IRAPs as a tool for molecular breeding of sunflower

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ABSTRACT

- The cultivated sunflower (*Helianthus annuus* L.) is one of the staple oilseed crops of the world. Information about the genetic diversity and relationships among breeding lines and varieties is not only useful for germplasm conservation and inbred line identification, but also for the selection of parental lines for hybrid breeding. The advent and application of molecular markers have provided effective tools to gain a better understanding of the diversity of crop germplasm. To date, diversity analysis of sunflower germplasm has been conducted using different isozymes and DNA markers such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), target region amplification polymorphism (TRAP), simple sequence repeat (SSR), and single nucleotide polymorphism (SNP). Retrotransposon insertional polymorphisms can be produced by a variety of PCR-based techniques such as inter-retrotransposon amplified polymorphisms (IRAPs), where outward-facing primers are designed for conserved domains such as long terminal repeats (LTRs) within the elements. From these conserved LTR regions, outward-facing primers for PCR amplification are designed that amplify the genomic DNA fragment lying between closely spaced retrotransposon
- Twenty nine sunflower inbred lines, including HA89, RHA274, 13 restorer and 14 maintainer lines, were analyzed using three different primers combinations of IRAPs and 14 well distributed SSR markers. The obtained polymorphic fragments were converted to binary notation and two database matrices were constructed, one for IRAP data and the other for the SSR polymorphisms. Using the simple matching coefficient, two similarity matrices were derived. Dendrograms were constructed based on them using the unweighted pair group method with arithmetic means (UPGMA). The degree of association of the relationships among inbred lines obtained by the two types of molecular markers was determined by a Mantel test.
- IRAP primers combinations yielded 48 polymorphic markers which clustered the 29 inbred lines into two major groups and an outlier (IB80, a maintainer line). One of the groups was composed by HA89, RHA 274, 13 maintainer and two restorer lines, while the other group included 12 restorer lines. The lowest similarity coefficient was 0.53 and the highest 0.96. SSR markers yielded 73 fragments which permitted to separate the analyzed lines into two groups and an outlier (RHA274). One cluster included HA89 and 14 maintainer lines; the other was composed by 13 restorer lines. In this case the lowest similarity coefficient obtained was 0.64 and the highest was 0.97. Relationships among the inbred lines utilized using both type of markers were associated given the significant correlation obtained by the Mantel Test (0.43, $p<0.008$). However, this association primarily reflects the divergence between the restorer and maintainer reproductive groups, since the internal relationships inside each of these groups were not conserved.
- The obtained results indicate that IRAP markers can be useful in sunflower genetics and breeding since they are cost effective multipoint markers that complement the information provided by SSR markers.
- This type of marker, together with other multipoint markers like TRAPs, are well suited for evaluating the descendants and recovering the genetic background of a given line during a marker assisted backcross procedure.

Key words: diversity, molecular diversity, molecular markers, polymorphism, retrotransposon.

INTRODUCTION

Sunflower (*Helianthus annuus* L. var. *macrocarpus* Ckll.) is grown all over the world with three main purposes: beauty (ornamental sunflower), direct consumption of the seeds (confectionary sunflower) and oil production (oilseed sunflower). By far, the last of them is the most important objective in terms of acreage and production (Miller and Fick, 1997). Sunflower oil has been traditionally viewed as a healthful vegetable oil and it is considered premium oil for salad, cooking, and margarine production (Dorrell and Vick, 1997) and also is being evaluated as a source of biodiesel (Bunta and Mario, 2008).

Information about the genetic diversity and relationships among breeding lines and varieties is not only useful for germplasm conservation and inbred line identification, but also for the selection of parental lines for hybrid breeding (Senior et al., 1998; Sun et al., 2001). Historically, germplasm characterization has been based on low and high heritability morphological traits. This phenotype germplasm characterization has many important drawbacks like the low polymorphism level of the characters under analysis, the impact of environmental interaction over them and the ontology of the plant during the description process, among others (Carrera et al., 2010).

Coancestry analysis has played an important role in the studies on genetic diversity or similarity when molecular markers were unavailable or expensive. However, incomplete pedigree data and inaccurate estimates of the contribution of each ancestor to a genotype may lead to inappropriate clustering of the genotypes involved, and some genotypes without clear pedigrees fail to cluster. On the other hand, molecular markers are stably heritable and abundant. Thus, the advent and application of molecular markers have provided effective tools to gain a better understanding of the diversity of crop germplasm. To date, diversity analysis of sunflower germplasm has been conducted using different isozymes (Carrera and Poverene, 1995; Cronn et al., 1997) and DNA markers such as restriction fragment length polymorphism (RFLP) (Berry et al., 1994; Gentzbittel et al., 1994), amplified fragment length polymorphism (AFLP) (Hongtrakul et al., 1997), simple sequence repeat (SSR) (Yu et al., 2002), and single nucleotide polymorphism (SNP) (Liu and Burke, 2006), and target region amplification polymorphism (TRAP) (Yue et al., 2009).

Retrotransposon insertional polymorphisms can be produced by a variety of PCR-based techniques such as inter-retrotransposon amplified polymorphisms (IRAPs), where outward-facing primers are designed for conserved domains such as long terminal repeats (LTRs) within the elements. From these conserved LTR regions, outward-facing primers for PCR amplification are designed that amplify the genomic DNA fragment lying between closely spaced retrotransposons (Teo et al., 2005).

The objectives of this study were to investigate the genetic diversity and relationship among a group of public and proprietary maintainer and restorer inbred lines using IRAPs and to compare these results with those obtained using SSRs.

MATERIALS AND METHODS

Twenty nine sunflower inbred lines, including the public maintainer line HA89 and the restorer line RHA274, 13 restorer and 14 maintainer proprietary lines were used (Table 1).

Table 1. Utilized sunflower inbred lines, their reproductive group and other features.

Inbred line	Reproductive Group	Features
HA89	Maintainer	Public*, conventional
Rha274	Restorer	Public, conventional
B14	Maintainer	Propietary**, conventional
B19	Maintainer	Propietary, conventional
B12	Maintainer	Propietary, conventional
B16	Maintainer	Propietary, conventional
B11	Maintainer	Propietary, conventional
B15	Maintainer	Propietary, conventional
IB192	Maintainer	Propietary, Imisun ***
IB14	Maintainer	Propietary, Imisun
OB3	Maintainer	Propietary, High Oleic ****
OB13	Maintainer	Propietary, High Oleic
OB17	Maintainer	Propietary, High Oleic
OB8	Maintainer	Propietary, High Oleic
IB9	Maintainer	Propietary, Imisun
IB113	Maintainer	Propietary, Imisun
R6	Restorer	Propietary, conventional
R11	Restorer	Propietary, conventional
R181	Restorer	Propietary, conventional

R9	Restorer	Propietary, conventional
IR183	Restorer	Propietary, Imisun
R10	Restorer	Propietary, conventional
R3	Restorer	Propietary, conventional
OR6	Restorer	Propietary, High Oleic
IR102	Restorer	Propietary, Imisun
R4	Restorer	Propietary, conventional
R19	Restorer	Propietary, conventional
IR91	Restorer	Propietary, Imisun

* Public line: line public available
** Propietary line: a line that belongs to a company
*** Imisun line: a line with herbicide tolerance conferred by the *Ahasl-1* allele
**** High Oleic line: a line with more than 85% oleic acid in its oil composition

Ten seedlings of each line were grown in a greenhouse. Total genomic DNA was isolated from about 50 mg (fresh weight) of leaf tissue sampled from 10-d-old plants using the method of Dellaporta (1994). DNA concentrations were adjusted to approximately 10 ng μL^{-1} for polymerase chain reaction (PCR) amplification. Three different primer combinations of IRAPs (Table 2, Vukich et al., 2009) and 14 well distributed SSR markers (Yu et al., 2003) were used (ORS008; ORS166; ORS495; ORS510; ORS595; ORS838; ORS844; ORS1024; ORS1039; ORS1045; ORS1095; ORS1097; ORS1128; ORS1256).

Table 2. Primer combinations of the IRAPs markers used in this study and their sequences.

Primer combination	Forward Sequence	Reverse Sequence
CF/CR1	GGTTTAGGTTCGTAATCCTCCGCG	ACAGACACCAGTGGCACCAAC
UF/UR1	TAACGGTGTTCTGTTTTGCAGG	AGAGGGGAATGTGGGGGTTTCC
UF/UR2	TAACGGTGTTCTGTTTTGCAGG	TTAACCAGGCTCCGGCGTGAG

In the case of IRAPs, high quality polymorphic fragments were manually scored as present (1), absent (0), and missing (−) across all genotypes to form a binary data matrix. The obtained polymorphic fragments were converted to binary notation and two database matrices were constructed, one for IRAP data and the other for the SSR polymorphisms. Using the simple matching coefficient, two similarity matrices were derived. The similarity coefficient of simple matching is calculated by the formula SM = *m*/*n*, where *m* = shared present fragments (11) + shared absent fragments (00), and *n* = the total of obtained fragments. Coefficients were computed using the NTSYS-pc software package, Version 2.02 (Rohlf, 1998). Each genetic similarity coefficient matrix was then used to construct a dendrogram with the unweighted pair-group method using the arithmetic averages (UPGMA) algorithm, employing the Sequential, Agglomerative, Hierarchical, and Nested (SAHN) clustering procedure. The degree of association of the relationships among inbred lines obtained by the two types of molecular markers was determined by a Mantel test (Mantel, 1967)

RESULTS

IRAP primers combinations yielded 48 polymorphic markers. The primer combination UF/UR1 is shown in Fig. 1. Multivariate analysis of this information permitted to obtain the dendrogram shown in Fig. 2. The multivariate analysis clustered the 29 inbred lines into two major groups and an outlier (IB80, a maintainer line). One of the groups was composed by HA89, RHA 274, 13 maintainer and two restorer lines, while the other group included only 12 restorer lines. The lowest similarity coefficient was 0.53 and the highest 0.96.

1 2 3 4 5 6 7

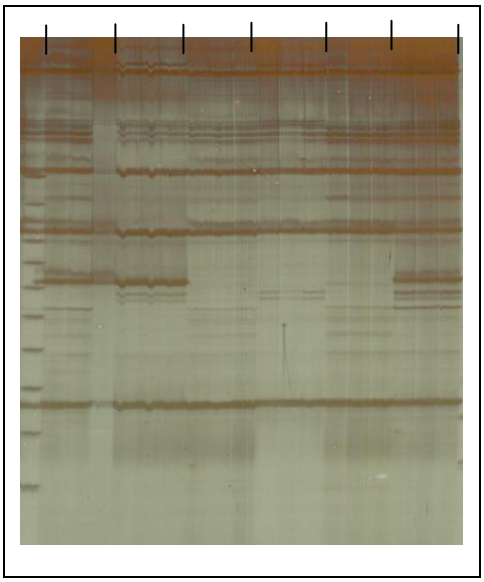

Fig.1. Silver stained polyacrylamide gel showing 6 genotypes analyzed using the UF/UR1 IRAP primer combination. Lane 1 molecular weight marker, lane 2: HA89, lane 3: RHA274, lane 4: B14, lane 5: B19, lane 6: B12 and lane 7: B16.

SSR markers yielded 73 fragments which permitted to separate the analyzed lines into two groups and an outlier (RHA274). One cluster included HA89 and 14 maintainer lines; the other one was composed by 13 restorer lines. In this case the lowest similarity coefficient obtained was 0.64 and the highest was 0.97.

A significant association between similarity matrixes obtained by using SSR and IRAPs markers was obtained by applying the Mantel test ($r=0.43$, $p<0.008$).

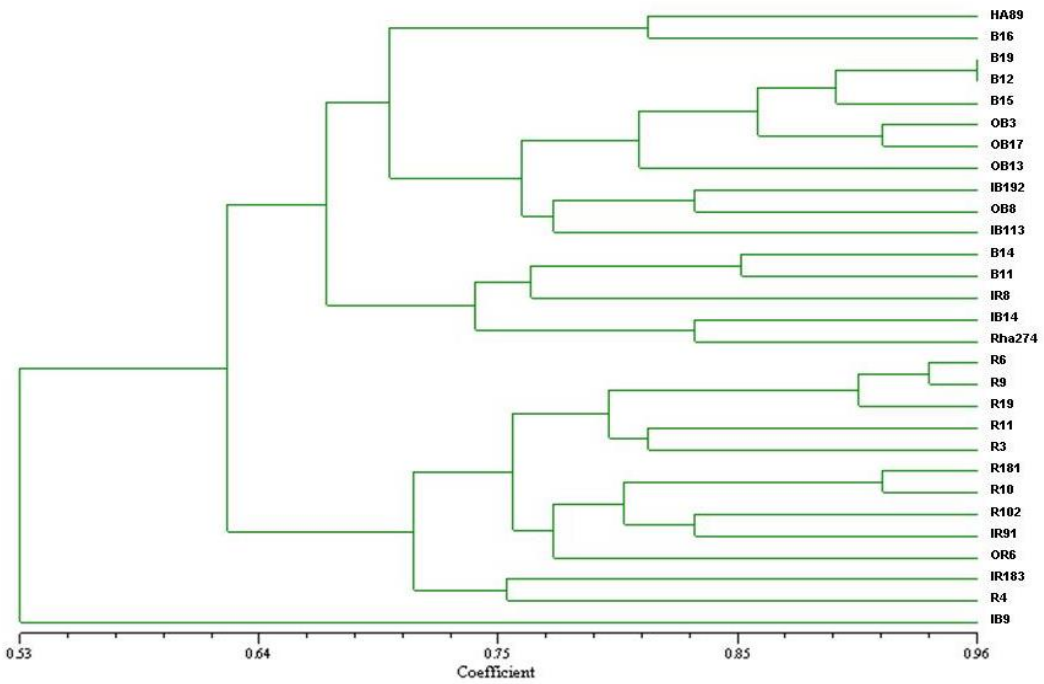

Fig. 2. Dendrogram obtained using IRAP marker analysis over 29 inbred lines, using the unweighted pair group method with arithmetic means (UPGMA).

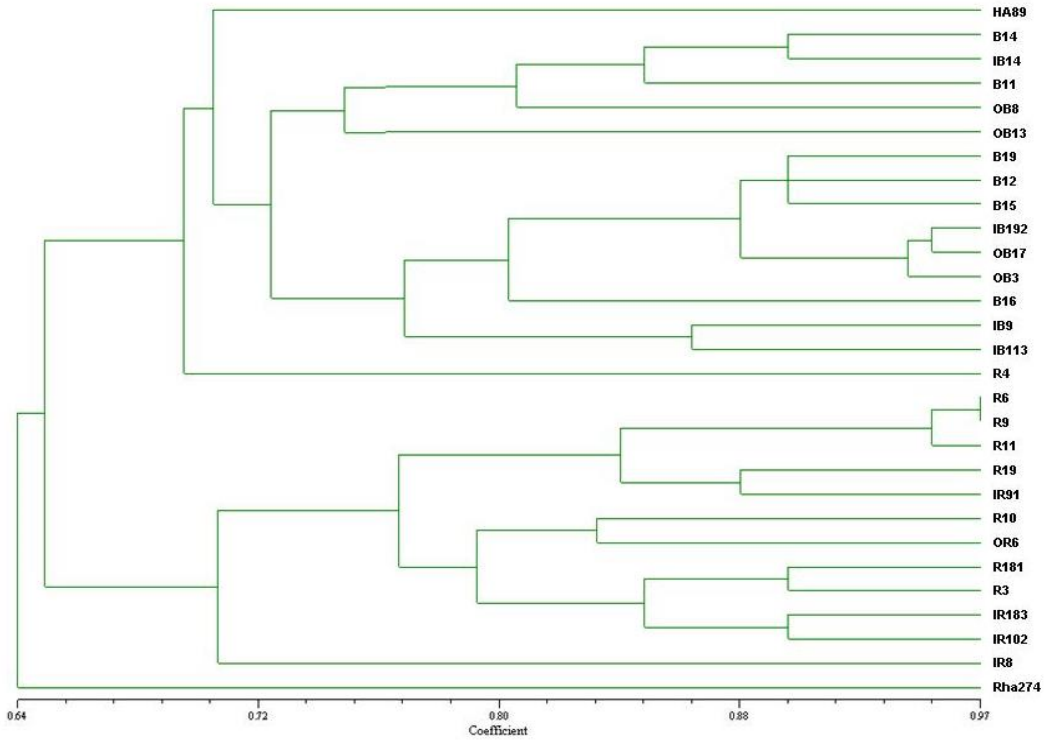

Fig. 3. - Dendrogram obtained using SSR marker analysis over 29 inbred lines, using the unweighted pair group method with arithmetic means (UPGMA).

DISCUSSION

Using AFLP markers, the genetic similarity among 25 oilseed sunflower lines was reported to range from 0.58 to 0.98 with an average similarity of 0.70 (Ronicke et al., 2005), and genetic similarity among 24 oilseed sunflower lines ranged from 0.70 to 0.91 (Hongtrakul et al., 1997). Using TRAP markers and 177 public inbred lines, an average genetic similarity of 0.58 was reported with a minimum of 0.30 and a maximum of 0.97 (Yue et al., 2009). The average genetic similarity in the present study (0.69, ranging from 0.33 to 0.96) was similar than that reported in previous studies using AFLPs and it was lower than that informed for TRAPs. In addition to the fact that different molecular markers were employed, this observation can be explained by the use of different inbred lines in this study.

As was previously shown using SSR markers (Yu et al., 2002; Smith et al., 2009) and also multipoint markers like TRAPs (Yue et al., 2009) sunflower restorer and maintainer inbred lines could be clearly separated into different groups. The same pattern of genetic distribution was obtained in this work using SSR and IRAP markers separately for inbred line analysis and clustering. Comparison of both analyses using Mantel test primarily reflects the divergence between the restorer and maintainer reproductive groups, since the internal relationships inside each of these groups were not conserved. Genetic relationships based on SSR markers have a better congruence with coancestry than IRAPs (data not shown).

IRAP markers could be implemented in molecular breeding programs to assist the selection for recovering the genetic background during the conversion of sunflower inbred lines by one or more traits, especially if it is coupled with other multipoint markers like TRAPs, AFLPs or ISSRs. The wide genome coverage of these types of markers and the great number of genomic sites sampled during each PCR reaction, permits to obtain reliable information in an easy, fast and cost-effective way.

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