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BRIEF COMMUNICATION

The content of light-harvesting complexes in different maize (*Zea mays* L.) genotypes

D. HOLÁ

Department of Genetics and Microbiology, Faculty of Science, Charles University, Viničná 5, CZ-128 44 Praha 2, Czech Republic

Abstract

Genetic analysis of the content of light-harvesting complexes of thylakoid membranes was accomplished for the first time during the study of intraspecific variation in photosynthetic characteristics. The existence of genetically determined differences between genotypes together with positive heterosis in F_1 generation was demonstrated.

Additional key words: genetic analysis; heterosis; intraspecific variation.

The light-harvesting complexes (LHC) of thylakoid membranes have been extensively studied during the last twenty years. Many details about their structure, gene expression, and function are already known (for reviews see, *e.g.*, Jansson 1994, Grossman *et al.* 1995, Simpson and Knoetzel 1996). Still, the intraspecific variation in the content of LHC has not been often analysed (Ranieri *et al.* 1995, Wang *et al.* 1998). This work is a part of a complex research project aimed at revealing the basis of inheritance of several photosynthetic characteristics of maize leaves. Its purpose was to examine the possibility of existence of genetically determined differences in the content of LHC in maize and to determine the basis of these differences.

The hybrid combinations CE704×CE810 and 2013×CE810 of maize were selected on the basis of the high heterotic effect in photochemical activity of isolated mesophyll chloroplasts (Holá *et al.* 1999) displayed in F_1 generation. They were formed by the parental inbred lines, their F_1 hybrid, F_2 generation (self-pollinated F_1), and two backcrosses of either of the parents on the F_1 hybrid (referred to as B_1

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e-mail: danahola@cesnet.cz
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and B_2). Seeds were obtained from the Maize Breeding Station CEZEA in Čejč (Czech Republic). The planting conditions were the same as described in Holá *et al.* (1999). The experiments were carried out during July.

The assimilation tissue samples were taken at 07:00 h of summer time from 8-10 plants. The middle part of the leaf blade (fourth or fifth leaf counting from the vegetative top) was used for the isolation of thylakoid membranes. Leaf tissue (2 g) in 50 cm^3 of medium A (0.3 M sorbitol, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM $Na_4P_2O_7$, 50 mM Hepes-KOH, pH 6.8) precooled on ice was homogenised for 18 s (*Thurmix 302*, Poland; maximum rotations). Homogenate was filtered through 8 layers of gauze, filtrate centrifuged at 1400×*g* for 8 min, and resulting pellet resuspended in medium A (10 cm^3) and centrifuged again at 3 560×*g* for 10 min. The pellet was resuspended in 10 mM $Na_4P_2O_7$, pH 7.4 (5 cm^3), kept 20 min on ice in dark and centrifuged at 3600×*g* for 10 min. It was then resuspended in 50 mM Tris-HCl, pH 7.4 (2.5 cm^3), kept 20 min on ice in dark, and centrifuged at 4120×*g* for 20 min. The final pellet was resuspended in 1.2 cm^3 of medium B (65 mM Tris-HCl, 10 % glycerol, pH 7.8) and stored at -76 °C. All the above steps were carried out at 0-4 °C. Chlorophyll (Chl) content in the suspensions of thylakoid membranes was determined spectrophotometrically in 80 % aqueous acetone extracts (Porra *et al.* 1989); the final concentration of Chl in the suspensions was 0.6-1.4 kg m^{-3}.

Pigment-protein complexes of thylakoid membranes were separated by SDS-PAGE (Laemmli 1970) on a vertical electrophoresis system (*Vývojové dílny ČSAV*, Czech Republic) with gel dimensions of 120×150 mm and thickness 0.7 mm. The stacking gel contained 6 % acrylamide, the resolving gel was based on 10-20 % linear acrylamide gradient (acrylamide : N,N'-methylenebisacrylamide ratio 40 : 1). Thylakoid membranes were solubilized by incubation with *n*-dodecyl-β-D-maltoside (DDM) for 45 min on ice, the DDM : Chl ratio in the suspensions was 10 : 1. The standard volume of suspensions, corresponding to approx. 12.5 μg Chl was loaded per each lane. Electrophoresis was run overnight at constant voltage (100 V) and 6 °C. Gels were fixed and stained for 2 h in 50 % methanol, 10 % acetic acid, 40 % water, 0.1 % *Coomassie Brilliant Blue-R 250* (CBB) and destained in the same solution without CBB for 24 h. The amount of pigment-protein complexes and individual proteins in gels was determined densitometrically as an area under the respective peaks using the programme "Gel scan" on spectrophotometer *Beckman DU 70* (Great Britain). Protein standards of M_r 116, 66, 46, 29, and 20.1 kDa were used for molecular mass determination. The content of LHC of M_r 24-28 kDa (their position in gels was previously ascertained also by immunodetection) was expressed per leaf area unit.

The existence of genetically determined differences between genotypes was analysed by standard analysis of variance, allowing the separation of genetic and environmental variation. The average values characterising each genotype on each experimental day were used for this analysis. A generation mean analysis according to Hayman (1958) was applied to determine the mechanisms of the inheritance of LHC content. This analysis enables to separate additive and dominance components of genetic variation as well as several kinds of non-allelic interactions. The statistical

computations were made with the aid of CBE computer program (Wolf 1996).

Genetically determined differences in the LHC content were found for both hybrid combinations examined, but they were much less prominent compared to differences in the photochemical activity of mesophyll chloroplasts (Holá *et al.* 1999). Generally, they were more pronounced in the beginning and at the end of experimental season, *i.e.*, in certain marginal stages of plant development. The effect of experimental days was also statistically significant.

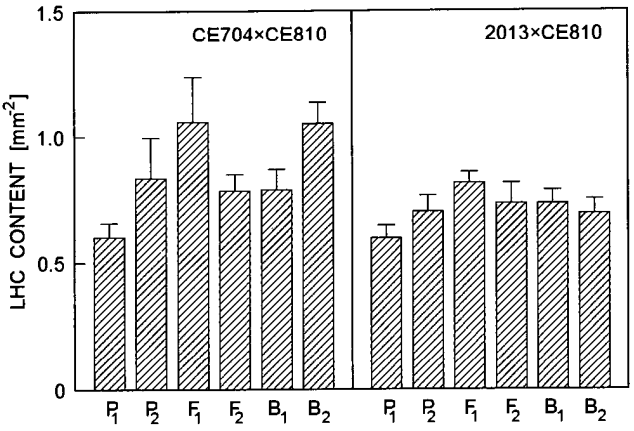

Fig. 1. The content of LHC in two hybrid combinations of maize, CE704×CE810 and 2013×CE810, as the area under respective peaks on densitogram expressed per leaf area unit (±SEM, $n = 6$).

Individual generations differed strongly in the LHC content. The positive heterosis in F_1 generation was observed for both hybrid combinations. It could be as high as 140 % of parental mean depending on the hybrid combination examined and on the ontogenic stage. High values of heterotic effect found for F_1 hybrids were accompanied by its decrease in F_2 generation. Both B_1 and B_2 usually followed the LHC content of their respective paternal genotype (Fig. 1). The presence of positive dominance was probably the main cause of positive heterosis observed in F_1 generation of 2013×CE810. Similarly, positive dominance together with the additive

Table 1. Genetic analysis of the LHC content in two hybrid combinations of maize, the non-allelic interactions (NAI) were or were not included into genetic model. Parameter μ represents mean of all generations, *a* represents additivity, *d* dominance, *aa, dd, ad* NAI between two homozygous, two heterozygous, or between homozygous and heterozygous loci, respectively. Statistical significance: $^{*}p = 0.05$, $^{**}p = 0.01$.

	CE704×CE810		2013×CE810	
	without NAI	with NAI	without NAI	with NAI
μ	8.65 ± 0.36**	7.82 ± 0.59**	7.17 ± 0.20**	7.27 ± 0.72**
a	-1.59 ± 0.61**	-2.62 ± 1.05**	-0.29 ± 0.32	0.39 ± 0.66
d	2.40 ± 1.37	8.77 ± 3.64**	1.68 ± 0.54**	0.82 ± 3.21
aa		5.36 ± 3.16		-0.82 ± 3.17
ad		-1.47 ± 1.32		0.90 ± 0.75
dd		-6.54 ± 6.03		1.57 ± 4.05

genetic effects was found for the hybrid combination CE704×CE810 (Table 1).

As a conclusion, the intraspecific variation in the content of LHC proteins of thylakoid membranes can result from the genetic causes. There can even be found a positive heterotic effect in F_1 generation, which is usually due to positive dominance.

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