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Investigation of different priming techniques on seed germination of *Papaver* species

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Abstract

Seed dormancy is important as an adaptive trait for weeds that help ensure the continuance of weed species in the soil seed bank. This study examined the effects of some priming treatments including hydropriming, gibberellic acid (GA_3), potassium nitrate (KNO_3), priming duration and light regime on seed dormancy breaking of *Papaver rhoeas* and *P. dubium*. The result showed that all treatments significantly stimulated germination of both *Papaver* species. Soaking seeds in distilled water led to significant increase in germination in both species. Maximum germination percentage was observed for 24 h hydro-priming treatments (32.4% for *P. dubium* and 34.6% for *P. rhoeas*) in light/dark condition. In general 0.5 $g.L^{-1}$ KNO_3 treatments resulted in more vigorous seed germination in both species at any duration compared to any other concentration of the potassium nitrate. In both species the highest seed germination was achieved when the seeds were treated 24 h with 0.5 $g.L^{-1}$ KNO_3 solution but it was decreased in 6 $g.L^{-1}$. Among the priming treatments with GA_3, the highest germination of both species was recorded in seed treated with 500 and 750 ppm GA_3 respectively in *P. dubium* and *P. rhoeas* for 48 h.

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Introduction

The *Papaver* genus in the Papaveraceae family comprises 100 species distributed in various countries around the world, from central and South Europe to temperate Asia, America, Oceania and South Africa (Holm *et al.,* 1997). *Papaver rhoeas* and *P. dubium* are a cross-pollinated dicot species, frequently has been reported as a common weed of winter wheat (*Triticum aestivum* L.) and other winter cereals (Kaloumenous and Eleftherohorinos, 2008). These species have a high reproductive potential through its seeds, which are dormant and have long viability, thus facilitating its persistence and spread (Lutman *et al.*, 2002). The non-deep simple morphophysiological dormancy described in these species determines the formation of persistent seed banks that make it a troublesome weed (Karlsson and Milberg, 2007).

Seed dormancy is one of the most important characteristics of weeds and extends the germination and emergence of weeds over time thus favoring the persistence of seeds in the seed bank (Baskin and Baskin, 2004). Several methods have been proposed for seed dormancy breaking. Seed priming is nowadays being extensively used to improve seed germination and braking seed dormancy in a wide range of plant species (Pill and Killian, 2000). Common priming techniques include hormonal priming (soaking seeds in regulators solutions such as gibberellins), halo priming (soaking seeds in salt solutions) and hydro-priming (soaking seeds in water) (Caseiro *et al.,* 2004; Basra *et al.*, 2005). Priming may improve germination by accelerating imbibitions, which in turn would facilitate the emergence phase and the manipulation of radicle cells (Shim *et al.*, 2008). During the priming, several processes including storage, material handling, activation and synthesis of a number of enzymes and nucleic acids, repair and build up, ATP synthesis, and the cytoplasmic membrane repair in treated seeds will all start to develop (Ghobadi *et al.*, 2012). Bailly *et al.* (2000) stated seed priming caused physiological and biochemical changes before germination.

Hydro-priming is a simple and inexpensive method of priming treatment, which it does not require any special technical equipment and owing to the use of distilled water as a priming medium (Farooq *et al.,* 2006). Artola *et al.* (2003) reported that hydro-priming resulted to be a valid physiological treatment that significantly improves the germination of *Lotus corniculatus* L. seeds. Improved seed germination due to hydro-priming may be explained by an increased water uptake and rate of cell division (Caseiro *et al.*, 2004).

Plant growth regulators such as gibberellic acid (GA_3) and chemicals such as KNO_3 have been recommended to break seed dormancy and enhance germination (Foly and Chao, 2008; Karlsson *et al.,* 2006; Chauhan *et al.,* 2006). GA_3 activate the synthesis of proteins and other metabolites required by the embryo for germination (Finch-Savage and Leubner-Metzger, 2006). Gashi *et al.* (2012) stated that positive effect of KNO_3 on breaking seed dormancy could be due to its role on balancing hormonal portion within seed which in turn results decrement of germination inhibitors ratio like ABA (Abscisic Acid). Bush *et al.* (2000) reported that *Axonopus affinis* and *Eremochloa ophiuroides* seeds primed with KNO_3 showed highest values of germination percentage.

To improve management system for specific species it is critical to have good information of germination requirements and dormancy breaking methods. Seed dormancy is major motive towards the success of this species, since there is not much evidence about seed priming of *Papaver* species, this research was aimed to investigate the effects of hydro, halo and hormonal priming on seed germination of this weed.

Material and method

Plant material collection

Seeds of *Papaver rhoeas* and *P. dubium* were collected in spring 2011 at wheat fields of Qaemshahr, Iran. Seeds were sieved to remove any extraneous materials and seeds were stored at room temperature in paper bags until required. Experiments were

carried out in Sari Agricultural Sciences and Natural Resources University, Sari, Iran, in october 2012.

General germination test

Before pretreatments commenced, seeds were sterilized by soaking in 1% sodium hypochloride for 1 min and subsequently rinsed with distilled water for several times. Seed priming treatments consisted of untreated seeds (control), hydro-priming (using distilled water, 4, 8, 12, 24 and 48 h), halo priming (0.5 , 2, 4 and 6 $g.L^{-1}$ KNO_3 for 12, 24, 48, 72 and 96 h) and hormonal priming (250, 500, 750 and 1000 ppm GA_3 for 12, 24, 48, 72 and 96 h). After priming, samples of seeds were removed and the seeds were rinsed in distilled water and then dried to the original moisture level. For seed germination test, fifty seeds of each species were placed on Whatman No. 1 filter paper in 9 cm Petri dishes. The filter paper was moistened with either 4 ml of distilled water. All Petri dishes were sealed with Parafilm to inhibit evaporation and water loss. To evaluated the effect of light on germination the study was performed under both light/dark and complete darkness regimes. The regimes were set up at 16/8 h (day/night) for light/dark condition and 24 h continuous dark for darkness treatment. Light was provided by fluorescent lamps to produce a light intensity of 300 $\mu mol\ m^{-2}\ s^{-1}$. For the treatments in darkness, the Petri dishes wrapped with two layers of aluminum foil before incubation. Thermo period was set at 25/15 °C (day/night) for all experiments. Seed germination was determined 4 wk after incubation. Seed were considered germinated when radicles emerged from the seed coat.

Statistical analysis

All experiments were conducted as a factorial experiment on completely randomized design with five replicates. ANOVA was performed on arcsine transformed data of germination percent. Means were tested by Duncan's Multiple Test Range at P=0.05. SAS program was used for statistical analyses.

Result and discussion

Effect of hydro-priming on seed germination

Hydro-priming duration and light regimes significantly influenced seed dormancy breaking of both species (Table 1). Also, the effect of interaction was significant in both species (Table 1). Untreated seeds (control) showed lowest germination percentage which might be due to seeds dormancy. Untreated seeds of *Papaver dubium* had 8.4% and 5.6% in light/dark and continuous darkness regimes respectively. Initial seed germination of *P. rhoeas* at starting the experiments was 13.6% and 7.2% in light/dark and continuous darkness, respectively (Fig 1A,B). Significantly, the higher germination percentage in hydro-primed seeds compared with no-primed seeds (control) in all duration indicated a positive effect of seed priming in synchronizing the seed germination process.

Table 1. Analysis of variance (Means of squares) of germination percentage of *Papaver* species under pre-soaking period and light period.

S.O.V.	d.f.	Germination percentage	
		Papaver dubium	*Papaver rhoeas*
Hydro-priming duration (A)	5	0.1655^{***}	0.1199^{***}
Light regime (B)	1	0.0921^{***}	0.09804^{***}
A×B	5	0.0005^{***}	0.00115^{*}
Error	48	0.00078	0.000552
Coefficient of Variance (%)	-	6.51	5.15

*** indicated significant at P≤0.001 respectively, and n.s. indicated no significant difference.

As 24 h hydro-primed seeds in *P.dubium* gave highest germination under light/dark regime (32.4%), the lowest germination was obtained from untreated seeds (control) from continuous darkness (5.6%). Similarly, the higher germination percentage in *P.rhoeas* was observed for 24 h hydro-priming

treatments (34.6%) in light/dark condition compared to the control (13.6%) (Fig 1a,b). In both species, seed germination in complete darkness was significantly lower than incubated seeds in light/dark.

The results of this experiment showed that the use of hydro-priming increase the seed germination in *Papaver* species. According to Farooq *et al.* (2006) the positive effects probably is due to the stimulatory effects of hydro-priming on the early stages of germination process by mediation of cell division in germinating seeds. Consistent with our results, similar findings were observed by Artola *et al.* (2003) and Dastanpoor *et al.* (2013) who reported improvement in germination, breaking seed dormancy and enhanced emergence in hydro-primed seed. Caseiro *et al.* (2004) reported that the promotive influence of distilled water on seed germination could be ascribed to the fact that most of the inhibitors might be washed away from the seeds.

Table 2. Analysis of variance (Means of squares) of germination percentage of *Papaver* species under KNO_3, priming period and light period.

S.O.V.	d.f.	Germination percentage	
		Papaver dubium	*Papave rhoeas*
KNO_3 concentrations (A)	3	0.5718^{***}	0.43005^{***}
Priming duration (B)	4	0.5323^{***}	0.5291^{***}
Light regime (C)	1	0.3357^{***}	0.4013^{***}
A×B	12	0.01204^{***}	0.01562^{***}
A×C	3	$0.000323^{n.s.}$	$0.00199^{n.s.}$
B×C	4	$0.000086^{n.s.}$	$0.001073^{n.s.}$
A×B×C	12	0001876^{*}	$0.000305^{n.s.}$
Error	160	0.000868	0.000812
Coefficient of Variance (%)	-	7.39	6.73

*, ** and *** indicated significant at P≤0.05, P≤0.01 and P≤0.001 respectively, and n.s. indicated not significant difference.

Effect of KNO_3 priming and duration on seed germination

Germination of *Papaver* species had significantly affected by KNO_3 concentration, priming duration and light regime. Analysis of variance showed that there is a significant three way interaction (KNO_3 concentration× priming duration× light regime) for germination percentage in *Papaver dubium* (Table 2). The highest seed germination (49.6%) was achieved when the seeds were treated 24 h with 0.5 $g.L^{-1}$ KNO_3 solution but it was decreased in 6 $g.L^{-1}$ (17.2%) in light/dark condition. When KNO_3 concentration increased to 6 $g.L^{-1}$, final germination for duration more than 24 h decreased even less than control (8.4%). Also similar results have been obtained in *P. rhoeas,* potassium nitrate at 0.5$g.L^{-1}$ had a positive interaction with both priming periods (12 and 24 h), the maximum seed germination was obtained with this treatment was 40.4% and 51.2% in light/dark regime respectively comparing unprimed seeds (13.6%). Whereas, the minimum seed germination percentage (3.6%) was achieved in priming periods of 96 h at 6 $g.L^{-1}$ KNO_3 in darkness condition (Table 3). The duration of priming with KNO_3 influenced germination percentages differentially, the results indicated that short-term priming had a more positive effect on germination. As illustrated in Table 3 among the species, germination percentage was higher in *P. rhoeas*.

Based on results, treatment with KNO_3 solution for 24 h showed practically acceptable germination percentage in the presence of light/dark regimes. With increasing of priming duration from 24 to 96 h germination percentage was decreased. Treated seeds with KNO_3 for 96 h found adversely effective on germination. Meanwhile, priming with KNO_3 solution of a minimum concentration of 0.5 or 2 $g.L^{-1}$ was deemed appropriate for increasing germination.

percentages under light/dark regimes in both species while high concentration had low effect on

germination. This leads to an assumption that higher concentrations exert decreasing effects on seed germination by causing death of cells and ultimately result in loss of seed viability (Tiryaki and Buyukcingil, 2009). This suggests that there is toxic effect of KNO_3 due to ion accumulation in the embryo (Song *et al.*, 2007).

Table 3. Interaction of KNO_3 concentration, priming period and light period on germination percentage of *Papaver* species.

KNO_3 concentrations	Priming duration	Light regime	Germination percentage	
			Papaver dubium	*Papaver rhoeas*
0.5	12h	Light	35.2c	40.4b
		Dark	29.6de	30.4c
	24h	Light	49.6a	51.2a
		Dark	40.8b	41.6b
	48h	Light	30.4d	30.4c
		Dark	212fg	21.2de
	72h	Light	20.8fg	21.6de
		Dark	14.8jk	15.6ghi
	96h	Light	12.4lm	13.2ij
		Dark	7.6pq	8.4no
2	12h	Light	26.8e	29.6c
		Dark	18.8ghi	20.4e
	24h	Light	36.8c	39.2b
		Dark	28.8de	30.4c
	48h	Light	19.2gh	20.4e
		Dark	13.6kl	13.2ij
	72h	Light	17.2hij	18.8ef
		Dark	10.8mn	11.6jk
	96h	Light	10.4mno	11.6jk
		Dark	7.6pq	7.6nop
4	12h	Light	18.8ghi	20.8e
		Dark	12.4m	14.4hi
	24h	Light	22.8f	24d
		Dark	15.2jk	16.4gh
	48h	Light	16ij	17.2fg
		Dark	9.2nop	11.8jk
	72h	Light	10.4mno	11.2jkl
		Dark	6.8q	6.8op
	96h	Light	7.6pq	8.8mn
		Dark	3.6rs	4.4q
6	12h	Light	15.2jk	18.8ef
		Dark	9.2nop	10.8klm
	24h	Light	17.2hij	21.6de
		Dark	13.6kl	15.2ghi
	48h	Light	8.4opq	15.2ghi
		Dark	6.8q	9.2lmn
	72h	Light	6.8q	8.4no
		Dark	3.2s	4.4q
	96h	Light	4.4r	6.4p
		Dark	1.2t	3.6q

Means in a column with the same letter are not significantly different at 5% based on Duncan's test.

Potassium nitrate solution has long been known as a suitable chemical approach for promoting germination in various plant species and generally as a priming agent for germination (Shim *et al.*, 2008). The positive effects of potassium nitrate might be due to its role in influencing the permeability of the

membranes which ultimately leads to activation of enzymes involved in protein synthesis and carbohydrate metabolism (Ghobadi *et al.,* 2012). In accordance with our results, Shim *et al.* (2008) found that priming of *Paspalum vaginatum* seeds with KNO_3 had significant effects on germination, also Shim *et al.* (2008) stated during halo-priming (KNO_3), ions from potassium nitrate solutions accumulate within the seeds, reducing water potential and increasing water absorption. Basra *et al.* (2005) reported that the presence of nitrate during imbibition may provide additional substrate for amino acid and protein synthesis for the enhancement of germination during priming.

Table 4. Analysis of variance (Means of squares) of germination percentage of *Papaver* species under gibberellic acid, priming period and light period.

S.O.V.	d.f.	Germination percentage	
		Papaver dubium	*Papaver rhoeas*
Gibberellic acid concentrations (A)	3	0.2381^{***}	0.1643^{***}
Priming duration (B)	4	0.6939^{***}	0.5528^{***}
Light regime(C)	1	0.4895^{***}	0.7519^{***}
A×B	12	0.0102^{***}	0.0225^{***}
A×C	3	0.004489^{*}	$0.00234^{n.s.}$
B×C	4	$0.00103^{n.s.}$	$0.0023^{n.s.}$
A×B×C	12	$0.00118^{n.s.}$	$0.00093^{n.s.}$
Error	160	0.000853	0.001599
Coefficient of Variance (%)	-	6.52	8.81

* and *** indicated significant at P≤0.05and P≤0.001 respectively, and n.s. indicated not significant difference.

Effect of GA_3 priming on seed germination

Gibberellins concentration, priming duration and light regime significantly influenced seed germination of *Papaver* species. Effect of interaction among gibberellic acid concentration and priming duration was significant in both species (Table 4).

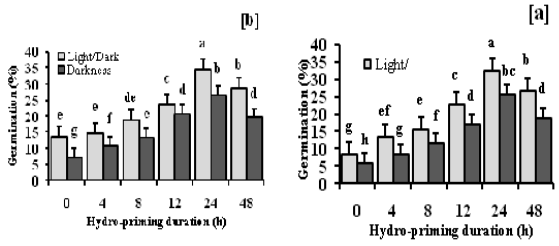

Fig. 1. Effect of hydro-priming duration and light regimes on germination of *Papaver dubium* (a) *Papaver rhoeas* (b). Bars within a priming duration followed by the same letter(s) are not significantly different at P=0.05.

Based on result for final germination, seed treatment by priming had statistically significant effect on increasing the germination percentage compared to unprimed seeds (8.4%) in *P. dubium,* the highest seed germination (47%) was achieved after 48 h of soaking in 500 ppm GA_3, however, 36.8% seed germination was achieved in the same duration at 750 ppm GA_3. In general 500 ppm GA_3 treatment resulted in more vigorous seed germination at any duration compared to any other concentration of the GA_3 (Fig. 2a). Also in *P. rhoeas*, priming duration had significant effect on germination synchrony, compared to priming period of 12 h, priming duration of 48 h significantly increased germination between 21.8% and 48.8% at 750 ppm GA_3. A higher germination percentage was observed in GA_3 750 ppm treatment for 48 h that was significant (48.8%) compared to the untreated seed (13.6%) (Fig 2B).

Gibberellic acid is known to play an essential role in breaking seed dormancy and enhanced seed germination. The results revealed that the regulation of endogenous GA_3 levels during seed imbibitions is a crucial factor in determining seed germination and duration of seeds priming with GA_3 is also important.

In this study, GA_3 increased germination percentage significantly depending on used concentration and priming duration. Germination percentage was reduced beyond 48 hours of soaking in GA_3 at all concentrations. Our results are in agreement with previous studies, which revealed that seed priming treatment with GA_3 was an effective method for stimulation of seed germination in different species (Conner, 2008; Ghobadi *et al.,* 2012). GA_3 activate the synthesis of proteins and other metabolites required by the embryo for germination (Eisvand *et al.,* 2010). Also Shouhui *et al.* (2010) reported that treated *Solanum rostratum* seeds in 2.4 mM GA_3 for 24 h increased germination (98%).

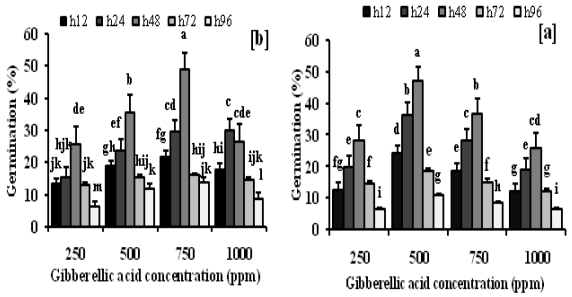

Fig. 2. Effect of gibberellic acid concentrations and priming duration on germination of *Papaver dubium* (a) *Papaver rhoeas* (b). Bars within a gibberellic acid concentration followed by the same letter(s) are not significantly different at P=0.05.

In conclusion, the results of this study confirmed that the *Papaver* seeds were in a dormant state. Priming as physiological treatment causes an increase in the seed germination of *Papaver dubium* and *P. rhoeas*. The response of seeds to priming has been found to be dependent on the priming treatments, duration of priming and species. The research findings of the study show that the best hydropriming period obtained from 24 h in light/dark regimes and KNO_3 priming increased seed germination percentage, it is suggested that the priming with GA_3 for 48 h are the best to break seed dormancy in *Papaver* species. The results of priming among species have been variable. Variation within a taxon may depend on genetic differences, local weather during growth of mother plants and maturation of seeds, seed position on the mother plant, soil quality, or other naturally occurring factors (Karlsson and Milberg, 2007). On the basis of findings information about seed dormancy regulation could improve our basis knowledge to understand weed behavior and better management strategies to control this species weed.

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