

LOOKING FOR PHYSIOLOGICAL AND MOLECULAR MARKERS
OF LEAF CUTICULAR TRANSPIRATION USING INTERSPECIFIC CROSSES BETWEEN

HELLANTHUS ARGOPHYLLUS AND *H. ANNUUS*

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SUMMARY

A selection of drought tolerant genotypes of sunflower was attempted. Starting from an interspecific cross with *H. argophyllus*, four cycles of divergent selection on a physiological criteria in relation with the cuticular transpiration of leaf (the Relative Water Loss) allow the production of two contrasted genotypes: T- (low level of leaf cuticular transpiration) and T+ (high level of leaf cuticular transpiration). Field experiments show better yield tolerance index combined with good potential yield for T- hybrids in some location. Physiological analysis done in the in field and in controlled conditions allow to distinguish the two genotypes for only one parameter : the osmotic adjustment. Molecular comparison revealed the existence of a cDNA differentiating T- from T+. This cDNA has high homology with a amino-acid transporter. A quantification of the amino-acid concentrations during water deficit in T- and T+ lines, show that the T- plant accumulate significantly more proline than T+ ones. RFLP and STS analysis using this cDNA allow to differentiate the two lines.

Key-Words : drought tolerance, transpiration, osmotic adjustment, physiological markers, molecular markers, *H. argophyllus*

INTRODUCTION

Drought is an environmental constraint very complex. Drought can be of different types (heat, water deficit, insolation...) and can occur through different ways (early or late in the season, continuously or by intermittence...). In general, the effect of drought on plant physiology results in a slowing down of cellular growth (division, enlarging, translocation), alterations of the energetic mechanisms (photosynthesis, respiration) and hormonal

desequilibrium (ABA, cytokinins). All those phenomena lead to a reduction of quality and quantity of products. To face those variety of type of stress, higher plants have developed different tolerance strategies (Cf. Blum, 1988):

1) the *avoidance* consists in the realisation of the life cycle during the favourable period (through earliness or development plasticity)

2) the *deshydration evitment* allows to maintain high hydric potential in the plant, using water saving mechanisms. This can be realised by maintaining water uptake, by reducing water wastes (transpiration) or by cellular modifications (osmotic adjustment, membrane elasticity).

3) The *deshydration* tolerance consists in the resistance *sensus stricto* to low water potential.

Breeding for drought tolerance necessarily implies focusing to one of those mechanisms, which are composed of several polygenic traits (see for a review, Belhassen *et al.*, 1995). The sunflower is a crop well preadapted to drought in comparison with other crops, essentially because of a powerful water uptake due to an efficient root system (Hattendorf *et al.*, 1988). This allow deshydratation evitements in water deficit conditions. However, the water loss are important through intensive stomatal and cuticular transpiration (Chartier and Berthenod, 1971). We have tried to focus on this last trait in order to decrease the water loss of sunflower crop in water limited conditions.

The examination of wild relative species of *Helianthus* genus lead to the identification of some species which are naturally adapted to drought. *Helianthus argophyllus*, a wild annual species growing spontaneously in drought areas of the Texas (USA), is the closest relative of the cultivated sunflower. This wild species had shown easy interspecific crosses abilities with the sunflower even in open pollinated conditions (Belhassen *et al.*, 1994).

Morphological and physiological studies suggest that *H. argophyllus* could be used to improve transpiration of the sunflower (Serieys *et al.*, 1988). Indeed, *H. argophyllus* presents highly hairy leaves and has a lower level of cuticular transpiration estimated by a criteria closed to the Relative Water Loss (RWL) defined on cereals by Clarke et McCaig (1982). *H. argophyllus* leaves had lower RWL than the sunflower ones.

MATERIAL and METHODS

An interspecific cross was realised under controlled conditions between *H. argophyllus* and a sunflower line. Two cycles of divergent selection using the RWL criteria were realised on the F2 genotypes; between each cycle, panmictic crosses were realised in order to encourage genetic mixing (Serieys *et al.*, 1988). Two further cycles of RWL selection followed by self-pollination were carried out for fixation. The genotypes selected for lower and higher RWL were called T- and T+ respectively.

Trial experiment

Crosses between a sunflower line and T- and T+ were realised in order to obtain F1 hybrids and multisite trials were realised in drought areas (10 European and Brazilian sites) under two levels of water supply (irrigated and water deficit conditions). The yield measured in irrigated and dry conditions allow the calculation of the drought tolerant index (DTI) as followed:

$$DTI = \text{water deficit yield} / \text{irrigated yield}$$

Physiological experiment

In order to determine if some physiological mechanisms differentiate the T- and T+ genotypes, lines of the two genotypes were grown in greenhouse in pots under 2 water status (fully irrigated and water deficit evaluated with tensiometers). Analysis of physiological parameters was realised for porometry (photosynthesis, conductance, foliar temperature), water status (RWC, predawn potential), ABA xylemic content and RWL. The RWL test consisted in the excision the uppermost expanded leaf, its weighting after water saturation (Wsat) and the weighting after 4 hours of desiccation (W4h) in a growing chamber where temperature and humidity were controlled. RWL is calculated as followed:

$$RWL = (W_{sat} - W_{4h}) / (W_{sat} - W_{dry}) * 100$$

Evaluation of osmotic adjustment (OA) was realised following the methodology of Chimenti and Hall, (1993). During the water stress period, measurements of osmotic potential (Po) and relative water content (RWC) of the uppermost fully expanded leaves were carried out. The degree of OA was expressed as the value of RWC (named RWCE) for a pre-defined value of Po following the function $\ln(RWC)/\ln(Po)$ fitted to the data.

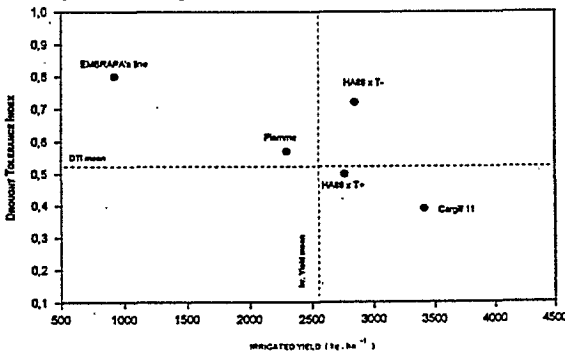
Molecular biology experiment

The molecular analysis consisted in a comparison of T- and T+ gene expression. At the difference with the analysis of drought induced genes, only the constitutive genes were compared in one plant of each genotype sampled in irrigated conditions. Two cDNA libraries of approximately 1.2 millions of clones were constructed in λ gt10. The T+ library was differentially screened on the T- one in order to identify clones which are qualitatively or quantitatively different between the two genotypes.

RESULTS

Trial experiment

In rainfed/irrigated conditions, the T- and T+ hybrids yield correctly in comparison with commercial hybrids (Griveau *et al.* in this issue). This suggest that important heterosis existed since no selection for productivity were realised out on the T- and T+ lines. Furthermore, in the Brazilian site, only T- hybrids present higher tolerance index combined with higher potential yield in comparison with T+ (Figure 1; Guimares *et al.*, 1995).



Physiological experiment

The process allowed to obtain differentiated genotypes for the RWL, supporting the existence of a high heritability of this trait (Figure 2).

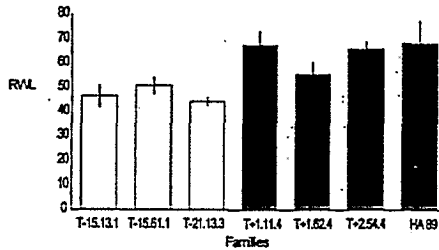


Figure 2: Comparison of T- and T+ families for RWL

While the T- and T+ genotypes were significantly different for the RWL criteria, no difference was observed for the other parameters (Jamaux *et al.*, 1995).

In field conditions, similar physiological evaluations were realised on hybrids of the 2 genotypes. No difference was observed between genotypes compared in similar water status conditions, except for RWL.

Evaluation of the degree of osmotic adjustment (OA) in semi-field conditions reveal significative differences between the T- and T+. The T- individuals present a higher level of OA than the T+ (Chimenti *et al.*, 1995). Analysis of the level of OA and of RWL on the same plants, during progressive water deficit, show the existence of a high correlation between both traits (Figure 3).

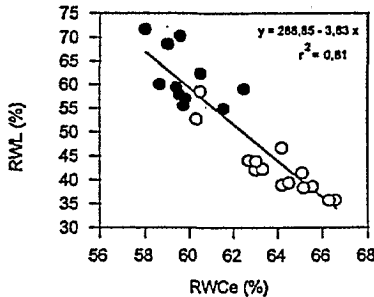


Figure 3: Relationship between level of OA and RWL in T- and T+ genotypes
T+: black dots T-: white dots

Molecular biology experiment

Three cDNA were found to be more expressed in T-. One of them (DRS26) present a high homology with an amino acid transporter isolated in animals. A quantification of the amino-acid concentrations during water deficit in T- and T+ lines, show that the T- plant accumulate significantly more proline than T+ ones.

The research of polymorphism between T- and T+ genotypes were carried out with RFLP tools (using the DRS26 as a probe), RAPD and STS (using DRS26 specific primers). In the

three cases, it was possible to distinguish the T- from the T+ genotypes (Jamaux *et al.*, 1995).

DISCUSSION AND CONCLUSION

The study of two genotypes T- and T+ issued from divergent selection for RWL, show that these two lines differ in the level of osmotic adjustment during water deficit. The higher level of OA has been associated with a higher level of drought tolerance in wheat (Morgan *et al.*, 1986) and in barley (Blum, 1989). OA mechanisms allow deshydration evitement by maintaining the symplasmic osmotic pressure by increasing solute concentration (amino-acids, ions, sugars...).

In our sunflower genotypes, the high correlation existing between the value of RWL and that of OA suggest that the RWL could be an interesting physiological marker in the selection for OA, at least in our interspecific population. Further analysis in other genetic background would provide the range of utilisation of this criteria. Because the RWL evaluation can be easily realised on large sample sizes (300 individuals per day), selection on this criteria could help to obtain « pre-bred populations ». On those populations of smaller sizes, finest evaluation of OA could then easily be done.

The hybrid issued from T- and T+ genotypes had showed in Brazilian location important differences of behaviour. The T- hybrids discarded better yield stability under drought, while irrigated yield were similar with T+ hybrids. Clarke et McCaig (1982) obtain similar results on wheat when selecting on the RWL criteria.

The molecular comparison of T- and T+ lead to the isolation of a cDNA (DRS26) homologous with an amino-acid transporter. In sunflower, the free amino-acid concentration increases significantly during water deficit, and explain 18% of the osmotic potential decrease (Jones *et al.*, 1981). Interestingly, the T- accumulate more proline during water deficit in controlled conditions. In the fields, correlations were found between proline accumulation and DII (El Midaoui, 1993). However, the intervention of proline in OA remain controversial.

The utilisation of the DRS26 cDNA for molecular polymorphism analysis, reveal RFLP and STS differentiation between T- and T+. The use of such molecular markers in selection could provide important help (cf. Monneveux *et al.*, 1996). Further works are necessary to determine the level of linkage between the RWL criteria and those molecular markers. RWL evaluation of genotypes with other genetic background combined with RFLP and STS analysis will provide interesting results.

The analysis of different levels of integration (molecule, cell, organ, whole plant) lead thus to the identification of several traits (DRS26 cDNA, OA, RWL, yield) putatively involved in drought tolerance. The determination of causal effects between traits and adaptation has to be analysed between level of perception. For each trait, we will have to study its effect at the upper level: from the molecule to the cell: is the DRS26 involved in the better degree of OA of T-? From the cell to the whole plant level: is this T- better degree of OA responsible of a better yield stability? Answers to those questions require interdisciplinary approaches, which reflect the complexity of drought tolerance mechanisms.

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