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Effect of storage, growth regulator treatment and seed priming on germination of *Eryngium foetidum*

S. N. Mozumder[1] M. I. Haque[1], M. M. Kamal[2], S. Akter[3] and B. R. Banik[3]
[1]Senior Scientific Officer, [2]Scientific Officer, [3]Chief Scientific Officer
Regional Spices Research Center
Bangladesh Agricultural Research Institute
Gazipur-1701, Bangladesh.
[4]Director General, Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh.
Corresponding Address: Dr. Shailendra Nath Mozumder
Senior Scientific Officer, Regional Spices Research Center, Bangladesh Agricultural Research Institute, Gazipur -1701, **Bangladesh**
E-mail: *shailenbari95@yahoo.com*

Keywords

Eryngium,
Germination,
Seed treatment,
Soaking,
Storage.

Abstract

Viability and germination of *Eryngium* seeds with growth regulator treatment and priming after one year of storage under low and ambient temperature were studied at the Regional Spices Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during November 2012 to March 2014. Seeds kept in low temperature (3-5 ^{0}C in refrigerator) were germinated but no seeds were germinated that stored under ambient temperature. Growth regulator enhanced and increased seed germination but short time soaking (12-24 hours) gave good germination compared to no soaking and longer period (36-96 hours) of soaking. Growth regulator treated with 12 hours soaking of seeds gave the highest seed germination (32.3%) of low temperature stored in the laboratory.

Introduction

Eryngium foetidum L. is a tropical annual herbaceous horticultural crop grown as condiments in Bangladesh. It is gaining popularity world wide due to excellent aromatic and medicinal value. *Eryngium* is mainly cultivated for its leaves as condiments and for its essential oils (Ignacimuthu *et al*., 2006). The essential oil from the leaves and stem is rich in aliphatic aldehyds, most of which are α, ß unsaturated that have remarkable demand in industrial as well as consumer's level (Leclerq, *et. al*., 1992). *Eryngium* is rich in calcium, iron, carotene, and riboflavin. Leaves are an excellent source of vitamin A, B_2, B_1, and C (Bautista *et al.* 1988). The aerial parts are rich in calcium iron and riboflavin with approximately 0.1-0.95% essential oil and a peculiar saponir (Anam, 2002). Increasing demand and high value attracts the farmers to cultivate this crop but they are facing some constraints. Asynchronized and un-uniform seed germinations as well as very low germination rate (6-10%) are the major problems for popularizing *Eryngium* cultivation throughout the country (Mozumder *et al*., 2010). In addition to this, unavailability of adequate amounts of seeds also limits its cultivation. On the other hand, all these criteria influence higher seed rate (40 kg/ha) of *Eryngium* which

negatively affects the cost of cultivation (Moniruzzaman *et al*., 2000). To overcome such problems the germination rate should be increased. The use of GA_3 and kinetin for enhancing germination of coriander seed is well documented (Moraes *et al.* 1998; Naidu and Rajendrudu, 2001). In *Eryngium*, combined application of GA_3 (1000 ppm) and Kinetin (50 ppm) proved effective for enhancing seed germination up to 28.54% (Mozumder, 2009). Increased germination may be reduced seed rate which directly influences the cost of production. Moreover, more area can be cultivated with a limited amount of seed. But previously developed technology is not sufficient to increased seed germination near 100% inhibiting the negative effect of 'Coumarin' presents in *Eryngium* seeds (Ekpong, and Sukprakran, 2008). Researches are required for complete removal of coumarin and increased germination percentage as well as identifying proper storage condition. An emphasis should be given to increase germination with application of growth regulators (GA_3 and Kinetin) with alternately seed soaking and drying lowering the coumarin level, increased α-amylase activities in seeds and to decrease seed rate that increase farmer's profitability decreasing the production cost in *Eryngium* cultivation. Therefore, the experiment was conducted to know the effect of storage condition, growth regulator treatment and priming for increasing germination rate.

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Methodology

The experiment was conducted at the Regional Spices Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh during November 2012 to March 2014. The experiments comprised with three different factors such as storage temperature, growth regulator treatment and soaking duration.

Germination test were conducted in normal room temperature with 24 treatment combinations of three factors. Factor A comprised of two levels of storage temperature viz. ambient and low (3-5 ^{0}C), factor B comprised of two seed treatment viz. growth regulator (GA_3 500ppm + Kinetin 50 ppm) and control (distilled water) and Factor C comprised of six soaking levels viz. 0, 12, 24, 48, 72 and 96 hours soaking having 8 hours consecutive soaking and 4 hours drying was used in the experiments. Seeds packed in air tight sealed polythene bag before storing in both normal and low temperature. Package is broken before one day of treatment application. 1000 ml of GA_3 500ppm + Kinetin 50ppm solution was prepared by mixing of previously prepared 500 ml 1000ppm GA_3 and 500 ml 100ppm kinetin solution. One milliliter growth regulator solution was

used for each gram of primed seeds before placing of seeds on petridis. Each petridis having 100 seeds were placed onto 3 layers of blotting paper that treated as unit treatment and replicated three times. Placed seeds were covered with a thin layer of tissue paper for keeping it stable on the blotting paper. Judicial watering was continued to keep the blotting paper moist till 10 weeks after placing. The first germination was recorded as days to germination because 50% germination was not found at all. Germinated seedlings were counted and displaced from the Petridis everyday. The data was recorded, compiled, calculated and analyzed statistically using MS Excel, MSTAT-C Program and mean separation was done following the Duncan's Multiple Range Test (Zaman, *et al.* 1987).

Results and Discussion

Effect of storage temperature

Most of the seeds kept in low temperature (3 - 5 ^{0}C in refrigerator) were germinated but not a single seeds were germinated from the lots that stored under ambient temperature (Table 1.). Seeds stored in low temperature took 15.5 days for first germination. The weekly germination started from the end of 2nd week and continued up to 8th week. After 8th weeks, no seeds were germinated at all. Comparatively higher germination was observed in the 3rd week (5.9) and declined thereafter. The total germination percentage was 18.4 in refrigerated seeds while seeds kept in ambient temperature showed total viability loss. The color of the soaked seeds became black which stored in normal temperature while it was grey that kept in low temperature.

Table 1. Effect of storage temperature on the germination of stored Eryngium seeds.

Seed treatment	Days to germin.	Weekly mean germination (number of seedlings/100 seeds in petridish)							Total germin.
		2nd	3rd	4th	5th	6th	7th	8th	(%)
Low (3-5 ^{0}C)	15.5	0.7a	5.9a	4.6a	3.5a	2.6a	1.0a	0.3a	18.4a
Normal	--	0.0b	0.0b	0.0b	0.0a	0.0b	0.0b	0.0b	0.0b
Signi.	NS	*	*	*	*	*	*	*	**
CV%	3.04	16.89	18.91	8.13	15.57	13.52	23.63	35.21	6.27

Means followed by same letter or without letter in a column are not differed significantly at 5% level. * and ** indicated the level of significance at 5% and 1% level, respectively.

This result disproved the myth that *Eryngium* seed are not storable in any condition for more than one month (Mozumder, 2003). Though the germination percentage of stored seeds was lower compared to fresh seeds (Mozumder 2009) but *Eryngium* seeds can be stored under low temperature in sealed polybag. As there no seeds were germinated those stored under ambient temperature, only the results of low temperature stored seeds were presented for priming and chemical treatments.

Effect of growth regulator treatment

Better germination observed in hormone treated seeds compared to untreated control (Table 2.). Though the time required for germination was not varied significantly but in all weekly count showed superiority in germination as well as total germination and germination percentage when seeds were treated with growth regulator. Third weeks to 6th week gave higher germination while 2nd, 7th, and 8th week gave lower germination. The number of seed germination after 8 weeks was negligible. The highest (7.5) number of seedlings were counted in third week from growth regulator treated seeds. The maximum germination percentage (23.8) was counted from growth regulator treated seeds while it was the lowest (12.9) in untreated control. GA_3 and Kinetin independently as well as in combination increase the germination of Eryngium seeds. This result conformed the reports of Dutt *et al.* (2000) who obtained increased seed germination in GA_3 treatment. Nadafi *et al.* (2005) found the highest germination rate and percentage of *T. polium* seeds at concentrations of 500–2500 ppm GA_3.

Table 2. Effect of hormone treatment on the germination of stored Eryngium seeds

Seed treatment	Days to germin.	Weekly germination (no. seedlings/petridish)							Total germin. (%)
		2nd	3rd	4th	5th	6th	7th	8th	
Hormone	15.4	0.9a	7.5a	6.1a	4.3	3.4a	1.3a	0.4	23.8a
Control	15.6	0.4b	4.2b	3.1b	2.7	1.7b	0.7b	0.2	12.9b
Signi.	NS	*	*	*	NS	*	*	NS	**
CV%	3.04	16.89	18.91	8.13	15.57	13.52	23.63	35.21	6.27

Means followed by same letter or without letter in a column are not differed significantly at 5% level. * and ** indicated the level of significance at 5% and 1% level, respectively.

The higher germination after one year of storage was when GA_3 and Kinetin was applied together. The cause of enhanced germination with GA_3 and Kinetin is enhanced amylase activity induced by applied GA_3 and Kinetin. Moraes *et al*. (1998) reported that coriander seeds germination increased when seeds were treated with gibberellic acid (GA_3 200ppm). Samaan *et al*. (2000, 2000a) reported that GA_3 and kinetin could increase seed germination replacing the effect of moist chilling. This result ensured the report of Khider (1999) and Ekpong (2009) that GA_3 promoted alpha-amylase activity which was further enhanced if GA_3 was applied together with Kinetin. Reducing sugars content increased as α-amylase activity increased. These reducing sugars are used for the development of new cell organelles during cell division for the growth of hypocotyl and epicotyl of embryo thus enhanced germination after imbibitions. Riley (1987) reported that auxins which control root formation and growth; the gibberellins which regulate protein synthesis and stem elongation; and cytokinins that control organ differentiation. Application of GA_3 and Kinetin together helped in germinating some vulnerable seeds after one year storage that might not be able to germinate without growth regulator treatment.

Effect of soaking duration

Increasing soaking duration significantly enhanced seed germination. Early germination (13.3 days) was observed when seeds are soaked for 96 hours and it was late (17.7 days) in control. Weekly germination trend was distinctly different compared to previous years (fresh seed) result. After one year of storage, shorter duration of soaking gave good better germination than longer time of soaking. In all periods of soaking, third and fourth week was better in respecting the number of seedling germination than declined with time and almost stopped after eight weeks. The maximum weekly germination (7.0) was found from 24 hours soaking in the third week. The total germination was higher (24.4%) from 12 hours soaking followed by control (21.2%) and 24 hours soaking (21.0%). The lowest germination (11.3%) of the stored seeds was observed when the seeds soaked for 96 hours

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Table 3. Effect of soaking duration on the germination of stored Eryngium seeds

Soaking duration	Days to germin.	Weekly germination (no. seedlings/petridish)							Total germin. (%)
		2nd	3rd	4th	5th	6th	7th	8th	
Control	17.7a	0.0c	4.7b	5.5ab	5.0a	3.5a	1.8a	0.7a	21.2b
12 hours	17.0b	0.0c	6.2a	6.0a	5.2a	3.8a	2.2a	1.0a	24.3a
24 hours	16.2c	0.0c	7.0a	5.8a	4.2ab	3.0ab	1.0b	0.0b	21.0b
48 hours	15.0d	0.0c	6.7a	4.7b	3.0bc	2.3bc	0.7bc	0.0b	17.3c
72 hours	14.0e	1.7b	6.2a	3.2c	2.2cd	1.8c	0.2cd	0.0b	15.2c
96 hours	13.3f	2.2a	4.5b	2.3c	1.5d	0.8d	0.0d	0.0b	11.3d
Signi.	**	**	**	**	*	**	**	**	**
CV%	3.04	16.89	18.91	8.13	15.57	13.52	23.63	35.21	6.27

Means followed by same letter or without letter in a column are not differed significantly at 5% level. * and ** indicated the level of significance at 5% and 1% level, respectively.

The lower number of seeds germination in longer duration of soaking might be the cause of loss of seed vigor and viability during long time soaking due to disruption of membrane, leakage of electrolytes and degradation of cell organelles in seeds. On the other hand, rapids dislocation of germination inhibitor coumarin's within short time of soaking resulted better germination. At the time of long time storage cell membrane was weakened and membrane permeability increased that caused rapid imbibitions that resulted enhanced seed germination with short time of soaking.

Combined effect of growth regulator and soaking duration

Days to germination did not differ significantly with the application of growth regulator or soaking duration. The earliest germination (13 days) was found from 96 hours soaking without growth regulator application while it took longer time (18 days) without soaking in control. Though it's ranged from 13 to 18 days but the trend was similar with the single application of two factors that resulted insignificant in interaction. Weekly germination rate showed significant variation with all the combination of two factors. For 72 and 96 hours soaking, germination started from the end of second week but in other treatments germination started from the commencement or middle of third week. The third and fourth week seems the most potential period for germination then declined gradually and stopped after 8 weeks in almost all the treatment combinations. The maximum weekly germination (9.3) was counted from 24 hours soaking closely followed by 12 hours soaking with growth regulator treatment.

The total germination percentage was significantly influenced by with the application of different combination of seed soaking and growth regulator treatment. The maximum germination percentage (32.3) was calculated from 12 hours soaking with growth regulator treatment while it was lowest (7.7) in 96 hours soaking without growth regulator treatment.

Table 4. Combined effect of GR and soaking duration on the germination of stored Eryngium seeds.

Treatments		Days to germin a-tion	Weekly germination (number of seedlings /petridish)							Total germin. (%)
ST *	Soaking		2nd	3rd	4th	5th	6th	7th	8th	
Hormone	Control	17.3	0.0d	6.0bc	6.7a	6.0a	4.7ab	2.7a	1.0	27.0b
	12 hours	16.6	0.0d	9.0a	7.7a	6.7a	5.3a	2.3a	1.3	**32.3a**
	24 hours	16.3	0.0d	9.3a	7.3a	5.0ab	4.0bc	1.3bc	0.0	27.0b
	48 hours	15.0	0.0d	8.3a	6.3a	3.3bcd	3.0cd	1.0cd	0.0	22.0c
	72 hours	14.0	2.3b	7.3ab	4.7b	2.7cde	2.3de	0.3de	0.0	19.7cd
	96 hours	13.3	3.0a	5.0cd	3.7bc	2.0cde	1.3ef	0.0e	0.0	15.0e
Control	Control	18.0	0.0d	3.3d	4.3bc	4.0bc	2.3de	1.0cd	0.3	15.3e
	12 hours	17.3	0.0d	3.3d	4.3bc	3.7bcd	2.3de	2.0ab	0.7	16.3de
	24 hours	16.0	0.0d	4.7cd	4.3bc	3.3bcd	2.0de	0.7cde	0.0	15.0e
	48 hours	15.0	0.0d	5.0cd	3.0c	2.7cde	1.7e	0.3de	0.0	12.7ef
	72 hours	14.0	1.0c	5.0cd	1.7d	1.7de	1.3ef	0.0e	0.0	10.7fg
	96 hours	13.0	1.3c	4.0cd	1.0d	1.0e	0.3f	0.0e	0.0	7.7g
Significance		NS	**	*	*	*	*	*	NS	**
CV%		3.04	16.89	18.91	8.13	15.57	13.52	23.63	35.21	6.27

* ST= seed treatment. Means followed by same letter or without letter in a column are not differed significantly at 5% level. * and ** indicated the level of significance at 5% and 1% level, respectively.

In all duration of soaking with growth regulator treatment showed better performance compared to the respective soaking duration in control (without GR). Growth regulator helped in the recovery of viability activating alpha amylase activities in seeds suppressing the activity of germination inhibitor coumarin's with all the soaking duration that resulted higher germination with growth regulator treatment.

Conclusion

Eryngium seed is storable under low temperature (4-60C) with air tight packaging for one year. 12 hours priming with GA_3 500 ppm plus Kientin 50ppm treatment gave good germination on of one year stored seeds.

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