

## Induction of Free Radicals in Seeds by High Intensive Flashes and the Relevant Phosphorus Metabolism in the Seedling

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**Abstract.** Influence of high intensive flashes on the yield of free radicals in intact seeds and excised embryonic axis, endosperm, and seed coat, and its resulting effect on seedling growth, total biomass production and phosphorus metabolism in wheat (*Triticum aestivum*), vetch (*Vicia sativa* L.) and onion (*Allium cepa* L.) was studied. Free radicals (f.r.) were formed mainly in seed coat and not in the endosperm. Vetch seeds after irradiation had  $20.76 \times 10^{13}$  f.r. g<sup>-1</sup> dry intact seed and  $17.30 \times 10^{13}$  f.r. g<sup>-1</sup> dry seed coat. Excised seed coats exposed to irradiation also yielded  $17.28 \times 10^{13}$  f.r. g<sup>-1</sup> dry matter. High irradiance "white light" flashes induced more f.r. than a monochromatic one of the same photon content. Red (650 nm), far-red (750 nm) and even infra-red (1100 nm) radiation did not initiated f.r. formation but resulted in their decay in samples irradiated earlier by "white", blue and green parts of the spectrum. Blue irradiation of seeds led to the decrease in the length of shoots and roots in comparison to "white", green and red irradiation but their biomass increased faster than in the seedlings obtained from non-irradiated or irradiated with "white" and green radiation. The quantity of total acid soluble phosphorus followed a sequence with respect to wavelength of radiation: 436 nm > 650 nm > 540 nm > non-irradiated > 300–800 nm. Quantity of inorganic phosphorus remained unaffected by different spectral character of radiation. The quantity of organic acid soluble nucleic phosphorus and acid insoluble polyphosphates was higher in samples irradiated with red beams (650 ± 6 nm).

Plants develop various radiation absorbing pigments to protect themselves against excess irradiation. Their protection is accomplished by free radicals (f.r.), singlet oxygen, and peroxide radicals (SOKINA *et al.* 1979). Recent interest in the field is in evaluating the influence of sources of high intensive constant or flash radiation (coherent and non-coherent) on photosynthetic and non-photosynthetic objects.

In this article the influence of high intensive radiation on the yield of f.r. in intact seeds and their parts (embryonic axis, endosperm and seed coat) exposed to various irradiances and sources was studied in relation to seedling growth, biomass production, and phosphorus metabolism in wheat, vetch and onion.

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## MATERIAL AND METHODS

Healthy air-dry seeds of wheat (*Triticum aestivum* L.) cv. Promin, cv. *Lutescence-72*, common vetch (*Vicia sativa* L.) cv. Balynaiskaya with light coloured seeds and cv. Nemchinovskaya with dark coloured seeds, and onion (*Allium cepa* L.) cv. Strigunovskii, collected from the Timiryazev Institute of Plant Physiology of the Academy of Sciences of the U.S.S.R., Moscow, were used for experiments.

Seeds were irradiated with "white" and various colour flashes at room temperature and humidity using universal spectral irradiator (ULSI) model-1 Zenit on long xenon lamp, and laboratory spectral irradiator (LSI) model 1 M. The ULSI-1 Zenit device produces "white" flashes from 250 to 2 000 nm, 0.2 s long, with dark intervals of 0.6 s. Seeds were irradiated with a definite number of flashes (50, 100, 200, 300, 500). The energy of the flash was 3.0 to  $10^5$  J m<sup>-2</sup>. The device LSI 1 M was used for studying the influence of constant "white" (300–800 nm), blue ( $436 \pm 9$  nm), green ( $540 \pm 7$  nm) and red ( $650 \pm 6$  nm) and ( $750 \pm 3$  nm) irradiation with the total energy of  $0.98 \times 10^5$  J m<sup>-2</sup> during 60 min of irradiation. The energy was determined using an F-18 irradiance meter.

Seeds were irradiated for 10 min and then the free radical (f.r.) yield was determined. ESR spectra were registered using the Radiospectra meter RE-1306 X-band under normal room temperature and humidity. The concentration of f.r. was measured in comparison to internal standard (Mn<sup>2+</sup>/MgO) of third line which corresponded to  $1.8 \times 10^{15}$  concentration of f.r.

Irradiated seeds were soaked in distilled water for 3 h, then planted on wet blotting paper placed in Petri dishes, and germinated in complete darkness at  $25 \pm 0.5$  °C. Length of shoots and roots was measured in 10 d-old seedlings and a part of the sample was fixed in liquid nitrogen for analysis.

Frozen shoots were extracted according to BELOKERSKIĭ and KULAEV (1957). The amount of phosphorus was determined by the technique of

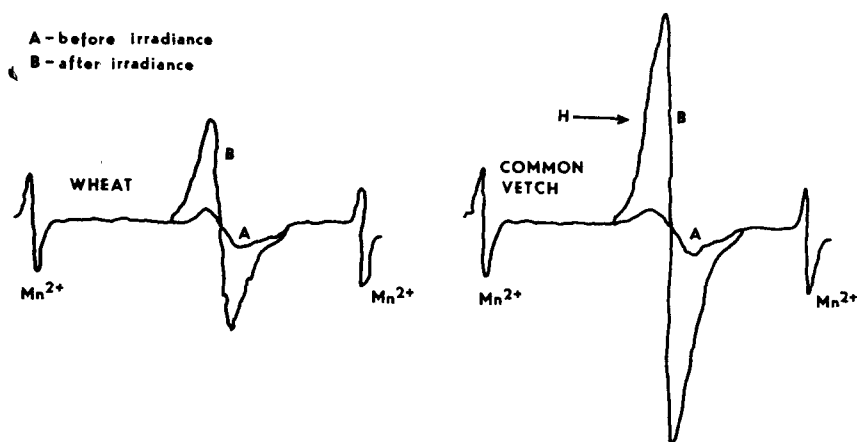


Fig. 1. ESR Spectra of wheat and common vetch seeds before and after irradiation by intensive "white" radiation.

WEIL-MOLHERBE and GREEN (1951). In the first acid-soluble fraction inorganic phosphorus ( $P_i$ ) was determined in the extract immediately, acid-labile phosphorus was determined as orthophosphate after hydrolysis of samples for 10 ( $P_{10}$ ) and 30 ( $P_{30}$ ) min by 1 M HCl in a boiling water bath. Total phosphorus ( $P_t$ ) was extracted by  $HClO_4$  at 20 °C. The amount of polyphosphates in this fraction was calculated according to the formula:

Amount of polyphosphates =  $(P_{10} - P_i) - (P_{30} - P_{10})$ . Quantity of organic phosphorus compounds ( $P_o$ ) in acid-soluble fraction was calculated using the formula

$$P_o = P_t - (P_i + \text{amount of polyphosphates}).$$

The amount of acid-insoluble polyphosphates was determined as the orthophosphate was formed at the expense of acid-insoluble polyphosphates during the extraction. Phosphorus of nucleic acids was determined by spectrophotometric method (SPIRIN 1958).

All the experiments were replicated thrice.

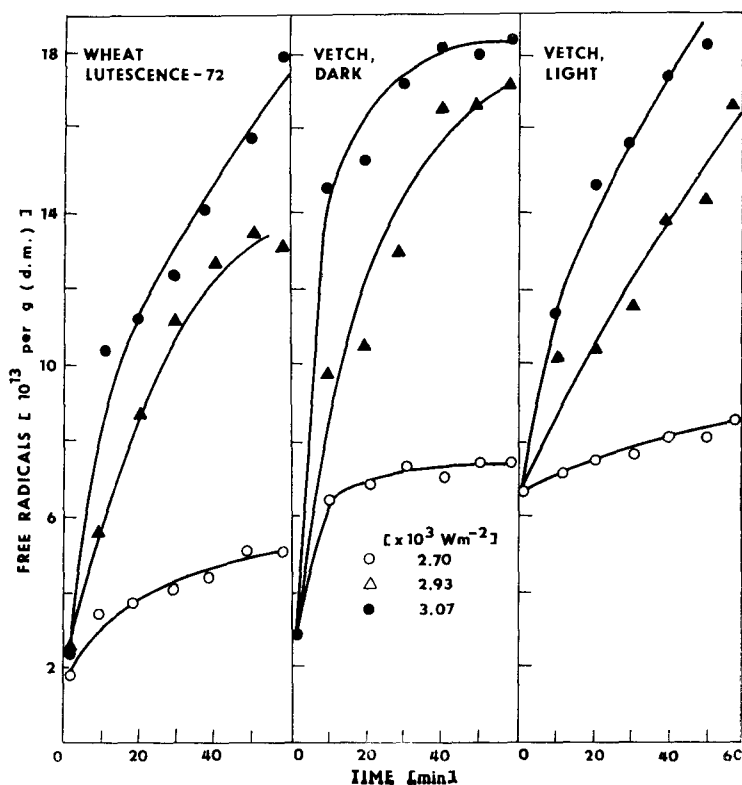


Fig. 2. Change of free radical concentration in wheat seeds and vetch, dark and light-coloured seeds, after various irradiances with integral "white light" (300–800 nm) using LSI 1M.

## RESULTS

## Photoinduction of Free Radicals in Seeds and Their Parts

Spectra of electronic spin resonance (ESR) of dry seeds appeared as an asymmetrical singlet with  $g$ -factor 2.005 and  $H_{1/2} = 8$  Gs (Fig. 1). F.r. yield in non-irradiated seeds of wheat cv. Promin was  $3.0 \times 10^{13}$  f.r. per 1 g dry matter (d.m.) of seeds, in cv. Lutescence-72  $4.0 \times 10^{13}$  f.r. per 1 g d.m., in common vetch cv. Balynaïskaya (light-coloured seeds)  $6.5 \times 10^{13}$  f.r. per 1 g d.m. and in cv. Nemchinovskaya (dark-coloured seeds)  $3.3 \times 10^{13}$  f.r. per 1 g d.m. Irradiation with "white intensive light" increased the f.r. concentration (with the exception of seeds of onion) in correspondence to the given irradiance (Fig. 2). In this case the form and the width of the ESR spectrum does not change even when the spectrum of "white light" contains the ultraviolet region.

F.r. yield saturation depended on the quantity of the pigment in the object while the time of saturation was determined by the irradiance (Fig. 3). During irradiation of onion seeds by intensive flashes there was no change in the f.r. concentration. This can be explained as that there are deep traps where electrons are caught under the photoexcitement and remain there during ripening of seeds in direct sunlight.

Photoinduced f.r. were formed mainly in seed coats and not in the endosperm (Fig. 4.). Polyphenol and melanin pigments are the main suppliers of f.r. concentrated in seed coats. After irradiation of common vetch seeds the concentration of free radicals was  $20.76 \times 10^{13}$  f.r. per 1 g dry intact

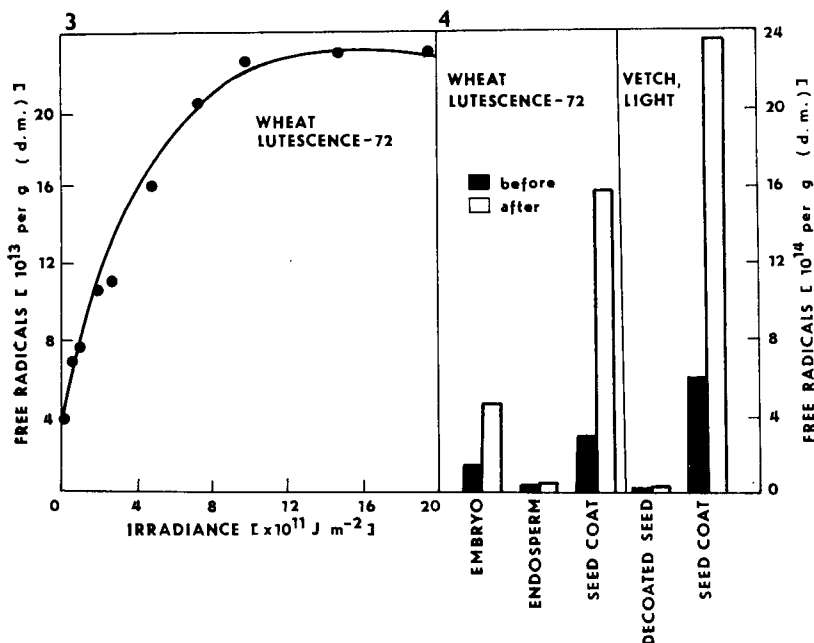


Fig. 3. Free radical yield in wheat seeds after irradiation by ULSI-1 Zenit with high irradiance.  
Fig. 4. Free radical yield after irradiation of different parts of wheat and vetch seeds, before and after irradiation.

seeds when calculated to the mass of seed coats only, the concentration of f.r. was  $17.30 \times 10^{13}$  f.r. per 1 g mass of coats. Almost similar results were obtained when only the seed coats separated from the seeds were irradiated, which was equal to  $17.28 \times 10^{13}$  f.r. per 1 g dry coat mass.

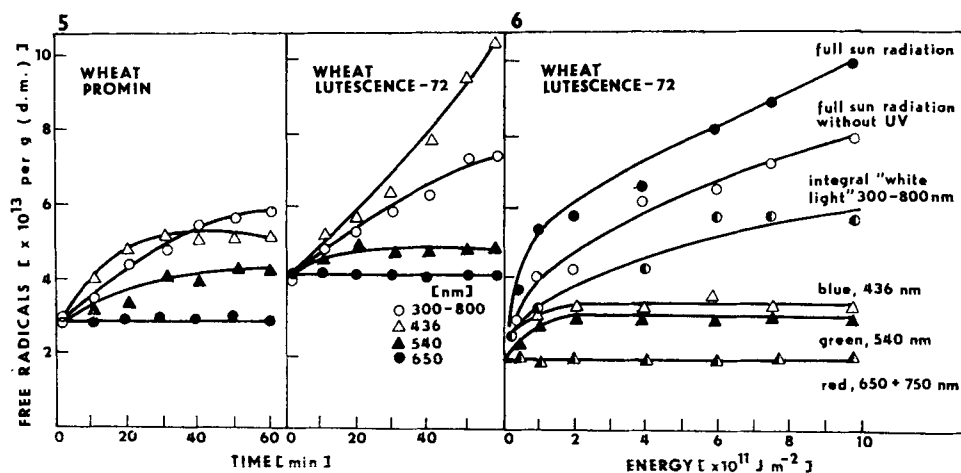


Fig. 5. Free radical yield in wheat seeds cv. Promin and cv. Lutescence-72 after irradiation with different spectral ranges ("white" 300–800 nm, blue- 436 nm, green- 540 nm, red- 650 nm) using the LSI-1 M with  $2.7 \times 10^3 \text{ Wm}^{-2}$ .

Fig. 6. Free radical yield in wheat seeds cv. Lutescence-72 after irradiation of different spectral composition using the LSI-1 M.

The yield of f.r. of seeds irradiated by different spectral range with equal energy varied for different cultivars (Fig. 5.), possibly because of different set of pigments and their ratio. Under high irradiance, "white light" induced more f.r. than monochromatic radiation of similar energy (Fig. 6.).

Red (650 nm), far-red (750 nm), and even infra-red (1100 nm) radiation did not cause f.r. formation but resulted in their death in samples irradiated earlier by "white", blue or green radiation (Fig. 7). The influence of red radiation can be initiated by heating the irradiated seeds up to  $100^\circ\text{C}$  (Fig. 8.). Quanta of red radiation or heating lead to strengthening of oscillatory processes in the crystal lattices of the pigments and promoting the outcome of electrons from traps and their recombination with holes. FRANTSEVICH *et al.* (1980) suggested that infra-red radiation generates more f.r. than "white light". The results may be explained by using carbonized seeds in which high condensed systems were formed containing many unpaired electrons. We did not succeed in producing f.r. under powerful infra-red irradiation without carbonization of seeds, while irradiation of these seeds by intensive "white" flashes resulted in the immediate induction of free radicals (Fig. 9.).

CHETVERIKOV *et al.* (1978) showed in seeds stored under normal room temperature and humidity that f.r. recombine under the influence of atmospheric moisture. We stated that preliminarily irradiated dry seeds or their coats containing high concentration of f.r. when placed into a moist chamber lost the majority of f.r. within 5 min (Fig. 10). During this procedure

the mass of both seeds and their coats did not increase. In this case the intracellular structure inside the seeds and their coats are probably covered by a monomolecular layer of water promoting the migration of free radicals.

### Photoinduced Seedling Growth and Biomass

Seedlings raised from seeds previously irradiated by high intensive flashes had an increased biomass (Table 1). Energy variation from  $1.5 \times 10^5$  to  $15.0 \times 10^5 \text{ J m}^{-2}$  did not influence the length of shoots. The roots of seedlings obtained from the irradiated seeds were longer and more branched than in

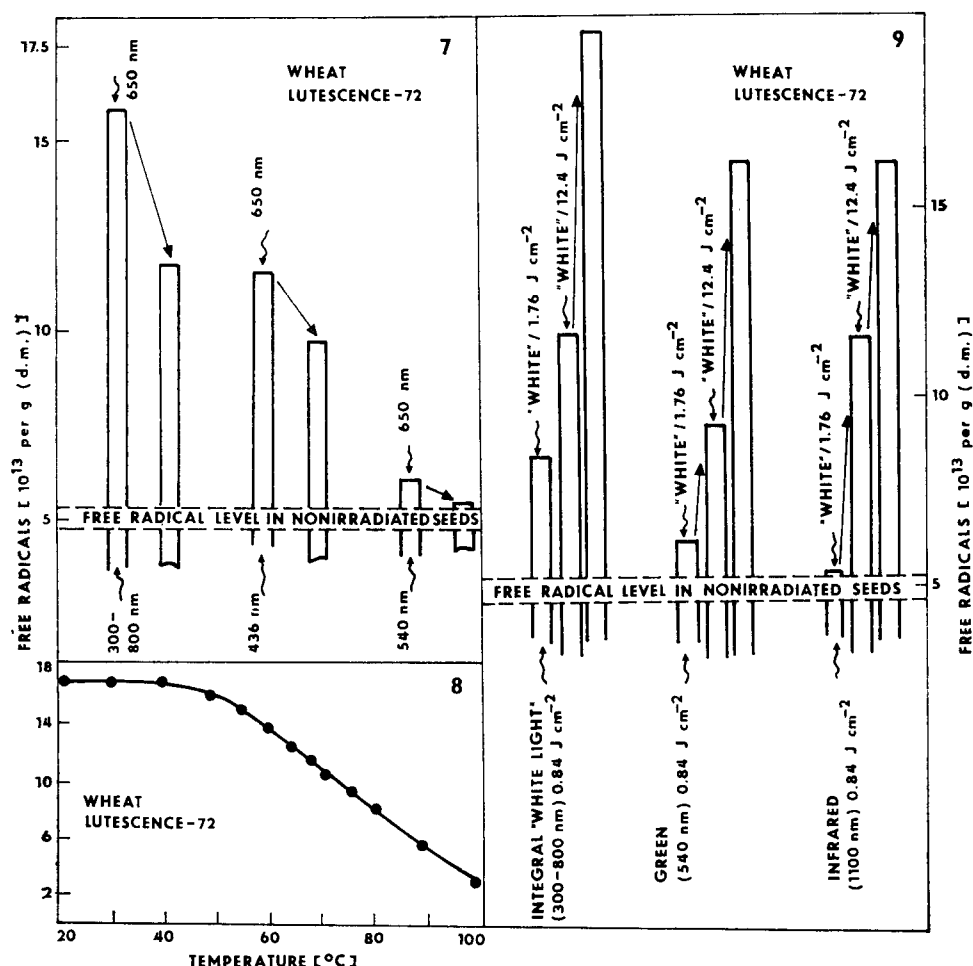


Fig. 7. Free radical recombination in wheat seeds after irradiation by white, blue or green radiation of  $2.70 \times 10^3 \text{ W m}^{-2}$  and after irradiation by red radiation of  $0.40 \times 10^3 \text{ W m}^{-2}$ .

Fig. 8. Recombination of photoinduced free radicals in wheat seeds after incubation at different temperatures for 5 min.

Fig. 9. Change of free radical concentration in wheat seeds, earlier irradiated by "white" green and infra-red radiation of  $0.84 \text{ J m}^{-2}$  power after second irradiation by "white light" flashes of  $1.76 \text{ J m}^{-2}$  and  $12.4 \text{ J cm}^{-2}$ . The time of irradiation was 10 min.

the seedlings from non-irradiated seeds. The root and shoot growth were correlated with dry matter accumulation.

The irradiation of seeds by blue radiation led to a decrease in the length of shoots and roots in comparison to "white", green and red radiation, but their biomass had increased faster than in the seedlings obtained from non-irradiated seeds or irradiated by "white" and green radiation. The green irradiation of seeds decreased the biomass of the seedlings (only a 9.3% increment). The red irradiation of seeds stimulated the dry matter accumulation but the length of the shoots and roots were similar to those of other seedlings.

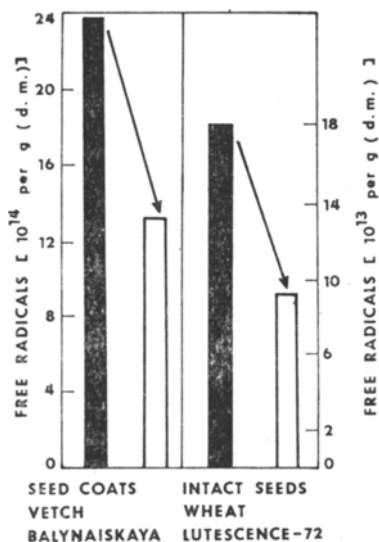


Fig. 10. Recombination of photoinduced free radicals in wheat seeds and seed coats of vetch seeds incubated under high humidity for 5 min.

#### Phosphorus Metabolism in Wheat Shoots Grown from Irradiated Seeds

During germination some part of endosperm phosphorus was used for growth of the seedlings, especially of shoots (Table 1.). Similar results were obtained for seedlings grown from seeds irradiated by "white", blue, green and red radiation (energy of the irradiation  $0.98 \times 10^5 \text{ J m}^{-2}$ ).

Seedlings from the seeds irradiated by "white" and blue light used the endosperm metabolites better. The amount of endosperm phosphates in seeds irradiated by green and red radiation was similar after 10 d of germination in treated and control seedlings.

The content of acid-soluble inorganic phosphate practically did not depend on the energy of the irradiation (Table 2), in contrast to the content of other phosphorus compounds. Total acid soluble phosphorus, organic acid-soluble phosphates, and nucleic phosphorus in shoots from irradiated seeds under  $1.5-3.0 \times 10^5 \text{ J m}^{-2}$  had increased, under  $6.0-9.0 \times 10^5 \text{ J m}^{-2}$  a plateau and a further increase was recorded under  $15.0 \times 10^5 \text{ J m}^{-2}$  (Table 2). The amount of acid-insoluble polyphosphates decreased with an increase in the energy of irradiation.

TABLE 1  
Growth parameters and the distribution of the endosperm phosphorus compounds in 10 day old etiolated wheat seedlings grown from irradiated seeds [mg P g<sup>-1</sup> (d.m.)]

Irradiation	Quantity of irradiation [ $\times 10^5$ J m <sup>-2</sup> ]	Length [cm]		Dry matter of 100 shoots [mg]	% of dry matter	Per cent of phosphorus in (intact seeds = 100%)		
		shoots	roots			shoots	roots	endosperm residue
Non-irradiated	0	11.0	4.0	515	11.8	47.1	15.7	37.2
High intensive flashes	1.5	10.6	4.3	526	12.1	51.3	14.9	33.8
	3.0	10.7	5.0	570	12.6	53.7	15.4	30.9
	6.0	10.2	4.3	520	12.0	50.5	14.8	34.7
	9.0	10.8	5.0	509	11.9	50.0	14.9	35.1
300—800 nm	15.0	9.9	4.1	459	10.6	46.2	16.4	37.4
	0.98	10.9	4.8	630	12.1	53.2	17.2	29.6
	0.98	8.4	3.3	783	12.7	52.3	16.6	31.1
	0.98	10.5	5.3	351	9.3	48.4	15.1	36.5
650 $\pm$ 6 nm	0.98	10.5	4.6	809	13.8	45.8	14.4	39.8



TABLE 2  
The distribution of phosphorus compounds in 10 d old etiolated wheat shoots grown from seeds irradiated with high intensive flashes or radiation of various wavelengths

Irradiation	Quantity of irradiation ( $\times 10^5 \text{ J m}^{-2}$ )	Total phosphorus	Acid-soluble phosphorus			Acid insoluble polyphos- phates	Nucleic phosphorus
			total	[mg P g <sup>-1</sup> (d.m.)]			
				inorganic	organic		
Non-irradiated	0	16.32	7.41	2.82	4.43	0.16	1.46
High intensive flashes	1.5	19.73	9.39	2.87	6.39	0.13	1.79
	3.0	18.78	9.94	2.85	6.92	0.17	1.87
	6.0	17.52	10.48	3.14	7.19	0.15	1.70
	9.0	17.09	10.13	3.01	7.01	0.11	1.87
	15.0	17.59	10.59	3.27	7.11	0.21	1.70
[mg P per 100 shoots]							
Dark	0	4 733.18	2 382.35	771.76	1 610.59	—	196.32
300—800 nm	0.98	4 835.10	2 309.78	643.48	1 666.29	—	234.49
436 $\pm$ 9 nm	0.98	5 282.08	2 823.53	668.23	2 118.59	36.71	268.43
540 $\pm$ 7 nm	0.98	4 647.59	2 794.11	828.23	1 926.35	39.52	214.58
650 $\pm$ 6 nm	0.98	5 472.17	2 812.50	636.36	2 176.14	—	278.53

Total acid soluble phosphorus in plant tissues from seeds affected by various spectral ranges of radiation changed in the sequence  $436\text{ nm} > 650\text{ nm} > 540\text{ nm} > \text{non-irradiated shoots} > 300\text{--}800\text{ nm}$ . The amount of organic acid-soluble phosphorus, nucleic phosphorus, and acid-insoluble polyphosphates was higher under red irradiation ( $650 \pm 6\text{ nm}$ ).

## DISCUSSION

The photoexcitement and subsequent formation of f.r. compounds can be a mechanism of action of high intensive flashes in various plant tissues (STANKO and CHETVERIKOV 1981). "White light" causes a higher f.r. yield in comparison with monochromatic light (including laser irradiation). When the ultraviolet range is omitted, this process can either result in the photo-sensitized formation of intermediate compounds (SHOLINA *et al.* 1969, BLYUMENFEL'D *et al.* 1969), or the photoexcitement of electrons in plant pigments such as melanins, polyphenols, *etc.* In analogous organic dyes  $\pi$ -electrons excited by irradiation subside in traps and then slowly recombine with holes (BLYUMENFEL'D *et al.* 1964). A portion of energy gets absorbed by electron wastes to oscillatory processes, and the other portion of energy is spent during electron recombination. The rate of death of photoinduced f.r. varies for different objects. CHETVERIKOV *et al.* (1978) reported that the concentration of f.r. in carrot seeds stored after irradiation by high intensive flashes in complete darkness under room temperature and humidity, decreased by only 10% per month. Photoinduced f.r. in wheat seeds did not recombine completely during storage for a year. In dependence on the dose of irradiation by high intensive flashes the f.r. concentration was 20 to 25% of that of f.r. in a moment of irradiation. The death of photoinduced f.r. may be induced by the contact of the stored seeds with water vapour. Absorbed water vapour enables the electrons to migrate from the traps, and a portion of these photoinduced free radicals can take part in oxidative — reductive reactions in stored seeds in the absence of free water.

Thus, high intensive flashes were able to generate excited electrons in pigments. These electrons in wet materials can either (1) form peroxide and hydroxide radicals, which are strong mutagens, (2) reduce quinoid compounds, or (3) intensify the local oscillatory processes (local heating). In turn it affects the enzyme reactions and hence metabolism and development. This will also inactivate the endogenous inhibitors (STANKO 1980).

In pea seeds (STANKO 1971) the stimulation of utilization of endosperm nitrogen compounds did not depend on the energy of irradiation; a similar stimulation of endosperm phosphorus utilization was observed in wheat seeds. Therefore, a stimulatory mechanism of metabolism and development of seedlings grown from seeds irradiated by high intensive flashes represents an active and more complete utilization of endosperm reserve compounds.

Monochromatic radiation of different spectral composition also increased the utilization of reserve compounds. The optimal energy of high intensive flashes stimulated an increase in biomass and growth of seedlings, which are associated with maximum utilization of polyphosphates. Acid-soluble polyphosphates are the more universal reserves of active phosphorus. The specific involvement of acid-soluble polyphosphates is in active transport of glucose and other sugars.

These polyphosphates are located on the outer surface of a cell. When concentrations of sugar and phosphorus are lowered, these polyphosphates perhaps break down and enter the cell, and the break-down products take part in different biochemical reactions (KULAEV 1975). The observed decrease in the quantity of acid-soluble polyphosphates indicates that polyphosphates favour the heterotrophic nutrition. Thus the different forms of irradiation of seeds can essentially modify phosphorus metabolism.

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