CHIMERA INDUCTION IN SUNFLOWER (HELIANTHUS ANNUUS L.). ANALYSIS OF SECTOR PATTERNS FOR DIFFERENT GENETIC MARKERS.

Franco Cecconi, Mauro Durante. *Pisa University, Dipartimento di Biologia delle Piante Agrarie sez. Genetica.Via Matteotti 1/b 56124 – Pisa Italy. Fax +39-050-576750 ; e-mail: fcecconi@agr.unipi.it*

Raul Srebernich, Nestor Luciani. *Agromania S.A., Estado de Israel 190 – 3100 Parana (E.R) Argentina.Fax +54-3434-350264*

Summary

In order to study the fate of cells during shoot meristematic growth of sunflower, clonal analysis was performed.

In sunflower the shape and the large size of capitulum facilitates the recovery and description of mutated sectors, furthermore as mutation breeding is considered, the knowledge of chimera development in relation to the capitulum differentiation, helps the work of sunflower breeders in order to decide about the most efficient system of collecting seeds from mutagen treated plants for the subsequent mutant screening

F1 seeds were obtained by crossing the cytoplasmic male sterile line AD-811 with 3 restorer lines carrying 3 different markers for epidermis pigmentation. All the genotypes used in this study were inbreed lines selected at the Department of Plant Biology of Pisa University.

About 3000 dry seeds for each cross were irradiated with 15 KR of hard x-rays, (200Kwp, 2kRad/min) which from preliminary experiments were known not to damage plant development. Chimerical plants were scored and classified considering the pigmentation of florets and pericarp. Mutation frequency was 8,14% for achene colour, 3,6% for floret colour, 2,2% for anthocyanin pigmentation of the stigma and 0,18% for fertility restoration.

The analysis of the different patterns of mutated sectors and their relative frequencies indicate that the apparent cell number (ACN) contributing to the capitulum development is 2 or 4, while the analysis of the shape and the size of mutated sectors indicates the possibility of cell rearrangement during cell meristematic growth.

Key words: Sunflower, Chimera, Clonal analysis, Diplontic selection

Introduction.

The induction of sectorial chimera and the analysis of the different patterns of mutated sectors have been successfully performed in order to determine the initial cell number contributing to the flower and/or inflorescence formation of various plant species. (Poethig R.S. 1987). The methods consist in the treatment of seeds and/or plantlets, heterozygous for visible markers, with either chemical or physical mutagens to induce a mutation of the dominant allele (generally the loss) in one of the initial meristematic cells. The subsequent comparative analysis of the shape and distribution of the mutated visible clones in the tissue where the mutation is expressed, indicate besides the initial cell number contributing to the formation of the considered organ, even the possible model of cell organization during its development and differentiation (Balkema G.H. 1971)

In sunflower the shape and the large size of capitulum facilitate the recovery and description of mutated sectors, furthermore as mutation breeding is considered, the knowledge of chimera development in relation to the capitulum differentiation, helps the work of sunflower breeders in order to decide about the most efficient system of collecting seeds from mutagen treated plants for the subsequent mutant screening (Hermelin T. et al. 1987).

In a previous work I indicated the possibility of layer rearrangement during shoot meristematic growth (Cecconi et al. 1992). On the basis of this hypothesis and with the aim to add more informations on the knowledge of M1 chimera formation of sunflower, clonal analysis was performed considering different genetic markers controlling epidermis pigmentation of pericarp and florets.

Materials and methods.

F1 seeds were obtained by crossing the cytoplasmic male sterile line AD-811 with 3 restorer lines carrying 3 different markers for epidermis pigmentation (tab.1). All the genotypes used in this study were inbreed lines selected at the Department of Plant Biology of Pisa University.

Tab.1: Phenotypic characterisation of the inbreed lines used.
C.m.s.:Cytoplasmic male sterile line. R: Restorer line

	Lines			
Character	D-811	R-GB223	R-GB11	R-GR234
Achen color	Black	White	Black	Black
Flower color	Yellow	Yellow	Lemon	Yellow
Stigma color	Yellow	Yellow	Yellow	Anthocian.
Fertility	C.m.s	R	R	R

About 3000 dry seeds for each cross were irradiated with 15 KR of hard x-rays , (200Kwp, 2kRad/min) which from preliminary experiments were known not to damage plant development.

The 3 sample of irradiating achenes were then seeded in 3 separated blocks on the open field. Emergence was good, M1 seedling initially grew slower than the control plants presenting somewhat deformations on the first leaves, however, at maturity plant height and capitulum diameter were similar to those of control plants (data not shown).

From the visible head stage to maturity of seeds, capitula were scored weekly for the

presence of mutated sectors considering each genetic marker introduced by the cross. The mutation frequency was calculated for each character, as the ratio of the total number of chimerical plants to the total number of scored plants.

In order to classify each mutated sector for its size and shape all chimerical capitula were photographed.

Since the genetic markers used in this experiment were expressed only in the capitulum and its edge represented the visible basal terminus of the sector, the initial cell number was calculated dividing the circumference of the head by the part of the circumference subtended by the mutated sector.

To verify the segregation ratio for each genetic marker utilised in the experiment, Chi-square test was performed.

Results and Discussion

Genetic analysis and mutation frequency.

Genetic analysis, reported in table 2, indicates that the genetic control of the 3 markers analysed in the experiment is of monogenic type: the F2 population has a good fit to the expected ratio 3:1.

Tab.2: Genetic analysis and percentage of Chimeric plants

Character	Female	Male	F1	F2 segreg.	Segr.Ratio	Chim.F1-Pl.
Achen color	Black	White	White	413W-132B	3:1 p<0,01	8,14%
Flower color	Yellow	Lemon	Yellow	149Y-37L	3:1 p<0,01	3,60%
Stigma color	Yellow	Anthoc.	Anthoc.	302A-98Y	3:1 p<0,01	2,20%
Fertility	Sterile	Fertile	Fertile	515F-163S	3:1 p<0,01	0,18%

According to Mosjidis J.A. (1982), the absence of pigmentation in the achene epidermal tissues resulted dominant over its presence: F1 plants produced colour less (white) achenes. In this case, since the pericarp tissues are of maternal origin, mutated sectors appeared with black coloured achenes (fig.1-a). Over 3120 M1 F1 plants, 214 were chimeric for this character resulting a very high mutation frequency of 8,14%.

As reported by Fick G.N. (1978) the yellow colour of ligulate and tubulate flowers resulted dominant over the lemon colour and at flowering M1 chimeric plants appeared with sectors of lemon ligulate flowers, the mutation frequency was 3,6% over 2891 M1 plants.

As expected the anthocyanin pigmentation of the stigma resulted dominant (tab.2), the monogenic control of this character, is not in agreement with previous studies of Fick G.N. (1978) and Mosjidis J.A. (1982), who found a genetic control determined by 3 independent genes with cumulative effects. In our case the stigma pigmentation of heterozygous F1 plants was not different from that of the male parent carrying this marker, furthermore mutated sectors of chimeric plants appeared always with yellow stigma as in fig.1-c This indicate the presence of a dominant major gene controlling this character, the mutation frequency of this marker was 2,2% over 2452 M1 plants.

Finally over the 8432 M1 plants resulting from the 3 crosses analysed, were found 15 plants with sectors of male sterility (0,18%), this result is in agreement with the loss of the dominant restorer gene being all M1 plants heterozygous for this character.

The frequency of chimeric plants was much higher than the mutation rate expected for a recessive change in a single gene, but in agreement with the probability to induce a break in a chromosome arm (Brock, 1979). The observed mutated sectors were thus probably due to

deletions and considering the differences in mutation frequency, it may be assumed that the 3 genes controlling epidermis pigmentation are probably located near telomers of different chromosomes, while the gene of the restoration fertility is more linked to the contromere and may be loss only when a large deletion occurs.

Cell lineage pattern of chimeric capitula.

The diagrammatic representation of chimeric capitula, classified for the shape and the relative size of mutated sectors is showed in tab.3. On the first column of the table are reported the expected patterns of chimerism,: when at the time of irradiation (dry seed) the initial cell number is 2, assuming that the sector arises from a single mutated cell, chimeric capitula have an half mutated part, this pattern was observed on 10% of the cases. When the initial cell number is 4, the sectors are extended for ¼ of the surface (17,5% of the cases). If the initial cell number is more than 4, the sectors appear smaller and may be extended from the centre to the edge of the capitula (15% of the cases) or not (10% of the cases) depending on the disposition of the initial cells on the shoot apex. This type of regular sectoring covers 54% of the cases (frequency sum of the first column), the remaining 46% of chimeric capitula may not be explained considering only this simple model of cell lineage organisation.

Tab. 3: Observed chimeric patterns

I.N.	Sector frequency and shape
2	10 % ; 15 % ; 5 %
4	17,5 % ; 5 % ; 10 %
+4?	15% ; 10 %
----	10 %

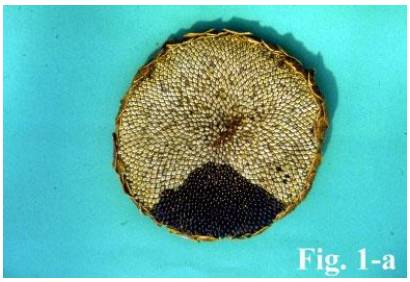


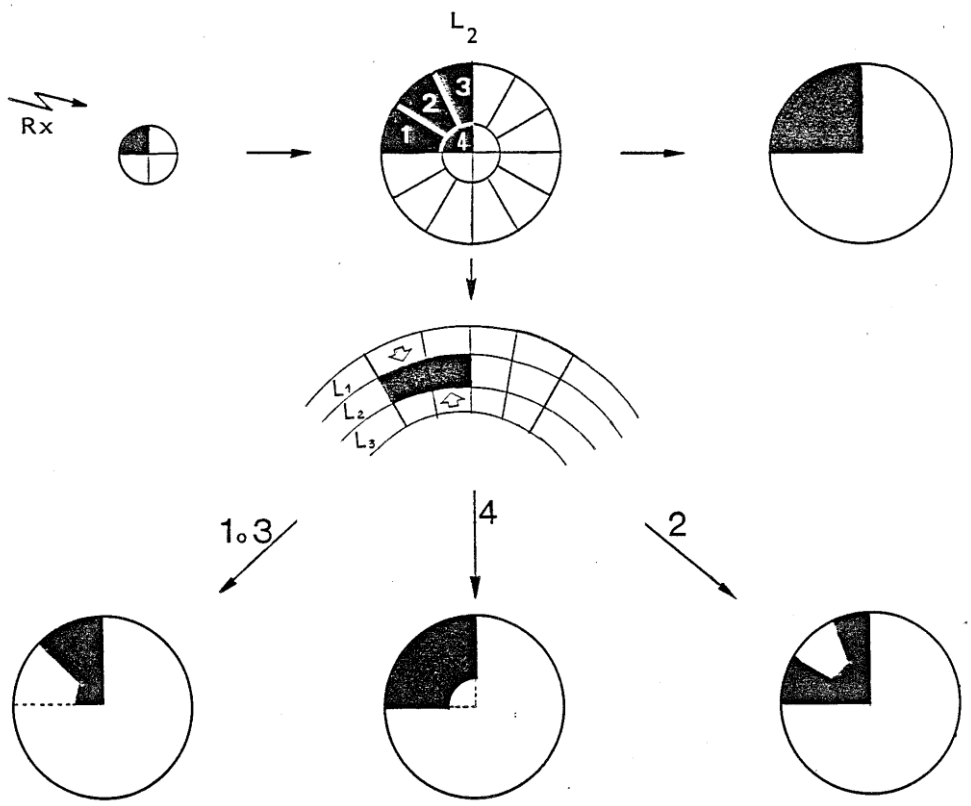
Fig. 1-a


Fig. 1-b


Fig. 1-c

The first row of this picture illustrated the normal sector development when mutation occurs in one of the four initial cells of the shoot apex at the moment of irradiation. If during the growth of the mutated clone, some cellular reorganisation takes place, different patterns of sectoring may be observed: the substitution of a mutated cell by a normal cell coming from L1 or L3 layer, results in a capitulum where the chimeric pattern of ¼ become irregular depending on which cell go to be substituted. On fig.1-d and fig.1-e are reported some photos of capitula with chimeric patterns very similar to those reported on the fig.2 when the cells n°1 or 2 are substituted by normal "white" cells during restructuration layers.

Fig. 2: Models of cell rearrangement of mericlinal chimera.



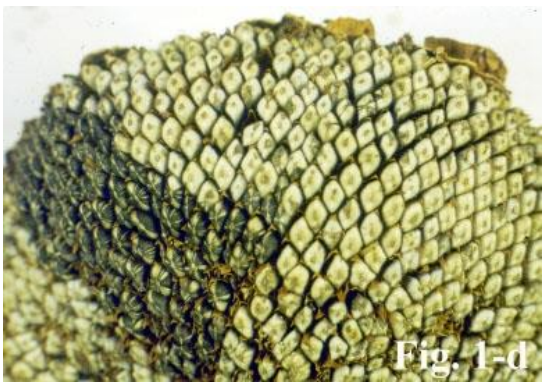


Fig. 1-d

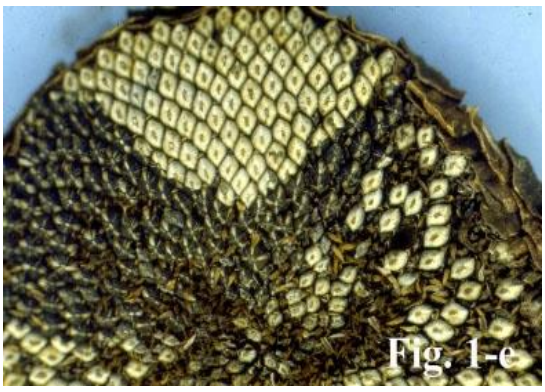


Fig. 1-e

Conclusion

The large population examined permitted an estimate of the number of cells in the apex of dry sunflower seed. Our observation that mutated sectors represent ½ to ¼ of the capitulum circumference can be interpreted as evidence that there are from two to four initial cells in the apex primordium which take part in capitulum development. A possible scenario is that the nucleus of the initial cell is damaged by the radiation while in the DNA pre-synthesis phase (G1). In a nucleus after DNA synthesis (G2) a mutation would be induced at the chromatid level and after two mitoses only one of the four daughter nuclei would show the genetic change. Our results are in agreement with those obtained by Balkema G.H. (1971) who found that chimeric capitula, developing after colchicine treatments, had polyploid sectors ranging from ¼ to ¾ of its total area. As to the disposition of the initial cells in the dry seed embryo, it is clear that in the case of four initials, the cells are arranged as quadrant considering also that the pattern of sectoring may be modified during development by intrusion of normal cells from the layers of the shoot apex in which originally there were two or four initial cells.

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