EFFECTS INDUCED BY SINGULAR AND COMBINED TREATMENTS WITH GAMMA RADIATION AND FLAVONOID COMPOUNDS ON *TRITICUM AESTIVUM* L.

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Abstract. The paper presents some aspects regarding the cytogenetic and phyto- biological effects induced by gamma radiations and by flavonoids compounds (rutin and quercetin at different concentrations) singularly and combined on *Triticum aestivum* L.

INTRODUCTION

The effects induced by ionizing radiation are multiple and depend on a wide range of factors like radiation debit and dose, ionizing density and, time and method of irradiation etc. Biological effects induced by gamma irradiation are represented by increase of chromosomal aberrations frequency in roots meristems, inhibition of irradiated seeds germination, inhibition of growing processes, fertility reduction, modification of biochemical traits etc. (Basi *et al*., 2006; Melki and Sallami, 2008; Singh and Balyan, 2009; Zaied *et al*., 2005; Zaka *et al*., 2002). Another important effect reffers to generation of free radicals also defined as reactive oxygen species (ROS), responsible for a large range of degenerative processes (Oshima *et al*., 1998). Excessive generation of ROS in biological systems include membrane lipids peroxidation, nucleid acids and carbohydrates oxidative damage (Jovanovic *et a*l., 1997). These oxidative processes can be controlled or reduced with exogenous antioxidants with free radical- scavenging activity (Kahkönen et al., 1999). The most important are polyphenols and flavonoids which act against oxidative effect of ROS.

Radiation protection is an area of great significance due to its possible applications in planned radiotherapy as well as unplanned radiation exposure. Research in the development of radioprotectors had focused on screening a variety of chemical and biological compounds. Various drugs from natural and synthetic origin have been evaluated extensively for their radioprotective potentials in both in vitro and in vivo models. Many flavonoids are already known for their antioxidant, and thus contribute towards radioprotection (Hosseinimehr and Nemati, 2006; Orsolic *et al*., 2006; Petrovic *et al*., 2008).

The purpose of our study was to identify natural chemical compounds with radioprotective properties against damages induced by gamma radiation, according to the current radioprotection research.

The two substances used are rutin and quercetin which belong to flavonoid class, principles with recognized antioxidative effects.

Tests were done on vegetal cell and the results can offer some cytogenetic and physiological information that can be used in further research on animal and human cells.

MATERIALS AND METHODS

In our experiments, seeds of *Triticum aestivum* L. (*Dropia* cultivar from SCAZ- Secuieni, Piatra- Neamt) were treated with gamma radiation (50, 100, 150, 250, 300, 400 Gy), rutin (0,01- R1 and 0,1%- R2, for 24 h) and quercetin (0,01- CV1 and 0,1%- CV2, for 24 h), singularly and combined. Rutin and quercetin were purchased from Merck. They were dissolved in distilled water at 80^0C.

The following parameters were analysed: the frequency of ana- telophases in root meristems with chromosomal aberrations; cariopses germination percent; root and shoot length; the fresh and dried weight of roots and shoot, respectively. The results were statistically interpreted.

RESULTS AND DISCUSSION

1. Frequency of ana- telophases in root meristem with chromosomal aberrations

Increasing of irradiation dose leads to increase of ana- telophases with chromosomal aberrations number from 6,62% (50 Gy) to 43, 59% (400 Gy) (table 1). Rutin and quercetin, singularly, have a cytogenetic effect, especially at 0,1% concentration, rutin at 0,01% also, aberrant cells number being double compared to control- 6,36- 6, 59%- or even higher in case of quercetin at 0,1% concentration- 8,69% instead of 3,33% in control (table 1).

At minimum radiation doses (50- 100 Gy), combined treatment with rutin leads to chromosomal aberrations induction- 8,3- 13, 78%, compared to 6, 62- 12, 40% in case of gamma irradiation, singularly; treatment with rutin in maximum concentration after gamma irradiation with 150- 250 Gy potentiate the action of gamma radiation- 24, 9- 28, 57%, compared with gamma irradiation, singularly, and at 0,01% concentration acts as a radioprotective substance. Rutin has a radioprotective effect against high doses of gamma radiation (300- 400 Gy) (table 1).

Table 1. Frequency of chromosomal aberrations in root meristem

Variants	**Total analysed A + T**	**Normal A + T**	**Aberrant A + T**	**x ± sx**	**Variants**	**Total analysed A + T**	**Normal A + T**	**Aberrant A + T**	**x ± sx**
Control	1416	1366	50	3,33 ± 0,02	Control	1416	1366	50	3,33 ± 0,02
50Gy	831	776	55	6,62 ± 0,03	50Gy	831	776	55	6,62 ± 0,03
100Gy	1145	1003	142	12,40 ± 0,03	100Gy	1145	1003	142	12,40 ± 0,03
150Gy	1297	1016	281	21,67 ± 0,03	150Gy	1297	1016	281	21,67 ± 0,03
250Gy	1382	1020	362	26,19 ± 0,03	250Gy	1382	1020	362	26,19 ± 0,03
300Gy	1166	778	388	33,28 ± 0,04	300Gy	1166	778	388	33,28 ± 0,04
400Gy	1035	667	358	34,59 ± 0,05	400Gy	1035	667	358	34,59 ± 0,05
0,01% (R1)	956	893	63	6,59 ± 0,03	0,01% (CV1)	1573	1509	64	4,07 ± 0,01
0,10% (R2)	1084	1015	69	6,36 ± 0,02	0,10% (CV2)	1381	1261	120	8,69 ± 0,02
50Gy + R1	711	652	59	8,30 ± 0,04	50Gy + CV1	1107	944	163	14,72 ± 0,03
50Gy + R2	771	667	104	13,49 ± 0,04	50Gy + CV2	1530	1386	144	9,41 ± 0,01
100Gy + R1	791	682	109	13,78 ± 0,04	100Gy + CV1	1249	1096	153	12,24 ± 0,02
100Gy + R2	1064	922	142	13,35 ± 0,03	100Gy + CV2	1524	1270	254	16,66 ± 0,02
150Gy + R1	495	394	101	20,40 ± 0,08	150Gy + CV1	1104	885	219	19,83 ± 0,03
150Gy + R2	743	558	185	24,90 ± 0,06	150Gy + CV2	1453	1131	322	22,16 ± 0,03
250Gy + R1	1007	768	239	23,73 ± 0,04	250Gy + CV1	802	626	176	21,94 ± 0,05
250Gy + R2	819	585	234	28,57 ± 0,06	250Gy + CV2	1115	745	370	33,18 ± 0,04
300Gy + R1	902	596	306	33,92 ± 0,05	300Gy + CV1	756	529	227	30,03 ± 0,06
300Gy + R2	810	591	219	27,04 ± 0,05	300Gy + CV2	1183	786	397	33,56 ± 0,04
400Gy + R1	703	499	204	29,02 ± 0,06	400Gy + CV1	866	479	387	44,69 ± 0,05
400Gy + R2	404	275	129	31,93 ± 0,12	400Gy + CV2	761	418	343	45,07 ± 0,06

At low radiation doses – 50 – 100Gy- quercetin amplifies the cytogenetic potential of gamma radiation- 9,41- 16,66% aberrant cells compared to 6,62- 12, 40%. These effect is less obvious in 100Gy + CV1 variant; we can do the same observation in combined treatment with high dose of radiation – 400Gy, the chromosomal aberrations percent being with about 10% higher than gamma irradiation, singularly (table 1).

Quercetin applied after irradiation with medium doses has a radioprotective effect in 300Gy + CV1 variant, compared to 150 – 250Gy and 300Gy, singularly (table 1).

2. Cariopses germination percent

In our experiment, the number of germinated cariopses was reduced in irradiated variants with 16% minimum at 150Gy with 34% maximum at 100Gy (table 2).

The two tested flavonoids also inhibited cariopses germination, but not very important compared to control, with one exception represented by rutin in maximum concentration (table 2).

We can observ a similar situation in case of combined treatment – gamma radiation and rutin, compared with gamma irradiation, singularly, but at the same dose of 150Gy the situation is not very clear and at maximum dose, the treament with rutin applied after irradiation was not efficient as radioprotector, cariopses germination values were smaller than those after gamma irradiation with 400Gy dose (table 2).

Table 2. Percent of cariopses germination

Variants	Germination (%)	Variants	Germination (%)
Control	98,33	Control	98,33
50Gy	76,00	50Gy	76,00
100Gy	66,00	100Gy	66,00
150Gy	84,00	150Gy	84,00
250Gy	72,66	250Gy	72,66
300Gy	70,66	300Gy	70,66
400Gy	82,66	400Gy	82,66
0,01% (R1)	94,66	0,01% (CV1)	93,33
0,10% (R2)	76,00	0,10% (CV2)	95,33
50Gy + R1	86,00	50Gy + CV1	80,66
50Gy + R2	88,66	50Gy + CV2	83,33
100Gy + R1	84,66	100Gy + CV1	86,66
100Gy + R2	84,66	100Gy + CV2	79,33
150Gy + R1	76,66	150Gy + CV1	82,00
150Gy + R2	84,00	150Gy + CV2	88,06
250Gy + R1	80,66	250Gy + CV1	80,66
250Gy + R2	84,00	250Gy + CV2	86,66
300Gy+ R1	77,33	300Gy + CV1	81,33
300Gy + R2	72,66	300Gy + CV2	84,00
400Gy + R1	75,33	400Gy + CV1	90,66
400Gy + R2	74,00	400Gy + CV2	86,00

Quercetin seems to be more efficient than rutin. But, in one experimental variant- 150Gy + CV1-, cariopses germination procent was insignificant reduced with 2% compared to gamma irradiation at 150Gy, singularly (table 2).

3. Root and shoot length

Growing processes of wheat treated with gamma radiation are affected by these. Root and shoot growth seems to be inhibited by mutagenic treatment, at high radiation doses especially. We can observ a reduction with 30 - 31% of root length and with 10 - 13% of shoot length at 300 and 400Gy. Some higher values are not important because we can not establish a dose- effect relation. But, root length was stimulated with 10- 12% at 100 Gy and even 250Gy. Shoot length was not significantly modified at similar gamma radiation doses (table 3, 4).

As concerning, singularly treatments with rutin and quercetin, root and shoot length was stimulated at maximum concentrations (table 3, 4).

Table 3. Effect of gamma radiation and of rutin treatments, singularly and combined on root and shoot length.

Variant	Root				Shoot			
	Length (mm)				Length (mm)			
	x ±	sx	s%	C=100	x ±	sx	s%	C=100
Control	111,46	1,15	11,53	100	126,40	1,82	16,05	100
50Gy	85,64	1,57	18,56	76	129,35	2,19	17,13	102
100Gy	123,10	2,38	15,82	110	130,30	2,98	18,72	103
150Gy	99,45	1,77	16,74	89	127,66	2,31	16,99	101
250Gy	125,22	2,58	21,47	112	127,39	2,13	17,49	101
300Gy	76,55	2,85	33,68	69	110,52	2,75	22,56	87
400Gy	78,07	2,99	35,14	70	114,33	2,45	19,67	90
0,01% (R1)	100,29	1,27	14,84	90	126,47	1,64	15,17	100
0,10% (R2)	160,61	2,25	15,84	144	130,83	1,85	15,97	104
50Gy + R1	110,51	1,65	16,12	99	141,76	1,83	13,93	112
50Gy + R2	132,85	1,99	16,92	119	129,75	2,01	17,59	103
100Gy + R1	120,46	1,83	16,25	108	139,47	1,77	13,54	110
100Gy + R2	128,62	1,90	15,55	115	132,93	1,80	14,19	105
150Gy + R1	84,47	1,53	20,03	76	128,68	2,08	18,14	102
150Gy + R2	84,38	1,36	16,56	76	132,57	1,93	14,96	105
250Gy + R1	90,86	2,01	24,18	82	121,87	1,90	17,03	96
250Gy + R2	65,42	1,55	25,25	59	121,53	2,06	18,06	96
300Gy + R1	104,06	2,56	25,50	93	122,70	2,28	19,21	97
300Gy + R2	66,46	2,08	31,72	60	113,81	2,65	23,63	90
400Gy + R1	83,37	3,61	42,02	75	111,14	3,04	26,52	88
400Gy + R2	46,52	1,90	42,04	42	91,19	2,71	30,32	72

Table 4. Effect of gamma radiation and of quercetin treatments, singularly and combined on root and shoot length.

Variants	Root				Shoot			
	Length (mm)				Length (mm)			
	x ±	sx	s%	C=100	x ±	sx	s%	C=100
Control	111,46	1,15	11,53	100	126,40	1,82	16,05	100
50Gy	85,64	1,57	18,56	76	129,35	2,19	17,13	102
100Gy	123,10	2,38	15,82	110	130,30	2,98	18,72	103
150Gy	99,45	1,77	16,74	89	127,66	2,31	16,99	101
250Gy	125,22	2,58	21,47	112	127,39	2,13	17,49	101
300Gy	76,55	2,85	33,68	69	110,52	2,75	22,56	87
400Gy	78,07	2,99	35,14	70	114,33	2,45	19,67	90
0,01% (CV1)	109,14	1,23	12,95	98	119,17	1,77	17,04	94
0,10% (CV2)	121,26	1,37	13,39	109	124,16	1,54	14,77	98
50Gy + CV1	98,43	1,42	15,45	88	128,45	1,79	14,95	102
50Gy + CV2	115,09	1,71	16,32	103	128,46	2,16	18,54	102
100Gy + CV1	97,14	1,56	17,59	87	127,92	1,95	16,72	101
100Gy + CV2	105,84	1,59	16,56	95	126,94	2,60	22,66	100
150Gy + CV1	98,08	1,23	13,58	88	125,68	2,06	17,73	99
150Gy + CV2	81,41	1,43	18,87	73	124,77	1,79	15,50	98
250Gy + CV1	74,23	1,46	19,94	67	114,71	2,11	18,63	91
250Gy + CV2	66,29	1,17	18,04	59	106,50	2,18	20,83	84
300Gy + CV1	65,30	2,12	36,56	59	104,93	2,26	24,23	83
300Gy + CV2	65,47	1,63	28,01	59	105,98	2,21	23,46	84
400Gy + CV1	59,02	2,13	37,38	53	99,14	2,29	23,96	78
400Gy + CV2	59,21	2,29	37,54	53	98,26	2,66	26,26	77

Treatment with rutin after gamma irradiation with 50- 100 Gy, slightly ameliorate the physiological damages involved in root and shoot growth. Rutin does not have a radioprotective effect from 150 Gy above for root and 250 Gy above for shoot, only for root partially, but only in minimum concentration (0,01%) (table 3).

Treatment with quercetin did not ameliorate the damages induced by irradiation, but only at 50Gy in case of root which had an increased length with 15 - 34% compared to 50Gy gamma irradiation, singularly. As for the rest, this parameter was reduced with 1 - 41%. The effect of quercetin- combined treatment was inefficient 50 – 150Gy in shoots and has intensified the irradiation effect with 5 - 16% at gamma high doses (table 4).

4. Fresh and dried weight of root and shoot

Root fresh weight of gamma irradiated variants is between 3,52 g at 400Gy and 7,33 g at 250Gy, and dried weight between 0,358 g at 300Gy and 0,600 g at 250Gy, compared to 8,0 g and 0,62 g respectively, in control; in the same variants, the values of shoot fresh weight were between 5,85 g at 100Gy and 9,51 g at 250Gy, and for dried weight between 0,981 g at 300Gy and 1,378 g at 250Gy, compared to 9,39 g and 1,267 g respectively, in control (table 5, 6).

In case of treatments with rutin and quercetin, the sequence of variable data for fresh and dried weight, respectively, even if we compare it to control, does not provide us enough information about biomass accumulation, because the obtained values are not comparable (table 5, 6).

Table 5. Values of fresh and dried weight on *Triticum aestivum* treated singularly and combined with gamma radiation and rutin

Variants	Root				Shoot			
	Fresh weight (g)		Dried weight (g)		Fresh weight (g)		Dried weight (g)	
	Total	C=100	Total	C=100	Total	C=100	Total	C=100
Control	8,00	100	0,620	100	9,39	100	1,267	100
50Gy	5,53	69	0,531	86	8,68	92	1,269	100
100Gy	3,92	49	0,414	67	5,85	62	0,814	64
150Gy	4,49	56	0,485	78	7,89	84	1,113	88
250Gy	7,33	91	0,600	97	9,51	101	1,378	109
300Gy	3,47	43	0,358	57	6,39	68	0,981	77
400Gy	3,52	44	0,377	61	6,51	69	0,986	78
0,01% (R1)	5,85	73	0,604	97	9,85	105	1,469	116
0,10% (R2)	8,41	105	0,729	118	9,97	106	1,406	111
50Gy + R1	6,50	81	0,701	113	11,10	118	1,511	119
50Gy + R2	7,75	97	0,791	128	11,09	118	1,524	120
100Gy + R1	6,64	83	0,701	113	10,24	109	1,433	113
100Gy + R2	7,40	92	0,629	101	9,58	102	1,338	106
150Gy + R1	6,08	76	0,588	95	10,94	117	1,506	119
150Gy + R2	5,79	72	0,565	91	9,42	101	1,285	101
250Gy + R1	5,91	74	0,515	83	9,49	101	1,298	102
250Gy + R2	5,01	63	0,541	87	9,45	101	1,328	105
300Gy + R1	5,58	70	0,511	81	8,89	95	1,290	102
300Gy + R2	3,23	40	0,420	68	8,24	88	1,271	100
400Gy + R1	3,91	49	0,419	68	6,80	72	1,039	82
400Gy + R2	3,30	41	0,372	60	6,19	66	0,975	77

In case of gamma radiation and rutin- combined treatment, root fresh weight is between 3,23 g (300Gy + R2) and 7,75 g (50Gy + R2); root dried weight is between 0,72 g (400Gy + R2) 0,791 g (50Gy + R2); shoot fresh weight is between 6,19 g (400Gy + R2) and 11,10 g (50Gy + R1), and dried weight of shoot between 0,975 g (400Gy + R2) and 1,524 g (50Gy + R2)(table 5).

In case of gamma radiation and quercetin- combined treatment, fresh weight values of roots are between 3,6 g (400Gy + CV2) and 9.8 g (50Gy + CV2), and between 7,05 g (400Gy + CV2) and 10,68 g (50Gy + CV2) for shoots, respectively; dried weight is between 0,367 g (400Gy + CV2) and 0,698 g (50Gy + CV2) for roots, and between 1,057 g (400Gy + CV2) and 1,336 g (50Gy + CV2) for shhots (table 6).

Compared to root and shoot length, we can affirm that dried mass accumulation in irradiated variants does not positively correlate with the dimensions of the two organs. High values of dried mass especially for shoot, are obtained in case of reduction of root and shoot length with 30 - 31% and 10 - 13%, respectively, at 300 and 400Gy.

It seems to be a response to irradiation stress induced by high doses, expressed by metabolism enhancement, therefore biosynthesis of different compounds which totalized (substances and structures) lead to the increase of dried mass.

Table 6. Values of fresh and dried weight on *Triticum aestivum* treated singularly and combined with gamma radiation and quercetin

Variants	Root				Shoot			
	Fresh weight (g)		Dried weight (g)		Fresh weight (g)		Dried weight (g)	
	Total	C=100	Total	C=100	Total	C=100	Total	C=100
Control	8,00	100	0,620	100	9,39	100	1,267	100
50Gy	5,53	69	0,531	86	8,68	92	1,269	100
100Gy	3,92	49	0,414	67	5,85	62	0,814	64
150Gy	4,49	56	0,485	78	7,89	84	1,113	88
250Gy	7,33	91	0,600	97	9,51	101	1,378	109
300Gy	3,47	43	0,358	57	6,39	68	0,981	77
400Gy	3,52	44	0,377	61	6,51	69	0,986	78
0,01 (CV1)	9,58	120	0,639	103	9,25	98	1,159	91
0,10 (CV2)	10,39	130	0,782	126	10,48	112	1,408	111
50Gy + CV1	8,70	109	0,648	104	10,20	109	1,262	99
50Gy + CV2	9,80	122	0,698	113	10,68	114	1,336	105
100Gy + CV1	7,95	99	0,556	89	10,04	107	1,207	95
100Gy + CV2	8,30	104	0,538	87	10,00	106	1,284	101
150Gy + CV1	7,35	92	0,571	92	10,35	110	1,317	104
150Gy + CV2	6,20	77	0,528	85	9,23	98	1,275	100
250Gy + CV1	5,20	65	0,437	70	8,24	88	1,150	91
250Gy + CV2	4,71	59	0,401	65	7,24	77	1,032	81
300Gy + CV1	5,85	73	0,403	65	9,14	97	1,223	96
300Gy + CV2	5,85	73	0,433	70	9,00	96	1,274	100
400Gy + CV1	4,42	55	0,389	63	7,60	81	1,099	87
400Gy + CV2	3,60	45	0,367	59	7,05	75	1,057	83

CONCLUSIONS

The investigations on Triticum aestivum L seeds and plants can be included in the research area regarding the screening and use of some vegetal products with radioprotective properties.

The results are accordingly with literature about biological effects induced by gamma radiation and the influence of flavonoids compounds against gamma irradiation damages.

Gamma radiation in doses of 50 – 400Gy produced chromosomal aberrations in root meristem, with an increased frequency according to irradiation dose. Rutin and quercetin have a slightly cytogenetic effect in tested concentrations (0,01% and 0,10%).

Thea treatment with rutin after gamma irdiation has a radioprotective effect on cytogenetic level; we can observe the same situation in case of quercetin treatment after gamma irradiation,but only at minimum concentration (0,01%), with the exception of high radiation doses. In this context, we can suppose that flavonoids compounds positively influence enzyme mechanisms of repair and recovery of chromosomal aberrations induced by gamma radiation.

Without a clear dose- effect relation, cariopsis germination was inhibited by gamma radiation. Treatment with flavonoids compounds slightly reduced cariopsis germination percent in case of quercetin, while rutin at maximum concentration (0,1%) strongly inhibited cariopsis germination. Treatment with flavonoids after irradiation has favorable effects on germination, improving implied and damaged physiological processes by gamma radiation, with the exception of rutin treatment after the higher dose of radiation.

At minimum and medium gamma radiation (50 – 250Gy), their influence on wheat plants growth is not very important;we can observ the negative effect of gamma radiation at 300 – 400Gy, with an inhibition of 30- 31% in case of root growth and with 10-13% for shoot growth, respectively. It seems that roots are more sensitive to gamma radiation.

The tested flavonoids differentially influenced wheat plants growth accordingly to their chemical structure and concentrations. We did not observ clear correlations between above-mentioned factors, but it seems that rutin has favorable effects on root and shoot growth, while quercetin slightly inhibited shoot growth.

Treatament with rutin after gamma radiation was efficient as radioprotector for root growth, in case of 50 and 100Gy doses, and 50 – 150Gy doses, for shoot growth, respectively; rutin has a similar effect at high radiation doses, but only in minimum concentration and for root growth.

Quercetin has a favorable effect only on root growth of wheat treated with 50 Gy. We can observe an amplification of radiation negative effects accordingly to radiation dose increase, about 1 - 24% in case of root growth and 1 - 16% in case of shoot growth, respectively.

Gamma radiation increase the amount of dried mass.

The results suggest a possibly water imbalance, and on the other side a general stimulation of metabolism, cell biosynthesis which lead to an increase of dried mass, as a response to gamma radiation stress.

Variantions of the fresh and dried mass in gamma radiation and flavonoids - combined treated variants are according to those obtained in growth test.

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