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CYTOGENETIC AND HISTOLOGICAL STUDIES OF A HIGH-OLEIC SUNFLOWER MUTANT

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SUMMARY

Numerous genetic studies have indicated the instability of the high oleic characteristic in sunflower. A high-oleic sunflower mutant was obtained using a dimethyl sulfate chemical treatment. There are several hypotheses about the number and action of genes that control high oleic acid content in sunflower oil. Since most mutations cause changes at the chromosome level, a cytogenetic study (analyses of meiosis and pollen viability) was undertaken to analyze a normal line, an isogenic mutant line and their F_1 hybrid. The results of the cytogenetic analyses showed no change in either the number or the structure of chromosomes in the mutant and the F_1 hybrid in relation to the inbred line. It was concluded that there were no chromosome aberrations such as heterozygous translocation or inversion. Histological analyses showed no difference in the content of the spongy and palisade parenchyma tissues of the cotyledons in the normal line, the mutant and their F_1 hybrid.

Key words: **Sunflower, high-oleic mutant, meiosis, pollen vitality, cotyledon tissue structure.**

INTRODUCTION

Attempts to increase the variability of sunflower in recent decades consisted of mutations induced by irradiation, chemicals or other agents. It has been established that the sunflower is more responsive to chemical mutagens than to irradiation (Anaščenko, 1977).

The high oleic mutant obtained by Soldatov (1976), who treated seeds of the variety VNIIMK 8931 with a 0.5% solution of dimethyl sulfate (DMS), had a great impact on sunflower breeding. In the M_3 generation, Soldatov obtained a material with a high content of oleic acid in oil. This material was used to develop the high-oleic variety Pervenets which is presently used in a number of breeding programs as a source material for the development of high-oleic hybrids.

Genetic studies of high-oleic sunflower mutations have produced several hypotheses concerning the number of genes controlling high oleic acid and their mode of action (Miller, 1992): 1 gene (Ol) - dominant, 2 genes (Ol and ml) - one dominant and one recessive (modifier), 3 genes (Ol_1, Ol_2, Ol_3) - complementary dominant genes.

Recent studies have shown that the mutation is genetically unstable. Demurin and Škorić, (1992b) demonstrated the instability of the Ol gene for high oleic acid in sunflower. They found that the percentage of heterozygous forms of this gene is genotype-dependent. Furthermore, they reported a non-Mendelian mode of inheritance and the occurrence of a patchy distribution of the oleic acid content in individual heterozygous seeds. A high-oleic mutant was tested for important agronomic characters, seed yield, oil yield and percentage of self-pollination by Fernandez-Martinez *et al.*, 1993.

Most mutations (both natural and induced) cause changes in DNA, genome and chromatin. Mutations cause changes in chromosome number (aneuploidy and polyploidy) and chromosome structure (deficiency, duplication, translocation and inversion).

Dimethyl sulfate (DMS) is a chemical mutagen. It is an alkyl agent that introduces a highly active alkyl group (CH_3-CH_3CH_2- etc.) into DNA nucleotides.

Due to the paucity of cytogenetical data about high oleic mutants, we examined meiosis and pollen viability in the isogenic lines (normal and mutant line) and their F_1 hybrids (normal x mutant line).

Histological analyses of the cotyledon tissue were also performed.

MATERIAL AND METHODS

The experimental materials used were the isogenic lines VK-66 (normal) and VK-66 Ol (mutant) and their F_1 hybrid. They were grown in a greenhouse during 1995 and 1996. Seeds for histological analyses were produced in field in 1995.

Five to ten plants of each line and the hybrid were analyzed. Meiosis was analyzed in pollen mother cells (PMC) using the acetocarmine method (Georgieva-Todorova, 1976). When anthers reached the desired stage of development, they were fixed in Carnoy I (3 parts ethyl alcohol : 1 part glacial acetic acid). The samples were fixed for 24 h at room temperature, transferred to 70% alcohol and refrigerated at 4°C. Prior to making preparations, the material was pretreated for 45 minutes with a 4% solution of Fe-ammonium sulfate. Acetocarmine squash preparations were made. The following meiotic phases were analyzed: diakinesis, metaphase I, anaphase I, telophase II and tetrads. Chromosome number and configuration of chromosome pairs were determined in diakinesis and departures from the normal meiotic cycle in the other phases. Thirty or more preparations were analyzed per plant. Meiotic phases were photographed (mf-matic Zeiss, mag-

nification 40 x 8). Pollen viability was estimated using the method of Alexander, (1969) which is based on differences in staining of fertile and sterile pollen grains. Pollen vitality was analyzed in 2-3 plants per line, with three slides for each plant. These were analyzed at 10 locations per slide. The values of pollen viability were expressed as percentages.

Tissue structure was analyzed by the conventional histological method. Samples were fixed with Carnoy II (6:3:1). Hematoxyline and safranin were used for staining. Cotyledon cross sections were analyzed with a light microscope (88x). Histological analyses were replicated five times.

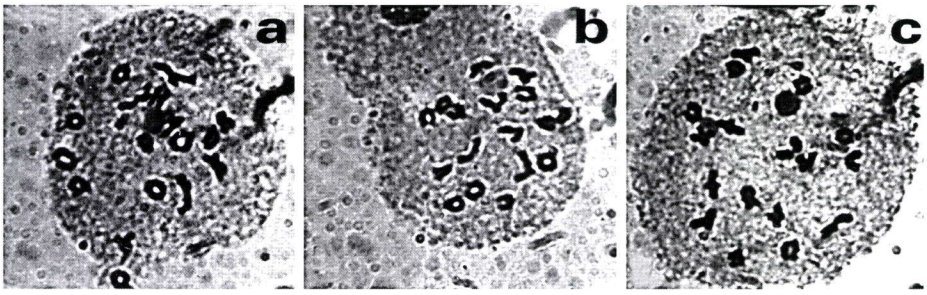

Figure 1. Diakinesis (17 bivalents - 2n=34) in:
a) normal line VK-66
b) mutant line VK-66 Ol
c) F_1 hybrid (VK-66 x VK-66 Ol)

Figure 2. Cross section of cotyledons in:
a) normal line VK-66
b) mutant line VK-66 Ol
c) F_1 hybrid (VK-66 x VK-66 Ol)

RESULTS AND DISCUSSION

The analysis of diakinesis showed that both lines as well as their hybrid had the normal number of chromosomes (2n=34). Seventeen bivalents were registered in diakinesis (Figure 1). Changes in chromosome number were expected in the mutant line (VK-66 Ol) or the F_1 hybrid (VK-66 x VK-66 Ol).

The analysis of meiosis (per phase) showed the absence of detectable chromosomal aberrations at the cytological level. Particular attention was paid to the analysis of diakinesis where a large number of meiocytes were analyzed in the mutant line and the F_1 hybrid. Chromosomal aberrations of the translocation type, which are frequent in mutants, may be detected in diakinesis as multivalent configurations (quadrivalents are clear indicators of translocation). However, all PMC's analyzed had only bivalents in diakinesis. Chromosomal aberrations of the inversion type may be detected in anaphase I as chromosomal bridges. This type of meiotic irregularity was not detected in the examined material.

Both of the above aberration types are difficult to detect without a genetic marker if they are in the homozygous state. Conversely, heterozygous translocations and inversions are detectable (Petrović, 1992). Since the tested material consisted of a normal line and its isogenic mutant line, it was possible to detect heterozygous chromosomal aberrations when they existed in the material.

Pollen viability was high in the normal line (88.17 - 93.26%) and the F_1 hybrid (93.22 - 95.42%). The lower viability in the mutant line, which ranged from 67.61 to 71.07%, might be due to mutation. This assumption cannot be confirmed because pollen viability was determined in a small number of plants. It is mentioned in the literature that pollen abortiveness is an indicator of large changes in chromosome structure. Pollen viability and the percentage of pollination remain to be studied in a larger number of plants of the high-oleic mutant.

The histological analysis of cotyledon cross sections showed that the high-oleic mutants differed neither in the portions of spongy and palisade parenchyma nor in the cell size of these tissues from the normal line (Table 1 and Figure 2). A similar histological analysis of the high-oleic variety Pervenets indicated that it had a less developed spongy tissue and much smaller cells than the normal variety Peredovic (Ključkin *et al.*, 1986).

Table 1: Sunflower cotyledon tissue structure (cross section)

Genotype	Spongy parenchyma (%)	Cell size (m()			
		Spongy parenchyma		Palisade parenchyma	
		Length	Width	Length	Width
VK-66 (a)	64	32	18	57	9
VK-66 Ol (b)	63	30	16	59	8
F1 (VK-66 x VK-66 Ol) (c)	62	32	17	55	8
LSD 0.05	10	10	8	8	8

CONCLUSION

The high-oleic mutation in sunflower caused no changes in the number and structure of chromosomes. There were no heterozygous translocations and inversions which are a frequent consequence of mutation. The low pollen viability in the mutant lines could not be explained. Further investigations are necessary. The genotypes examined did not differ in the portions of spongy and palisade parenchyma in the cotyledon tissue.

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ESTUDIOS CITOGENÉTICOS Y HISTOLÓGICOS DE UN MUTANTE DE GIRASOL CON ALTO CONTENIDO OLÉICO

RESUMEN

Se ha obtenido un mutante de girasol con un alto contenido oléico a partir de un tratamiento guímico con DMS. Existen varias hipótesis sobre el número y la actuación de los genes que controlan el alto contenido de ácido oléico en el aceite de girasol. Numerosos estudios genéticos han indicado la inestabilidad del alto carácter oléico en el girasol.

Puesto que la mayoría de las mutaciones provocan alteraciones al nivel cromosomal, se ha considerado que sería interesante llevar a cabo un estudio citogenético (análisis de la meiosis y de la vitalidad del polen) de una linea normal, la linea mutante isogénica y el híbrido F_1 de las mismas.

Los resultados de los análisis no demostraron ninguna alteración ni en el número ni en la estructura de los cromosomas en el mutante y en el híbrido F_1 con respecto a la linea endogámica. Se ha llegado a la conclusión de que no hubo aberraciones cromosomales, tales como la translocación heterocigotosa o la inversión, detectables al nivel citológico.

Los análisis histológicos no revelaron diferencia alguna en la estructura del tejido cotiledóneo, es decir, en el contenido del parénquima esponjoso y palizado, en la linea normal, en el mutante o en el híbrido F_1 de éstos.

ETUDES CYTOGÉNÉTIQUES ET HISTOLOGIQUES D'UN MUTANT DE TOURNESOL RICHE EN OLÉIQUE

RÉSUMÉ

Un mutant de tournesol riche en acide oléique a été obtenu par traitement chimique au DMS. Il y a plusieurs hypothéses sur le nombre et le mode d'action des gènes qui contrôlent la teneur élevée d'acide oléique dans l'huile de tournesol. De nombreuses études génétiques indiquent l'instabilité des caractéristiques de forte teneur en oléique chez le tournesol.

Etant donné que la plupart des mutations provoquent des modifications au niveau chromosomique, on estime intéressant de développer une étude cytogénétique (analyse de méiose et de viabilité pollinique) chez une lignée normale, la lignée mutante isogénique et leur hybride F_1.

Les résultats de l'analyse cytogénétique ne montrent aucune modification dans le nombre ou la structure des chromosomes chez le mutant et l'hybride F_1 avec la lignée fixée. On en conclut qu'il n'existe pas d'aberrations chromosomiques, de type translocation hétérozygote ou inversion, détectables au niveau cytologique.

Les analyses histologiques ne montrent aucune différence de structure du tissu cotylédonaire, i.e. dans la proportion de parenchyme spongieux et palissadique, chez la lignée normale, le mutant et leur hybride F_1.