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The Roles of Gibberellins and Ethylene on the Germination Physiology of the Malting Barley, Variety Puma (*Hordeum valgare* cv Puma)

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The separate roles of ethylene and gibberellins on seed germination have not been clearly established. This has limited full utilisation of these hormones in barley malting. This study was carried out to investigate the roles of gibberellins and ethylene on the germination physiology of 'Puma' barley seeds. Germination curves, percent germination, rootlets length and respiration rates of seeds treated with ethylene, 1-methylcyclopropane (1-MCP), gibberellic acid (GA_3), daminozide (B-nine), GA_3+ethylene, GA_3+1-MCP, ethylene+B-nine, 1-MCP+B-nine and the control were compared after 24, 48 and 72 h from soaking, respectively. GA_3 and ethylene treatments were used to investigate the effects of increasing endogenous ethylene and GA_3 levels, respectively. B-nine and 1-MCP treatments were used to assess the effects of inhibiting gibberellins synthesis and ethylene perception, respectively. Combination treatments were used to assess hormonal interactions. GA_3 and ethylene treatments suppressed germination after 24 h, but, had no effect after 48 and 72 h, respectively. B-nine, ethylene+B-nine and 1-MCP+B-nine suppressed germination, rootlet elongation and respiration after 24, 48 and 72 h, respectively. 1-MCP and GA_3+1-MCP stimulated germination after 24 h, but, had no effect after 48 and 72 h, respectively. GA_3+ethylene treatment suppressed germination and rootlet elongation but stimulated respiration after 24 h. Ethylene suppressed rootlets elongation after 24, 48 and 72 h from soaking, respectively. GA appeared to be the dominant germination hormone, and ethylene, to regulate the rate of germination through suppression of rootlets elongation.

Keywords: 1-MCP, B-nine, percent germination, respiration rate, rootlet growth.

Abbreviations: 1-MC, 1-Methylcyclopropane; B-nine, Daminozide; GA, Gibberellin; GA_3, Gibberellic acid

Introduction

Gibberellin (GA) and ethylene are prominent seed germination promotion hormones (Corbineau et al. 2014). The germination promotion effect of GA appears to be universal, but the germination promotion effect of ethylene varies with plant species (Matilla and Matilla-Vázquez 2008). In barley, both GA and ethylene are synthesised during seed

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germination (Locke et al. 2000; Hartman et al. 2011). Since both hormones are present, it is difficult to attribute the germination stimulation effect to one hormone and not the other.

In plant species whose seed germination is promoted by ethylene, it is assumed that ethylene either stimulate GA synthesis or affects GA signal pathway, which then promotes seed germination (Corbineau et al. 2014). This is partly because GA promotes germination of many plant species whose germination is also promoted by ethylene (Kucera et al. 2005). For example, gibberellic acid (GA_3) and ethylene promotes germination of dormant seeds of *Arabidopsis* (Matilla and Matilla-Vázquez 2008). This was also because ethylene promotes stem elongation of submerged rice *(Oryza sativa)* through stimulation of GA synthesis (Taiz and Zeiger 2010). Moreover, treatment of ethylene insensitive *(etr1)* mutant *Arabidopsis* seeds with GA_3 stimulates germination (Corbineau et al. 2014).

Conversely, GA was reported to promote germination in some plant species by stimulating ethylene production (Corbineau et al. 2014). For example, incubation of embryos of beechnut seeds in GA_3 solution resulted in increased ethylene production (Calvo et al. 2004a; Corbineau et al. 2014). The puzzle was further complicated by the fact that ethylene and GA share common seed germination promotion mechanisms, e.g., antagonism of abscisic acid (ABA, a germination inhibiting hormone) action, promotion of biosynthesis and release of germination enzymes and promotion of cell elongation (Kucera et al. 2005; Taiz and Zeiger 2010; Miransari and Smith 2014). It is therefore clear that the separate roles of ethylene and GA in seed germination have not been clearly established.

GA is routinely used to accelerate germination and grain modification (breaking down of cell walls and enzymatic conversion of insoluble starch and proteins to soluble forms) during malting of barley (Briggs 1998). Although not currently being used in malting, ethylene was also reported to promote germination and synthesis of grain modification enzymes in barley seeds (Locke et al. 2000). Therefore, understanding the separate roles of ethylene and GA on barley seed germination may have the potential to improve the way they would be manipulated in barley malting. This study was, therefore, carried out to investigate the separate roles of GA and ethylene, as well as their possible interaction during germination of 'Puma' barley, a variety that is widely malted in South Africa. Inhibitors of hormone synthesis or action were used to separate the roles of one hormone from the other.

Material and Methods

Plant material and sample preparation

'Puma' barley grain of the 2014 winter production season was used. The grain was obtained from SAB-Alrode malting (SAB-Alrode, 2 Johnson Street, Alrode, South Africa). The experiments were carried out in September 2015 ensuring that the grain attained enough after-ripening. The grain was screened to remove kernels that were less than 2.2 mm so as to reduce variations in germination that could result from seed size differences.

After screening, the grain was surface sterilised with 1% sodium hypochlorite solution and rinsed 5 times with distilled water. After sterilisation, the grain was air dried to its original moisture content (12%).

Germination test

Treatments and germination conditions

Nine treatments were compared, *viz;* ethylene, GA_3, GA_3 + ethylene, daminoside (B-nine), 1-methycylopropene (1-MCP), ethylene + B-nine, GA_3 + 1-MCP, 1-MCP + B-nine and the control. In each treatment, six replicates of 100 seeds each were germinated on two layers of Whatman No. 1 filter papers moistened with 5 ml distilled water (except in the B-nine containing treatments) in a 9 cm diameter Petri-dish. For all treatments, Petri-dishes were placed in hermetically sealed 60 cm^3 glass tanks and the glass tanks were placed in a growth chamber (germinator) where temperature and light were controlled. Seeds were germinated at 20 ± 1 °C under continuous darkness (except during evaluations). Out of the six replicates, three replicates were used in assessing percent germination and the remaining three replicates were used for assessment of rootlets length.

Preparation of treatments

For ethylene containing treatments (ethylene, ethylene + B-nine, ethylene + GA_3), six open/unclosed Petri-dishes containing 100 seeds each per treatment were placed in 60 cm^3 glass tank. The glass tank was closed with a size fitting glass sheet with two 1 cm diameter holes on it and sealed air tight with a sole tape. A plastic pipe was connected from a 1 cm diameter hole on the closing glass sheet though a 5 l glass jar containing ethylene gas and out of the germinator to the air pump. Air was pumped at a rate of 11.1 ml/h though the 5 l glass jar containing ethylene gas and again though the glass tank in which the Petri-dishes were placed and out though the other 1 cm diameter hole on the closing glass sheet; forming a flow though system. After 24 h, commensurate to the first 24 h of incubation, the pump was stopped, and the glass tank was opened. The glass tank was then kept open until the end of the germination experiment period (72 h). However, the Petri-dishes were closed to avoid rapid moisture loss. For 1-MCP (Smartfresh™, Agro Fresh, Pennsylvania, USA) containing treatments (1-MCP, 1-MCP + B-nine and GA_3 + 1-MCP), six open Petri-dishes containing 100 seeds each per treatment were placed in 60 cm^3 glass tank (same as for ethylene containing treatments). A 35 ml beaker containing 0.1 g 1-MCP powder was placed in the glass tank. The tank was closed with size fitting glass sheet with a 1 cm diameter hole on it and sealed air tight with a sole tape. After sealing, 1-MCP was volatilized by addition of 30 ml distilled water into the 1-MCP containing beaker using a syringe connected to a sizeable plastic pipe through the 1 cm diameter hole on the glass tank covering glass sheet. The hole was immediately closed with a stick staff. After 24 h of incubation, the closing glass sheet was removed, the Petri-dishes were closed, and the glass tank was kept open until the end of the germination experiment

period. For GA_3 containing treatments (GA_3, GA_3 + ethylene, GA_3 + 1-MCP), seeds were treated with GA_3 solution before being divided into sets of 100 seeds each. GA_3 solution was prepared by diluting 6.7 mg GA_3 (Sigma Aldrich, Johannesburg, South Africa) in 100 ml 48% ethanol (50 ml 96% ethanol + 50 ml distilled water) and the volume was adjusted to one litre by distilled water. Three millilitres of GA_3 solution was sprayed to 100 g seeds. The seeds were left to air dry for 1 h at room temperature and then divided into sets of 100 seeds each. In the GA_3 + ethylene treatment, seeds were treated with GA_3 before being incubated in the ethylene containing atmosphere as explained for ethylene containing treatments. Again, in the GA_3 + 1-MCP treatment, seeds were incubated in the 1-MCP containing atmosphere after treatment with GA_3 solution. In the B-nine containing treatments (B-nine, ethylene + B-nine, 1-MCP + B-nine), filter papers in the Petri-dishes were moistened with 5 ml B-nine solution. B-nine solution was prepared by dissolving 10 g B-nine powder (Uniroyal Chemical (Pvt) Ltd, USA) in one litre distilled water. In ethylene + B-nine treatment, seeds were incubated in the ethylene containing atmosphere as explained for ethylene containing treatments and in the B-nine + 1-MCP treatment, seeds were incubated in the 1-MCP containing atmosphere as explained for 1-MCP containing treatments. The control seeds were also germinated on two layers Whatman No. 1 filter papers moistened with 5 ml distilled water in 9 cm diameter Petri-dishes.

Measurement of percent germination and rootlet growth

Germinated seeds were removed and counted after 24, 48 and 72 h of incubation, respectively. Germination percentages were calculated as averages of germinated seeds from three replicates of each treatment. The other set of three replicates per treatment was used to assess rootlets length. At each evaluation interval, the lengths of the primary rootlets were measured on five randomly selected germinated seeds per replicate per treatment. The average rootlets length per treatment was calculated.

Determination of the respiration rate

Treatments and the germination conditions

The respiration rate of the germinating 'Puma' barley seeds was assessed on seeds treated with ethylene, GA_3, 1-MCP, GA_3 + ethylene, GA_3 + 1-MCP, B-nine, ethylene + B-nine, 1-MCP + B-nine and, the control. In all treatments, 3 replicates of 9 g seeds each were used. Each set of 9 g seeds (replicate) was incubated on two layers of Whatman No. 1 filter papers wetted with 8 ml distilled water (except for B-nine containing treatments) in a 250 ml air-tightly closed container placed in a germination chamber. The germination chamber was set at 20 + 1 °C and continuous darkness (except during evaluations). For ethylene containing treatments, 25 µl ethylene was injected in each tightly closed 250 ml container using a syringe. For 1-MCP containing treatments, 100 µl 1-MCP gas was injected in each tightly closed 250 ml container using a syringe. For GA_3 containing treatments, 100 g seeds were sprayed with 3 ml GA_3 solution and then divided into sets of 9 g

each. In the B-nine containing treatments, filter papers were wetted with 8 ml B-nine solution. Preparation of B-nine and GA_3 solutions was done following the procedures described under germination test (above). The control seeds were germinated on two layers Whatman No. 1 filter papers wetted with 8 ml distilled water.

Measurement of the respiration rate

Respiration was measured as the amount of carbon dioxide evolved. Carbon dioxide was measured using F-950- three gas analyser (CID Bio-Science, Inc., 1554 NE 3rd Ave, Camas, WA 98607, USA). Respiration was measured at 24, 48 and 72 h of incubation, respectively. After the first 24 h, the sample containers were removed from the germination chamber, air-tightly closed containers opened and blown with fresh air to flash out gases. The sample containers were then air-tightly closed and left to stand for 30 min before measuring carbon dioxide evolved during this period. After carbon dioxide measurement, seeds were taken out, blotted with a paper towel and weighed. The average weight of all treatments by replicates per evaluation period was used in calculating the respiration rate. After 24 h evaluation, the sample containers were closed, but, not air-tightly. The respiration rate was calculated using the formula:

$$R = [(c - r) \div 4]/g \times 2$$

where R is the respiration rate, c is the carbon dioxide evolved over a period of 30 min in µl per litre, r is the carbon dioxide level of the experimental environment, 4 is a factor for converting one litre to the volume of the sample container (250 ml = 1/4 of a litre), g is the seed weight per replicate per evaluation time in grams and 2 is a factor for converting 30 min to one hour.

Experimental design and data analysis

Data for both experiments was analysed as factorial design with treatment and incubation period as factors. The data was applied to analysis of variance (ANOVA) using General Linear Models (GLM) in the Statistical Analysis System (SAS) computer programme (SAS Enterprise Guide 4.0; SAS Institute, 2006, Cary, NC 27513, USA). Means were separated using least squares difference (LSD) at 5% level of significance. Graphs were drawn using Microsoft Office Excel 2007 (Microsoft Corporation, One Microsoft way, Redmond, WA 98052-7329, USA) computer programme.

Results

Germination curves

The germination curves for 'Puma' barley seeds treated with GA_3, ethylene, 1-MCP, GA_3 + 1-MCP, GA_3 + ethylene, B-nine, 1-MCP + B-nine, B-nine + ethylene and the con-

trol are shown in Figure S1*. The control, ethylene, GA_3, GA_3 + 1-MCP and GA_3 + ethylene treated seeds had few seeds that germinated after 24 h, the highest number of seeds that germinated after 48 h and again few seeds that germinated after 72 h. Besides, GA_3 + 1-MCP treated seeds had more seeds that germinated after 24 h compared to the control, ethylene, GA_3 and GA_3 + ethylene, respectively. Therefore, GA_3 + 1-MCP treatment had lower number of seeds that germinated after 48 h compared to the control, ethylene, GA_3 and GA_3 + ethylene treatments, respectively. Ethylene, GA_3 and GA_3 + ethylene treatments had fewer seeds that germinated after 24 h and more seeds that germinated after 48 h compared to the control treatment. This shows that although ethylene, GA_3, GA_3 + 1-MCP and GA_3 + ethylene treatments had similar triangular like shaped curves, the curves varies with treatments. 1-MCP treatment had more seeds that germinated after 24 h, fewer seeds that germinated after 48 h, and far few seeds that germinated after 72 h. In B-nine containing treatments, no seed germinated after 24 h, less than 20% of the seeds germinated after 48 h and less than 30% germination after 72 h. Generally, germination increased with incubation time for the B-nine containing treatments.

Cumulated percent germination

The effects of GA_3, ethylene, 1-MCP, GA_3 + 1-MCP, GA_3 + ethylene, B-nine, 1-MCP + B-nine, ethylene + B-nine and the control on cumulated percent germination of 'Puma' barley seeds after 24, 48 and 72 h of incubation are shown on Table 1.

There was a significant interaction between incubation time and treatments on cumulated percent germination of 'Puma' barley seeds, $P = 0.000$. Moreover, there was a significant percent germination differences between treatments after 24 ($P = 0.000$), 48 ($P = 0.000$) and 72 h ($P = 0.000$), respectively. Ethylene, GA_3 and GA_3 + ethylene treated seeds had significantly lower percent germination (5, 24 and 8%, respectively) compared to the control (31%) after 24 h. 1-MCP and GA_3 + 1-MCP treated seeds had significantly higher percent germination (61 and 38%, respectively) compared to the control after 24 h. Again, 1-MCP treated seeds had significantly higher percent germination (61%) after 24 h compared to GA_3 + 1-MCP (38%), GA_3 (24%) and ethylene (5%) treated seeds, respectively. GA_3 treated seeds had significantly higher percent germination (24%) compared to ethylene treated seeds (5%) after 24 h. There was no germination recorded for all the B-nine containing treatments after 24 h. There were no significant cumulated percent germination differences between treatments for the control, ethylene, GA_3, 1-MCP, GA_3 + 1-MCP and GA_3 + ethylene treated seeds after 48 and 72 h, respectively. The germination percentages for the control, ethylene, GA_3, 1-MCP, GA_3 + 1-MCP, GA_3 + ethylene treated seeds were 95.3, 88.7, 93.3, 94.3, 98.0 and 98.7%, respectively, after 48 h and 96.3, 97.0, 95.0, 96.3, 99.0 and 99.3%, respectively, after 72 h. The germination percentages for the B-nine containing treatments were significantly low compared to non-B-nine containing treatments after 48 and 72 h, respectively. The germination percentages of B-nine, ethylene + B-nine, B-nine + 1-MCP treated seeds were 10.3, 16.3 and 16.3%,

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

Table 1. The effect of GA_3, ethylene, 1-MCP, GA_3 + 1-MCP, GA_3 + ethylene, B-nine, 1-MCP + B-nine, ethylene + B-nine and the control treatments, respectively, on cumulated percent germination of 'Puma' barley seeds incubated for 72 h from soaking and evaluated after 24, 48 and 72 h, respectively

Treatment	% Germination		
	24 hrs	48 hrs	72 hrs
Control	31.0^{b}	95.3^{a}	96.3^{a}
Ethylene	5.0^{d}	88.7^{a}	97.0^{a}
1-MCP	61.0^{a}	93.3^{a}	95.0^{a}
GA	23.0^{bc}	94.3^{a}	96.3^{a}
B-nine	0.0^{d}	10.3^{b}	31.3^{b}
GA + Ethylene	8.3^{cd}	98.7^{a}	99.3^{a}
GA + 1-MCP	38.3^{b}	98.0^{a}	99.0^{a}
Ethylene + B-nine	0.0^{d}	16.3^{b}	40.0^{b}
1-MCP + B-nine	0.0^{d}	16.3^{b}	42.0^{b}
P values	0.000	0.000	0.000
Treatment			0.000
Incubation period			0.000
Treatment × incubation period			0.000

Means with similar superscript within a column are not significantly different at 5% level.

respectively, after 48 h and 31.3, 40.0 and 42.0%, respectively, after 72 h. The germination suppression effect of B-nine was also shown on Figure S2. B-nine containing treatments suppressed germination, but, GA_3 treated and the control seeds germinated well after 72 h of incubation.

Rootlets length

The effect of GA_3, ethylene, 1-MCP, GA_3 + 1-MCP, GA_3 + ethylene, B-nine, 1-MCP + B-nine, ethylene + B-nine and the control on rootlets length of germinating 'Puma' barley seeds after 24, 48 and 72 h of incubation are shown on Table 2.

There was a significant interaction between incubation time and treatments on rootlets length of the germinating 'Puma' barley seeds, P = 0.000. Moreover, there was a significant rootlets length differences between treatments after 24 (P = 0.000), 48 (P = 0.000) and 72 h (P = 0.000), respectively. There were no significant rootlets length differences between the control (2.9 mm), GA_3 (3.2 mm) and GA_3 + 1-MCP (3.6 mm) treated seeds after 24 h. The rootlets of 1-MCP treated seeds were significantly longer (4.2 mm) compared to the control seeds (2.9 mm) after 24 h. There were no significant rootlets length differences between 1-MCP (4.2 mm) and GA_3 + 1-MCP (3.6 mm) treated seeds after 24 h. The rootlets of ethylene and GA_3 + ethylene treated seeds were significantly short (1.1 and 1.3 mm, respectively) compared to the control, 1-MCP, GA_3 and GA_3 + 1-MCP

Table 2. The effect of GA_3, ethylene, 1-MCP, GA_3 + 1-MCP, GA_3 + ethylene, B-nine, 1-MCP + B-nine, ethylene + B-nine and the control treatments on rootlets length of germinating 'Puma' barley seeds after 24, 48 and 72 h of incubation, respectively

Treatment	Rootlet length (mm)		
	24 hrs	48 hrs	72 hrs
Control	2.9^{b}	12.4^{b}	22.5^{a}
Ethylene	1.1^{c}	8.5^{c}	16.2^{b}
1-MCP	4.3^{a}	13.3^{ab}	23.6^{a}
GA	3.2^{b}	11.9^{b}	21.4^{a}
B-nine	0.0^{d}	3.0^{d}	5.1^{c}
GA + Ethylene	1.3^{c}	9.5^{c}	20.5^{b}
GA + 1-MCP	3.6^{ab}	14.6^{a}	20.7^{b}
Ethylene + B-nine	0.0^{d}	1.5 ^{d}	3.1^{c}
1-MCP + B-nine	0.0^{d}	2.3^{d}	4.0^{c}
P values	0.000	0.000	0.000
Treatment			0.000
Incubation period			0.000
Time × Incubation period			0.000

Means with similar superscript within a column are not significantly different at 5% level.

treated seeds after 24 h. There were no rootlets length recorded for B-nine containing treatments after 24 h. There were no significant rootlets length differences for the control (12.4 mm), 1-MCP (13.3 mm) and GA_3 (11.9 mm) treated seeds after 48 h. GA_3 + 1-MCP treated seeds had significantly longer rootlets (14.6 mm) compared to the control (12.4 mm). However, the rootlets length of GA_3 + 1-MCP and 1-MCP treated seeds were not significantly different after 48 h. The rootlets for ethylene and GA_3 + ethylene treated seeds were significantly short (8.5 and 9.5 mm, respectively) compared to the control, 1-MCP, GA_3 and GA_3 + 1-MCP treated seeds, respectively, after 48 h. However, the rootlets length of ethylene and GA_3 + ethylene treated seeds were not significantly different after 48 h. The rootlets length for B-nine, ethylene + B-nine and 1-MCP + B-nine treated seeds were significantly short (3.0, 2.3 and 1.5 mm, respectively) compared to the control, 1-MCP, GA_3, GA_3 + 1-MCP, ethylene and GA_3 + ethylene treated seeds, respectively, after 48 h. However, the rootlets length for B-nine, ethylene + B-nine and 1-MCP + B-nine were not significantly different after 48 h. After 72 h, the rootlets length of the control (22.5 mm), GA_3 (21.4 mm) and 1-MCP (23.6 mm) treated seeds were not significantly different. The rootlets for ethylene, GA_3 + ethylene and GA_3 + 1-MCP treated seeds were significantly short (16.2, 20.5 and 20.7 mm, respectively) compared to the control, GA_3 and 1-MCP treated seeds. The rootlets of B-nine, ethylene + B-nine and 1-MCP + B-nine treated seeds were significantly short (5.1, 4.0 and 3.1 mm, respectively) compared to the control, 1-MCP, GA_3, ethylene, GA_3 + ethylene and GA_3 + 1-MCP treated seeds, respec-

tively. However, the rootlets length of B-nine, ethylene + B-nine and 1-MCP + B-nine treated seeds were not significantly different.

The effect of ethylene on rootlets growth of the germinating 'Puma' barley seeds was further emphasised in Figure S3. The rootlets lengths of ethylene treated 'Puma' seeds were shorter than 1-MCP treated and the control seeds after 48 and 72 h of incubation, respectively.

Respiration

The effects of GA_3, ethylene, 1-MCP, GA_3 + 1-MCP, GA_3 + ethylene, B-nine, 1-MCP + B-nine, ethylene + B-nine and the control treatments on the rate of respiration of the germinating 'Puma' barley seeds are shown on Table S1. There was no significant interaction between incubation time and treatment on the respiration rate of the germinating 'Puma' barley seeds, $P = 0.079$. However, there was a significant respiration rate differences between treatments, $P = 0.000$. The respiration rates for the seeds treated with ethylene (107.7 µl/g/hr), 1-MCP (110.5 µl/g/hr), GA_3 (111.2 µl/g/hr), GA_3 + ethylene (123.5 µl/g/hr), GA_3 + 1-MCP (121.1 µl/g/hr) and the control (104.2 µl/g/hr) were not significantly different. However, the respiration rates of B-nine, ethylene + B-nine and 1-MCP + B-nine treated seeds were significantly low (66.8, 70.4 and 67.3 µl/g/hr, respectively) compared to non-B-nine containing treatments. Again, there were significant respiration rate differences between incubation times, $P = 0.000$. The respiration rate of the germinating 'Puma' barley seeds was significantly low (68.9 µl/g/hr) after 24 h compared to 48 (112.2 µl/g/hr) and 72 h (113.1 µl/g/hr), respectively. However, there was no significant respiration rate difference between 48 and 72 h of incubation.

Discussion

During germination, the barley seed produces GA (Palmer 2018) and ethylene (Locke et al. 2000; Hartman et al. 2011) which regulates embryo growth and enzyme synthesis. Since both ethylene and GA are produced by the germinating barley seed and their presence either in or around the seed affects germination, the separate roles of these hormones on barley seed germination are not clear. This study, therefore, used hormone inhibitors to study the effects of one hormone in the absence of the other. 1-MCP was used to inhibit ethylene action and daminozide (B-nine) to inhibit GA synthesis. 1-MCP inhibits ethylene action by competing with ethylene for membrane bound receptors (Taiz and Zeiger 2010) and B-nine inhibits GA synthesis by inhibiting 2-oxoglutrate-dependent dioxygenase, an important enzyme in the synthesis of bioactive GAs (Rademacher 2000). Comparisons were made on germination curves, percent germination, rootlets length and the respiration rates of 'Puma' barley seeds treated with GA_3, ethylene, 1-MCP, GA_3 + 1-MCP, GA_3 + ethylene, B-nine, 1-MCP + B-nine, ethylene + B-nine and the control.

The germination curves showed that, both ethylene and GA regulates germination of 'Puma' barley seeds. Inhibiting ethylene perception with 1-MCP as well as inhibiting GA biosynthesis with B-nine changed the shape of the germination curve of 'Puma' barley

(the control). The germination curve of 'Puma' barley seeds showed that few seeds germinate after 24 h, the highest number of seeds after 48 h and again few seeds after 72 h. Conversely, seeds treated with 1-MCP had large number of seeds that germinated after 24 h, few seeds after 48 h, and far fewer seeds after 72 h. The B-nine treated seeds showed no germination after 24 h, less than 20% germination after 48 h and less than 30% germination after 72 h. GA_3, ethylene and GA_3 + ethylene treated seeds, though with slight variations, have normal germination curve shapes (where few seeds germinate after 24 h, large number of seeds after 48 h, and again, few seeds after 72 h).

The germination curves also suggested that ethylene is the dominant hormone that determines the rate of germination of 'Puma' barley seed. Considering that ethylene is synthesised by germinating barley seeds (Locke et al. 2000; Hartman et al. 2011), the curves showed that supplementing ethylene synthesised by seeds (control) by exogenous ethylene treatment further reduces the number of seeds that germinates after 24 h and increases the number of seeds that germinates after 48 h. Moreover, inhibiting ethylene perception increased the number of seeds that germinated after 24 h. It could be concluded that ethylene treatment delays germination of 'Puma' barley and inhibiting its perception hastens germination.

The germination curves also suggested that GA_3 treatment enhances the negative effect of ethylene on the rate of 'Puma' barley seed germination. Compared to the control, GA_3 in GA_3 + ethylene combination treatment did not increase the number of seeds that germinated after 24 h. Again, GA_3 in GA_3 + 1-MCP combination treatment reduced the germination stimulation effect of 1-MCP after 24 h. Moreover, though less than ethylene, increasing endogenous GA (control) with exogenous GA_3 treatment decreased the number of seeds that germinated after 24 h and increased the number of seeds that germinated after 48 h (a trend that was observed with ethylene treatment).

Although ethylene delayed germination of 'Puma' barley seeds, it had no effect on ultimate cumulated percent germination. Germination is deemed to be complete when the seed's embryonic axis (radical or plumale) have protruded out of the structures surrounding the embryo (Bewley and Black 1994; Bewley 2001). In the current study, seeds were considered to have germinated after radical protrusion from both testa and coleorhiza. Although ethylene treated seeds had significantly low percent germination compared to the control and 1-MCP treated seeds after 24 h, the cumulated percent germination of ethylene treated seeds was not significantly different from both the control and 1-MCP treated seeds after 48 and 72 h, respectively. However, in barley germination technologies such as malting, a delay in germination may cause poor malt modification (Briggs 1998). Again, delayed germination may mean delayed enzyme development and reserve mobilisation hence delayed seedling development.

The cumulated percent germination results also showed that GA is the dominant hormone that regulates germination in 'Puma' barley seeds. All the B-nine containing treatments had significantly lower percent germination compared to all non-B-nine containing treatments. Again, although ethylene was reported to restore germination of GA deficient mutant *ga1-3* in *Arabidopsis* (Corbineau et al. 2014), ethylene in B-nine + ethylene treatment did not rescue B-nine induced germination inhibition in 'Puma' barley seeds. This

suggests that GA synthesis is important for 'Puma' seed germination. Matilla and Matilla-Vázquez (2008) also reported that GA and ethylene work together, but, GA is qualitatively and quantitatively more important. However, GA_3 treated seeds had significantly lower percent germination after 24 h, but, no significant cumulated percent germination differences compared to the control after 48 and 72 h, respectively. This may suggest that after-ripened 'Puma' barley seeds produce enough GA to promote germination and hence, increasing endogenous GA delay germination, but, has no effect on ultimate percent germination of 'Puma' barley seeds. Very low GA_3 rates (0.2 – 0.25 ppm) are generally used to treat barley grain during malting (Briggs 1998; Palmer 2018). Again, optimum levels of GA_3 are generally known to promote rapid seed germination (Briggs 1998).

Analysis of rootlets length showed that ethylene suppresses rootlets elongation. Ethylene is generally known to inhibit and GA to promote rootlet elongation (Taiz and Zeiger 2010). In the present work, inhibiting ethylene action by 1-MCP, exogenous application of GA and exogenous application of a combination of GA and 1-MCP had significantly longer rootlets compared to ethylene treatment after 24, 48 and 72 h, respectively. However, there were no significant rootlets length differences between ethylene and GA_3 + ethylene treatment after 24, 48 and 72 h, respectively. This suggests that GA treatment cannot rescue germinating 'Puma' barley seeds from ethylene induced suppression of rootlets growth.

Rootlets elongation results also indicated that GA synthesis is important for rootlet growth. The rootlets lengths of B-nine containing treatments were significantly short compared to the control, 1-MCP, GA_3, GA_3 + 1-MCP, ethylene and GA_3 + ethylene, respectively, after 48 and 72 h. The rootlets length of B-nine + ethylene treated seeds, though not significantly short, were slightly shorter compared to other B-nine containing treatments after 48 and 72 h, respectively. These results suggest that the rootlets elongation suppression effect of ethylene is less effective if GA synthesis is not inhibited. Again, the results suggest that ethylene and B-nine may have additive effects on suppression of rootlets elongation.

Analysis of the rate of respiration showed that the respiration rate of the germinating 'Puma' barley seeds increased with incubation time and plateaus after 48 h. However, the respiration rate remains high after 72 h. Ethylene had no significant effect on the respiration rate of germinating 'Puma' barley seeds. Both, ethylene treated and 1-MCP treated seeds had no significant respiration rates differences compared to the control. However, the results suggested that GA synthesis is important for respiration of the germinating 'Puma' barley seeds. Inhibiting GA synthesis significantly decreased the respiration rate of the germinating 'Puma' barley seed. However, the respiration rate of GA_3 treated seeds was not significantly different from the control. This further suggest that after-ripened 'Puma' barley seeds produce enough GA to promote normal respiration during germination and, hence, increasing GA level has no effect on respiration, but, low levels of GA due to inhibition of biosynthesis reduce respiration. Germinating B-nine treated seeds in the presence of ethylene did not rescue 'Puma' barley seeds from B-nine induced reduction of the respiration rate. Generally, respiration rate correlates with the rate of metabolism (Bewley and Black 1994) and normal seed metabolism is important for seed germi-

nation. These results therefore suggest that endogenous GA synthesis is important for 'Puma' barley seed metabolism during germination.

Conclusion

The results suggested that GA is the dominant hormone that regulates germination of 'Puma' barley seeds. However, ethylene regulates the germination rate of 'Puma' barley seeds through suppression of rootlets elongation. Besides, the rootlet growth suppression effect of ethylene could not inhibit germination of 'Puma' barley seeds if GA synthesis is active. This could be partly because GA is important for the respiration process. However, increasing GA level by exogenous GA_3 treatment could also delay germination of 'Puma' barley seeds. However, this increased GA level induced delay in germination is less compared to that of ethylene treatment. It could be hypothesised that increased level of GA cause seed stress, hence, delayed germination. Since germination delaying was found to be the effect of ethylene treatment, future studies should investigate if the germination delaying effect of GA_3 treatment is not a result of GA induced ethylene synthesis.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Table S1*. The effect of GA_3, ethylene, 1-MCP, GA_3+1-MCP, GA_3+ethylene, B-nine, 1-MCP+B-nine, ethylene+B-nine and the control treatments on the respiration rate of germinating 'Puma' barley seeds after 24, 48 and 72 h of incubation, respectively

Electronic Supplementary *Figure S1*. The germination curves of 'Puma' barley seeds treated with ethylene, GA_3, 1-MCP, GA_3+ethylene, GA_3+1-MCP, B-nine, B-nine+ethylene, 1-MCP+B-nine and the control, respectively, and evaluated at 24 h intervals over a period of 72 h from soaking. The markers how the number of seeds that germinated after 24, 48 and 72 h of incubation, respectively

Electronic Supplementary *Figure S2*. B-nine, ethylene+B-nine and 1-MCP+B-nine treatments caused poor germination of 'Puma' barley seeds compared to GA_3 and the control treatments after 72 h of incubation

Electronic Supplementary *Figure S3*. The rootlets lengths of ethylene treated 'Puma' seeds were shorter than 1-MCP treated and the control seeds after both 48 and 72 h from soaking