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Impact of Seed Maturation and Drought on Seed Germination and Early Seedling Growth in White Clover (*Trifolium repens* L.)

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ABSTRACT

Background: Understanding the drought tolerance of white clover seeds during germination and early seedling growth and how that is linked to seed maturation in legume species, is crucial to developing new stand establishments.

Methods: An experiment was conducted in Randomized Block design in two factorial concept to explore how seed maturation and drought tolerance influence seed germination and early seedling growth using the two factors: seed coat color (yellow and brown, two levels as one factor) and drought intensity (control: 0 MPa [distilled water], mild drought: -0.2 MPa, moderate drought: -0.4 MPa and severe drought: -0.6 MPa, four levels as another factor).

Result: In contrast to the yellow-coated seeds, the mean germination percentage, germination index, seedling vigour index and radicle length of the brown-coated seeds were lowered by 7.9%, 23.1%, 20.5% and 12.9%, respectively. Compared to the control, introducing drought conditions reduced the mean of germination percentage, germination index, seedling vigour index and radicle length of yellow-coated seeds by 52.4%, 64.1%, 41.9% and 23.2%, respectively. Meanwhile, drought affected brown-coated seeds more than yellow-coated seeds. The results indicating that the seed quality of white clover seed lots with yellow-colored coats higher than the seed lots with brown-coloured coats. In agronomic practice, a larger proportion of yellow-coated seeds should be harvested to improve new stands successful establishment under environment easy to dry.

Key words: Drought, Germination, Maturation, Seedling growth, Seedling vigour index, White clover.

INTRODUCTION

Frequent droughts caused by global climate change are the major issues influencing plant growth and crop production worldwide (Fan *et al*. 2019). Soil moisture directly affects seed germination and seedling growth (Yu *et al*. 2019), two of the most critical steps in the life cycle of plants (Kuriakose and Prasad, 2008; Ellison, 2001). Consequently, this relationship influences the vegetative and reproductive growth in the later stages of plant development, ultimately leading to a decline in crop yield and quality (Yu *et al*. 2013; Yu *et al*. 2015; Oliveira *et al*. 2017). Additionally, seed germination and early seedling growth are closely linked to seed maturity (Atis *et al*. 2011). That is, well-developed seeds can easily germinate under suitable water conditions and grow into seedlings (Li *et al*. 2013). As the seed matures, its coat changes colour at different developmental stages (Velijević *et al*. 2017). Typically, different coat colours represent variations in seed maturity based on their correlation to water uptake (Ertekin and Kirdar, 2010), gas diffusion (West and Harris, 1963), seed dormancy (Baskin *et al*. 2000) and other characteristics in of *Trifolium pratense* (Atis *et al*. 2011), *Arabidopsis thaliana* (Debeajun *et al*., 2000) and *Cyamopsis tetragonoloba* (Liu *et al*. 2007). Normally, darker-colored seeds are more matured than the lighter-coloured seeds (Wang *et al*. 2008). Also, darker-colour seeds germinate more successfully in both suitable and disturbed habitats.

White clover is a perennial legume species, considered an important legume forage species in many regions of the world, with a long green period, beautiful shape, high forage value and palatability (Abbasi and Khan, 2004; Harp and Pulatie, 2008). Seed lots harvested in the same year present as two different colors: brown and yellow (Bortnem and Boe, 2003). Currently, there is no literature discussing the relationship between the seed colors to seed germination and seedling growth. In the study, we used polyethylene glycol (PEG) to simulate various drought conditions and identified the response of brown- and yellow-coated seeds to drought intensities. We hypothesized that: (i) the germination and early seedling growth of seeds with brown-coloured coats was superior to yellow-coloured coats in both

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control and stress conditions; (ii) the difference in germination and early seedling growth between two different coat colours will increase with greater drought intensity.

MATERIALS AND METHODS

Experimental material

Huia white clover is a perennial legume species commonly planted in temperate regions. The growth stage begins in early spring and flowers from mid-May to late-October. Typically, the Full-blooming stage lasts from mid-June to mid-August and the seeds are maturing at the same time with flowering. In this experiment, the seeds were purchased from Lvchuang Technology Co. Ltd., in the Gansu province of China in 2013. These seeds were harvested in early August 2012 and used in a germination experiment at the Institute of Grassland Science, Northeast Normal University. Prior to beginning the experiment, the seeds were stored for one year, at room temperature, without use of any heating equipment. Brown-coated seeds accounted for 55% of total seeds and yellow-coated seeds accounted for 45%.

Seed germination tests

An experiment in randomized block design with two factorial concepts was designed using the following two factors: seed coat color (yellow and brown) as first factor and drought intensity (control [distilled water], 10% [-0.2 MPa], 15% [-0.4 MPa], 20% [-0.6 MPa] as another factor). We firstly divided seeds into yellow and brown two groups (Fig 1). Prior to the experiment, the seeds were surface-sterilized in 0.1% $HgCl_2$ solution for 5 minutes, rinsed with distilled water until all residual disinfectant was removed and air-dried to avoid fungal infections (Hui *et al*. 2013). The seeds were placed into 28 petri dishes, 9 cm in diameter and the bottom was covered with two layers of filter paper.

Drought stress treatments were simulated using PEG 6000 solutions of different concentrations. There were a total of eight treatment combinations. Each treatment was replicated four times with 100 seeds in each replication. PEG solutions were added to distilled water at room temperature (20°C) to ensure they were fully dissolved. The petri dishes were then placed in growth chamber and maintained at 28°C/18°C with a 14 h photoperiod to facilitate seed germination (Sylvania cool white fluorescent lamps, 200 μmol/[m^2·s], 400-700 nm, HPG-400, Haerbin, China). Additionally, germinated seeds were counted daily until germination ceased for three consecutive days. We considered a seed germinated when the length of the radicle was more than 2 mm. At the end of the 3-week experiment, we randomly selected ten germinated seeds from each replication to measure the seedling and radicle length. The straightened seedling and radicle were measured beside a ruler and recorded values were accurate to four decimal places, or one micrometer. The germination percentage, germination index and seedling vigour index were calculated using the following formulations:

$$\text{Germination percentage (GR(\%)\%} = n/nT \times 100$$

"n" is the number of germinated seeds and "nT" is the total number of seeds.

$$\text{Germination index (GI)} = \sum(Gt/Dt)$$

"Gt" is the number seeds germinated a time "t" days and "Dt" indicates the corresponding number of seed germination days.

$$\text{Seedling vigour index (VI)} = S \times GI$$

"S" is the total length of the radicle and shoot (mm) and "GI" is the germination index.

Data analysis

The data was analyzed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). A two-way analysis of variance (ANOVA) using a general linear model was applied to test the effects of maturity and drought on seed germination and seedling growth indexes. Tukey's test, a single-step multiple comparison procedure, were performed to determine significant (P<0.05) differences between treatments. An independent sample t-test was used to compare the difference between seed germination and seedling growth in brown- and yellow-coated seeds.

RESULTS AND DISCUSSION

Seed maturity effect

The seed germination percentage, seed germination index, seedling vigour index and radicle length of white clover were affected by the maturity main factor (Table 1). Under the control, light drought, moderate drought and severe drought conditions, the germination percentage of yellow-coated seeds were 9%, 14%, 32% and 23% higher than corresponding brown-coated seeds (Fig 2), the germination index was 19%, 27%, 26% and 15% higher than brown-coated seeds, respectively (Fig 3). Additionally, under the control, light drought and moderate drought conditions, the seedling

Table 1: Two-way ANOVA for effects of drought stress (D), seed coat colour (C) and their interactions on seed germination and seedling growth of white clover.

	Germination percentage		Germination index		Seedling vigor index		Radicle length	
	df	*F*	df	*F*	df	*F*	df	*F*
Color (C)	1	202.9***	1	127.3***	1	0.19***	1	0.14***
Drought (D)	3	5673.6***	3	2327.4***	2	227.29***	2	3.46***
C×D	3	19.6***	3	21.5***	2	11.93***	2	12.89***

***P<0.001.

vigour index of yellow-coated seeds was 13%, 28% and 28% higher than that of brown-coated seeds (Fig 4), the radicle length was 18%, 11% and 8% higher than that of brown-coated seeds, respectively (Fig 5).

The yellow-coated seeds had considerably higher germination success rate and growth ability than brown-coated seeds in white clover. The results did not consistent to the results found in studies of the cowpea (Marwanto, 2004), pea (Atak *et al*. 2008) and other legumes, which concluded that the darker the colour, the higher the seed vigour (Velijević *et al*. 2017). Several previous researches documented that, delayed harvest could largely decrease seed germination success and subsequent seedling growth, which results from reduction in seed protein percentage (Darby and Lauer, 2002; Gaile, 2008; Sallam and Ibrahim, 2014). In white clover, the blooming stage usually lasting three months in growing season. Thus the individuals flowered early or later could largely functioning to seed maturation at harvest time. So, in the first, owing to non-uniformed flowering time, seeds with different coat colour results a big different in protein and other nutrients content, then made the performance in germination of the brown-coated seeds worse than yellow-coated seeds. Secondly, the color of the seed coat is perhaps related to other factors like genetics. In some species like red clover, a previous research reported that the seed coat colour is determined by two loci. The yellow-coated seed is homozygous and recessive at both loci. The light purple seed is heterozygote and dominant at two loci, but the purple seed is homozygous and dominant at either loci (Bortnem and Boe, 2003). There are no direct evidences to support this probably reason in white clover. However, genetic factor is potentially affects the seed coat colour, as well as both seed germination and seedling growth.

Drought effect

The seed germination percentage, seed germination index, seedling vigour index and radicle length of white clover were affected by the drought main factor (Table 1). In contrast to control, the light drought, moderate drought and severe drought conditions were significantly reduced seed germination percentage by 10%, 53% and 96% in yellow-coated seeds, was reduced by 14%, 61% and 96% in brown-coated seeds, respectively (Fig 2); the seed germination index was significantly reduced by 23%, 65% and 87% in yellow-coated seeds, was reduced by 28%, 64% and 86% in brown-coated seeds, respectively (Fig 3). Meanwhile, as compared to each control, the light drought and moderate drought conditions were significantly decreased seedling vigour index of yellow-coated seeds by 18% and 66%, were reduced brown-coated seeds by 27% and 70%, respectively (Fig 4). Furthermore, the radicle length of yellow-coated seeds was significantly reduced by 24% and 20%, respectively. The reduction in radicle length was greater in brown-coated seeds (Fig 5).

Introduced drought condition, no matter with intensity, all negatively impacts both germination and subsequent

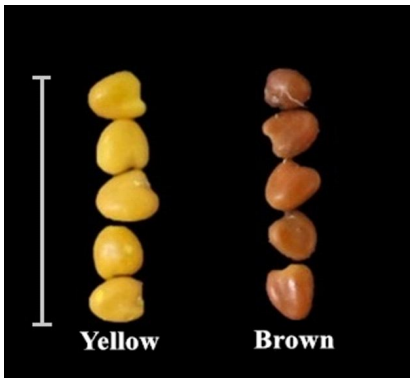

Fig 1: Seeds were used in the experiment. Bar represents the 0.5 cm in length.

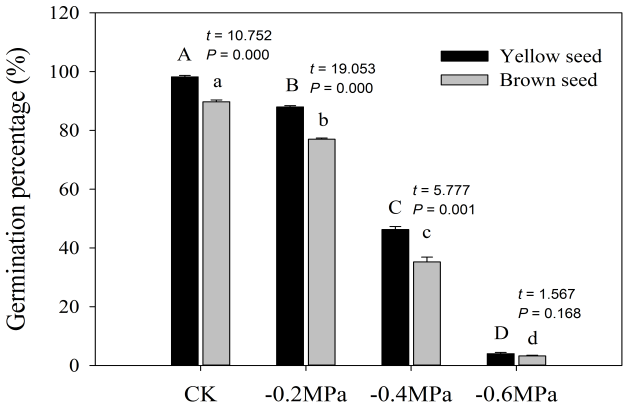

Fig 2: Germination percentage in relation to seed coat colour and drought intensity. The data are presented as the mean ±1 SE. *P*-value and *t*-value marked on the picture indicate that there was a significant difference between yellow seeds and brown seeds. Uppercase and lowercase letters indicated significantly differences ($P<0.05$) between drought intensity under seed with yellow and brown coat colour, separately. CK=Control.

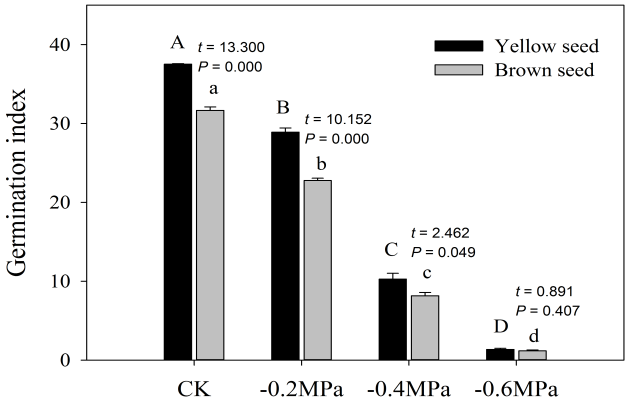

Fig 3: Germination index in relation to seed coat colour and drought intensity. The data are presented as the mean ±1 SE. *P*-value and *t*-value marked on the picture indicate that there was a significant difference between yellow seeds and brown seeds. Uppercase and lowercase letters indicated significantly differences ($P<0.05$) between drought intensity under seed with yellow and brown coat colour, separately. CK= Control.

seedling growth. It is also did not consistent with previous results in other species, which light drought normally improve seed germination and subsequent seedling growth (Ao *et al*. 2019). It is seems because of the sensitivity of white clover seeds to drought much higher than other species like grass. Therefore, any great stress could affect seed germination and seedling growth ability of white clover. Note that, under the same drought intensity, the influence of drought stress on yellow-coated seeds was much lighter than brown-coated seeds, which may also link low protein percentage in darker-coated seeds (Marwanto, 2004; Sallam and Ibrahim, 2014). Drought together with low protein content limits the water absorb, thus made the maturity × drought interaction has a significance effect on germination percentage, germination index, seedling vigor index and radicle length (Table 1). The results suggest that, due to white clover seeds sensitive to drought, in new stands establishment, the farmers should adopt more efficiency irrigation management to improve seed germination and seedling survival during their early growth stage.

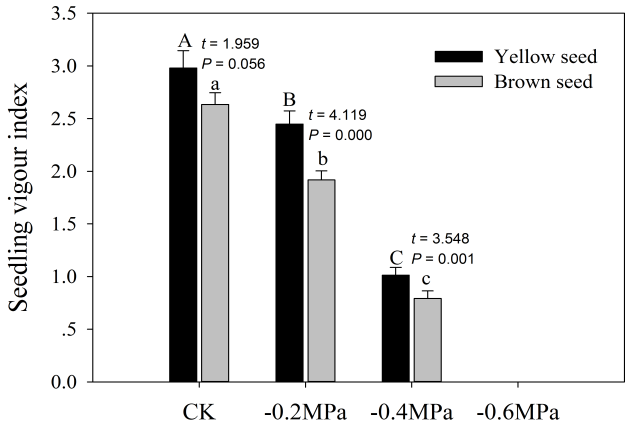

Fig 4: Seedling vigour index in relation to seed coat colour and drought intensity. The data are presented as the mean ±1 SE. *P*-value and *t*-value marked on the picture indicate that there was a significant difference between yellow seeds and brown seeds. Uppercase and lowercase letters indicated significantly differences (*P*<0.05) between drought intensity under seed with yellow and brown coat colour, separately. CK=Control.

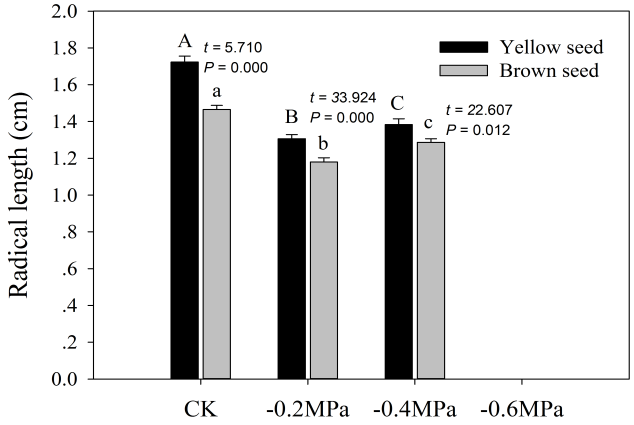

Fig 5: radicle length in relation to seed coat colour and drought intensity. The data are presented as the mean ±1 SE. *P*-value and *t*-value marked on the picture indicate that there was a significant difference between yellow seeds and brown seeds. Uppercase and lowercase letters indicated significantly differences (*P*<0.05) between drought intensity under seed with yellow and brown coat colour, separately. CK=Control.

Summaries, the germination performance of yellow-coated seeds is superior to brown-coated seeds. In agronomic practices, the colour of seed coats can be considered as an important indicator to determine the quality and optimal harvest time of white clover seeds. Farmers should select a harvest time when the most seeds are yellow-coated to improve quality of seeds. This process avoids spending excess time determining the internal physiological index, is useful to harvest crop of white clovers for seed purpose.

CONCLUSION

This experiment found that, 1): Yellow-coated seeds outperformed brown-coated seeds. 2): As drought conditions intensified, the difference of germination and early seedling growth did not increase between brown- and yellow-coated seeds. In agronomic practice, farmers should select a harvest period when white clovers have the highest proportion of yellow-coated seeds in the sample as an index of harvest crop of clovers for seed purpose.

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