DER ZÜCHTER

33. BAND 1963 HEFT 2

Agricultural Experimental Institute, Iregszemcse, Hungary

Triploidy induced by gamma irradiation during meiosis in sunflower

By B. I. POZSÁR, E. KURNIK and J. PARRAGH

With 5 figures

Hydratation and submicroscopic structure of the living plasm undergo considerable changes under the effect of X-ray or gamma irradiation respectively, even in case of small dosages. The damage by ray injury is the most important in the nucleus, as known from data reported by NEWCOMBE (12), SAX (14) and LEA (9). The X-ray irradiation and the gamma irradiation — which in physical sense is entirely identical with it — decompose the ultra-structure and micromorphology of chromosomes in the first place and inhibit their movement during the cell division but their action comes to effect also on the longitudinal constriction and division of the chromosomes as demonstrated also by experimental results of SAX and KING (15). The cytomorphologic effect induced by X-ray or gamma irradiation respectively, is in general not analogous with the ionizing irradiation which is justified by the great velocity of penetration through the living plasm against the other ionizing radiations. Alpha ray has a maximum range of action of 10 cm in the air and only a fraction of it in body-sap. On body sap after 1 to 2 cm layer-thickness the beta ray suffers a complete absorption. Penetration of the quick neutron does not approximate the velocity of the X-ray or of gamma ray respectively and its efficiency is likewise different. The penetration speed of the slow neutron is inferior to that of the quick neutron while its participation in nucleus-reactions is far larger. Its biologic role, significance and application are restricted by the secondary activation caused by the neutron fluxus to a narrower field.

The physical character and biologic action of X-ray or gamma irradiations respectively are specifically different from that of the other ionizing radiations and this affords them a differential possibility of application. Due to their influence on the nucleus the X-ray and gamma radiation respectively have an outstanding importance from cytologic, genetical and breeding points of view alike and form therefore a subject of extensive studies. As known on the basis of experimental results obtained by DEUFEL (4), THODAY (17) and WILSON and SPARROW (18) the frequency of mitosis in tissues stimulated to a considerable extent already by a small dosage (0,3 to 3,0 Kr) of X-ray or gamma irradiation respectively. According to SWANSON (16) and WILSON and SPARROW respectively (18) in later cytomorphologic examinations it came to light also that among the phases of cell-division the prophase displays the highest sensitivity to radiation. On the evidence of last years researches it became known that X-ray or gamma irradiation respectively have an analogy with the effect of colchicine since polyploidy appears after both types of treatment. The polyploidy-inducing action of X-ray or gamma radiation respectively is confirmed by a large number of more recent studies. LEWIS (10) observed an increase of ploidy in the root meristem under effect of X-ray or gamma radiation respectively. DAVIDSON (3) applied a small dose X-ray radiation (300 r) jointly with colchicine treatment and under the effect of radiation demonstrated a considerable increase of polyploidy after 2 to 10 days. The phenomenon of polyploidy induced under effect of X-ray or gamma irradiation respectively is attributed by some authors, e. g. RIEGER and MICHAELIS (13) to the formation and accumulation of automutagen substances produced after the irradiation. In the mutation-inducing action of the X-ray or gamma irradiation respectively, a role is quite certainly played also by the phenomenon of aneuploidy together with the polyploidy. CROUSE (2) demonstrated chromosome deficiencies during the meiosis under effect of a small X-ray radiation dosage already. In the diaster stage a marked chromosome shortage can be demonstrated from the two new nuclei produced which involves or can involve a genetic divergence with the phenomenon of aneuploidy. Through polyploidy and aneuploidy the X-ray or gamma irradiation respectively causes alterations, well visible also cell-morphologically, which in part account for the experimental mutations. In their meiosis studies HAQUE (7), CROUSE (1, 2) and MITRA (11) apply small doses of X-ray irradiation which is justified by the fact that from the point of view of breeding the effect of small dosages may be considered by far more favourable. The structural and kinetical anomaly of chromosomes may be well demonstrated also by means of the applied small dosages, but a study of cytomorphologic alterations caused by the larger dosages (5 to 20 Kr) leads to a far broader knowledge of the phenomenon.

In the phenomenon of polyploidy induced under effect of X-ray or gamma irradiation respectively, the authors generally rather neglect the significance of cell organs and operating forces which take part in the cell-division, further in the movement of chromosomes which on the other hand is also directly justified by the decomposition resulting from the high energy-content of the radiation. In the kinetics of chromosomes a very important role must be attributed to the reorganization of the spindle fibre and to its solification taking place under the effect of X-ray or gamma irradiation respectively. In inhibi-

tions of chromosome movements induced under the effect of the larger (5 to 20 Kr) doses, a primary role may be ascribed to the abnormal dissolution of the gelificated cytoplasm filaments of the spindle fibre which directly accounts for the increase of the degree of ploidy and through the increasing of the cytoplasm hydratation can be brought into a close connection with the solificating influence of irradiations.

Experimental material and methods

The selected 'Iregi csikos' (Striped of Ireg) sunflower variety was irradiated in our Institute prior to the onset of flowering, in the early phase of microsporogenesis, with a 20 Kr dose by means of a gamma ray-source (Co-60) of 1 C activity closed in a portable case giving the possibility for the performance of field irradiations. The applied large dose caused profound alterations, the cytologic, morphologic and physiologic study of which gave results well comparable with the untreated ones. Part of samples

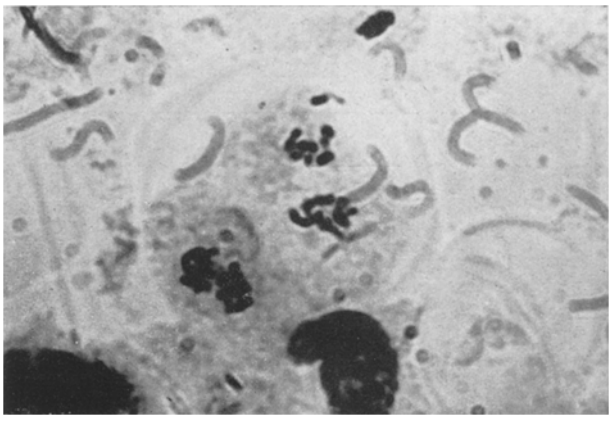

Fig. 1. Regular tetrad formation of pollen mother cell in untreated sunflower.

taken after the irradiation was worked up cytologically while the remaining samples served, with isolation, for the production of selfed progenies. The chromosome numbers of pollen mother-cells studied directly after irradiation as well as of tissue elements taken from the root-meristem of progenies deriving from selfed crops could be assessed after staining with acetic acid carmine, according to Heitz.

Experimental results

In untreated flowers microsporogenesis is regular, in consequence of which four haploid cells — tetrad — are produced during the mitosis following the meiosis. The chromosome number of tetrads produced through a synchronous process is the same as shown in fig. 1. Though the chromosome sets disintegrate under the effect of a 20 Kr dose gamma irradiation, still not all of them will move away one from the other. According to the anomaly shown in fig. 2 the four haploid chromosome garnitures will be formed regularly, but the process of the two mitoses taking place in the cell is no longer synchronous. Compared with the regularly disintegrating two chromosome sets the other two chromosome sets which are hindered in moving away remain conspicuously near one to the other, even at the end of the diaster stage. In fig. 3 it is still more striking that during the tetrad-producing process of microsporogenesis the chromo-

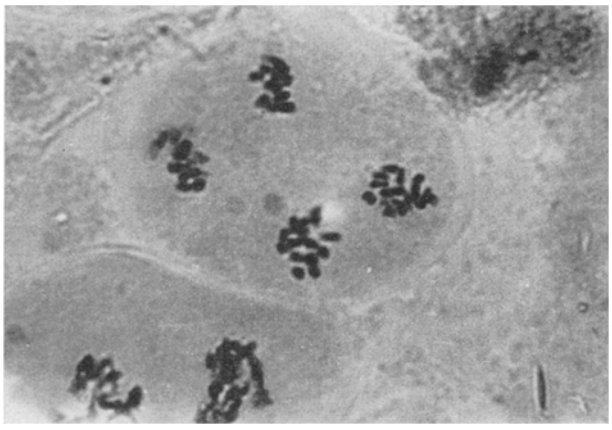

Fig. 2. Second maturity division of pollen mother cell in sunflower irradiated with 20 Kr dosage, in the falling out diaster stage.

somes are not arranged kinetically into sets synchronously but the movement of two haploid garnitures drops out abnormally and they occupy a near place one to the other in the cell space. On account of the synchronous divergence and their abnormally near spatial arrangement the last two chromosome sets which took place one near the other can be considered from the point of view of ray injury as characteristically damaged. It is clearly visible on fig. 4 that during advancement of the spirem stages not a tetrad is produced in the cell but a triad, due to the synchronous divergence. In the triad the first and second chromosome sets are haploid garnitures and enable the production of normal pollens, while the third member of the triad is of diploid garniture and leads therefore to the formation of pollen of abnormal shape and function. The production of the triad is accompanied by the further anomaly that in case of fecundations caused by pollens with diploid chromosome set — which are present in a not insignificant number — embryoes, fruits of triploid (3 n) chromosome will develop in the selfing sunflower disc. During examination with acetic acid carmine of samples taken from the root tips of seedlings developing from selfed fruits, among the plants a rather large number could be found with triploid chromosome number. According to results of mitosis examinations in root tips a large number of triploid plants could be demonstrated (fig. 5), which may be regarded as a consequence of triad production. An irradiation of plants which originally had a chromosome number of 34 ($2n = 34$) with

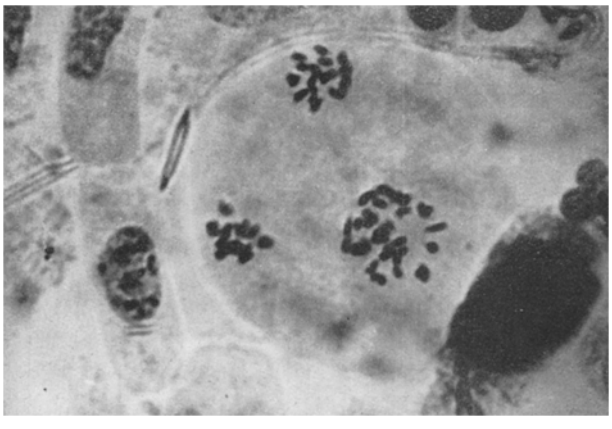

Fig. 3. Triad formation in the pollen mother cell of sunflower irradiated with 20 Kr dosage, with development of a diploid nucleus.

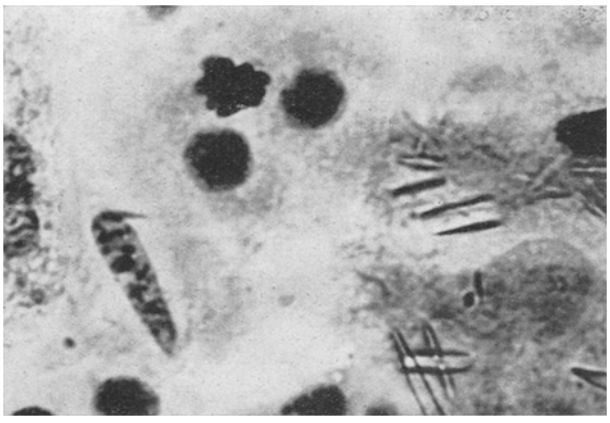

Fig. 4. Spirem stage of triad of sunflower pollen irradiated with 20 Kr dosage, with delayed abnormal daughter cell of diploid set.

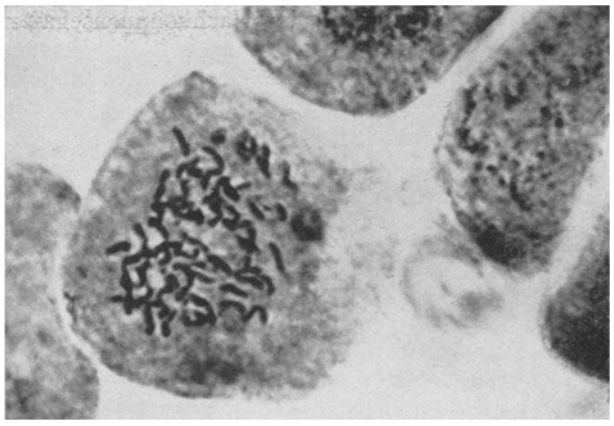

Fig. 5. Mitosis of meristems isolated from root growing points of seedlings of crops developed selfed from flowers irradiated with a 20 Kr dosage during microsporogenesis of sunflower, with triploid chromosomes.

a 20 Kr dose, with selfing, led to the production of a considerable number (3,86 per cent) of triploid progeny. (17 plants are triploid out of examined 441.) Among the chromosome sets deriving from the presented four different individuals the chromosomes in three sets were remarkably thinned and stained rather poorly, while in the fourth group they were shortened and more intensely stained in comparison to the control. This staining — relatively poor compared with the control — may be attributed to the decrease in the quantity of nucleic acids or nucleoproteids respectively due to the large dose of irradiation, which may be regarded as a general symptom. At the same time the — relatively to the control — bright colouration is likewise a symptom of ray injury but may be considered as a consequence of a slighter injury.

Evaluation of experimental results

On the evidence of experimental results it became known that during the microsporogenesis of sunflower with 34 chromosome number (2 n) the production of abnormal triads takes place due to a 20 Kr dosage of gamma irradiation. As regards proportion the production of pollen with diploid chromosome number is not unsignificant. The abnormal solification of the gelificated plasm-filaments of the spindle fibre may be regarded as a very likely cause of triad organization leading to the production of diploid pollen. It may be deemed very probable that the hydratation of plasm increases directly under effect of X-ray or gamma irradiation respectively, while the spindle fibre of gelified condition will be completely dissolved due to the injury symptom of irradiation. The solificated spindle fibre loses its indirect role in the kinetics of chromosomes, in consequence of which the disintegrated chromosomes remaining together enable the production of diploid cells. The gelified plasm-filaments of the spindle fibre take also directly part in the active movement of the chromosomes [GIMESI (5, 6)] through the adhesion of kinetochors. On basis of the above-said it must be considered highly probable that the cause of cytologically abnormal triad formation can be led back to the spindle fibre dissolving direct influence of the X-ray or gamma irradiation respectively. The phenomenon of triploid zygosis may be regarded as a consequence of the fecundation caused by diploid pollens forming in the abnormal triads. The production of triploid progenies finds its explanation in the increase of affinity, the presumption of which is probable also on account of the relatively numerous occurrences. The smaller nucleic acid or nucleoproteid content respectively in the poorly stainable chromosomes of a relatively thinning structure which can be observed in the mitotic division of triploid individuals may be regarded as a symptom of ray injury caused by X-ray or gamma irradiation. A decrease due to the lower nucleoproteid level can be presumed also in the intensity of protein synthesis.

Summary

1. Under effect of a large dose (20 Kr) of gamma irradiation performed during the microsporogenesis of sunflower the formation of tetrads is affected by ray injury which not unfrequently results in production of triads.

2. Also a nucleus with diploid chromosome number takes place among the triads. Under the first and second maturity divisions a synchronous divergence can be demonstrated which becomes only stronger towards the final phase of microsporogenesis.

3. As a result of fecundation caused by diploid pollen a possibility exists also for the production of triploid progenies. According to examinations the number of triploid progenies (3 n = 51) varies at about 4 per cent.

4. In a large part of progenies with triploid chromosome number the chromosomes are thinned and weakly stainable which may be regarded as a symptom of ray-injury.

Zusammenfassung

Als Folge einer starken Gamma-Bestrahlung (20 Kr) während der Mikrosporogenese der Sonnenblume zeigten sich Strahlenschädigungen bei der Tetradenbildung, es ergaben sich nicht selten Triaden.

Unter ihnen wurde auch ein Nukleus mit diploider Chromosomenzahl beobachtet. Bei der 1. und 2. Reifeteilung konnte eine synchrone Divergenz nachgewiesen werden, die gegen Ende der Mikrosporogenese stärker wurde.

Eine Möglichkeit für das Auftreten triploider Nachkommenschaften kann auch in der Befruchtung durch diploiden Pollen gesehen werden. Bei unseren Untersuchungen schwankte die Zahl der triploiden Nachkommenschaften (3 n = 51) um 4%.

Bei einem großen Teil der Nachkommenschaften mit triploider Chromosomenzahl sind die Chromosomen dünn und schlecht färbbar, was als Symptom einer Strahlenschädigung angesehen werden kann.

Literature

1. CROUSE, H. V.: X-ray breakage of lily chromosomes at first meiotic metaphase. Science **119**, 485—487 (1954). —2. CROUSE, H. V.: Irradiation of condensed meiotic chromosomes in *Lilium longiflorum*. Chromosoma **12**, 190—214 (1961). — 3. DAVIDSON, D.: Mechanisms of reorganisation and cell repopulation in meristems in roots of *Vicia faba* following irradiation and colchicine. Chromosoma **12**, 484—504 (1961). — 4. DEUFEL, J.: Investigations into the influence of chemicals and X-rays on mitosis of *Vicia faba*. Chromosoma **4**, 239—272 (1951).— 5. GIMESI, N.: The double breakage of spindle fibre. Acta Bot. Hung. **1**, 27—35 (1954). — 6. GIMESI, N.: Studies in the field of cytology. Bot. Közl. **45**, 193—198 (1954). — 7. HAQUE, A.: The irradiation of meiosis in *Tradescantia*. Heredity, Suppl. **6**, 57—75 (1953). — 8. KURNIK, E.: Verwendung von haploiden Fruchtzwillingen bei der Züchtung von Sonnenblumen. Kisérletügyi Közl. **54** (A), 1—18 (1962). — 9. LEA, D. E.: Actions of radiations on living cells. London: Cambridge Univ. Press 1955. — 10. LEWIS, D.: Production of polyploidy by colchicine and X-rays. Nature **167**, 891—892 (1951). — 11. MITRA, S.: Effects of X-rays on chromosomes of *Lilium longiflorum* during meiosis. Genetics **43**, 771—789 (1958). — 12. NEWCOMBE, H. B.: The action of X-rays on the cell. I. The chromosome variable. Jour. Genet. **43**, 145—171 (1942). — 13. RIEGER, R., and A. MICHAELIS: Comparative examinations on distribution of breakages of chromosome sets in *Vicia faba* L. induced by different mutagens. Chromosoma **10**, 163—178 (1959). — 14. SAX, K.: The effect of X-rays on chromosome structure. Jour. Cell. Comp. Physiol. **35**, 71—81 (1950). — 15. SAX, K., and E. D. KING: An X-ray analysis of chromosome duplication. Proc. Nat. Acad. Sci. **41**, 150—155 (1955). — 16. SWANSON, C. P.: Differential sensitivity of prophase pollen tube chromosome to X-rays and ultraviolet radiation. Jour. Gen. Physiol. **26**, 485—494 (1943). — 17. THODAY, J. M.: The effect of ionizing radiations on the broad bean root. Brit. Journ. Radiol. **24**, 572—576, 622—628 (1951). — 18. WILSON, G. B., and A. R. SPARROW: Configurations resulting from isochromatid and iso-subchromatid unions after meiotic and mitotic prophase irradiations. Chromosoma **11**, 229—244 (1960).

Aus dem Institut für Pflanzenzüchtung Groß-Lüsewitz der Deutschen Akademie der Landwirtschaftswissenschaften zu Berlin

Rohverfärbungsuntersuchungen an bekannten Sorten und aussichtsreichen Kartoffelzuchtstämmen der DDR

Von **D. ROTHACKER** und **J. VOGEL**

Sowohl bei Speisekartoffeln als auch bei Industriekartoffeln ist die Rohverfärbung des Fleisches nach dem Schälen, Schneiden, Reiben usw. eine unerwünschte Eigenschaft. Über die vermutlichen chemischen Ursachen dieser Erscheinung liegen verschiedene Arbeiten vor (vgl. SCHREIBER 1961, dort weitere Literatur). Mit Hilfe chemischer Mittel können zwar die Verfärbung verhindert bzw. verfärbte Kartoffeln gebleicht werden, doch dürfen diese in vielen europäischen Ländern wegen der geltenden Lebensmittelgesetze nicht angewendet werden.

Die Kartoffelzüchtung hat sich u. E. bisher noch relativ wenig mit der Aufgabe befaßt, nicht oder nur gering verfärbende Sorten zu schaffen, obwohl SCHMALFUSS (1938), SCHMALFUSS, STELZNER und KRÖNER (1938), SCHMALFUSS und STELZNER (1942) und neuerdings auch FIRBAS (1961) und ROTHACKER (1962) bereits ein umfangreiches Material an *S. tuberosum*-Sorten sowie wilder und kultivierter mittel- und südamerikanischer Arten auf ihre Rohverfärbung untersucht haben und Unterschiede bei den verschiedenen Formen feststellen konnten.

Die dringende Forderung nach Kartoffeln ohne oder mit nur geringer Rohverfärbung ergibt sich aus der zunehmenden Spezialisierung und den ständig steigenden Qualitätsansprüchen bei der Kartoffelverwertung und -veredelung. Zum Beispiel wird der Verkauf geschälter roher Speisekartoffeln nur dann an Umfang zunehmen, wenn sie bis zum Kochen ohne bedeutende Qualitätsminderung und ohne zu verfärben aufbewahrt werden können. Auch die Stärkeindustrie würde nicht verfärbende, stärkereiche Kartoffelsorten vorrangig verarbeiten, weil sie aus diesen Stärke noch besserer Qualität bei geringerem Produktionsaufwand gewinnen könnte. Ebenfalls sind die Produzenten anderer Speisekartoffel-Veredelungsprodukte daran interessiert.

Versuchsmaterial

Im Dezember 1961 prüften wir in Groß-Lüsewitz insgesamt 28 Sorten und Kartoffelzuchtstämme mittelfrüher bis später Reifezeit aus der Ernte 1961 (Tab. 1). Da wir bei anderen Untersuchungen nachweisen konnten, daß an gekochten Kartoffeln die Verfärbungsintensität u. a. durch die Herkunft modifiziert wird (MÖLLER und VOGEL 1961, VOGEL 1961), wurden alle Sorten von jeweils 3 Herkünften mit unterschiedlichem Boden geprüft, und zwar aus Bernburg (Lößboden), Groß-Lüsewitz (sandiger Lehmboden) und Wentow (Sandboden).

Das geprüfte Material war an diesen Versuchsorten aus einheitlichem, gesundem Pflanzgut bei annähernd gleichen Kulturbedingungen aufgewachsen. Nach der Ernte wurde es in Groß-Lüsewitz bis zur Durchführung der Untersuchungen unter einheitlichen Bedingungen gelagert.

Versuchsmethodik

a) Vorversuche

Bisher versuchten wir, die Rohverfärbung mit verschiedenen Methoden zu bestimmen. Das einfachste Verfahren bestand darin, die Kartoffeln zu schälen, vom Kronen- zum Nabelende zu teilen und in Glasschalen auszulegen. Daraufhin verfärbten sich die Schnittflächen, und die Intensität der Verfärbung wurde nach 4 Stunden visuell mittels einer von HANSEN entwickelten Farbskala eingestuft, die eigentlich zum Messen der Verfärbung an gekochten