Lethal chlorophyll change resulting in a light-violet colour in winter rye (*Secale cereale* L.) seedlings

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Abstract

This paper describes a lethal chlorophyll change characterized by light-violet seedlings, obtained after self-fertilization of a dwarf winter rye form from Jeleniec. The manner of inheritance of this mutation has been established. Genetic analysis of green and light-violet plant segregating frequencies in inbred generations S_3 and S_4 has showed that the obtained split ratio approximates the theoretical ratio 3:1. This proves that the feature in question is determined by the recessive gene cl_3 . The gene cl_3 is lethal as it results in the death of the seedlings 4 weeks after germination. In contrast to the membrane system characteristic for the chloroplasts, the mesophyll cells of mutants (light-violet in colour) contained plastids devoid of granal and intergranal thylakoids. Only fragments of lamellae, vesicles or light areas were present in the granular matrix of these plastids.

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

INTRODUCTION

During self-fertilization and selecting of winter rye, recessive genes, undetected in the heterozygotic state, segregate in inbred generations. One of the more frequently segregating features in inbreeding besides male and female sterilities are chlorophyll changes (Kubicki and Kubicka 1981, Kubicka et al. 1986, 1988). These changes are lethal or sublethal and in most cases determined by recessive genes (Kubicka et al. 1986, 1988).

This paper describes another of the lethal chlorophyll changes — the light-violet colour of young seedlings, occurring in an inbred line of the winter rye. The way of inheritance of this change has been determined.

MATERIAL AND METHODS

Young light-violet seedlings (I 10) were found in the inbred generation S_2 obtained from dwarf form from Jeleniec in 1975. Since these plants die 4 weeks after germination, they have been reproduced in further inbred generations by means of self-fertilization and sister-breeding of the fertile plants of the same population. For that reason the genetic analysis of the violet colour was conducted on the basis of the frequency of the segregating generations S_3 and S_4 in relation to the nonsegregating ones as well as on the basis of the ratio of young light-violet seedlings to green ones (control). The reliability of the results has been checked by means of the chi-square test.

The plastid ultrastructure was examined on 1 mm² sections cut off from the middle part of five day old leaves (control material) or coleoptiles (mutants). The material was fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 6.8, for 12 h at 4°C. After washing several times with 0.1 M cacodylate buffer the material was postfixed in 2% OsO₄ in the same buffer for 2 h and dehydrated in increasing concentrations of ethanol, followed by propylene oxide after which it was embedded in Epon.

The fragments of leaves or coleoptiles prepared as described above were sectioned on a LKB ultratome. Ultrathin sections were stained with uranyl acetate and lead citrate according to the Reynolds (1963) procedure and examined in a Tesla BS-500 electron microscope.

RESULTS

The difference in the colour of seedlings became evident when they reached the length of 1 cm. Seedlings with green or light-violet coleoptiles were observed. The growth rates of both seedlings types varied markedly and the difference increased with time. The light-violet seedlings did not produce leaves, remaining in the state of weak, thin coleoptiles and ceased to grow after two weeks. Three weeks after germination the primarily light-violet seedlings with an average length of 5.3 cm turned white and died within the next several days. In contrast, 3 weeks after germination the green seedlings reached a length of 27 cm and had 3 leaves.

The light-violet seedlings were observed in the inbred generations S_3 and S_4 . Among 1489 plants, 1123 were green and 366 light-violet. The obtained results approximate the theoretical ratio 3:1 (for $\chi^2 = 0.13$, where $p = 0.70$). These data indicate that the feature of the light-violet colour of young seedlings is determined by a recessive gene. Since the feature of the light-violet colour of young seedlings is one of the chlorophyll changes described in inbred generations of winter rye (Kubicka et al. 1986, 1988), the gene responsible for its appearance has been designated by the symbol *cl* for

chlorophyll lethal, whereas the digit "3" indicates the order in which the gene was detected.

The ultrastructure of plastids from the leaves of green seedlings (control) and coleoptiles of light-violet mutants was entirely different. Plastids in green plants had a structure typical for chloroplasts. They were characterized by the presence of granal and intergranal thylakoids (Fig. 1). In such plastids besides starch grains, several plastoglobuli dispersed in matrix appeared very often.

In the mutated plants, the plastids were almost completely deprived of the internal lamellar system (Figs. 2-4). In these plastids only fragments of lamellae (Fig. 2) or a few lamellae packed with electron dense material (Figs. 3-4) could be seen in the granular matrix. Sporadically, besides single vesicles, electron transparent areas of various dimensions were visible in the plastids matrix (Fig. 4).

DISCUSSION

The chlorophyll change manifested as an light-violet colour of young seedlings similarly as the pink or orange ones described earlier by Kubicka et al. (1986, 1988) — was selected after self-fertilization of the dwarf form of winter rye from Jeleniec.

The conducted genetical analysis of the light-violet colour of young seedlings has shown that this feature is determined by one recessive gene, cl_3 . This gene is lethal resulting in the deaths of young seedlings 4 weeks after germination. Our earlier observations (Kubicka et al. 1986, 1988) as well as the studies by Fiedorov et al. (1971) indicate that the action and the way of inheritance of other genes determining the occurrence of chlorophyll mutations in rye are the same as in the case of the light-violet seedlings.

Since it has been well documented that chlorophyll mutations are characterized by disturbances in plant growth and development (Kubicka et al. 1986) it is not surprising that the light-violet rye seedlings survive only four weeks. The lack of thylakoids in these plastids allow us to suppose that the gene cl_3 responsible for this feature acts at an early stage of the development of the internal plastid membranes. Investigations of chloroplast proteins showing that the mutant plants lacked a number of lamellar proteins in early stages of development (Jhamb and Zalik 1973) seem to prove the above suggestions.

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Letalna zmiana chlorofilowa uwidaczniająca się jasnofioletowym zabarwieniem siewek u żyta ozimego (Secale cereale L.)

Streszczenie

W obecnej pracy opisano letalną zmianę chlorofilową, charakteryzującą się jasnofioletową barwą młodych siewek otrzymanych po samozapyleniu formy karłowej żyta ozimego z Jeleńca oraz określono jej sposób dziedziczenia. Analiza genetyczna przeprowadzona na podstawie częstości segregacji roślin zielonych i jasnofioletowych w pokoleniach wsobnych S_3 i S_4 wykazała, że uzyskany stosunek rozszczepień jest zbliżony do stosunku teoretycznego 3:1, co dowodzi, że cecha ta uwarunkowana jest genem recesywnym cl_3 . Gen ten jest letalny, ponieważ powoduje zamieranie siewek po czterech tygodniach od wschodów. W przeciwieństwie do systemu membranowego typowego dla chloroplastów, komórki mezofilu mutantów (jasnofioletowej barwy) zawierają plastidy pozbawione tylakoidów gran i międzygranowych. W ziarnistej matriks tych plastydów obecne są jedynie fragmenty lamelli, pęcherzyki lub elektronowo-przejrzyste obszary.

Fig. 1. Mesophyll chloroplast of a green leaf. $\times 24\ 000$. Figs. 2-4. Plastids of light-violet seedlings. Internal plastid membranes are absent (Fig. 2), visible in the form of single lamellae (Fig. 3) or as connected with osmophilic material (Fig. 4). In some of the plastids of the latter type, variously sized vesicles with single membranes are present in the matrix near the plastid membrane. $\times 24\ 000$