Identification of Gene Loci Encoding for 11S Globulin of Sunflower Seed

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Abstract

11S globulin, or helianthinin is a major storage protein of sunflower seed. It is a 300 kD hexameric holoprotein every subunit of which consists of large acidic and small basic polypeptides linked by disulphide bonds. The subunit and polypeptide composition of helianthinin is heterogeneous due to heterogeneity of the corresponding gene family. Screening by sodium dodecylsulphate polyacrylamide gel electrophoresis of more than 20,000 genotypes situated at the Sunflower World Collection have revealed a number of molecular variants of helianthinin protein. Several variants were used as markers of the three heterogeneous gene subfamilies. Analysis of segregation by helianthinin polypeptide compositions in F_2 and BC populations of ten cross combinations showed that helianthinin genes are clustered in at least three loci. Locus *Hel B* comprises three genes for major subunit group B. Locus *Hel C* codes for small subunit pairs of helianthinin. Locus *Hel A* corresponds to the largest helianthinin subunits. Loci *Hel B* and *Hel C* were inherited jointly with a recombination frequency of about 22%. Locus *Hel A* segregated independently on the loci *Hel B* and *Hel C*. Locus *Hel C* showed loose linkage with isozyme loci *Est 1* and *Gpi 1* and locus *Vs* (increased leaf venation).

Introduction

Sunflower seed accumulates two major groups of storage proteins, the salt soluble 11S globulin designated helianthinin and the water soluble 2S albumins (DERBISHIRE et al. 1976). Helianthinin is known to be an oligomeric protein composed of six subunits (SCHWENKE et al. 1979) each of them consisting of disulphide linked large (acidic) and small (basic) polypeptides. Some work on the biochemical and physical properties of helianthinin are available (PLIETZ et al. 1983, DALGALARRONDO et al. 1984, KORTT and CALDWELL 1990). The results of these studies indicated that helianthinin polypeptides are heterogeneous both in charge and molecular masses (M_r). No M_r polymorphism was observed among helianthinin fractions isolated from different sunflower varieties (DURANTE et al. 1989, RAYMOND et al. 1991, 1994). Nevertheless, the homologous proteins from other species (i.e. legume plants) was known to display variability in the M_rs of constituting polypeptides. In the preliminary study we revealed the polymorphic variants of helianthinin proteins in some sunflower lines and varieties (ANISIMOVA and GAVRILYUK 1989). The purpose of the present work was identification of mutant variants and analysis of inheritance of helianthinin protein in interline crosses.

Materials and Methods

The seed materials comprised 300 inbred lines and 100 varietal populations from VIR Sunflower World Collection. Helianthinin was cryoprecipitated with water from salt soluble extracts of individual seeds (SCHWENKE et al. 1975). Electrophoresis (SDS-PAGE) was carried out in 12.5% polyacrylamide gels in Laemmli system (LAEMMLI 1970). The procedure used for isolation of proteins and running gels was described in details earlier

(ANISIMOVA et al. 1991). The isozyme analyses were carried out as described in LOSKUTOV et al. (1988). The Chi-square test was perfomed to examine fitness of the frequencies of the marker alleles against expectation from Mendelian segregation.

Results and discussion

SDS-PAGE analyses of more than 20,000 genotypes from Sunflower World Collection showed that the majority of them had identical helianthinin banding patterns. The pattern of reduced protein (in presence of β-mercaptoethanol) comprised three groups of acidic polypeptides and a number of basic ones. The major acidic polypeptides (bands 32 and 33, 28-30, 22 and 23) derived after reduction of subunits A, B and C respectively, which were identified earlier (RAYMOND et al. 1991). The most common (normal) helianthinin pattern included major polypeptide variants 1, 3, 4, 7, 8, 10, 12, 20, 22, 23, 28, 30, 32, 33 (see Figure). Screening of sunflower gene pool also revealed a number of mutant polypeptides which differed from the normal ones by their electrophoretic mobilities and hence by the M_rs. The frequencies of mutant polypeptides varied among the varieties or groups of lines. Several lines with mutant helianthinin polypeptides were used as parents in crosses to produce segregating F_2 and BC populations (Table 1). The following cross combinations were examined: F_2(1×6), F_2(4×1), F_2(1×8), F_2(3×2), F_2(3×7), BC(4×1)×1, BC(3×5)×5, BC(3×4)×4, BC(3×4)×1, BC(3×5)×1. The number of individual seeds analysed in different segregating populations varied between 77 and 434. Frequencies of segregation in the polypeptide pairs 12 (normal) - 9 (mutant), 12 (normal) - 11 (mutant), 29 (mutant) - 30 (normal), 33 (normal) - 34 (mutant) corresponded to those expected for monogenic inheritance. Variant 9 co-segregated with polypeptide 12 null-variant. Thus, several allelic pairs of helianthinin polypeptides, 12-9, 12-11, 12-9, 29-30, 33-34 have been identified.

The M_r heterogeneity of helianthinin polypeptides is known to be a result of heterogeneity within the corresponding gene family. Although there is no available data on the number of helianthinin encoding genes, the studies of cDNA give a molecular evidence for existence of two genes subfamilies for helianthinin (VONDER HAAR et al. 1988). These results are supported by the data of genetic control of other 11S globulin proteins, for example legumin protein of pea seed (GATEHOUSE et al. 1988). However, legumin lacks the 30 kD acidic polypeptides which correspond to the subunit group C of helianthinin. The fact that helianthinin is composed of three distinctive subunit groups indicated the presence of at least three encoding gene subfamilies, or loci of linked genes, *Hel A*, *Hel B*, *Hel C*. The mutant polypeptides were further considered as markers for those subfamilies for estimation of linkage relationships. Variant 34 was considered as a marker of locus *Hel A*, and variant 29 as a marker of locus *Hel B*. The biggest basic variant 12 was shown to be paired with smallest acidic one (32 kD protein bands 22 and 23) into the subunit C in the native protein (RAYMOND et al. 1991). Hence, the allelic variants 11-12-9 were considered as markers for locus *Hel C*. Analysis of segregation in the pairs of allelic variants (Table 2) have demonstrated a genetic linkage of loci *Hel B* and *Hel C* (derived recombination frequency about 22%) and absence of linkage between loci *Hel A* and *Hel B* or *Hel A* and *Hel C*. No linkage between loci *Hel B* and *Est 1* was found. Very loose linkage was demonstrated for the pairs of loci *Hel C-Est 1*, *Hel C-Gpi 1* (glucose phosphate isomerase), and *Hel C-Vs* (increased leaf venation). No linkage was found between locus *Hel C* and those for morphological characters *Ep* (striped pericarp epidermis) and *P* (presence of armour layer in pericarp).

Thus, the results of study allowed identifications of new gene loci in sunflower genome and may be useful for construction of a genetic linkage map of cultivated sunflower.

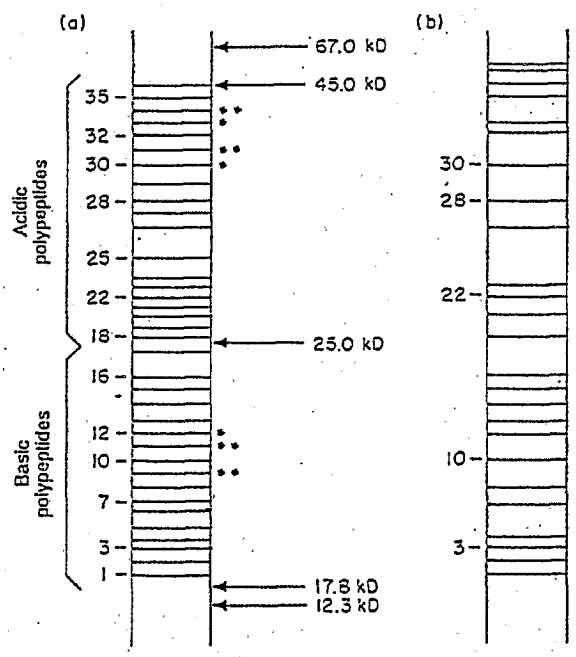

Figure. Schemes of distribution of variant forms in helianthinin banding patterns. (a) The total pattern (all the polypeptide variants revealed in sunflower gene pool are included). The polypeptides are numerated from 1 (the smallest basic variant) to 35 (the largest acidic variant). At the right the positions of normal variants 12, 30, 33 (one asterisk) and mutant ones 9, 11, 29, 34 (two asteriks) are indicated. (b) The normal banding pattern of helianthinin.

Table 1. Characteristics of parental lines

Number	Line	Presence of helianthinin polypeptides							Genotypes for other characters
		9	11	12	29	30	33	34	
1	VIR104	+	-	-	-	+	+	-	
2	VIR122	-	-	+	-	+	+	-	*Est1(SS);Gpi1(SS);Vs/Vs;ep/ep;P/P*
3	VIR130	-	-	+	+	-	+	-	*Est1(FF);Gpi1(FF);vs/vs;Ep/Ep;p/p*
4	VIR131	-	-	+	-	+	-	+	
5	VIR302	-	-	+	-	+	-	+	
6	VIR369	-	-	+	-	+	+	-	
7	CM144	-	+	-	-	+	+	-	*Est1(vF/vF);Gpi(SS);Vs/Vs*
8	469802	-	-	-	-	+	+	-	

"+" - presence of polypeptide
"-" - absence of polypeptide
Vs - increased leaf venation
Ep - striped pigmentation of pericarp epidermis
P - armour layer of pericarp
S - "slow" and "fast" allozyme variants

Table 2. Segregation by the pairs of helianthinin alleles

Pairs of alleles A,b/a,b	Phenotypes of progeny AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	Expected segregation	χ^2	χ^2_L	Derived frequency recombination
12,29/11,30	1	8	16	9	36	4	10	15	1	1:2:1:2:4:2:1:2:1	40.00	37.36	21.8±4.1
33,29/34,30	0	0	22	31	23	0	0	24	0	1:1:1:1	2.00	1.36	55.0±8.9
33,29/34,30	0	0	33	37	39	0	0	28	0	1:1:1:1	2.07	0.36	47.4±4.2
33,29/34,30	33	39	0	30	42	0	0	0	0	1:1:1:1	2.50	0.25	52.0±4.2
34,12/33,9	3	9	5	8	25	11	4	9	3	1:2:1:2:4:2:1:2:1	3.25	1.79	44.0±5.7
34,12/33,9	0	0	0	24	23	0	27	36	0	1:1:1:1	3.81	0.90	54.5±4.7

References

Anisimova, I.N. and Gavrilyuk,I.P. (1989) *Genetika* (in Russian) 25, 1248.

Anisimova, I.N., Gavrilyuk, I.P. and Konarev, V.G. (1991) *Plant Varieties and Seeds* 4, 133.

Dalgalarrondo, M., Raymond, J. and Azanza, J.L. (1984) *J.Exp.Bot.* 35, 1618.

Derbishire, E., Wright, D.J. and Bolter, D. (1976) *Phytochemistry* 15, 3.

Durante, M., Bernardi, R., Lupi, M. and Sabelli, P. (1989) *J.Agric.Food Chem.* 37, 852.

Kortt, A.A. and Caldwell, J.B. (1990) *Phytochemistry* 29, 1389.

Laemmli, U.K. (1970) *Nature* 227, 680.

Loskutov, A.V., Borovkova, I.G. and Borovkov, A.Y. (1988) *Scientific Bull.VNIIMK* (in Russian) 4, 22.

Plietz, P., Damashun, G., Muller, J.J. and Schwenke, K.D. (1983) *Eur.J.Biochem.* 130, 315.

Raymond, J., Inquello, V. and Azanza, J.L. (1991) *Phytochemistry* 30, 2849.

Raymond, J., Mimouni, B. and Azanza, J.L. (1994) *Pl.Syst.Evol.*193, 69.

Schwenke, K.D., Schultz, M. and Linow, K.J. (1975) *Nahrung* 19, 425.

Schwenke, K.D., Pahtz, W., Linow, K.J., Raab, B. and Schultz, M. (1979) *Nahrung* 23, 241.

Vonder Haar, R.A., Allen, R.D., Cohen E.A., Nessler, C.L. and Thomas, T.L. (1988) *Gene* 74, 433.

escarificadas y descascaradas produjeron un número similar de semillas germinadas. Sin embargo la técnica del escarificado es más sencilla por lo que es aconsejada.

La utilización de la técnica de germinación de semillas inmaduras en los planes de mejoramiento es por lo tanto factible. Ella sería una herramienta útil de trabajo en, por ejemplo, la evaluación de la resistencia del girasol frente a los estrés bióticos y abióticos. Trabajos posteriores darán validez a dicha hipótesis.

Conclusión

Se detectaron efectos promedios significativos del genotipo, de la edad de la semilla y del tipo de acondicionamiento sobre el porcentaje de semillas inmaduras germinadas luego de 7 días de incubación en cajas de Petri no esterilizadas.

Las semillas de 15 días escarificadas del híbrido G100 germinaron en su totalidad.

Estos resultados sugieren la posibilidad de incluir esta sencilla y poco costosa técnica en los planes de mejoramiento de girasol. El costo de producción de líneas endocriadas e híbridos se vería disminuido.

Bibliografía

CARABACA, L.; IZQUIERDO, N.; ARANA, D.; CASTAÑO, F. & AGUIRREZABAL, L., 1995. *In:* Proceedings, 2nd Latin American Meeting on Plant Biotechnology/REDBIO 95, Puerto Iguazú, Argentina, Abstract A23

CHANDLER, J. y JAN, C., 1985. *Crop Sci.*, 25: 356-358

MAEDA, M. y UNGARO, H., 1985. *In:* Proceedings, XI International Sunflower Conference, Mar del Plata, Argentina, I, pp 73-79

MOUTOUS J. y MOUTOUS, C., 1994. *In:* International Sunflower Yearbook 1994, (Abstract) 57

PAUL, M. y BARTHOU, H., 1994. *agronomie*, 14: 281-284

TABLA N° 1. Porcentajes ($) de germinación obtenidos mediante el cultivo de semillas inmaduras de girasol.

GENOTIPOS =>	PIONEER 6440				DEKALB G100			
EDAD =>	10		15		10		15	
GIBERELINAS =>	NO	SI	NO	SI	NO	SI	NO	SI
Tipo de Semilla								
ENTERAS	0	0	0	0	0	0	0	0
ESCARIFICADAS	0	49,9	90,2	88,3	0	0	100	100
DESCASCARADAS	26,6	39,9	91,8	89,2	36,3	12,5	86,8	0

($)= Promedio de dos repeticiones
CV%= 18,6