the shoots in the medium, are important for the mass propagation of potato tubers using a jar fermentor. We also reported that the hormonal conditions in step 1 affected tuberization in step 2[14]. Thus, the culture scheme should be selected based not only on the conditions related to the induction and development of tubers but also on the conditions for the multiplication of shoots.

The total number and weight of tubers can be increased by continuously avoiding submerged conditions in step 1 but the percentage of tuber weight for the total weight did not vary significantly between 1 - L, 2 - L treatment (approx. 50. 1% (w/w)) and 1 - L, 2 - H treatment (approx. 54. 1% (w/w)). If the shoots have sufficient potential to induce tubers, the growth of shoots and the development of tubers are stimulated in a larger volume of medium, because the shoots are supplied with more nutrients. This may be one of the reasons why maximum tuberization was observed with the 1-L, 2-H treatment.

The induction and development of tubers was clearly stimulated just above the medium surface area as compared with the other areas of the jar fermentor (**Fig. 4**). The number of tubers decreased in accordance with the distance from the stimulative area and the tuberization was inhibited in the liquid medium. The tubers weighing more than 2-3 g, rarely above 10 g, could be observed only in this narrow area. Especially, on the shoots which were continuously cultured in liquid medium (1-H, 2-H), the induction and development of tubers was not observed in the liquid phase except for the area just below the medium surface (**Fig. 5**).

This inhibition of tuber development might also cause significant variation in weight (**Fig. 6, Fig. 7**), because the development of tubers was stimulated only in the narrow area even if the buds throughout in the jar fermentor had the potential for having tubers induced as 1-L (**Fig. 4**). Up to now, it is not clear why tuberization was inhibited in liquid medium. In the future, it will be necessary to further investigate the physiological effects of liquid medium on tuberization and to establish culture methods to avoid such inhibitory effects.

3. *Characteristics of propagated tubers*

The weights of tubers varied significantly as shown in **Fig. 7**. The largest tuber was above 7 g (FW) but most of the tubers were below 0. 2 g (FW). Each tuber contained about the same percentage of dry matter irrespective of size. The dry matter content was not reduced in the tubers which were grown under completely submerged conditions as shown in **Fig. 8**. The dry matter content

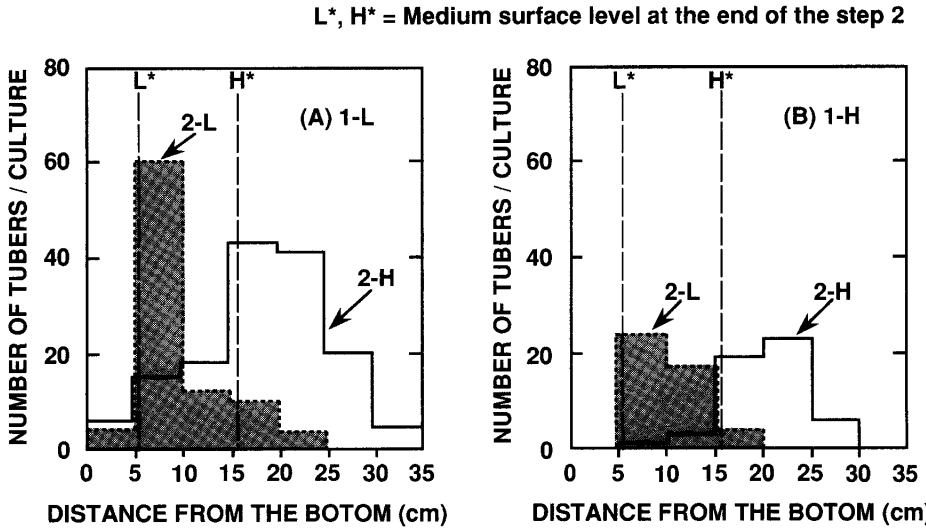

Fig. 4 Effect of medium surface level control on vertical distribution of tubers in the jar fermentor.
Every symbol represents the same as shown in **Fig. 3**. The medium surface level at the end of the step 2 (L=2000 m*l*, H=6000 m*l*) are shown by broken lines. The number of tubers in a vertical range of every 5 cm was counted (n=2).

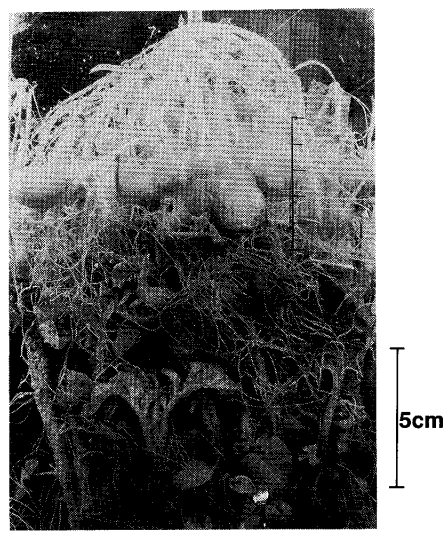

Fig. 5 Tubers in a 1-H, 2-H jar fermentor after the medium was discarded.
Arrow indicates the medium surface level.

(about 20% (w/w)) of tuber is almost the same as that of field grown tubers. Thus it is thought that these tubers can be directly transplanted to field conditions without any acclimatization. In the future, it will be necessary to investigate the field performance of tubers propagated in jar fermentors.

Acknowledgements

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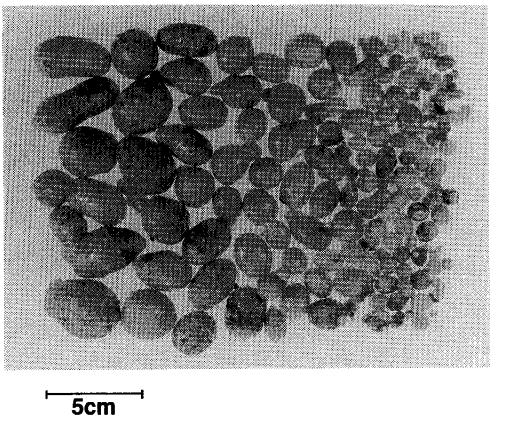

Fig. 6 Tubers propagated in a jar fermentor.
Tubers propagated in 1-H, 2-H jar fermentors are shown.

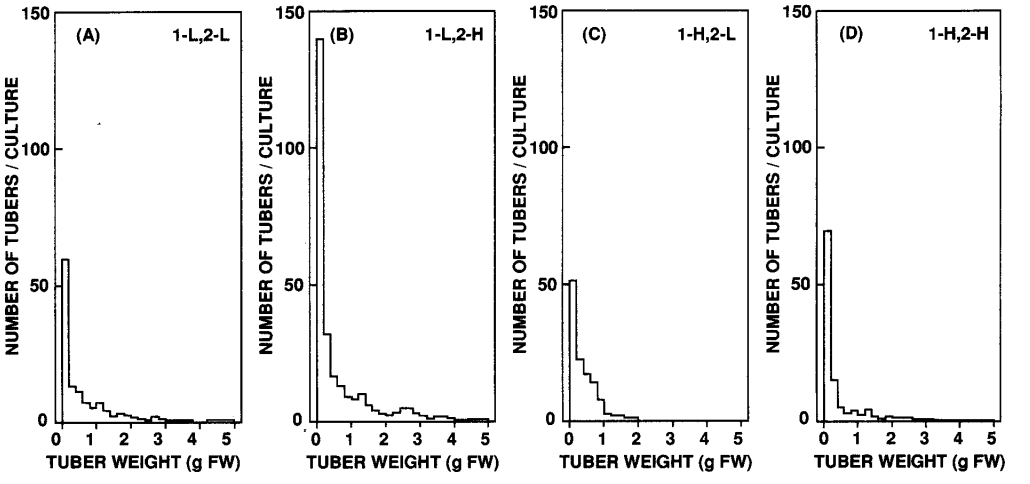

Fig. 7 Effect of medium surface level control on weight distribution of tubers.
Tubers weighing less than 5 g (FW) are shown. Every symbol represents the same as shown in **Fig. 3**. Bars indicate mean number of tubers at every 0.2 g (FW) ; (n=2).

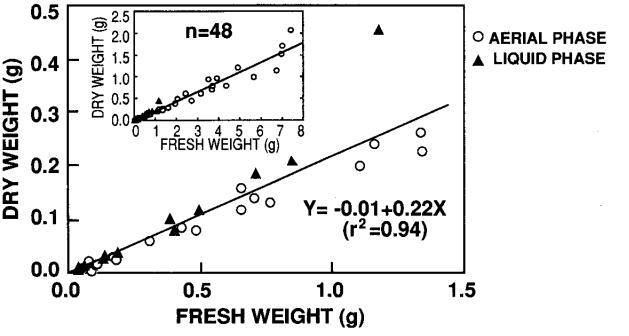

Fig. 8 Relationship between dry weight and fresh weight of tubers.
Tubers derived from 1-L, 2-H jar fermentors were randomly selected. Closed triangles represent the tubers which were developed under continuous submerged condition in the medium. Dry weights of tubers were measured after drying at 80°C for 1 day.

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《和文要約》

ジャーファーメンターを用いたジャガイモ塊茎大量培養に対する培地液面位置調節効果

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** 東海大学開発工学部

植物体を予め増殖させたのちに培養条件を変更してジャガイモ塊茎を誘導する2段階培養法を，小型ジャーファーメンターによる培養に応用した．液体培地中で増殖させた植物体上には塊茎がほとんど誘導されず，培地液面位置を調節することで塊茎形成数を著しく増大させることができた．塊茎の肥大は液体培地表面付近に著しく偏っておこり，このことによって，塊茎重に大きなばらつきを生じた．一方，形成された塊茎の乾物率は，形成場所や大きさに係わらずほぼ一定であった．