Cytogenetic Studies in *Avena*, X The Artificial Culture of Albino Sand Oats¹⁾

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There has often been found genic or chromosomal lethality in higher plants and animals. In some cases their death is directly caused by visible abnormal developments of organs, but in others by invisible abnormalities, probably due to physiological disturbances. Little is known, however, regarding the detailed mechanism of lethality, especially in the latter cases.

The albinism in plants is lethal, which occurs due to the deficiency of chlorophyll. In recent years von Wettstein (1958, 1960) and Maruyama (1961) reported by their electron microscopic observations on albino leaves that the chlorophyll deficiency is associated with the abnormal development of the submicroscopic structure of the chloroplast. Albino or chlorophyll mutants are useful materials not only for analyzing the genetic system of chlorophyll formation but also for studying the chemistry or physiology of chlorophyll.

The authors have obtained many kinds of chlorophyll mutants, including albino plants, of oats through spontaneous or induced mutations and are engaged in the genetic investigation of these chlorophyll mutants. For the genetic and physiological studies of the albino mutants it is desirable to grow them to maturity under artificial conditions in order not to let them die in young seedling stages.

Spoehr (1942) successfully brought albino maize plants to maturity by feeding them with artificial nutrient solution containing sucrose, the solution being supplied through the cut ends of leaves. Wallace and Habermann (1959) succeeded in growing albino *Helianthus* by means of grafting them on green plants.

In the present study it was attempted to cultivate albino plants of diploid oats until the flowering stage by supplying mineral and organic nutrients through the roots.

Materials and Methods

Albino and green segregates from seven different strains of diploid oats ($2n=14$) were used in this study.

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Strain I is a spontaneous mutant with 14 chromosomes which was found in the progeny of trisomics ($2n+a'_4$, 58-602) derived from the triploid hybrid between *Avena barbata* ($2n=28$) and *A. strigosa* ($2n=14$). The albino is due to a single gene mutation (Nishiyama 1934, 1957).

Strains II, III, IV, V, VI and VII (59-A-2556, 60-A-3074, -3079, -3084 and 61-A-23040) are albino mutants which were obtained by X-irradiating early ripening mutants derived from the triploid *Avena* hybrid as mentioned above (Nishiyama 1934, 1941, 1957; Nishiyama and Amano, unpublished). Heterozygotes of these strains segregated for greens and albinos in an approximately 3:1 ratio in the self-pollinated progenies.

The basic culture medium consisted of White's mineral solution (White 1943) and 0.9% agar as shown in Table 1. Glucose or sucrose and powdered brewer's

Table 1. Composition of the White's mineral solution used for the cultivation

Inorganic salts	Milligrams per 1000 ml	Inorganic salts	Milligrams per 1000 ml
MgSO ₄	360.0	Fe ₂ (SO ₄) ₃	2.5
Ca(NO ₃) ₂	200.0	MnSO ₄	4.5
Na ₂ SO ₄	200.0	ZnSO ₄	1.5
KNO ₃	80.0	H ₃ BO ₃	1.5
KCl	65.0	KI	0.75
NaH ₂ PO ₄ ·H ₂ O	16.5		

yeast, "Ebios", were added to the basal medium in order to enrich the culture medium. After autoclaving, the pH of the culture medium was adjusted to 7.0 with 0.1 N KOH solution. About 10 ml of the medium were dispensed per test tube and again autoclaved. The seeds were sterilized in a saturated solution of calcium hypochloride for 40 minutes, followed by washing several times in sterilized distilled water. Every one of the seeds was put on the agar medium in each test tube and germinated at a room temperature. When the coleoptile grew about 1 cm long, the test tube was placed in an incubator or temperature-controlled room in the light. It was already shown that the form and growth rate of plants were strikingly affected by light (Spoehr 1942; Sugino 1957; Mizobuchi who tried an artificial culture of albino sand oats in 1959, unpublished).

Results

Culture experiment I

A. Artificial culture under different temperature and light intensity

Preliminary experiments were first made to know what conditions of temperature and light would be favorable for the artificial culture of albino plants. Seeds from the albino heterozygotes of strain I were used in this experiment. The culture

media used consisted of White's mineral nutrient solution with 0.9% agar, 0.3 M sucrose, and 0.5 g 'Ebios' per liter. Every seed in each test tube was germinated at the room temperature, until the coleoptiles grew about 1 cm. Then the seedlings were grouped into three lots, each of which consisted of both the albino and normal sibs. Both the first and the second lot, which were kept in the incubators at 9~18°C and 20~23°C respectively, were illuminated by 500~700 lux light with the daylight fluorescent lamps. The third was kept in the room at 21~31°C and illuminated by 900~1300 lux light with the daylight fluorescent lamps and incandescent lamps. On 40th day after sowing it was examined whether each plant survived or died. Percentage of survivals at 40th day is shown in Table 2. On 80th day after sowing all the plants were taken out of test tubes and measurements were made on plant height, length of the longest root, number of leaves on the primary stem, number of roots per plant, and development of flower-primordia (Table 2). In the above measurements dead plants were also included to know how far they had grown before they died under the given culture conditions. From these results it is found that favorable growth conditions may be different between albino and green plants. For instance, all albino plants died within 80 days after sowing when cultured at 9~18°C under 500~700 luxes of light, while green plants survived under the same conditions.

Albino plants cultured at 20~23°C under 500~700 lux light showed more vigorous growth in both plant height and root length than those cultured under other culture conditions, though the maximum numbers of leaves per primary stem and of roots per plant were obtained at 21~31°C under 900~1300 lux light. Normal plants cultured at 21~31°C under 900~1300 lux light and at 9~18°C under 500~700 lux light grew more vigorously as measured in plant height, root length of the longest root, number of leaves per primary stem and number of roots per plant than albino plants cultured under the same culture conditions. However, at 20~23°C under 500~700 lux light, albino plants showed more vigorous growth than normal plants. These results seem to show the difference in growth response between albino and normal plants.

It should be especially noted that the first leaves of albino plants at the temperatures of 20~23°C and 21~31°C were slightly tinged with a pale green color one week after germination. About 40 days after sowing the pale green parts of albino plants became as green as the color of the standard number J-4 or K-5 in plate 18 in "A dictionary of color" (Maerz and Paul 1950). However, the green color did not appear in any parts of albino plants at 9~18°C.

B. Artificial culture in media with different sugar concentrations

A preliminary experiment was carried out to know the optimum concentrations of sucrose and glucose, and nutritive effects of these two kinds of sugars on the plant growth. Agar media were prepared from the basal medium (listed in Table

Table 2. Growth of albino and green plants of strain I cultured under three different conditions

Culture conditions	No. of plants cultivated	Percentage of survivals*		Plant height (mm)**	Length of the longest root (mm)**	No. of leaves on primary stem**	No. of roots per plant**	No. of plants with flower-primordia
		40 days after sowing	80 days after sowing					
Albino plants								
9~18°C (500 ~700 lux)	20	100.0 (20)	0.0 (0)	72±27	41±40	2.2±0.8	4.9±1.5	0
20~23°C (500 ~700 lux)	12	83.3 (10)	58.3 (7)	133±11	66±28	3.1±1.1	5.7±1.3	0
21~31°C (900 ~1300 lux)	11	100.0 (11)	36.4 (4)	103±21	64±10	3.5±1.8	8.4±2.6	1
Green plants								
9~18°C (500 ~700 lux)	18	100.0 (18)	100.0 (18)	173±32	76±36	4.2±0.9	7.7±1.6	9
20~23°C (500 ~700 lux)	9	100.0 (9)	22.2 (2)	104±11	61±42	2.5±0.7	5.9±1.4	0
21~31°C (900 ~1300 lux)	10	100.0 (10)	90.0 (9)	113±40	87±16	5.9±2.1	8.8±1.8	9

* Figures in parentheses show the actual number of plants survived.

** A part of measurements was made on dead plants.

1) supplemented with 0.5 g Ebios per liter, to which sucrose was added at the concentration of 0.1, 0.2, 0.3 or 0.4 M. The basal medium containing only the same amount of Ebios was used as the control.

Sterilized seeds of strain I were germinated on the culture media in test tubes, and then kept in the incubator adjusted to $21\pm1^{\circ}\text{C}$ and illuminated by 500~700 lux light from daylight fluorescent lamps at the plant level. On 80th day after sowing, the growth of plants was measured as in the previous experiment (Table 3). The results seem to show that the higher the concentration of sugar, the higher was the survival rate in both albino and green plants so far examined, excepting green plants grown in the media with 0.4 M sucrose. In green plants the percentage of survivals was highest with 0.2 M sucrose, decreasing with higher or lower concentrations of sucrose. In the medium deficient for sugar no plant was able to survive longer than 80 days after sowing. In short, the superior growth of both albino and green plants were observed in the culture media with 0.3~0.4 M glucose. The formation of spikelet was not observed in any plants in these cultures, but flower-primordia were formed in some albino and green plants grown in the media containing 0.3~0.4 M glucose, 0.4 M glucose appearing most effective on flower-primordia formation. Glucose seems to be more effective than sucrose, although the effects varied with the concentrations.

Changes in color of albino leaves were also observed in this culture experiment. Albino plants grown in the media with 0.3~0.4 M glucose or sucrose produced white leaves, which gradually become slightly greenish from the tip to the lower

Table 3. Effects of sugar concentrations on growth of cultured albino and normal oat plants of strain I on 80th day after sowing ($21 \pm 1^\circ\text{C}$, 500~700 lux illumination)

Concentration of sugar	No. of plants observed	Percentage of survivals*		Plant height (mm)**	Length of the longest root (mm)**	No. of leaves on primary stem**	No. of roots per plant**	No. of plants with flower-primordia**
		40 days after sowing	80 days after sowing					
Albino plants								
Without sugars	18	0.0 (0)	0.0 (0)	107 ± 5	38 ± 11	1.9 ± 0.5	3.5 ± 0.8	0
Glucose								
0.1 M	19	36.8 (7)	5.3 (1)	118 ± 18	47 ± 11	2.4 ± 0.5	4.1 ± 1.3	0
0.2 M	18	83.3 (15)	33.3 (6)	134 ± 41	74 ± 18	3.3 ± 1.7	5.0 ± 1.6	0
0.3 M	18	94.4 (17)	44.4 (8)	149 ± 33	89 ± 61	4.0 ± 1.5	4.8 ± 2.0	3
0.4 M	13	100.0 (13)	84.6 (11)	141 ± 15	79 ± 40	4.8 ± 2.0	7.1 ± 2.6	7
Sucrose								
0.1 M	13	15.4 (2)	7.7 (10)	117 ± 22	33 ± 12	2.1 ± 0.6	3.3 ± 0.5	0
0.2 M	16	31.3 (5)	12.5 (2)	129 ± 29	51 ± 11	2.2 ± 0.7	3.4 ± 1.2	0
0.3 M	15	80.0 (12)	26.7 (4)	122 ± 34	54 ± 24	2.7 ± 0.8	3.7 ± 0.9	0
0.4 M	6	100.0 (6)	66.7 (4)	94 ± 12	45 ± 1	2.7 ± 0.8	4.3 ± 1.0	0
Green plants								
Without sugars	11	0.0 (0)	0.0 (0)	102 ± 17	36 ± 9	1.7 ± 0.4	3.8 ± 0.8	0
Glucose								
0.1 M	16	37.4 (6)	6.3 (1)	109 ± 11	54 ± 16	1.8 ± 0.6	3.2 ± 0.5	0
0.2 M	15	86.7 (13)	16.7 (4)	105 ± 16	59 ± 22	2.5 ± 1.1	4.5 ± 1.4	0
0.3 M	14	100.0 (14)	64.3 (9)	136 ± 30	83 ± 50	3.9 ± 1.5	5.3 ± 2.3	1
0.4 M	13	92.3 (12)	69.2 (9)	115 ± 31	61 ± 27	3.2 ± 1.4	5.4 ± 1.0	0
Sucrose								
0.1 M	17	35.3 (6)	11.8 (2)	117 ± 17	65 ± 22	2.9 ± 1.0	4.0 ± 1.1	0
0.2 M	13	76.9 (10)	38.5 (5)	109 ± 17	53 ± 19	2.0 ± 0.5	3.7 ± 0.7	0
0.3 M	12	58.3 (7)	33.3 (4)	110 ± 18	47 ± 20	2.6 ± 1.0	4.6 ± 1.2	0
0.4 M	14	92.9 (13)	28.6 (4)	103 ± 15	47 ± 16	2.3 ± 0.8	4.9 ± 1.4	0

* Figures in parentheses show the actual number of plants survived. **A part of measurements was made on dead plants.

part of leaves, and in 40 days old seedlings the green color was comparable with the standard color number J-4 or K-5. On the other hand, albino plants grown in the media with 0.1~0.2 M glucose or sucrose were very poorly colored. It should be noted that albino plants grew usually as good as or sometimes better than green plants under these conditions.

Considering the results obtained so far, the followings would provide excellent conditions for the artificial culture of albino oats: (1) a temperature higher than 20°C, (2) continuous illumination by 900~1300 luxes of light, and (3) basal nutrient medium with 0.4 M glucose.

As albino plants of strain I are able to produce a slight amount of chlorophyll, it is uncertain whether the above culture conditions could be available for albinos of other strains.

Culture experiment II: Comparative culture of albino plants of two different strains

A further culture experiment was made using albino mutants of strain I and X-ray induced albino mutants of strain II by the same method as in the experiment I. Seeds from strains I and II were sown on the media in test tubes. Just after germination, they were placed in an incubator at $25\pm 1^\circ\text{C}$ in the dark for 24 hours. Then each seedling was pushed into the agar medium with the help of a nichrome loop so that the mesocotyl or the lower part about 1 cm long of the coleoptile was immersed in the agar medium. This procedure was taken in an attempt to in-

crease the number of roots which would secondarily promote a better plant growth. After this treatment the plants were grown at $25\pm 1^\circ\text{C}$ and illuminated by 1000~1300 lux light in an incubator during the whole culture period.

Fig. 1. Albino (a) and normal plant (b) of strain II. $\times 0.6$.

Fig. 2. Anthers and pistils produced by albino and normal plants of strain II. a: anthers and pistil of a normal plant. b: those of an albino plant.

In strain I, the first leaves of albino plants gradually became slightly green as observed in the preliminary culture. In albino plants of strain II, on the contrary, the first and second leaves were at first very slightly yellow or green, but they were gradually discolored later. The third and higher order leaves were quite white. Albino and green plants of either strain grew very well, especially better in the plants of strain II. It was remarkable to find heading in albino and green plants of strain II, although each panicle that headed had only one spikelet (Fig. 1). Both the albino and green plants produced normal anthers and pistils (Fig. 2).

Table 4. Comparison of growth between two albino mutant strains, I and II, on 80th day after sowing ($25\pm 1^{\circ}\text{C}$, continuous 1000~1300 lux illumination)

Strains	No. of plants observed	Plant height (mm)	Length of the longest root (mm)	No. of leaves on primary stem	No. of roots per plant	No. of plants headed
I Albino	12	119 ± 30	46 ± 16	3.4 ± 1.1	6.4 ± 1.3	0
I Normal	13	132 ± 26	61 ± 12	3.8 ± 1.9	7.4 ± 2.8	0
II Albino	22	138 ± 31	69 ± 9	4.5 ± 1.1	6.6 ± 1.7	5
II Normal	21	160 ± 24	56 ± 15	4.4 ± 1.6	7.8 ± 1.8	5

Culture experiment III: Effects of "Ebios" on growth of albino and green plants

In an effort to obtain more satisfactory growth of albino seedlings, Ebios was added to the culture media in various concentrations. Albino seedlings of strain II grown in these media showed characteristic changes in leaf color. That is, the first and second leaves were slightly pale green or pale yellow, tips of the first leaves being more or less green, whereas leaves were almost white. In a concentration higher than 2g Ebios per liter inhibited the elongation of roots which turned to brown in color. Plants, albino or green, grown in the media with 10 g Ebios per liter produced no root longer than 0.5 cm and died at two- or three-leaf stages. Some of plants grown under these culture conditions shot panicles 45 to 60 days after sowing as shown in Table 5. Albino plants headed most frequently when 0.5 g Ebios per liter was added to the basal medium. In green plants, however, the frequency of heading was highest in the nutrient media without Ebios and even a small amount of Ebios decreased the frequency. Usually only one spikelet was formed on each panicle shot from the primary stem of the albino plant. In these spikelets the first florets developed well but the second ones were poorly developed. A spikelet having two well developed florets was found only in an albino plant grown in the medium with 0.5 g Ebios per liter. Panicles with two spikelets were observed in two albino plants cultured in the media containing 0.5 g per liter or no Ebios, although the second florets developed very poorly.

Table 5. Effects of Ebios on heading of albino and normal oat plants of strain II under culture conditions ($25 \pm 1^\circ\text{C}$, 1000~1300 lux illumination)

Amounts of Ebios (g/l)	Albino plants			Normal plants		
	No. of plants observed	No. of heading plants	Percentage of heading plants	No. of plants observed	No. of heading plants	Percentage of heading plants
0	11	3	27.3	9	8	88.9
0.5	11	7	63.6	12	8	66.7
2	14	4	28.6	11	8	72.7
5	14	4	28.6	11	2	18.2
10	8	0	0	15	0	0

In most cases of this culture experiment green plants produced panicles more frequently than albino plants, these panicles had usually two spikelets, each possessing two well developed florets (Fig. 1). These florets had pistils and stamens as shown in Fig. 2. Some of the anthers were fixed in a mixture of 3 parts 95% ethanol and 1 part glacial acetic acid within five days after heading. Pollen grains smeared on a slide were stained with propionocarmine. By this method pollen grains at various developmental stages were microscopically observed in the anthers of both albino and green plants. Normal metaphase figures in the first pollen mitosis were occasionally found and seven chromosomes were clearly counted. Their mitotic behaviors were apparently normal. A metaphase figure of a pollen mitosis is illustrated in Fig. 3e. The frequencies of pollen grains showing different develop-

Fig. 3. Pollen grains produced by an albino plant. a: pollen grain containing only one nucleus. b: pollen grain containing one vegetative and one generative nucleus. c: pollen grain containing one vegetative nucleus and two sperm nuclei. The sperm nuclei are abnormal in shape. d: pollen grain having normally developed nuclei. e: seven chromosomes at the metaphase of pollen mitosis in an albino plant.

ment stages are shown in Table 6 and their sketches in Fig. 3.

Culture experiment IV: Comparative culture of albino plants of five different strains

For the comparison of growth response between different albino strains, both albino and green plants from five strains (III, IV, V, VI and VII) of different

Table 6. Frequencies of pollen grains at various development stages in the anthers of three albino oat plants

Plants of strain II	Pollen development*			
	a	b	c	d
Albino 1	28	67	455	290
Albino 2	23	20	138	325
Albino 3	6	4	29	380

* Refer to Fig. 3 for a, b, c and d.

genetic origins were tested under artificial culture conditions. Seeds were germinated in test tubes at room temperature under diffused sun light. When the coleoptiles grew about 1 cm long they were moved into an incubator adjusted to $25 \pm 1^\circ\text{C}$ and continuously illuminated by daylight fluorescent lamps. The luminosity at

Figs. 4~6. 4: albino plants of strain III. None of the albino plants in this strain headed during the culture period. $\times 0.6$. 5: an albino plant of strain IV having four abnormal leaves (?) like an empty glume in shape. $\times 1.25$. 6: an albino plant of strain V, which has two spikelets. $\times 0.6$.

the plant level varied from 1000 to 1300 luxes. The results from growth measurements made on 80th day after sowing and of pollen examinations made one week after heading are shown in Tables 7 and 8, respectively. Plants grown under these conditions showed a very uniform growth, although some characteristic differences were observed among different strains. The formation of panicle was observed in

Table 7. Comparison of growth among five albino mutant strains on 80th day under the culture condition in test tubes
(25±1°C, 1000~1300 lux illumination)

Strains	No. of plants observed	Plant height (mm)	Length of the longest root (mm)	No. of leaves per stem*	No. of roots per plant	No. of stems per plant	No. of plants headed	No. of plants with one spikelet	No. of plants with two spikelets	No. of spikelets with two florets
III Albino	19	28±27	77±29	4.3±2.1	8.5±2.5	2.6±1.3	0(0.0)	0(0.0)	0(0.0)	0
III Normal	18	138±18	66±22	3.8±1.7	9.3±2.5	1.2±0.4	6(33.3)	5(27.8)	1(5.6)	4
IV Albino	16	97±11	72±20	4.6±1.3	9.1±1.9	3.4±0.8	9(56.3)	9(56.3)	0(0.0)	0
IV Normal	16	131±14	79±30	4.5±1.8	10.1±2.8	2.2±1.1	7(43.8)	6(37.1)	1(6.3)	6
V Albino	12	103±16	64±27	5.0±1.5	10.5±2.3	2.0±0.7	7(58.3)	6(50.0)	1(8.3)	5
V Normal	10	137±32	71±30	5.0±0.2	13.0±4.0	1.7±0.8	6(60.0)	6(60.0)	0(0.0)	5
VI Albino	6	122±10	13±1.5	5.8±1.6	14.8±1.8	1.0±0.0	0(0.0)	0(0.0)	0(0.0)	0
VI Normal	10	140±9	62±14	5.2±0.4	12.1±2.1	1.6±0.5	6(60.0)	3(30.0)	3(30.0)	3
VII Albino	7	120±17	59±10	3.9±0.6	9.3±2.7	1.4±0.5	1(14.3)	1(14.3)	0(0.0)	0
VII Normal	11	148±24	76±36	4.5±0.7	9.7±2.0	1.3±0.4	6(54.5)	5(45.5)	1(9.1)	2

* The number of leaves was counted in stems with the highest number of leaves.

all the five strains, except in albino plants of strains III and VI which did not show particularly inferior vegetative growth. It seems that panicles were more easily formed in strains IV and V than in others. In these experiments panicles were produced by tillers but not by primary stem. The albino plants of strains III, IV

Table 8. Frequencies of pollen grains in various developmental stages in the anthers of albino and normal plants of strains IV and V

Strains	Pollen development*			
	a	b	c	d
Strain IV albino—1	111	105	90	201
" albino—2	120	67	46	161
" normal-1	137	65	50	225
Strain V albino—1	98	123	91	209
" albino—2	61	50	38	121
" normal-1	102	38	32	287

* Refer to Fig. 3 for a, b, c and d.

and V are shown in Figs. 4, 5 and 6, respectively. As shown in Table 7, albino plants of strains III and IV were distinctly shorter in plant height but produced more tillers than those of other strains. Of nine albino plants with panicles in strain IV, three had besides normal leaves some deformed leaves like an empty glume in size and shape in which blade and sheath were indistinguishable (Fig. 5). The frequencies of pollen grains in different stages as shown in Fig. 3 were given in both normal and albino plants of strains IV and V (Table 8). The leaf color of albino plants of strain III remained white during the culture period. The first and second leaves of albino plants of strains IV and V were slightly yellow or pale green, but others were usually colorless. In albino plants of strain VI, the basal part of the first and second leaf-blades were tinged with pale green but the upper parts were white or slightly yellow. Other leaves did not well develop and varied in color from pale green to pale yellow or white. It was quite unexpected that these albino plants which produced a small amount of chlorophyll were unable to form panicles. All albino plants of strain VII were white even in the first leaves. They showed a good development of vegetative parts but heading was observed in only one plant.

Discussion

It has been reported by Wallace and Habermann (1959) that albino plants of *Helianthus* grafted on the green seedlings were grown to maturity and set seeds by selfing or crossing with the pollen of green plants. In the species in which grafting

is difficult, however, a number of attempts have been made to grow albino plants by means of artificial cultivation, *e.g.* in maize by Knudson and Lindstrom (1919) and in *Artemisia vulgaris* by Rischkow and Bulanowa (1931). A successful cultivation of albino maize plants to maturity was made by Spoehr (1942) who supplied the plants with nutrient solution through the leaves.

In the present study albino mutants of diploid oats were successfully grown to heading in various artificial culture media and some of them formed normal pollen grains. Since photosynthesis did not play a major role in the growth of these albino plants due to the deficiency of chlorophyll they had to depend mainly on sugars taken up by roots. In the culture experiment using strain I, the mineral nutrient media with 0.4 M glucose was more effective for the vegetative growth and the formation of flower-primordia than any other media containing less amount of glucose. Sucrose used as the organic nutrient proved to be less effective for the growth of both albino and green plants than glucose. More satisfactory results were obtained with artificial culture when the nutrient medium was supplemented with 0.5 g Ebios per liter. In summarizing the data mentioned above, the mineral nutrient medium with 0.4 M glucose, 0.5 g Ebios per liter and 0.9% agar was very effective for the efficient culture of albino and green plants of strains I, II, IV, V and VII. Besides, a temperature higher than 20°C and continuous illumination by 900~1300 lux light were required. However, no albino plants of strains III and VI showed heading under these conditions, so that other favorable conditions must be found for them.

It is remarkable that some leaves of albino plants of certain strains became slightly colored with pale green when grown in the media containing a high concentration (0.3~0.4 M) of glucose or sucrose at a temperature higher than 20°C.

Langridge (1955, 1958 a, b) found that some of lethal mutants in *Arabidopsis* required different nutrient or temperature in artificial culture, and that another mutant restored a normal growth even at a sublethal temperature of 23°C if the osmotic pressure of the medium was higher than 2.5 atmosphere. In the present experiment characteristic responses to the culture conditions were often observed in different albino strains. These phenomena may be caused by their different genetic backgrounds although no definite evidence has been obtained yet. The electron microscopic observations on chloroplasts in the albino mutant (the same strain I used in this study) and a pale green mutant (3250) of *Avena* have been conducted by Maruyama (1961). According to him the normal structural development of chloroplast was not observed at all and various degrees of abnormalities were seen in pale green, yellowish or white parts of leaves of albino plants. He suggested that these structural blockings may be associated with the failure of chlorophyll synthesis. From these facts it may be assumed that these albino plants examined here show different responses to aging, temperature or chemical con-

ditions, possibly owing to their different genetic backgrounds.

Summary

Albino plants from seven different strains (I~VII) obtained by spontaneous or X-ray induced mutations in the diploid progenies of triploid hybrids between *Avena barbata* ($2n=28$) and *A. strigosa* ($2n=14$), were successfully grown to maturity in artificial culture media in test-tubes.

Based on the results obtained from several culture experiments the following conditions were determined as most adequate for the plant growth: (1) White's mineral nutrient solution with 0.4 M glucose and 0.5 g brewer's yeast "Ebios" per liter and 0.9% agar, (2) the temperature of $25\pm 1^{\circ}\text{C}$, and (3) the continuous illumination by 1000~1300 lux light at the plant level during the culture period.

Some albino plants produced panicles with usually one or occasionally two spikelets. The florets had normal stamens and pistils. The pollen mitosis took place in the normal manner. Pollen grains with two sperm nuclei and one vegetative nucleus were often observed.

Some leaves of albino plants of strains I, II, IV, V and VI showed characteristic changes in color, being usually tinged with slight green, pale green or pale yellow, but those of other strains, III and VII, remained quite white while they were alive.

By detailed observations it was further found that in albino plants from different strains or, even in the same strains, certain plant organs showed various degrees of growth in response to the culture conditions. These characteristic responses to the culture conditions seem to occur due to different genetic backgrounds in these strains.

Green plants consisted of both heterozygous and homozygous green segregates were cultured as control by the same method as used for albino plants. In most case they grew well and produced panicles more frequently. However no remarkable difference in plant growth including pollen formation was observed between green and albino plants although more or less different responses to the culture conditions could usually be observed.

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