SOMATIC HYBRIDIZATION BETWEEN FERTILE AND CMS "OGURA" WINTER OILSEED RAPE

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ABSTRACT

Culture of PEG-fused protoplasts isolated from fertile and sterile (CMS) "Ogura" lines of winter oilseed rape resulted in regeneration of 71 plants (14 fertile and 57 male-sterile plants). Progeny of at least 15 sterile plants showed variation of chlorophyll content in cold treated leaves. Originally synthesis of chlorophyll in CMS "Ogura" lines was restricted to very low level.

INTRODUCTION

Maternal inheritance of cytoplasmic DNA among angiosperms during sexual reproduction precludes independent recombination of organellar genomes. Such recombination however is possible as a result of independent sorting-out of organelles during mitotic divisions of cells - products of protoplasts fusion.

Recombination of cytoplasmically inherited traits: CMS vs. malefertility (inherited by mitochondria) and normal vs. retarded chlorophyll synthesis in low temperature (inherited by chloroplasts) has been already presented by others (Pelletier at al. 1983, Jarl at al. 1988, Morgan and Maliga 1987). The aim of this work was to apply this method to generate novel cytoplasmic recombinants between certain, chosen by breeders lines and introduce them to breeding projects.

EXPERIMENTAL

Seeds of winter oilseed rape cv "Bolko" (male-fertile, double improved) and CMS "Ogura" line were kindly supplied by Dr. I. Bartkowiak-Broda (IHAR-Poznan). Protoplasts were isolated from aseptic leaves (first fusion parent) and hypocotyls (second parent).

Prior to fusion hypocotyl protoplasts were stained with FDA (FDA was added during final 30 minutes of enzyme digestion, 25ug.ml^{-1} final concentration) and inactivated with iodoacetamide (IOA) (5mM final concentration, 20 minutes at 26°C). PEG-fusion was performed according to procedure presented by Sundberg and Glimelius (1986).

Protoplasts after fusion were cultured on 8p medium (Kao, Michayluk 1975) with 0.4M glucose, 2,4-D (1mg.dm^{-3}), NAA (0.1mg.dm^{-3}) and BAP (0.1mg.dm^{-3}). Shoot regeneration medium was based on K3 (Nagy, Maliga 1976) supplemented with IAA (0.1mg.dm^{-3}), zeatin (0.5mg.dm^{-3}) and BAP (0.5mg.dm^{-3}).

Culture of IOA treated hypocotyl protoplasts showed that the growth of practically all protoplasts was retarded. Culture conditions elaborated for hypocotyl protoplasts did not support growth of leaf protoplasts. FDA stain allowed tracking the cells during 3 days of culture after fusion showed that the majority of regenerating (and undergoing first division) cells displayed double fluorescence - fluorescence of FDA originating from hypocotyl and chlorophyll fluorescence from leaf protoplasts.

Culture of fusion derived calli resulted in regeneration of 71 plants. Table 1 presents the number of plants (with fertile or sterile flowers) regenerated after either type of fusion.

Table 1. Number of fertile and male-sterile plants regenerated from PEG-fused protoplasts.

Type of protoplasts used for fusion	Number of regenerated plants (R_0) with fertile flowers	Number of regenerated plants (R_0)with male-sterile flowers
hypocotyl ppts: Bolko leaf ppts: CMS	14	15
hypocotyl ppts: CMS leaf ppts: Bolko	-	42 (18*)

* - number of cold treated (+4°C, 6 weeks) plants which had at least one chimeric leaf with yellowish to dark green sectors

Fig. 1. Chlorophyll content in cold treated (+4°C, 6 weeks) leaves of R_1 (R_0 x Bolko) plants.

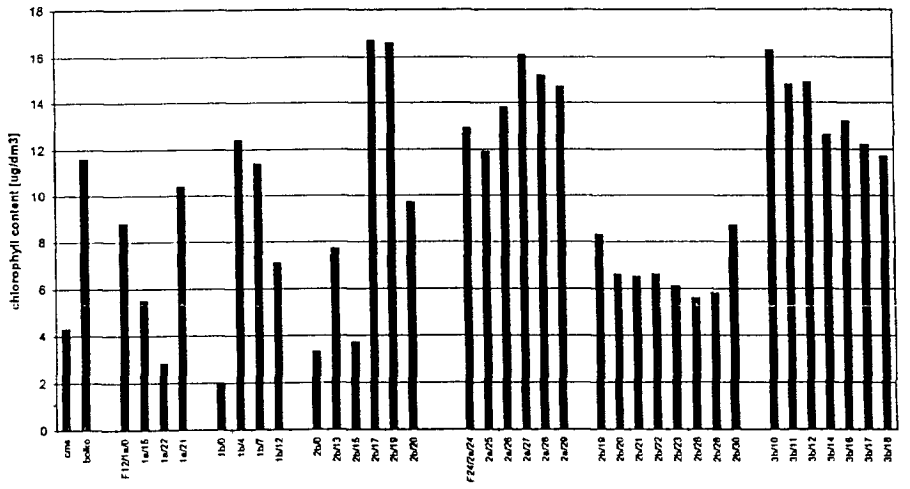

Fig. 1 presents variation of chlorophyll content in leaves of R_1 plants - the progeny of six regenerated plants from two different fusion experiments. All six R_0 plants were male-sterile and showed chimeric type of leaves. Chlorophyll level was heterogenous among progeny of plants from F12 fusion and almonst homogenous with slightly higher level comparing with one parent among progeny of plants from F24 fusion ...

Number of chromosomes counted in root tips of R_1 plants varied from 38 to 72. Analysis of cpDNA and mtDNA is under the way.

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