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CONDITIONS OF ROOTING FROM SHOOT APICES FOR MASS PROPAGATION IN ASPARAGUS*

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Introduction

In the cultivation of asparagus (*Asparagus officinalis* L.), when an excellent plant has been found and the plants genetically identical with it are required on a large scale, seed propagation cannot be used because asparagus is a dioecious plant, while vegetative propagation by tissue culture is useful. In other words, efficient mass propagation of a specified plant is possible by means of apex culture using the apices of the lateral shoots of spear tops or the shoots obtained by shoot-apex culture as starting materials, from which shoots and roots are formed *in vitro*.

In this case, however, two types of roots are formed : one is a white and vigorous root, the other is a transprarent and less active root. The latter is easily formed but has no function as a root ; accordingly, the conditions necessary to raise the rate of the formation of white-rooted plants were investigated in this study.

Materials and Methods

The asparagus spears used for *in vitro* culture were taken from the plants grown in the field of Experiment Farms, Faculty of Agriculture, Hokkaido University. The top of a spear was cut off to a length of 3-5 cm, dipped in 70 per cent ethanol for a few seconds, rinsed with tap water, subsequently immeresed in a 10 per cent aqueous solution of sodium hypochlorite (active chlorine 1 per cent, added with a surfactant : Tween 20) for 15-20 minutes for surface sterilization and rinsed with sterilized water. Lateral-shoot apices including a growing point each were cut out of the spear top under an anatomy microscope and used as

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culture material. With regard to environmental conditions in the course of culture in every experiment, the temperature was held at 25 °C and fluorescent lamps were used for lighting of which intensity was set at 4,000 lx and lighting duration was 16 hours a day.

The survey on rooting was made as follows : the plant which formed both white roots and transparent roots was regarded as a white-rooted plant, and in this case the number and the lenghth of roots were surveyed on white roots alone. To calculate the mean root length, indication values shown in TABLE 1 were used as a scale of root lengths for the convenience of calculation.

Table 1. Indication values of root lengths

Root length (RL)	Indication value
0 <RL<1cm*	0.5
1cm≦RL<3cm	2
3cm≦RL<5cm	4
5cm≦RL	6

* In Experiment V., root lengths less than 5mm were represented as 0.25, and those equal to or more than 5mm and less than 1cm were represented as 0.75.

Results and Discussion

Experiment I. Effect of ancymidol

Since it is reported that ancymidol which acts as a GA-synthesis inhibitor, promotes the formation of white roots simillar to storage roots in the culture of shoot apices and stem nodes of asparagus[1,2], and experiment was made to confirm adequate concentrations in application.

Shoot tips were cut off to a length of 1-3 mm from the spears of 'Mary Washigton 500 W' and cultured on four kinds of media which contained the Murashige and Skoog (abbreviated to MS hereafter) basal medium, 0.1 mg/l NAA, 0.1 mg/l kinetin, 0.1 M sucrose and 2 g/l gellum-gum and were added with none (control), 0.1 mg/l, 0.3 mg/l or 1.0 mg/l ancymidol corresponding to the kind of media. The pH value was adjusted to 5.8.

Untill after 30 days from the start of culture, rooting was not observed, while after 70 days of culture, transparent roots were formed, and the rooting rates were slightly higher on the media added with 0.1 mg/l or 0.3 mg/l ancymidol. However, all of the root lengths were less than 1 cm and white roots were not formed (TABLE 2). On the other hand, CHIN[1] reported that 100 per cent rooting from shoot nodes was observed on a medium added with ancymidol after culture for four weeks, and both shoots and roots grew vigorously. The reason why results similar to that of the above report was not obtained in the present experiment, is considered to be that the concentration of ancymidol might be inadequate, and furthermore, the material used was not nodes but tips 1-3 mm long. In addition, the observation by SATO and others[6] that the rooting rates

Table 2. Effect of ancymidol on rooting from lateral-shoot apices (after 70 days of culture)

Concentration of ancymidol (mg/l)	Number of explants cultured	Number of rooting explants	
		White-rooted explants	Transparent-rooted explants
0	20	0 (0)*	6 (30)*
0.1	20	0 (0)	8 (40)
0.3	20	0 (0)	8 (40)
1.0	20	0 (0)	4 (20)

* Values in parenthese are the rates of rooting (%).

were higher when smaller tips as long as 0.2-0.5 mm were used, may also account for this discrepancy.

Experiment II. Effect of ancymidol in connection with sugars

According to DESJARDIN and others[2], the rates of white-root formation in node culture are higher on media containing sucrose with higher concentrations, when added with ancymidol. Based on this information, an experiment was undertaken to confirm whether the same result would be obtained in apex culture as well.

The preparation of materials and common methods used were the same as in Experiment I. Two kinds of sugars, sucrose and glucose, were used with concentrations of 0.1 M, 0.2 M and 0.3 M, and ancymidol was added with a concentration of 5 μM, not added to control.

The rooting rates were as low as 0-35 per cent in all plots after culture for 30 days as shown in Fig. 1-A, while after culture for 70 days, the rates reached 60-80 per cent levels irrespective of the kind and the concentration of sugars and the addition of ancymidol. However, most of the roots formed were transparent roots and the rate of white-root formation was less than 20 per cent and was lowered as the sugar concentration increased regardless of the kind of sugars and the addition of ancymidol (Fig. 1-B). The average number of roots was 1.0-5.5 per explant as to white-rooted explants, and that of transparent-rooted explants was 4.6-8.5, while the mean root length of white roots was 1.0-4.0, and that of transparent roots was 0.5-0.6 (TABLE 3). In this experiment, the rate of white-root formation was not more than 20 per cent and the effect of sugar concentrations and ancymidol on rooting was hardly recognized, whereas considering that the rooting rate reached 60-80 per cent as a whole after culture for 70 days, the lateral-shoot apex of a spear top is judged to have a rather high rooting potency. Therefore, if white-root formation is possible by any means, a probable increase in the existence rate of rooted explants would be anticipated.

Besides, the result that two groups of explants on the culture mdeia with the same composition (one is in Experiment I., the other in Experiment II.) showed a large difference in rooting rates, suggests the difference in rooting potency among

plants or spears within a cultivar.

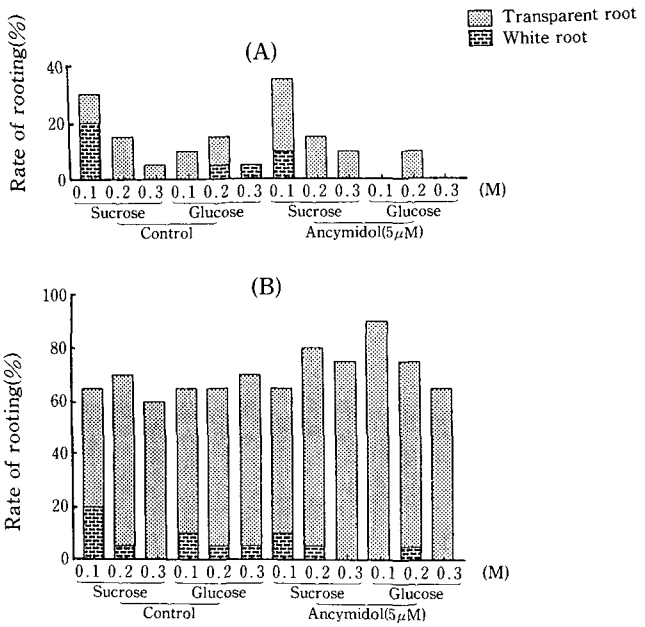

Fig. 1. Effect of sugars and ancymidol on rooting from the lateral-shoot apices of spear tops.

Table 3. Effect of sugars and ancymidol on rooting from lateral-shoot apices (after 70 days of culture)

Conc.of sugars(M)		Conc. of	Number of	Number of	Number of	White-root formation			Transparent-root formation		
Sucrose	Glucose	ancymidol (μM)	explants cultured	survivals	rooting explants	Number of explants	Number of roots per explant	Mean of lengths	Number of explants	Number of roots per explant	Mean of lengths
0.1	0	0	20	15(75)[z]	13(65)[y]	4(20)[x]	5.5	2.7	9(45)[w]	4.8	0.5
0.2	0	0	20	16(80)	14(70)	1(5)	2.0	2.0	13(65)	4.6	0.5
0.3	0	0	20	13(65)	12(60)	0(0)	—	—	12(60)	6.4	0.5
0	0.1	0	20	16(80)	13(65)	2(10)	1.0	1.3	11(55)	5.7	0.5
0	0.2	0	20	13(65)	13(65)	1(5)	1.0	1.0	12(60)	8.5	0.5
0	0.3	0	20	16(80)	14(70)	1(5)	5.0	2.0	13(65)	6.8	0.5
0.1	0	5	20	14(70)	13(65)	2(10)	1.0	2.0	11(55)	8.4	0.5
0.2	0	5	20	16(80)	16(80)	1(5)	1.0	1.0	15(75)	8.0	0.5
0.3	0	5	20	15(75)	15(75)	0(0)	—	—	15(75)	6.7	0.5
0	0.1	5	20	18(90)	16(80)	0(0)	—	—	18(80)	4.8	0.5
0	0.2	5	20	16(80)	15(75)	1(5)	3.0	4.0	14(70)	8.1	0.5
0	0.3	5	20	13(65)	13(65)	0(0)	—	—	13(65)	7.4	0.5

Values in parenthese are the rates of

z: survivals (survivals/all explants×100).

y: rooting (explants with roots/all×100).

x: white-rooted explants (explants with white roots/all×100).

w: transparent-rooted explants (explants with transparent roots/all×100).

Experiment III. Difference in rooting among cultivars or strains

In order to clarify whether rooting potency of apices depends on cultivars or strains, an investigation was made on the following 13 cultivars or strains : 'MM1', 'MM2', 'MM3', 'MM5', 'MM7', 'Rhum von Braunscheig', 'NJ322', 'KBF×3-9', 'Goldschatz', 'Limburgia', 'Viking', 'Eden' and 'Zuiyo'.

The medium used was composed of the MS modified basal salt mixture (the concentration of nitrogen resources, NH_4NO_3 and KNO_3, were half of those of the standard medium), 10^{-4}M adenine, 10^{-6} M NAA, 10^{-6} M BA, 0.1 M sucrose and 2 g/l gellum-gum. Varietal difference in rooting was recognized ; the rooting rates of 'MM3', 'MM5', 'Limburgia', 'Viking' and 'Eden' were 80 per cent or more, while those of 'MM1', 'MM2', 'Rhum von Braunschweig' and 'NJ322' were 10-30 per cent, and 'KBF×3-9' did not form any roots. The roots formed were mostly white roots (Fig. 2). As judged from the comparison of the result of this experiment with that of another experiment, higher rates of white-root formation in this experiment may be explained by the fact that the concentrations of nitrogen resources in the medium was half those of the standard, and adenine was added.

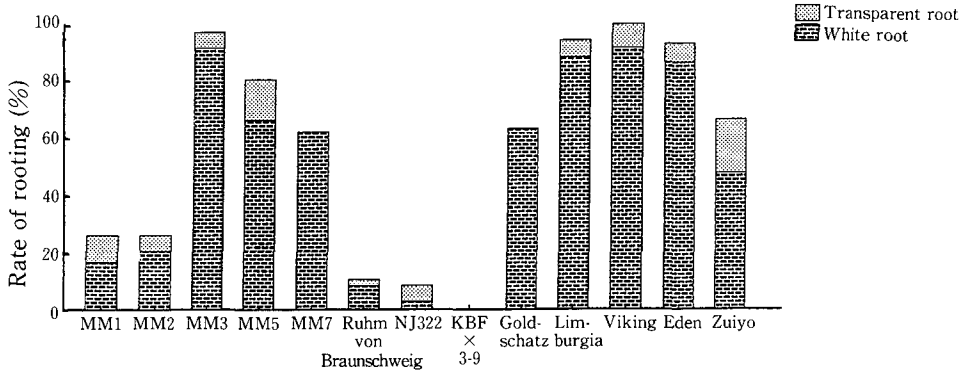

Fig. 2. Difference in rooting among cultivars or strains in the culture of the lateral-shoot apices of spear tops.
The cultural medium used in this experiment contained the MS modified medium (the concentration of nitrogen source is half of the standard), 1 μM NAA, 0.1 mM adenine, 0.1 M sucrose and 2 g/l gellum-gum, and pH was adjusted to 5.8.
The survey was made after 60 days of culture.

Experiment IV. Effect of desiccation treatment to materials

Spear tops of 'Mary Washington 500W' 3-5 cm in length were sterilized and subsequently received desiccation treatment in the desiccators in which relative humidity was maintained with saturated solutions of four kinds of chemicals at 90, 50, 30 or 10 per cent respectively. The period of treatment was 24, 72 or 120 hours with respect to each humidity level.

Lateral-shoot apices about 1 mm long cut out of treated spear tops were cultured on a medium containing the MS basal medium, 0.1 mg/l NAA, 0.1 mg/l kinetin, 0.1 M sucrose and 2 g/l gellum-gum.

After culture for 30 days, white-root formation was observed on all plots of 24-hour treatment and those of 72-hour treatment at 10 or 30 per cent humidity. The rooting rates ranged from 6 to 45 per cent. Furthermore, after culture for 60 days the rate of white-root formation in those plots reached 12-65 per cent. Although transparent-root formation was seen in allmost plots of 24-hour treatment and 72-hour treatment, the rate was as low as 5-15 per cent. The distinct difference in the average number of roots between white-rooted explants and transparent-rooted explants was not recognized, while the mean root lengths of the former was 1.7-3.4 contrary to 0.5, that of the latter (TABLE 4). The result indicated that desiccation treatment was successful for white-root formation from the lateral-shoot apices of spear tops, and the treatment for 24-72 hours at 10-30 per cent humidity was probably adequate. In the case where the treatment is applied to spear tops, however, the treatment condition may be not uniform depending on the thickness of the spears. Therefore, some improvement of treatment methods, such as a treatment of excised shoot apices, is expected.

Table 4. Effect of desiccation treatment to spear tips on rooting (after 60 days of culture)

Duration of treatment (hour)	Relative humidity (%)	Number of explants cultured	Number of survivals	Number of rooting explants	White-root formation			Transparent-root formation		
					Number of explants	Number of roots per explant	Mean of lengths	Number of explants	Number of roots per explant	Mean of lengths
24	10	20	15(75)[z]	10(50)[y]	7(35)[x]	3.0	2.6	3(15)[w]	3.3	0.5
	30	20	20(100)	16(80)	13(65)	2.5	2.0	3(15)	2.3	0.5
	50	17	17(100)	3(18)	2(12)	1.5	1.7	1(6)	1.0	0.5
	90	11	8(73)	3(27)	2(19)	4.5	1.7	1(9)	1.0	0.5
72	10	20	20(100)	10(50)	8(40)	4.8	3.4	2(10)	2.5	0.5
	30	20	5(25)	5(25)	4(20)	3.5	2.0	1(5)	5.0	0.5
120	10	20	0	—	—	—	—	—	—	—
	30	20	0	—	—	—	—	—	—	—

Values in parenthese are the same as in Table 3.

Experiment V. Effect of a high concentration of IBA and transplanting to a medium containing no growth regulators

Since FUJIME[4] et al. reported that rooting is promoted when transplanted to a medium containing no growth regulators after preculture on a medium containing auxins with a high concentration and the result of experiments by TOBISE[7] suggested the same possibility, an experiment was carried out to explore the possibility of elevating rooting rates by transplanting to a medium lacking in auxins after preculture on a medium containing a high concentration of an auxin

(IBA) for a short period. Shoot apices 0.5-1 mm long were cut out of 'Mary Washington 500 W' spears and cultured on a medium containing the MS modified mixture, 0.1 M sucrose and 10 mg/l or 100 mg/l IBA for 12, 24, 48, 96 or 192 hours. After that, they were transplanted to a medium without auxins. Check explants (control) were cultured on an auxinless medium from the beginning and represented as an 0-hour plot. After two, three and four weeks of culture, the number of rooting explants, the number of roots and root lengths were surveyed. The rooting rate of explants precultured on a medium added with 10 mg/l IBA was higher than the control irrespective of hours of preculture.

After culture for four weeks, rooting rates reached 85 per cent or more on all plots except control. This result indicates that IBA addition to a preculture medium is effective for rooting. A rooting rate of 90 per cent was obtained after culture for two weeks by adding 100 mg/l IBA in case of 12-hour preculture,

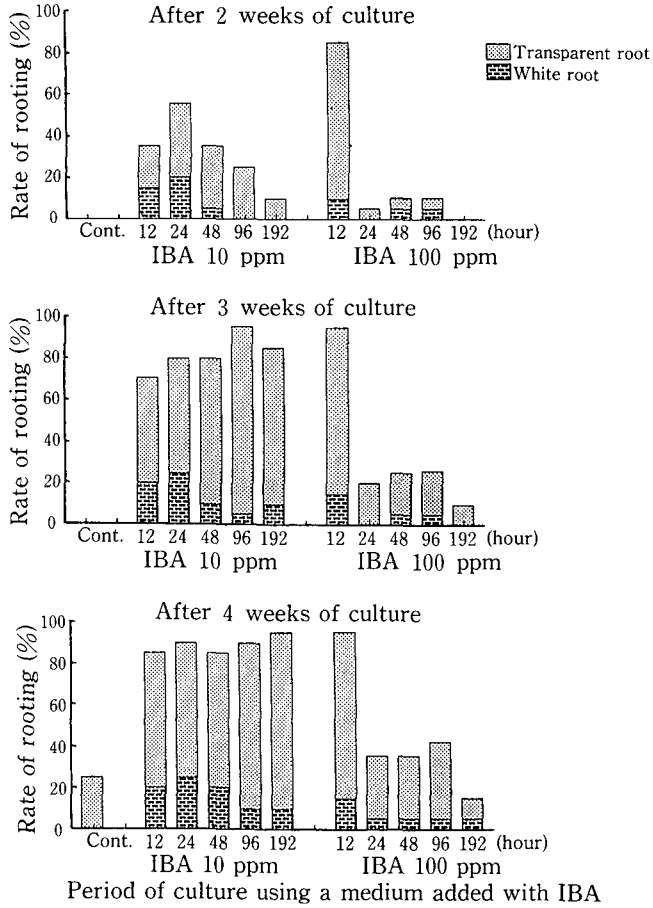

Fig. 3. Effect of the period of preculture using a medium added with IBA on rooting from the lateral-shoot apices of spear tops.

whereas as to the preculture of 24 hours or more, existence rates were lower, and the rooting rates were as low as 10-42 per cent likewise. White-root formation was observed on all plots except control after culture for four weeks, and the rooting rate on the medium added with 100 mg/l IBA was 30 per cent, the highest value in all plots (Fig. 3). In the course of preculture, however, rooting was not seen on any plots.

No difference in the average number of roots was recognized between white-rooted explants and transparent-rooted explants, and there was a tendency that as preculture period was extended, the average number of transparent roots increased and their average length decreased. The average length of white roots was 0.5-3.3, while that of transparent roots was 0.3-0.9 after culture for four weeks (TABLE 5). Auxins are generally known as rooting promoters, and rooting promotion with IBA was also confirmed in this experiment. Since the explants precultured on a medium added with 10 mg/l IBA showed high rates of shoot and root formation, the treatment with this concentration of IBA for eight days in the course of preculture was found to be not harmful to explants. However, the concentration of 100 mg/l is considered to exceed an adequate level. On the other hand, it is reported that the rooting of fruit-tree explants was promoted by immersing them in a solution containing 100 mg/l IBA for 20 minutes[5]. In addition, in the present experiment extremely high rates of shoot and root formation were observed when precultured on the medium with the above concentration of IBA for 12 hours. These facts suggest that the application of such a high concentration of IBA is probably useful to shorten the period

Table. 5 Effect of preculture period on a medium added with IBA at a high concentration on the rooting from lateral-shoot apices (after four weeks of culture)

Conditions of culture on IBA-added media		Number of explants cultured	Number of survivals	Number of rooting explants	White-root formation			Transparent-root formation		
Conc. of IBA (mg/l)	Period (hour)				Number of explants	Number of roots per explant	Mean of lengths	Number of explants	Number of roots per explant	Mean of lengths
0	0	20	20(100)[z]	5(25)[y]	0(0)[x]	—	—	5(25)[w]	1.4	0.9
10	12	20	20(100)	17(85	4(20)	2.5	1.8	13(65)	2.3	0.8
10	24	20	20(100)	18(90	5(25)	1.8	3.3	13(65)	3.2	0.8
10	48	20	20(100)	17(85	4(20)	2.8	1.0	13(65)	4.5	0.8
10	96	20	20(100)	18(90	2(10)	3.5	0.8	16(80)	5.1	0.6
10	192	20	20(100)	19(95	2(10)	1.5	0.8	17(85)	5.1	0.5
100	12	20	20(100)	19(95	3(15)	3.3	1.7	16(80)	4.8	0.9
100	24	20	7(35)	7(35	1(5)	4.0	0.8	6(30)	2.2	0.8
100	48	20	7(35)	7(35	1(5)	7.0	2.0	6(30)	3.8	0.6
100	96	19	7(37)	8(42	1(5)	3.0	1.2	7(37)	3.3	0.6
100	192	20	4(20)	3(15	1(5)	2.0	1.1	2(10)	7.0	0.4

Values in parenthese are the same as in Table 3.

for obtaining regenerated plants. For this purpose, the transfromation of transparent roots into white roots by means of the desiccation treatment proposed by URAGAMI and NAGAI[8] or in any way is necessary.

Experiment VI. Effect of the concentration of the MS basal salt mixture and the addition of adenine

The result of Experiment III. indicated the possibility that the concentration of the MS basal salt mixture and adenine were related to white-root formation. Furthermore, calcium is generally recognized as an element for root growth. Considering from this point of view, therefore, an experiment was made in order to verify the relation of these factors to rooting. Shoot apices 0.5–1 mm long cut out of 'Mary Washington 500 W' spear tops were cultured on 12 kinds of media shown in TABLE 6 and the rooting rate was surveyed after two, three and four weeks from the start of culture. The result obtained is exhibited in Fig. 4, which indicates that rooting started earlier on the media numbered as 7 through 12 than on those of 1 through 6. The former on which 72–80 per cent of explants formed roots, most of which were white roots, contained the MS basal medium with half the standard concentration. On the adenine-added media, numbered as 4, 5, 6, 10, 11 and 12, more transparent roots were formed than on the adenine-free media, 1, 2, 3, 7, 8 and 9. The effect of the ratio of calcium concentration to potasium concentration in media on rooting was not clearly recognized.

Table 6. Composition of culture media used in the nutrition experiment

Plot number	Concentration of the MS basal salts with minimal organics*	Conc. of nitrate salts		Adenine sulfate (mg/l)
		KNO_3 (mg/l)	$Ca(NO_3)\cdot 4H_2O$ (mg/l)	
1	Standard	1900	0	0
2		950	1100	
3		0	2200	
4		1900	0	40
5		950	1100	
6		0	2200	
7	1/2	950	0	0
8		475	550	
9		0	100	
10		950	0	40
11		475	550	
12		0	1100	

* Concentration of the mixture from which KNO_3 was removed.

Common components of media used in this experiment included the MS basal salts with minimal organics, 0.1mg/l NAA, 0.1mg/l kinetin, 0.1M sucrose and 2g/l gellun-gum. The value of pH was adjusted to 5.8.

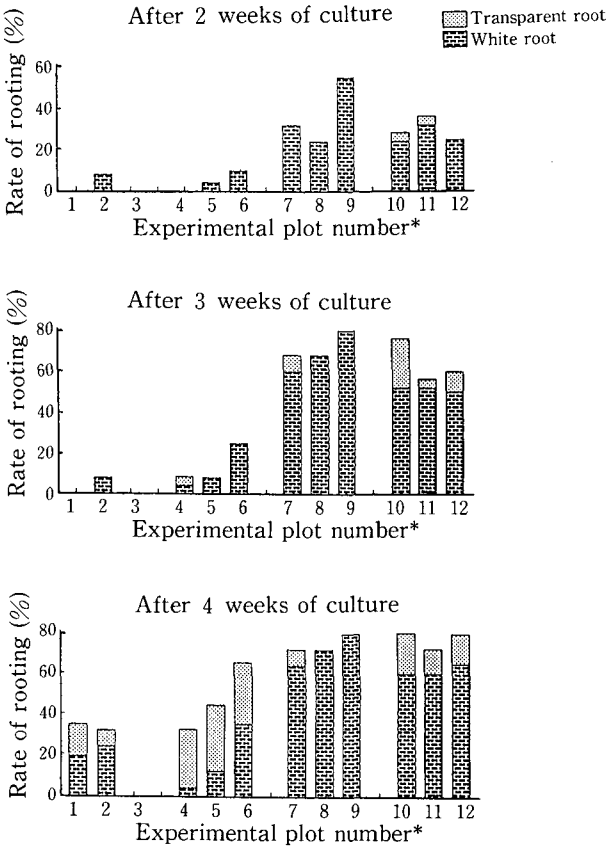

Fig. 4. Effect of medium compositions on rooting from the lateral-shoot apices of spear tops.

* Refer to Table 6.

In the case where the medium with the standard concentration of the MS basal salt medium was used, shoot formation exceeded rooting, whereas rooting exceeded on the medium whith half the standard concentration and furthermore almost all roots formed were white roots. These results suggest that explants are probably in a state of nutritional deficiency under the condition of half the standard concentration and rooting becomes superior to shoot formation as a result of a reaction adaptive to the situation. The same phenomenon is reported on the rooting from the explants of fruit trees as well[3,5]. Based on the fact that desiccation treatment promotes white-root formation from the lateral-shoot apices of spear tops and the roots formed do not change into transparent roots after transplanting, it is postulated that water content in spear tissues is connected with white-root formation at the early step of rooting, in other words, white-root formation is suppressed under such a condition that the tissues used contain sufficient water. Besides, white-root formation is presumably promoted

in a state of nutritional deficiency induced by the reduction of medium concentration.

It is concluded that the stress imposed on the tissues by desiccation preceding apex culture, and/or nutritional deficiency in the course of culture, induces an adaptative reaction resulting in rooting and white-root formation ; therefore, these treatments can be possibly used for the promotion of rooting and white-root formation.

Summary

To establish a method of mass propagation in asparagus, the conditions necessary for white-root formation *in vitro* were investigated, and the following results were obtained :

1. An addition of ancymidol to the medium at a concentration of 0.1, 0.3 or 1.0 mg/l was not effective for white-root formation in contrast to the result described in the report on shoot-node culture.
2. The rate of white-root formation was lowered as the sugar concentration increased regardless of the type of sugars used and the addition of ancymidol.
3. The difference in rooting among cultivars including some strains was recognized. The rooting rates of five out of 13 cultivars investigated were more than 80 per cent, while those of four ranged from 10 to 30 per cent, and one did not form any roots.
4. The desiccation treatment to a spear top before culture was effective for white-root formation, and the treatment for 24-72 hours under 10-30 per cent relative humidity was adequate.
5. Rooting was better when precultured on a medium added with IBA at a concentration of 10 mg/l for 12-192 hours or at 100 mg/l for 12 hours than when not precultured. However, the existence rate was low when precultured at 100 mg/l for more than 12 hours.
6. White-root formation was promoted on a medium with a concentration of half of the concentration of the MS basal salt mixture.

These results indicate that rooting rate is increased by preculturing on a medium containing IBA, and white-root formation from the shoot apices of spear tops can be possibly promoted by imposing stress arising from the treatments of desiccation before culture and/or nutritional deficiency applied to the apex tissue in the course of the culture.

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