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EFFECT OF SOME SEED HARDENING TREATMENTS ON CHEMICAL COMPOSITION OF SUDAN GRASS GROWN UNDER SALINE CONDITIONS

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SUMMARY

The effect of some seed hardening treatments on chemical composition of sudan grass (Sorghum vulgare var. sudanense) was investigated in two pot experiments under saline conditions.

Salinity induced a progressive increase in total carbohydrates and proline. However, protein content decreased with increasing salinity level during the two cuts. The application of different pre-sowing treatments caused an enhansive effect on total carbohydrates and protein content according to the applied salinity level as well as the period of growth. The amount of proline diminished greatly by using most of the applied pre-sowing treatments throughout the two cuts under different salinity levels.

The increase of salinity has a depressive effect on chlorophyll a & b during the 2nd cut and the reverse was true at the 3rd cut. On contrast, carotene content showed a progressive increase with increasing salinity level throughout the two cuts. On the other hand, the application of pre-treatments was effective for correcting the adverse effect of salinity on photosynthetic pigments.

Generally, salinity induced a pronounced increase in both Na^+ and Cl^- as well as ash contents while, it decreased Ca^{++}, Mg^{++} and K^+ contents in sorghum shoots. The application of different pre-sowing treatments eliminated the depressive effect of salinity and corrected to great extent ions absorption. However, the applied seed treatments differed greatly among their effectiveness on ion uptake and accumulation.

INTRODUCTION

In previous investigation, the same authors (in press) studied the effect of some hardening treatments i.e irradiation, soaking in water, succinic acid, CCC, $CaCl_2$ and $ZnSo_4$ beside untreated dry seeds on germination, growth and yield of sudan grass grown under saline conditions. They recorded that salinity induced a significant decrease in both germination rate and percentage. Growth of sorghum plants, as being indicated from the values of fresh and dry weight of shoots, shoot height, leaf area, number of leaves and number of tillers have been adversely affected by salinity. Dry-and wet-seed irradiation, $ZnSo_4$ and CCC were the most effective treatments for counteracting the adverse effect of salinity on plant growth. Such pre-treatments induced a marked increase in all growth characters except CCC which depressed shoot height comparing with the control (water soaking).

Considering yield, salinity at any of the applied level decreased forage yield (green and dry matter). The application of most of pre-sowing treatments can eliminate the adverse effect of salinity on yield. The effect of seed hardening treatments on chemical composition that has been disturbed under saline conditions has been pointed out in some plant species (Khafagi et al. 1984 & 1986, Kishk 1984, Ozoris et al. 1984 and El-Agamy et al. 1991).

The aim of the present study was to evaluate the effect of some pre-sowing hardening treatments on chemical composition of sudan grass grown under different salinity levels.

MATERIALS AND METHODS

Two pot experiments were carried out under a fenced area at the Faculty of Science (Girls), AL-Azhar University, Cairo, to investigate the effect of some pre-sowing treatments on chemical composition of sudan grass (<u>Sorghum vulgare</u> var. sudanense) at three levels of salinity 2000, 4000 and 6000 ppm mixtures of NaCl and $CaCl_2$ (2:1, W/W) in addition to the control. The first was performed in 1988/1989 and the second was in 1989/1990 growing seasons.

The studied pre-sowing treatments were :

1- Untreated dry seeds (control for irradiated dry seeds and water soaking treatments)

2- Exposure of dry seeds to gamma rays irradiation (3000 rad).

3- Soaking in distilled water (control for wet-seed irradiation and different soaking treatments).

4- Exposure of soaked seeds to gamma rays irradiation (3000 rad).

5- Soaking seeds in 0.09% succinic acid solution.

6- Soaking seeds in 2000 ppm CCC (Cycocel) solution.

7- Soaking seeds 0.25% calcium chloride solution.

8- Soaking seeds in 100 ppm Zinc sulphate solution.

All seed soaking treatments were performed for 4 hours and subsequently dried before planting. Gamma-ray irradiation was performed in the National Center of Radiation Research and Technology, Nasr City, Cairo, Egypt at 3000 rad level using Co^{-60} source. In all treatments, seeds were cultivated after 24 hours from seed hardening treatment.

All seeds soaked or unsoaked, were planted in pots (30X25cm) filled with 13.5 Kg HCl treated sand. The experiment was represented by 256 pots, statistically arranged in randomized complete block design. The pots were irrigated with tap water for 10 days from planting and after complete germination, plants were thinned to ten plants/pot. Then irrigation with Hoagland solution (Hoagland & Arnon, 1950) was applied in both seasons until the establishment of seedling emergence. Each treatment was irrigated with a particular salinity level, including the control (tap water), 2000, 4000 and 6000 ppm till the end of the experiment.

Four cuts were taken throughout the growth period while, chemical analysis of sorghum shoots was carried out on the second and third cuts, 105, 135 days from sowing respectively **during the last growth season (1989) .**

The photosynthetic pigments (chlorophyll "a", "b" and carotenoids) were determined in fresh leaves of each treatment using the method described by Vernon and Selly (1966). Proline was determined in fresh material according to Bates et al. (1973).

Shoot samples were dried in an oven at 70°C and ground to fine powder. The following chemical analysis were conducted :

1- Total carbohydrates were extracted according to Smith et al. (1964) and estimated colorimetrically by phenol-sulphuric acid method as described by Dubois et al. (1951).

2- Total nitrogen content was determined by using the modified micro-Kjeldahl method adopted by Paech and Tracay (1956). The nitrogen content was multiplied by 5.7 to obtain the total protein content.

3- Total ash content was determined following A.O.A.C.(1960) methodology. The chloride content was determined by titration with silver nitrate after ashing the plant material.

4- After digestion of dry samples by using the wet-ashing procedure of Johson and Ulrich(1959), sodium and potassium were photometrically determined. Calcium and magnesium were determined using Atomic Spectrophotometer "Pye Unicum SP1900".

RESULTS AND DISCUSSION

1- Metabolic Aspects:

1-1 Total carbohydrate contents.

The results in tables (1&2) illustrate that a marked increase in carbohydrate content was observed with the progress of plant growth and development for both salinized and non-salinized plants. Also, increasing salinity stress induced a considerable increase in total carbohydrate content in sorghum shoots. From previous investigation (Ismaeil et al. in press) data revealed that there was a marked decrease in shoot dry weight by increasing salinity level throughout the growth period. This result may be due to the their rapied rate of accumulation of carbohydrates than its utilization for the formation of new cells and tissues (Strogonov,1962). These results are in agreement with those obtained by Ebad et al. (1992).

Concerning the pre-sowing treatments under non-saline conditions data show that the different applied treatments induced an enhansive effect on carbohydrate accumulation as compared with the corresponding controls for the two cuts. Zinc sulphate and $CaCl_2$ pre-treatments were rather superlative for the two cuts.

Under saline conditions, the application of most pre-sowing treatments tended to increase total carbohydrate content. This is more obvious at the 2nd cut than at the 3rd cut.

1-2 Protein content

Tables (1&2) show that there was a noticeable increase in the crude protein content with age for either salinized and non-salinized plants. Increasing salinity levels in irrigated solution caused a marked reduction in protein content throughout the two cuts. The depressive effect of salinity on protein content has been noted by Khafagi et al. (1986) and Ebad et al. (1992) on different plant species. The depression in protein content under saline conditions could be attributed to the reduction of RNA and DNA required for protein synthesis and increased their degradation by affecting hydrolyzing enzymes (Garg and Garg,1980 and Ebad et al., 1992).

Most of the pre-sowing treatments revealed more or less similar effect on protein content as compared with the corresponding controls under non-saline conditions.

The interaction effects between different salinity levels and pre-sowing treatment showed that succinic acid and wet-seed irradiation treatments induced a stimulative effect on protein accumulation during the 2nd cut under low salinity level (2000ppm.). On the other hand, under moderate salinity level (4000ppm.), CCC and wet-seed irradiation showed a marked increase in protein accumulation at the 2nd cut for the former pre-treatment and at the 3rd cut for the latter compared with the control. Moreover, under highest salinity level, CCC was the most effective treatment for improving protein accumulation at the 2nd cut.

Similar effect was observed by using dry-seed irradiation and water soaking treatments under high salinity level (6000ppm.). The stimulative effect of some pre-sowing treatment on protein accumulation under saline condition was also confirmed by El-Agamy et al. (1991). The enhancing effects of CCC have been recorded by Salim (1984) and Khafagi et al. (1984 & 1986). Uprety and Sarin (1975) reported that application of growth regulators caused salt tolerance by supporting protein synthesis and preventing its hydrolysis.

1-3 Proline content:

Data presented in table (1 & 2) reveal that proline content in sorghum leaves exhibited a progressive increase with the advancement of plant growth for either salinized and non-salinized plants. Additionally, proline content showed a progressive increase with increasing salinity levels in the soil media throughout the two cuts. These results agree with some investigators (Storey and Wyn Jones 1978 and Salim. 1984). Handa et al. (1985) suggested that proline accumulation was an adaptive response to stress conditions and added that proline at high levels acts as cytoplasmic osmotic solute.

Under non saline conditions, dry-seed irradiation and water soaking treatments induced a depressive effect on proline content in leaves as compared with the control (dry seeds) at the 2nd cut. However, at the 3rd cut all the different pre-sowing treatments caused an inhibitive effect on proline accumulation as compared with the corresponding controls.

The interaction between different salinity levels and pre-sowing treatments revealed that dry-seed irradiation, $CaCl_2$ as well as water soaking treatments diminished greatly the amount of proline in leaves during the two cuts under different salinity levels. Moreover, $ZnSo_4$ induced such effect under moderate and high salinity levels throughout the two cuts.

1-4 Photosynthetic pigment content:

The results in tables (1 & 2) show that there was a marked decrease in chlorophylls a & b with age for either salinized and non-salinized plants. In addition, salinity caused a progressive reduction in chlorophylls a & b at the 2nd cut. Wherese, at the 3rd cut a pronounced increase was observed under low and moderate salinity levels, while the reverse was true under high salinity level. On the other hand, carotene content showed a progressive increase with increasing salinity level during the two cuts. The increase in carotene content was considered as an adaptive response for the continuity of metabolic activities, particularly at the high levels of soil salinity (Adel-Rahman and Abdel Hadi, 1984). The reduction in chlorophyll contents by salinity is due to the decrease in absorption of magnesium needs for chlorophyll synthesis and to the destruction of chlorophyll "a" which restricted the photosynthetic rate (Poljakoff Mayber and Gale, 1975).

The obtained data indicate that under non-saline conditions, water soaking treatment showed a pronounced increase in chlorophyll a and carotene contents during the two cuts as well as in chlorophyll b at the 3rd cut. In addition, dry-seed irradiation showed a marked increase in photosynthetic pigments at the 3rd cut. CCC and $CaCl_2$ pre-treatment induced a stimulative effect on chlorophylls a & b at the 2nd cut only.

The interaction between soil salinity and pre-sowing treatments illustrated that dry-seed irradiation induced a pronounced stimulative effect on photosynthetic pigments throughout the 2 cuts under the highest salinity level while, wet-seed irradiation caused such effect under different salinity levels at the 2nd cut only. In addition, CCC, $CaCl_2$ and $ZnSo_4$ induced such effect throughout the two cuts under high salinity level (6000ppm.).

2- Ash and mineral contents:

Results in tables (1 & 2) show that Na^+ and ash contents exhibited a pronounced increase with the progress of age and development in both salinized and non-salinized plants while, Ca^{++} show the same effect for non-salinized plants. The reverse was true for K^+, Mg^{++} and Cl^- contents. Generally, the increase in salinity level in the soil was coupled with the increase in total ash, Cl^- and Na^+ contents as well as decrease in Mg^{++}, Ca^{++} and K^+ contents throughout the two cuts. In this regard, Kaddah (1962) indicated that the increasing content of one or more cations in the plant tissues to some extent compensated by a decrease in the content of other cations.

Regardless salinity levels, some of the applied pre-sowing treatments increased the accumulation of K^+, Ca^{++}, Mg^{++} as well as ash content, while Na^+ and Cl^- were depressed. Such effect varied according to the period of growth. In this concern, $ZnSo_4$ treatment caused an enhansive effect on K^+, Ca^{++}, Mg^{++} as well as ash content and induced a reduction in Na^+ accumulation at the 2nd cut. On the other hand, such treatment exhibited a stimulative effect on K^+, Ca^{++} and Mg^{++} contents at the 3rd cut. Also, $CaCl_2$ treatment proved to be the most effective treatment for accumulation of K^+, and Ca^{++} at the 3rd cut.

The application of different pre-sowing treatments could eliminate the adverse effect of salinity and correct to great extent ions uptake in sorghum shoots. However, the application of different pre-sowing treatments differed greatly among their effectiveness on ion uptake and accumulation. Similar conclusion was obtained by Khafagi et al. (1984 & 1986), KishK (1984), Ozoris et al. (1984) and El-Agamy et al. (1991). Similarly, Ali and Robishy (1983) indicated that plant mineral content was disturbed under saline conditions, whereas gamma rays tended to correct such effect by enhancing the absorption of divalent cations and decreasing that of monovalent.

From the above results, it is obvious that potassium is the major cation in sorghum because sorghum is a sodium excluder, while sodium was the major cation in roots except at low sodium/potassium ratios in culture media (Weimberg et al., 1984). This result may be due to that plants cope to some degrees with the excess of Na by excluding its uptake or secreting it into vacuoles (Rains, 1972). The data also confirm the idea that the application of CCC induces salt-tolerance by supporting the synthesis of protein and preventing its hydrolysis (StarcK and KarvowosKa, 1978) as well as by restoring the mineral balance in plant tissues.

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Table 1: Effect of different seed hardening pre-sowing treatments on total carbohydrate, crude protein, proline and pigments contents of sorghum plant grown under different salinity levels at second cut.

Salinity levels (ppm)	pre-sowing treatments	Carbohydrate content % dry wt.	Protein content % dry wt.	Proline content µmole/g fresh wt.	Pigments (mg/g fresh wt.)		
					Chl. a	Chl. b	Carotene
Tap Water	Control	13.0	8.19	0.24	1.05	1.11	0.98
	Irradiation (dry)	18.5	5.80	0.16	0.97	0.88	0.69
	Water soaking	18.5	8.39	0.20	1.24	0.75	1.11
	Irradiation (wet)	20.0	8.39	0.32	0.81	0.62	0.82
	Succinic acid	19.5	8.99	0.30	0.58	0.79	0.75
	C C C	25.0	8.39	0.28	1.32	0.84	1.08
	$Ca\ Cl_2$	28.0	7.69	0.23	1.40	0.94	1.04
	$Zn\ SO_4$	28.0	8.99	0.22	1.20	1.03	1.14
2000 ppm	Control	18.0	7.39	0.37	1.12	1.00	0.97
	Irradiation (dry)	25.5	8.69	0.15	1.09	0.98	0.95
	Water soaking	20.0	7.69	0.28	1.11	0.73	1.00
	Irradiation (wet)	28.0	13.99	0.35	1.18	0.92	1.08
	Succinic acid	25.0	18.79	0.18	0.88	0.89	0.79
	C C C	27.0	8.99	0.35	1.32	0.95	1.09
	$Ca\ Cl_2$	19.5	5.80	0.25	1.13	0.87	0.93
	$Zn\ SO_4$	18.0	7.69	0.28	1.32	0.98	1.01
4000 ppm	Control	20.5	6.09	0.48	0.94	1.10	1.07
	Irradiation (dry)	28.0	6.59	0.27	0.97	0.81	0.82
	Water soaking	22.5	8.99	0.35	0.97	1.01	0.98
	Irradiation (wet)	28.5	8.39	0.17	1.87	1.15	1.30
	Succinic acid	28.0	8.29	0.28	0.67	0.94	0.83
	C C C	21.0	11.19	0.28	1.18	0.57	1.19
	$Ca\ Cl_2$	25.5	8.39	0.18	1.05	0.64	0.88
	$Zn\ SO_4$	18.0	6.99	0.17	1.17	1.72	0.83
6000 ppm	Control	22.0	8.69	0.78	0.80	0.95	1.05
	Irradiation (dry)	19.5	5.60	0.24	1.20	1.24	1.09
	Water soaking	24.0	8.29	0.49	0.85	0.60	0.81
	Irradiation (wet)	25.5	8.39	0.32	0.97	0.81	0.93
	Succinic acid	28.0	7.69	0.19	0.78	0.70	0.73
	C C C	22.5	11.29	0.31	1.40	1.11	0.98
	$Ca\ Cl_2$	28.5	9.79	0.23	2.25	1.34	1.82
	$Zn\ SO_4$	17.5	5.80	0.16	1.55	1.00	1.03

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Table 2: Effect of different seed hardening pre-sowing treatments on total carbohydrate crude protein, proline and pigments contents of sorghum plant grown under different salinity levels at third cut.

Salinity levels (ppm)	pre-sowing treatments	Carbohydrate content % dry wt.	Protein content % dry wt.	Proline content µmole/g fresh wt.	Pigment (mg/g fresh wt.)		
					Chl. a	Chl. b	Carotene
Tap Water	Control	15.0	15.63	0.67	0.37	0.42	0.51
	Irradiation (dry)	25.0	12.70	0.62	0.95	0.80	0.75
	Water soaking	17.0	16.59	0.56	1.17	0.98	0.90
	Irradiation (wet)	27.5	12.78	0.39	0.58	0.58	0.98
	Succinic acid	27.0	13.31	0.59	0.44	0.42	0.57
	CCC	22.0	16.98	0.45	0.42	0.33	0.61
	CaCl2	21.0	16.58	0.36	1.66	0.47	0.91
	ZnSo4	28.0	16.79	0.54	1.31	0.53	0.85
2000 ppm	Control	20.5	15.31	0.79	0.54	0.69	0.64
	Irradiation (dry)	26.0	13.37	0.11	0.38	0.40	0.57
	Water soaking	23.5	15.31	0.49	0.82	0.67	0.47
	Irradiation (wet)	16.0	11.40	0.14	0.73	0.39	0.48
	Succinic acid	22.0	16.58	0.69	0.92	0.58	0.84
	CCC	24.0	17.86	0.43	0.82	0.44	0.49
	CaCl2	23.0	12.76	0.16	0.83	0.52	0.83
	ZnSo4	23.0	15.89	0.63	0.95	0.83	1.41
4000 ppm	Control	22.0	12.76	0.64	0.43	0.59	0.68
	Irradiation (dry)	25.5	17.85	0.15	0.68	0.60	0.99
	Water soaking	24.0	15.11	0.58	0.76	0.43	0.53
	Irradiation (wet)	28.0	28.07	0.26	0.26	0.35	0.41
	Succinic acid	28.0	12.79	0.58	0.62	0.43	0.86
	CCC	22.0	16.58	0.45	0.92	0.74	0.65
	CaCl2	26.5	14.03	0.13	0.73	0.66	0.56
	ZnSo4	27.0	16.93	0.54	0.83	0.39	0.68
6000 ppm	Control	23.0	11.49	0.53	0.32	0.40	0.59
	Irradiation (dry)	22.0	15.31	0.56	0.42	0.45	0.75
	Water soaking	26.0	14.03	0.67	0.36	0.40	0.42
	Irradiation (wet)	24.0	12.78	0.14	0.28	0.32	0.42
	Succinic acid	20.0	11.48	0.84	0.46	0.29	0.69
	CCC	23.0	12.78	0.22	0.54	0.47	0.76
	CaCl2	25.0	15.31	0.20	0.38	0.45	0.61
	ZnSo4	27.0	15.91	0.43	0.61	0.46	0.70

Table 3: Effect of different seed hardening pre-sowing treatments on ash content (%) and mineral content (meq/100 g dry wt.) of sorghum shoot plant grown under different salinity levels at second cut.

Salinity levels (ppm)	pre-sowing treatments	Ash	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-
Tap Water	Control	8.8	26.6	128.8	78.5	41.1	40.9
	Irradiation (dry)	8.6	23.7	125.2	109.7	37.0	35.2
	Water soaking	9.4	26.8	132.3	71.1	49.3	17.0
	Irradiation (wet)	15.3	29.5	128.8	81.1	57.8	15.1
	Succinic acid	11.4	20.7	132.3	88.6	40.1	16.8
	C C C	12.1	20.3	139.4	51.1	44.2	10.9
	$Ca\ Cl_2$	13.0	17.7	111.1	61.1	46.3	18.9
	$Zn\ SO_4$	10.0	23.7	135.8	170.9	65.8	17.5
2000 ppm	Control	9.8	20.7	128.4	89.7	45.2	31.6
	Irradiation (dry)	11.4	20.4	128.6	114.7	42.7	41.7
	Water soaking	9.9	23.7	128.8	84.6	39.1	37.4
	Irradiation (wet)	12.5	23.3	132.3	212.1	100.7	18.3
	Succinic acid	11.0	20.7	125.2	65.6	38.0	24.4
	C C C	18.0	20.5	132.8	43.7	51.4	17.9
	$Ca\ Cl_2$	11.6	23.7	132.3	58.6	42.2	18.6
	$Zn\ SO_4$	8.3	23.6	132.9	113.5	43.2	19.7
4000 ppm	Control	8.1	23.8	125.2	78.5	38.0	47.8
	Irradiation (dry)	10.9	29.6	128.6	97.3	43.2	15.4
	Water soaking	9.7	32.5	130.5	79.8	32.9	32.3
	Irradiation (wet)	9.9	29.1	134.1	88.6	51.4	37.7
	Succinic acid	13.7	23.7	128.8	69.9	40.1	33.1
	C C C	18.9	35.5	132.3	91.1	54.5	23.4
	$Ca\ Cl_2$	9.8	29.6	142.9	63.6	56.5	27.8
	$Zn\ SO_4$	12.9	23.7	121.7	128.5	40.1	18.3
6000 ppm	Control	9.4	33.7	121.7	73.5	41.1	49.2
	Irradiation (dry)	14.8	26.4	139.4	87.3	38.0	25.9
	Water soaking	9.9	26.8	132.3	79.8	41.4	24.9
	Irradiation (wet)	11.1	20.7	128.0	53.9	33.9	16.8
	Succinic acid	12.8	26.1	134.1	74.9	44.2	15.1
	C C C	15.3	21.8	125.2	64.9	52.4	9.7
	$Ca\ Cl_2$	12.9	32.5	139.4	51.1	45.2	16.5
	$Zn\ SO_4$	12.7	28.1	121.7	164.7	38.0	18.1

Table 4: Effect of different seed hardening pre-sowing treatments on ash content (%) a mineral content (meq/100 g dry wt.) of sorghum shoot plant grown under differe salinity levels at third cut.

Salinity levels (ppm)	pre-sowing treatments	Ash	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-
Tap Water	Control	10.7	35.5	125.2	113.4	37.0	21.0
	Irradiation (dry)	11.8	32.5	107.8	106.5	26.7	22.2
	Water soaking	10.6	25.6	111.1	102.3	45.2	25.5
	Irradiation (wet)	12.6	32.5	112.9	57.4	44.2	17.9
	Succinic acid	13.4	26.6	114.7	78.1	47.3	27.3
	C C C	12.9	28.1	121.7	102.9	46.3	35.1
	$Ca\ Cl_2$	13.9	29.8	128.6	141.0	44.2	32.3
	$Zn\ SO_4$	9.3	28.1	128.8	121.0	49.6	29.6
2000 ppm	Control	11.4	41.4	118.2	79.8	28.8	23.4
	Irradiation (dry)	13.1	35.5	125.2	93.5	37.0	35.1
	Water soaking	12.1	44.4	114.7	69.9	33.5	23.8
	Irradiation (wet)	12.2	39.4	104.1	46.2	66.9	18.7
	Succinic acid	13.5	26.6	102.3	74.9	42.2	20.7
	C C C	14.1	26.1	125.6	82.3	48.3	39.7
	$Ca\ Cl_2$	13.9	26.6	125.2	139.7	32.9	30.4
	$Zn\ SO_4$	10.2	32.5	118.2	144.7	33.9	22.6
4000 ppm	Control	11.2	38.4	118.2	83.4	27.6	27.1
	Irradiation (dry)	11.7	38.4	107.8	84.8	30.8	19.1
	Water soaking	13.0	29.6	125.2	73.6	29.3	34.5
	Irradiation (wet)	14.9	58.2	130.5	81.1	43.2	27.3
	Succinic acid	12.1	29.6	125.4	66.1	39.1	36.2
	C C C	18.0	26.6	128.3	154.7	41.1	48.7
	$Ca\ Cl_2$	14.6	32.5	128.8	155.9	35.0	34.7
	$Zn\ SO_4$	9.2	38.4	134.1	157.2	33.9	27.7
6000 ppm	Control	13.8	44.4	108.8	73.50	31.9	40.5
	Irradiation (dry)	12.2	41.4	107.6	63.58	26.7	23.2
	Water soaking	13.7	29.6	121.7	72.38	31.4	31.2
	Irradiation (wet)	13.7	47.3	104.1	59.92	36.0	32.0
	Succinic acid	11.1	26.6	118.2	81.10	48.3	37.0
	C C C	13.7	29.6	107.6	147.20	36.0	37.8
	$Ca\ Cl_2$	10.7	32.5	142.9	102.30	37.0	42.1
	$Zn\ SO_4$	14.0	41.4	125.2	170.50	28.8	42.5