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SEM Observation of Regenerating Shoots on *Asparagus officinalis* L. Stems Cultured in vitro

Yasuko KANEKO* and Hisashi MATSUSHIMA

Department of Regulation Biology, Faculty of Science, Saitama University, Urawa, Saitama 338, Japan

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Extensive investigations on shoot induction in *Asparagus officinalis* L. tissue cultures were reviewed 10 years ago.[1] However, there have been few reports on fine structural studies on the asparagus shoot formation. A new method for direct SEM-viewing of fresh plant specimens by LT-SEM[2–4] was applied to the fine structural study of shoot formation on asparagus stems cultured in vitro.

Young *Asparagus officinalis* L. stems were sterilized in 0.5% NaClO for 10 min. The 2–3 mm stem sections were cultured on Murashige and Skoog nutrient agar medium[5] with 0.01 ppm indole-3-acetic acid and 0.1 ppm benzylaminopurine. The cultures were maintained at 27 ± 2°C under continuous fluorescent lamp (Toshiba FL40SD/NL) of 7,000 lux intensity at the level of cultures. They were daily examined under a Hitachi S-430 scanning electron microscope equipped with a Hitachi cryo-system. The fresh cultures were put directly onto the specimen holders and pre-evacuated in the cryo-system for 10 sec. They were then set on a cryo-stage cooled to -15°C in advance. The specimens were examined and photographed within 10 min at an accelerating voltage of 10 kV.

Observations using this new method are shown in **Figs. 1–11.** Shoots were induced to form on the young asparagus stem cultures (**Fig. 1**). The stomata and the neighboring regions on the stem segments continued rising in the first week (**Figs. 3–6**). Nascent shoot apices appeared upon the rising regions in the 2nd week (**Figs. 7 and 8**) and developed into shoots (**Figs. 9–11**).

Three dimensional information on plant materials by scanning electron microscope (SEM) is of great importance to studies on plant morphology and morphogenesis. However, ordinary specimen preparation for SEM viewing is still complicated, and therefore, takes much time. The advantage of the new SEM method introduced in this study is that it is free from chemical fixation and additional procedures of the ordinary SEM method, which simplifies and expedites examination and photography. Thus, a process of the shoot formation on asparagus stem cultures was revealed by use of this method. However, the fine structure of the cells involved in the shoot formation has not been made clear yet. Recently, the authors succeeded in TEM observation of the same specimens examined under the LT-SEM in advance. There-

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* Present address: Department of Botany, University of Wisconsin-Madison, 132 Birge Hall, 430 Lincoln Drive, Madison, WI 53706, U.S.A.

Abbreviations: SEM (scanning electron microscope); LT-SEM (low-temperature SEM); TEM (transmission electron microscope).

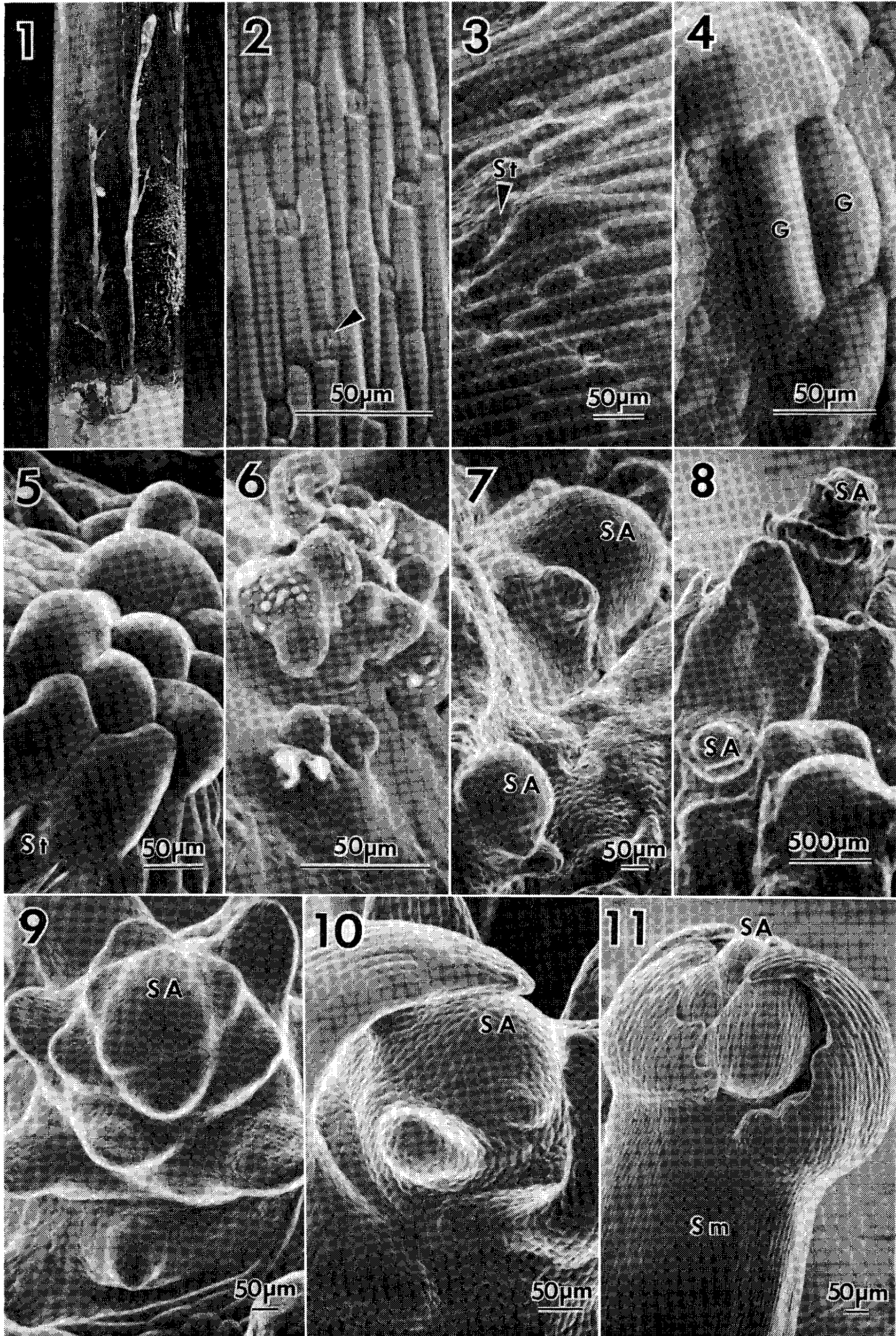

Fig. 1. Shoots induced to form on an asparagus stem cultured for 7 weeks. Two of them are well developed.
Fig. 2. SEM-image of the stem surface just before cultivation. Arrow head: guard cell.
Figs. 3–6. Rising stomata and the neighboring regions in the 1st week. St: stomata, G: guard cell.
Figs. 7 and 8. Nascent shoot apices upon the rising regions in the 2nd week. SA: shoot apex.
Figs. 9–11. Developing shoots after a few weeks. SA: shoot apex, Sm: stem.

fore, this method holds promise for further fine structural studies on the cells underlying the swelling guard cells and the neighboring regions.

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《和文要約》

アスパラガス再生シュートの走査型電子顕微鏡による観察

金子康子*・松島　久
埼玉大学理学部生体制御学教室
*(現)　ウィスコンシン大学植物学教室

アスパラガス (*Asparagus officinalis* L.) の若い茎をインドール酢酸 0.01 ppm とベンジルアデニン 0.1 ppm を含む Murashige & Skoog の寒天培地で培養した結果，shoot が形成された．培養直後から，日を追って，低温走査電子顕微鏡で観察を続けた結果，培養1週間で，培地に近い位置のいくつかの気孔の周辺部の細胞群が大きくなり，全体として盛り上り，そこから shoot が現われることが観察された．

低温走査電子顕微鏡は，植物の生試料を直接観察することが可能であり，化学固定やその他の複雑な処理を必要とせず，簡単で，速やかに観察結果が得られるので，培養系の形態形成の観察に有効であることが示された．