EFFECTS OF DIASPORE BRACTS AND CATIONS ON GERMINATION OF *CERATOIDES ARBORESCENS* (LOSINSK.) TSIEN ET C.G. MA UNDER *IN VITRO* CULTURE

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Abstract

Ceratoides arborescens (Losinsk.) Tsien et C. G. Ma, a species endemic to China, is widely used in ecological restoration and as fine forage due to its high drought resistance and nutrient content, respectively. Its bracts are often removed from diaspores during planting because their dense hair covering is not conducive to sowing. However, it is not clear whether the removal of bracts is beneficial to *C. arborescens* seed germination. A seed germination experiment was conducted to measure how bracts influence seed germination. Three diaspore treatments were used: seed bracts removed (BR), bracts removed and recombined with seeds (BRB); and seed bracts not removed (B). To further explore whether bract nutrient content affects seed germination, seeds without bracts (BR) were tested with additions of three different nutrient (Mn, Ca, and Fe) in a culture solution. It was found that BR accelerated average germination time but had no detectable effect on seed germination rate. Further, the BR significantly decreased seed germination potential and germination index. Additionally, seed germination potential and the germination index were improved by BRB, while the seedling vigor index was also enhanced. Moreover, it was found that the elements Ca, Mn, and Fe were higher in bracts than those in seeds. However, nutrient addition manipulation showed that the addition of Mn promoted seed germination. The findings of this experiment suggest that *C. arborescens* bracts are beneficial during planting, because they contain essential elements for germination. Removing bracts from the diaspore has no potential impact on the reduction of costs and improvement of the seed germination characters of *C. arborescens*.

Introduction

Ceratoides arborescens (Losinsk.) Tsien et C. G. Ma, a perennial sub shrub, is endemic to China's sandy grasslands and deserts. It is an important grassland restoration species because of its high drought tolerance (Tong *et al.* 2010), and is used for the supplementary feeding to grazing livestock as it is nutrient-rich (Aalata *et al.* 2012). Its diaspore comprises 2 united, sub tending bracts (dead layer) that are covered in white, 2 to 6 mm long hairs. Within the bracts is a pubescent-covered utricle which encloses a seed (Fig. 1). The dense hairs covering the bracts make it difficult to sow *C. arborescens* seeds. In order to improve sowing efficiency, bracts are often detached from the seed, which may increase planting costs, and potentially reduce the survival rate of seedlings (Sun *et al.* 2010). The impact of removing bracts on seed germination is not well understood. Therefore, the role of bracts in *C. arborescens* seed on germination was studied . Also effect of different nutrients was observed.

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The dead coverings of seeds from different species are very diverse and reflect the adaptive characteristics of seed dispersion in a variety of different environments (Webb *et al.* 2009, Wang *et al.* 2019). Previous studies have exported the effect of dead layers on seed germination was species-specific, and showed that dead coverings could increase (Ueno and Miyoshi 2005), decrease (Fandrich and Mallory-Smith 2006), or have no influence on seed germination characters. These positive effects on seed performance and survival may be attributed to the rich storage of nutrients, such as potassium, calcium, phosphorus, and sulfur in dead layers, which provide an immediate nutritional supply for germinating seeds (Booth 1989, Raviv *et al.* 2018).

In the present study, seed germination experiments was designed to investigate the impact of bracts, and the nutrients in bracts, on seed germination. Specifically, to address the following questions: (1) How do bracts influence seed germination characters? (2) How do the elements stored in bracts impact germination characters?

Fig.1. *Ceratoides arborescens* diaspore.

Materials and Methods

Diaspores of *C. arborescens* were collected from the desert steppe of Siziwang Banner, Inner Mongolia, China, in October 2020. Dry seeds were stored at 4°C before being used in the germination experiment.

Twelve, 50 capsule samples were randomly selected from a thoroughly mixed collection of mature diaspores. The samples were sterilized by 5% sodium hypochlorite, then rinsed 3–5 times with distilled water. Four samples were individually threshed, and the bracts were removed (BR). Another four samples were individually threshed, and the bracts were recombined with the seeds (BRB). The remaining four samples were not threshed, and the seeds and bracts remained intact (B). Germination was conducted in 90 mm Petri dishes lined with filter paper and filled with 6 ml of distilled water. distilled water (1 ml) was added to each Petri dish daily. Four replicate dishes, each with 50 seeds, were used for each treatment. The dishes were incubated in programmed growth chambers adjusted for 16 hrs dark and 8 hrs light cycles, at 25°C. A seed was considered to have germinated when radicle emergence reached 2 mm in length. Germination was inspected daily for a total of seven days. The length of the hypocotyl and radicle of each seedling was measured 7 days after emergence, and average length was calculated as the repeated measurement value (Ranal and Santana 2006).

The germination characters of the three treatments were calculated as follows:

(1) Germination rate = Final normal seedling number / Number of seeds tested × 100

2) Average germination day: MGT = $\sum$(gd·d) / D, where gd is the number of seeds germinated on day d, and D is the total number of germinated seeds in each Petri dish

3) Germination potential = Maximum germination number / Number of seeds tested $\times$ 100 (where the maximum germination number is the maximum number of seeds germinated on any day during the test period)

4) Germination index: Gi = Σ (Gt / Dt), where GT is the number of germinations on day t, and Dt is the corresponding germination days

5) Seedling vigor index: Vi = Gi $\times$ L, where L is seedling length

Three 5 g samples were randomly selected from a thoroughly mixed collection of diaspores. The samples were individually threshed, and bracts and seeds were separated before being analyzed for cations. This involved briefly placing the samples into an oven at 65°C and, once dried to constant weight, grinding them with a high-throughput tissue grinder (SCIENTZ-192, Ningboxinzhi). Approximately 0.5 g of each ground seed and bract sample was digested in 4 ml of HNO_3 at 37°C for 3 d. Samples were subsequently centrifuged (5430 R, Eppendorf) at 4,000 rpm for 20 min, and the supernatant was collected to determine its mineral element content using a TXRF spectrometer (S4 T-STAR, Bruker) (Hönicke *et al.* 2020).

To explore the effects of bract nutrients on seed germination, mineral contents which occurred at significantly higher levels in the bracts compared to the seeds of *C. arborescens* were determined.

Four 50 capsule samples were randomly selected from a thoroughly mixed collection of diaspores, and the samples were individually threshed (BR). A solution with the same concentration of cations contained in *C. arborescens* bracts (Mn 298 mg/kg, Fe 1.8 mg/kg, and Ca 12 mg/kg) was added to treat BR. A weighing method was used to check that the distilled water added to the Petri dishes did not alter the osmotic potential of the solution. All the other aspects of the germination assay were the same as for treatment BRB.

A one-way ANOVA analysis followed by multiple comparisons through the Duncan test was used to compare the response differences in seed germination characters among the three treatments. The differences between the mineral element content of seeds and bracts were measured using paired samples *t* tests. These statistical analyses were performed in R version 3.5.3 (R Core Team 2018). Treatment effects were considered to be statistically significant at p < 0.05.

Results and Discussion

Bract removal prompted seed germination, thus increasing seed germination potential and the germination index, while it did not influence the seed germination rate (Table 1). Compared to seed with bracts (B), seed germination was 1.12 and 1.34 days ahead for treatments BR and BRB, respectively. However, seedling vigor only significantly increased for treatment BRB (p < 0.001).

There were seven elements which showed a significant difference in content between the seeds and bracts of *C. arborescens*. The phosphorus, barium, sulfur, and zinc content of seeds was higher than that of bracts. Whereas the Ca, Mn, and Fe content of *C. arborescens* bracts were 9.2, 4.7, and 8.5 times higher than that of seeds, respectively. There was no difference in copper, mercury, potassium, nickel, silver, and chlorine content between bracts and seeds (Table 2).

Effects of cation addition on seed germination varied between elements. The addition of Mn ions increased the seed germination rate and seedling vigor index of *C. arborescens* (Fig. 2). The addition of Ca ions did not affect the seed germination rate, average germination days, germination potential, and seedling vigor index of *C. arborescens* (Fig. 3). The addition of Fe ions increased average germination days but reduced the *C. arborescens* germination potential and seedling vigor index (Fig. 4).

Table 1. The effect of *C. arborescens* bracts on seed germination.

Treatment[*]	N	Seed germination rate	Seed average germination day (d)	Germination potential	Germination index	Seedling vigor index
B	4	0.41±0.046 a	2.48±0.17 a	0.19±0.013 b	11±1.35 b	23.21±3.06 b
BR	4	0.44±0.015 a	1.36±0.07 b	0.33±0.026 a	19±1.08 a	22.06±1.34 b
BRB	4	0.45±0.017 a	1.14±0.03 b	0.41±0.026 a	21±0.95 a	39.79±1.92 a

Values are mean ± SE. [*]B: seed with bracts; BR: bracts removed; BRB: bracts removed then recombined with seeds.

Table 2. Element content of *C. arborescens* seeds and bracts.

Cation nutrients	N	Content (mg/kg)		P-value	Remark
		bract	seed		
Calcium	3	298.845	31.722	<0.001	bracts > seed
Manganese	3	1.876	0.394	<0.001	
Iron	3	12.259	1.445	0.002	
Phosphorus	3	Not detected	475.385	<0.001	seed > bracts
Barium	3	Not detected	21.831	0.012	
Zinc	3	0.806	1.355	0.0026	
Sulfur	3	227.981	338.120	0.047	
Copper	3	0.063	0.149	0.088	No significant difference between seeds and bracts
Mercury	3	2.533	0.506	0.249	
Potassium	3	1923.634	1079.157	0.324	
Nickel	3	0.029	0.020	0.355	
Silver	3	1.397	1.549	0.865	
Chlorine	3	27.764	27.037	0.948	

Bracts are commonly considered a passive barrier for embryo protection and a means for seed dispersal (Grafi 2020). In the present study the previously unrecognized role of dead bracts in regulating seed germination and seedling establishment was studied. Results showed that bracts delayed seed germination but did not change the germination rate. This might be that bracts and their accessories inhibit seed water absorption (Cousens *et al.* 2010), gaseous exchange (Adkins *et al.* 2002, Zhang *et al.* 2012), and radicle protrusion (Miyajima 1996), which slows down seed germination. It was also found that bracts enhanced the seedling vitality index. This finding is supported by Grafi (2020) who demonstrated that intact dispersal units can improve the seedling establishment rate compared to naked seeds. Previous studies reported that for grasses, such as *Brachypodium hybridum* Catalan (El-Keblawy *et al.* 2019), *Festuca rubra* L. (Bülow-Olsen 1983), and *Lolium perenne* L. (Brown 1931), the main role of husks is to enhance seedling growth. Similarly, the pericarps of pea plants (*Pisum sativum* L.) possess nutritional and antioxidant compounds that are further enhanced by plant growth-promoting microbes (Jain *et al.* 2015). Raviv *et al.* (2017) suggested that storing nutrients within the dead organs enclosing embryos is a general theme of plant reproductive biology that has evolved to increase germination success and

seedling establishment. Thus, dead organs that enclose seeds/caryopses might serve as an immediate nutritional supply during germination.
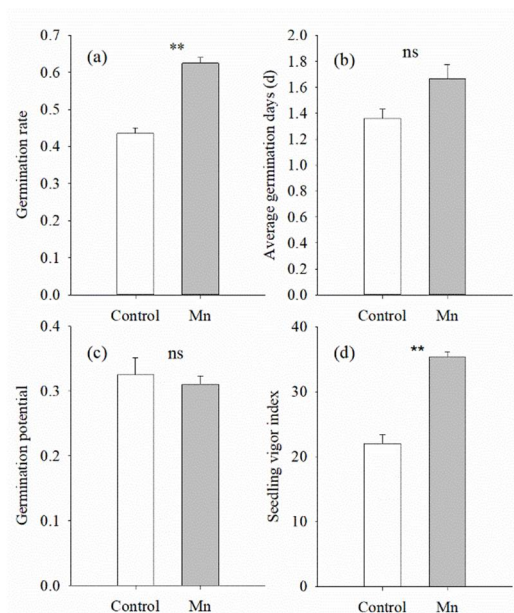

Fig. 2. Effect of Mn ions on the (a) germination rate, (b) average germination days, (c) germination potential, and (d) seedling vigor index of *C. arborescens* seeds.
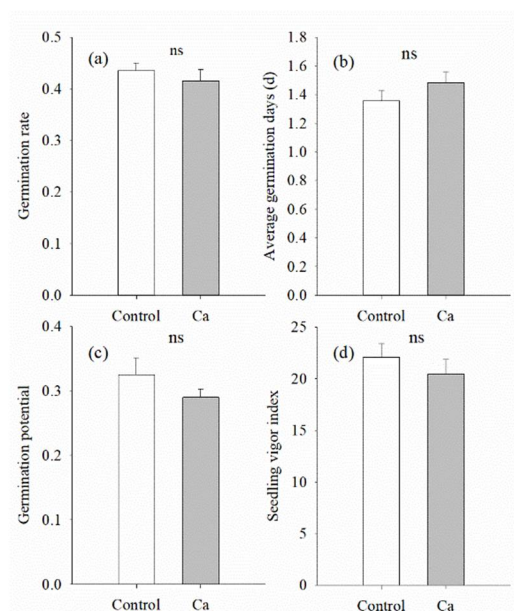

Fig. 3. Effect of Ca ions on the (a) germination rate, (b) average germination days, (c) germination potential, and (d) seedling vigor index of *C. arborescens* seeds.
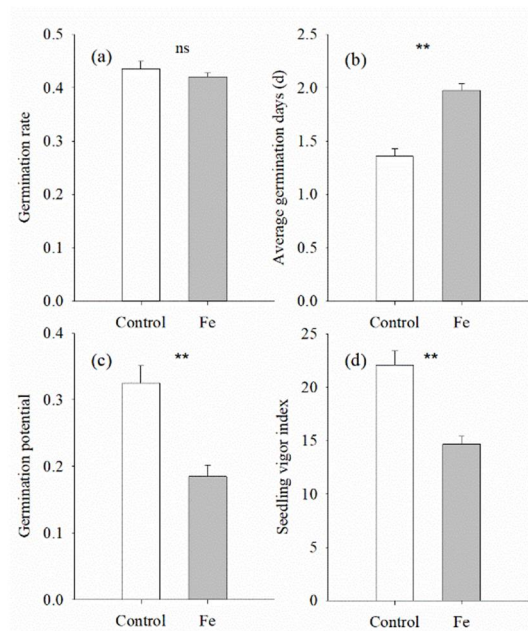
Fig. 4. Effect of Fe ions on the (a) germination rate, (b) average germination days, (c) germinate potential, and (d) seedling vigor index of *C. arborescens* seeds.

Results showed that the mineral element content of seeds and bracts is different, with higher quantities of Ca, Mn, and Fe present in bracts. Moreover, the mineral element content of bracts plays a role in seed germination. Notably, bracts consist of multiple minor elements that are involved in germination and seedling development (Booth 1989).The findings also showed that Mn ion addition can increase the germination rate and germination index of *C. arborescens*. This observation is more or less similar to the findings of a study on lettuce (*Lactuca sativa* L.) reported by Liu *et al.* (2016). Zhou *et al.* (2003) have widely reported that manganese seed soaking treatment can increase the germination rate and vitality index of *Glycine max* (L) Merrill seeds. Although it has been demonstrated that the application of Mn can improve the germination of plants (Das *et al.* 2015), its effectiveness is determined by the concentration, form, or oxidation state of Mn (Socha and Guerinot 2014). It might be that low manganese concentration activates the enzyme system inside the seed, promotes metabolic activity, and provides suitable conditions for breaking dormancy, thereby, promoting seed germination (Zhang 2004).

Calcium ion addition had no significant effect on germination for naked seeds of *C. arborescens*. Hepler (2005) observed that calcium plays an important role in the processes of plant growth and development. However, other studies have showed that excessive calcium ions have an inhibitory effect on the germination of species, such as *Sophora tonkinensis* Gagnep. and *Raphanus sativus* L. (White and Broadley 2003, Cai and Gao 2011, Tan and Ma 2017). Some plants resist calcium ion stress by accumulating calcium ions, and transporting them via the cytosol to the vacuole, endoplasmic reticulum, mitochondria, plastids, and cell walls.(Harper 2001, Volk *et al.* 2004). *Lysionotus pauciflorus* Maxim. accumulates calcium ions in its palisade and spongy tissues to deal with calcium stress (Li *et al.* 2014). Islam and Kawasaki (2014) found that excess calcium ions are stored in the form of calcium oxalate crystals to deal with calcium stress.

Fe ion addition reduced the seed germination potential, germination index, and seedling vigor index of *C. arborescens* (Fig.4). This result is consistent with the resultsreported by Zheng who found excessive iron inhibits the growth of seed radicles (Zheng et al. 2009) . Excess iron leads to ectopic binding of metals to proteins, thus disturbing seed germination (Yang *et al.* 2006). However, the addition of iron has been found to increase the germination rate of rice and *Panicum virgatum* L. seeds (Sun 2021). This might be related to the iron absorption mechanism of gramineous plants (Roberts *et al.* 2004). Iron uptake occurs through different mechanisms in grasses and nongraminaceous monocots (Curie and Briat 2003). AtNRAMP3 and AtNRAMP4 participate specifically in Fe mobilization from vacuolar metal stores during seed germination (Lanquar *et al.* 2005).

Common agricultural practices for enhancing seed performance include the application of chemicals (coating) to protect germinating seeds from pathogens and adding substances that increase germination success to these coatings. The findings of this experiment suggest that the addition of bracts can enhance the seedling vitality of *C. arborescens*, even though diaspores with bracts are more difficult to sow. Storage of mineral elements within the dead bracts that enclose embryos appear to be a general theme in plant reproductive biology, providing embryos with nutrition for increasing successful seedling establishment. In particular, bracts have a relatively high content of Mn ions that can promote *C. arborescens* germination.

Acknowledgements

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