SOME EVIDENCE REGARDING REPAIRING, RECOVERY AND OVER–COMPENSATING PROCESSES DURING ONTOGENESIS, AFTERX–RAY–IRRADTATION OF BEAN SEEDS

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Abstract

Exposing plant organs to high doses of ionizing irradiation, penetrating into the plant tissues and cells, along the track structure of particles, lesions, and sublesions are formed on the molecules and organelles. As a result, disorders in the growth and development as well as chlorophyll–deficiency symptoms occur. The time scale of their reparation, recovery and over compensation during ontogenesis, constitutes a question of high theoretical and practical importance, with special regard to nuclear fallout.

With an aim to model the "ut supra" stated phenomena, the seeds of bean, Echo elit licensed variety, were irradiated by 300 Gy dose of X–ray–irradiation (120 kV:4.5 mA). According to the data obtained, the biosynthesis of photosynthetic pigments, will have been completed by the beginning of flowering.

In consequence of the overcompensation of the repairing processes, the organs of plants developed from irradiated seeds, showed a partly differing correlative growth, compared to those of control plants.

In order to characterize the on vivo response of radiation–injured plants, a new method and approach were used. The changes of the electric capacitance of the plants during their ontogenesis, were continously monitored and recorded via a computer–aided and controlled measurement. In view of the data collected in such a way, the repairing plants may respond more quickly and intensively to the changes of environmental factors.

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INTRODUCTION

After treating the plant organs, tissues and cells with ionizing irradiation physical, physicochemical, chemical, biochemical and physiologicl chain effects occur(Kōrösi—Pāl, 1987). When high doses are delivered or in the radius of fallout from a nuclear catastrophe, key questions are the repairing processes, their levels, e.g. molecular, gene, chromosome, biochemical, morphological, biological production as well as manifestation during ontogenesis (Tobias, 1985;Ustumi—Elkind, 1979;Weber, 1988;Kōrösi—Pāl, 1989).

Another rather disputed phenomenon requiring more supporting data and interpretation constitutes the socalled overcompensation (frequently referred to as stimulations after intermedicate or high—dose irradiation) (Kōrösi, 1990)

This processes can be related to the changes of hormonal background of the plants (Skoog, 1935;Sax, 1963;Vilenskij, 1980;Jezierska—Szabó et al., 1986;Krjukova, 1975; Kuzin—Vagabova, 1980). In consequence, an alteration in the correlative development and growth may occur (Kōrösi—Pāl, 1989).

There is a burning lack of the in vivo detecting and following the extent of irradiation injuries of the plants.

In view of the "ut supra" described statements, the aims of our experiments can be summarized as follows:

1. Biological production of bean under the influence of X—ray—irradiation;
2. Modification in the correlative conditions of the organ growth;
3. Alteration in the concentration and chemical composition of the photosynthetic pigments during the ontogenesis;
4. Computer—aided registration of the alterations in the electric capacitance of the plants developed from irradiated seeds.

MATERIAL and METHODS

For the irradiation, air—dried bean (water content 9.3%) seeds, Echo elit licensed variety, were used (thousand wight 186.5g).

For treatment and control, ten repetitions were used, the number of plants per pot set at three. The pots were filled with earthworm humus in order to ensure the proper plant growth and development. To asses the biological production of the plants, the above ground organs, e.g. stem leaves and pods, were separated and measured.

For statistical evaluation of the data, confidence, limits of the mean, regression analysis as well as hierarchic cluster analysis were used (Winer, 1962;Anderberg, 1973;Jardine—Sibson, 1971).

Irradiation treatment

The X—ray irradiation was carried out by Liliput 140 X—ray emitting apparatus (Medicor, Hungary) at the Central Laboratory of the Agricultural University of Gödöllö. The X—ray apparatus was set to 4.5mA current at 120 kV.

According to our preliminary experiments, 300 Gy was the dose that causes such injuries to the exposed seeds, from which the developing plants are being repaired during ontogenesis. On the other hand to bring about chlorophyll—deficiency symptomes and make them visualized as high as 180 Gy does seems to be appropriate. Therefore 180 Gy and 300 Gy doses were applied.

Determination of Chlorophyll—a, —b and carotenoids

To measure the chlorophyll—a, —b and carotenoids concentrations the homogenized leaves were extractedby aceton. The corresponding pigment quantities were calculated according to Mayer—Bertenrath (1966). The following equations were used:

Chl—a = /11.75x(e662—e720)—2.35x(e664—e720)/xV/1000xDW

Chl—b = /18.16x(e664—e720)—3.96x(e662—e720)/xV/1000xDW

Car. = (4.695xE440)—(0.268xz)xV/1000xDW

where z = 5013xE662 + 0.41xE664

DW = dry weight

E = measured extinctions

Chl—a = chlorophyll—a (mg/g DW)

Chl—b = chlorophyll—b (mg/g DW)

Car. = carotenoids (mg/g DW)

The measuring of the capacitance of the plants

The bioelectrical properties of the plants as active characteristics result from the metabolism and various stimuli. These properties as passive characteristics illustrate the changes of the vegetal dielectricum. The dielectrical features of plants (e.g. permittivity, quality factor, phase of impedance, capacitance) may outline the physical state in vivo.

In our previvouly done tests the change of capacitance tested by low level sinusoidal voltage described the water supply and environmental temperature of the plants (Lászlő, 1982a ; 1982b Lászlő—Kristőf, 1982 ; Lászlő, 1983).

Platinum needle electrodes are connected to the plants at the consequently defined points, thereby providing information about the plants state during ontogenesis. Data collection is carried out by a computer every twenty minutes. This method supports reliability and data processing.

RESULTS

As seen from Table 1. 300 Gy X−ray irradiation retarded the emergence of bean by more than 40%. This means a severe hampering effect to be repaired and restored during ontogenesis.

The organ growth was higher as a result of irradiation. This either relates to the leaf, stalk, yield formation (Table2., 3.). the overcompensation was as high as 505 in the leaf and stalk formation, and meant about a 28% increment in dry yield). this trend was reflected in the above ground biological production and constituted a 37% surplus (Table 4).

Modification in the correlative conditions of organ growth

In order to evaluate the connections between the growth of various organs, a concerning correlation matrix was established (Table 5).

As evidenced from Table 5. the biological production shaped in a close relation with the dry weight of the wield (r=0.97), fresh weight of leaf (r=0.85), and of yield (r=0.88). It also infers from the presented calculations that the higher the ratio of the dry yield, the lower the ratios of dry leaf (r=−0.93) and of dry stem (r=−0.78).

So as to estimate the changes in the correlating properties of organ growth during the repairing and overcompensation processes, hierarchic cluster analysis was applied. As it is well demonstrated in fig. 1. the organ formation and growth can be grouped into three distinct clusters. 1.) Similar behaviour was observed between the the dry weight of stem, leaf and fresh weight of stem. 2.). Connection also appeared between the biological production, dry weight of yield and yield index. 3.) A connection was noticed between the ratio of the dry weight of the stem and of dry leaf as well as fresh weight of the yield.

Due to the repair and possibly to the overcompensation processes taking place in the correlative condition of organ formation, either a similar pattern or distinctly different ones from the control came into being. The same connection manifested itself between the dry weight of stem and leaf as well as fresh weight of stem. Possibly as a result of repair, a closer connection between the yield index and dry weight of yield was noted. In comparison with the control, an entirely new connection was formed between the fresh weight of leaf as well as the bilogical production of the above ground organs (Fig. 2).

A possible manifestation of repairing and over compensation in the biosynthesis of photosynthetic pigments during ontogenesis

In the plants developed from seeds irradiated with higher doses (>180 Gy), various chlorophyll deficiency symptoms were observed. Frequently encountered were white spots on the leaf and leaf sectors showing light green colours. As the plants developed, the severity of deficiency symptoms disappeared gradually.

So as to monitor the repairing of the biosynthetic pathways of photosynthetic pigments, the cloropghyll−a, −b and carotenoid concentration were determined at three phenological stages. It is clear cut from the data presented in Table 6 that during the period commencing at the beginning of budding and lasting till the beginning of pod formation, both the chlorophyll−a, −b and carotenoid synthetizing capacity of the leaves were repaired and restored. Further on, from flowering until pod formation an overcompensation took place. this resulted in a surplus of more than 8% in the pigment concentration. However it should be noted that during either the repairing or over compensation period the chlorophyll−a/b and clorophyll−(a+b)/carotenoid ratios did not change significantly.

A peculiar (?) pattern in the electric capacity of bean plants as affected by X−ray−irradiation

By inserting platina electrodes into the stem after about 5−7 day period the mechanically injured tissues formed calli and the connections within the vascular system were assumed to be restored. When the changes in the electric capacity of the control and treated plants compared, it may be stated that the treated plant responded more sensitively to the changes, e. g., temperature and water supply (Fig. e.). These responses relate both to the responses vs time and the magnitude of the amplitudes.

DISCUSSION

It is a question of utmost importance after a nuclear catastrophe to determine to what extent plants are capable of repairing the primary lesions that cause injuries in the macro molecules during the energy deposition along the track structures of primary and secondary ionizing particles (Sontag, 1987 ; Kiefer, 1988).

Another frequently encountered and as yet, not fully supported and interpreted phenomenon, constitutes the socalled overcompensation after treating the plant organs with high doses of irradiation (ó, 1990). This may result in a higher biological production.

In our experiment, in order to bring about a severe irradiation injury, the bean seeds were exposed to 300 gy X−ray dose. This treatment, setting off a considerable hampering effect, reduced the emergence percent by about 50%, compared to that of control (Table 1.).

As it has been proved, during the post irradiation period, alterations in the phytohormones and their ratios ae changed (Sax, 1963 ; Jezierska−Szabó et al., 1986). Also there are many results indicating that the impeding dffect of irradiation has been released by exogenously applied plant growth regulating substances (masahiro−Hirotoshi et al., 1974 ; Ahmad−Trifu, 1980). These results are likely to support the involvement of phytohormones in the repairing processes.

By judging higher biological production, the repairing processes resulted in a complete recovery and further on an overcompensation (Tabel 2., 3., 4.). On the other hand this overcompensation can be termed as a special type of "stimulation" possible taking place after irradiation of seeds with higher doses.

The repairing goes together with partial changes in the correlative connections among the organs growth (Figs. 1., 2.).

The repair relates to the biosynthetic pathways of chlorophyll—a, —b and carotenoids, either, as it is evidenced from Table 6.

According to the photosynthetic pigment concentrations a reparation period lasting from the beginning of emergence to the beginning of flowering are postulated. By this phenological state, the recovery will have been completed. Thereafter overcompensation occurred resulting in higher chlorophyll —a, —b, carotenoid and naturally higher total pigment concentration (Table 5.).

There is some evidence that both the repairing, recovery and overcompensation may be in vivo described and followed via measuring the electric capacitance of the plants developed from seeds irradiated with 300 Gy (Fig. 3.). The electric capacitance changes followed a pattern, with the amplitude of the capacity being higher and more sensitive in a time scale (ontogenesis) to the environmental factors. Whether this response is specific or aspecific remains a subject for further studies.

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Table 1

Effect of 300 Gy-irradiation on the emergence percent of the bean, variety Echo elit licenses

Treatment	average(%)	confidence interval (P=5%)	in % of control
Control	57.00	42.12~71.87	100.00
irradiated	33.33	15.65~51.01	58.47

Table 2

Leaf and stalk formation as affected by X-ray-irradiation(300 Gy)

organ	control		irradiated		
	average (weight g/plant)	confidence interval (p=5%)	average (weight g/plant)	confidence interval (p=5%)	in % of control
fresh leaf	5.52	5.09~5.95	8.24	6.31~10.18	149.28
dry leaf	0.66	0.61~0.70	1.01	0.73~ 1.27	153.03
fresh stalk	1.55	1.45~1.58	2.42	1.92~ 2.92	159.21
dry stalk	0.26	0.23~0.28	0.41	0.29~ 0.52	157.63

Table 3

Yield formation of bean after recovery form 300 Gy of X-ray-irradiation injury

yield properties	control		irradiated		
	average	confidence interval (p=5%)	average	conficence interval (p=5%)	in % of control
N⁰ of pods/plant	3.30	3.03~ 3.56	4.71	4.12~ 5.30	142.73
fresh yield/plant(g)	9.45	8.59~10.30	13.66	11.63~15.68	144.55
dry yield/plant(g)	1.82	1.66~ 1.97	2.33	1.92~ 2.74	128.02

Table 4

The above ground biological production of bean as influenced by 300 Gy of X-ray-irradiation

Treatment	average* (g/plant)	confidence interval (P=5%)	in % of control
Control	2.74	2.55~2.93	100.00
irradiated	3.75	3.10~4.39	136.86

* =dry matter of (stem+leaf+yield)

Table 5

A general correlation between organ formation at "Echo Elit" bean variety

Biological production property	Column number	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
N⁰ of pod/plant	(1)	1.00	0.47	0.49	0.62	0.54	0.51	0.51	0.55	−.10	0.11	0.03
fresh weight of leaf	(2)	0.47	1.00	0.85	0.88	0.96	0.84	0.85	0.92	−.20	0.24	0.04
fresh weight of stem	(3)	0.49	0.85	1.00	0.87	0.87	0.93	0.69	0.80	−.38	0.28	0.38
fresh weight of yield	(4)	0.62	0.88	0.87	1.00	0.88	0.87	0.81	0.88	−.20	0.16	0.17
dry weight of leaf	(5)	0.54	0.96	0.86	0.88	1.00	0.87	0.80	0.91	−.33	0.37	0.11
dry weight of stem	(6)	0.51	0.84	0.93	0.87	0.87	1.00	0.73	0.84	−.38	0.23	0.46
dry weight of yield	(7)	0.51	0.85	0.69	0.81	0.80	0.73	1.00	0.97	0.26	−.23	−.25
biological production	(8)	0.55	0.92	0.80	0.88	0.91	0.84	0.97	1.00	0.05	−.03	−.09
ratio of dry yield	(9)	−.10	−.20	−.38	−.20	−.33	−.38	0.26	0.05	1.00	−.93	−.78
ratio of dry leaf	(10)	0.11	0.24	0.28	0.16	0.37	0.23	−.03	−.03	−.93	1.00	0.47
ratio of dry stem	(11)	0.03	0.04	0.38	0.17	0.11	0.46	−.09	−.09	−.78	0.47	1.00

Table 6

Changes of the pigment concentrations and compositions during repairing and overcompensation processes in bean leaves developed from seeds irradiated by 300 Gy of X-ray

Photosynthetic pigment s/mg·g(DW)$^{-1}$/ and their ratios %	Pheno-phase	control		treated		
		average	confidence interval (P=5%)	average	confidence interval (P=5%)	in % of control
Chlorophy 11-a	+	15.23	14.12～16.33	10.36	8.66～13.06	68.02
	++	16.48	14.50～18.45	15.10	12.06～18.13	91.62
	+++	10.89	8.84～12.94	11.81	10.07～13.53	108.45
Chlorophy 11-b	+	4.45	4.20～ 4.71	3.17	2.60～ 3.74	71.23
	++	5.75	4.90～ 6.59	5.54	4.27～ 6.81	96.35
	+++	4.09	3.47～ 4.71	4.71	3.93～ 5.48	115.16
Chlorophy 11-a+b	+	19.69	18.37～20.99	13.54	11.27～15.80	68.77
	++	22.24	19.41～25.05	20.65	16.34～24.94	92.85
	+++	14.99	12.32～17.65	16.52	14.03～18.99	110.21
Carotenoids	+	4.30	3.91～ 4.67	3.00	2.57～ 3.42	69.76
	++	5.26	4.84～ 5.66	4.63	3.92～ 5.34	88.02
	+++	3.77	3.23～ 4.30	4.15	3.46～ 4.84	110.08
Total pigment concentration *	+	23.98	22.33～25.63	16.54	13.86～19.22	68.97
	++	27.49	24.28～30.69	25.28	20.27～30.27	91.96
	+++	18.76	15.57～21.94	20.81	17.63～23.99	110.93

	+	3.42	2.35～3.57	3.29	3.20～3.36	96.19
Chlorophy 11-a/b%	++	2.87	2.78～2.95	2.73	2.60～2.85	95.12
	+++	2.65	2.54～2.74	2.52	2.38～2.66	95.09
	+	4.61	4.40～4.80	4.49	4.29～4.67	97.40
Chlorphy11-(1+b)/ carotenoids%	++	4.23	3.97～4.47	4.44	4.17～4.71	104.96
	+++	3.96	3.74～4.16	3.86	3.74～3.97	97.47

DW=dry weight
* =chlorophyl1-a+b+carotenoids
+ =beginning of budding
++ =beginning of flowering
+++=pod formation

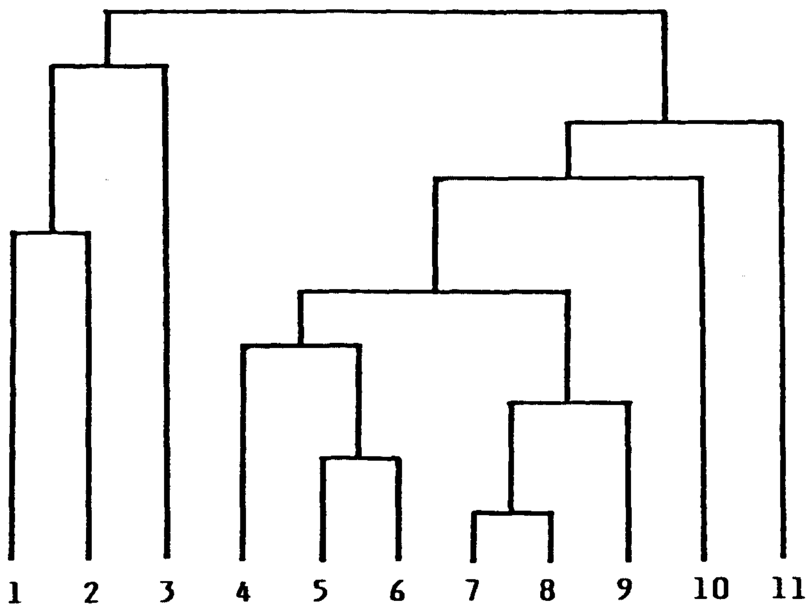

Fig. 1
Hierarchic cluster analysis of the correlative organ formations of the control bean plants

1=dry weight ratio of stem
2=dry weight ratio of leaf
3=fresh weight of yield
4=yield index
5=biological production(dry weight of stem+leaf+yield)
6=dry weight of yield
7=dry weight of stem
8=dry weight of leaf
9=fresh weight of stem
10=fresh weight of leaf
11=number of pods

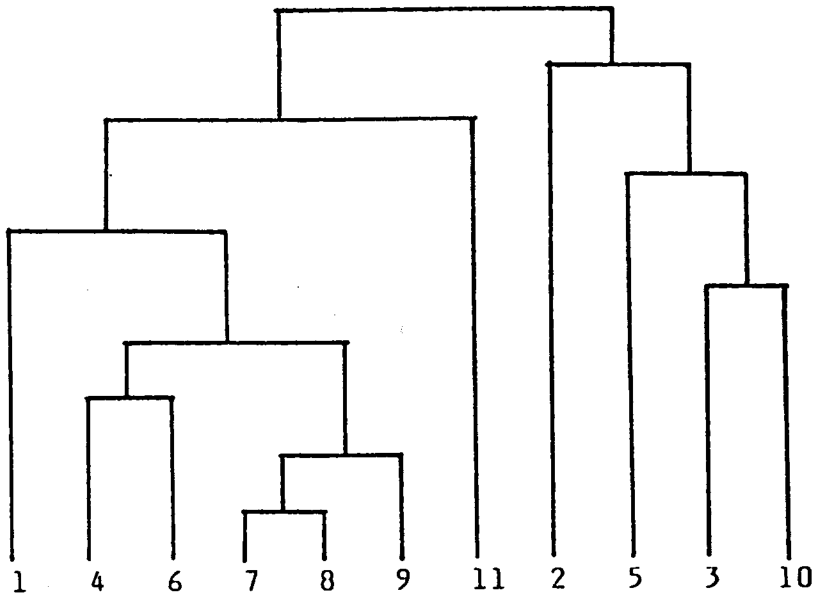

Fig. 2
Hierarchic cluster analysis of organ formation of beans, developed from seeds treated by X-ray(300Gy)
(The meaning of the numbers are the same as for Fig. 1)

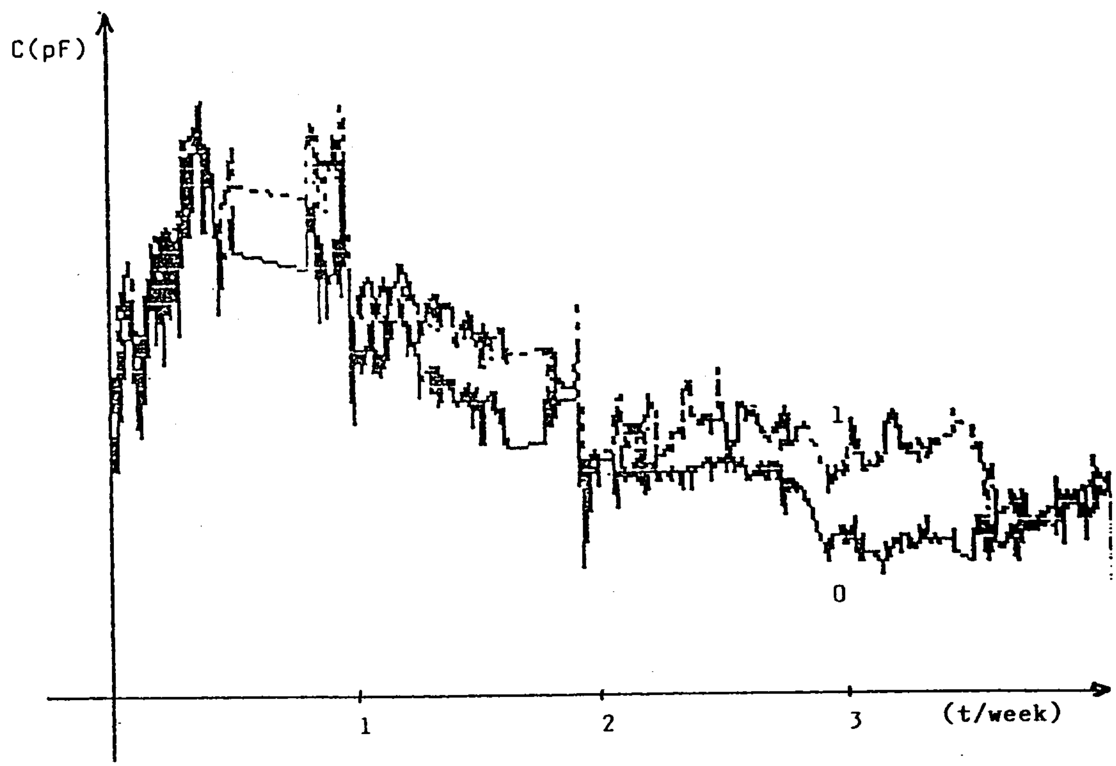

Fig. 3
A 4-week capacitance measuring vs time
(0=control plant; 1=irradiated by X-ray)