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Inhibition of germination of barley seeds by inhibitors of gibberellic acid synthesis: reversal by oxygen or hydrogen peroxide

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RÉSUMÉ

FONTAINE-ROUX, O., J.-P. BILLARD, T. GASPAR & C. HUAULT (1997). Inhibition de la germination de l'orge par des inhibiteurs de la synthèse de l'acide gibbérellique: réversion par de l'oxygène ou de l'eau oxygénée. *Saussurea* 28: 59-64. En anglais, résumés français et anglais.

La germination de l'orge est inhibée par des inhibiteurs de monooxygénases dépendantes du cytochrome P-450 (tetcyclasis et triadimefon) et des dioxygénases dépendantes du 2-cétoglutarate (Ca-prohexadione). Quel que soit l'inhibiteur utilisé, l'inhibition de la germination est réversée par l'acide gibbérellique, l'oxygène (O_2) ou l'eau oxygénée (H_2O_2). Il est conclu que la disponibilité en oxygène contrôle l'activité des oxygénases impliquées dans les changements hormonaux conduisant à la germination des semences d'orge dormantes.

ABSTRACT

FONTAINE-ROUX, O., J.-P. BILLARD, T. GASPAR & C. HUAULT (1997). Inhibition of germination of barley seeds by inhibitors of gibberellic acid synthesis: reversal by oxygen or hydrogen peroxide. *Saussurea* 28: 59-64. In English, French and English abstracts.

Germination of barley seeds was inhibited both by inhibitors of cytochrome P-450-dependent monooxygenases (tetcyclacis or triadimefon) and of 2-ketoglutarate-dependent dioxygenases (prohexadione-Ca). Whatever the inhibitor used, the inhibition of germination was reversed by gibberellic acid, O_2 or H_2O_2. It was assumed that oxygen availability controls the activity of oxygenases involved in the hormonal changes leading to germination of dormant barley seeds.

Key words – *Hordeum vulgare* – Prohexadione – Seed germination – Tetcyclacis – Triadimefon.

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Abbreviations – ABA = abscisic acid; GA = gibberellic acid; H_2O_2 = hydrogen peroxide; KGD = 2-ketoglutarate-dependent dioxygenase; P-450 = cytochrome P450-dependent monooxygenase; PHD = prohexadione-Ca; TDF = triadimefon; TET = tetcyclacis.

1. Introduction

The biochemical basis of cereal dormancy remains unclear. A variable period of after- ripening is generally necessary for seeds to become fully germinable [2]. Oxygen has a crucial role in the initiation of germination. Dormancy of cereal seeds has been explained by a limitation of the oxygen supply to the embryo by the seed coats. Indeed, it has been shown that the seed coats fix oxygen through polyphenol oxidase-mediated oxidation of phenolic compounds [6]. Cereal dormancy can be broken by oxygen [3, 7], or by oxygen-generating compounds such as hydrogen peroxide [8] or hypochlorite [11]. Furthermore, the transient supply of inhibitors of cytochrome oxidase, such as azide and cyanide, led also to a rapid germination of dormant cereal seeds [5]. Moreover, it has been proposed that the resulting spare of oxygen would become available for key oxidases involved in the initiation of germination [15].

In another connection, it has been shown that the very early stages of barley seed germination are characterized by a drop in abscisic acid (ABA) level in embryo and by a concomitant increase of gibberellic acid (GA) level [1]. This hormonal change might be counteracted by the seed envelopes since isolated cereal embryos can fully germinate [17].

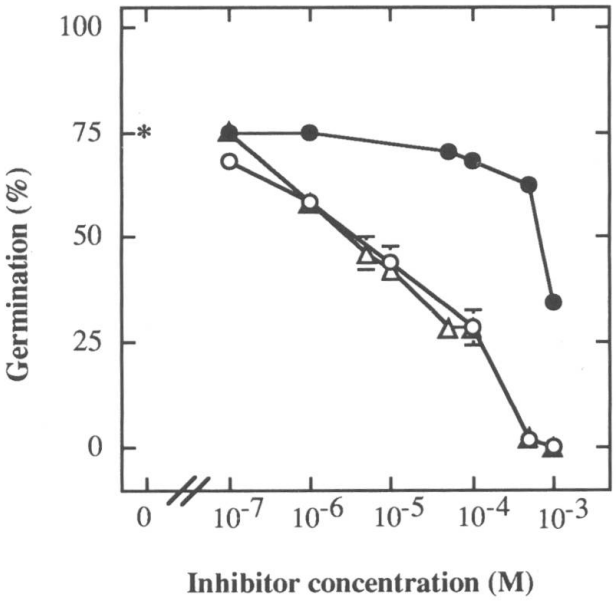
One can wonder whether a common factor, such as oxygen, might trigger the rapid and inverse evolutions of ABA and GA concentrations occurring during the first hours following seed imbibition. A relationship between oxygen and hormones has already been considered. Indeed, low oxygen tensions suppress, like ABA, precocious germination of immature embryos of cereals [9, 13]. Moreover, mature oat embryos cannot germinate under anoxia (N_2 gas), but if GA is added in the medium they can [16]. In our opinion, the seed envelopes might prevent, during dormancy, oxidative events leading to ABA degradation and to synthesis of the active (s) form (s) of GA. This would explain the rapid germination of embryos isolated from dormant wheat seeds [17]. In this context, it is striking that both ABA degradation [18] and the first steps of GA synthesis leading from ent-kaurene to ent-kaurenoic acid [14] require oxygen and involve cytochrome P_{450}- dependent monooxygenases (P-450s). Moreover it has to be noticed that the late steps of GA synthesis need also oxygen and involve 2-ketoglutarate-dependent dioxygenases (KGD).

The present work was designed to study, in relation to oxygen and hydrogen peroxide, the effects of inhibitors of P-450s and of KGD on germination of barley seeds.

2. Material and methods

For the germination assays, non-dormant seeds were surface-sterilized (8 min) with Na-hypochlorite solution (commercial Javel water diluted 5 times) and then rinsed with distilled water. Seed pretreatments and germination tests were performed with lots of 50 seeds, in temperature-controlled incubator at 20°C, under continuous white light

Fig. 1. – Effect of concentration of tetcyclacis (TET), triadimefon (TDF) or prohexadione (PHD) on barley seed germination measured after 3 days. Seeds were imbibed for 24 h on filter paper moistened with TET (o), TDF (Δ) or PHD (•). Seeds were then transferred for 48 h on filter paper moistened with distilled water. Vertical bars represent SD (n = 4). * Control without inhibitor.



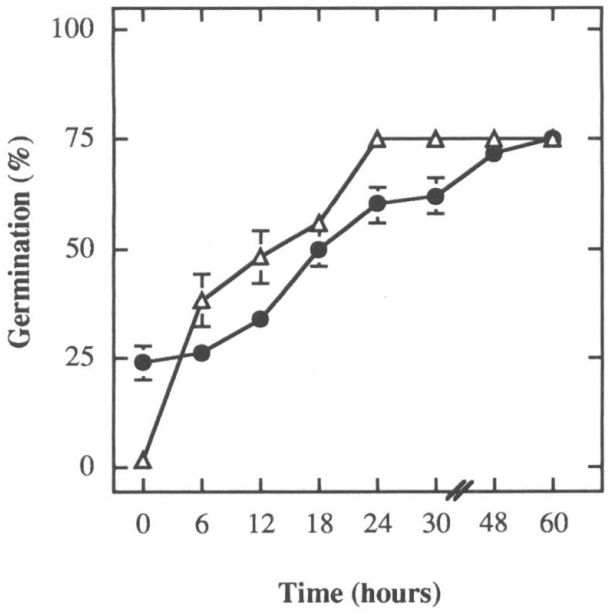


Fig. 2. – Effect of a 24 h-transfer of seeds on 0.5 mM triadimefon (Δ) or on 1mM prohexadione (•) at various times during water imbibition of seeds on filter paper. Germination percent was measured 72 h after sowing. Vertical bars represent SD (n = 4).

(15 µmol PAR $m^{-2}s^{-1}$). Seed pretreatments (24 h) with inhibitors of P-450s (tetcyclacis, TET ; triadimefon, TDF) or of KGD (prohexadione-Ca, PHD) were carried out, in plastic boxes (90 × 60 mm) containing three layers of filter paper moistened with 8 ml of the different solutions. For reversal of inhibitors effects, the seeds (carefully washed with distilled water) were transferred on filter paper wetted with GA or hydrogen peroxide (H_2O_2), or flushed periodically (15 min every 6 h) with 100% O_2 gas in a flow-through dessicator containing the plastic boxes. Germination percentage was determined, seeds with radicles grown in excess of 1 mm were considered germinated. All experiments were performed four times. The results were expressed as mean values ± SD.

3. Results and discussion

3.1. Effect of concentration of tetcyclacis, triadimefon or prohexadione on germination

Barley seeds were pretreated during 24 h with different concentrations of inhibitors of P-450s (TET, TDF) or of KGD (PHD). Germination percentage was measured 48 h after the transfer of seeds on filter paper moistened with distilled water. Germination was totally blocked with 0.5 mM TDF or 0.5 mM TET (Fig. 1). The inhibitory effect of these two P-450s inhibitors might be explained both by an inhibition of degradation of ABA into phaseic acid [18] and by a blockage of the first steps of GA synthesis leading to kaurenoic acid [14]. TDF and TET might prevent the establishment of the suitable ABA/GA ratio needed to initiate germination. A weaker inhibition was obtained with PHD which is a compound known to block the late steps of GA synthesis [12, 14].

3.2. Effect of inhibitors during seed imbibition

Seeds have been exposed to a 24 h treatment with 0.5 mM TDF at any time (0 to 48 h) during seed imbibition. These experiments have been performed in order to determine the duration after sowing beyond which TDF has no more effect on germination. A delay in TDF supply to barley seeds reduced the inhibitory effect of TDF on germi-

	Germination %			
Pretreatment[a]	**24 h reversal treatment with**			
(24 h)	H_2O	O_2	H_2O_2	GA_3
Control (H_2O)	75 ± 3	96 ± 2	94 ± 3	95 ± 3
TDF 0.5 mM	0 ± 0	85 ± 3	85 ± 3	82 ± 3
TET 0.5 mM	4 ± 2	75 ± 4	75 ± 4	69 ± 2
PHD 1 mM	32 ± 3	98 ± 2	94 ± 3	92 ± 0

[a]Seeds were imbibed for 24 h on filter moistened with water (control) or with inhibitors of cytochrome P-450-dependent monooxygenases (TET or TDF) or with PHD, inhibitor of 2-ketoglutarate-dependent dioxygenases. Seeds were then transferred for the following 24 h on water, 1 mM GA3, or 20 mM H_2O_2 or placed under pure oxygen. Germination percent was determined at the end of the treatment.

Table 1. – Reversal effect of gibberellic acid (GA_3), hydrogen peroxide (H_2O_2) and oxygen (O_2) on inhibition of barley seed germination induced by triadimefon (TDF), tetcyclacis (TET) or prohexadiane (PHD).

nation. No more effect was observed when TDF was supplied 24 h after sowing (Fig. 2). It has to be noticed that TDF did not prevent the further growth of seedlings. Thus it may be assumed that the 24 h period following the onset of seed imbibition leads to the change of the hormonal balance required for germination. Similar experiments were undertaken with 1mM PHD. It appeared that this other inhibitor, which did not block germination totally when applied at sowing, had still an effect between 24 h and 48 h after sowing contrary to TDF. The transformation of kaurenoic acid into GA may need a longer time to be achieved.

3.3. Reversal effect of gibberellic acid, hydrogen peroxide and oxygen on inhibition of seed germination induced by inhibitors

Seeds were treated, after a 24 h-inhibitor(s) pretreatment on filter paper, for another 24 h period either with 20 mM H_2O_2, pure oxygen or 1 mM GA_3. In all conditions, and whatever the inhibitor used, the reversal of inhibition was complete and observed immediatly after the end of the treatment (Table 1). The reversal of germination inhibition by GA_3 supply showed that the induction of germination actually involves a change in the hormonal balance which can be prevented by inhibitors of P-450s and of KGD. The reversal of TET or TDF by oxygen may be due to a competition at the level of the haem moiety of P-450s between inhibitors and oxygen [10]. On the other hand, the reversal of PHD by oxygen remains unsolved.

The reversal effect of H_2O_2 is worth thinking over, if we consider that H_2O_2, might be converted into O_2 by catalase which is present in dry seeds [4], which would promote ABA degradation and GA synthesis. In another connection, it has to be noticed that the inhibition of germination by ABA was completely suppressed by a short-term treatment with pure oxygen or with H_2O_2 (results not shown).

The overall results suggest that one of the effects of oxygen (and/or hydrogen peroxide) at the start of germination might lie in the adjustement of the hormonal balance of seeds. The temporary inefficiency of dormant cereal seeds to germinate might therefore really due to the impermeability of seed envelopes to oxygen as previously assumed [6].

Our further aim will be to estimate the effect of oxygen and of inhibitors on ABA and GA contents of barley seeds during the imbibition phase preceeding radicle protrusion. In order to strengthen our hypothesis, it will be also important to evaluate the extent of the competition towards oxygen between the oxidase(s) involved in respiration and those involved in the change of hormonal balance.

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