Dimorphic fruit color is associated with differences in germination of Calligonum comosum

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Dimorphic fruit color is associated with differences in germination of *Calligonum comosum*

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**Running Head:** Dimorphic fruit color of *Calligonum comosum*

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Abstract

Calligonum comosum L'Hér is a perennial evergreen shrub that produces dimorphic fruits of distinctly yellow or red color. The species is found on sand dunes of the Middle East, is valued as a highly palatable livestock feed that survives extreme drought and is suitable for desert restoration of non-saline sandy soils. The association of fruit color with germination was assessed in laboratory conditions under two temperature (15/20 and 20/30°C) and two light (0 and 12 hours per day) treatments. Additionally, the association of fruit color with emergence and seedling growth up to 90 days from sowing was assessed in shade house conditions. Germination percentage in the laboratory was the only variable that exhibited significant variation by seed color. Germination was highest for red seeds grown in the dark at the higher temperature but the influence of seed color on germination was not repeated in the shade house study. The dimorphic fruit color of C. comosum is thus associated with differing germination rates but further study is needed to determine if morphs employ differing recruitment strategies.

Keywords: Germination; arid desert; heteromorphism; seedling survival; Polygonaceae
Introduction

Seed heteromorphism is common among plant species adapted to challenging environmental conditions (Imbert 2002) and is found in 87% of species adapted to arid and semi-arid conditions (Wang et al. 2010). Seed heteromorphism may be expressed as a difference in size, mass, shape, color or dispersal structure and can be linked to differences in dormancy (Childs et al. 2010), dispersal capacity (Imbert 1999), seed bank persistence (Cao et al. 2012), viability during storage (Bhatt and Santo 2017) and germination behavior (Bhatt and Santo 2016; Bhatt et al. 2017a, b; Bhatt and Pérez-García 2018). Heteromorphism is believed to be associated with recruitment success in unpredictable environments via each morph exhibiting a different recruitment strategy.

*Calligonum comosum* L'Hér (Polygonaceae) is a self-pollinating perennial evergreen shrub with fruits that are morphologically adapted for wind dispersal (Koller 1956; Dhief et al. 2012). Fruit color is dimorphic, being either red or greenish yellow (Mandaville 1990). Self-pollination is believed to be a means of reproductive assurance under pollination-uncertain environments, such as desert habitats (Shivanna 2015). Production of color in flowers and fruits is metabolically costly (Lev-Yadun and Gould 2009) but attracts pollinators and seed dispersing agents, thus improving reproductive success for plant species that use these vectors (Carlson and Holsinger 2010). However, flower and fruit color polymorphism has been reported in other wind dispersed and/or pollinated desert species such as *Haloxylon salicornicum*, *Salsola rubescens* and *S. vermiculata* (El-Keblawy et al. 2013; Bhatt et al. 2017a, b). These studies reported that both morphological and physiological heteromorphism exist in seeds of these species, and correspond to differences in germination requirements.

*Calligonum comosum* is a highly palatable desert species (Heneidy 1996) used for forage, medicine, fuel, dune stabilization and to boost soil organic matter content (Tao 2000; Ren 2001), found in Northeast Africa, Egypt, Sinai, Palestine, Middle east and Pakistan (Taia and El-Etaby 2006). The species is tolerant to severe water stress but intolerant to salinity (Mao and Pan 1986; Zhang 1992; Western 1989). Water stress adaptations include the discarding of leaves and entire branches during periods of high stress (Dhief et al. 2009) and morphological modification of the wood (Al-Khalifah et al. 2006). This species is phenotypically highly variable (Taia and El-Etaby 2006; Taia and Moussa 2011) with a macromorphology that ranges from a shrub to a small tree (Western
Species of the genus have recently diverged (Li et al. 2014), resulting in some authors referring to the taxon at the species (*C. comosum*) and others at the sub species (*C. polygonoides* subsp *comosum*) level.

Previous studies of the species have examined the effects of temperature, dormancy breaking treatments and salinity on seed germination and seedling growth (Bahrani and Kazempour 2007; Dashti et al. 2011; Al-Otaibi and Ebid 2015). However, to the best of our knowledge the effect of seed dimorphism on germination and seedling survival has not yet been investigated. The aims of the present study were to assess whether fruit color dimorphism affects seed germination, seedling survival or growth. We expected that seed color would influence germination response (amount and time) under shade house and laboratory conditions. Additionally, we expected that seedling survival and growth would differ between red and yellow seeds.

**Materials and methods**

**Seed collection**

Mature *C. comosum* yellow and red fruits (hereafter referred to as seeds) (Fig. 1) were collected during the last week of April 2017 from Sulaibiya, Kuwait (29°9'45.88''N; 47°39'39.66''E). Each plant produced seeds of one morph only and approximately 80% of the plants of the population sampled produced yellow seeds. Geographic distribution of morphs within the site appeared random though this was not tested. The seeds of both colors matured and dispersed at the same time. Seeds of each color were collected, kept in two separate bags, and pooled from 15 plants that had been selected to ensure maximum genetic variation within the population, the selected plants were at least 2-3 m apart from each other. Soils were collected randomly from five different points of seed collection site at a depth of 0-25 cm and mixed together. Seed mass of each color was determined by weighing three replicates of 25 seeds each at the time of seed collection.

**Laboratory germination**

Seeds of each color morph were germinated one week after collection using four replicates of 25 seeds for each of two temperature (12/12 hours of 15/20 and 20/30°C) and two light (0 and 12 hours of light per day - with a light intensity of 200 μmol/m²/s) treatments. Each 25-seed replicate was placed in one petri dish, which was placed in one of two incubators. One incubator was set at 15°C / dark for 12 hours and 20°C / light for the other 12 hours,
and the other incubator at 20°C / dark and 30°C / light. Petri dishes of seeds undergoing dark (0 hours light per day) treatment were wrapped in aluminium foil before placing them in the incubators and germination was assessed at the conclusion of the 28-day trial. Germination of light (12 hours per day) treatment seeds was recorded daily for 28 days with germination being defined as the emergence of a radical by at least 2 mm.

Shade house emergence and growth

Four replicates of 50 seeds of each color morph were individually sown during the first week of May (2017) in 8 cm diameter plastic pots at a depth of 0.3 cm and were placed in a shade house. The local soil (collected at the provenance of the seeds) was used to fill the pots and pots were given 50 ml water every second day. Emergent seedlings were counted every second day for 28 days. Surviving seedlings (after 28 days) were individually transplanted to 15 cm diameter plastic pots. Seedling survival was evaluated 28 (n=200 for each seed color) and 90 (n=88 and n=106 for yellow and red seeds, respectively) days after sowing. After 90 days, ten seedlings were randomly selected from each color morph and assessed for length of shoots and roots then the seedlings were oven dried at 80 °C to constant weight and mass of shoots and roots was determined. Shade house climatic conditions were recorded with a Thermo-Hygrometer (Electronic Temperature Instrument Ltd, UK). Average daily minimum / maximum temperatures for May, June and July were 27.6 / 40.7, 30.4 / 44.7 and 32.9 / 47.5°C and relative humidity was 12.4 / 30.4, 8.0 / 19.1 and 9.0 / 34.1%.

Data analysis

A t-test was used to compare the mass of yellow and red seeds. A generalized linear model (GLM) with binomial error structures was used to analyse the germination under lab conditions as a function of seed colour, light condition (cycles of light and darkness vs. complete darkness) and temperature of incubation (15/20°C and 20/30°C). The emergence recorded in the shade house was also analysed with GLM with binomial error structures with seed colour as the only explanatory variable.

Mean germination time was calculated following the formula: MGT = ΣDN / ΣN; where D is the number of days counted from the date of sowing and N is the number of seeds germinated on day D (Ellis and Roberts 1981).
Mean germination time (MGT) under lab conditions and mean emergence time (MET) under shade house conditions were analysed with GLM with Gaussian error structures. Mean germination time (MGT) under lab conditions was analysed as a function of seed colour and temperature of incubation. The effect of light was not considered since MGT was not assessed for seeds incubated in darkness. Mean emergence time under shade house was analysed as a function of seed colour. The growth variables were analysed with GLM with Gaussian error structures and seed colour as explanatory variable. The MGT and growth variables were log transformed to achieve normality and homogeneity of variance prior the analysis. For each variable analysed, first the full model was fitted (all the factors and the interactions included), next a model simplification was achieved by dropping one non-significant interaction and explanatory variable at a time. Following Zuur et al. (2009), Chi square tests were performed each time a variable was dropped to ensure the model fit did not significantly decrease. All the statistical analysis were performed using R version 3.5.0 (R Core Team, 2018).

Results

Germination and mean germination time under lab and shade house conditions

The average seed mass was 37.11 mg (±0.415 standard error) for yellow seeds and 43.87 mg (±2.021 standard error) for red seeds. The seed mass did not vary significantly as a function of seed color (t = 3.277, df = 2.168, p-value = 0.073). Germination under lab conditions was only significantly affected by individual factors (i.e.; seed colour, light and temperature of incubation) and not by their interactions (Table 1). Germination was 34.5% and 24.5% for red and yellow seeds, respectively. Additionally, germination decreased from 33% to 26% from the complete darkness treatment to the cycles of light and darkness treatment. Finally, the germination increased with increasing temperature from 26.6% to 32.3% under the 15/20°C and the 20/30°C treatment, respectively (Table 1 and Fig. 2). Mean germination time was not significantly influenced by any of the individual factors analysed (seed colour and temperature) or their interaction (Table 1). Emergence percentage and the mean emergence time under shade house conditions were not significantly influenced by the seed colour (Table 1 and Fig. 2).
Seedling survival and growth under shade house conditions

Seedling survival after transplant was very similar for both seed morphs. Seedlings produced by yellow seeds exhibited a survival of 75% and seedlings from red seeds 74%. No growth variables analysed in this study were influenced by seed colour (Table 2).

Discussion

The present study suggests that seed color dimorphism in *C. comosum* is associated with germination success. The association between seed color morph and germination / emergence differed in the two experiments. Germination percentage was strongly influenced by seed color, temperature and light conditions in the laboratory study but the influence of seed color was not repeated in the shade house study, where overall emergence was double. The higher emergence recorded in the shade house might be due to conditions being slightly closer to natural conditions, in that the trial was performed in local soil, at higher absolute temperatures and at higher diurnal temperature variation (Ooi et al 2012; Baskin and Baskin 2014) than the conditions in the laboratory trial. The lack of differences between emergence of red and yellow seeds in the shade house might be linked to the leaching of inhibitory chemicals under regular watering, which might thus eliminate differences between morphs. However, other species in the genus typically exhibit physical rather than chemical dormancy (Tao et al. 2000; Ren and Tao 2004), thus further study is needed to determine if this species differs.

The mechanism causing variation of germination according to seed color is, to the best of our knowledge, not known. Anatomically different seeds may differ in their water uptake and oxygen diffusion (Powell et al. 1986; Baskin et al. 2000; Atis et al. 2011) as has been reported in other heteromorphic species (Lu et al. 2010; Yao et al. 2010). The higher temperature of 20/30°C increased germination percentages of both morphs in the laboratory trial. This temperature corresponds to typical field conditions of Kuwait in November and March (Omar et al. 2007) but seed germination (emergence) was significantly higher in shade house, which might be due to higher temperatures in the shade house that increase germination.

The photo inhibition of germination demonstrated in the laboratory trial supports findings of previous experiments on *C. comosum* (Al-Otaibi and Ebid 2015) and other species adapted to sand habitats (Zheng et al. 2005; Lai et al. 2010). Seed burial is common in sand habitats, particularly in shifting dunes, resulting in darkness. The germination difference among morphs might be due to photo inhibition by the darker seed coat of red seeds.
compared with yellow seeds. Darker seeds of *Trifolium resupinatum* and *Cyamopsis tetragonoloba* have been reported to exhibit greater germination (Liu et al. 2007; Atis et al. 2011). Maternal environmental conditions can influence seed heterogeneity (Wright et al. 1999; Luzuriaga et al. 2006) but seeds of the present study were collected at the same location and time. However, micro-environmental variation may play a role but if it does, the mechanism is unknown.

Mean germination time was unaffected by seed color, supporting previous studies that showed no link between this variable and either the color or size of seeds (Valencia-Díaz et al. 2015; Bhatt and Pérez-García 2018). Seedlings did not differ among morphs in shoot or root mass or in shoot or root length which is consistent with observations from other heteromorphic species such as *Heterotheca latifolia* and *Leontodon saxatilis* (Venable and Levin 1985; Brandel 2007). These findings indicate that the influence of seed color is limited to the first stages of recruitment (germination) with no effect on subsequent stages (survival and growth). In conclusion, the dimorphic fruit color of *C. comosum* is associated with differing germination rates. However, additional studies that include broader genotypic sampling and a wider range of environmental conditions will be required to confirm any association between seed color and environment-specific patterns of germination and establishment.

**Acknowledgments**

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**References**


Table 1. Germination and mean germination time under laboratory and shade house conditions.

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<thead>
<tr>
<th>Variables</th>
<th>Factors</th>
<th>N</th>
<th>LRT/scaled dev.</th>
<th>Pr(&gt;Chi)</th>
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<tr>
<td>Germination - laboratory conditions</td>
<td>Colour</td>
<td>200</td>
<td>9.760</td>
<td>0.002 **</td>
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<td></td>
<td>Light</td>
<td></td>
<td>4.803</td>
<td>0.0284 *</td>
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<td></td>
<td>Temperature</td>
<td></td>
<td>4.144</td>
<td>0.0418 *</td>
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<td>0.008</td>
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<td></td>
<td>Temperature</td>
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<tr>
<td></td>
<td>Colour*Temperature</td>
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<td>400</td>
<td>1.229</td>
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<td>0.187</td>
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Significance codes: n.s.: $P > 0.05$; * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. 
Table 2. Seedling growth variables as a function of seed colour

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<thead>
<tr>
<th>Growth variables</th>
<th>scaled dev.</th>
<th>Pr(&gt;Chi)</th>
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<td>Root length</td>
<td>4.991</td>
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<td>Root fresh weight</td>
<td>0.595</td>
<td>0.440 n.s.</td>
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<tr>
<td>Root dry weight</td>
<td>1.209</td>
<td>0.271 n.s.</td>
</tr>
<tr>
<td>Shoot length</td>
<td>0.006</td>
<td>0.939 n.s.</td>
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<tr>
<td>shoot fresh weight</td>
<td>3.78E-05</td>
<td>0.995 n.s.</td>
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<tr>
<td>shoot dry weight</td>
<td>0.107</td>
<td>0.744 n.s.</td>
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*Bonferroni corrected p-values n.s.: \( P > 0.0083 \)
Figure Caption

**Fig. 1.** Dimorphic fruit of *C. comosum*; yellow (left) and red (right)

**Fig. 2.** Mean +/- SE of seed mass at time of collection (a), seedling root length 90 days after sowing (b) for each seed color morph, and for significant factors affecting germination percentage in laboratory conditions (c).