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Influence of Priming with Exogenous Selenium on Seed Vigour of Alfalfa (*Medicago sativa* L.)

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ABSTRACT

Background: Selenium (Se) is an essential trace element to higher plants, animals and humans, but low Se levels are a global public health concern. Seed priming has become a basic strategy for the production of Se-riched agricultural products, but its application is still not clear in the production of Se-enrich alfalfa, hence this study was conducted for the production of Se-enriched alfalfa by seed priming with different concentrations and time on seed vigour.

Methods: Seeds were primed with 0, 0.5, 1.0, 2.0, 4.0 and 8.0 mmol L^{-1} of sodium selenite solution for 0, 3, 6, 9 and 12 h at 20°C and their germination percentage, germination index, seedling vigour index and mean germination time were analyzed.

Result: Seed vigour of alfalfa was improved by priming with low selenium (Se) concentration (0.5 and 1.0 mmol L^{-1}), but was inhibited by high Se concentration (> 2.0 mmol L^{-1}). Hence, it must be necessary to carefully select appropriate concentration and time for the application of Se priming in alfalfa seeds. The optimal manner of Se priming in alfalfa seeds might be at 1.0 mmol L^{-1} concentration for 9 h.

Key words: Alfalfa, Germination, Seed priming, Seed vigour, Selenium.

INTRODUCTION

Selenium (Se) is an essential trace element to maintain the homeostasis of endocrine and immunity in humans and animals and is also considered beneficial to higher plants (Naziroðlu, 2009; Silva *et al*. 2019), its deficiency can cause many diseases such as Keshan disease, Kashin-Beck disease and most brain disease (Du *et al*. 2019). Se levels in plants are closely related to human dietary Se status (Du *et al*. 2019), but Se availability are at low levels in most soils of the world, which can result in a lack of Se in plants and consequently insufficient Se intake in human diets (Silva *et al*. 2019). Therefore, low Se levels in humans are a global public health concern (Du *et al*. 2019). Fortunately, proper artificial supplementation of Se, as a basic biofortification strategy, can effectively improve the yield and qualityof plants and enhance the Se levels in human body (Motesharezadeh *et al*. 2019; Gu *et al*. 2020). However, the safe range of Se intake is very narrow (Schiavon and Pilon-Smits, 2017), the high level of Se is toxic to human health (Hadrup and Ravn-Haren, 2020) and also becomes a major concern for terrestrial and aquatic ecosystems (Etteieb *et al*. 2020). Hence, it must be care for using to produce Se-riched agricultural products (Hadrup and Ravn-Haren, 2020). Seed priming is an attractive and easy physiological strategy in micronutrients application (Arun *et al*. 2017), which is also widely used in Se application of many plants (Du *et al*. 2019). Se priming can improve seed germination, seedling quality and the photosynthesis and antioxidative responses of plants (Moulick *et al*. 2018). Therefore, seed priming has become a basic strategy for the production of Se-riched agricultural products.

Alfalfa (*Medicago sativa* L.) is a primary forage species that has much excellent features like high adaptability, rapid regeneration, high yield and rich in high protein (Zhang *et al*. 2019). Thus, it is widely cultivated around the world for animal feeding and green manure crops (Zhang *et al*. 2017; Yang *et al*. 2019). Traditionally, direct additives of Se to animals are frequently associated with high toxicity, non-uniform intake and low bioavailability (Bai *et al*. 2019). Therefore, the production of Se-enriched alfalfa has also been paid more and more attention and has been carried out in many forms such as soil application, inoculant of seleno-bacteria and foliar application (Motesharezadeh *et al*. 2019). However, the response of alfalfa growth to Se application is still with few studies (Bai *et al*. 2019) and rarely focuses on the influence of Se priming on seed vigour of alfalfa. Therefore, it is still not clear that how to use the technology of seed priming for the production of Se-enrich alfalfa. This study was designed to evaluate the influence of Se concentration and priming time on seed vigour of alfalfa, to eventually provide an effective and economic strategy for the production of Se-enrich alfalfa.

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MATERIALS AND METHODS

Alfalfa var. Pianguan seeds were collected by the Grass Seed Laboratory of Shanxi Agricultural University on August 2018, which was sealed in plastic bags and stored at -20°C. This experiment was carried out on July 2019 in the Grass Seed Laboratory of Shanxi Agricultural University. The seeds were soaked in different concentration (0.5, 1.0, 2.0, 4.0 and 8.0 mmol L^{-1}) of sodium selenite solution at 20°C for 0, 3, 6, 9 and 12 h. Thereafter, seeds were rinsed three times with deionized water and then wiped the water off their surface with a clean filter paper and air-dried for three days at 25°C and 45% relative humidity in the dark (moisture content reached approximately 10 % on fresh weight basis). Each treatment had four replicates.

Germination tests were executed by following the ISTA rules (2017). One hundred seeds from each treatment were selected and placed into petri dishes that were lined with three layers of filter papers wetted with 10 ml deionized water. The petri dishes were placed in constant temperature incubators at 20°C. Germination was recorded daily based on 2 mm radicle growth through seed coat. The number and length of normal seedlings were counted in each petri dish on 10^{th} day. Germination percentage was computed and expressed in percentage. Germination index and seedling vigour index were calculated according to Abdul-Baki and Anderson (1973). Mean germination time was counted according to Ellis *et al*. (1982). The final calculations were evaluated as below:

$$\text{Germination percentage (\%)} = (G_{10}/N) \times 100$$

Where

G_{10} was the number of normal seedlings on the 10^{th} day.

N was the total number of seeds in the test.

$$\text{Mean germination time (day)} = \Sigma(nd)/\Sigma n$$

Where

n was number of germinated seeds (2 mm radicle growth through seed coat) in day, d, of counting seed germination, Σn was total germinated seeds.

$$\text{Germination index} = \Sigma(n/d)$$

where n was the number of germinated seeds in day d, d was day of counting seed germination.

$$\text{Seedling vigour index} = [\text{germination percentage (\%)} \times \text{average seedling length (cm)}] / 100$$

Data was analysed using the Kolmogorov-Smirnov test and the homogeneity test of variances, which was conformed to normal distribution and homoscedasticity. Mean difference of comparisons were performed by an analysis of variance (ANOVA), which was conducted using SPSS for Windows ver. 13.0 followed by Duncan's multiple range test (P = 0.05).

RESULTS AND DISCUSSION

The changes in germination percentage, germination index, mean germination time and seedling vigour index of alfalfa seeds were related to priming time and solution concentration (Xia *et al*. 2019), this was similar to the results of alfalfa seeds primed with Se. There were highly significant (P < 0.01) differences between the Se concentration, priming time and their interaction on the germination percentage, germination index, mean germination time and seedling vigour index of alfalfa seeds, (Table 1), these results showed that the effect of Se priming on germination percentage, germination index, mean germination time and seedling vigour index of alfalfa seeds were closely related to the Se concentration and priming time.

At the same priming time, germination percentage (Table 2), germination index (Table 3) and seedling vigour index (Table 4) of alfalfa seeds had increased firstly and

Table 1: Variance analysis of Se concentration and priming time on alfalfa seed vigour.

Source	Degree of freedom	Germination percentage		Germination index		Mean germination time		Seedling vigour index	
		F	*P*	F	*P*	F	*P*	F	*P*
Concentration	4	25.686	0	23.145	0	8.882	0	15.133	0
Time	4	7.785	0	35.817	0	14.397	0	14.484	0
Concentration×Time	16	5.731	0	5.797	0	2.576	0	4.992	0

Table 2: Effect of Se priming on germination percentage of alfalfa seeds.

Time/h	Sodium selenite concentration/mmol L^{-1}				
	0.5	1.0	2.0	4.0	8.0
0 (Control)	84±1.26Ab	84±1.26Ab	84±1.26Aa	84±1.26Aa	84±1.26Aa
3	84±3.30ABb	87±1.7Aa	86±3.30Aa	84±2.36ABa	81±1.50Bb
6	84±2.63ABb	87±2.08Aa	85±1.29ABa	81±1.04Ba	65±0.50Cc
9	88±2.87Aa	87±2.38Aa	84±1.83ABa	80±2.99Ba	63±4.50Cc
12	91±1.91Aa	86±0.96ABa	83±3.04Ba	79±1.91Ba	62±3.46Cc

Note: Means in the same row with different capital letters are significant difference (P<0.05), in the same column with different small letters are significant difference (P<0.05).

Table 3: Effect of Se priming on germination index of alfalfa seeds.

Time/h	Sodium selenite concentration/mmol L^{-1}				
	0.5	1.0	2.0	4.0	8.0
0 (Control)	20.69±0.40Ad	20.69±0.40Ac	20.69±0.40Ac	20.69±0.40Ac	20.69±0.40Ab
3	27.83±2.84Bc	33.30±2.92Ab	32.05±0.87Ab	29.89±1.40ABab	28.96±1.92Ba
6	35.85±1.57Ab	34.80±2.17Ab	34.43±2.00Aab	32.26±3.78Aa	22.12±2.12Bb
9	38.24±0.53Aa	40.45±1.45Aa	37.94±1.21Aa	25.63±2.96Bb	18.82±4.71Cc
12	37.17±0.36Aab	35.52±2.70Ab	35.59±1.69Aab	21.49±0.91Bc	18.36±0.86Cc

Note: Means in the same row with different capital letters are significant difference (P<0.05), in the same column with different small letters are significant difference (P<0.05).

Table 4: Effect of Se priming on mean germination time (d) of alfalfa seeds.

Time/h	Sodium selenite concentration/mmol L^{-1}				
	0.5	1.0	2.0	4.0	8.0
0 (Control)	2.31±0.09Aa	2.31±0.09Aa	2.31±0.09Aa	2.31±0.09Aa	2.31±0.09Ab
3	2.00±0.23Ab	1.61±0.18Cb	1.61±0.07Cb	1.81±0.08Bc	1.88±0.11Bd
6	1.52±0.09Bc	1.40±0.11Bc	1.62±0.18Bb	1.65±0.20Bc	2.28±0.13Ac
9	1.46±0.05Cc	1.39±0.07Cc	1.42±0.08Cc	1.73±0.26Bc	2.35±0.35Ab
12	1.59±0.08Dc	1.47±0.10Dc	1.64±0.07Cb	2.00±0.29Bb	2.61±0.27Aa

Note: Means in the same row with different capital letters are significant difference (P<0.05), in the same column with different small letters are significant difference (P<0.05).

Table 5: Effect of Se priming on seedling vigour index of alfalfa seeds.

Time/h	0.5	1.0	2.0	4.0	8.0
0 (Control)	4.04±0.36Ac	4.04±0.36Ac	4.04±0.36Aa	4.04±0.36Aa	4.04±0.36Aa
3	3.92±0.27Bc	4.35±0.08Ab	3.94±0.12Ba	3.67±0.20Cb	3.05±0.13Db
6	4.21±0.08Bb	4.81±0.22Aa	3.88±0.13Cab	3.04±0.26Dc	2.64±0.30Ec
9	4.99±0.18Aa	4.32±0.20Ab	3.44±0.16Cb	2.39±0.21Dd	2.18±0.29Ed
12	3.69±0.23Ad	3.47±0.45Bd	2.87±0.43Cc	1.93±0.33De	1.94±0.35De

Note: Means in the same row with different capital letters are significant difference (P<0.05), in the same column with different small letters are significant difference (P<0.05).

then decreased with the enhancement of Se concentration, but their mean germination time was opposite (Table 5). Moreover, the promoting effects of low Se concentration needed more time on germination percentage, germination index, mean germination time and seedling vigour index of alfalfa seeds primed with same Se concentration, but their inhibiting effects of high Se concentration took a shorter time. These results showed that priming with low concentration of Se could promote the germination of alfalfa seeds, but priming with high concentration of Se could restrain their germination. Similar phenomenon was found in rice seeds primed with Se (Du *et al*. 2019). Appropriate Se priming for seeds not only enhanced their energy metabolisms (Chen and Arora, 2013), but also increased the contents and activities of their antioxidant and defensive substance (Roqueiro *et al*. 2012). However, high concentration of Se solution not only delayed the cells activation by declining water potential (Xia *et al*. 2017), but also caused the aggravation of peroxide damage by reducing antioxidant capacity (Bai *et al*. 2019). Overall, the optimal manner was at 1.0 mmol L^{-1} concentration for 9 h during Se priming of alfalfa seeds in terms of their germination percentage, germination index, mean germination time and seedling vigour index.

Conclusion

Alfalfa seed vigour was highly significantly (P < 0.01) related to Se concentration, priming time and their interaction and it was promoted by priming with low Se concentration (0.5 and 1.0 mmol L^{-1}), but was restrained by high Se concentration (> 2.0 mmol L^{-1}). Overall, the optimal manner was at 1.0 mmol L^{-1} concentration for 9 h during Se priming of alfalfa seeds.

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