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Effects of Drought Stress on Seed Germination and Seedling Growth of Alfalfa with Different Seed Coat Colors

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ABSTRACT

Background: Because of the close relationship between seed coat color and forage seed quality, it is of great significance to reveal the effect of seed coat color on seed germination and seedling growth under drought stress.

Methods: We explore the effect of different seed coat colors (yellow, dark brown) on the seed germination and seedling growth of alfalfa under simulated drought stress (polyethylene glycol (PEG-6000) with eight osmotic potentials: 0 MPa, -0.1 MPa, -0.2 MPa, -0.3 MPa, -0.4 MPa, -0.6 MPa, -0.8 MPa, -1.0 MPa).

Result: Under control conditions, the germination rate and germination energy of yellow seeds (91.6% and 91.2%, respectively) were higher than dark brown seeds (46.2% and 42.0%). The maximum seed germination rate and germination energy were observed in the -0.1MPa treatment for both yellow and dark brown seeds and were higher than control by 1.53% and 0.66% (yellow) and 9.96%, 14.76% (dark brown), respectively. The root length and seedling growth of two kinds of seeds were significantly inhibited when drought intensity increased. In summary, the germination and seedling growth traits of yellow seeds were much better than dark brown ones. Agronomically, selecting seed with high proportions of yellow coat color is more beneficial for seed storage and establishment of new alfalfa grasslands.

Key words: Alfalfa, Drought resistance, Osmotic potential, Seeds of different coat color.

INTRODUCTION

Seed germination and early seedling growth are the key stages that affect the successful establishment of plant populations. Seed germination is not only dramatically impacted by seed morphology, genetic characteristics and physiological structure, but also closely related to environmental factors such as soil moisture. In the process of seed formation, the seed coat appears in various colors due to different stages of seed maturity and other factors (Avcý *et al*., 2020). Indeed, at different stages of seed development, seed coat color can undergo remarkable successive changes from green to light yellow, to yellow, brown and black (Wang *et al*., 2008). Normally, seeds with dark coat colors have better germination ability than those with light colors; however, this is not always true (Velijevic *et al*., 2017).

Alfalfa, as a significant forage crop, plays an extremely important role in the development of animal husbandry worldwide (Atumo *et al*., 2021). It is susceptible to drought (Diatta *et al*., 2021) and is generally more sensitive during seed germination and early seedling growth (Li *et al*., 2013). Previous studies have shown that the seed coat colors of leguminous plants such as Red clover, White Clover and *Melilotus suaveolens* were closely correlated with seed vigor (Wang *et al*., 2008). However, for alfalfa, the effect of different seed coat color on germination and growth under drought stress has been ignored because of its high germination rate overall (Sharavdorj *et al*., 2021). Therefore, we compared the drought resistance between seeds with different seed coat colors under different drought intensities. This study aimed to clarify how seed coat color and drought impact the seed germination and seedling growth of alfalfa. Based on previous studies, we assumed: 1) that the drought resistance of two kinds of seeds was significantly different and specifically that dark brown seeds had stronger drought resistance than yellow seeds because of the former's higher level of maturity; 2) that the germination and growth of the two kinds of seeds were promoted under low drought stress and then inhibited as drought stress increased. This study provide theoretical and practical evidence for the selection and breeding of drought-resistant varieties and have great significance to the establishment of alfalfa grasslands.

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MATERIALS AND METHODS

Experimental material

The experiments were conducted in Changchun University in 2020. The alfalfa variety used in the experiment was Gongnong No. 1. This variety has strong cold resistance, drought resistance. The seeds of this experiment came from Jinong Grass Technology Development Co., Ltd., which were collected in 2018.

Experiment design

The experiment used a two-factor fully factorial design, set two factors of seed coat color (yellow and dark brown) and drought intensity (0 MPa, -0.1 MPa, -0.2 MPa, -0.3 MPa, -0.4 MPa, -0.6 MPa, -0.8 MPa, -1.0 MPa) and was repeated five times. Before the experiment, 4000 yellow and 4000 dark brown alfalfa seeds of uniform size, plumpness and no diseases were selected (Fig 1). The seeds were soaked and disinfected with 0.1% $HgCl_2$ solution for 10 minutes, then rinsed with distilled water six times.

One hundred seeds were evenly placed in each dish and 10ml of PEG-6000 solution was used to treat the seeds. Distilled water was used as control. The seeds were placed into a culture incubator set to 16°C/28°C (14 h illumination /10 h darkness) and regular weighing of the dish was used to replenish water lost to evaporation so that the osmotic potential of the PEG was maintained.

Measurement

The daily germination number and total germination number were counted according to the method of the "International Seed Inspection Code" (Liu *et al*., 2012). The calculation formulas of each index were as follows:

$$\text{Germination rate} = \frac{\text{Germination number within 10 d}}{\text{Number of seeds tested}} \times 100\%$$

$$\text{Germination energy} = \frac{\text{Germination number within 4 d}}{\text{Number of seeds tested}} \times 100\%$$

$$\text{Germination index} = \frac{\Sigma Gt}{Dt}$$

Where,
Gt = Number of germinated seeds on day t.
Dt = Germination days.

$$\text{Drought resistance index} = \frac{\text{Seed germination index under drought stress}}{\text{Seed germination index under control}}$$

$$\text{Relative water content} = \frac{\text{Fresh weight of seedlings - Dry weight of seedlings}}{\text{Fresh weight of saturated water absorption of seedlings - dry weight of seedlings}} \times 100\%$$

$$\text{Seed vigor index} = \frac{S \times \Sigma Gt}{Dt}$$

Where,
S = Average seedling biomass (fresh weight of seedlings).
Σ(Gt/Dt) = Germination index.

To measure the biomass, seedlings were baked for 30 minutes at 105°C and then weighed after 24 h of drying at 65°C.

Statistical analysis

Statistical analysis of the data was undertaken with SPSS25.0 (SPSS Inc., Chicago, IL, USA). We used a two-way ANOVA of the GLM model to test the effect of drought intensity and seed coat color on germination and seedling growth indexes. One-way ANOVA was used to analyze the germination rate, germination energy and germination index of seeds and the root length, fresh dry weight, relative water content and seed vitality index of seedlings from seeds with different seed coat colors. Multiple comparisons between treatments were performed with *Duncan's post host* test. The statistical values were expressed as mean±1SE. Statistically significance was set at the 0.05 level.

RESULTS AND DISCUSSION

Seed germination

The germination rate, germination energy and germination index were significantly affected by the seed coat color, drought main factors and the coat color×drought interaction (Table 1). With the decrease in osmotic potential, the germination rate and germination energy of yellow and dark brown seeds initially increased but then decreased (Fig 2A; Fig 2C). When the osmotic potential was -0.1 MPa, the germination rate and germination energy of both yellow seeds and dark brown seeds reached their highest values. In comparison to the control, the germination rate was increased by 1.53% and the germination energy of yellow seeds and dark brown seeds increased by 0.66% and 14.76%, respectively. The germination energy of dark brown and yellow seeds was 0 when the osmotic potential was lower than -0.8 Mpa (Fig 2). The above results indicated that mild drought stress could promote seed germination, while moderate and high drought stress significantly inhibited seed germination. This is consistent with other studies (Kintl *et al*., 2021). The changes in relative germination rate and germination energy of the two kinds of seeds were similar to the germination rate under low drought conditions. Both the germination rate and energy were higher in yellow seeds than dark brown seeds under moderate and severe drought conditions (Fig 2B; Fig 2D).

Drought stress reduced the germination index and drought resistance index (Table 2). In this study, the germination index and drought resistance index were higher in yellow seeds of alfalfa than in the dark brown seeds. This means that yellow seeds germinate faster with greater uniformity, drought resistance and vitality in response to drought stress. Seed coat color might reflect the maturity of the seeds and it has been highly correlated to water

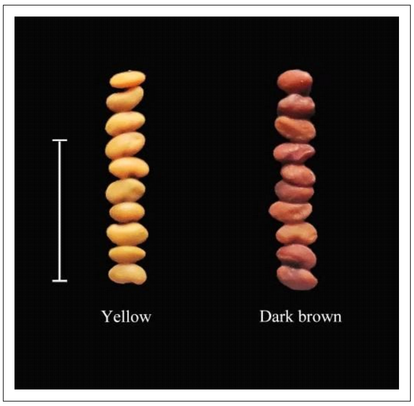

Fig 1: Alfalfa seeds were used in the experiment. Bar = 1 cm.

absorption (Ertekin *et al*., 2010). The morphology of seeds is also closely related to seed germination (Liu *et al*., 2007; Gao *et al*., 2012). Some previous studies have shown that water permeability and water absorption is higher in small seeds than large seeds. Smaller seeds have thinner shells and relatively larger surfaces and this characteristic ensures that small seeds have greater water permeability (Souza *et al*., 2014). In the current study, yellow seeds were generally smaller than dark brown seeds, which accounts for the stronger germination ability of yellow seeds (Quan *et al*., 2016). Under severe drought, dark brown seeds were the first to reach the tolerance limitation to drought stress, but yellow seeds maintained resistance to severe drought.

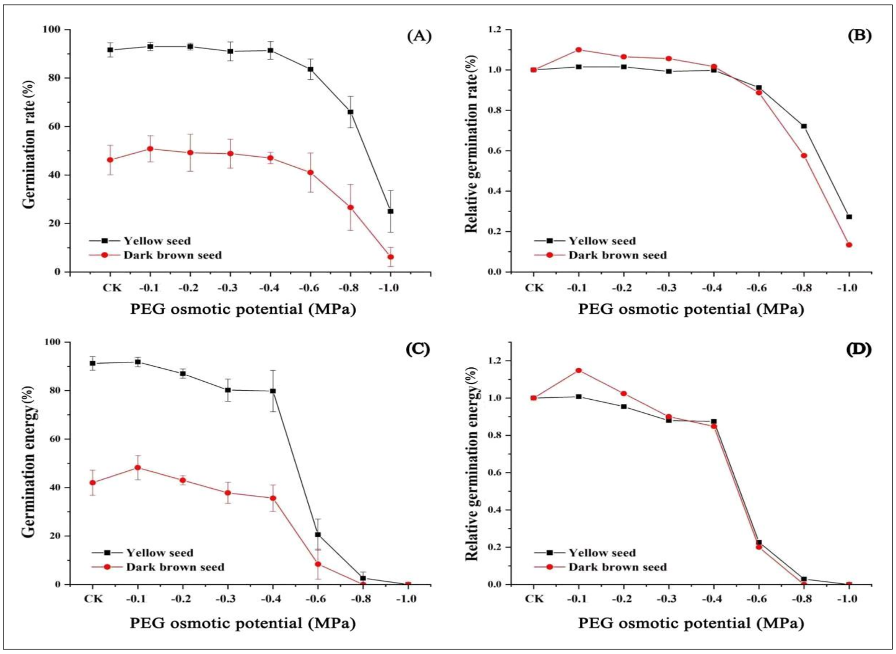

Fig 2: Effect of drought stress on germination rate, germination energy, relative germination rate and relative germination energy of seeds with different coat colors.

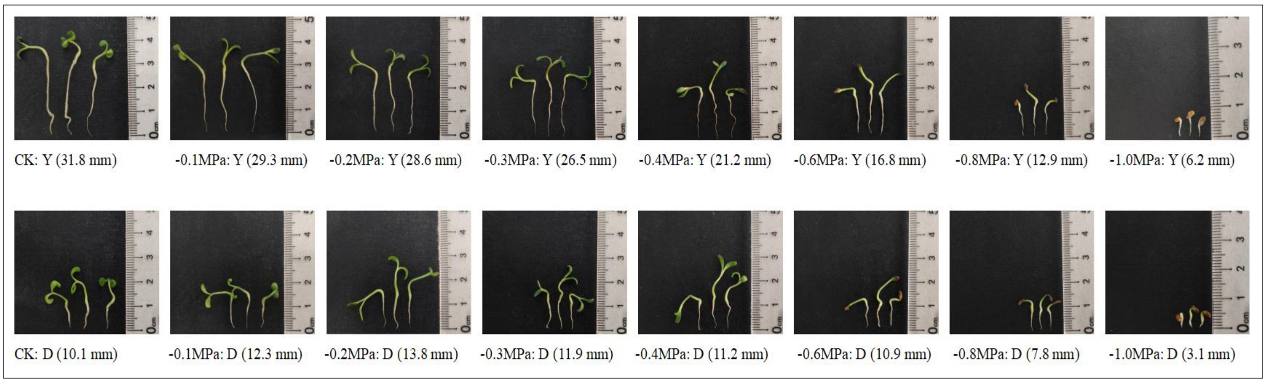

Fig 3: Root lengths of two kinds of seeds with different coat colors under drought stress (Y: Yellow seeds, D: Dark brown seeds).

Table 1: Two-way ANOVA of the effects of seed coat color and drought on seed germination and seedling growth.

	Germination rate		Germination energy		Germination index		Root length		Fresh weight		Dry weight		Relative water content		Seed vigor index	
	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Color (C)	796.251	*P*<0.001	758.749	*P*<0.001	655.617	*P*<0.001	1261.517	*P*<0.001	43.362	*P*<0.001	60.654	*P*<0.001	11191.358	*P*<0.001	2150.995	*P*<0.001
Drought (D)	6.276	*P*<0.001	405.299	*P*<0.001	300.508	*P*<0.001	180.170	*P*<0.001	129.391	*P*<0.001	0.825	*P*>0.05	5030.247	*P*<0.001	1241.381	*P*<0.001
C×D	4.737	*P*<0.001	46.952	*P*<0.001	39.258	*P*<0.001	52.148	*P*<0.001	3.414	*P*<0.01	4.523	*P*<0.01	4053.354	*P*<0.001	183.020	*P*<0.001

Table 2: Germination index and drought resistance index of two kinds of seeds with different coat colors under drought stress.

Seed coat color	Osmotic potential (MPa)	Germination index	Drought resistance index
Yellow	0	72.05±3.10 a	1.000
	-0.1	54.94±6.35 b	0.763
	-0.2	41.00±1.05 c	0.569
	-0.3	32.14±3.57 d	0.446
	-0.4	25.57±1.64 e	0.355
	-0.6	15.28±0.81 f	0.212
	-0.8	10.63±1.86 fg	0.148
	1.0	5.61±1.93 g	0.078
Dark brown	0	34.28±5.14 a	1.000
	-0.1	23.74±3.11 b	0.693
	-0.2	18.51±0.96 c	0.540
	-0.3	14.47±1.76 cd	0.422
	-0.4	11.96±1.42 d	0.349
	-0.6	7.24±1.47 e	0.211
	-0.8	4.28±1.46 ef	0.125
	1.0	1.83±0.63 f	0.053

Note: The above data are presented as mean±1SE. Different lowercase letters within each column for the yellow and dark brown seed coat colors indicate significant differences at the 0.05 level.

Seedling growth

The root length, fresh weight and dry weight of seedlings were significantly affected by the seed coat color, drought main factors and the coat color×drought interaction (Table 1). Drought stress significantly inhibited the growth of seedlings to the point that the final root length of yellow seeds was significantly constrained when the osmotic potential was lowered to -0.4MPa (33.33% lower than control) (Table 3; Fig 3). The hypocotyl length of yellow seeds was significantly inhibited when the osmotic potential decreased to -0.3MPa. However, for dark brown seeds, the hypocotyl length initially showed an increasing trend, then it decreased with lower osmotic potential. The root length, hypocotyl length and seedling fresh weight of the two seed types were significantly inhibited with increases in the level of drought stress. However, the root and hypocotyl length of yellow seeds did not show an increasing trend under mild drought. This may be linked to the fact that yellow seeds store more nutrients (Ma *et al*., 2016) and these may have met the needs of root and hypocotyl growth during the experimental period.

The root length, hypocotyl length and seedling fresh weight were greater in yellow seeds than dark brown seeds under each drought intensity (Table 3). The growth ability was stronger in yellow seeds than dark brown seeds in response to drought stress. In addition, the rate of reduction in the fresh weight of dark brown-seed seedlings was higher than in yellow-seed seedlings and this indicated that drought stress has a greater inhibition effect on the growth of dark brown-seed seedlings.

Table 3: Root length, hypocotyl length, fresh and dry weight, relative water content and seed vigor index of two kinds of seeds with different coat colors under drought stress.

Seed coat color	PEG osmotic potential (MPa)	Root length (mm)	Hypocotyl length (mm)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Relative water content	Seed vigor index
Yellow	0	31.8±0.3 a***	10.73±0.46 a**	0.2950±0.0064 a	0.0160±0.0018*	0.7904±0.0011 g***	21.2548±0.4602 a***
	-0.1	29.3±1.2 ab***	10.67±0.17 a***	0.2888±0.0106 a***	0.0156±0.0007**	0.8591±0.0003 f***	15.8667±0.5798 b***
	-0.2	28.6±0.7 ab***	10.57±0.21 a	0.2650±0.0253 ab	0.0165±0.0020*	0.8584±0.0010 f***	10.8650±1.0388 c***
	-0.3	26.5±1.0 b***	8.53±0.33 b	0.2388±0.0144 bc	0.0176±0.0021*	0.8785±0.0010 e**	7.6757±0.4642 d***
	-0.4	21.2±1.5 c***	8.77±0.19 b**	0.2270±0.0307 cd	0.0150±0.0014*	0.8848±0.0007 d***	5.8042±0.7849 e***
	-0.6	16.8±2.4 c***	8.67±0.09 b	0.2058±0.0079 d**	0.0141±0.0013	0.9068±0.0006 c***	3.1446±0.1205 f***
	-0.8	12.9±2.1 d***	7.37±0.17 c	0.1684±0.0104 e**	0.0142±0.0010	0.9747±0.0002 a***	1.7898±0.1110 g***
	1.0	6.2±1.7 e***	4.77±0.57 d**	0.1366±0.0145 f**	0.0140±0.0008	0.9616±0.0002 b***	0.7669±0.0816 h***
Dark brown	0	10.1±0.7 d	8.67±0.12 b	0.3010±0.0057 a	0.0113±0.0005	0.8140±0.0003 f	10.3183±0.1939 a
	-0.1	12.3±0.4 bc	8.60±0.08 b	0.2340±0.0167 b	0.0102±0.0005	0.8357±0.0003 e	5.5556±0.3973 b
	-0.2	13.8±0.3 a	10.40±0.16 a	0.2324±0.0150 b	0.0108±0.0004	0.8762±0.0002 b	4.3022±0.2772 c
	-0.3	11.9±0.2 b	7.87±0.09 c	0.2350±0.0115 b	0.0107±0.0008	0.8741±0.0004 b	3.4005±0.1668 d
	-0.4	11.2±0.2 bc	7.70±0.22 c	0.2104±0.0147 c	0.0106±0.0003	0.8560±0.0002 d	2.5154±0.1752 e
	-0.6	10.9±0.2 c	8.43±0.12 b	0.1794±0.0081 d	0.0123±0.0012	0.8855±0.0007 a	1.2989±0.0584 f
	-0.8	7.8±0.2 e	7.00±0.14 d	0.1400±0.0064 e	0.0140±0.0021	0.8131±0.0025 f	0.5991±0.0272 g
	1.0	3.1±0.3 f	3.10±0.08 e	0.1010±0.0095 f	0.0139±0.0016	0.8675±0.0021 c	0.1848±0.0174 h

Note: The above data are presented as mean±1SE. Different lowercase letters in each column of the yellow and dark brown seed coat colors indicate significant differences at the 0.05 level. *, ** and ***Represent statistical significance within the yellow and dark brown seed coat colors under the same osmotic potential at P<0.05, P<0.01 and P<0.001, respectively.

Relative water content and seed vigor index were also significantly affected by the seed coat color, drought main factors and the coat color×drought interaction (Table 1). The relative water content of yellow-seed seedlings was greater under each drought intensity (Table 3) and the biomass of yellow-seed seedlings became greater as the level of drought increased. It is known that cultivars with stronger drought tolerance have lower transpiration, which manifests as better water retention and hence greater drought resistance (Quan *et al*., 2016). The seed vigor index of the two kinds of seeds decreased significantly with the decrease in osmotic potential. This has been associated with high vigor seeds that have obvious growth advantages and growth potential (Finch-Savage *et al*., 2016) and this might improve the biomass of seedlings in terms of root length and fresh weight. The seedlings with strong growth momentum had enhanced stress resistance and this laid down a foundation for their continued growth.

CONCLUSION

Irrespective of the presence of drought or not, the ability to germinate, the rate of growth and water retention and drought resistance were greater in yellow seeds than in dark brown seeds. Seed germination and seedling growth in the two kinds of seeds decreased as drought stress increased and the inhibitory effect of drought on dark brown seeds was more obvious. For this reason, during agronomic production, we should harvest alfalfa seeds when the proportion of yellow seeds is the highest. The resulting reduction in the proportion of dark brown seeds that are harvested should improve the success rate of grassland establishment and would be conducive to cost savings during planting.

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