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IMPROVEMENT SEEDLING GROWTH OF CELERY (*Apium graveolens* L.) USING HUMIC ACID, POTASSIUM SILICATE AND LOW GAMMA IRRADIATION DOSES

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ABSTRACT

The effects of different priming treatments either at room temperature or at 5°C in an incubator for 16 hours were studied. An experiment of pots was planted using hydro priming celery (*Apium graveolens* L.) seeds for comparison with those primed in different solutions. Seeds were soaked in Petri dishes containing: tap water, different concentrations of potassium silicate (2, 4, 8, 16 mM Si) and humic acid concentrations (5, 20,100, 200 mM) at room temperature and 5ºC in an incubator before planting for 16 hours. Also, some dry seeds were exposed to gamma rays at different low doses (20, 40, 60Gy) before priming in tap water. This work aimed to improve the tolerance of celery seeds subjected to chilling stress by priming seeds in different concentrations of humic acid and potassium silicate in addition to use low doses of ionizing radiation to accelerate plants proliferation, growth, and leaves yield. Humic acid and potassium silicate were effective as priming solutions for alleviating the chilling stress, stimulating celery growth, and proliferation. In addition, using of low doses of ionizing radiation had a stimulant effect on plants after 120 days from planting.

Key words: Celery, humic acid, potassium silicate, gamma irradiation, growth.

INTRODUCTION

Celery (*Apium graveolens* L.) is a member of plant species of the family Apiaceae; it is one of the oldest cultivated plants to be used for medicinal and dietary purposes. Today it is mostly used as food and condiment (Mielke and Schöber-Butin, 2007). Also, leaf celery(*Apium graveolens* L. var. secalinum), known as cutting celery, is a variety in which the usable parts are the dark-green, glossy leaves on long, thin leaf petioles, presenting a strong celery flavor. They may eaten as fresh or processed, mainly frozen or dried, with which their aroma is not lost (Mielke and Schöber-Butin, 2007; Rozek, 2007). Humic acids aid in correcting plant chlorosis, increase the permeability of the plant membranes and intensify enzyme systems of plants. They accelerate cell division, show greater root development, and decrease stress deterioration. Under the influence of humic acids, plants grow stronger and better resist plant diseases. The stimulation of ions uptake in the applications of humic materials led many investigators to proposing that these materials affect membrane permeability (Zientara, 1983). This is related to the surface activity of humic substances resulting from the presence of both hydrophilic and hydrophobic sites (Chen and Schnitzer, 1978). Therefore, the humic substances may interact with the phospholipids structures of the cell membranes and react as carriers of nutrients through them.

The benefits of silicon (Si) amendments have been well documented in plants. These include enhanced productivity and tolerance to various biotic and abiotic stresses, such as freezing, drought (Jacob *et al.*, 2009), pests and diseases. Potassium silicate (K silicate) is a source of highly soluble K and Si. It is used in agricultural

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The benefits of silicon (Si) amendments have been well documented in plants. These include enhanced productivity and tolerance to various biotic and abiotic stresses, such as freezing, drought (Jacob *et al.*, 2009), pests and diseases. Potassium silicate (K silicate) is a source of highly soluble K and Si. It is used in agricultural

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production systems primarily as a silica amendment, and has the added benefit of supplying small amounts of K. Silicon is an essential micronutrient, and deficiencies affect significantly plant health. Silicon was used in the form of the potassium (K) salt. Foliar spray with K silicate showed increments in chlorophyll content and plant growth. Silicon can reduce salinity stress and reduce transpiration in plants (Tisdale *et al.,* 1993; Epstein, 1994; Marschner, 1995). Furthermore, in sugarcane, there was evidence that Si may play an important role in protecting leaves from ultraviolet radiation damage by filtering out the harmful ultraviolet rays (Tisdale *et al.,* 1993). Thus, Si had been shown to ameliorate abiotic stresses in several ways.

This work aims to improve celery seed face up to chilling which is necessary for surviving in winter by priming seeds in different concentrations of humic acid and potassium silicate in addition to use of low doses of ionizing radiation to accelerate plants proliferation, growth, and leaves yields.

MATERIALS AND METHODS

Main Treatments

This experiment was conducted at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt during December 2012 season. Seeds of local variety of celery (*Apium graveolens* L.) obtained from the Agricultural Research Center, Giza, Egypt were used. A pots experiment was conducted using celery seeds that primed in water for comparison with those primed in different concentrations of humic acid or potassium silicate. Seeds were soaked in Petri dishes containing: normal water, potassium silicate in concentrations (2, 4, 6, 8, 16 mM Si) and humic acid in concentrations (5, 20, 100 and 200 mM). Soaking process was achieved at ambient room temperature (20°C ± 2) or in an incubator at 5°C for 16 hours before planting. Some seeds were irradiated on gamma cell at NCRRT by 20, 40 or 60 gamma rays (Gy) and soaked in tap water, other seeds (un-irradiated seeds) were soaked in tap water to serve as control. Pots were kept under greenhouse conditions.

Measurement of Growth

Five plants were washed gently with tap water for few minutes, wiped with paper, shoot length cm^{-1} was measured from culms base to the tip of the longest leaf and root length cm^{-1} was measured from the root-shoot junction to the tip of the longest root, leaf number (unit $plant^{-1}$), the plant samples were weighed freshly then oven dried at 70°C till constant weight and the dry weight per each plant was recorded.

Measurement of Photosynthetic Pigments

Chlorophyll a, chlorophyll b and carotenoids were calorimetrically determined in plants according to Metzner *et al.* (1965). Fresh leaves (0.5 g) were extracted twice with 5 ml of 80% acetone, the combined extract made to constant volume after filtration. The absorbance of chlorophyll a, chlorophyll b and carotenoids were colorimetrically measured on shimadzu 120-02 UV/V spectrophotometer and the concentrations were calculated using Metzner equations.

Measurement of Mineral Concentrations

Dried plants parts were ground into fine powder and used after digestion of samples according to AOCS (1984) for measurement of mineral concentrations. The minerals Ca, Mg, Mn, Fe, and Zn were estimated on Atomic Absorption Spectrometer (SOLAR- UNICAM 989) in NCRRT laboratory after complete digestion according to an appropriate dilution.

Statistical Analysis

Statistical analysis was carried out using SAS Computer Program (1988) according to general linear methods procedure (SAS Computer Program, 1988).

RESULTS

The concentrations of K silicate used as soaking solution at room temperature or at 5°C increased length of plant, length of shoots, length of root, number of leaves and fresh weight of plants and their parts (shoots and roots) in comparison with control as shown in Table 1. Priming in silicate solution with concentration 8 mM before planting in an incubator increased most of the aforementioned

Table 1. Effect of seed priming in potassium silicate solutions at room temperature or in an incubator for 16 hours on some growth parameters of celery plants after 120 days of planting date

Treatments (mM) K silicate	Room temperature(20°C ±2)						5°C					
	Length of shoot (cm)	Length of root (cm)	No. of leaves	Plant fresh Wt. (g)	Wt. of leaves (g)	Wt. of root (g)	Length of shoot (cm)	Length of root (cm)	No. of leaves	Plant fresh Wt. (g)	Wt. of leaves (g)	Wt. of root (g)
Control	15.48f	5.64f	3.19d	0.99d	0.75e	0.24f	10.27f	3.91f	3.04f	0.57f	0.35f	0.22e
2	17.27e	6.73e	4.74b	1.55c	1.00d	0.55e	11.68e	4.39e	3.68d	0.64e	0.38e	0.26c
4	20.14d	9.61d	3.08d	2.84b	2.07b	0.77d	13.69d	5.79c	5.28b	1.27d	0.76d	0.51b
6	25.34a	11.58c	5.73a	3.1a	2.23a	0.87b	14.89c	5.13d	3.46e	1.34c	1.14c	0.20d
8	23.39b	14.20a	3.20d	2.93b	2.08b	0.85c	17.98a	6.85a	4.27c	2.13a	1.37b	0.76a
16	21.56c	13.70b	3.53c	2.68b	1.76c	0.92a	17.38b	6.11b	5.46a	1.64b	1.47a	0.17f
F. test	**	**	*	*	*	**	**	**	**	**	**	**

*, ** significant at $p \leq 0.05$

growth parameters and the difference was highly significance. Meanwhile, priming in silicate solution with concentration 6m M at room temperature and 8 mM at 5°C before planting were considered as the best treatment where they gave the highest values of the measured growth parameters. The longest shoot part reached to 25.34 and 17.98 cm at room temperature and at 5 C, respectively.

Also, all concentrations of humic acid stimulated significantly all studied plant growth parameters (Table 2). The high significance differences were noticed after priming seeds in 100 or 200 mM humic acid at room temperature or under low temperature. The same trend was noticed in concern to elements in shoots or roots length and weight.

Data obtained after radiation treatments by 20, 40, 60 Gy and primed in tap water before planting are represented in Table 3. All used doses increased length of shoot, length of root, number of leaves and fresh weight of shoot, either at room temperature or at 5°C. Celery seeds that exposed to 40 Gy achieved the highest significance.

Most seeds that primed at room temperature produced plants had high copper and zinc concentration, most of seeds that primed at 5°C produced plants had variation in elements concentrations, some treatments increased copper and zinc concentrations and another decreased them as shown in Figs. 1, 2 and 3. In respect to copper concentration, 60 Gy dose, 200 mM humic acid and 8 or 10 mM potassium silicate recorded the highest concentration at room temperature. Meanwhile, at low temperature (5°C), 20 Gy for shoot and 60 Gy for roots, 20 mM for shoots and 200 mM humic acid, 10mM for shoot and 2 mM potassium silicate had the highest copper content. Roots of plants produced from priming in 200 mM humic acid at room temperature or at 5°C gained the highest concentrations of estimated elements except manganese and iron only in samples from 200mM at 5°C. While 20, 100 mM humic acid at room temperature and 5, 20 mM at 5 C produced significant results with the most measurements.

Zinc concentration behaved like copper, but 5mM humic acid in shoot instead of 20 mM in case of room temperature and 2 mM silicate instead of 10mM in shoot at room temperature had gained the highest concentration of zinc as compared with the control. The highest dose (60Gy) that primed in an incubator was not tested for minerals.

Perusal to Figs., the ions concentration on dry weight basis mg kg^{-1} in celery plants grown after seeds priming for 120 days at room temperature or at 5°C after 16 weeks from planting date, it was noticed that most of priming treatments used at room temperature produced plants had high concentrations of copper and zinc.

Table 2. Effect of seed priming in humic acid solutions at room temperature or in an incubator for16 hours on some growth parameters of celery plants after 120 days of planting date

Treatments	Room temperature(20°C ±2)						5°C					
Humic acid (mM)	Length of shoot (cm)	Length of root (cm)	No. of leaves	Plant fresh Wt.(g)	Wt. of leaves (g)	Wt. of root (g)	Length of shoot (cm)	Length of root (cm)	No. of leaves	Plant fresh Wt.	Wt. of leaves (g)	Wt. of root (g)
Control	15.21c	6.85d	3.49d	0.94	0.87	0.07	10.28d	4.59d	3.11e	0.46	0.34	0.12
5	21.7a	9.17c	4.56a	2.76b	1.36c	1.40a	17.49a	5.93a	4.68b	1.31b	1.11a	0.20c
20	22.23a	13.62a	3.82c	2.43c	1.91b	0.52d	15.65b	5.03c	4.98a	1.98a	1.21a	0.77a
100	20.99ab	10.05c	3.49d	2.97a	2.27a	0.70c	13.80bc	5.80a	4.29c	1.37b	0.91b	0.46b
200	22.53a	11.49b	4.18b	2.64b	1.83b	0.81b	14.34b	5.46b	3.93d	1.21c	1.00ab	0.21c
F. test	*	*	*	*	*	**	*	*	**	**	*	**

*, ** significant at $p \leq 0.05$

Table 3. Effect of gamma irradiation before sowing and hydro priming at room temperature or in an incubator for 16 hours on some growth parameters of celery plants after 120 days of planting date

Gamma irradiation	Room temperature(20°C ±2)						5°C					
	Length of shoot (cm)	Length of root (cm)	No. of leaves	Plant fresh Wt. (g)	Fresh Wt. of leaves (g)	Wt of root (g)	Length of shoot (cm)	length of root (cm)	No. of leaves	Plant fresh Wt. (g)	Fresh Wt. of leaves (g)	Wt. of root (g)
Control	11.11c	7.11d	3.08d	2.14c	1.92d	0.22a	9.25d	8.13d	2.58d	2.02d	1.61d	0.41a
20	14.15b	10.10b	4.18b	2.99b	2.82b	0.17c	11.53b	10.04b	3.63b	2.54b	2.37b	0.17d
40	18.32a	11.03a	5.01a	3.71a	3.50a	0.21b	15.62a	11.17a	4.61a	2.94a	2.72a	0.22c
60	13.03b	7.48c	3.81c	2.42c	2.29c	0.13d	10.51c	9.14c	2.78c	2.18c	1.94c	0.24b
F. test	*	**	**	*	**	**	**	**	**	**	**	**

*, ** significant or significant at $p \leq 0.05$

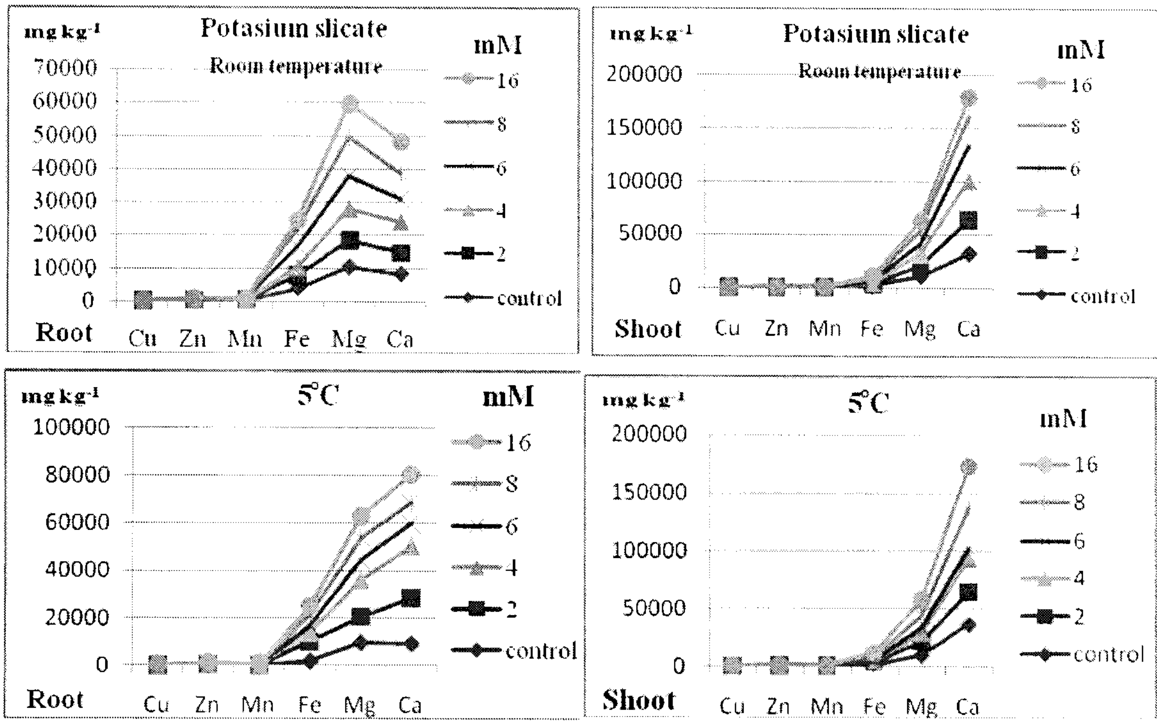

Fig.1. Ions concentration in celery plants grown after seeds priming in potassium silicate for 16 hours at room temperature or at 5°C on dry weight basis (mg kg^{-1}) after 120 days of planting

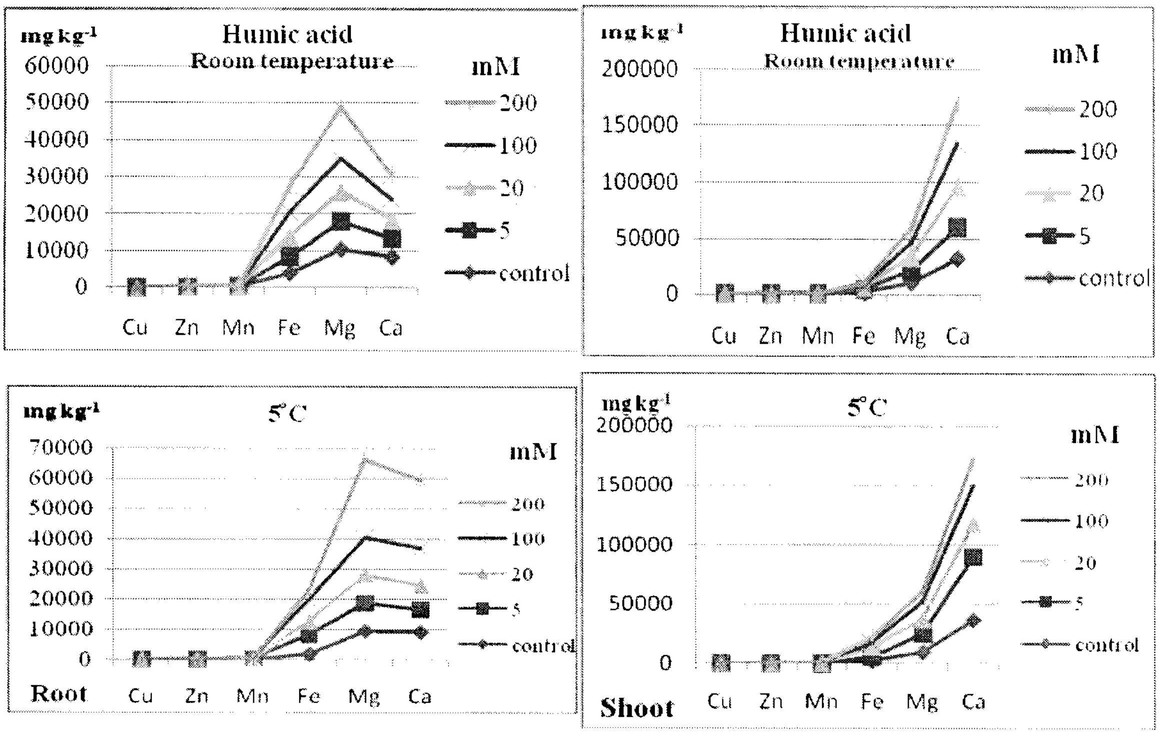

Fig. 2. Ions concentration in celery plants grown after seeds priming in humic acid for 16 hours at room temperature or at 5°C on dry weight basis (mg kg^{-1}) after 120 days of planting

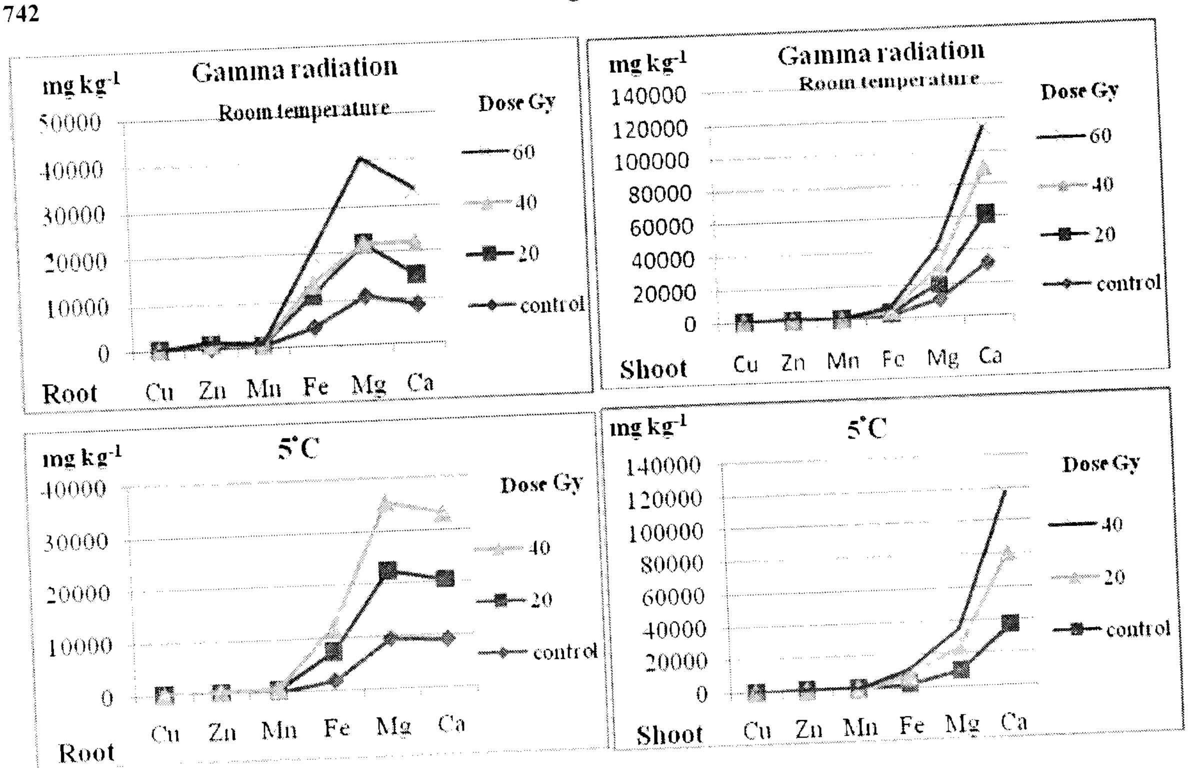

Fig. 3. Ions concentration in celery plants grown after seeds irradiation and priming in tap water for 16 hours at room temperature or at 5°Con dry weight basis (mg kg^{-1}) after 120 days of planting

Using of potassium silicate as priming solution in concentrations of (2, 4 and 6 mM) at room temperature increased chlorophyll (a) and chlorophyll (b). While the concentrations 4, 6, 8 and 16 mM at an incubator increased chlorophyll a and (2 and 6 mM) increased chlorophyll (b) above the control values (Table 4). Humic acid treatments at room temperature or at incubator cut down on the photosynthetic pigments, except chlorophyll (a) or chlorophyll (b) which raised up above its corresponding control at 200 mM humic acid. Also, carotenoids were raised up but at 5 °C only (Table 5).

Only chlorophyll (a) in plants produced from seeds irradiated before sowing by 40 or 60Gy and primed in water at 5°C and chlorophyll b were increased at 20, 40 Gy as compared with their corresponding controls. The rest of treatments (20, 40 and 60 Gy) at room temperature cut down on the photosynthetic pigments (Table 6).

DISCUSSION

The plant responses to different priming solutions together with priming in water to serve as control were studied. Also, seeds irradiated by different treatments of gamma rays were primed in tap water. The overall performance of grown celery subjected to pre sowing soaking treatments were evaluated on growth, photosynthetic pigments (chlorophyll a, b and carotenoids) and minerals after 120 days from planting date. Silicate and humic acid improves plant growth and their minerals content mainly calcium.

Using of silicates in new seedlings helps speed up the rate of growth by as much as 90%. Also, Si is consider as benefit element in higher plants, its deposition in cell walls of several organ such as leaf and stem can promote benefit effects, for this reason it has been frequently linked to physiological, morphological nutritional and molecular aspects in plants. High silica uptake has been shown to improve drought

Table 4. Photosynthetic pigments (mg g^{-1}) in celery plants from seeds primed in potassium silicate solutions at room temperature and in an incubator after 120 days of planting date

Pigments (mg g^{-1})	Potassium silicate (mM)											
	Room temperature(20°C ±2)						5°C					
	Control	2	4	6	8	16	Control	2	4	6	8	16
Chlorophyll (a)	14.56	19.89	18.85	15.50	11.19	10.98	10.83	9.57	12. 7	14.32	13.18	18.37
Chlorophyll (b)	6.70	14.68	8.96	6.88	3.27	3.53	5.23	8.09	3.14	5.59	2.39	4.02
Carotenoids	5.80	4.49	4.71	4.25	4.16	3.09	5.26	5.07	3.99	4.61	3.44	3.63

Table 5. Photosynthetic pigments (mg g^{-1}) in celery plants from seeds primed in humic acid solutions at room temperature and in an incubator after 120 days of planting date

Pigments (mg g^{-1})	Humic acid (mM)									
	Room temperature (20°C ±2)					5°C				
	Control	5	20	100	200	Control	5	20	100	200
Chlorophyll (a)	14.56	11.54	7.67	7.79	15.44	10.83	1.274	7.73	10.81	17.36
Chlorophyll (b)	6.70	6.52	3.82	3.98	8.16	5.23	1.23	3.38	4.59	13.21
Carotenoids	5.80	4.49	2.60	2.51	4.00	5.26	1.35	2.76	3.61	7.37

Table 6. Photosynthetic pigments (mg g^{-1}) in celery plants treated by gamma irradiation from seeds primed in water at room temperature and in an incubator after 120 days of planting date

Pigments	Room temperature(20°C ±2)				5°C			
	Control	20	40	60	Control	20	40	60
Chlorophyll (a)	14.56	10.00	11.39	12.33	10.83	9.62	12.67	13.68
Chlorophyll (b)	6.70	3.88	5.03	4.64	5.23	6.96	6.27	5.78
Carotenoids	5.80	4.08	3.86	5.47	5.26	5.60	4.13	5.85

resistance and increase plant growth rate and yield (Marschner, 1995; Belanger *et al.*, 1995). Silicon nutrition has several beneficial effects on plant growth largely due to its unique physiological role (Takahashi *et al.*, 1990). Adatia and Besford (1986) reported number of positive effects of Si on the growth of cucumber plants such as more leaf thickness, more dry matter per unit area of leaf, a small but significant added increment in root fresh and dry weight, and less propensity of leaves to wilt.

The beneficial effects of Si on crop growth are well documented especially under biotic and abiotic stress conditions, Several mechanisms have been proposed for these responses, Crop yield responses to Si may be associated with improved resistance against biotic and abiotic stresses, such as disease and pest resistance, Al, Mn and Fe toxicity alleviation, increased P availability, reduced lodging, improved leaf and stem erectness, freeze resistance and improvement in plant water economy (Aziz *et al.*, 2002).

Foliar application of humic acids increased the uptake of P, K, Mg, Na, Cu and Zn. Humic acids can significantly reduce water evaporation and increase its use by plants in arid and sandy soils. Furthermore, they increased the water holding capacity of soils. Humic acids increased the permeability of the plant membranes and intensify enzyme systems of plants and they accelerated cell division(Khaled and Fawy, 2011).

The absorption of humic substances into seeds has a positive influence on seed germination and seedling development. The application of humic or fulvic acids to seeds will increase seed germination; resulting in higher seed germination rates. Application rates of humic acids or fulvic acids, required for improving seed germination, range from 20 to 100 mg/liter of seed. In order to improve germination to occur the humic substances must be present within the cells of seeds. As the humic substances enter the seed cells, respiration rate increases, and cell division processes are accelerated. These same respiratory processes enhance root meristems development and activate other growing points within the seedlings. Humic substances have been demonstrated to enhance mitotic activity during cell division under carefully controlled experiments. Placement of these humic substances on seed (seed treatment) or within the seed furrow will improve significantly seed germination and seedling development (Chen *et al.*, 2004 a,b). Adani *et al.* (1998) concluded that humic acid stimulated plant growth and mineral nutrition of tomato plants (*Lycopersicon esculentum* L.). They added that, from evidence in the previous literature, reduction of Fe_3 to Fe_2 by humic acid is considered as a possibility to explain a higher Fe availability for the plants.

Pre-sowing seed irradiation is one of the most effective methods to improve plant production, yield components and chemical composition (Selenia and Stepanenko, 1979). The previous studies reported that low doses of gamma rays stimulated seed germination, plant growth and oil production as peppermint (Zheljazkov *et al.*, 1996) and *Mathiola incana* and *Delphinium ajacis* (Mahmoud, 2002).

Conclusion

Humic acid and potassium silicate were effective as priming solution for stimulating celery growth, and proliferation. In addition, using low doses of ionizing radiation had the same stimulant effect.

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تحسين نمو بادرات الكرفس باستعمال حمض الهيوميك، سليكات البوتاسيوم وجرعات منخفضة من أشعة جاما

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قسم بحوث المنتجات الطبيعية ، المركز القومى لبحوث وتكنولوجيا الإشعاع، ص. ب. ٢٩ مدينة نصر ، مصر

أجريت تجربة أصص مملوءة بالتربة لزراعة بذور الكرفس المنقوعة فى الماء والتى استخدمت للمقارنة بالبذور التى تم نقعها فى حمض الهيوميك أو سليكات البوتاسيوم، حيث تم نقع البذور فى أطباق بترى تحتوى على ماء الصنبور وتركيزات من كل من حمض الهيوميك (٥، ٢٠، ١٠٠، ٢٠٠ملليمول) وسليكات البوتاسيوم (٢، ٤، ٨، ١٦ملليمول) فى درجة حرارة الغرفة وفى الحضان على درجة ٥°م لمدة ١٦ ساعة قبل الزراعة، وقد تم أيضا تعريض جزء من البذور إلى أشعة جاما بجرعات (٢٠، ٤٠، ٦٠ جراى) قبل النقع فى الماء، وأجريت هذه الدراسة بهدف زيادة تحمل نباتات الكرفس للبرودة الشديدة فى الشتاء وذلك باستعمال تركيزات مختلفة من حمض الهيوميك أو سليكات البوتاسيوم بالإضافة إلى تعريض البذور لجرعات منخفضة من أشعة جاما ثم النقع فى الماء قبل الزراعة لتحفيز التفريع والنمو، وقد وجد أن النقع قبل الزراعة فى محلول حمض الهيوميك أو سليكات البوتاسيوم قد أثر إيجابياً فى تنشيط النمو والتفريع فى نبات الكرفس، كما وجد أيضا أن استخدام أشعة جاما بجرعات منخفضة كان منشطاً للنمو.

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