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# CHLOROPHYLL-DEFICIENT MUTANTS IN SUNFLOWER

## I. Mendelian inheritance

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## Summary

Chlorophyll-deficient sunflower genotypes occur spontaneously in the course of selfing. High frequencies of color alteration were recorded in the following populations: Novosadski 4, Chernyanka 11, VNIIMK 4966, VNIIMK 8883, Nain Noir, Saratovsky, Volgar, Cordobes INTA, Armavirski 1813, and especially Impira INTA and Progress. A genetic analysis of the progenies produced by crossing five viable and fertile, phenotypicaly distinct mutants with standard green-leaved genotype showed no difference between the direct and the reciprocal crosses. All $F_1$ crosses had green leaves. The segregation ratios 3:1, 13:3, 55:9 and 45:19 observed in the $F_2$ generation, indicated that the changes were due to a single recessive gene (in NCMS-128 and NCMS-181), inhibition (in RHA-CD-YLW), and complementarity and inhibition with homozygous and heterozygous genes (in AG-111 and RFPH-45). Segregation ratios in the set of test crossing (1:1) supported the previous finding. A preliminary functional allelism test showed that all $F_1$ plants had normal green leaves. This is an indication of the non-allelic nature of the genes which are inherited independently but which may cause different interactions and segregation ratios in the $F_2$ generation.

## Introduction

Chlorophyll-deficient mutants have been studied in numerous plant species. In sunflower, most mutants studied had been developed by means of physical or chemical agents. With a few exceptions, chlorophyll-deficient mutants occurring in the course of inbreeding have received small attention, probably because of lethal and semilethal gene effects. Marked examples of these effects are the lutescens, hlorina, virescens, xantha and albina mutants (Leclercq, 1968; Beletsky & Lyashenko, 1968; Skaloud & Kovacik, 1978). Lethality is a serious limitation to a genetic analysis of chlorophyll mutants. Such defects can be overcome by grafting or, rarely, by alteration of cultural conditions (greenhouse, climatic chambers). Selfed varieties, hybrids, parental lines, plants from gene pools, and interspecific hybrids also render vital and fertile mutants with characteristic chlorotic changes of the leaf (Mihaljcevic, 1992). The paper reports the presence of phenotypically stable and conditional chlorophyll mutants with altered leaf pigmentation in the Novi Sad sunflower breeding program as well as the mode of inheritance of some chlorotic changes caused by nuclear genes.

## Material and Method

In a search for chlorophyll-deficient mutants, the entire breeding material included in the Novi Sad breeding program has been checked. Varietal populations, experimental and commercial hybrids, their parental lines, and interspecific hybrids were selfed in order to establish a collection which included about twenty chlorophyll-deficient mutants. The following genotypes have been selected for the analysis of the mode of inheritance: NCMS-128 (with the full interveinal chlorosis of all leaves), NCMS-181 (with yellow apical leaves), RHA-CD-YLW (leaves with yellow spots which differ in size and have diffuse margins), RFPH-45 (with a dark yellow transversal crescent zone on the leaves developed after the phenocritical phase), and AG-111 (with lemon yellow leaves to the beginning of budding). The inbred V-8931-3-4 with normal green leaves was used as the check. Conclusions on the genetic nature of chlorotic changes were drawn on the basis of the analysis of parental lines, $F_1$ and $F_2$ progenies of direct and reciprocal crosses, and test crosses. The reliability of the assessment of the assumed segregation ratios was checked by the Chi-square test.

## Results and Discussion

A continued selfing of 91 varietal populations of different origin confirmed the existence of lutescens-type mutants (Nain Noir, Vympel), hlorina-type mutants (Saratovsky, Impira INTA), virescens-type mutants (Novosadski 4, Impira INTA), and albina-type mutants (all populations selfed). High frequencies of color alteration (1-3% plants/sample) were recorded in the following populations: Novosadski 4, Chernyanka 11, VNIIMK 4966, VNIIMK 8883, Nain Noir, Saratovsky, Volgar, Cordobes INTA, Armavirski 1813, and especially Impira INTA and Progress. A lethal gene effect (albinism), reduced vitality, and sterility were present in most of these varieties. The classification of chlorophyll-deficient mutants according to the color of cotyledons and true leaves, proposed by Lyashenko (1966) and later amended by Lyashenko & Vilor (1971), was corroborated by the finding of the genotype RHA-271-ACOT, with pale yellow cotyledons and normally green leaves. This genotype had been made by selfing the restorer line RHA-271.

In a few instances, conditional chlorophyll-deficient mutants (those in which the expression of mutant phenotype is controlled by environmental conditions such as light intensity and temperature) have been obtained in the course of selfing. The seed samples of the varieties Impira INTA (PI 378895) and Progress (VIR 2699) are reliable sources of genotypes whose leaves change color in the course of vegetation from light yellow (the first pair of leaves) to a uniform green color. A contrary phenomenon, i.e., a change of the color of the lamina from normal green to light green after the stage of budding, occurred in the genotype FRM-48-LG selected from the cross V-8931-3-4 x FRM-45 (normal leaf color and pale yellow ray flowers).

The direct and reciprocal crosses between phenotypically dissimilar genotypes AG-111, RFPH-45, RHA-CD-YLW, NCMS-128, and NCMS-181 on one side and the fertile analogue of the inbred line V-8931-3-4 on the other produced plants with normally green leaves.

The exclusive occurrence of green leaved plants in the $F_1$ generation proves the recessiveness of the genes which control chlorophyll deficiency in sunflower leaves as well as the absence of the effect of heritable determinants located in cytoplasm on the expression of this characteristic.

The analysis of phenotypic ratios in the $F_2$ generation further confirmed the hypothesis on the action of nuclear genes. The values of average probability ranged from 50% to 90%.

Phenocritical phase (growth stage at which a chlorotic change becomes apparent) of individual mutants and phenotypic classes in the segregating generation affected decisively the assessment of the mode of inheritance of the characteristic studied. The first pair of leaves is the phenocritical phase for the mutants NCMS-128 and AG-111, fourth to seventh leaf for the deficient lines NCMS-181, RHA-CD-YLW, and RFPH-45.

The most intensive changes of leaf color (complete interveinal chlorosis and the chlorosis of all apical leaves), observed in the mutants NCMS-128 and NCMS-181, were due to a single recessive gene. The segregation ratios 3:1 in the $F_2$ generation of the direct and reciprocal crosses (P=70-95% and P=50-90%, respectively) and 1:1 in the test crosses (P=30-70%) seem to validate the hypothesis.

The chlorophyll-deficient mutant of RHA-CD-YLW, with diffuse-edged yellow spots of different sizes, belongs to the variegata type. Beletsky & Lyashenko (1968), crossing the inbred line M-1-12, which is phenotypically almost identical with the mutant RHA-CD-YLW, with a normally green check, proved the monohybrid nature of the examined alterations in leaf pigmentation. The segregation ratios obtained in this investigation, 13:3 in the $F_2$ generation (P=90-95% for the direct and P=50-70% for the reciprocal crosses) and 1:1 in the test crosses (P=30-70%), indicate the inhibitory gene action. The inhibitory gene action was reported by Skaloud & Kovacik (1978). According to these authors, spotted leaves of the variegata type itself are controlled by four genes, while in the chlorinovariegata type (segregation ratio 13:3) the gene St2 is inhibited by St1.

The mutant RFPH-45 was obtained by selfing a Rumanian hybrid Select for five years. The segregation ratios of 55:9 (50-99%) and two cases of 45:19 (P>50%) in the $F_2$ generation, recorded in the progenies of the crosses RFPH-45 x V-8931-3-4 and V-8931-3-4 x RFPH-45, may be considered as due to the action of two complementary genes and an inhibitory gene which, being dominant, prevents the expression of chlorosis. The shift to the ratio 45:19 results from the heterozygosity of the inhibitory gene. Skaloud & Kovacik (1978) associate the ratio 55:9 to a brunescensvariegata-type mutant (phenotypically similar to the mutant RFPH-45), i.e., to the complementarity of the genes St3 and St4 and the inhibitory action of the gene St1. Nevertheless, the mode of inheritance of this deficiency appears to be more complex. In the $S_7$ generation, the phenotypically uniform subline RFPH-45-10 produced about 8% of dwarf plants with light green leaves. In the $F_2$ generation, there was one cross (RFPH-45 x V-8931-3-4) with the segregation ratio of 9 green to 7 lethal albino plants (P>70%) at the cotyledon stage. All leaves of the surviving progenies were green. Although the segregation ratio obtained may be interpreted differently (complementarity, double recessive epistasis), which should be clarified in further research, it was important to note the occurrence of two distinct phenotypic classes at the cotyledon stage and the complete absence of the mutant phenotype at later stages. Since $S_7$ plants were used in this crossing, heterozygosity of certain loci may also be the cause for the different segregation ratios.

The inbred line AG-111 selected in the $S_5$ generation from a Bulgarian variety Kolos had light yellow leaves till the beginning of budding. The later leaves had a normal green color. The segregation ratios 55:9 and 45:19 in the $F_2$ generation follow the interpretation for the mutant RFPH-45, but only when the number of differential plants is scored to the stage of budding. At the stage of full flowering, it is difficult to discriminate between the plants with chlorotic leaves and those with normally green leaves. This trait classified the inbred line AG-111 and the mutant progenies of the varieties Impira INTA and Progres among conditional mutants.

The segregation ratios in the $F_2$ generation of the mutant genotypes crossed to the green-leaved check are presented in Table 1.

Tab. 1 - Segregation ratios in $F_2$ and BCPm crosses between mutant genotypes and a green-leaved genotype

| Cross | Type | Total no. of plants | Green leaf | Chl-def. leaf | Segr. ratio | P % |
|---|---|---|---|---|---|---|
| NCMS-128 x V-8931-3-4 | Dir. | 149 | 110 | 39 | 3:1 | 70-90 |
| $F_1$ x NCMS-128 | BCPm | 132 | 69 | 63 | 1:1 | 50-70 |
| V-8931-3-4 x NCMS-128 | Rec. | 153 | 116 | 37 | 3:1 | 70-90 |
| $F_1$ x NCMS-128 | BCPm | 129 | 68 | 61 | 1:1 | 50-70 |
| NCMS-181 x V-8931-3-4 | Dir. | 106 | 80 | 26 | 3:1 | 90-95 |
| $F_1$ x NCMS-181 | BCPm | 134 | 64 | 70 | 1:1 | 50-70 |
| V-8931-3-4 x NCMS-181 | Rec. | 162 | 118 | 44 | 3:1 | 50-70 |
| $F_1$ x NCMS-181 | BCPm | 143 | 76 | 67 | 1:1 | 30-50 |
| RHA-CD-YLW x V-8931-3-4 | Dir. | 162 | 132 | 30 | 13:3 | 90-95 |
| $F_1$ x RHA-CD-YLW | BCPm | 130 | 62 | 68 | 1:1 | 50-70 |
| V-8931-3-4 x RHA-CD-YLW | Rec. | 142 | 113 | 29 | 13:3 | 50-70 |
| $F_1$ x RHA-CD-YLW | BCPm | 129 | 69 | 60 | 1:1 | 30-50 |
| RFPH-45 x V-8931-3-4 | Dir. | 98 | 84 | 14 | 55:9 | 95-99 |
| $F_1$ x RFPH-45 | BCPm | 135 | 71 | 64 | 1:1 | 50-70 |
| V-8931-3-4 x RFPH-45 | Rec. | 147 | 124 | 23 | 55:9 | 50-70 |
| $F_1$ x RFPH-45 | BCPm | 142 | 76 | 66 | 1:1 | 30-50 |
| AG-111 x V-8931-3-4 | Dir. | 138 | 99 | 39 | 45:19 (55:9) | 70-90 |
| $F_1$ x AG-111 | BCPm | 151 | 91 | 70 | 1:1 | 30-50 |
| V-8931-3-4 x AG-111 | Rec. | 144 | 103 | 41 | 45:19 (55:9) | 70-80 |
| $F_1$ x AG-111 | BCPm | 116 | 63 | 53 | 1:1 | 30-50 |

A preliminary functional allelism test (mutant x mutant) for the genotypes AG-111, RFPH-45, RHA-CD-YLW, NCMS-128, and NCMS-181 showed that all $F_1$ plants had normal green leaves. This is an indication of the non-allelic nature of the genes which are inherited independently but which may cause different interactions and segregation ratios in the $F_2$ generation.

## References


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