SUNFLOWER SEED 11S GLOBULIN (HELIANTHININ) AS GENETIC MARKER

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Summary

The major seed storage globulin helianthinin from diverse sunflower species, intraspecific forms and individual genotypes was studied using the method of sodium dodecylsulphate-polyacrilamide gel electrophoresis (SDS-PAGE). Comparative analyses of 10 Helianthus L. species have revealed the significant divergence of helianthinin polypeptide composition between annual and perennial sunflowers. Considerable interspecific differences within these two taxonomic groups were also found. Among the 40 analyzed varieties of H.annuus L., the helianthinin of the variety Gigant 549 only had the distinctive varietal character. All other varieties were distinguishable based upon the frequences of certain polymorphic polypeptides. In breeding lines, as well as in the majority of genotypes within the varieties, the standard helianthinin types were predominated. However, in lines marked on morphological characters, the marker helianthinin types were fixed more frequently. Helianthinin markers can be used for line identification, registration and purity checking. Helianthinin SDS-PAGE supplemented with the isozyme analysis give more exact estimations of line homogeneity and interline differences.

Introduction

Plant seed storage proteins possess genome and genotype specificity so they are extensively used as markers in genetic, phylogenetic, evolutionary, taxonomic and breeding studies (Konarev, 1983). In sunflower, works using protein markers were initiated at the end 1970's and were devoted to comparative immunochemical analysis of total seed protein fractions of

Helianthus species with reference to problems of systematics, phylogeny and genome analysis (Anashchenko and Gavriljuk, 1979; Anisimova, 1984a,b). Further, we have isolated the major storage protein of sunflower seed 11S globulin (helianthinin), characterized it by sodium dodecylsulphate polyacrilamide gel electrophoresis - SDS-PAGE (Anisimova et al., 1986) and shown the genotype specificity in the cultivated species H.annuus L. (Anisimova and Gavriljuk, 1989). Here, we summarize data on interspecific and intraspecific helianthinin variability and show possibilities of using this protein for the identification and registration of sunflower species, varieties and lines.

Material and Methods

Seed accessions of 10 wild species, 40 varieties and 220 lines from the Vavilov Institute (VIR) Sunflower World Collection were used.

Helianthinin was isolated from single seeds or seed samples using the method of cryoprecipitation (Schwenke et al., 1975). Isolated helianthinin was dissociated to polypeptides in presence of SDS and β-mercaptoethanol and then fractionated in 12,5% SDS-polyacrilamide gel according to the method of Laemmli (1970) with some our modifications (Anisimova, 1991a).

Results

Helianthinin SDS-PAGE patterns of all analyzed sunflower species, intraspecific forms and individual genotypes included three main groups of polypeptides - the basic polypeptides with molecular masses of about 20 kDa and two groups of acidic polypeptides (30 kDa and 40 kDa), however, within these groups, polypeptide compositions were highly variable. Polypeptide composition was independent of growing conditions suggesting the possibility of using this character as a marker for the identification and registration of sunflower germplasm.

The more essential differences in the polypeptide numbers and electrophoretic mobilities were revealed between annual and perennial sunflowers. Within the two taxonomic groups the interspecific variability was also apparent (Fig.1).Helianthinin

variability in the cultivated sunflower H.annuus was parallel with that in wild annual species (H.lenticularis Ckll., H.praecox E & G, H.debilis Nutt.). Thus, polypeptide variants characteristic of wild forms were frequent in the cultivated sunflower genepool. Moreover, the majority of polypeptides in patterns of wild annual and cultivated forms were displayed as identical or allele variants. Such overlapping variability in the closely related taxons is probably accounted for by their genetic similarities. It should be noted that seed non-globulin fraction of H.lenticularis, which is the closest to H.annuus, have the marker antigen easily identified in immunochemical reactions and distinguishing H.annuus from the wild relative (Anisimova, 1984a).

Within the cultivated species H.annuus the helianthinin polypeptide composition was very varied. Analyses of random samples of 50 or 100 seeds of native and foreign varieties and hybrid varieties have shown that the majority of genotypes were mainly of standard (variety Peredovik) type. According to the way of recording the helianthinin formula (Anisimova et al., 1991a) the standard helianthinin polypeptide composition can be presented in the next form: $\overline{1}$ $\overline{2}$ 3 4 7 8 10 12 $\overline{18}$ $\overline{20}$ 22 23 28 30 32 33 were $\overline{1}$, $\overline{2}$, $\overline{18}$, $\overline{20}$ are the minor polypeptides and 3, 4, 7, 8, 10, 12, 22, 23, 28, 30, 32, 33 are the major polypeptides. Among the 40 analyzed varieties the helianthinin of the variety Gigant 549 only had the distinctive varietal feature that is lacking polypeptide 4. All other varieties were classified into several groups differing by the presence of common polypeptides. A number of polypeptides including "wild" variants were polymorphic in all the variety accessions. Their frequences varied between 10 and 30%. In hybrid varieties, such as Fransol, Soldor, Penyigei "A" toifaita, polymorphism was conditioned by Mendelian segregation on certain polypeptides.

In a line analysed, all seeds as a rule had similar, inherited in self-pollination, helianthinin polypeptide composition. This could be the standard or differing from the standard type. The marker helianthinin characters included presence of additional or allele variants, absence of certain major polypeptides, alterations in polypeptide intensities. It is notable, that in

breeding VIR lines with valuable quantitative traits, the proportion of genotypes marked by helianthinin polypeptide composition was smaller than in lines with marker morphological characters (20% and 80% respectively). In the group of 19 analyzed lines of foreign breeding, the eight lines HA-6, HA-61, HA-298, CM 144, RHA-265, RHA-273, Dark Stripe, Do 264 had helianthinin markers. However, almost all lines of this group could be differentiated by the simultanous analyses of helianthinin and six isozyme systems (Anisimova et al., 1991b). Method of helianthinin SDS-PAGE was also useful for testing of line homogeneity and genetic purity. The more perfect and exact estimations were obtained by comparisons of data of helianthinin and isozymes analyses.

Discussion

The results suggest the helianthinin specificity on the level of species or groups of closely related species. Hence, the helianthinin polypeptide composition along with protein antigens (Anisimova, 1984a) and DNA markers (Choumane and Heizmann, 1988; Perez and Berville, 1988) can be used in phylogenetic and taxonomic studies and also for control of gene and chromosome transmission in interspecific crosses. Helianthinin can be also used as a marker character for the identification and registration of the cultivated sunflower genefund. Method of helianthinin SDS-PAGE is more accessible if compared with other known methods of biochemical identification such as for instance with the analyses of sunflower leaf phenol compounds (Sanlaville et al., 1988), so far as it allows identifying genotypes yet in rest (seed) stage. Coomassie stained helianthinin SDS-PAGE patterns have no background and contaminations of other proteins. Unlike the extremly heterogenic patterns of total proteins (Koranyi, 1988), the helianthinin patterns are easily readable and clearly show differences in helianthinin encoding loci.

Sunflower varieties representing complex heterozygous populations can be distinguished by frequences of certain polypeptide variants. Predominance of standard helianthinin types among the varieties can hardly be explained by the common origin

because the examined group included genetically heterogenous material from different Institutes. Meantime, the prevalence of standard helianthinin types in breeding lines suggests that in sunflower the selection on quantitative characters was probably accompanied by the co-selection on molecular characters and fixation of certain helianthinin encoding alleles that of primary standard type. Thus, for example, in maize, the selection on 15 isozyme loci, comprising 30 - 40% of genome and correlating with quantitative traits was the same effective as the selection on phenotypic markers covered the whole genome (Brown et al., 1989). Correspondence between phenotypic and helianthinin characters in sunflower lines also indicated efficiency of using the helianthinin SDS-PAGE analysis to seed purity testing especially in combination with isozyme electrophoresis.

Conclusions

Helianthinin polypeptide composition is highly polymorphic in genus Helianthus. It is greatly divergated in different sunflower species. The degree of differences correspond to the degree of species phylogenetic closety. Certain conservation of helianthinin variability in cultivated annual forms is apparently explained by the constraints resulting from the selection on quantitative traits. Nevertheless, the sunflower varieties can be differentiated by the frequences of polymorphic variants. Lines can be identified by the helianthinin SDS-PAGE in 20 - 80% instances but implication of isozyme marker make possible almost 100% identification.

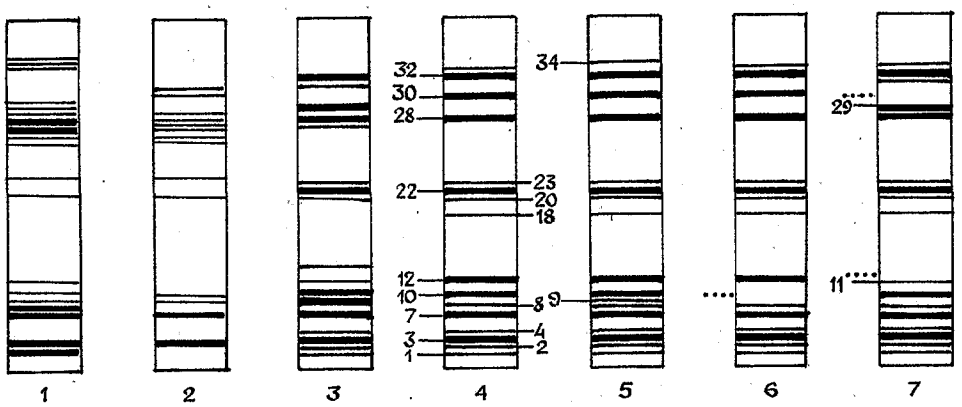

Fig.1. Schemes of distribution of polypeptide variants in helianthinin SDS-PAGE patterns of H.tuberosus (1), H.mollis (2), H.lenticularis (3) and H.annuus (4 - 7). 4 -standard type; from 5 to 7 - marker types with additional (5), lacking (6) and allelic (7) polypeptides.

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