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INHERITANCE OF A *Chlorina-apicalis* MUTANT OF SUNFLOWER

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SUMMARY

The present study was conducted to genetically characterize a pigment-deficient mutant of sunflower induced by *in vitro* tissue culture. This mutant, designed as *chlorina-apicalis*, shows pale yellow cotyledons; the first true leaves are initially green, then they became yellow starting from the apical end. This phenomenon of photobleaching causes the plantlets to die in the first stage of development. Segregation data from heterozygous progenies indicate that the trait is controlled by one recessive gene. The symbol *ch-ap* is proposed for the gene that controls this trait.

Key words: *Helianthus annuus*, *in vitro* culture, pigment mutant, genetic analysis.

INTRODUCTION

Several approaches have been used in attempts to define the genetic control and biochemical nature of the photosynthetic apparatus in plants. One of the most frequently used involves the use of mutants that are defective in chloroplast structure, function, and/or pigment biosynthesis (Levin, 1969; Taylor et al., 1987). On the other hand, the physiological and biochemical characterization of these mutants can provide the essential information to construct genetically plants with an increased photosynthetic efficiency.

Chlorophyll-deficient variants occurred frequently in progeny derived from *in vitro* tissue culture (Mathews et al., 1986; Barwale and Widholm, 1987; Barotti, et al., 1995) and many variations described among regenerated plants are heritable (Meins, 1983). These include numerical and structural chromosome variations, point mutations, transpositions of DNA sequences, and changes in mitochondria and chloroplast genomes (Karp, 1991). This variability has been termed "somaclonal variation" by Larkin and Scowcroft (1981) and it results

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from genetic differences pre-existing in somatic cells and genetic changes occurring during the *in vitro* tissue culture (D'Amato, 1985).

Generally, somaclonal variants have not direct value for crop improvement (Karp, 1995); however they are a useful plant material to investigate some aspect of plant biochemistry and physiology. It is the case, for example, of carotenoid-deficient mutant *nd-1* of sunflower (Fambrini et al., 1993, 1995).

The aim of this paper was to determine the inheritance pattern of the pigment mutant, *chlorina-apicalis*, obtained by *in vitro* tissue culture of sunflower (Barotti et al., 1995).

MATERIALS AND METHODS

One R_2 progeny of the inbred line GB had segregated 15 *chlorina-apicalis* plants (Barotti et al., 1995), classified according to the colour of cotyledons and true leaves as proposed by Blixt (1961). The mutant is not viable, and can be maintained only in heterozygous conditions. Thus, normal R_2 plants were selfed and the genetic analysis was conduced in R_3 segregating progenies that were scored for the trait in the field, 14-18 days after planting.

We used a chi-square test to determine the goodness-of-fit of the observed ratios to a hypothesized 3:1 ratio for a trait controlled by a single recessive allele. A homogeneity chi-square test was used before the progenies were pooled.

Table 1: Chi-square analysis of the *chorina-apicalis* mutant

Progeny of heterozygous plants	Normal	Mutant	χ^2 (3:1)	*P*
1	176	52	0.584	0.30 - 0.50
2	30	9	0.077	0.70 - 0.80
3	222	67	0.508	0.30 - 0.50
4	110	36	0.008	0.90 - 0.95
5	180	37	7.313	0.001 - 0.01
6	90	44	4.387	0.02 - 0.05
7	158	61	0.951	0.30 - 0.50
8	281	93	0.036	0.95 - 0.98
9	300	90	0.769	0.30 - 0.50
10	281	87	0.363	0.50 - 0.70
11	181	67	0.537	0.30 - 0.50
Pooled	2009	643	0.804	0.30 - 0.50
Heterogeneity (DF=10)			14.697	0.10 - 0.20

RESULTS AND DISCUSSION

Soon after germination, cotyledons of the *chlorina-apicalis* seedlings, appear pale yellow (Figure 1A), sometimes mixed with green areas (Figure 1B). The first true leaves are initially green (Figure 1B), but after few days, they grow yellow from the apical end. In the same time, cotyledons are wholly lacking chlorophyll pigments and they show spots of necrotic tissues (Figure 1C). Afterward, the yellowing affect the median and basal part of leaves, while the tips become necrotic (Figure 1D). A this stage, 3-4 true leaves, the growth of seedlings is arrested and a little time after, the plantlets die.

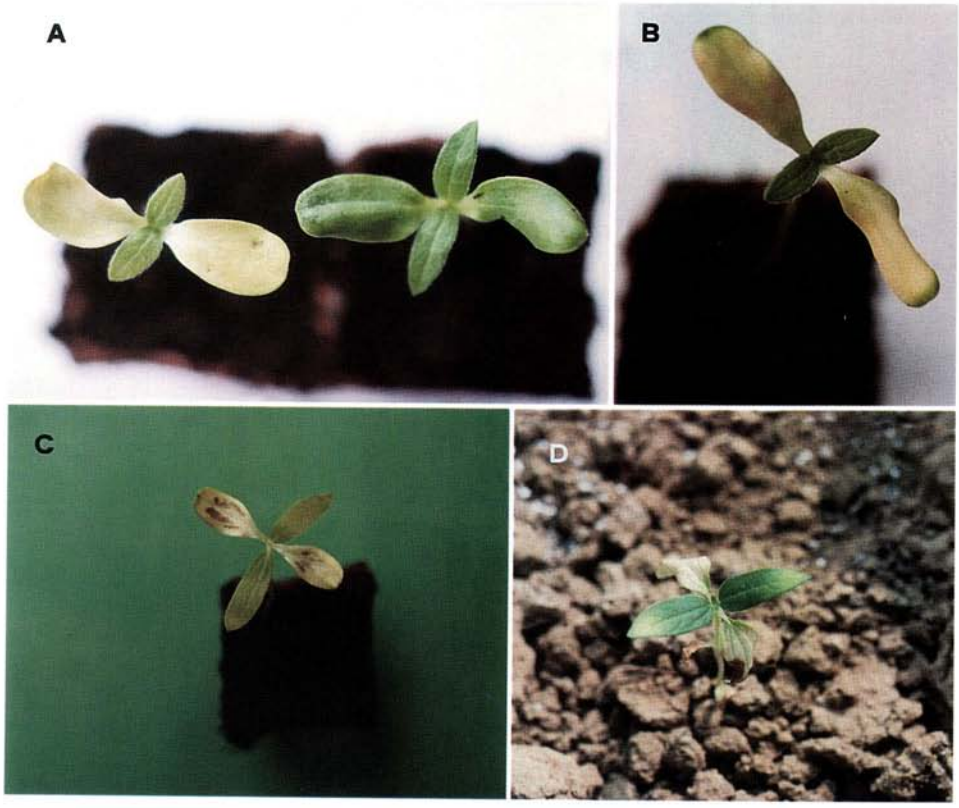

Figure 1. Phenotype of chlorina apicalis mutant of sunflower. (A) chlorina apicalis mutant: (left) and wild type (right). (B) Mutant plantlet showing pale yellow cotyledons with green areas and first two leaves initially green. (C). chlorina apicalis mutant with necrotic areas on cotyledons. (D) Mutant at the stage of 3-4 true leaves: first leaves wholly pale yellow with necrotic tips; youngest true leaves chlorotic at the apical end.

The genetic analysis has been conduced in heterozygous progenies when plants could easily be classified as either normal or mutant (20-25 days after germination). The mutant trait segregated into mutant and normal types fitting a monogenic 3:1 ratio (Table 1). However, two progenies (5 and 6) showed an unexpected segregation ratio that can be ascribed to the small number of plants in each progeny (Table 1). On the other hand, the chi-squares of all population (c2 = 0.804) and the heterogeneity test (Table 1), fitting the expected 3:1 ratio.

We propose the symbol *ch-ap* for the gene that controls this recessive trait.

Although spontaneous chlorophyll-deficient mutations are common in a wide variety of cultivated and natural plants' species and they can be a major component of inbreeding depression (Leclercq, 1968; Mihaljčević, 1992; Willis, 1992), in sunflower most pigment deficient mutants had been induced by means of physical and chemical agents (Beletzky and Liashchenko, 1968; Razoriteleva et al., 1970), or by *in vitro* tissue culture (Fambrini et al., 1993).

The high frequency of pigment variants observed in R_2 progenies of sunflower tissue culture (Barotti, et al., 1995) agree with previous reports on other regenerated species (Mathews et al., 1986, Barwale and Widholm, 1987) where mutations most frequently met are those affecting the pigmentation.

In sunflower, besides single nuclear gene mutation (Pugliesi et al., 1991; Fambrini et al., 1993) and repetitive DNA frequency variation (Natali et al., 1995), significant changes in morphological and biochemical characters have also been reported from progenies of regenerated plants (Roseland et al., 1991; Encheva et al., 1993; Barotti et al., 1995). It is likely that in sunflower many variations are already present in the plant tissue used for culture initiation, as observed in cytological studies of callus and regenerated plants (Cavallini and Lupi, 1987). On the other hand, mutations in somatic cells are not easily recognized and in most cases do not interfere with normal plant development. However, difference in the occurrence of somatic mutation could explain the influence of genetic background in determining somaclonal variations (Pugliesi et al., 1991).

Studies on *chlorina-apicalis* mutants are in progress to understand if the genetic lesion affects the chlorophyll and/or carotenoid biosynthesis.

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HERENCIA DE UN MUTANTE *Chlorina-apicalis* DE GIRASOL

RESUMEN

El presente estudio se llevó a cabo para caracterizar genéticamente un mutante de girasol con una deficiencia de pigmentos inducidos por *in vitro*. Este mutante designado como *Chlorina-apicalis* muestra cotiledones amarillo pálido las primeras hojas verdaderas son inicialmente verdes y después se ponen amarillas empezando por el final del ápice. Este fenómeno de fotoblanqueado causa que las plantas mueran en el primer estado del desarrollo. Los datos de segregación de descendencias heterogéneas indican que el carácter es controlado por un gen recesivo. Se propone el símbolo *ch-ap* para el gen que controla este carácter.

HÉRÉDITÉ D'UN MUTANT *Chlorina-apicalis* DE TOURNESOL

RÉSUMÉ

Cette étude été conduite pour caractériser la génétique d'un pigment mutant déficient de tournesol induit par culture de tissus *in vitro*. Ce mutant, dénommé *chlorina-apicalis*, présente des cotylédons jaune pale, les premières vraies feuilles sont initialement vertes, puis deviennent jaune à partir de l'extrémité apicale. Ce phénomène de décoloration à la lumière provoque la mort des plantules dès les premiers stades de développement. Les données de ségrégation à partir de descendances hétérozygotes indiquent que le caractère est sous contrôle d'un gène récessif. Le symbole *ch-ap* est proposé pour le gène qui contrôle cette caractéristique.