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Genetic and ultrastructural studies on an orange coloured chlorophyll mutant of winter rye (*Secale cereale* L.)

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Abstract

The feature of the orange colour of young seedlings, which appeared in inbred generation S_2 of a short straw form of winter rye cultivated in Jeleniec is described. Genetic analysis revealed that this feature is determined by one recessive gene, marked with the symbol cl_2 (chlorophyll lethal), because the plants with a double dose of this gene (recessive homozygotes) die four weeks after germination. In contrast to typical chloroplasts in normal plants (green), mesophyll cells of mutants (of orange colour) contain plastids devoid of grana thylakoids. The lamellar system of these plastids is composed only of more or less parallel single thylakoids. Short thylakoids often demonstrate a "staircase" arrangement forming in this way long thylakoids. Moreover, such plastids are characterized by the presence of 1-4 clusters of osmophilic globules. Starch grains were lacking in plastids of mutated plants.

Key words: *rye*, *chlorophyll mutant*, *inheritance*, *plastid structure*

INTRODUCTION

The homozygotyzation of inbred lines of winter rye reveals the lethal recessive genes which in the parental populations are invisible due to the heterozygosity of these lines. Phenotypic changes are visible effects of the operation of these genes. One of the earliest changes occurring in inbred lines of winter rye are chlorophyll mutations (Kubicka et al. 1986).

In the present paper the chlorophyll mutations in the form of orange colour winter rye seedlings are presented and the morphology of these plants as well as the ultrastructure of plastids from leaf mesophyll are described and the way of inheritance of this feature is determined.

MATERIAL AND METHODS

The first orange in colour seedlings were discovered in 1975 in the inbred S_2 generation derived from the form with short straw cultivated in Jeleniec. As these plants died four weeks after germination they can be regained only as segregants in further inbred generations by means of self-pollination of fertile plants of the same population. On the basis of the frequency of segregating generations S_3 and S_4 in relation to non-segregating ones and basing on the frequency of plants of orange colour in relation to normal ones (green) in the segregating populations the possible way of inheritance the studied feature has been assumed and tested with the chi-square test.

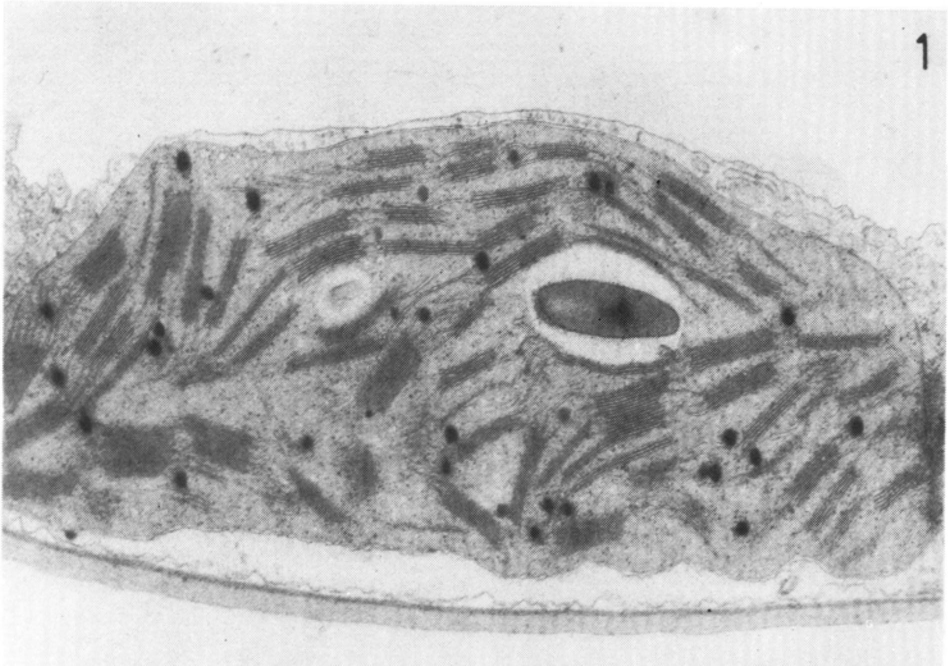
Preliminary analysis of three lethal chlorophyll rye mutants (Kubicka et al. – in prep.) has revealed that plastids in 1 cm long seedlings as well as in 2-week-old leaves exhibit the same ultrastructure. Therefore, for electron microscopy, small pieces (1 mm square) were taken from 2-week-old leaves of normal (green) and mutated (orange) seedlings and prepared as reported previously (Kubicka et al. 1986). Thin sections, cut with a LKB Ultratome III, were stained with uranyl acetate and lead citrate and examined with a Tesla BS-500 electron microscope at 60 kV.

RESULTS

Morphology of plants. The difference in the colour of seedlings was marked when they attained a length of 1 cm. At that point, two types of seedlings were observed: 1) with green and 2) with orange coleoptyles. The growth rate of both types already differed to a considerable degree during their germination. Later, the difference in the growth rate gradually intensified. After 10 days the first orange leaf stopped growing when it was 9 cm long, at which point the green leaves were 23 cm long and still growing attaining a length of 29 cm after a further 3 days.

Similar changes were observed in the growth rate of the length of the next leaf. Green plants produced the second leaf on the fifth day after germination and after 10 days the leaf was much longer than the first one. In orange plants, the second leaf was formed about 3 days later and it attained the length of the first one 3 days later as well. Three weeks after germination when a further two leaves appeared in green plants, no leaves developed in the orange plants at all.

PLATE I



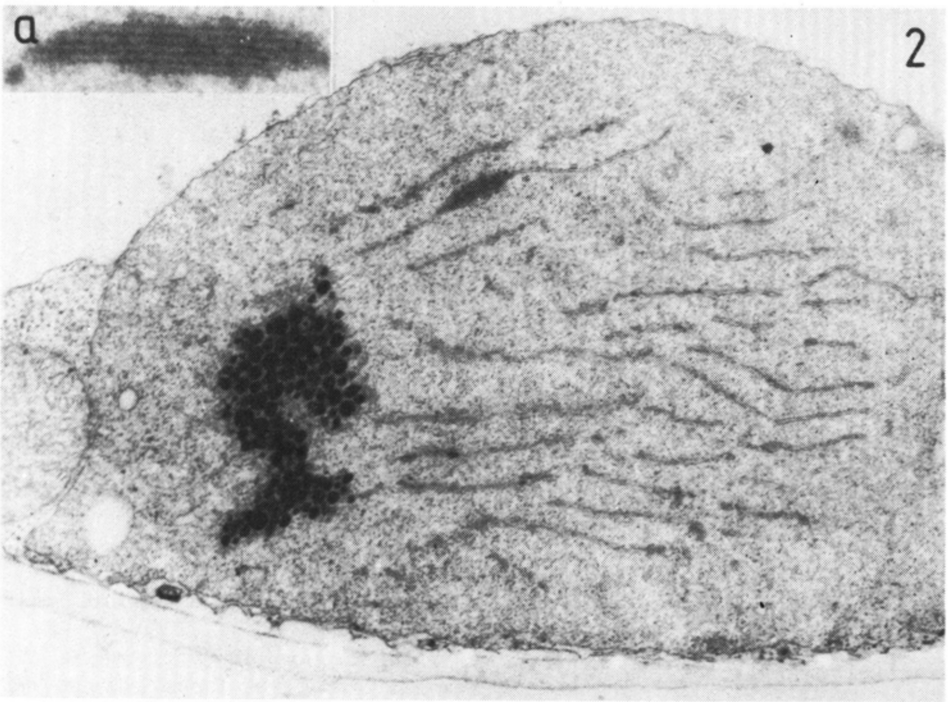
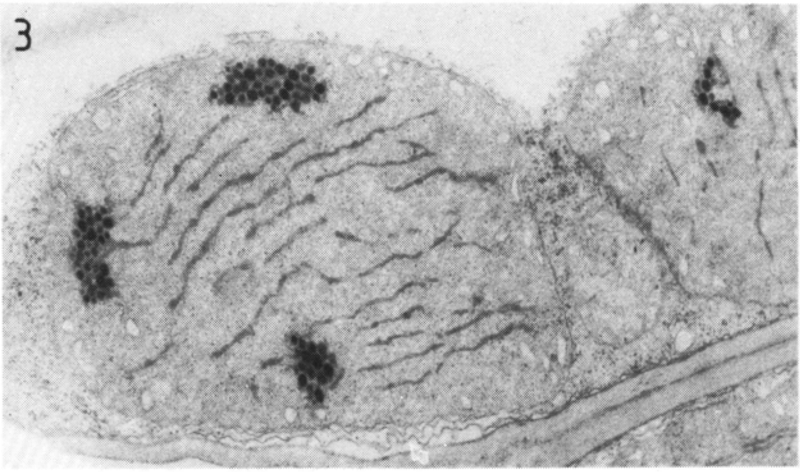


Fig. 1. Mesophyll chloroplast of a control leaf. ×20 000

Fig. 2. Mesophyll plastids of an orange mutant. Thylakoids of various length and an aggregate of osmophilic globules are visible. ×20 000. Sporadically, a stack of parallel thylakoids appears (Fig. 2a, ×72 000)



Figs. 3-5. Mesophyll plastids of an orange mutant

Fig. 3 Numerous, variously sized vesicles with single membranes are present in the matrix, located near plastid membrane. ×20 000

Fig. 4. A few osmophilic globules are seen among the group of vesicles. ×20 000

Fig. 5. Plastid showing a DNA area. ×20 000. "Staircase" arrangement of some thylakoids (Fig. 5a, ×72 000)

However, the first two leaves that were once orange lost their colour turning white and died after four days.

Genetic analysis. Orange winter rye seedlings were observed in inbred generations S_3 and S_4. In all, from a total number of 1905 green plants, 662 plants were orange (Table 1). Convergence of the experimental data with the expected ones for the ratio 3:1 is very high ($x^2 = 0.21$) when $p = 0.56$. These data indicate that the feature of orange colour in young seedlings is determined by one recessive gene, marked with the symbol cl_2 (cl – chlorophyll lethal, Kubicka 1986).

Ultrastructural analysis of plastids. Plastids in normal plants (green) were characterized by the presence of typical chloroplasts (Fig. 1). Their inner lamellar system was differentiated into grana thylakoids and the inter-grana thylakoids. The arrangement of grana and stroma thylakoids was rather irregular in the most of the chloroplasts. DNA areas were present. Very often, besides numerous osmophilic globules dispersed in the plastid matrix, single starch were visible.

Table 1

Genetic analysis of the feature of orange colour of young seedlings

Total number of generation S_3 and S_4			
After self-pollination		After crossing of plants from the same generation	
54		28	
Segregating	non-segregating	segregating	non-segregating
37	17	16	12
Chi² for the ratio 2:1 = 0.08 p = 0.90		chi² for the ratio 5:4 = 0.14 p = 0.70	
Total number of plants in segregating generations			
Normal (green)	orange	normal (green)	orange
703	230	1202	392
Chi² for the ratio 3:1 = 0.07 p = 0.90		Chi² for the ratio 3:1 = 0.14 p = 0.70	
Total value of Chi² for the ratio 3:1 when the experimental ratio 1905:622 = 0.21, p = 0.65			

Plastids from orange winter rye seedlings differed significantly from chloroplasts observed in green plants (Figs. 2-5). Their inner lamellar system was very poor. It consisted of several lamellae of various lengths, oriented more or less paralell to the long diameter of the plastids. In many plastids short thylakoids demonstrated a "staircase" arrangement (Fig. 5a) and in this way they formed long thylakoids. Sporadically in plastids of mutated plants the initials of 1-3 grana thylakoids (Fig. 2a) could be seen. As a rule, the plastids contained 1-4 clusters of osmophilic globules (Figs. 2-4). Moreover, numerous, electron transparent vesicles limited with a single membrane were present in the matrix of plastids. They were deposited around the plastid membrane (Figs. 2, 3, 5), or they formed aggregations in the plastid matrix (Fig. 4). Usually a few osmophilic globules were visible in the central part of such an aggregation. Mutated plastids showed starch grain deficiency. DNA areas were present (Fig. 5).

DISCUSSION

Chlorophyll changes are features which occur relatively often in inbred lines of winter rye. These changes are most frequently determined by recessive genes (Fiedorov et al 1971, Kubicka et al. 1986). Genetic analysis of the feature of orange colour of young seedlings detected in the inbred generation S_2 of the winter rye derived from the form with short straw cultivated in Jeleniec has shown that the obtained ratio of splittings amounted to 3:1. The obtained values are very close to the theoretical one for $chi^2 = 0.21$, when $p = 0.65$. These data indicate that the described chlorophyll change of the orange coloured young seedlings is determined by one recessive gene. We have marked it with the symbol cl_2 (in contrast to cl_1 evoking pink colour in the seedlings of winter rye – Kubicka et al. 1986). Observations of the plants from the generations F_1 and F_2 obtained as a result of crossing plants with gene cl_1 with those with gene cl_2 indicate that these genes are non-allelic (Kubicka et al. – unpublished).

As was mentioned in a previous paper (Kubicka et al. 1986) chlorophyll mutations are correlated with plant growth and development. Mutated plants very often cannot attain maturity. We observed such a situation in a chlorophyll mutant characterized by the presence of anthocyanins in cells (Kubicka et al. 1986) as well as in the mutant, orange in colour, which is the object of the present study. Both these winter rye mutations mentioned above are lethal.

The striking feature of the mutant chloroplasts is the poor development of their membrane system. Mutant leaves exhibit a greatly reducet pigment level in thylakoids protein (Sangeetha et al. 1986).

The lack of grana thylakoids accompanied by a parallel system of single lamellae characteristic of the orange rye mutant was also observed in mutants of soybean (Crang and Noble 1974) and corn (Paolillo and Reighard 1968, Millerd et al. 1969). Moreover, a parallel system of single thylakoids was noticed in wheat plastids treated with herbicide SAN-9789 which causes inhibition of carotenoid synthesis (Axelsson et al. 1982). In the opinion of Ryberg et al. (1980), the almost complete absence of carotenoids is accompanied by an almost complete lack of grana, however, the explanation of this relationship remains unclear.

The lack of grana thylakoids in the studied orange mutant was accompanied by the presence of 1-4 aggregates of osmophilic globules. Some authors have proved that carotenoids liberated from thylakoids during leaf senescence are deposited in plastoglobuli (Tevini and Steinmüller 1985). Moreover, electron microscopic studies reveal that the increase in number of plastoglobuli strongly corresponds to a minimum of thylakoid development (cf. Tevini and Steinmüller 1985). It cannot be excluded that osmophilic globules in the orange mutant contain carotenoids responsible for the organe colour of these plant seedlings. The appearance of a high number of osmophilic globules in mutated plastids also corresponds to poor thylakoid development. On the other hand, the globules appearing in plastids are considered to be not only the structures collecting the breakdown products of the photosynthetic apparatus, but also the reservoir of components necessary for the organization of this apparatus (Steinmüller and Tevini 1985, cf. Woźny 1985).

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Badania genetyczne i ultrastrukturalne pomarańczowej barwy mutanta chlorofilowego żyta ozimego (Secale cereale L.)

Streszczenie

Opisano cechę pomarańczowej barwy siewek żyta ozimego, która ujawniła się w pokoleniu wsobnym S_2 pochodzącym z formy krótkosłomej z Jeleńca. Analiza genetyczna wykazała, że cecha ta uwarunkowana jest jednym genem recesywnym oznaczonym symbolem cl_2 (chlorophyll lethal), ponieważ rośliny zawierające ten gen w podwójnej dawce (homozygoty recesywne) po czterech tygodniach od wykiełkowania giną.

W przeciwieństwie do typowych chloroplastów w roślinach normalnych (zielonych) komórki mezofilu mutanta zawierają plastydy pozbawione tylakoidów gran. System lamelarny tych plastydów składa się jedynie z mniej lub bardziej równolegle ułożonych pojedynczych tylakoidów. Często krótkie tylakoidy ułożone są schodkowo, tworząc w ten sposób długie tylakoidy. Cechą charakterystyczną plastydów pomarańczowego mutanta jest obecność 1-4 skupień osmofilnych globul. Nie obserwuje się natomiast w tych plastydach ziaren skrobii.