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ORIGINAL ARTICLE

Exogenous putrescine increases the responsiveness of thermodormant *Avena fatua* L. caryopses to karrikinolide and gibberellic acid

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Abstract A natural feature of freshly harvested *Avena fatua* L. caryopses is primary dormancy which, however, was relieved partially by putrescine (Put) (10^{-2} M) and completely by karrikinolide (KAR_1) (3×10^{-9} M) or gibberellic acid (GA_3) (10^{-5} M). The sensitivity of *A. fatua* caryopses to these stimulators was adversely affected by supraoptimal temperature (SOT) (30 °C). A reduced germinability of caryopses due to high temperature even after transferring them to lower temperatures (10 or 20 °C) indicated the induction of thermodormancy. The maintenance of relatively constant levels of abscisic acid (ABA) in embryos but not surrounding tissues during SOT treatment was observed. The application of Put either during the SOT treatment or afterwards counteracted the effects of high temperature but had no significant impact on ABA content. The action of exogenous Put in alleviating the loss of responsiveness to KAR_1 and GA_3 imposed by SOT treatment in *A. fatua* PD caryopses is discussed in reference to the interconnection between ABA and GA metabolism and signaling pathways.

Keywords *Avena fatua* · Fluridone · Gibberellin · Karrikinolide · Paclobutrazole · Putrescine · Primary dormancy · Thermodormancy

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Introduction

Avena fatua L. (wild oat) is a cosmopolitan grass species listed among the most serious weeds in the world (Holm et al. 1977; Krähmer 2016). An effective weed control is severely hampered by the persistence of *A. fatua* caryopses stored in soil (Naylor and Fedec 1978; Fennimore et al. 1998). Research concerning mechanisms that control the germination of *A. fatua* caryopses may help to improve methods of reducing their soil stored reservoirs.

Weed seeds persist in agricultural soils owing to seed dormancy, the degree of which defines what conditions should be met to make the seed germinate (Thompson and Ooi 2010). Dormancy is usually acquired already during seed maturation on the mother plant (primary dormancy) or can be imposed after seed dispersal (secondary or induced dormancy) by unfavorable environmental cues (Bewley and Black 1994). One of them may be supraoptimal temperature (SOT) which may prevent germination either through thermoinhibition or thermodormancy (Hills and van Staden 2003). The former ceases as soon as the temperature is reduced; the latter exemplifies a type of secondary dormancy and requires a dormancy-breaking condition to be overcome.

A response of plants to environmental cues comprises substantial changes in active regulatory compounds in different cellular compartments and organs, among them polyamines (PAs). Putrescine (Put), spermidine (Spd) and spermine (Spm) are most often detected PAs in plants in relatively large quantities (Minocha et al. 2014). Variations in their cellular contents are often connected with different phases of growth activity and to varied responses of plants to different forms of stress. PA levels and activities of their biosynthetic enzymes may increase in reaction to plant stresses, including high-temperature stress (Das et al. 1987).

The aim of our studies was to compare the efficacy of Put with karrikinolide (KAR_1) and gibberellic acid (GA_3) in releasing primary dormancy and preventing the induction of thermodormancy in *A. fatua* caryopses. Using fluridone (FL) and paclobutrazol (PBZ), well-known biosynthesis inhibitors of abscisic acid (ABA) and gibberellins (GAs), respectively, and also by analyzing the content of ABA, we tried to explain the mode of action of Put in alleviation of SOT impact on the germinability of wild oat caryopses.

Materials and methods

Avena fatua L. spikelets were collected in Poland near Szczecin in July 2011. Spikelets contain two or three florets covered with glumes. The floret is a single caryopsis (fruit) covered by the lemma and palea (Simpson 2007). After collection, spiklets were dried at room temperature for 7 days to a constant moisture content (ca 11%) and then stored at −20 °C to maintain primary dormancy. In the experiments, only the caryopses were used so both lemma and palea were removed from the florets.

Primary dormant (PD) caryopses, 25 in each of three replicates, were incubated in darkness, in 6-cm Petri dishes on one layer of filter paper (Whatman No. 1) moistened with 1.5 mL deionized water or a solution of the tested compound at 10, 20 or 30 °C. Germinated caryopses (with a radicle protruded through the coleorhiza) were counted during up to 14 days of incubation. In some experiments, caryopses were preincubated at 30 °C (SOT treatment), then rinsed with 100 mL deionized water and then incubated at 10 or 20 °C.

Compounds used in the experiments were purchased from biochemical suppliers: GA_3 (Sigma-Aldrich, USA), Put and FL (Fluka, Germany), PBZ (Duchefa Biochemie). KAR_1 was synthetized (Cembrowska-Lech et al. 2015). They were applied at optimal concentrations determined in preliminary experiments.

To quantify the endogenous level of ABA in *A. fatua* L. caryopses, in each of 5 replicates 25 caryopses were incubated at 20 or 30 °C in darkness, in 6-cm Petri dishes on one layer of filter paper (Whatman No. 1) moistened with deionized water or with Put (10^{-2} M). After 1 or 4 days, embryos were dissected from caryopses. Both dissected embryos and endosperms with bran left after embryo excision were frozen immediately in liquid nitrogen and stored at −80 °C prior to ABA analysis conducted according to the procedure described by Cembrowska-Lech et al. (2015) with [$^2H_6$]-ABA (100 ng/sample) as an internal standard obtained from ICON ISOTOPES (USA).

Results

Germination of PD caryopses of *A. fatua* in water was very limited (Fig. 1). Only up to 40% of them germinated at 10 °C (Fig. 1A) whereas at 20 °C they could hardly germinate during 14-day incubation (Fig. 1B). Exogenous Put (10^{-2} M) caused ca 60% or about 20% germination at 10 or 20 °C, respectively (Fig. 1). Both KAR_1 and GA_3 were highly effective at 10 °C, causing about 90% germination (Fig. 1A). Due to KAR_1 or GA_3 applied at 20 °C, nearly 90% or over 75% of PD caryopses germinated, respectively (Fig. 1B). However, FL (10^{-4} M) had no significant effect on their germination at 10 or 20 °C compared to the control treatment (Fig. 1).

PD caryopses of *A. fatua* were totally unable to germinate at 30 °C in water or in the presence of Put, but their germination due to KAR_1 or GA_3 was only a little less than 50% (Table 1). Application of Put in combination with KAR_1 or GA_3 further

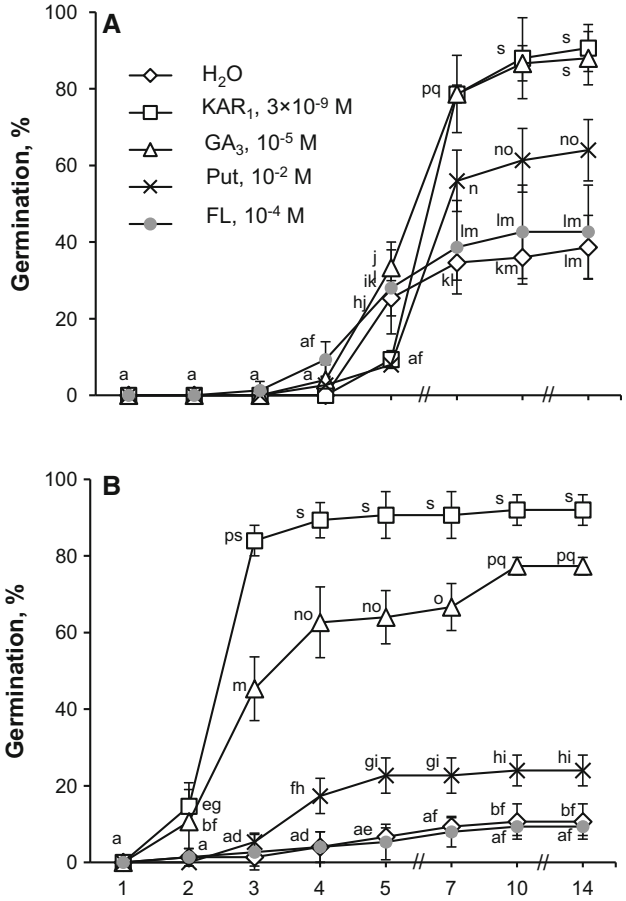

Fig. 1 The effect of KAR_1, GA_3, Put and FL on the germination of PD *A. fatua* caryopses at 10 (**A**) and 20 °C (**B**). % germination was determined during 14 days of incubation. The vertical bars indicate ± SD. Two-way ANOVA with the Duncan's post hoc test was used to identify significant differences. Means with *different letters* (*a–s*) are significantly different ($P < 0.05$, $n = 3$)

Table 1 The effect of Put or FL on the germination of PD *A. fatua* caryopses in the absence or presence of KAR_1 or GA_3 at 30 °C

Compound		Putrescine (Put)		Fluridone (FL)	
Concentration, M	0	10^{-2}	2×10^{-2}	10^{-4}	3×10^{-4}
Water	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
KAR_1, 3×10^{-9}	42.7 ± 8.5 b	73.3 ± 8.3 d	78.7 ± 5.7 d	88.0 ± 8.0 d	85.3 ± 5.7 d
GA_3, 10^{-5}	46.7 ± 4.6 b	80.0 ± 9.8 d	74.7 ± 9.6 d	61.9 ± 5.4 c	65.3 ± 6.1 d

% Germination was determined after 7 days of incubation

Data displayed as mean values ± SD

Two-way ANOVA with the Duncans's post hoc test was used to identify significant differences

Means with different letters (a–d) are significantly different ($P < 0.05$, $n = 3$)

increased the germination of caryopses by ca 30%. Similarly to Put, FL alone was ineffective as a germination stimulator at 30 °C. Nevertheless, if applied in combination, FL improved the final percentage of germination due to KAR_1 or GA_3, up to over 80 or 60%, respectively.

When PD caryopses were preincubated at 30 °C (SOT treatment) in water and after transfer to 10 or 20 °C further incubated in water, their percentage of germination was around 10%, irrelevant to the length of preincubation time (Fig. 2A, B). Application of Put during SOT treatment had no significant effect on subsequent germination at 10 or 20 °C, in water (Fig. 2A, B). Around 60 or 40% germination on KAR_1 at 10 or 20 °C, respectively, was observed for SOT-treated caryopses for 1 day in water (Fig. 2C, D). If the preincubation period was longer, 4 or 8 days, about 30 or 40% of caryopses germinated in response to KAR_1 at 10 or 20 °C, respectively. However, Put applied during SOT treatment increased subsequent germination of caryopses in the presence of KAR_1 of about 20–30% (Fig. 2C, D). Irrespective of the absence or presence of Put during 1- or 4-day SOT treatment, up to 90% caryopses germinated subsequently at 10 °C in the presence of GA_3 (Fig. 2E). Even after prolonged to 8 days SOT treatment in water, there was still nearly 60% germination due to GA_3 at 10 °C. If SOT-treated caryopses were subsequently incubated at 20 °C, their germination in response to GA_3 was around 40%, irrespective of the length of preincubation time in water (Fig. 2F). Put improved the response to GA_3 at 10 °C of 8-day SOT-treated caryopses and at 20 °C of those preincubated at 30 °C for any period of time (Fig. 2E, F).

The presence of FL during 4-day SOT treatment had no effect on the germination of caryopses in water after transfer to 10 or 20 °C (Fig. 3). However, if SOT-treated caryopses were subsequently incubated at 10 or 20 °C on KAR_1, FL applied alone or in combination with Put during 4-day preincubation at 30 °C caused higher germination percentage in comparison to Put used alone. If GA_3 was used instead of KAR_1, the stimulatory effect of FL used alone or in combination with Put during SOT treatment on subsequent germination was evident at 20 but not 10 °C.

If *A. fatua* caryopses were SOT treated in water for 4 days, they germinated poorly at 10 or 20 °C either in the absence or presence of Put (Fig. 4). Their germination, however, in response to KAR_1 applied in combination with Put was nearly two (Fig. 4A) or two and a half (Fig. 4B) times higher in comparison to the effect of KAR_1 alone. These caryopses achieved very high, ca 80%, germination percentage in response to GA_3 at 10 °C and simultaneous addition of Put did not improve their response (Fig. 4A). In contrast, when these caryopses were subsequently incubated at 20 °C, fewer than 40% germinated in the presence of GA_3 alone, whereas over double as many germinated due to GA_3 applied in combination with Put (Fig. 4B). Application of FL during subsequent incubation at 10 °C increased by over two times the final germination percentage of caryopses SOT treated in water (Fig. 4A). FL improved by nearly 40% their responsiveness to KAR_1 but had no effect on the response to GA_3 (Fig. 4A). The response of SOT-treated caryopses to KAR_1 or GA_3 was not changed by FL when they were transferred to 20 °C (Fig. 4B).

The content of ABA was determined in dry and imbibed PD *A. fatua* caryopses, separately in isolated embryos and endosperms with bran (Table 2). The highest level of endogenous ABA (387.1 ± 41.9 ng per g FW) was detected in embryos isolated from dry caryopses. After 1 day of incubation in water at 20 or 30 °C, the content of ABA in embryos decreased by about one half and did not change after 4 days. Put had no influence on ABA content in embryos isolated from imbibed caryopses. Endosperms with bran contained approximately 20 times less ABA compared to embryos isolated from dry caryopses (Table 2). Their ABA content also decreased by about one half after 1-day incubation either at 20 or 30 °C; extended incubation up to 4 days further reduced ABA level over two times. Like in embryos, also in endosperms with bran, endogenous ABA was not affected by Put.

Application of PBZ (10^{-4} M) served to eliminate the action of endogenous GAs and caused total inhibition of PD caryopses germination at 30 °C, also in the presence of KAR_1 but had no effect in combination with GA_3 (Table 3). Addition of Put to the combination of KAR_1 and PBZ did not improve the germination ability of caryopses. However, Put applied in a mixture with both GA_3 and PBZ

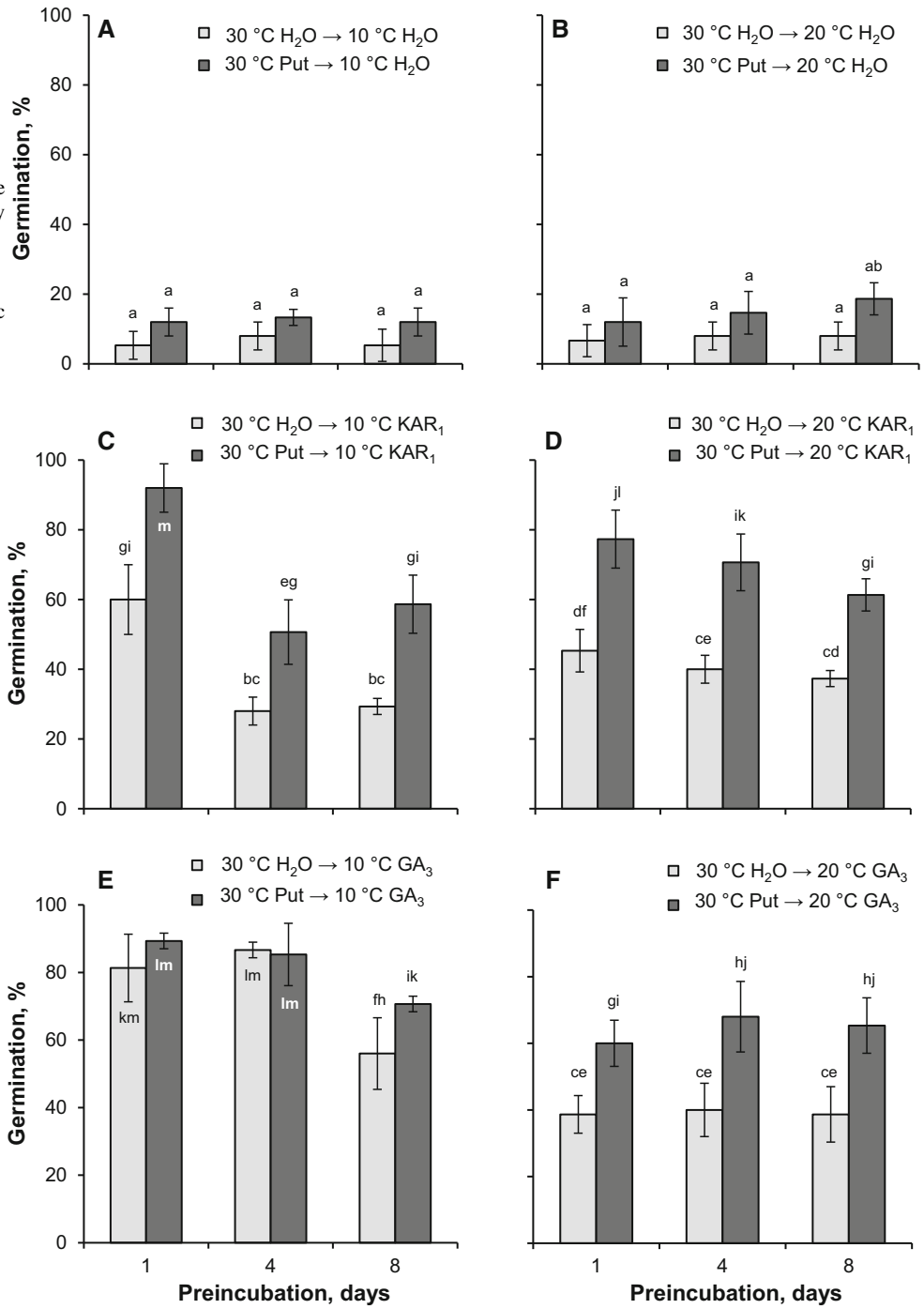

Fig. 2 The effect of Put (10^{-2}M) applied during SOT treatment (preincubation at 30 °C) on the subsequent germination of PD *A. fatua* caryopses in water (**A**, **B**), KAR_1 (3×10^{-9}M) (**C**, **D**) or GA_3 (10^{-5}M) (**E**, **F**) at 10 or 20 °C. % germination was determined after the transfer from 30 to 10 or 20 °C at day 14 (**A**, **C**, **E**) or 7 (**B**, **D**, **F**) of incubation, respectively. Vertical bars indicate ± SD. Two-way ANOVA with the Duncan's post hoc test was used to identify significant differences. Means with *different letters* (*a–m*) are significantly different ($P < 0.05$, $n = 3$)

increased the percentage of germination as compared to GA_3 and PBZ alone.

Discussion

Primary dormant *A. fatua* caryopses were hardly able to germinate at 20 °C (Fig. 1B), the temperature considered as optimal for germination of non-dormant wild oat caryopses (Sawhney et al. 1984; Symons et al. 1987). The state of primary dormancy in *A. fatua*, which develops during the maturation of caryopses on the parent plant, can be effectively released by gibberellins (Adkins et al. 1986; Kępczyński et al. 2013; Cembrowska-Lech and Kępczyński 2016) and as shown recently also by karrikinolide (Daws et al. 2007; Stevens et al. 2007; Kępczyński et al. 2010, 2013; Kępczyński and van Staden 2012; Cembrowska-Lech et al. 2015; Cembrowska-Lech and

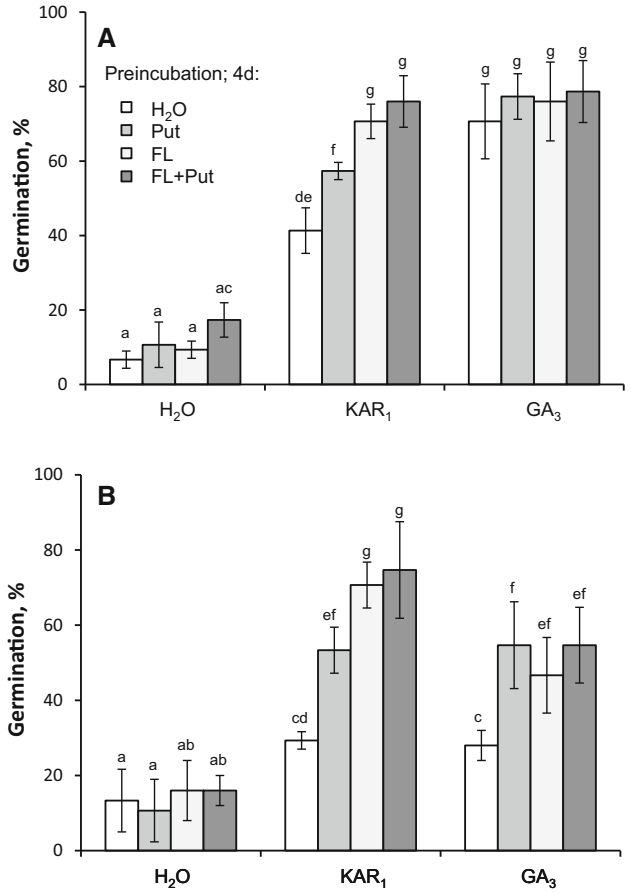

Fig. 3 The effect of FLD (10^{-4}M) and/or Put (10^{-2}M) applied during SOT treatment (4-day preincubation at 30 °C) on germination of *A. fatua* PD caryopses in water, KAR_1 (3×10^{-9}M) or GA_3 (10^{-5}M) at 10 (**A**) or 20 (**B**) °C. % Germination was determined at day 14 of incubation at 10 °C (**A**) or at day 7 of incubation at 20 °C (**B**). *Vertical bars* indicate ± SD. Two-way ANOVA with the Duncan's post hoc test was used to identify significant differences. Means with *different letters* (*a–g*) are significantly different ($P < 0.05$, $n = 3$)

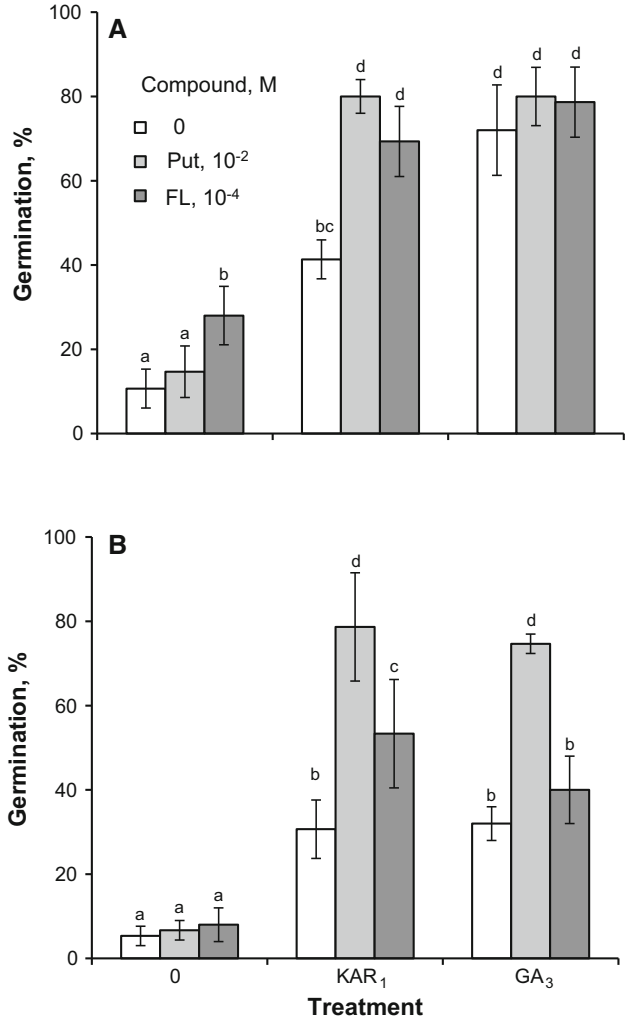

Fig. 4 The effect of KAR_1 (3×10^{-9}M) or GA_3 (10^{-5} M) in combination with Put or FL on the germination at 10 or 20 °C of SOT-treated PD *A. fatua* caryopses (preincubated at 30 °C in water for 4 days). % Germination was determined at day 14 of incubation at 10 °C (**A**) or at day 7 of incubation at 20 °C (**B**). *Vertical bars* indicate ± SD. Two-way ANOVA with the Duncan's post hoc test was used to identify significant differences. Means with *different letters* (*a–d*) are significantly different ($P < 0.05$, $n = 3$)

Kępczyński 2016). Similarly, in our experiments, both KAR_1 (3×10^{-9} M) or GA_3 (10^{-5} M) were able to break the primary dormancy of *A. fatua* caryopses at temperatures ≤20 °C (Fig. 1A, B). Moreover, both KAR_1 and GA_3 also markedly stimulated their germination at alleviated temperature of 30 °C (Table 1).

Supraoptimal temperatures impose the suspension of germination, either causing thermoinhibition or inducing thermodormancy (Hills and van Staden 2003). If PD *A. fatua* caryopses were transferred from 30 to 10 °C, the temperature at which their dormancy should be less expressed, only around 10% of them germinated (Fig. 2A) instead of about 40% which would be the case if no SOT treatment occurred (Figs. 1A, 2A). A similar phenomenon was described in closely related species of *Avena sativa*, in which a loss of germinability at 20 °C (a temperature suitable for the germination of PD grains) followed their exposure to 30 °C (Corbineau et al. 1993). In addition, the responsiveness of SOT-treated PD *A. fatua* caryopses to otherwise highly effective dormancy-breaking agents (KAR_1 or GA_3) was reduced (Fig. 2C–F). Such germination impairments may be interpreted as a manifestation of primary dormancy reinforcement, which in turn can be considered as an induction of thermodormancy.

Although seed dormancy may be released by PAs, the type of PA, most active as an enhancer of germination, varies in different plant species (Matilla 1996). There are several reports on Put promoting seed germination, even under adverse conditions (Sińska and Lewandowska 1991;

Table 2 ABA content in embryos and endosperms with bran isolated from dry or imbibed PD *A. fatua* caryopses

Time (day)	Compound, M	Embryos		Endosperms with bran	
		Water	Put, 10^{-2}	Water	Put, 10^{-2}
	Temperature (°C)	PD	PD	PD	PD
0		387.1 ± 41.9 d		20.4 ± 3.3 c	
1	20	220.2 ± 24.5 bc	263.1 ± 22.9 c	11.5 ± 2.2 b	12.5 ± 2.0 b
	30	205.1 ± 56.2 ab	247.9 ± 55.6 bc	10.0 ± 1.2 b	10.6 ± 1.0 b
4	20	237.1 ± 28.4 bc	228.2 ± 38.2 bc	6.0 ± 0.6 a	5.8 ± 1.1 a
	30	156.3 ± 13.2 a	192.2 ± 51.1 ab	3.7 ± 0.9 a	4.4 ± 0.9 a

Caryopses were incubated at 20 or 30 °C for 1 or 4 day in water or Put. ABA content expressed in ng g^{-1} FW

Data displayed as mean values ± SD

Two-way ANOVA with the Duncan's post hoc test was used to identify significant differences*

Means with different letters (a–d) are significantly different ($P < 0.05$, $n = 5$)

* Statistical tests were performed separately for embryos and endosperms with bran

Table 3 The effect of Put alone or in combination with KAR_1 or GA_3 on the germination of PD *A. fatua* caryopses in the absence or presence of PBZ at 30 °C

Compound	Paclobutrazol (PBZ)	
Concentration, M	0	10^{-4}
Water	0 ± 0 a	0 ± 0 a
Put, 10^{-2}	0 ± 0 a	0 ± 0 a
KAR_1, 3×10^{-9}	41.3 ± 2.3 b	0 ± 0 a
GA_3, 10^{-5}	42.7 ± 6.1 b	32.0 ± 4.0 b
KAR_1, 3×10^{-9} + Put, 10^{-2}	77.3 ± 10.1 d	0 ± 0 a
GA_3, 10^{-5} + Put, 10^{-2}	84.0 ± 5.6 d	62.7 ± 4.6 c

% Germination was determined after 7 days of incubation

Data displayed as mean values ± SD

Two-way ANOVA with the Duncan's post hoc test was used to identify significant differences

Means with different letters (a–d) are significantly different ($P < 0.05$, $n = 3$)

Szczotka et al. 2003; Zeid and Shedeed 2006, Krasuska et al. 2014; Li et al. 2014). In our experiments, exogenous Put (10^{-2} M) could also release *A. fatua* caryopses from primary dormancy at lower temperatures although less effectively in comparison with KAR_1 or GA_3 (Fig. 1). There are some reports on improving germination of seeds exposed to SOT by simply presoaking them in Put solution (Cavusoglu and Kabar 2007; Sedaghat and Rahemi 2011; Khan et al. 2012). Moreover, compelling evidence indicates the involvement of PAs in stress responses. Therefore, we applied exogenous Put during incubation of *A. fatua* caryopses at SOT to assess its efficacy in preventing thermodormancy. Interestingly, although unable to prompt the germination of the caryopses incubated at 30 °C, Put could increase their sensitivity to GA_3 or KAR_1 (Table 1). Either applied during SOT treatment or afterwards, Put enhanced caryopses germination in the presence of KAR_1 or GA_3, after transferring them to lower temperature (Figs. 2, 3, 4). These findings raise questions about the mechanism by which Put improves germinability of PD caryopses that underwent SOT treatment. The participation of PAs in stress responses as antioxidants, free radical scavengers and membrane stabilizers (Minocha et al. 2014) may account for Put action in alleviating the loss of sensitivity to dormancy-breaking regulators in thermodormant *A. fatua* caryopses (Table 1; Fig. 2). However, exogenous Put exerted similar effects, either applied during or after the SOT treatment (Figs. 2, 3). Additionally, high temperature (30 °C) had no effect on electrolyte leakage, H_2O_2 content or catalase activity in *A. fatua* PD caryopses (data not shown) which would indicate membrane damage as well as some oxidative stress that Put could counteract. This would suggest that the effect of Put was probably not due to its physico-chemical protective role but quite possibly comprised the triggering of some repair mechanisms.

ABA plays a major role in inducing and maintaining seed dormancy (Kermode 2005) and there is also vast evidence of its involvement in plant responses to environmental stresses, especially caused by abiotic factors (Verma et al. 2016). There are several reports proving the connection between ABA signaling, polyamines metabolism and plant defense system (Toumi et al. 2010; Marco et al. 2011; Saha et al. 2015). Therefore, we attempted to elucidate a probable connection between exogenous Put effects and endogenous ABA in thermodormancy induction and alleviation in *A. fatua* caryopses. It is well documented that high temperature induces ABA synthesis in seeds and this fact may account for their inability to germinate (Yoshioka et al. 1998; Tamura et al. 2006; Toh et al. 2008). In contrast to these reports, there was no change in ABA content in *A. fatua* PD caryopses due to SOT treatment after 1 day of incubation, either in embryos or remaining

tissues (Table 2). Moreover, 4-day incubation of caryopses at 30 °C resulted in a reduction of ABA level in embryos in comparison to those incubated at 20 °C. This, however, remains in agreement with the report of Leymarie et al. (2009) concerning thermodormancy induction in PD barley grains, in which dormancy expression during seed imbibition at 30 °C has been associated not with the increase but the maintenance of ABA at relatively high levels in the embryo. High ABA content and sensitivity are often related to both thermoinhibition and thermodormancy in seeds (Yoshioka et al. 1998; Argyris et al. 2008; Leymarie et al. 2009). The results of several studies, however, indicate that there exists no correlation between ABA content and the induction of secondary dormancy by the exposure to high temperature (Ozga and Dennis 1991; Corbineau and Côme 2003; Hoang et al. 2012). Even if an absolute level of ABA may be of not much importance, its continuous synthesis is necessary to prevent germination at a wide range of temperatures (Yoshioka et al. 1998; Toh et al. 2008; Leymarie et al. 2009; Hoang et al. 2012). The inhibition of ABA biosynthesis by fluridone restored the seed germination of different plant species at high temperatures (Yoshioka et al. 1998; Toh et al. 2008). In *A. fatua* caryopses, during or after induction of thermodormancy, the application of FL also increased their germination but only in the presence of KAR_1 or GA_3 (Table 1; Fig. 4). Mere blocking ABA synthesis was not sufficient to release *A. fatua* caryopses from dormancy irrelevant to the temperature of incubation (Fig. 1; Table 1) which would strongly suggest the necessity of additional dormancy-breaking cues.

An antagonism between ABA and GAs in the control of both dormancy and germination seems to be an axiom nowadays. An interconnection between ABA and GA metabolism and signaling pathways most probably also determines whether or not thermodormancy will be induced (Gonai et al. 2004; Toh et al. 2008; Bahin et al. 2011). Transcriptomic analysis has shown that *Arabidopsis thaliana* seeds imbibed at SOT activate ABA biosynthetic genes, while repressing GA biosynthetic and signaling ones (Toh et al. 2008; Chiu et al. 2012). ABA may be the inhibitory agent in dormant and thermodormant seeds that represses GA biosynthesis (Pérez-Flores et al. 2003; Toh et al. 2008) and also blocks GA signaling (Gubler et al. 2002). Thus, probably in SOT-treated *A. fatua* PD caryopses the maintenance of relatively constant levels of ABA (Table 2) might also prevent the germination promotive action of endogenous GAs in a similar manner. The application of exogenous GA_3 partially complements that blockage (Table 1). If KAR_1 is used, the signaling route may be different although this regulator requires endogenous GAs for its action (Kępczyński et al. 2013); therefore, the germination under high temperature in the presence of KAR_1 is also reduced (Table 1). Further decrease in GA biosynthesis by PBZ totally abolishes KAR_1 germination promotive effect at SOT (Table 3). Such a relationship might support the notion of an ABA-mediated reduction in both GA biosynthesis and GA signal transduction as a basis for thermodormancy induced in *A. fatua* PD caryopses.

Based on the Put modulation of ABA biosynthesis at the transcriptional level in response to low temperature, a novel mode of action for PAs as regulators of hormone biosynthesis has been proposed by Cuevas et al. 2009. In our work, however, there was no significant effect of exogenous Put on the amount of ABA in PD caryopses incubated at 20 or 30 °C for 1 or even 4 days (Table 2). The results of the experiment with PBZ applied in combination with either KAR_1 or GA_3 and Put simultaneously (Table 3) strongly suggest that exogenous Put could not have affected endogenous GAs synthesis/content as it was unable to support the KAR_1 promotive effect. More probably, Put might be involved in unblocking the signaling pathways of dormancy-breaking regulators.

Summing up, the temperature of 30 °C induces thermodormancy in PD *A. fatua* caryopses, which manifests itself in reduced germinability at low temperature (10 °C) subsequently to SOT treatment as well as generally decreased responsiveness to KAR_1 or GA_3. Exogenous Put, either applied during or after the SOT treatment, improves *A. fatua* caryopses germination combined with KAR_1 or GA_3, probably by triggering some yet unidentified repair mechanisms. Although, no change in ABA content due to Put in SOT-treated *A. fatua* PD caryopses was observed, it cannot be ruled out that Put affects ABA-mediated GA signaling pathway as well as interrelated KAR_1 signal transduction.

Author contribution statement JK initiated the research, interpreted the results and revised the manuscript. IR conducted experiments and statistical analysis, interpreted the results and wrote the manuscript. The authors declare that they have no conflict of interest.

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