Environmental and Experimental Biology (2016) 14: 145–150 *Original Paper*
DOI: 10.22364/eeb.14.20

Dormancy release of wild barley seed germination by using plant growth regulators

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Abstract

Plant growth regulators play an important role in control of seed germination. This experiment was conducted to investigate the effect of lant growth regulators (indole-3-acetic acid, gibberellin and kinetin) on seed germination of *Hordeum spontaneum*. Seeds of wild barley were soaked in distilled water (control) or different concentrations of gibberellin (25, 50, 75, 100, 125 and 150 mg L^{-1}), indole-3-acetic acid and kinetin (5, 10, 15, 20, 25 and 30 mg L^{-1}). The following germination parameters were determined: germination percentage, germination index, mean germination time, germination energy, germination value, seedling length and seed vigour index. The results showed that plant growth regulators, depending on the used concentration, effectively improved all germination parameters except the mean rate of germination, which was increased only by indole-3-acetic acid at concentrations of 20 and 25 mg L^{-1}. Gibberellin was generally shown to be more effective on all evaluated traits except the mean rate of germination. The highest values in germination percentage, germination index, germination energy, germination value, seed vigor index were obtained by using gibberellin at 75 mg L^{-1} and followed by 100 mg L^{-1}. Gibberellin also at concentration of 50 and 125 mg L^{-1} led to the greatest mean germination time and seedling length, respectively.

Key words: germination, *Hordeum spontaneum*, plant growth regulator, seeds, wild barley.
Abbreviations: ABA, abscisic acid; GE, germination energy; GI, germination index; GP, germination percentage; GV, germination value; IAA, indole-3-acetic acid; MGT, mean germination time; MRG, mean rate of germination; PGR, plant growth regulator; SVI, seed vigour index.

Introduction

Wild barley (*Hordeum spontaneum* [K. Koch] Thell.) is a weedy winter annual, dominantly self-fertilizing diploid plant species from the Poaceae family. Wild barley is known to have been used in Neolithic food production in the Near East. Wild barley is considered as the progenitor of cultivated barley (*Hordeum vulgare* subsp. *vulgare* L.) and is a part of the primary gene pool with valuable sources of beneficial genes and largely unexploited resources for improving the narrowing genetic base of cultivated barley (Nevo 1992; Fu, Horbach 2012).

Seed dormancy is a mechanism that can be described as the inability of an intact, viable and mature seed to complete germination under optimal conditions (Linkies, Leubner-Metzger 2012). This mechanism helps the plant to adapt the timing of germination to the surrounding environmental conditions to prevent germination during seasons with unsuitable ecological conditions for the subsequent seedling establishment and plant growth (Linkies, Leubner-Metzger 2012). Dormancy can be caused by different seed tissues, such as embryo, endosperm or seed coat (Linkies, Leubner-Metzger 2012).

In wild barley, dormancy is much stronger than in cultivated barley, and freshly harvested caryopses of wild barley do not germinate in a range of temperatures (10, 20 and 30 °C), in continuous white light or darkness (Gutterman et al. 1996). Primary dormancy in wild barley seeds is caused by covering structures (glumellae as seed covering tissue and pericarp) (Gutterman et al. 1996; Gutterman, Gozlan 1999). The dormancy seems to be largely due to the increased abscisic acid (ABA) diffusion from the seed (Wang 1997). It has been proposed that the covering structures of the seed may reduce the availability of oxygen to the embryo and prevent germination (Gutterman 1996). Moreover, hypoxia condition may also interfere with ABA activity in the seed (Bench-Arnold et al. 2000). It has been found that the removal of glumellae and husk or after-ripening greatly increased the seed germination even in low O_2 conditions (Gutterman et al. 1996; Wang 1997). Thus, wild barley seeds require after-ripening for breaking of dormancy; this usually occurs during storage in dry conditions at 35 °C or in the natural habitat during summer (Gutterman 1996). Seed dormancy and germination are regulated by interaction of plant hormones. After-ripening leads to changes in seed hormone content and/or

sensitivity (Liu et al. 2013). Similarly, it has been suggested that the balance between ABA and gibberellin (GA) is a major regulator of seed dormancy and germination in cereal, *Arabidopsis thaliana* and other species (Bradford et al. 2008). In this case, GA and ABA have antagonistic action; GA breaks dormancy and promotes germination while ABA maintains dormancy and inhibits germination (Chaudhuri et al. 2013). It has been shown that the ABA content in barley embryos was greater in a more dormant variety than in a less dormant variety (Bradford et al. 2008).

The effects of other PGRs, such as brassinosteroids, ethylene, cytokinins, salicylic acid (Kucera et al. 2005; Wang, Irving 2011), jasmonate and auxin (Liu et al. 2013) on the regulation of seed physiological processes have been previously investigated.

High degree of dormancy in wild barley can have a negative effect on the rate and uniformity of germination and also ultimately causing poor seedling establishment. Furthermore, these flaws could hinder their application in breeding programs for cultivated barley. The available data about the effect of PGRs on germination of wild barley seeds are scarce and most studies are mainly limited to cultivated barley (Jacobsen et al. 2002; Gubler et al., 2008). The present investigation was designed to study the effect of different PGRs (indole-3-acetic acid, gibberellic acid and kinetin) on seed germination of wild barley.

Materials and methods

The experiment was conducted in Laboratory of Faculty of Agriculture, University of Kurdistan. Caryopses of wild barley *Hordeum spontaneum* were harvested on 23 July 2015 from the Experimental Station Farm of Kurdistan University. In order to maintain dormancy, wild barley seeds were stored at 5 °C , for a period of 6 months (Shahmoradi et al. 2014).

The seeds were surface sterilized after washing with tap water for 1 h in 1% sodium hypochloride follwed by three washes with sterile distilled water for 5 min. To determine the effect of PGRs on overcoming dormancy, 25 seeds were placed in Petri dishes (90 mm) on filter paper (Whatman No. 1) moistened with test solution (distilled water or different concentrations of PGRs). The treatments included a control (distilled water) and treatments with gibberellic acid (GA_3, 25, 50, 75, 100, 125 and 150 mg L^{-1}), indole-3-acetic acid and kinetin (5, 10, 15, 20, 25 and 30 mg L^{-1}). The above mentioned concentrations were obtained by dissolving of appropriate amounts of respective substances in 1 mL of 1M NaOH, and final volume was made up to 100 mL with distilled water, followed by filter sterilization, and stored at 4 °C. During germination, filter papers were kept moist with the above mentioned solutions. The experiment was carried out for eight days at 20 ± 2 °C, relative humidity of 55 to 65% and continuous darkness in a growth chamber. The number of germinated seeds was recorded daily to determine the required time to achieve the final number of germinated seeds. Seed germination was recorded when the radicle was approximately 2 mm long or more (Rahimi et al. 2013). The germination parameters were calculated by the following formulas.

$$\text{Germination percentage (GP)} = n / N \times 100,$$

where n is a number of seeds germinated, N is a total number of seeds (Mousavizadeh et al. 2013).

$$\text{Germination index (GI)} = \Sigma (ni / ti),$$

where ni is a mumber of germinated seeds in a specified interval, ti is a number of days after germination (Shahmoradi et al. 2014).

$$\text{Mean germination time (MGT)} = \Sigma (ni \times ti) / \Sigma n,$$

where ni is a number of germinated seeds in a specified interval, ti is a number of days after germination, n is a number of seeds planted (Shahmoradi et al. 2014).

Mean rate of germination (MRG) = 1 / MGT (Gholinejad 2012).

$$\text{Germination energy (GE)} = Mng / N \times 100,$$

where Mng is a maximum cumulative percentage of germinated seeds, N is a number of seeds planted (Ahmadloo et al. 2009).

Germination value (GV) = peak value × germination %, where peak value is cumulative percent germination on each day/number of days elapsed since initial imbibition (Ramana et al. 2002).

$$\text{Seed vigour index (SVI)} = Ls \times Pg / 100,$$

where Ls is average length of seedling, Pg is germination percentage (Ghalmbaz, Fateh 2011).

Seedling length was measured for all germinated seeds on the 8th day of the germination test. Also, at the end of the germination test, dry weight of seedlings were determined after oven drying at 70 °C for 48 h.

The experiment was established in a completely randomized design with four replications. Data were analyzed using the MSTATC program and the mean values were compared by Duncan's test at $p \leq 0.05$.

Results

All concentrations of used PGRs effectively increased germination of *H. spontaneum* seeds (Table 1). Among all treatments, the highest GP (64 and 62%) was obtained in 75 and 100 mg L^{-1} GA_3 treatments, respectively. The lowest GP (5%) were recorded in the control treatment. GA_3 at 75 and 100 mg L^{-1}, IAA at 10 mg L^{-1} and kinetin at 10 and 15 mg L^{-1} were more effective to increase GP than other concentrations, and increasing PGR concentration led to a decline in GP (Table 1).

H. spontaneum GI was significantly ($p < 0.01$) affected by the treatments (Table 1). Soaking of seeds in all doses of PGRs significantly increased the GI, compared with control treatment. In addition, the highest GI (3.94 and 3.75) was obtained by applying 75 and 100 mg L^{-1} GA3, respectively. The optimum PGR doses to achieve maximum rate of GI

Table 1. Comparison of means of *Hordeum spontaneum* seed germination traits as affected by growth regulators. GA_3, gibberellic acid; IAA, indole-3-acetic acid; GP, germination pecentage; GI, germination index; MGT, mean germination time; MRG, mean rate of germination; GE, germination energy; GV, germination value; SL, seedling length; SW, seedling dry weight; SVI, seed vigour index.

Treatment	GP (%)	GI	MGT (days)	MRG	GE (%)	GV	SL (cm)	SW (g)	SVI
Control	5 ± 1 h	0.23 ±0.00 g	6.0 ± 2.1 a	0.173 ± 0.001 c	0.26 ± 0.00 j	0.150 ± 0.001 h	5.25±0.86f	0.011 ± 0.002 gh	0.26 ± 0.00 h
GA_3 25 mg L^{-1}	35 ± 2 b-f	2.06 ± 0.40 cd	5.0 ± 1.5 abc	0.198 ± 0.003 abc	2.24 ± 1.70 def	0.790 ± 0.005 c-f	14.98±1.87cde	0.036 ± 0.001 b-e	5.21 ± 2.33 d-e
GA_3 25 mg L^{-1}	41 ± 1 bcd	2.93 ± 0.51 b	4.5 ± 0.9 bc	0.223 ± 0.004 abc	2.75 ± 1.97 bc	1.340 ± 0.080 b	17.6±1.94a-e	0.028 ± 0.002 c-g	6.45 ± 2.46 c-f
GA_3 25 mg L^{-1}	64 ± 3 a	3.94 ±0.66 a	5.2 ± 0.9 abc	0.188 ± 0.002 bc	3.98 ± 2.03 a	3.545 ± 0.750 a	24.4±2.3ab	0.066 ± 0.005 a	15.59 ± 4.73 a
GA_3 25 mg L^{-1}	62 ± 4 a	3.75 ± 0.72 a	5.3 ± 1.3 ab	0.183 ± 0.003 bc	3.83 ± 2.10 a	2.392 ± 0.710 a	20.92±2.1a-d	0.048 ± 0.004 ab	13.20 ± 4.33 ab
GA_3 25 mg L^{-1}	38 ±1 b-e	2.48 ± 0.65 bc	4.8 ± 1.1 abc	0.208 ± 0.003 abc	2.49 ± 2.00 bcd	0.950 ± 0.002 b-e	25.63±2.73a	0.043 ± 0.005 bcd	9.74 ± 3.87 bc
GA_3 25 mg L^{-1}	31 ± 3 c-g	1.90 ± 0.22 c-f	4.8 ± 1.3 abc	0.210 ± 0.004 abc	1.97 ± 1.10 e-h	0.610 ± 0.001 d-g	21.8±2.7labc	0.035 ± 0.003 b-f	6.91 ± 3.12 cde
IAA 5 mg L^{-1}	27 ± 2 efg	1.85 ± 0.21 def	4.8 ± 1.0 bc	0.223 ± 0.004 abc	1.85 ± 1.01 f-i	0.500 ± 0.003 e-h	17.13±1.78b-e	0.022 ± 0.001 e-h	4.58 ± 2.11 d-g
IAA 10 mg L^{-1}	46 ± 2 b	2.84 ± 0.47 b	4.9 ± 1.1 abc	0.205 ± 0.004 abc	2.92 ± 1.83 ±b	1.372 ± 0.070 b	17.2±1.88b-e	0.034 ± 0.002 b-f	7.85 ± 2.37 cd
IAA 15 mg L^{-1}	38 ± 2 b-e	1.98 ± 0.36 cde	4.9 ± 1.3 abc	0.198 ± 0.002 abc	2.06 ± 1.09 d-g	0.790 ± 0.004 c-f	11.35±1.04ef	0.033 ± 0.003 b-f	4.24 ± 2.56 fg
IAA 20 mg L^{-1}	26 ± 3 fg	1.83 ± 0.24 def	3.9 ± 0.9 c	0.253 ± 0.005 a	1.72 ± 1.00 ghi	0.450 ± 0.002 fgh	14.33±1.54cde	0.013 ± 0.001 gh	3.63 ± 1.95 e-h
IAA 25 mg L^{-1}	24 ± 2 fg	1.61 ± 0.20 def	4.3 ± 1.2 bc	0.233 ± 0.004 ab	1.57 ± 1.10 hi	0.382 ± 0.001 fgh	18.02±1.92a-e	0.024 ± 0.001 d-h	4.19 ± 2.17 efg
IAA 30 mg L^{-1}	24 ±2 fg	1.49 ± 0.10 def	4.8 ± 1.3 bc	0.205 ± 0.004 abc	1.54 ± 1.30 hi	0.362 ± 0.003 fgh	11.93±1ef	0.025 ± 0.001 d-h	2.86 ± 1.03 gh
Kinetin 5 mg L^{-1}	27 ± 2 efg	1.79 ± 0.2 def	4.5 ± 1.1 bc	0.228 ± 0.005 abc	1.61 ± 1.50 ghi	0.455 ± 0.002 fgh	10.70±1.03ef	0.011 ± 0.001 gh	2.87 ± 1.06 gh
Kinetin 10 mg L^{-1}	42 ± 2 bc	2.77 ± 0.3 b	5.8 ± 1.3 ab	0.180 ± 0.003 bc	2.74 ± 1.97 bc	1.150 ± 0.090 bc	14.38±1.86cde	0.027 ± 0.002 d-h	6.02 ± 2.14 d-e
Kinetin 15 mg L^{-1}	42 ± 2 bc	2.44 ± 0.31 bc	5.5 ± 1.5 ab	0.178 ± 0.003 bc	2.35 ± 2.10 cde	0.995 ± 0.008 bcd	15.6±1.66cde	0.031 ± 0.002 b-f	6.32 ± 2.64 c-g
Kinetin 20 mg L^{-1}	30 ±2 d-g	1.66 ± 0.2 def	5.1 ± 1.6 abc	0.193 ± 0.002 bc	1.66 ± 1.80 ghi	0.500 ± 0.002 e-h	15.5±1.91cde	0.046 ± 0.003 bc	4.35 ± 1.79 d-g
Kinetin 25 mg L^{-1}	23 ± 2 g	1.46 ± 0.1 def	4.6 ± 1.0 bc	0.218 ± 0.003 abc	1.44 ± 1.08 i	0.320 ± 0.005 gh	13.23±1.09def	0.016 ± 0.002 gh	3.01 ± 2.19 fgh
Kinetin 30 mg L^{-1}	26 ± 1 fg	1.32 ± 0.12 f	5.5 ± 1.4 ab	0.180 ± 0.002 bc	1.55 ± 1.44 hi	0.390 ± 0.006 fgh	13.23±1.1def	0.029 ± 0.003 c-g	3.51 ± 2.31 e-h

were 75 and 100 mg L^{-1} in GA_3 and 10 mg L^{-1} in IAA and kinetin. With increasing the concentration of PGR, the GI was decreased, but it was significantly higher that in the control.

There was a a significant difference ($p < 0.05$) for MGT of *H. spontaneum* seeds exposed to different PGRs (Table 1). All PGR concentrations tested shortened the time necessary to start germination; but the effects of 50 mg L^{-1} GA_3, 5, 20, 25 and 30 mg L^{-1} IAA and 5 and 25 mg L^{-1} kinetin treatments significantly differed only from that in the control. The lowest (3.93 day) and highest (6 day) MGT were obtained in 20 mg L^{-1} IAA and control treatments respectively.

PGRs treatment improved MRG (Table 1). In this case, the highest MRG was obtained when seeds were treated with 20 and 25 mg L^{-1} IAA. Other treatments although increased MRG, but with no significant ($p > 0.05$) difference with the control (Table 1). Generally, IAA was more effective in increasing MRG, compared to GA_3 and kinetin (Table 1).

GE improved dramatically in all seeds treated with PGRs (Table 1). The lowest GE (0.26) was observed in the control treatment, while the highest GE (3.98 and 3.83) was recorded in 75 and 100 mg L^{-1} GA_3 treatments, respectively. IAA and kinetin (10 mg L^{-1}) treatments and 75 and 100 mg L^{-1} GA_3 treatments were suitable doses to obtain maximum rate of GE, compared to other concentrations. In PGR treatments, there was a decreasing trend in GE with increase of PGR concentration.

All concentrations of GA_3 treatments were effective in increasing GV, compared to the control (Table 1). Moreover, seeds treated by GA_3 at 75 and 100 mg L^{-1} resulted in significantly higher GE (3.55 and 2.39) than in other treatments. Both IAA and kinetin treatments had significant effect on GV at 10 and 15 mg L^{-1} concentrations. Other concentrations of IAA and kinetin increased GV, but with no significant difference ($p > 0.05$) from that in the control (Table 1).

GA_3 at all used concentrations dramatically increased seedling length, compared to the control (Table 1). IAA at concentrations of 5, 10, 20 and 25 mg L^{-1} and kinetin at 10, 15 and 20 mg L^{-1} significantly increased seedling length compared with the control treatment. The highest seedling

length (25.63 mm) occurred when seeds were soaked with 125 mg L^{-1} GA_3, and the lowest length (5.25 mm) for untreated seeds (control).

All concentrations of GA_3, except 50 mg L^{-1}, considerably improved seedling dry weight compared with the control (Table 1). Also, all concentrations of IAA (except 10 and 15 mg L^{-1}) and kinetin (except 15 and 20 mg L^{-1}) were effective in increasing seedling dry weight. The highest (0.066 g) and the lowest (0.011 g) weight were obtained in 75 mg L^{-1} GA_3 and control treatments, respectively.

GA_3 at all used concentrations, IAA at concentrations of 5, 10, 15 and 25 mg L^{-1}, and kinetin at concentrations 10, 15 and 20 mg L^{-1} dramatically increased SVI, compared with the control treatment. The highest SVI (15.59) was occurred in 75 mg L^{-1} GA_3, and the lowest SVI (0.26) for untreated seeds.

Discussion

All germination parameters of *H. spontaneum* seeds except mean germination time (MGT) were significantly affected by GA_3. Soaking of seeds at all doses of GA_3 effectively improved germination percentage (GP), germination index (GI), germination energy (GE), germination value (GV), seedling length and seed vigour index (SVI). GA_3 at 50 mg L^{-1} significantly shortened the time necessary to start germination and at all concentrations, except 50 mg L^{-1}, increased seedling dry weight. Gibberellic acid can overcome seed dormancy in several types of seeds (Jacobsen et al. 2002; Gubler et al. 2008). In previous studies, the positive effect of gibberellins on improving seed germination in several plants have been shown, including barley (Jacobsen et al. 2002), *Vigna mungo* and *Macrotyloma uniflorum* (Chauhan et al. 2009), *Teucrium ploium* (Kocheki, Azizi, 2006), and *Secale montanum* (Afrigan et al. 2013).

The GA/ABA balance determines germination and dormancy maintenance in seeds (White et al. 2000; Chibani et al. 2006; Jacobsen et al., 2002). ABA has a positive role in inducing and maintaining dormancy, while GA releases dormancy and stimulates germination (Matilla, Matilla-Vazquez 2008; Chaudhuri et al. 2013). Embryo elongation requires cell expansion in defined regions of the radicle and lower hypocotyl, which is promoted by GA and inhibited by ABA (Linkies, Leubner-Metzger 2013).

GA promotes seed germination by inhibiting ABA activity and decreasing ABA concentration, via activation of catabolizing enzymes and inhibition of the related biosynthesis pathways (Toyomasu et al. 1994; Atia et al., 2009), as well as by the suppressing effects of excess ABA on embryo expansion (Liu et al. 2013).

GA stimulates the synthesis and production of mannanase (Wang et al. 2005) and hydrolytic enzymes, such as α-amylase, which are necessary for germination (Miransaria, Smith 2014). In this case cleared that Gibberellins are able to induce a range of genes, which are necessary for α-amylase, protease and glucanase production (Yamaguchi 2008). GA upregulation of photosystem II oxygen production may enhance the efficiency of energy pathways in plant tissues. This may also be the case in germinating seedlings (Miransaria, Smith 2014).

We showed that IAA at all concentrations was significantly effective for increasing GP, GI and GE. It also improved other germination parameters, depending on the used dose. The role of auxin in germination has been described by many authors. Auxin by itself may not be a necessary hormone for seed germination, but it interferes with other PGPs like gibberellins and ethylene, and may influence the processes of seed germination and establishment (Fu, Harberd 2003; Chiwocha et al. 2005). IAA can stimulate the production of ethylene (Arteca, Arteca 2008) and ethylene plays a positive role in dormancy release and seed germination (Hermann et al. 2007; Finkelstein et al. 2008; Chaudhuri et al. 2013). IAA can affect seed germination by altering the activity of enzymes; for example, in germinating pea seeds, increase of glyoxalase I activity by IAA leads in higher rates of cell growth and development (Miransaria, Smith 2014). Furthermore, auxin is necessary for the growth of young seedlings (Hentrich et al. 2013).

In contrast to the above, some reports have suggested the potential involvement of auxin in maintenance of seed dormancy in *Arabidopsis* (Brady et al., 2003; Liu et al., 2007; Liu et al 2013) and also in delay of seed germination of wheat (Morris et al., 1988; Ramaih et al., 2003). Auxin promotes dormancy and inhibits germination by enhancing ABA action, but together auxin and ABA act synergistically to inhibit seed germination and the auxin-mediated inhibition of seed germination is dependent on ABA (Liu et al. 2013). As a result, an equal amount of IAA did not inhibit seed germination in the absence of ABA, but auxin can influence seed germination when ABA is present (Brady et al. 2003). Thus, it seems that the mechanism of auxin function in seed germination is not well understood yet.

As previously mentioned, seed treatment with all concentrations of kinetin considerably promoted GP, GA and GE, compared with the control. Furthermore, it also improved other evaluated traits (except MRG) depending on the applied dose. Other studies showed that cytokinins have stimulatory effects on the seed germination of a wide range of plant species (Jones, Stoddart 1977; Thomas 1997). In *Gossypium barbadense* treatment with kinetin improved seed viability and seedling vigour, as shown by increased length of hypocotyl, radicle and the entire seedling, as well as seedling fresh weight (Sawana et al. 2000). Kinetin application can be useful for breaking the strong dormancy commonly observed in the dehusked seeds of indica rice (Miyoshi, Sato 1997). Cytokinins are able to promote seed germination especialy under stress conditions (Peleg, Blumwald 2011). Cytokinins and their negative interaction

with ABA can positively regulate seed germination and break seed dormancy (Miransaria, Smith, 2014).

In conclusion, our results demonstrated that all PGRs, depending on the used concentration, improved the seed germination in *Hordeum spontaneum*. Overall, GA_3 especially at 75 mg L^{-1} and followed by 100 mg L^{-1}, was most effective in improving seed germination parameters, except MGT, which was not influenced by GA_3. Thus, the present study recommends that application of PGRs like GA_3, IAA and kinetin can be useful for overcoming dormancy and in promotion rapid germination in *Hordeum spontaneum*.

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Received 2 July 2016; received in revised form 9 September 2016; accepted 20 September 2016