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Chimerism of the tomato plant after seed irradiation with fast neutrons*

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Summary

Mutagenic irradiation of tomato seeds leads to a chimeric structure of the M_1 plants, also within the generative or sporocyte tissue. This chimerism was studied by harvesting seeds from the first two trusses of the main stem and of all side shoots below the second truss. The initial cells of each of the lower 5–7 side shoots are already laid down in the embryo, resulting in different mutant types in each of these side shoots. The plant part above approximately the 7th side shoot originates from the apical initial cells of the embryo, and from the 7th side shoot upwards sectors are formed from each cell in the growing-point. The higher in the plant, the smaller the number of cells forming that plant part. The number of sectors in the plant diminishes from 2–3 sectors at the level of the 7th leaf up to 1 and occasionally 2 sectors in the second truss. This suggests that the main growing-point contains 3 initial cells for forming the sporocyte tissue. To obtain the largest possible number of mutant types, seed should be collected from the lower plant parts.

Introduction

After mutagenic treatments of tomato seeds with ionizing irradiation, a number of cells in the embryos will be mutated, including the meristematic cells which form the M_1 plants. If these mutated meristematic cells are capable of growing and dividing along with the surrounding cells, each cell will give rise to a mutated part or sector of the M_1 plant and this sector is labelled by the mutation. In the sporocyte or sub-epidermic layer, which forms the pollen and egg cells of the M_1 plant, mutations can be scored from the segregation of recessive mutants in the next or M_2 generation. Several different types of mutants can be found in the progeny of a single M_1 plant, indicating the M_1 plant to be highly chimeric. The aim of the present research was to study this chimerism by following the sectors. It is a more detailed continuation of the research of Hildering and Verkerk (1965). Hildering and Verkerk harvested only fruits of the trusses along the main stem of the M_1 plants to study the M_2 generation. It was concluded that if a chimeric structure occurs, it is generally found in the lower part of the plant, up to the second truss. Most sector differences were found between truss one and two. As this is a reflection of the sympodial growth habit of the tomato plant, in

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which truss one is the top of the main stem, truss two the top of a side shoot of the main stem, and so on, further research on all side shoots below the second truss seemed worthwile.

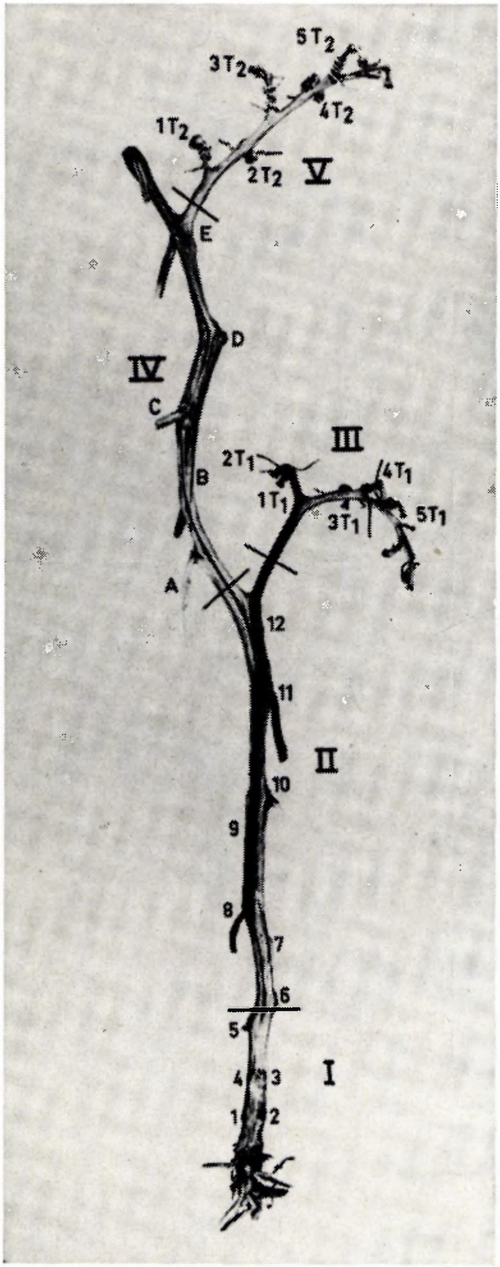

Fig. 1. M_1 plant with sampling-points and blocks.

Materials and methods

Seeds of the tomato cv. 'Moneymaker' were irradiated with 3 or 4 krad of fast neutrons from reactor BARN of the ITAL at Wageningen, at a dose rate of 1 krad/h and an 8 % gamma contamination. Some seeds were left untreated to serve as controls.

The *M_1 generation* was grown in a greenhouse during summer and good growth took place. The plants were topped at some leaves above the second truss to induce the formation of many side shoots. When these side shoots were 10–15 cm long they were removed, labelled for position on the 'mother plant' and put in a mixture of soil and sand for rooting. Two to three weeks later the rooted cuttings were planted out.

The seed harvest consisted of the first 5 fruits of trusses 1 and 2 of the mother plant separately and of 3–5 fruits together of each of the first two trusses from each of the side shoots (Fig. 1).

The side shoots below the first truss, including the 2 cotyledon side shoots, are numbered upwards as 1, 2, 3, etc. The side shoots in the axils of the leaves between the first and second truss are denoted upwards by A, B, C, D and E. In the first and second truss of the mother plant the fruits are numbered from the stem side $1T_1$, $2T_1$, $3T_1$, $4T_1$, $5T_1$, and $1T_2$, $2T_2$, $3T_2$, $4T_2$, $5T_2$, respectively. Groups of these so-called 'sampling-points' were combined into 'so-called' blocks (Fig. 1).

The *M_2 generation* was studied during the next winter. From each seed lot 24 seeds were sown, if available. The seedlings were grown for a period of three weeks, because during this period the M_2 plants were small enough to grow large numbers. Ten days after sowing, the cotyledons and after another ten days, the first two to three leaves were scored for visible mutants. The types of morphological and colour mutants found were topographically labelled on the M_1 mother plants. M_1 progenies from 167 mother plants of treated seeds and 8 control progenies were tested.

Results and discussion

M_1 generation. We can be brief about the M_1 generation for aside from a somewhat retarded growth, the M_1 plants looked normal.

M_2 generation. Mutated tissue within the plant. The frequency of mutated tissue from the different side shoots and fruits of the main trusses was the same, about 33 % (highest 39 %, lowest 24 %), which seemed to indicate that all the cells in the embryo giving rise to the generative tissue, were equally sensitive.

Singles and sectors

A mutant type found only in one side shoot or mother plant fruit sampling-point is without 'vertical distribution' and will be called single; if the same mutant type is found in different sampling-points along the mother plant, such a mutant type has a vertical distribution and will be called a sector. This distinction between mutant types forming singles and sectors has been made because mutant types without vertical distribution, the singles, cannot give us any information about different plant parts arising from one cell, while the mutant types with vertical distribution, the sectors, can. The sectors give more detailed information on the morphogenesis of the mother plant. If singles are found, this means in general, that a mutated cell has given rise only to that particular plant part or sampling-point, where that mutant type has been found. A sec-

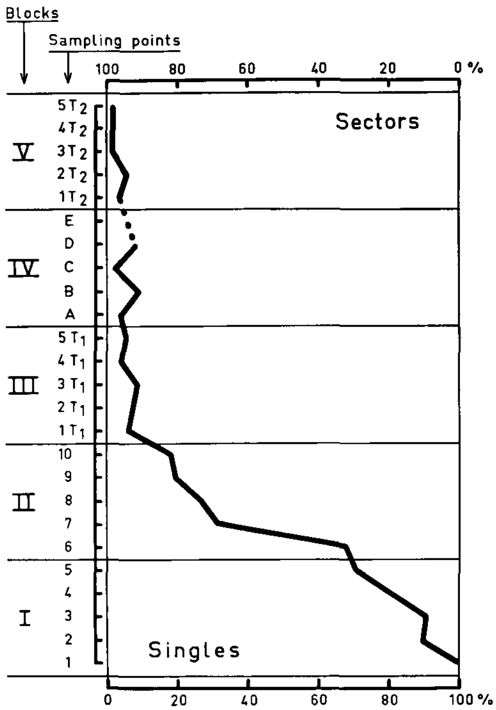

Fig. 2. Distribution of singles (left) and sectors (right).

tor, however, shows that all those sampling-points, where the sector is found, originally came from one mutated cell in the embryo.

Fig. 2 gives the distribution of the singles and sectors in the plant as the percentage of the total amount of mutant types found at each sampling-point. We can roughly distinguish three zones in the plant:

a. sampling-points 1–5 (block I), mainly showing singles;
b. sampling-points 6–10 (block II), showing a transitional stage to
c. sampling-points in and above the first truss (blocks III, IV and V), showing nearly 100 % sectors.

Where do the sectors start?
The majority of the sectors start in the transitional stage of the sampling-points 6–9 (block II), which is clearly demonstrated in Fig. 3.

Where do the sectors go to?
Each sector was scored in the block or combination of blocks in which it was found and those blocks or combination of blocks containing more than 5 % of the total number of sectors are given in Fig. 4. The percentages of the different columns of this figure are expressed by their widths. Differences between block IV and V were not found, so the whole part 'above T_1' can be considered as one double block (IV + V). It should be noted that:

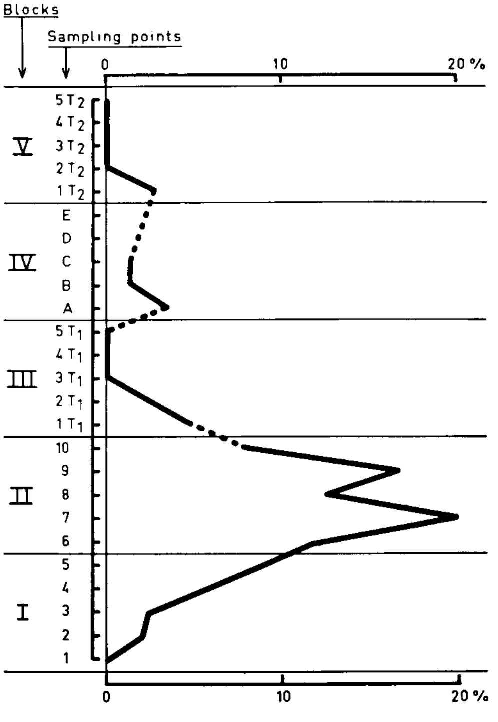

Fig. 3. Sectors starting in the different sampling-points (%).

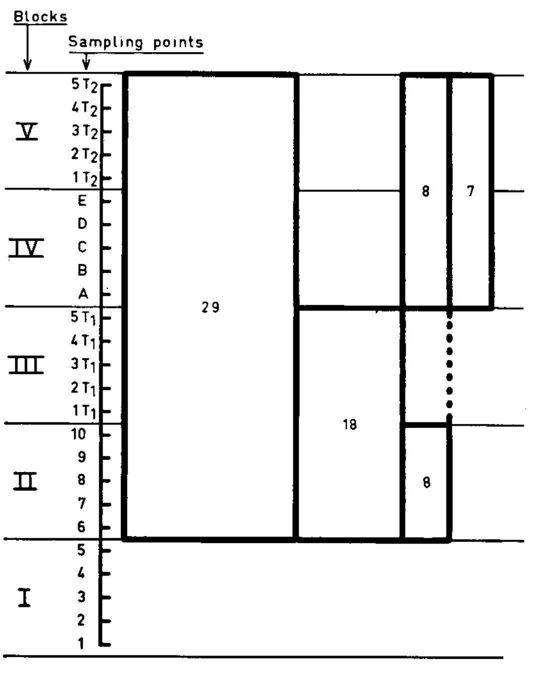

Fig. 4. Block distribution of sectors (%).

- the first block is not found in this figure as it did not contain many sectors;
- there are two wide and two narrow columns, namely:

a. sectors found below, in and above truss one (29 %);
b. sectors found below and in truss one (18 %);
c. sectors found below and above truss one (8 %);
d. sectors found only above truss one (7 %).

First truss
The percentages of sectors found in fruits $1T_1$, $2T_1$, $3T_1$, $4T_1$ and $5T_1$ of truss one, also found in the main side shoot (blocks IV + V), are 82, 61, 58, 55 and 50, respectively. This shows that the first fruit of truss one, $1T_1$, is more closely related to the main side shoot than the other fruits of the first truss.

Estimation of the number of initial cells forming the genetic tissue above the transitional stage
The number of sectors in the different plant levels which contained mutated sectors, was estimated. This estimate was converted to a percentage distribution and is shown in Table 1. Table 1 indicates that the smaller number of sectors was scored higher in the plant.

A second conclusion is that the terminal growing-point in the embryo contains three initial cells for the formation of the generative or sub-epidermic layer in the plant, since the highest number of sectors found is three.

Table 1. Plant levels containing 1, 2 or 3 sectors (as percentages of all plants with sectors at that level).

	Number of sectors		
	1	2	3
Above truss one	70	27	3
In truss one	49	48	3
Below truss one	2	72	26

Conclusions

1. Irradiation treatment of seeds of tomato results in chimeric M_1 plants with several sectors in the generative tissue.
2. At the time of the irradiation, the apical meristem or terminal initiation point is formed in the middle of the apical dome between the cotyledons. This terminal initiation point forms all of the plant parts from the 6–9th leaf onwards, depending on the development of the embryo involved. From there on sectors are found in upward direction. Surrounding the terminal initiation-point are cells forming the lower side shoots of the plant, which do not have mutant types in common. They result in singles only, because each of these side shoots is formed from a different cell or cell group in the apical dome situated in diverse directions relative to the terminal initiation point.

3. The higher in the plant, the fewer sectors that remain, and so the uniformity increases. This can be understood from the sympodial growth habit.
4. Seeds taken from the lower part of the plant produce the greatest number of different mutant types.
5. The terminal growing-point in the embryo, originating the generative tissue in the plant above the 6th–9th leaf, consists of a low number of initial cells, probably only three.

Acknowledgments

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Reference

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