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Germination response of Arabian desert species to gibberellic acid and potassium nitrate seed treatment

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ABSTRACT

Desert plant species commonly use seed dormancy to prevent germination during unfavorable environmental conditions and thus increase the probability of seedling survival. Seed dormancy presents a challenge for restoration ecology, particularly in desert species for which our knowledge of dormancy regulation is limited. In the present study the effect of gibberellic acid (GA_3) and potassium nitrate (KNO_3) on seed dormancy release was investigated on eight Arabian desert species. Both treatments significantly enhanced the germination of most species tested. GA_3 was more effective than KNO_3 in enhancing germination percentage, reducing mean germination time and synchronizing the germination in most of the studied species. Light requirement during germination was species-specific, but in general the presence of light promoted germination more effectively when combined with KNO_3 and GA_3. The wide variation in dormancy and germination requirements among the tested species is indicative of distinct germination niches, which might assist their co-existence in similar habitat/environmental conditions. Seed pre-treatments that optimise germination in this habitat must therefore be assessed for individual species to improve the outcomes of ecological restoration.

KEYWORDS: Desert, dormancy, GA_3, KNO_3, germination

Introduction

Seed dormancy contributes to successful plant propagation by limiting germination to times when environmental conditions are favorable for successful establishment (Gremer and Venable 2014; Baskin and Baskin 2014). Seed dormancy is particularly important in hyper-arid deserts, where environmental conditions are rarely suitable for seedling survival at the time of seed dispersal (Bewley 1997; Bhatt et al. 2016). Dormancy is determined genetically and modified by environmental signals. It is classified according to the mechanism that prevents germination (Finch-Savage and Leubner-Metzger 2006). Species adapted to hyper-arid deserts commonly exhibit a physiological mechanism that requires endogenous chemical changes before germination can occur (Baskin and Baskin 2003).

Gibberellic acid (GA_3) and potassium nitrate (KNO_3) are known as efficient chemicals to release physiological seed dormancy (Hartmann et al. 1997; Bao and Zhang 2011; Shen et al. 2012). Gibberellic acid counteracts the effect of abscisic acid, which induces dormancy during seed formation (Kermode 2005) and may persist after maturation (Debeaujon and Koornneef 2000). Abscisic acid stimulates the production of ά-amylase and other hydrolytic enzymes that release soluble sugars and amino acids, enabling the embryo to commence growth (Mayer and Poljakoff-Mayber 1989). Potassium nitrate also stimulates germination but the mechanism is poorly understood. Exogenous nitrate application appears to reduce the light intensity required to trigger germination (Batak et al. 2002) and reduces the abscisic acid concentration within imbibed seeds (Ali-Rachedi et al. 2004).

Dormancy can improve population persistence by ensuring variation of germination timing, thus maintaining a soil seed bank, and also by delaying germination until favorable conditions appear. Delayed germination has greater importance in arid systems with unpredictable timing of precipitation (Tliget al. 2008). For annual species of this habitat, the seed bank is their only living form for most months of the year (Brown 2002). Thus germination events must be timed for favorable conditions across multiple seasons, in case the favorable conditions of one season are insufficient for seedling establishment. However, dormancy type and the requirements for release are species-specific and dependent on phylogeny, geographical distribution, habitat preference and life cycle of the species (Vandelook et al. 2008). In arid desert environments where water availability is the most limiting factor (Schwinning and Kelly 2013), plant growth occurs only during one specific season, usually winter. However, each

species varies in its resource use (e.g., spatiotemporal location of water and nutrients acquired), thus species do not usually acquire resources at levels that are unbearable for co-occurring species (MacArthur and Levins 1967), which assures the existence of a plant community. Thus, inter-specific variation in germination strategies may facilitate species' co-existence by allowing for temporal partitioning of resources and providing a buffer against extinction (Chesson 2000).

Seed dormancy of hyper-arid desert species has been shown to respond to the environmental cues of temperature, moisture and light (Bhatt and Pérez-García 2016; Bhatt et al. 2018; Bhatt and Santo 2018). Temperature influences dormancy release through the deterioration of morphological barriers such as the softening of a hardened seed coat (Kebreab and Murdoch 1999) and influences the timing of germination within a season (Baskin and Baskin 2014). Moisture is normally essential for dormancy release, acting chemically by degrading inhibitory enzymes or facilitating the translocation of enzymes within the seed (Beyranvard et al. 2013). Light can affect germination rate in species that are adapted to be sensitive to seed burial (Karlsson and Milberg 2007).

Seed dormancy is a major obstacle to effective use of native seeds in restoration (Merritt et al.2007; Merritt and Dixon 2011). Up to 90% of seeds used in restoration programs fail to germinate due to the use of dormant seeds (Merritt and Dixon 2011). Therefore, knowledge of effective pretreatments for releasing seed dormancy is important to achieve successful desert re-vegetation (Merritt et al. 2007). Thus, it is necessary to investigate the effect of different dormancy releasing chemical in order to achieve maximum germination rapidly and uniformly. The aim of the present study was to assess the influence of GA_3 and KNO_3 during seed germination of eight species from the hyper-arid deserts of Kuwait, and on the interaction between these chemicals and light availability.

Materials and Methods

Study area and seed collection

Kuwait has a hot, arid climate with two distinctive seasons; dry (April to October) and wet (November to March). Temperature low/high averages are 10/21°C in January and 29/41°C in July. Temperatures can exceed 50°C in summer (Annual Statistical Report 2006) and precipitation is infrequent and unpredictable, with an annual average of 114 mm falling mostly during the wet season (Omar et al. 2007).

Six annual and two perennial species were selected for this study for being common and ecologically or economically important (Table 1). Most of these species are preferred natural fodder plants for herbivores including sheep, goats and camels (Omar et al. 2007; Norton et al.2009). *Farsetia aegyptia* Turra is a sand stabilizer that is important for maintaining habitat structure, and other species have medicinal value. All species are used intensively by local communities and are therefore at risk of decline through over exploitation (Omar et al. 2004). Better knowledge of restoration success is therefore needed to assist population recovery and persistence. In this habitat where annual species' life spans are ephemeral, excessive herbivory has a proportionately greater impact on perennial species' populations (Gallacher and Hill 2006; Gallacher and Hill 2008). Nevertheless, annual species can be affected indirectly through loss of habitat as well as directly through herbivory.

For each species, seeds were collected at the time of their natural dispersal to ensure seed maturity (Table 1) from multiple maternal plants to sample genetic diversity of the population. Collection sites were from the inland urban periphery, at an altitude of 110-115m. Seeds of each species were collected from 25-30 plants along a 150-180 m transect, leaving at least 2m between adjacent plants. Immediately after collection, seeds were cleaned and stored in paper bags at room temperature (20 ± 2°C) until early June 2018. Seed mass for each species was determined by weighing three 50-seed replicates.

Germination

Four 25-seed replicates of each species were germinated under two light regimes (0, 12 hours per day) in each of four concentrations of gibberellic acid (1.0, 1.5, 2.0 and 3.0 mM GA_3) and potassium nitrate (5, 10, 15 and 20 mM KNO_3), and one control treatment (0 mM). Hence, 14400 seeds were used (8 species x 9 treatments x 2 light regimes x 4 replicates x 25 seeds). Germination was conducted in 9-cm tight-fitting Petri-dishes containing one disk of Whatman No. 1 filter paper, wetted with 10 ml of the test solution. Petri-dishes were placed in a germination incubator at a 12/12-hour temperature of 20/30°C. These conditions were selected based on previous experience by the authors, but might not be optimal for germination of these species. The incubators were illuminated with a 50-W white fluorescent lamp with the light period coinciding with the higher temperature. Petri-dishes containing seeds undergoing dark treatment were wrapped in aluminium foil. Germination was defined as emergence of the radicle (≥2 mm). Germination of light exposed seeds was assessed daily, while the germination of the

dark-treatment seeds was only assessed at the termination of the trial after 26 days. At the end of 26 days, all un-germinated seeds were dissected and observed under a binocular microscope to determine embryo status, where a white cross-section indicated viability and a turgid or brown cross-section indicated an inviable embryo.

Data Analysis

Seed mass of each species was log-transformed and compared using analysis of variance. Germinability (percent germination), mean germination time (days) and synchronization index were calculated and analyzed using Germina Quant software version 1.0 (Marques et al. 2015) for each growth regulator (GA_3 and KNO_3) and species. Mean germination time calculates the average number of days that it takes seeds to germinate, while the synchronization index assesses the extent to which germination is spread across days, returning 0 when seeds germinate each on different days and 100% when seeds germinate all on the same day (see Marques et al. 2015 for formulae). Analyses of variance were applied within species to each growth regulator concentration (GA_3 or KNO_3), light treatment (0 and 12 hours light) and their interaction, and to mean germination time and synchronization index within growth regulator concentration using IBM SPSS version 23.

Results

Seed mass of species varied from 7.3 mg for *Silene arabica* Boiss. to 253 mg for *F. aegyptia* ($P = 2.19x10^{-17}$, Figure 1). Germination percentage varied considerably among species, from 3.2% in *Malva parviflora* L. to 90.1% in *Silene villosa* Forsk. (Figure 2). Application of both GA_3 and KNO_3 significantly increased germination percentage in most species, the exceptions being no effect for *M. parviflora*, and a negative effect of KNO_3 for *S. arabica* and *Haplophyllum tuberculatum* (Forssk.) Ad. Juss. (Table 2). In general, GA_3 had a stronger positive effect on germination percentage than KNO_3, and in most species the lowest concentration of GA_3 tested (1.0 mM) was sufficient to produce the highest germination percentage.

Presence of light either increased or had no effect on germination percentages. Germination was greater in *Savignya parviflora* (Delile) Webb and *Diplotaxis tenuifolia* (*L.*) DC. when treated with GA_3 in the presence of light, but other species were unaffected by the interaction between GA_3 and light. Germination was greater for most species when treated with KNO_3 in the presence of light rather than dark. Significant first-order interactions (Table 2)

indicate that the degree to which light increases germination differs with different growth regulator concentrations (Figure 2).

Mean germination time was significantly reduced by higher GA_3 concentrations in five species. It was also reduced by higher KNO_3 concentrations in *S. villosa* and *Gypsophila capillaris* (Forssk.) C.Chr, but *S. parviflora* exhibited the opposite response. Synchronization of germination was mostly not influenced by growth regulator concentrations, the exceptions being an increase with GA_3 in *G. capillaris* and *H. tuberculatum*, and with KNO_3 in *D. tenuifolia*.

Discussion

Knowledge of a species' seed dormancy is essential for an efficient restoration program (Turner et al. 2013), since the use of dormant seeds can result in fractional germination and emergence (Larson et al. 2015). Co-occurring species investigated in the present study exhibited a variety of dormancy/germination responses. Freshly matured seeds of *D. tenuifolia*, *M. parviflora* and *S. parviflora* showed poor germination (high levels of innate dormancy), whereas *F. aegyptia*, *G. capillaris*, *H. tuberculatum* and *S. villosa* showed an intermediate level of dormancy and *S. arabica* showed little dormancy. This interspecific variation of physiological dormancy among freshly collected desert seeds has been reported previously (see Baskin and Baskin 2014). It suggests community variation in seed bank strategies (Karlsson et al. 2008) that has also been observed in other plant communities (Li and Foley 1997; Smýkal et al.2014).

Both GA_3 and KNO_3 facilitated the release of innate dormancy in most of the tested species. There was no response in *M. parviflora*. This supports previous findings that the species exhibits physical or morpho-physiological dormancy via a hard seed coat that can be alleviated through scarification and treatment with 5 mM KNO_3 (Chauhan et al.2006a).

Germination of most species was significantly enhanced by KNO_3, as has been found for other desert species (AOSA 2009; ISTA 2009; Zaman et al. 2009; Nadeem et al. 2012), but the concentration that achieved maximum germination was species specific. Potassium nitrate stimulates germination by (i) decreasing the endogenous levels of abscisic acid in embryos (Wang et al.1998), (ii) causing vacuolation and cell wall weakening of the aleurone layer (Bethke et al.2007) and (iii) involvement in the pentose phosphate pathway due to increased oxidation of NADPH to NADP or enzymes that have been encoded to provide nutrients for germination (Finkelstein et al. 2008).

Gibberellic acid was found to be most effective in alleviating the dormancy of most of the studied species, as previously reported for species with physiological dormancy (Baskin and Baskin, 2014). Response to dosage varied, but a concentration of 1.50 mM was the most effective for many species. It is a commonly used hormone for alleviating seed dormancy in many species (Ali and Helal 1996; Aghilian et al. 2014; Bhatt and Pérez-García 2016; El-Keblawy and Gairola 2017) and its associated role with abscisic acid to regulate seed dormancy and germination is well established (Finkelstein et al. 2008; Hoang et al. 2014). Dormancy is maintained under high levels of abscisic acid and lower levels of GA_3 (Kucera et al. 2005; Finkelstein et al. 2008), and germination is triggered by the change in their balance (Yamauchi et al. 2004). In the present study the exogenous application of GA_3 might be responsible for changing this balance, thus triggering germination (Shu et al. 2016; Finch-Savage and Footitt 2017). These results are in agreement with previous studies that reported variation in the concentrations of KNO_3 and GA_3 required to promote germination among species (Cerabolini et al. 2004; Nurse and Cavers 2008; Wei et al. 2010; Gashi et al. 2012).

Action of both KNO_3 and GA_3 to release dormancy was enhanced in the presence of light for most species; *G. capillaris, S. parviflora, S. arabica* and *S. villosa*. *D. tenuifolia* responded to light under GA_3 treatments, and *F. aegyptia* responded under KNO_3 treatments. These results are consistent with previous studies on *Sisymbrium orientale* L., *Solanum nigrum* L. and *Solanum ptychanthum* Dun. (Bithel et al. 2002; Zhou et al. 2005; Chauhan et al. 2006b). The exception was *H. tuberculatum*, which showed no overall response to light. In general, GA_3 was effective in reducing mean germination time and promoting synchronized germination in most of the studied species. This indicates that GA_3 could be used to obtain faster germination and uniform aged seedlings, which are integral to rapid establishment under field condition compared to $KNO_3$and control, where favorable season for germination and seedling establishment is restricted to winter.

The effects of KNO_3 and GA_3 on alleviating seed dormancy were species-specific. Exogenous application of both KNO_3 and GA_3 enhanced the germination in the studied species, but GA_3 was more effective in *D. tenuifolia*, *F. aegyptia*, *H. tuberculatum, S. parviflora* and *S. arabica.* However, *G. capillaris* and *S. villosa* seeds showed similar results with both chemicals. Gibberellins are plant hormones and thus they are more effective in alleviating the dormancy in most of the species as compared to other chemicals (Amen 1968). Our results indicate the

existence of different types and levels of dormancy among species, being morpho-physiological dormancy that is responsive to light in *M. parviflora* and physiological dormancy in the other species.

This study shows that many desert species exhibit seed dormancy at the time of maturation, which prevents their immediate germination of at least a proportion of the seeds. There is large variation in dormancy/germination responses among co-occurring species which indicates the presence of a profuse 'regeneration niche' within the community that might assist in co-existence of different species. Exogenous application of KNO_3 and GA_3 produces rapid germination, and is, therefore, recommended for desert restoration/ rehabilitation utilizing these species.

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Table 1: General description of the species studied and of seed collection.

Species	Family	Distribution [1]	Collection month (2018) and location	Life form	Uses	Reference
Diplotaxis tenuifolia (*L.*) DC.	Brassicaceae	Bh, Kw, Om, Qa, KSA, UAE	Apr, Sulaibia 29° 9' 41.22" N; 47° 41' 35.27" E	Annual	Medicinal	Pedras et al. 2014
Farsetia aegyptia Turra	Brassicaceae	Kw, Om, Qa, KSA, UAE	Apr, Sulaibia 29° 9' 51.26" N; 47° 41' 42.62" E	Perennial	Sand-stabilizing, Fodder	Bidak et al. 2015
Gypsophila capillaris (Forssk.) C.Chr.	Caryophyllaceae	Kw, KSA, UAE	May, Sulaibia 29° 9' 44.59" N; 47° 41' 26.80" E	Annual	Medicinal, Fodder	Elgamal et al. 1995; Omar and Zaman 1998
Haplophyllum tuberculatum (Forssk.) Ad.	Rutaceae	Bh, Kw, Qa, KSA, UAE	May, Julaia 29° 9' 51.95"	Perennial	Medicinal	Said et al. 2002; Al-Burtamani et al. 2005

Juss.			N;47° 41' 5.53" E			
Malva parviflora L.	Malvaceae	Bh, Kw, Om, Qa, KSA, UAE	May, Sulaibia 29° 9' 34.23" N; 47° 41' 34.70" E	Annual	Medicinal, Fodder	Boulos 1977; Afolayan et al. 2008
Savignya parviflora (Delile) Webb	Brassicaceae	Bh, Kw, Om, Qa, KSA, UAE	Apr, Sulaibia 29° 9' 46.57" N; 47° 41' 28.59" E	Annual	Medicinal, Fodder	Akbar and Al - Yahya 2011; Middleditch 2012
Silene arabica Boiss.	Caryophyllaceae	Kw, Qa, KSA	Apr, Sulaibia 29° 9' 47.78" N;47° 41' 28.53" E	Annual	Fodder	Omar and Zaman 1998
Silene villosa Forsk.	Caryophyllaceae	Bh, Kw,Om, Qa, KSA, UAE	May, Sulaibia 29° 9' 35.91" N;47° 41' 32.88" E	Annual	Fodder	Omar and Zaman 1998)

[1]: Bh Bahrain; UAE United Arab Emirates; KSA Kingdom of Saudi Arabia; Kw Kuwait; Om Oman; Qa Qatar

Table 2: Significance (P) of gibberellic acid (GA_3) and potassium nitrate (KNO_3) concentrations, exposure to light (light / dark), and concentration / light exposure interaction on germination percentage of eight species, and the significance of concentrations on mean germination time and synchronization index.ns and ns* indicate a non-significant value without ($\alpha = 0.05$) and with the Bonferroni correction ($\alpha = 0.05/80$).

Species ordered by lifespan and seed size (small to large)	Germination percentage						Mean germination time		Synchronization index	
	Regulator concentration		Light / Dark		Interaction (Concentration x Light)		Regulator concentration		Regulator concentration	
	GA_3	KNO_3	GA_3	KNO_3	GA_3	KNO_3	GA_3	KNO_3	GA_3	KNO_3
Annual species										
Silene arabica	$6.4X10^{-5}$	$3.0X10^{-8}$	ns*	$5.8X10^{-13}$	ns	$9.3X10^{-5}$	$1.4X10^{-7}$	ns*	ns*	ns*
Silene villosa	$1.2X10^{-15}$	$9.5X10^{-13}$	ns*	$4.6X10^{-12}$	ns	$4.9X10^{-8}$	$1.4X10^{-8}$	$1.3X10^{-8}$	ns*	ns
Savignya parviflora	$3.1X10^{-18}$	$2.0X10^{-7}$	$2.4X10^{-13}$	$4.5X10^{-9}$	$1.1X10^{5}$	$4.5X10^{-5}$	ns*	$2.7X10^{-5}$	ns*	ns*
Diplotaxis tenuifolia	$2.1X10^{-29}$	$9.1X10^{-11}$	$3.5X10^{-09}$	$7.5X10^{-7}$	$2.9X10^{6}$	ns	ns	ns*	ns*	$5.5X10^{-5}$
Malva parviflora	ns	ns	ns	ns	ns	ns*	ns	ns	-	-
Gypsophila capillaris	$6.7X10^{-16}$	$4.6X10^{-13}$	ns	$2.5X10^{-7}$	ns	$8.6X10^{-6}$	$8.4X10^{-10}$	$1.2X10^{-7}$	$1.8X10^{-5}$	ns*
Perennial species										
Haplophyllum tuberculatum	$2.4X10^{-13}$	$6.6X10^{-6}$	ns*	ns	ns*	$1.2X10^{-5}$	$5.2X10^{-9}$	ns*	$1.3X10^{-5}$	ns
Farsetia aegyptia	$1.4X10^{-16}$	$4.0X10^{-11}$	ns*	$6.2X10^{-6}$	ns*	ns*	$1.1X10^{-6}$	ns	ns*	ns

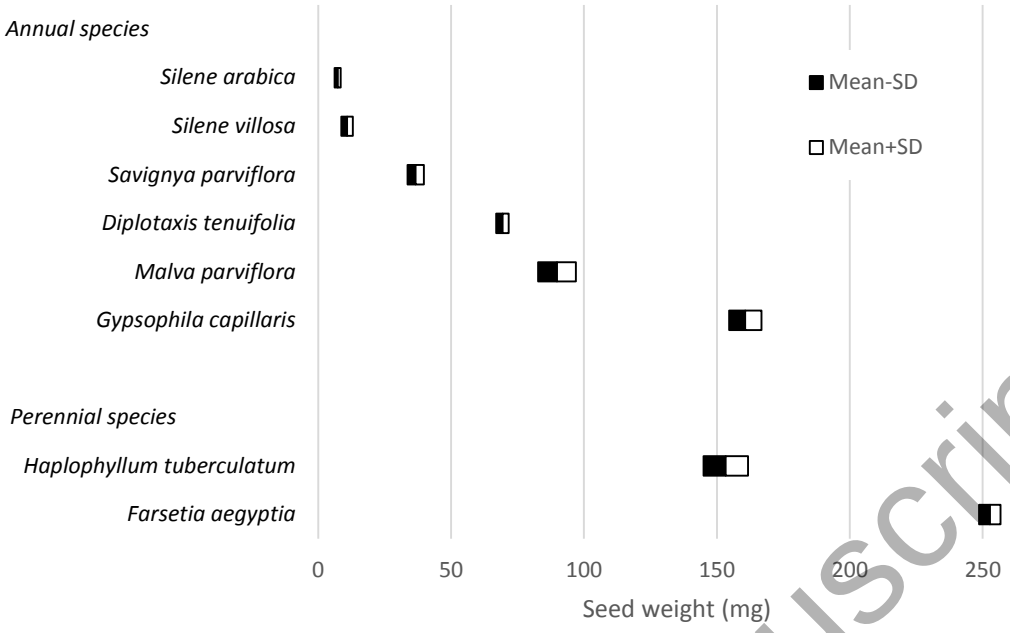
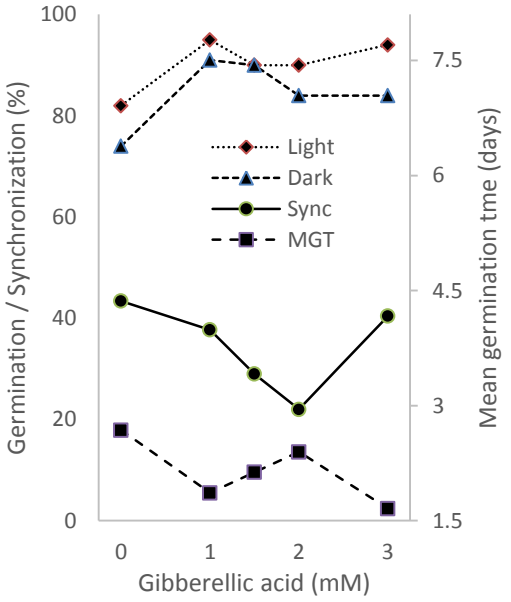
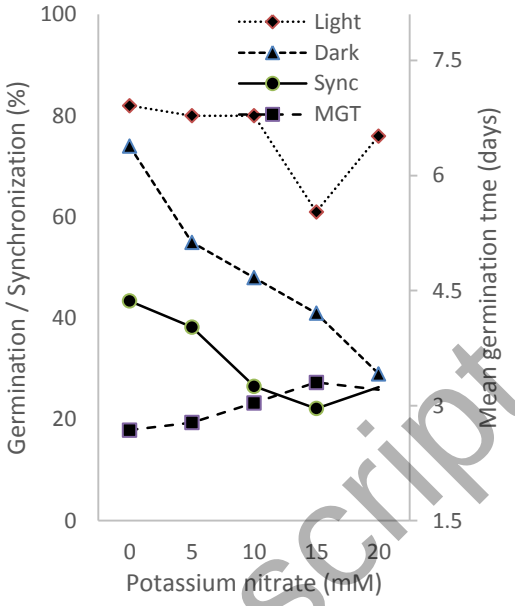


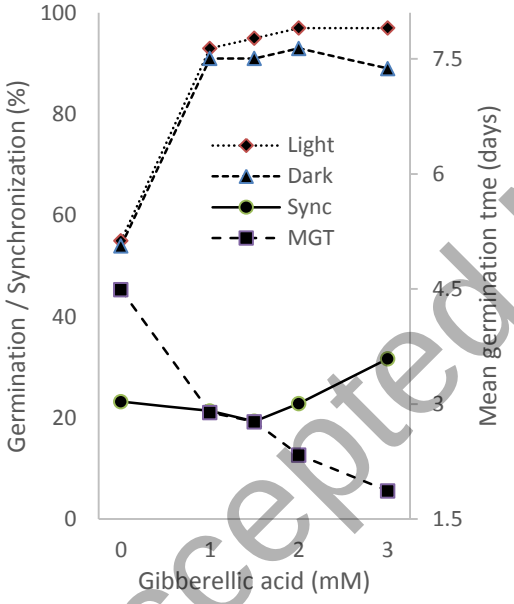
Figure 1: Seed mass (mg) of species studied, calculated from three 50-seed replicates

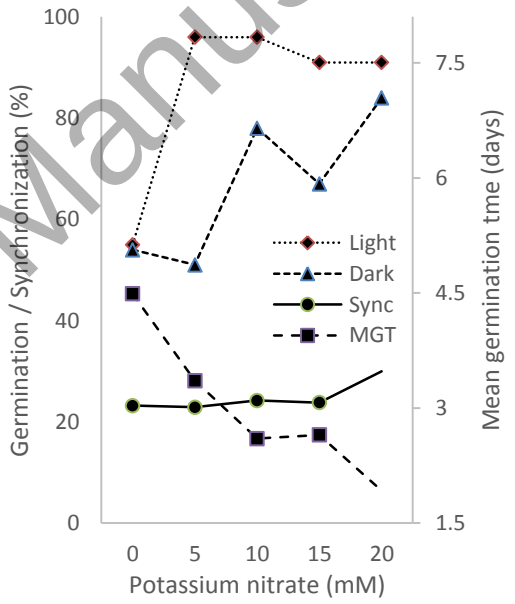
Silene arabica



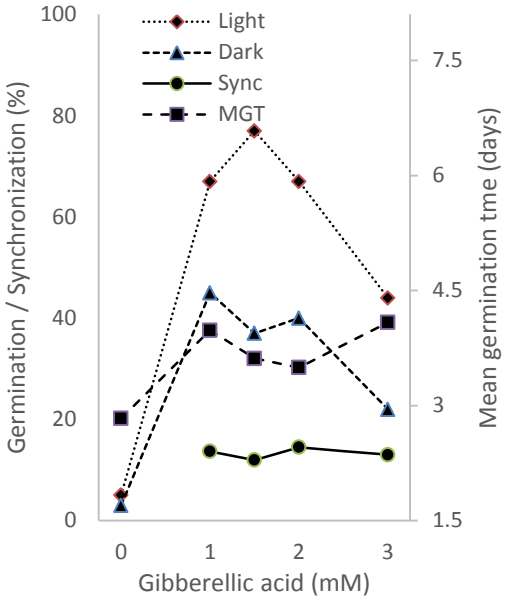


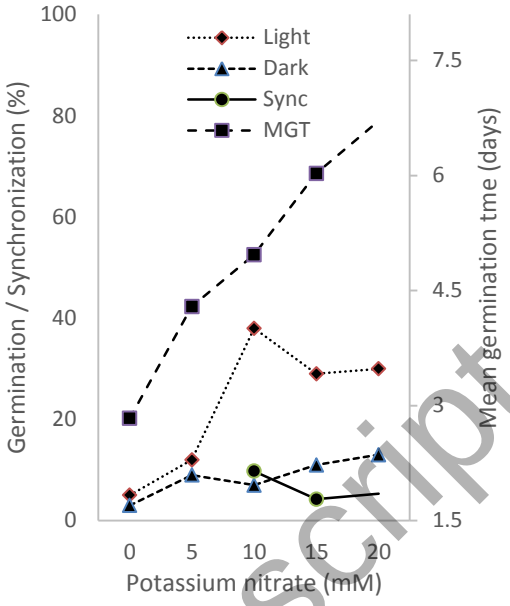
Silene villosa



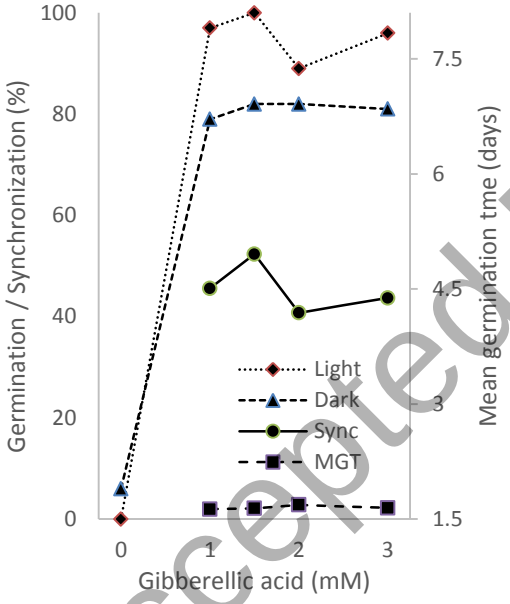


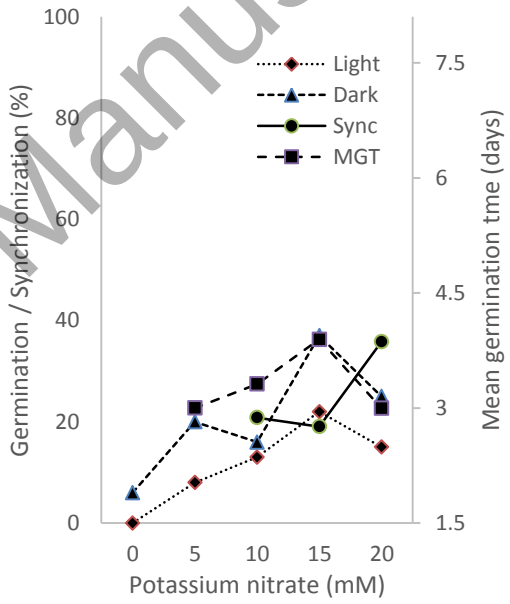
Savignya parviflora



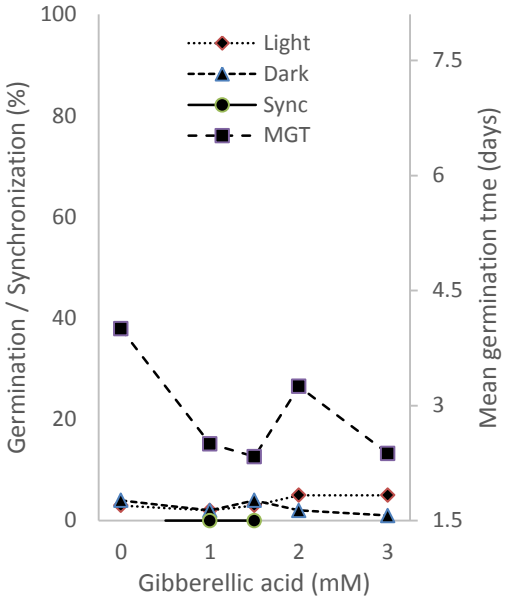


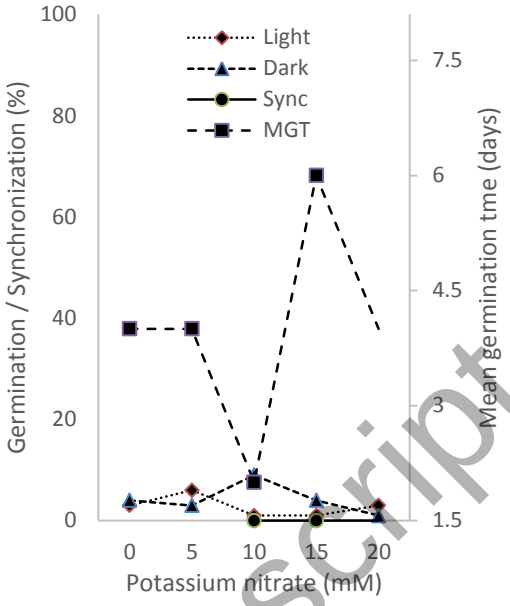
Diplotaxis tenuifolia



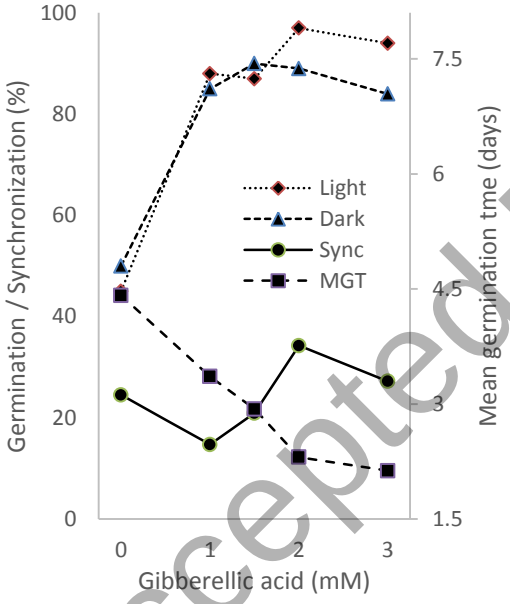


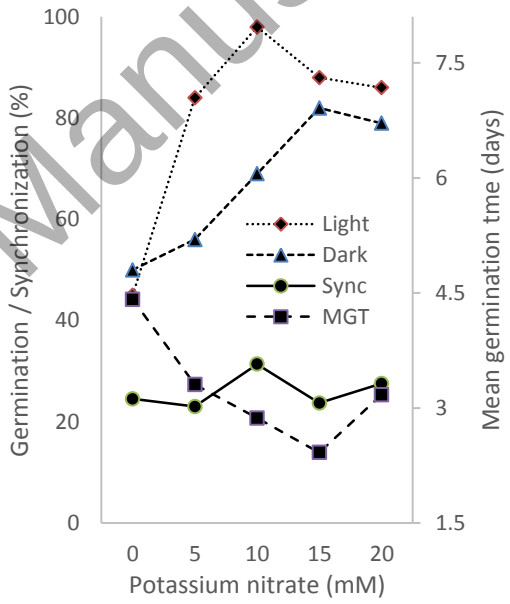
Malva parviflora





Gypsophila capillaris





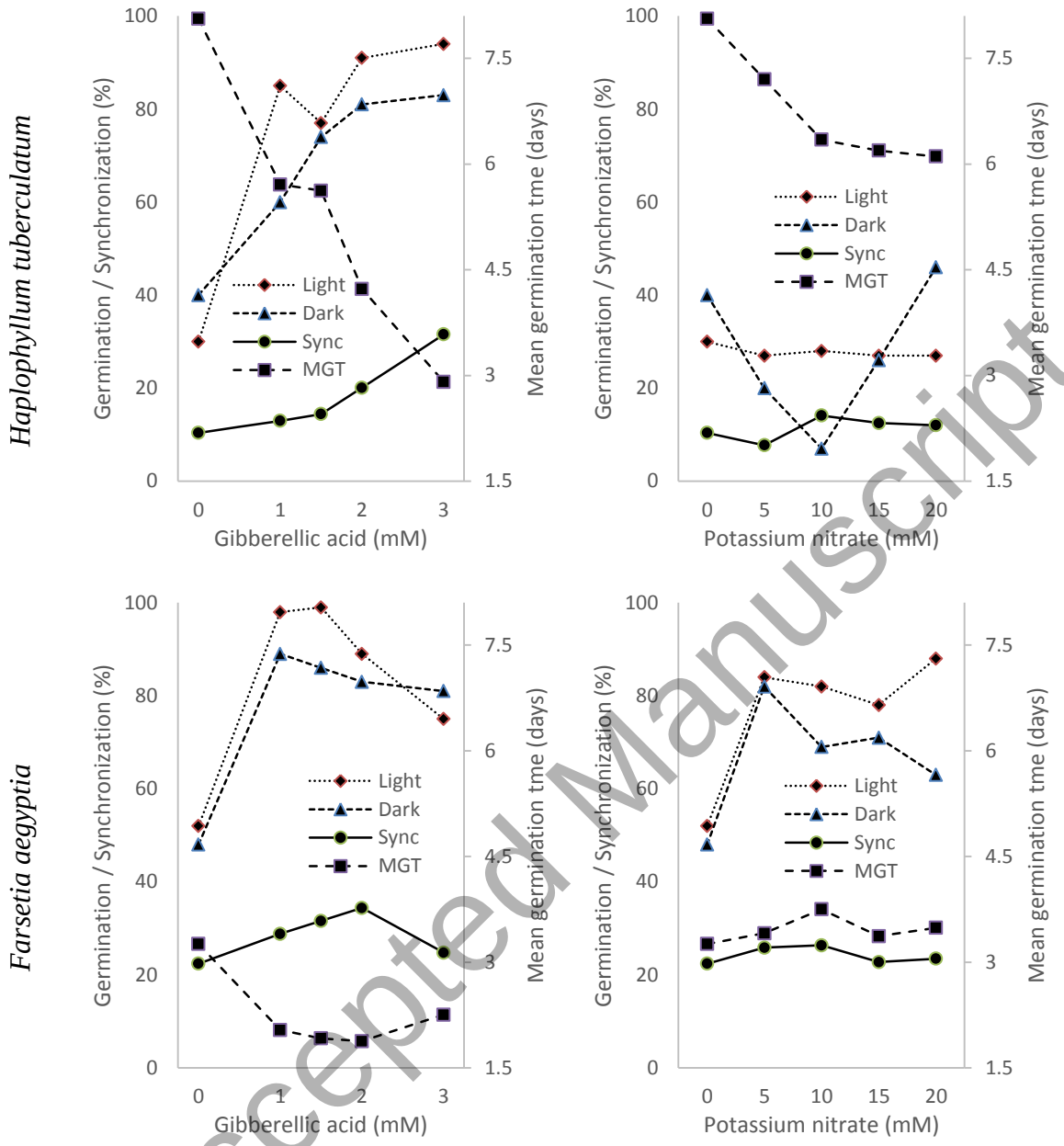


Figure 2: Change in germination under light and dark conditions, and the mean germination time and synchronization index under light conditions, with a change in concentration of gibberellic acid and potassium nitrate.