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SPECIES-SPECIFIC CLONES VERSUS DNA-FINGERPRINTING: DIFFERENTIATION IN THE GENUS *HELIANTHUS* ON A MOLECULAR LEVEL

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Summary

In this study, two techniques were compared as a means of differentiation in the genus *Helianthus*. Of these, the DNA-fingerprinting method exhibited polymorphic bands from the species level (six species being investigated) down to the level of individual plants from one *H. annuus* line. The number of differences was lowest within a line, as expected, and showed a steady increase from different lines and populations to the species examined. An identification of interspecific hybrids as differing from both parents was clearly possible.

This could not be achieved with the species-specific *H. annuus* clones investigated. However, a rather good differentiation between the *Helianthus* species was feasible. A more detailed analysis of the clones by Southern hybridization indicated the presence of the respective sequences in every species, although the copy number in *H. annuus* was several times higher than in the other species.

Introduction

Until recently, the degree of uniformity within lines and the relatedness between different lines, populations or species in the genus *Helianthus* was mainly based on morphological and chemical traits, as well as on isozyme analysis; this also holds true for the determination of the hybrid character in interspecific crosses.

Making use of the latest developments in molecular biology, much more specific tools of differentiation are nowadays at hand. As a rather rapid technique, species-specific clones can be used for the determination whether a wide hybridization between different species was

successful, e.g. by performing squash dot tests (JUNGHANS & METZLAFF 1988). The usefulness of such clones, mainly being constituted by repetitive sequences, was especially demonstrated in different cereal species (JUNGHANS & METZLAFF 1988, METZLAFF *et al.* 1986, ZHAO *et al.* 1989), sometimes exhibiting even chromosome-specifity (HARCOURT & GALE 1991).

In respect to examinations especially in the closer related categories, the 'fingerprinting' techniques have been established as one of the leading methods in differentiating even between individuals of one line. Besides PCR-fingerprinting (WELSH & MCCLELLAND 1991, WELSH *et al.* 1991, DEHMER & FRIEDT 1992), the more commonly used, 'classical' DNA- or oligo fingerprinting using oligonucleotide-probes can be employed for the revelation of characteristic banding patterns of the respective plants (BEYERMANN *et al.* 1992, WEISING & KAHL 1990, DEHMER *et al.* 1992).

Material

Populations of *Helianthus* wild species, i.e. *H. mollis* (MOL 1948), *H. resinosus* (RES 1545), *H. rigidus* (RIG 1848), *H. strumosus* (STR 1974), and *H. tuberosus* (TUB 5, 1700, 1705), were kindly provided by Dr. D. SKORIC, Novi-Sad, Yugoslavia, except *H. mollis* RH (Gießen). Interspecific hybrids (HA89(cms) x TUB 1705) were developed by M. DAHLHOFF, Gießen. HA89(maintainer) and RHA 271 were a gift from Dr. J. FERNANDEZ-MARTINEZ (Cordoba, Spain); Baso(cms) and RK320/86(cms) were propagated in the experimental station at Groß-Gerau.

Methods

Genomic *Helianthus* DNA was extracted according to BERNATZKY & TANKSLEY 1986 with several modifications. The DNA for the construction of the species-specific clones was first digested with DraI or SstI, followed by a TaqI digest of the upper (repetitive) bands resuspended from a LMP agarose gel.

Isolation of plasmid DNA in a midi scale, ligation of sunflower DNA with the BscI restricted pBSC vector, transformation into competent DH 5α cells and the blue/white screen on selective medium was conducted under standard conditions.

Genomic DNA was digested with HinfI for the dot blots, and BamHI, EcoRI and HindIII for Southern blots; hybridization with the postive clones was performed at 68°C, followed by several washing steps under stringent conditions.

Genomic DNA for the DNA-fingerprints was restricted using frequently cutting enzymes (AluI, DraI, HinfI, NdeII, RsaI and TaqI). After Southern Transfer to nylon membranes (Hybond N+), hybridization with three different, digoxigenin labeled oligonucleotides (Fresenius) was performed according to J.MATHE, with slight modifications (pers. comm.). Fingerprint bands were visualized by the Anti-DIG-AP catalyzed NBT/BCIP color reaction (Boehringer).

Results and Discussion

Out of several repetitive clones examined, most of these did not demonstrate any species-specifity. Mainly two HA89(cms) clones (ADT1 36, ADT1 55) showed a rather strong signal hybridized against *H. annuus* DNAs, a certain degree of cross-reaction with TUB 1705 and more or less no signal with the other species (including TUB 5 and TUB 1700, see fig. 1).

These finding were confirmed by Southern blot hybridization of the clones against the respective DNAs, although no difference in the banding patterns was shown. This indicates no strict specifity of the HA89 clones for *H. annuus*, but the presence of a partially significantly higher number of copies of the cloned sequences in the *H. annuus* plants does allow a differentiation. The experiments with ANNxTUB hybrids did not fulfil the expectations: no intermediate strength of signal was expressed in the hybrid lanes; however new bands did appear. Future hybridization experiments of the two ADT1 clones against DNA from crosses between HA89(cms) and parents other than TUB 1705, probably will allow a distinction between the two parental and the F_1 plants.

Especially the $(GATA)_4$ fingerprints of different species, populations and lines always resulted in a clear differentiation, and even the examination of single plants of one line did show differences in the banding pattern (compare fig. 2). Here, the degree of homology was the highest, decreasing rapidly towards the species level. A determination of the hybrid character in the ANNxTUB crosses was possible without problems, although the F_1 plants resembled much more the HA89(cms) parent than the TUB 1705 parent.

Conclusion

In the comparison between species-specific clones and DNA-fingerprints, the latter method proved to have the much greater potential of differentiation: in all categories examined, a distinction between the banding patterns of individuals was feasible.

Species-specific clones can serve as a fast means of distinguishing between species, and can allow in most of the cases a confirmation for a postulated hybrid character; more detailed information - at least with the clones presented here - will hardly be obtainable.

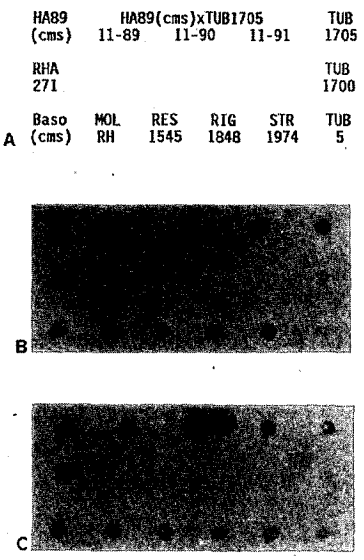

Figure 1: dot blots of HinfI digested genomic DNAs (A, 50 ng each), hybridized against B) ADT1 36, and C) ADT1 55.

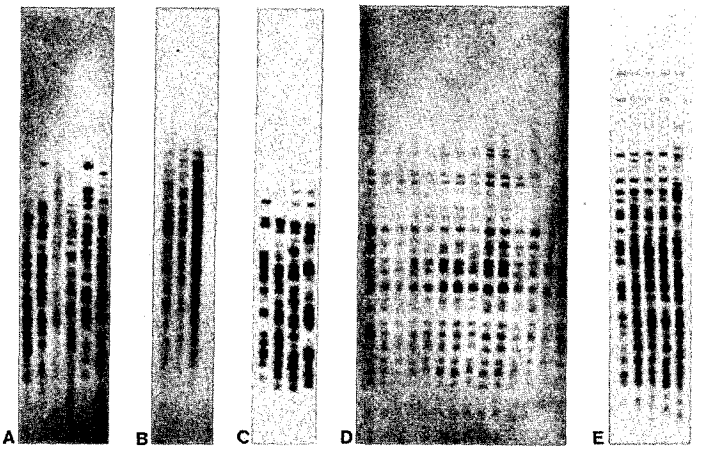

Figure 2: DNA fingerprint pattern of

A) *Helianthus* species: HA89(cms), MOL 1948, RES 1545, RIG 1848, STR 1974, and TUB 1705 (from left to right; TaqI digests),

B) *H. tuberosus* populations: TUB 5, TUB 1700, TUB 1705; (AluI),

C) *H. annuus* lines: RHA 271, HA89(cms), Baso(cms), RK320/86(cms); (TaqI),

D) 14 individual HA89(maintainer) plants; (HinfI), and

E) interspecific ANNxTUB hybrids: HA89(cms), ANNxTUB 11-89, ANNxTUB 11-90, ANNxTUB 11-91, TUB 1705; (DraI).

Restriction fragments were separated on 1.2% agarose gels and hybridized with $(GATA)_4^{DIG}$ at 39°C.

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