Inheritance and fate of translocations in *H. argophyllus* T.&G. x *H. annuus* progenies.

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SUMMARY

Translocations were inherited in Mendelian ratios in the progenies of the interspecific cross *H. annuus* L. cv RHA 274 (cultivated sunflower) x *H. argophyllus* n°92 and their subsequent BC, TC and TC1 selfed progenies. They led to decreased pollen viability rates according to the expected value of one-half for one translocation and three-fourth for two translocations. After selfing, translocations segregated and each translocation was isolated in a progeny. Their transmission occurred with severe distortion not predicted by the model. Moreover tetravalent frequency - another estimate for presence of a translocation - was not correlated with pollen viability. Tetravalent frequencies varied in sister progenies of this interspecific cross, based upon the size of translocated fragments leading to suspect rapid rearrangements around translocation points. This was supported by a molecular study of translocation ends marked with RAPD in the interspecific progenies. We observed that pollen variability variance explained by one marker in BC1 was no more effective in further generations , whereas some neutral markers in BC_1 were further linked with translocations. This means that recombination occurred between translocation ends and the marker loci. Concerning plant breeding goals, introgression patterns are proposed to optimise gene transfer from wild to cultivated forms.

RÉSUMÉ

Les translocations sont héritées comme des facteurs mendéliens dans le croisement interspécifique *H. annuus* L. cv RHA 274 (tournesol cultivé) x *H. argophyllus* n°92 dans les BC, et dans les descendances en autofécondation des TC et TC1. Elles diminuent la viabilité pollinique de 50% pour une translocation, et de 75% pour deux translocations. Après une autofécondation les deux translocations se séparent dans les descendances. Elles se transmettent avec une distorsion forte qui n'est pas prévue par le modèle de transmission. De plus, la fréquence des tétravalents, une autre façon d'estimer le nombre de translocation, n'est pas corrélée à la viabilité pollinique. La fréquence des tétravalents est différente pour les descendances sœurs d'un croisement, ce qui laisse supposer que des réarrangements se produisent au voisinage des points de translocation. Ceci est en accord avec le marquage moléculaire par des RAPD des translocations. Nous avons observé qu'un marqueur lié à la viabilité pollinique en BC_1, ne l'est plus dans les générations suivantes. Ceci suggère donc qu'une recombinaison se soit produite entre le marqueur et le point de translocation. Compte tenu de ces mécanismes un schéma d'introgression est proposé pour transférer un caractère favorable de l'espèce sauvage au tournesol.

INTRODUCTION

Cultivated sunflower and eleven annual species belong to the genus *Helianthus*, section *Helianthus*. All annual species and cultivated *H. annuus* L. are diploid (2*n* = 34) and outcross. The structural relationships of chromosomes in sect. *Helianthus* were defined by chromosomal rearrangements between species revealed by interspecific hybridization (HEISER 1951a, 1951b, 1961; WHELAN 1979; FERRIERA 1980, CHANDLER *et al*. 1986). Some chromosomal rearrangements such as tandem fusions, reciprocal translocations, centric fusion or fission, pericentric and paracentric inversions have the capacity to drastically reduce cross fertility because they completely disrupt meiosis.

In the present study, we modelized the segregation patterns of translocations in the interspecific cross between *H. annuus* L.cv RHA274 (cultivated sunflower) and *H. argophyllus* and their impact on hybrid fertility of progenies. The two translocations were mapped. These regions induced post-zygotic reduction of pollen viability and prezygotic delay in flowering time. Moreover, we followed the translocations and their inheritance in progenies to understand the behaviour as a reproductive barrier and their resistance to introgression in the heterozygous and the homozygous state.

MATERIAL AND METHODS

Plant material: The BC_1 population was obtained by crossing a unique F_1 interspecific hybrid plant used as the female parent with RHA274. Testcross (TC1) and testcross (TC2) progenies were derived from the BC_1 [(*H. argophyllus* n°92 x *H. annuus* cv RHA274) x *H. annuus* cv RHA274] hybrid plant used as the male parent crossed onto cultivated CMS PET1-HA89 line. Selfing the TC1 plants led to TC1_S1 generation.

Selection of low pollen viability individuals: We exerted a selection pressure on pollen viability variability by selecting seven BC_1 plants with low pollen viability for further backcrossing. Four populations produced enough individuals to be studied. Forty genotypes per TC1 family derived from BC_1 plants were scored for pollen viability and screened for presence/absence of markers. TC1 genotypes with variable pollen viability displaying one or several marker(s) involved in pollen viability were chosen for backcrossing with cultivated sunflower (CMS-HA89) and selfing.

Statistical analysis: Pollen viability, quadrivalent (IV) numbers and interaction effects were tested by an analysis of variance ($P<0.001$), and estimated translocation number was defined as Pollen viability + IV + Interaction. Variation in multivalent occurences in the BC_1 PMCs was checked using the Chi-square analysis. In progenies, the expected number of translocations was estimated using the pollen viability distribution of each progeny. Phenotypic classes were first arbitrarily determined using one mode per class. Mean value and standard deviations for each class were calculated for pollen viability values (and not frequencies). Conformity t-test was performed between each mean value and expected value which was 25% for class I, 50% for class II and 100% for class III. The association between each genetic marker and pollen viability was detected by analysis of variance in all progenies using the SAS GLM procedure. A multiple-regression analysis, including all the significant markers was performed using the SAS REG procedure. Linkage between loci was tested in progenies using SAS FREQ (SAS Institute) procedure with the CHISQ option. Effects were determined to be significant when the probability levels associated with F-values were less than 0.0001. Pollen viability distributions differed significantly from normality based on the value of Shapiro-Wilk statistic calculated using the SAS univariate procedure (SAS Institute).

We thus used a log or arcsin transformation according to phenotypic distribution.

RESULTS

Pollen viability observations: In parental genotypes, RHA274 and *H. argophyllus* pollen viability were approximately 95% (Table 1). The F_1 hybrid plant displayed about 25% pollen viability, whereas BC_1 progenies derived from F_1 hybrid plant and cultivated RHA274 had pollen viability values ranging between 27 to 93%. Pollen viability distribution in BC_1 plants and progenies was measured. These distributions deviated from normality by at least two standard deviations.

Model for translocation inheritance: In the F_1 pollen mother cells (PMC), we observed either quadrivalent pairing or rod bivalents pairing at meiosis (Table 1). Considering the plurimodal distribution of pollen viability in the progenies, mean value for each phenotypic class (displaying one mode) has been tested for its conformity with theoretical values, 25% for class I, 50% for class II and 100% for class III. Thus, using the positive conformity test, we assumed progenies, that displayed trimodal, bimodal, and unimodal pollen viability distributions carried two, one, and zero translocations, respectively.

TABLE 1 : Expected number of translocations according to theoretical estimates.

Pollen viability percent was obtained by scoring 400 pollen grains per genotype. Confidence limits for proportions at 1-α = 99% are reported in parenthesis. Frequency of one quadrivalent (IV) and two quadrivalents (IV) per cell in BC_1 hybrid plants in comparison with F_1 and parents are reported. PMC = pollen mother cells.

Genotype	Number of PMC	Pollen viability (%)	IV	2IV	Expected number of translocations
RHA274	21	93 (89-96)	0	0	0
H. argophyllus	30	95 (92-97)	0	0	0
F1	23	25 (20-31)	9	4	2
BC1-1	16	27 (22-33)	19	0	2
BC1-3	11	32 (25-38)	18	9	2
BC1-4	17	54 (50-60)	12	0	1
BC1-5	29	90 (85-95)	0	0	0
BC1-10	23	88 (83-92)	0	0	0
BC1-14	15	83 (77-88)	0	0	0
BC1-16	13	36 (30-42)	8	0	1 or 2
BC1-17	27	93 (89-96)	4	0	0
BC1-21	20	75 (69-80)	10	0	0
BC1-22	11	63 (57-69)	0	0	0 or 1
BC1-25	11	40 (34-44)	27	0	1 or 2
BC1-29	31	27 (22-33)	3	0	2
BC1-31	27	59 (52-63)	22	0	0 or 1

Conversely, the number of translocations in the BC_1 plants was estimated using the pollen viability value and its confidence limits encompassing the theoretical values. Thus, the variability in the frequency of quadrivalents is questionable.

Observations in TC1_S1: In the TC1_S1 progenies derived from BC_1 selfed plants, pollen viability distributions were described. Distributions were unimodal in five progenies, bimodal in three progenies (TC1_1-41S1, TC1_1-62S1 and TC1_16-31S1) and trimodal in one progeny (TC1_2-37S1). The phenotypic classes were arbitrarily defined and a conformity t- test allowed an estimation of translocations for five progenies (TC1_1-27S1, TC1_1-35S1, TC1_1-41S1 TC1_1-43S1 and TC1_16-51S1) (Table 2). In other progenies, mean pollen

viability value for each class was significantly different from theoretical values.

TABLE 2 : Translocation distribution in progenies based on theoretical estimates.
Mean pollen viability and standard deviation are reported for each phenotypic class.

Genotype	Pollen viability Mean number per class (Standard deviation)	Expected number of translocations
TC1_1-27S1	95 (7.5)	0
TC1_1-35S1	100 (0)	0
TC1_1-41S1	58 (9.6) -98 (3.8)	1
TC1_1-43S1	89 (8.2)	0
TC1_1-62S1	89 (13.9)	0 or 1
TC1_3-37S1	40 (0)-60 (7.4)-95 (7.6)	1 or 2
TC1_16-3S1	50 (0)-89 (13.6)	0 or 1
TC1_16-31S1	65 (10.9)-93 (5.1)	0 or 1
TC1_16-35S1	70 (10.0)-99 (1.0)	0 or 1
TC1_16-51S1	87 (12.9)	0

Following molecular markers genetically linked to translocations (Table 3): Multiple regression analysis revealed significant phenotypic variation for the A11_8 and B10_12 loci suggesting that they were near translocation breakpoints. TC1_3-37S1 should carry two translocations, TR1 and TR2, identified by loci A11_8 and B10_12, respectively. It thus appeared that linkage group1 characterised by A11_8 was involved in TR1 and both linkage groups 2 and 3 characterised by P5_8 and C7_5/B10_12 respectively, were involved in TR2.

TABLE 3 : Selected loci in selfed TC1_S1 progenies
The mean pollen viability values of each segregating class are reported. Values in parenthesis represent the distribution of the number of plants in each segregating class. To determine molecular markers selected in progenies, segregation at each locus was checked against the expected 3:1 ratio (except for A11_8, 1:1 ratio) using a chi-square test, $P<0.001$. R^2 is phenotypic variation explained by multiple regression analysis. Asterisks represent probability levels associated with F-values (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

Genotype	Pollen viability	Selected loci in progenies	R^2	Number of translocations	Other selected markers	R^2
TC1_1-27S1	95 (7.5)	P5_8	ns	0		
TC1_1-35S1	100 (0)	P5_8	ns	0		
TC1_1-41S1	58 (9.6) -98 (3.8)	A11_8	26	1		
TC1_1-43S1	89 (8.2)	P5_8	ns	0		
TC1_1-62S1	89 (13.9)	P5_8/C7_5	ns/52*	1		
TC1_3-37S1	40 (0)-60 (7.4)-95 (7.6)	A11_8/P5_8/B10_12	48*/ns/12*	2	A11_7	ns
TC1_16-3S1	50 (0)-89 (13.6)	A11_8	47*	1		
TC1_16-31S1	65 (10.9)-93 (5.1)	A11_8	ns	0 or 1		
TC1_16-35S1	70 (10.0)-99 (1.0)	P5_8/B10_12	ns	0 or 1		
TC1_16-51S1	87 (12.9)	P5_8/C7_5	ns	0		

Following molecular markers of other linkage groups: A11_7 was likely selected whereas other markers were eliminated in TC1_3-37S1.

Observations in TC1 : In the three TC1 progenies (TC1_1, TC1_3 and TC1_16), pollen viability distribution was trimodal which lead to the description of three phenotypic classes (Table 4).. Class I was not statistically different from 25% (t- test for p=0.99), class II was not statistically different from 50% (t- test p=0.99) and finally class III was not statistically different from 100%. These results inferred the existence of two translocations in TC1_1, TC1_3 and TC1_16. TC1_31 pollen viability distribution that was bimodal leading to the description of two phenotypic classes, that inferred the existence of one translocation.

TABLE 4 : Selected loci in backcrossed TC2 progenies.

The mean pollen viability values of each segregating class are reported. Values in parenthesis represent the distribution of the number of plants in each segregating class. To determine molecular markers selected in progenies, segregation at each locus was checked against the expected 1:1 ratio (except for A11_8, 1:3 ratio) using a chi-square test, P<0.001. R^2 is phenotypic variation explained by multiple regression analysis. Asterisks represent probability levels associated with F-values (* P<0.05, **P<0.01, ***P<0.001).

Genotype	Pollen viability Mean number per class (Standard deviation)	Translocation linked markers	R^2	Number of trans-locations	Other selected markers	R^2
TC1_1	30(11)-56(13)-90(8)	A11_8/P5_8/B10_12/C7_5	34*/34*/ns/13*	2	A11_7	ns
TC1_3	27(11)-58(11)-90(9)	A11_8/ P5_8/C7_5	36*/15*/ns	2	B10_9/A11_7	ns
TC1_16	36(2)-56(10)-87(11)	A11_8/P5_8/B10_12	15*/ns/14*	2	B10_9/C7_7	ns
TC1_31	61(13)-93(6)	B10_12/P5_8	19**/ns	1	B10_9	ns

Observations in TC2:

Pollen viability distribution was trimodal in the TC2 progenies which lead to the description of three phenotypic classes. These results suggest the existence of two translocations. Markers positively selected in these progenies supported these estimates (Table 5). A11_8 and P5_8/B10_12 explained highly significant pollen viability variation in the two progenies.

TABLE 5 : Selected loci in backcrossed TC1 progenies.

The mean pollen viability values of each segregating class are reported. Values in parenthesis represent the distribution of the number of plants in each segregating class.To determine molecular markers selected in progenies, segregation at each locus was checked against the expected1:1 ratio (except for A11_8, 1:3 ratio) using a chi-square test, P<0.001. R^2 is phenotypic variation explained by multiple regression analysis. Asterisks represent probability levels associated with F-values (* P<0.05, **P<0.01, ***P<0.001).

Genotype	Pollen viability distribution	Translocation linked markers	R^2	Number of translocations
TC2_3	32(5)-53(8)-93(3)	A11_8/P5_8	64***/15**	2
TC2_16	38(1)-69(10)-94(8)	A11_8/B10_12/P5_8	69***/26***/ns	2

BC_1 expected according to progenies observations: The number of translocations in BC_1 plants was first estimated according to pollen viability values and their confidence limits as described below which did not allow estimates of translocations in all BC_1 plants (Table 1). Based on inheritance of translocation-linked markers in progenies, the number of translocations should have been predicted (Tables 4-5). This confirmed the previous estimate according to the pollen viability value (32%) and was supported by the two quadrivalents observed. Markers linked to the two translocations were present in TC2_16 (A11_8, B10_12 and P5_8) and TC1_16 (A11_8, B10_12 and P5_8) progenies derived from the BC1_16 plant with a significant effect on pollen viability variation indicating the existence of two translocations in BC1_16. This clarified the estimate based on the pollen viability value (36%), but did not agree with the one quadrivalent observed. Markers linked to TR2 were present in the TC1_31 population derived from the BC1_31 plant indicating the existence of one translocation in BC1_31. This clarified the estimate based on the pollen viability value (59%) and agreed with the one quadrivalent observed.

DISCUSSION

Theoretical behaviour of translocations and their impact on hybrid fertility. Translocations affecting two chromosome pairs leads to three segregating classes of heterozygous and homozygous progenies (1:2:1). Class I (double heterozygote progenies) results in 25% of unbalanced gametes, class II (simple heterozygous progenies) in 50% of unbalanced gametes, and class III (homozygous progenies) in 100% of balanced gametes, respectively. The multimodal distribution of pollen viability observed in progenies results from the segregating classes.

Genomic localization of translocations and their environment: It is assumed that TR2 is located on LG2 and LG3, and markers used to follow TR2 inheritance encompass this translocation. Moreover, adaptative and/or phenology loci such as those for divergence in flowering time (BLM), leaf number (LEAN), or plant height (PHEI) were identified on the BC_1 map (QUILLET 1995). Linkage groups explaining the highest variation were LG1 (13% for BLM), LG2 (25% for LEAN, 24% for BLM and 18% for PHEI) and LG3 (20% for LEAN), the same linkage groups as those linked to translocations.

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