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ORIGINAL ARTICLE

Promotive effect of irradiated sodium alginate on seed germination characteristics of fennel (*Foeniculum vulgare* Mill.)

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Radiolytically derived oligomers of sodium alginate are considered to act as signal molecules, affecting growth, development and defense mechanisms of plants through gene regulation. Since germination is a critical stage in the life cycle of plants, the objective of this study was to determine the effect of irradiated sodium alginate (ISA) on the characteristics of seed germination of *Foeniculum vulgare* Mill. Soaking the seeds in an aqueous solution of ISA showed significant improvement in various seed germination parameters, *viz.* germination, viability and relative water content of seed, nitrate reductase activity, protease activity, α and β amylase activities in germinated seedlings of fennel. GPC study revealed formation of lower molecular weight oligomer fractions in irradiated samples which could be responsible for plant growth promotion in the present work.

Key words: Fennel, ISA, seed germination, enzymes activity

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Key words: Fennel, ISA, seed germination

Germination is a critical stage in the life cycle of grasses, medicinal plants and agriculture crop plants, and often controls the population dynamics with major practical implications (Ali *et al*. 2010). Biologically active oligosaccharides, derived from sodium alginate, have been known to act as signal molecules that govern plant growth and development and defense reactions by regulating gene expression (Hien *et al*. 2000; Aftab *et al*. 2011; Idrees *et al*. 2011). Gamma-rays irradiation degrades the sodium alginate into smaller oligomers with comparatively low molecular weight. Application of these oligomers on plants brings about a beneficial change in various biological and physiological activities in plants (Aftab *et al*. 2011; Idrees *et al*. 2011; Sarfaraz *et al*. 2011). The objective of this study was to determine the effect of irradiated sodium alginate (ISA) on seed germination characteristics of *Foeniculum vulgare* Mill.

MATERIALS AND METHODS

The samples of sodium alginate were irradiated in a Gamma Chamber (Cobalt-60,GC-5000) made by BRIT, Mumbai, India. The samples were irradiated to 520 kGy gamma radiation dose at a dose rate of 2.4 kGy/h. Different aqueous concentrations of irradiated sodium alginate (ISA) were finally prepared using double distilled water as spray treatments. GPC of sodium alginate samples were done on Hitachi-EMerck HPLC/GPC system using RI detector. The experimental conditions were as follows: Mobile phase-water, flow rate-1.5mL/min, column PL-Aquagel, mixed bed column, 300 mm × 10 mm, 20 micro liter injection loop. The average molecular weight of the un-irradiated sodium alginate samples were estimated to be about 6,95,131. Polyvinyl alcohol polymers of known molecular weight were used as standards. As per treatments, 25 fennel seeds were soaked for 15 h in DDW, 20, 40, 60, 80 and 100 mg L^{-1} of ISA. Each treatment was replicated five times. Relative water content (RWC) of seeds was measured according to Purcell and Sinclair (1995).

Seed viability (%) = (number of viable seeds/number of total seeds per treatment) × 100

Seed germination (%) = (number of germinating seeds/number of total seeds per treatment) × 100. The activities of nitrate reductase (NR) and α-amylase and β-amylase were estimated according to Jaworski (1971) and Das and Sen-mandi (1992), respectively. The data were analyzed statistically according to one-way ANOVA. The treatment means were separated by Duncan's multiple range test (P<0.05).

Table 1. Effects of different concentrations of irradiated sodium alginate (ISA) on germination, seed viability, relative water content of fennel (*Foeniculum valgare* Mill.). LSD (p ≤ 0.05) was employed to separate the means in the Table 1. Means within a column followed by the same letter(s) are not significantly different (*p* ≤ 0.05). The data shown are means of five replicates.

ISA (mg L^{-1})	Germination (%)	Seed viability	Relative water content (%)		
			After 24 h	After 36 h	After 48 h
Control	77^{c}	80^{c}	38.8^{c}	39.5^{c}	39.8^{c}
20	81^{bc}	81^{c}	39.5^{c}	40.2^{c}	40.5^{c}
40	83^{b}	85^{b}	40.2^{c}	41.2^{c}	42.5^{c}
60	84^{b}	87^{b}	42.6^{b}	43.3^{b}	44.6^{b}
80	89^{a}	96^{a}	45.6^{a}	46.2^{a}	46.9^{a}
100	82^{b}	82^{c}	44.8^{b}	44.9^{b}	43.9^{b}
LSD P = 0.05	4.1	4.5	3.2	3.1	2.5

Table 2. Effects of different concentrations of irradiated sodium alginate (ISA) on NR activity, protease, α and β amylase activities of fennel (*Foeniculum valgare* Mill.). LSD (p ≤ 0.05) was employed to separate the means in the Table 2. Means within a column followed by the same letter(s) are not significantly different (p ≤ 0.05). The data shown are means of five replicates.

ISA (mg L^{-1})	NR Activity (nm NO_3 $g^{-1}h^{-1}$ FW)	Protease activity (mg min^{-1})	α-amylase (mg min^{-1})	β-amylase (mg min^{-1})
Control	437.58^{d}	84.52^{e}	10.4^{e}	58.3^{d}
20	446.72^{d}	86.23^{d}	11.5^{c}	59.4^{c}
40	456.81^{c}	88.34^{c}	12.4^{b}	60.5^{c}
60	579.94^{b}	89.34^{b}	12.9^{b}	62.5^{b}
80	662.42^{a}	91.24^{a}	14.5^{a}	65.4^{a}
100	444.84^{d}	85.18^{d}	11.7^{c}	61.4^{b}
LSD P = 0.05	14.84	1.05	1.04	01.08.12

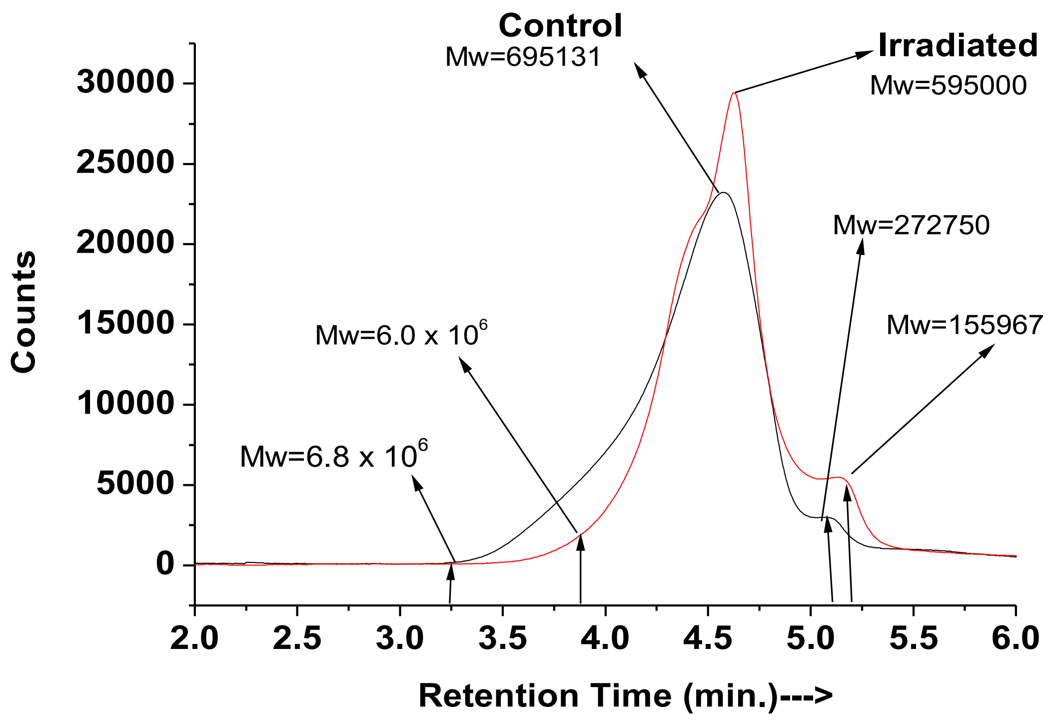

Figure 1. This shows the molecular weight distribution of un-irradiated and irradiated sodium alginate. The average molecular weight of the un-irradiated sodium alginate samples were estimated to be about 6,95,131. The distribution curve in the GPC profile shows shifting of whole graph to higher retention time indicating radiation degradation of sodium alginate on irradiation and forming lower molecular weight oligomers. This average molecular weight of 6,95,131 was observed in the control and 5,95,000 for the irradiated samples. However, considering the molecular weight values in the Fig. 1, it may be said that these value may fall in natural variation of SA. The lower molecular weight fraction (less than 100,000) which is coming at the end of the profile is very small.

RESULTS AND DISCUSSION

Figure 1 shows the molecular weight distribution of un-irradiated and irradiated sodium alginate. The distribution curve in the GPC profile shows the elution of different molecular weight fractions w.r.t to time. The profile shows shifting of whole graph to higher retention time indicating radiation degradation of sodium alginate on irradiation and forming lower molecular weight oligomers. This average molecular weight of 6,95,131 was observed in the control and 5,95,000 for the irradiated samples. However, considering the molecular weight values in the (*Figure* 1), it may be said that these value may fall in natural variation of SA. The lower molecular weight fraction (less than 100,000) which is coming at the end of the profile is very small. Therefore, it is difficult to say which molecular weight fraction of SA acts as a stimulant and investigations on this aspect is in progress. Soaking the seeds with ISA solution (20 to 80 mg L^{-1}) favored percent seed germination. The ISA solution at 80 mg L^{-1} proved the best, soaking the seeds in which resulted in the highest percentage of seed germination and viability (*Table* 1). The results of this study are in accordance with Hu *et al*. 2004, who reported promotion of seed germination in maize by alginate derived oligosaccharides. The relative water content (RWC) of the seeds increased as the germination percentage progressed. Treating the seeds with various concentrations of ISA increased the RWC values significantly over the control (*Table* 1). In this regard, the ISA possibly increased the permeability of the cell membranes for the water diffusion into the cells (Ma *et al*. 2010). A cumulative response of ISA-soaked seeds in terms of increased water content and enzyme activity might have, perhaps, speeded up the process of seed germination. As the seed germination progressed, the activity of NR increased consistently (*Table* 2). Application of ISA at 80 mg L^{-1} gave the best results, registering the highest extent of NR activity compared to the other concentrations.

Except 100 mg L^{-1}, all the ISA concentrations increased the protease activity, with 80 mg L^{-1} proving the best concentration (*Table* 2). Presumably, ISA increased the protein content (substrate to be hydrolyzed by protease) in the ISA-treated seedling. Both α- and β-amylase activities were increased as a consequence of ISA treatment, with 80 mg L^{-1} resulting in the highest values of the enzyme activities (*Table* 2). As an important starch-degrading enzyme, β-amylase enables the release of the fermentable sugars for the embryonic development of the seeds and is much more abundant than the α-amylase in the seeds (Hu *et al*. 2004). β-amylase activity of the maize seeds, treated with alginate derived oligosaccharides was markedly higher than that of the control (Hu *et al*. 2004), which indicates that ISA leads to an increase in the activity of β-amylase. The results suggest that ISA probably enhanced germination via promotion of the activity of the amylase and the resultant acceleration of the metabolic activities of the seeds.

CONCLUSION

Gamma-rays irradiation degrades the sodium alginate into smaller oligomers with comparatively low molecular weight. It is difficult to say which molecular weight fraction of SA acts as a stimulant and investigations on this aspect is in progress. Soaking the seeds with ISA solution (20 to 80 mg L^{-1}) favored percent seed germination, viability,the relative water content. As the seed germination progressed, the activity of NR increased consistently. Presumably, ISA increased the protein content (substrate to be hydrolyzed by protease), both α- and β-amylase activities. The results suggest that ISA probably enhanced germination via promotion of the activity of the amylases and the resultant acceleration of the metabolic activities of the seeds.

REFERENCES

Aftab, T., Khan, M.M.A., Idrees, M., Naeem, M., Moinuddin, Hashmi, N., and Varshney, L. (2011) Enhancing the growth, photosynthetic capacity and artemisinin content in *Artemisia annua* L. by irradiated sodium alginate. *Radiat. Phys. Chem.* **7**, 833-836.

Ali,T., Hossein, P., Asghar, F., Salman, Z. and Ali Z.C.M. (2010) The effect of different treatments on improving seed germination characteristics in medicinal species of *Descurainia sophia* and *Plantago ovata*. *African J. Biotechnol*. **9**, 6588-6593.

Das, G. and Sen-Mandi, S. (1992) Scutellar amylase activity in naturally aged and accelerated aged wheat seeds. *Ann. Bot.* **69**, 497–501.

Hien, N.Q., Nagasawa, N., Tham, L.X., Yoshii, F., Dang, H.V., Mitomo, H., Makuuchi, K. and Kume T. (2000) Growth promotion of plants with depolymerised alginates by irradiation. *Radiat. Phys. Chem.* **59**, 97-101.

Hu, X., Jiang, X., Hwang, H., Liu, S. and Guan, H. (2004) Promotive effects of alginate-derived oligosaccharide on maize seed germination. *J. App. Phyco*. **16**, 73–76.

Idrees, M., Naeem, M., Alam, M., Aftab, T., Hashmi, N., Khan, M. M. A., Moinuddin and Varshney, L. (2011) Utilizing the gamma irradiated sodium alginate as a plant growth promoter for enhancing the growth, physiological activities and alkaloids production in *Catharanthus roseus* L. *Agricul. Sci. China* **10**, 1213-1221.

Jaworski, E.G. (1971) Nitrate reductase assay in intact plant tissues. *Biochem. Biophys. Res. Commun.* **43**, 1274–1279.

Ma, L.J., Zhang, Y., Bu, N. and Wang, S.H. (2010) Alleviation effect of alginate-derived oligosaccharides on *vicia faba* root tip cells damaged by cadmium. *Bull. Environ. Contam. Toxicol*. **84**, 161–164.

Purcell, L.C. and Sinclair, T.R. (1995) Nodule gas exchange and water potential response to rapid imposition of water deficit. *Plant Cell Environ*. **18**, 179–187.

Sarfaraz, A., Naeem, M., Nasir, S., Idrees, M., Aftab, T., Hashmi, N., Khan, M. M. A., Moinuddin, and Varshney, L. (2011) An evaluation of the effects of irradiated sodium alginate on the growth, physiological activity and essential oil production of fennel (*Foeniculum vulgare* Mill.). *J. Med. Plant Res.* **5**, 15-21.