Molecular markers associated with leaf expansion response to water deficit conditions

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ABSTRACT

- Water deficit is one of the major constraints to crop yields worldwide. In this context, adaptation of genotypes to different water regimes is a key factor to ensure yield stability. An underlying response that seems to regulate the degree of decrease in yield is leaf expansion before anthesis. Progeny studies together with molecular marker technology would allow for this character to be mapped. The present work aims at studying the relationship between a set of molecular markers (AFLP and SSR) and leaf expansion parameters under water deficit conditions in a cross of two public sunflower lines of contrasting response, in its F_2 (data from a previous work) and $F_{2:3}$ progenies, and in an independent F_8 recombinant inbred line (RIL) population.

- Phenotyping trials were carried out in three different experiments, two of them in growth chambers (F_2 and $F_{2:3}$) and one in a greenhouse (RIL population). Plants were grown in cylindrical 2.7 l pots filled with soil, and a standardized method was used to generate water deficit conditions. Leaf expansion data were collected, and then processed to obtain parameters of leaf growth. DNA was extracted from F_2 individuals and RILs. A set of 60 SSR and 41 AFLP markers was evaluated in the F_2 population, whereas 68 SSR where evaluated for the RILs. Single marker analysis was performed associating genotype data from F_2 individuals and phenotype data from F_2 individuals and $F_{2:3}$ families

- A genomic region was identified which contains one stable marker significantly associated with the response of leaf growth to water deficit in the three generations of the cross (maximum significance: $p=0.02$, $p=0.03$ and $p=0.003$ in F_2, $F_{2:3}$ and RILs, respectively).

- These results confirm the previous findings, and provide evidence on the stability of a region of the genome associated to the response of leaf expansion to water deficit in sunflower. The nature of an F_2 population (individuals with unique genotypes) imposes limitations to characterize responses to water deficit, so a single-plant approach had to be used for that generation, while an experimental design with control and stressed plants could be used for RILs and $F_{2:3}$. The consistency of the identified genetic markers suggests that outputs of both phenotyping methods could be comparable.

- This information could be useful to develop molecular makers for assisted selection in breeding programs oriented to generate new cultivars with improved adaptation to water stress conditions. A dissection of this analysis into a single leaf approach is being carried out in order to achieve a more specific phenotype characterization under water stress conditions. Association of leaf expansion parameters of leaves at different growth stages at the moment of the stress treatment might allow a more precise identification of QTL associated with leaf expansion response.

Key Words: sunflower – water deficit – leaf expansion – molecular markers

INTRODUCTION

Plants respond to soil drying with different mechanisms which ultimately lead to reduced transpiration rate (e.g., stomatal closure, wilting, leaf rolling, reduced growth, senescence). When water deficit occurs before anthesis, the main mechanism that regulates water use by the crop is reduced leaf growth. Reduction of leaf growth results in less surface exposed, which allows for total plant transpiration to be reduced but reducing also the ability to capture radiation, with a consequent decrease in carbon assimilation and yield.

Water deficit is one of the major constraints to crop yields worldwide. In this context, adaptation of genotypes to different water regimes is a key factor to ensure yield stability. Breeding for different degrees of sensitivity of leaf growth to water deficit according to the expected scenario is a promising approach for improving yield stability (Reymond *et al*, 2003). Phenotyping for such a trait is, however, laborious and time -and resource-consuming. Identification of molecular markers associated with the response of leaf growth to water deficit could therefore help breeders identify favourable genotypes more easily.

Two contrasting genotypes (HAR2 and HA64) for characters involved in leaf expansion under water deficit have been previously screened in a sample of 18 inbred lines (Pereyra Irujo et al, 2008). An F_1 population resulting from the cross of these genotypes was used to obtain a set of F_8 recombinant inbred lines (RILs), an F_2 and its $F_{2:3}$ progeny, where variability for the characters was expected to be expressed. Data already published for the F_2 revealed the presence of variability for the character and molecular markers associated with the response of leaf expansion rate to water deficit (Pereyra Irujo, 2009).

The aim of this work was to analyze the stability of the association of molecular markers to the response of leaf growth to water deficit in the three mentioned populations.

MATERIALS AND METHODS

For each population under study, a trial to obtain phenotypic data was carried out (Table 1). To assess the response of plants to water deficit, a standardized method to impose drought was used according to Pereyra-Irujo *et al*, 2007. Plants were grown in cylindrical 2.7 l pots filled with soil of known water retention capacity parameters. Soil water content was measured gravimetrically and adjusted with daily irrigations. Soil was kept at field capacity until plants reached ten visible leaves on average; soil water content was then adjusted to -0,56MPa (water stress treatment).

Area of all leaves was measured every 2-3 days. For experiments involving $F_{2:3}$ families or RILs, the response of leaf growth to water deficit was quantified as the change in total leaf area between the beginning and end of the treatment of plants under stress, relative to that of control plants. For the F_2 population, where there were no control plants, the response was quantified as the change in leaf growth rate during the stress treatment relative to that before the stress. In all cases, this variable was corrected using leaf area at the beginning of the treatment as a covariate, therefore obtaning values around 1.

DNA was extracted from all F_2 individuals and RILs. A set of 60 SSR and 41 AFLP markers was evaluated in the F_2 population, whereas 68 SSR where evaluated for the RILs. Single marker analysis was performed associating genotype and phenotype for each population. In the case of $F_{2:3}$ families, the genotype of the F_2 parent was used.

Table 1: Details of the three phenotyping trials

Experiment	Generation	Conditions	Number of Genotypes	Plants per genotype	Duration of Stress Conditions
1	F_2	Growth chamber	102 individuals	1	8 days
2	$F_{2:3}$	Growth chamber	12 families	8	14 days
3	F_8	Greenhouse	68 RILs	4	7 days

RESULTS

Phenotypic variability for responses of leaf area and absolute leaf expansion rate at the whole plant level was found in all three generations, as well as transgressive segregation for the F_2 population and RILs (Figure 1). This could not be verified for the $F_{2:3}$ families because parental lines were not included in the experiment.

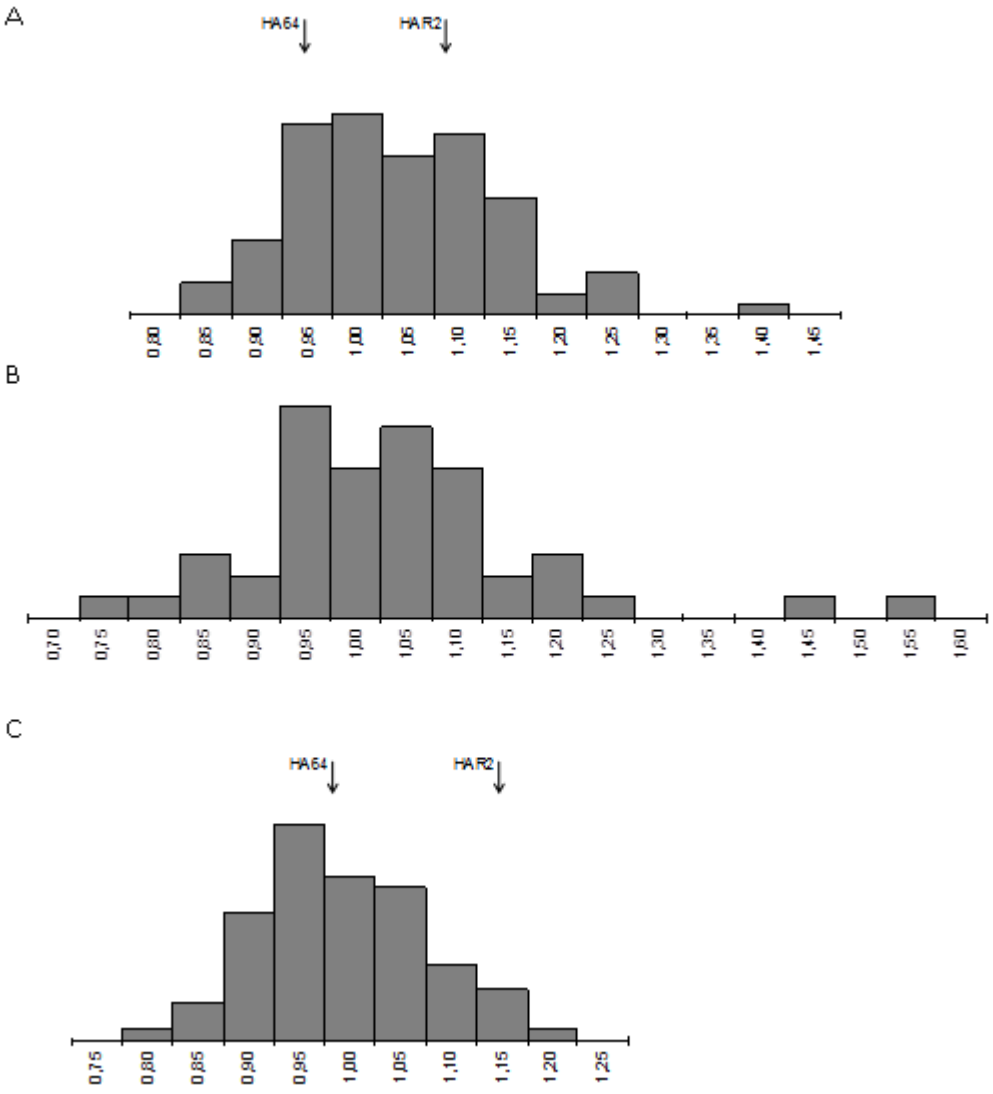

Figure 1: Frequency distributions of the responses of leaf growth to water deficit for the three generations under study. A: F_2. B:, $F_{2:3}$. C: RILs.

From the single marker association analysis, one marker was found to be significantly associated to leaf growth under stress in all three generations (Table 2). This marker was linked to the same parental allele in all cases (all coefficients of determination are negative), *i.e.*, plants with the allele from HAR2 shown a higher leaf area under stress than plants with the allele from HA64.

Table 2: Details of the association of the marker with the leaf area response to water deficit. N, number of data points used; *r*, coefficient of correlation between genotype and phenotype; R^2, coefficient of determination between genotype and phenotype, *p-value*, significance of the association between genotype and phenotype.

Generation	N	*r*	R^2	*p-value*
F_2	97	-0,23	0,05	0,02
$F_{2:3}$	48	-0,31	0,09	0,03
F_8	68	-0,35	0,12	0,003

DISCUSSION

These results confirm previous findings, and provide evidence on the stability of a region of the genome associated to the response of leaf expansion to water deficit in sunflower. Not only did the approach consider different generations of the progeny of the two contrasting genotypes, but also different environments, since experimental conditions ranged from controlled growth chambers to less controlled conditions in the greenhouse. Nonetheless, significant association of one marker through all generations under study could be proved.

Water stress affects leaf expansion through its effects on cell division and cell expansion processes. It is therefore expected to affect younger leaves (in which both processes are taking place) differently than older leaves (where only cell expansion occurs). The variables under study in this work were calculated on the basis of the leaf area of the whole plant, therefore confounding the responses of these underlying processes.

A dissection of this variable into a single leaf analysis would allow for a more precise mapping of the character, since an association of leaf expansion parameters only of leaves that were undergoing each process at the moment of the stress treatment could be achieved. This analysis is currently under way. This kind of detailed analysis would be expected to yield results similar to those of Reymond et al (2003) in maize, where distinct QTLs were found for the different parameters of the response model.

The nature of an F_2 population (individuals with unique genotypes) imposes limitations to characterize responses to water deficit so that a novel, single-plant approach had to be used for that generation. Plants were kept at field capacity during a considerable period before the stress treatment and measurements of leaf area were used to characterize control conditions. On the other hand, an experimental design with control and stressed plants, with replicates, could be used for the $F_{2:3}$ and RIL population. The consistency of the results suggests that outputs of both phenotyping methods could be comparable.

CONCLUSIONS AND PERSPECTIVES

So far, one stable molecular marker consistently associated to leaf expansion response to water deficit conditions has been found. Additionally, the results of this work prove that an unconventional and original method can be efficient to obtain phenotypic data. This information could be useful to develop molecular makers for assisted selection in breeding programs oriented to generate new cultivars with improved adaptation to water stress conditions.

A dissection of the present analysis into a single leaf approach is being carried out in order to achieve a more specific phenotype characterization under water stress conditions. Association of leaf expansion parameters of leaves at different growth stages at the moment of the stress treatment might allow a more precise identification of QTL associated with the response of leaf expansion to water deficit.

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