YYÜ TAR BİL DERG
(YYU J AGR SCI)
2018, 28(2): 192-199

Geliş tarihi (Received): 03.03.2017
Kabul tarihi (Accepted): 18.05.2018
doi: 10.29133/yyutbd.382330

Araştırma Makalesi/*Research Article (Original Paper)*

Effect of Seed Priming With Salicylic Acid on Germinability and Seedling Vigor Fenugreek (*Trigonella Foenum-Graecum*)

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Abstract: In orther to the effects of seed priming with salicylic acid (SA) on germinability and seedling vigor of fenugreek was used three salicylic priming treatments (control, 1700 and 2800 µM) and accelerated aging test for 24 and 48 hours including control with no aging at 95% relative humidity at at 45°C. It was found that aging for 48 hours without priming resulted in significant loss of mean germination percentage, seedling length vigor, seedling weight vigor, germination speed, coefficient of germination speed, mean daily germination, coefficient of germination uniformity, germination vigor, maximum mean daily germination, and germination value. But, when the aged seeds were primed with 2800 µM SA, it significantly improved these parameters so that germination percentage reached to 100% from 41%. In contrast to these germination speed were observed in unprimed aged seeds for 48 hours. Moreover, priming with 2800 µM SA of aged seeds for 48 hours increased their seedling tissue water percentage to 94.48%. Accelerated aging for 48 hours reduced seedling length and weight significantly. The highest seedling length, plumule dry weight and seedling dry weight were observed in seeds treated with 2800 µM SA.

Keywords: Accelerated aging, Germination parameters, Priming, Salicylic acid, Seedling vigor

Salisilik Asit Uygulamasının Çemen (*Trigonella Foenum- Graecum*) Yaşlandırılmış Tohumların Çimlenme Yeteneği ve Fide Canlılığı Üzerine Etkisi

Özet: Yaşlı çemen otu (*Trigonella foenum-graecum)* tohumlarında farklı salisilik asit uygulamalarının (kontrol, 1700 ve 2800 µN) çimlenme yeteneği ve fide canlılığı üzerine etkileri, 24 ve 48 saat 1hızlandırma ile yaşlanmaya bırakılmış tohumlar ve yaşlandırma uygulanmamış kontrol grubunda %95 nispi nem ve 45 °C sıcaklıkta incelenmiştir. Priming olmadan 48 saat yaşlandırmanın ortalama çimlenme yüzdesi, fide uzunluğu, fide ağırlığı, çimlenme hızı, çimlenme hızı katsayısı, ortalama günlük çimlenme, çimlenme tekdüzeliği katsayısı, çimlenme gücü, maksimum günlük çimlenme ve çimlenme değerlerinde önemli kayıplara neden olmuştur. Ancak, yaşlandırılmış tohumlar 2800 µM SA ile priming olduğunda, çimlenme yüzdesi % 41'den %100'e ulaşarak parametreler önemli ölçüde gelişmiştir. Bu parametrelerin aksine, en yüksek günlük çimlenme hızı, ortalama çimlenme süresi ve ortalama çimlenme hızı, 48 saat yaşlandırılmış priming yapılmış tohumlarda gözlenmiştir. Ek olarak, 2800 µM SA ile priming yapılmış tohumlarda, 48 saat süreyle yaşlandırma, fide doku su yüzdelerini % 94.48'e yükseltmiştir. 48 saat süre ile hızlandırılmış yaşlandırma, fide uzunluğu ve ağırlığını önemli ölçüde azaltmıştır. En yüksek fide uzunluğu, plumule kuru ağırlığı ve fide kuru ağırlığı 2800 µM SA ile priming uğramış tohumlarda gözlenmiştir.

Anahtar kelimeler: Hızlandırılmış yaşlanma, Çimlenme parametreleri, Priming, Salisilik asit, Fide gücü

Introduction

Medicinal herbs are a key component of health systems around the world. Fenugreek (*Trigonella foenum-graecum*) is an annual herb, family fabaceae leaves and seeds have a high medicinal value, and its leaves are consumed in fresh or dry form in Iran (Omidbbaigi, 2004). This species has a high nutritional value and contain high-value elements like Ca, P, Fe, carotene, vitamin C, and protein in its leaves (Ebubekir et al., 2005; Nazar et al., 2007). Fenugreek seeds contain alkaloid, trigonelline, choline and steroid saponines that have medicinal impacts in reducing blood glucose level (Omidbbaigi, 2004; Sandor et al., 2004).

Seed priming is a major method of improving seed germination and vigor (Farooq et al., 2006). Priming enhances germination percentage, germination speed (Asgedom and Becker, 2001), plant cover establishment and alleviates intrinsic physiological non-uniformity of germination (Rowse, 1995). Salicylic acid (SA) is a main substance used in priming. It is a phenolic compound produced by plants. These compounds can regulate the growth (Raskin, 1992). SA is used as an important molecular signal in plant responses to environmental stresses (Sairam et al., 1997). Its application may effect many plant processes including seed germination, membrane permeability, and growth rate (Khan et al., 2003).

Germination and emergence are significantly affected by environmental factors, especially by soil moisture and seed deterioration (De Figueiredo et al., 2003). The maximum seed vigor of most crops occurs at physiological maturity. As seeds deteriorate, their vigor is the first component of seed quality that starts to fall and then, germinability and viability start to decline (Basra et al., 2003).

A number of studies showed that seed deterioration reduces germination, emergence and growth of seedlings significantly and priming can partially compensate these losses. Moosavi and NabaviKalat (2011) reported that higher aging temperature resulted in the loss of germination and germination speed, the extent of which increased with seed moisture. In a study on bitter gourd, Mohammadi and Shekari (2015) found that aging decreased germination and increased mean germination time of the seeds and seed priming with salicylic acid reduced the impact of aging. According to a study on milk thistle, Parmoon et al. (2014) reported that accelerated aging reduced germination percentage, germination speed, seedling growth, seed vigor, dynamic reserves use efficiency, reservoir fraction, and the activity of antioxidants. They, also, found that priming with 1000 mg l^{-1} SA alleviated the detrimental impact of aging. The objective of the present work was to study the impact of accelerated aging on germination, seedling growth, seed vigor and to find the best SA concentration for reducing the deleterious impact of accelerated aging on fenugreek seeds.

Materials and Methods

The study was carried out as a factorial experiment based on a Randomized Complete Block Factorial Design with four replications in Agronomy and Seed Technology Laboratory (Faculty of Agriculture) of Urmia University during 2015. The first factor was assigned to different rates of accelerated aging (no aging, 24 and 48 hours) at 95% relative humidity at 45°C. The second factor was priming with SA at two rates of 1700 and 2800 µM as well as one unprimed treatment as control. To apply accelerated aging, the seeds were placed in nets, and soaked in a glass container (desiccator for protecting water vapour) with the predetermined moisture, then, were located in oven at 45°C. For seed priming, the desired number of seeds were placed inside two layers of filter papers, treated with the SA solution, and placed in darkness at 25°C for 24 hours. For germination test, the seeds were first disinfected with 1% sodiumhypochlorite. Subsequently, 50 seeds each were placed in two layers of filter papers in a 100 mm × 10 mm Petri dish. Thereafter, they were placed in an incubator at constant temperature of 20°C. The seeds germination was counted on daily basis from day 5 to day 14 according to the International Seed Testing Association (ISTA) guidelines (Ghassemi-Golezani and Dalil, 2012). The seeds were considered as germinated if their radicle was visible with the length of 2 mm (Moosavi and NabaviKalat, 2011). On the last day of germination tests, the seedlings were oven-dried at 70°C for 48 hours.

The seedling weight and length were measured by a 0.001 g precision scale and 1 mm precision ruler, respectively. Finally, the weight and length of 10 normal seedlings per experimental unit were recorded. Subsequently, according to the counts and measurements, the germination indices were calculated by Eq (Table 1). (1)-(15) in which N = total number of sown seeds, t = days after sowing, T = duration of germination period (day), D = days after germination, MCGP = maximum cumulative germination percentage, SFW = seedling fresh weight (g), SDW = seedling dry weight (g), SL = seedling length (g), PL = plumule length (cm), and RL = radicle length (cm).

Table 1. Equations of germination indices

Equation Number	Index	Equation	References
(1)	Germination percentage	$GP = \sum n / N \times 100$	Saberi and Tarnian, 2012
(2)	Seedling length vigor	$SLV = GP \times SL$	Reddy and Khan, 2001
(3)	Seedling weight vigor	$SWV = GP \times SDW$	Reddy and Khan, 2001
(4)	Germination speed	$GS = \Sigma(n/t)$	Kotows, 1926., Nicholas and Heydecker, 1968
(5)	Mean germination speed	$MGS = \Sigma(nt)/\Sigma n$	Kotows, 1926., Nicholas and Heydecker, 1968
(6)	Coefficient of germination speed	$CGS = \sum n.100 / \sum (nt)$	Kotows, 1926., Nicholas and Heydecker, 1968
(7)	Mean germination time	$MGT = \sum Dn / \sum n$	Kotows, 1926., Nicholas and Heydecker, 1968
(8)	Mean daily germination	$MDG = GP / T$	Kotows, 1926., Nicholas and Heydecker, 1968
(9)	Coefficient of germination uniformity	$CGU= GP / MGT$	Kotows, 1926., Nicholas and Heydecker, 1968
(10)	Germination vigor	$GE = MCGP/N\times100$	Agarwal, 1980
(11)	Daily germination speed	$DGS = 1/MDG$	Maguire, 1962
(12)	Maximum mean daily germination	$PV_{peak\ value} = MCGP/D$	Ranai and De Santana, 2006
(13)	Germination value	$GV = MDG\times PV$	Panwar and Bhardwaj, 2005
(14)	Seedling tissue water percentage	$STWP = SFW– SDW/ SFW \times100$	Tsonev et al., 1998
(15)	Allometry coefficient	$AC = PL/RL$	ISTA, 1979

N = total number of sown seeds, t = days after sowing, T = duration of germination period (day), D = days after germination, MCGP = maximum cumulative germination percentage, SFW = seedling fresh weight (g), SDW = seedling dry weight (g), SL = seedling length (g), PL = plumule length (cm), and RL = radicle length (cm).

Data were statistically analyzed by SAS 9.1 Software Package (Soltani 2007). In addition to analysis of variance, the means of data were compared by LSD test.

Results and Discussion

Germination indices and seedling vigor

According to the results of analysis of variance, the main effect of accelerated aging was significant on all germination parameters including germination percentage, seedling length vigor, seedling weight vigor, germination speed, mean germination speed, coefficient of germination speed, mean germination time, mean daily germination, coefficient of germination uniformity, germination vigor, daily germination speed, maximum mean daily germination, and germination value.The main effect of priming through SA was also found to be significant on most of these parameters (except mean germination speed, coefficient of germination speed, mean germination time, and coefficient of germination uniformity). In addition, the interaction between accelerated aging and priming with SA was significant for all studied parameters at the 1% probability level (Table 2).

Means comparison revealed that 48-hour aging without priming was associated with the lowest mean germination percentage (41%), seedling length vigor (2.430), seedling weight vigor (0.805), germination speed (3.202), and coefficient of germination speed (15.482), mean daily germination (4.10), coefficient of germination uniformity (16.610), germination vigor (0.82), maximum mean daily germination (10.917), and germination value (50.233). However, these parameters exhibited improvements in aged seeds primed with SA, thereby germination percentage reached 100%. In contrast to these parameters, the highest daily germination speed (6.467), mean germination time (2.467) and mean germination speed (0.293) were observed in unprimed seeds aged for 48 hours.

Seedling length and weight

Analysis of variance indicated that accelerated aging of fenugreek seeds significantly influenced radicle length, seedling length, and plumule fresh weight at the 5% probability level and radicle dry weight, seedling dry weight, and radicle: plumule weight ratio at the 1% probability level. Furthermore, priming significantly impacted seedling length and seedling dry weight at the 1% probability level and plumule fresh weight and plumule dry weight at the 5% probability level. The interaction between accelerated priming and priming with SA was significant for seedling fresh weight (at the 1% probability level) and plumule fresh weight, radicle fresh weight and seedling tissue water percentage (at the 5% probability level) (Table 3).

After analysis of variance, the means were compared among different levels of traits for which the interactions were significant. Other traits for which the interactions were insignificant, means comparison was carried out only for main effects. Accordingly, the lowest mean plumule fresh weight (205.25 mm), seedling fresh weight (230.00 mm) and seedling tissue water percentage (86.60%) were related to seeds aged for 48 hours and primed with 1700 μM SA, whilst priming with 2800 μM SA increased seedling tissue water percentage to 94.48% in 48-hour aged seeds. Besides, the highest radicle fresh weight (61.75 g) and seedling fresh weight (410.50 g) were obtained from control (no aging and no priming) (Table 3). Means comparison for the main effect of accelerated aging indicated that the lowest mean radicle length (2.022 cm), seedling length (7.449 cm), radicle dry weight (1.917 mm), seedling dry weight (26.500 mm) and radicle: plumule weight ratio (0.103) were obtained from the treatment of accelerated aging for 48 hours. In other words, these traits exhibited a descending trend with the increase in aging time (Table 4). The highest mean seedling length, plumule dry weight, and seedling dry weight were associated with seeds primed with 2800 μM SA (Table 5).

Table 1. Analysis of variance (means of squares) of the impact of accelerated aging and SA priming on germination parameters and seedling vigor of fenugreek

S.O.V.	df	Means of squares												
		GP	SLV	SWV	GS	MGS	CGS	MGT	MDG	CGU	GE	DGS	PV_{peak}	GV
Aging	2	5728.444**	59.732**	9.377**	48.464**	0.669**	5.480**	0.669**	57.284**	2648.806**	0.291**	0.047**	1017.694**	152889.952**
Priming	2	1469.778**	27.490**	5.438**	7.657**	0.021^{ns}	0.277^{ns}	0.021^{ns}	14.698**	88.712^{ns}	0.588**	0.013**	144.699**	24484.297**
Aging × priming	4	1511.111**	11.400**	2.038**	13.068**	0.336**	2.886**	0.366**	15.111**	85.706**	0.604**	0.013**	529.687**	72238.147**
Error	27	44.296	2.029	0.383	0.330	0.053	0.431	0.053	0.443	54.771	0.018	0.003	30.132	3055.404
C.V. (%)		7.69	19.23	22.74	7.88	3.79	3.97	11.12	7.69	16.72	7.69	17.98	18.37	19.61

ns: non-significance; *: significance at $p < 0.05$; **: significance at $p < 0.01$.

Table 2. Means comparison of accelerated aging × SA priming interaction for germination parameters and seedling vigor of fenugreek

Accelerated aging	SA priming	GP	SLV	SWV	GS	MGS	CGS	MGT	MDG	CGU	GE	DGS	PV_{peak}	GV
	Control (no priming)	99.00 a	9.468 a	3.902 a	8.335 ab	6.069 bc	16.503 c	2.069 bc	9.900 a	48.425bc	1.990 a	0.101 b	29.667bc	293.467 c
Control (no aging)	1700 µM	100.00 a	9.025 ab	3.075 ab	8.445 ab	5.970 cd	16.754 bc	1.970 cd	10.000 a	50.866 bc	2.000 a	0.100 b	33.333bc	333.333bc
	2800 µM	100.00 a	8.620 ab	3.475 ab	8.084 b	6.270 abc	15.962 cd	2.270 abc	10.000 a	44.327 c	2.000 a	0.100 b	27.083 c	270.833 c
	Control (no priming)	99.00 a	9.147 ab	3.144 ab	8.016 b	6.202 abc	16.130 cd	2.202 abc	9.900 a	45.131 c	1.980 a	0.101 b	33.000 bc	326.800 bc
Aging for 24 hours	1700 µM	99.00 a	7.128 b	2.865 b	8.792 ab	5.734 de	17.490 ab	1.734 de	9.900 a	58.821 ab	1.980 a	0.101 b	35.083 b	347.633 bc
	2800 µM	98.00 a	8.682 ab	2.776 b	8.967 a	5.510 e	18.188 a	1.509 e	9.800 a	66.966 a	1.960 a	0.102 b	49.000 a	480.800 a
	Control (no priming)	41.00 b	2.430 ab	0.805 c	3.202 c	6.467 a	15.482 d	2.467 a	4.10 b	16.610 d	0.82 b	0.293 a	10.917 d	50.233 d
Aging for 48 hours	1700 µM	43.00 b	3.468 c	1.003 c	3.441 c	6.314 ab	15.851 cd	2.313 ab	4.30 b	18.791 d	0.86 b	0.237 a	13.417 d	58.767 d
	2800 µM	100.00 a	8.695 ab	3.450 ab	8.264 ab	6.070 bc	16.477 c	2.070 bc	10.000 a	48.377 bc	2.000 a	0.100 b	37.500 b	375.000 b

The means with similar letter(s) in each column did not show significant differences at $p < 0.05$ according to the LSD test.

Table 3. Means comparison of accelerated aging × SA priming interaction for fenugreek seedling length and weight traits

Accelerated aging	SA priming	Plumule fresh weight	Radicle fresh weight	Seedling fresh weight	STWP
Control (no aging)	Control (no priming)	348.750 ab	61.750 a	410.500 a	90.280 abc
	1700 µM	371.000 a	65.000 a	436.000 a	92.922 ab
	2800 µM	327.000 ab	15.500 c	342.500 ab	89.586 bc
Aging for 24 hours	Control (no priming)	310.000 ab	46.250 abc	356.250 ab	90.909 abc
	1700 µM	269.000 bc	38.500 abc	307.500 bc	90.472 abc
	2800 µM	326.250 ab	43.250 abc	369.500 ab	92.716 ab
Aging for 48 hours	Control (no priming)	373.500 a	50.250 ab	423.750 a	91.573 ab
	1700 µM	205.250 c	24.750 bc	230.000 c	86.605 c
	2800 µM	305.705 ab	37.750 abc	343.500 ab	94.484 a

The means with similar letter(s) in each column did not show significant differences at $p < 0.05$ according to the LSD test.

Table 4. Means comparison of the main effect of accelerated aging on fenugreek seedling length and weight traits

Accelerated aging	Radicle length	Seedling length	Radicle dry weight	Seedling dry weight	Radicle: plumule weight ratio
Control (no aging)	2.573 a	9.067 a	5.333 a	35.000 a	0.184 ab
Aging for 24 hours	2.305 ab	8.427 ab	6.250 a	29.583 ab	0.276 a
Aging for 48 hours	2.023 b	7.449 b	1.917 b	26.500 b	0.103 b

The means with similar letter(s) in each column did not show significant differences at $p < 0.05$ according to the LSD test.

Table 5. Means comparison of the main effect of accelerated aging on fenugreek seedling length and weight traits

SA priming	Seedling length	Plumule dry weight	Seedling dry weight
Control(nopriming)	8.516 a	24.417 b	27.250 b
1700 µM	7.262 b	24.853 b	28.583 b
2800 µM	9.165 a	30.750 a	35.250 a

The means with similar letter(s) in each column did not show significant differences at $p < 0.05$ according to the L test.

The study showed significant impact of accelerated aging time on germination parameters which resulted in the loss of seed quality. However, the priming of seeds with SA inhibited their aging-induced quality loss. Similar results have been reported by other researchers. In Hsu et al. (2003)'s study, aging decreased germination and increased mean germination time of the seeds, and priming alleviated the influence of aging. In Moosavi and NabaviKalat (2011), as aging temperature was increased, germination and germination speed were lost. This effect was sever in seeds with higher moisture content. Parmoon et al. (2014), also, reported the loss of germination percentage and seedling growth parameters due to accelerated aging of milk thistle seeds. In MohssenNasab et al. (2010), the loss of germination percentage, seed vigor and germination speed were much lower under aged condition compared to non aged seeds. In a study on seed aging for 0, 48, 72, 96 and 144 hours at 43°C and 100% relative humidity, Soltani et al. (2009) concluded that vigor, germination and growth of wheat were linearly decreased with the increase in accelerated aging period under all environmental conditions. Ansari and Sharifzadeh (2012) regarding reported the significant loss of germination parameters and seedling growth occurred with the increase in aging period and the treatment of gradual loss of moisture facilitated the improvement of germination parameters during aging of mountain rye. They observed the highest germination percentage in seeds primed with SA and gibberellins without aging treatment. Moreover, it has been reported that the loss of germination speed is likely to be induced by the interruption that happens in aged seeds at the beginning of the process. This interruption may be related to the fact that seeds require time to repair the damages to membrane and other parts of cells, to reactivate antioxidant system and to avoid oxidative stress. The damages can be repaired only after the uptake of enough water by seeds. Thus, aged seeds need longer time for completing germination process, entailing the loss of germination parameters (Bailly et al. 2000). It has been reported that priming enhances germination parameters and the seed vigor decreases with the increase in aging, and that post-priming treatments like gradual loss of moisture with the increase in aging improves germination parameters (Chavoshinasab et al. 2010). Similar results about different plants show that priming enhances germination parameters (Demir Kaya et al. 2006; Murungu et al. 2003; Ansari et al. 2012; Kang et al. 2007).

Conclusion

Loss of seed vigor is common problem in fenugreek with aging. This could be easily overcome using 2800 µM SA priming treatment. This protocol will help fenugreek farmers to improve seed germination in fields and increase their yields significantly.

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