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Dormancy in Garlic Shoot Apices for *in vitro* Culture

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Dormancy, expressed in apices of the garlic plant, *Allium sativum* L. cv. Katei harvested in May, 1984 and 1985, was studied in *in vitro* culture. The bulbs were kept at room temperature, except for a period from August 22 to the end of September, during which they were kept at 20℃, and then were transferred to a refrigerator at 3℃. Apex culture was done from February to March, 1985, using bulbs harvested in 1984. From May 1985 until January 1986, those harvested in May 1985 were used. About 20% of apices cultured immediately after harvest in May sprouted by the end of August, whereas 45-80% sprouted from those cultured from September to February. The period required for sprouting in the cultures started in July and early September was 16.8 and 6.9 days, respectively. Sprouting gradually increased from those cultured in October. Growth of plantlets was retarded when apices were planted from May to the end of August, but was normal when planted after mid-September.

Although the garlic plant is a diploid, it is sterile under natural conditions due to abortion of pollen grains before tetrad formation thus it is vegetatively propagated with bulbs. Therefore, most garlic plants are known to be infected with viruses which cause the reduction of their productivity. It is necessary to produce virus free plants to increase bulb size and yield.[1,2] Virus-free plants of garlic have been obtained by culturing shoot apices of 0.2-0.3 mm on MS medium containing 0.01 $mg \cdot l^{-1}$ NAA and BA.[3] Since one garlic shoot apex usually produces only one plant, callus culture for proliferation of plants has been studied by many researchers.[4-7] However, because of karyological abnormalities that appear among callus cells,[8] shoot bud is thought to be more suitable for the proliferation of virus free plants with normal karyotype.[1,9] For the efficient propagation of garlic plants by shoot apex culture, it is desirable to clarify the difference in growth of apices depending on the seasons which affect the dormancy of apices.[10]

The present paper reports the dormancy in apices studied by using *in vitro* culture during a whole year.

Materials and Methods

Bulbs of garlic plant 'Katei' were harvested in Kagawa prefecture on May, 1984 and 1985 and kept at room temperature under hanging condition in mesh bags except for a period from 22 August to the end of September, and then transferred to a refrigerator at 3℃ under dark condition until used for culture. Dissection of apices from the bulbs harvested in 1984 was made four times from February to the end of March, 1985. From the bulbs harvested in 1985, culture of shoot apices was made 12 times in either 15- or 30- day intervals from the end of May to January. Bulbs were halved transversally after sterilization with 70% ethanol for 1 min and then sodium hypochlorite (available chlorine 1%) for 15 min. Apices of 0.2-0.3 mm in length were dissected by stripping leaf primordia from basal halves under microscope. Then each apex was planted on 10 ml of the medium in a test tube. The basal medium consisted of Murashige and Skoog inorganic salts,[11] 3% sucrose, 0.8% agar, 2.0

mg/*l* *myo*-inositol, 0.5 mg/*l* nicotinic acid and pyridoxine·HCl, and 0.1 mg/*l* thiamine·HCl. Phytohormones, 0.01 mg/*l* NAA and BA, were added. Maintenance of explants was carried out by transferring onto fresh medium with the same ingredients at 60-day intervals. Cultures were placed in a cabinet controlled at 25℃ under 16 hr daylength of 1,500 lx incandescent lamps. The number of leaves was indicated with the mean number per explant sprouted. The longest length of leaves and roots was measured with the mean lengths of the longest leaf and root in each explant sprouted, respectively. The leaf length was measured until 60 days after planting when leaves began to show yellowing. Percentages of sprouting and rooting were counted within 2 months after planting apices.

Results

Eighty percent of apices that were planted from mid-February to early March, 1985, sprouted (**Fig. 1**) and showed vigorous growth with 2–4 leaves having the longest leaf length of 6–7 cm (**Fig. 2**). Fifty percent of the shoots formed a single root (**Fig. 2**). Although the sprouting rate decreased in the explants inoculated at mid-March (**Fig. 1**), the growth rate of the sprouted explants was as good as those planted earlier (**Fig. 2**). On the other hand, sprouting rate was as low as 20% in the culture started from May to mid-August except for the apices planted in the mid-July (**Fig. 1**). Growth rate of the explants was also low, having 1–2 leaves per explant and showing only 2 cm in the longest leaf (**Fig. 2**). The apices excised in September 1985 showed considerably high percentages of sprouting and rooting after only five days of culture (**Fig. 1**). The percentage of sprouting was as high as 85% and that of rooting was 75%. Shoots thus obtained showed vigorous growth with 2 leaves per explant and the longest leaves measured 2 cm after 60 days.

Forty-five to sixty-five percent of apices cultured during the period from early October to early February sprouted soon and showed vigorous growth with 1.7–2.5 leaves, the longest leaf being 4–

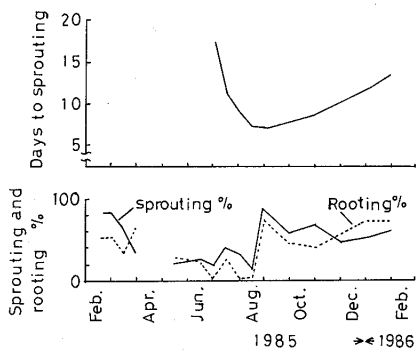

Fig. 1. Sprouting and rooting behavior of apices planted at 15- or 30-day intervals during a whole year.

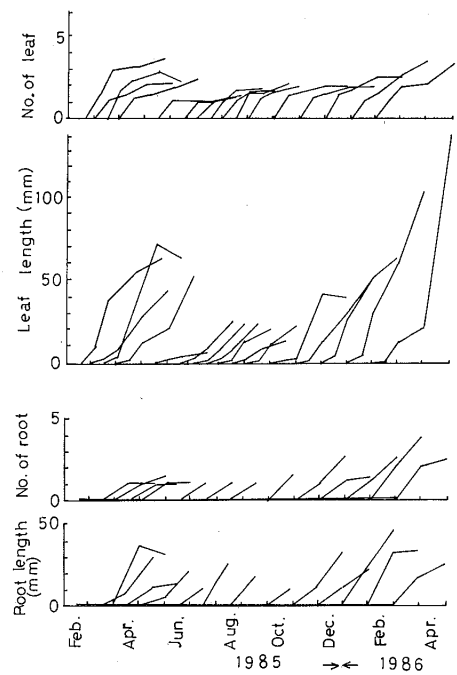

Fig. 2. Top and root growth of apices planted at 15- or 30-day intervals during a whole year. Twenty apices were planted for each treatment.

5 cm. About fifty percent of shoots rooted and the mean number of root per plantlet was 1.3–2.0.

The days required for apex sprouting within 30 days after planting clearly decreased with the time of initiation of culture; 16.8 days in culture started on early July and 6.9 days in that of early September (**Fig. 1**); however, gradual increase in the number of days required was seen in the culture from October and returned again to 13.7 days in the culture of early February.

Discussion

Dormancy in garlic plants starts after yellowing of leaves and the true dormancy that was referred to by Vegis[12] continues for about 2 weeks after harvest.[10] Then it gradually decreases[13] and terminates in early September under normal condition.[10] In the present study, 20% of apices were found to sprout even during the true dormancy period, and incomplete dormancy may be induced by the dissection of apices from bulbs. Inhibition of sprouting in June, July and August might be due to an imposed dormancy induced by high summer temperature in which growth is impossible as a consequence of lack of the necessary external factors in the environment.[12] However, the time required for sprouting from planting decreased gradually and the percentage of sprouting increased rapidly from early September. Gradual increase of the period for sprouting after October is not explained by the dormancy in apices.

It is desirable to use the terminated dormant state of apices after September or apices in which dormancy has been overcome by some artificial treatments, such as temperature treatment[10] or phytohormone application such as gibberellin or thiourea. After dormancy has been suppressed, material for apex culture should be stored in a refrigerator until use, because they may be infected with fungi under storage condition at room temperature in the summer season. For the successful establishment of plants in the field, it is recommended to initiate the apex culture during winter (January and February),[3] because plants grow within the first two months of culture and bulbs are then formed within the following two subcultures for each two months. Bulbs are easily acclimatized and planted in the field in September or October.

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≪和文要約≫

ニンニクの *in vitro* 培養での茎頂の休眠

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1984年，1985年のそれぞれ5月に収穫されたニンニク *Allium sativum* L. の，茎頂の休眠について *in vitro* 培養での影響を調べた．収穫した球は室温におき，8月22日から9月末までは 20℃，その後 3℃ の冷蔵庫に貯蔵した．茎頂培養は1984年産の球は1985年2月から3月まで，1985年産の球は収穫直後から翌年1月まで供試した．培養は15または30日ごとに行い，茎頂の萠芽率，生長量，植え付けから萠芽までの日数を調べた．

5月から8月までに培養した茎頂は20%が萠芽し，9月以降は45から80%の萠芽率となった．植え付け日から萠芽までの日数は，7月1日に植え付けた茎頂は16.8日なのに9月1日に植え付けた茎頂では6.9日と最短となり，10月1日に植え付けた茎頂からはまた徐々に長く15日に近くなった．外植体の生長は，5月から8月までは伸長不良であったが，9月以降は正常になった．ニンニクの休眠は，9月には完全に破れると思われた．