Cereal Research Communications 47(4), pp. 689–700 (2019)
DOI: 10.1556/0806.47.2019.38

The Potential of Ethylene as an Alternative to GA_3 Treatment during Malting of Barley *(Hordeum valgare)*

P. Zanamwe*

Department of Botany and Plant Biotechnology, University of Johannesburg,
P.O. Box 524, Auckland Park, Johannesburg, 2006, South Africa

(Received 12 May 2019;
Accepted 12 July 2019)

Treatment of barley grain with gibberellic acid (GA_3) during malting promotes abnormal proteolysis and rapid rootlets growth affecting malt quality. This study investigated the potential of ethylene treatment as an alternative by comparing the amylase activity, total starch and total reducing sugars of germinating 'Puma' barley seeds treated with ethylene, 1-methylcyclopropane (1-MCP), GA_3, daminozide (B-nine), GA_3 + 1-MCP, ethylene + B-nine and the control after 24, 48 and 72 h from soaking. Ethylene had no effect on amylase activity. B-nine reduced amylase activity by 16 and 9.6% compared to the control after 48 and 72 h, respectively. The amylase activity of ethylene + B-nine treated seeds was higher (13.3 and 4%) than B-nine treated seeds after 48 and 72 h. This suggest that endogenous GA is important for normal amylase activity and, ethylene stimulates amylase activity where GA synthesis is inhibited. Ethylene and GA_3 treatments reduced starch (83.3 mg g^{-1} and 76.7 mg g^{-1}, respectively) and increased reducing sugars (16.0 and 17.1 µg ml^{-1}, respectively) compared to the control (115.3 mg g^{-1} starch and 12.1 µg ml^{-1} reducing sugars) after 72 h. It was concluded that, ethylene may replace GA_3 treatment without interfering with starch changing processes during barley malting.

Keyword: barley, germination, starch, amylase, total reducing sugars, malting

Abbreviations: 1-MCP: 1-methylcyclopropane; B-nine: daminozide; GA: gibberellin; GA_3: gibberellic acid

Introduction

Ethylene, a gaseous plant hormone with germination promotion and rootlets growth restriction effects during germination of seeds of many plant species, was thought to be a potential alternative to gibberellic acid (GA_3) treatment during malting of barley (Hartman et al. 2010; Corbineau et al. 2014). When used in barley malting, GA_3 has both desirable (rapid germination and grain modification) and undesirable effects (promotion of abnormal proteolysis, increased respiration and stimulation of rapid rootlets growth) (Briggs et al. 2004). Because of its undesirable effects, GA_3 combinations and/or alternatives that reduce the undesirable effects, while maintaining the desirable effects, are being

*Corresponding author; E-mail: zanamwep@gmail.com

sought (Yamada 1985). Although ethylene was thought to be a potential alternative, its effects on storage reserve mobilisation relative to GA_3 treatments has not been clearly established.

Changes that occur to starch grains during seed germination have profound influence on biochemical attributes of malt quality (Briggs et al. 2004). Insoluble starch is converted to soluble starch during malting. Soluble starch is converted to fermentable sugars during the mashing stage of the brewing process (Briggs et al. 2004; Kunze 2004; Kleinwächter et al. 2012). Hydrolysis of the endosperm cell walls and the protein matrix that surrounds starch grains is the major objectives of malting (Woonton et al. 2005; Perveen et al. 2008). Little but significant starch should be hydrolysed during malting (MacGregor and Matsuo 1982; Briggs 1998; Frank et al. 2011).

During germination, the barley seed (grain) produces the plant growth regulator, GA_3 (Kleinwächter et al. 2012), which diffuses to the aleurone tissue, stimulating it (aleurone tissue) to synthedize enzymes important in reserve starch mobilisation (Bewley 2001). The grain starch level decreases, and simple sugars increases (Bewley 2001; Kunze 2004; Palmer 2018). Because of its influence on storage reserve mobilisation during seed germination, GA_3 is routinely used in barley malting (Briggs et al. 2004). However, it also imparts some undesirable effects which include promotion of abnormal proteolysis, increased respiration and stimulation of rapid rootlets growth (Yamada 1985; Briggs et al. 2004). Potential alternatives are, therefore, being sought.

The aim of this study was, therefore, to investigate the potential of ethylene as an alternative treatment to GA_3 by comparing the effects of ethylene and GA_3 treatments on reserve (starch) mobilisation during germination of 'Puma' barley; a variety which is widely malted in South Africa (Roux 2011). Since both GA_3 and ethylene are synthesised by a germinating barley seed, this study used hormone synthesis or hormone perception inhibitors to study the effects of one hormone in the absence of the other. 1-MCP was used to inhibit ethylene action and daminozide to inhibit GA synthesis.

Material and Methods

Plant material and its preparation

'Puma' barley grain (2014 harvest) was used in this study. The grain was kindly donated by AB-InBev (AB-InBev, Johannesburg, South Africa). The experiments were carried out in 2015 ensuring that the grain has after-ripened. The grain was screened with a 2.5 mm slit width sieve (Graintec, Toowoomba, Australia) to remove kernels that were less than 2.5 mm in length to reduce germination variation that may result from seed size differences. After screening, the grain was surface sterilised with 1% sodium hypochlorite solution, rinsed 5 times with distilled water and air dried to its original moisture content (12%).

Experimental design

Two germination experiments were carried out. The first experiment was carried out to assess amylase activity and the second to assess starch and total reducing sugars contents of germinating 'Puma' barley seeds. In each experiment, a two-factor factorial design with incubation period and treatment as factors was used. For both experiments, the seeds were treated with ethylene, GA_3, 1-methylcylopropene (1-MCP), daminozide (B-nine), GA_3 + 1-MCP, ethylene + B-nine, and the control. 1-MCP is a competitive ethylene perception inhibitor and daminozide inhibits GA synthesis. For each treatment, nine replicates of 50 seeds in the amylase activity experiment and 100 seeds in the starch and reducing sugars contents experiment were incubated at 20 ± 1 °C and continuous darkness in a germination chamber and evaluated at 24 h interval over a period of 72 h, i.e. after 24, 48 and 72 h, respectively. Each seed lot (equal to a replicate) was germinated on two layers of Whatman No. 1 filter papers moistened with 4 ml distilled water (expect for B-nine containing treatments) in a 9 cm diameter Petri-dish. In the amylase activity experiment, three replicates of 50 seeds each per treatment per evaluation interval were removed and 10 randomly selected seeds per treatment were used to assess amylase activity (also see; amylase activity below). In the starch and reducing sugars contents experiment, three replicates of 100 seeds each per treatment per evaluation interval were removed from the germination chamber, dried at 80 °C for 14 h in an oven, ground in a laboratory mortar and sieved through a 5 µm sieve. The samples were then kept in a –80 °C freezer before determination of starch and total reducing sugars.

Treatment procedure

For ethylene containing treatments (ethylene and ethylene + B-nine), the seeds were exposed to a flowing (11 mL h^{-1}) air stream containing 10 µL L^{-1} ethylene in a 60 cm^3 glass box placed in a germination chamber during the first 24 h of a 72 h incubation period. For 1-MCP containing treatments (1-MCP and GA_3 + 1-MCP), the seeds were exposed to 1000 nL L^{-1} 1-MCP (Smartfresh™, Agro Fresh, Pennsylvania, USA) in a sealed 60 cm^3 glass box in a germination chamber during the first 24 h of a 72 h incubation period (same as for ethylene containing treatments). GA_3 + 1-MCP treatment seeds were treated with GA_3 solution before incubation in 1-MCP containing atmosphere. For GA_3 containing treatments (GA_3 and GA_3 + 1-MCP), 100 g of seeds were treated with 6.7 ppm GA_3 solution an hour before incubation. The seeds were then divided into nine lots of 50 seeds each per treatment for amylase activity experiment and 100 seeds each per treatment for starch and reducing sugars contents experiment and incubated in a sealed 60 cm^3 glass box in a germination chamber during the first 24 h of a 72 h incubation period (same as for ethylene and 1-MCP containing treatments). For the B-nine containing treatments (B-nine and ethylene + B-nine), filter papers in the Petri-dishes were moistened with 4 ml 10,000 ppm B-nine solution. B-nine treatment seeds were also incubated in a sealed 60 cm^3 glass box in a germination chamber during the first 24 h of a 72 h incubation period

(same as for ethylene, 1-MCP and GA_3 containing treatments). The ethylene + B-nine treatment seeds were incubated in ethylene containing atmosphere as explained for ethylene containing treatments above. The control seeds were also incubated in a sealed 60 cm^3 glass box in a germination chamber during the first 24 h of a 72 h incubation period (same as for ethylene, 1-MCP, GA_3 and B-nine containing treatments); however, no air or chemical treatments were applied.

Amylase activity

Amylase activity was assessed using a modified method of Johnson (2007). Briefly, ten seeds per replicate per treatment were homogenised in 15 ml cold citric acid-sodium citrate buffer (10 mM, pH 5) and centrifuged at 15,000 g for 10 min at 4 °C in an Eppendorf centrifuge (Hamburg, Germany). The supernatant (enzyme extract) was kept on ice and the pellet was discarded. The enzyme extract (0.5 ml per replicate per treatment) and 1 ml starch (0.5 mg ml^{-1} 10 mM citric acid-sodium citrate buffer, pH 5) were mixed, allowed to react for 5 min and stopped by 3.5 ml 1N HCI. The blank (where 1 ml distilled water instead of starch solution was added) and the reaction time zero (where the reaction was not allowed to occur by addition of 3.5 ml 1N HCI to the starch solution before the enzyme extract) controls were included. Half a millilitre iodine solution (5 g KI and 0.36 g KIO_3 dissolved in one litre 2 mM NaOH) was added to all stopped reactions (including the blank control). The amount of starch that remained after each reaction time was determined by measuring light absorption of the resultant solution at 580 nm using a spectrophotometer (Shimadzu, Milton Keynes, UK). Starch concentrations were determined by means of a standard graph according to the method of Knee (1973). Amylase activity was calculated as a percentage using the formula: (Reaction time zero starch concentration – 5 min reaction starch concentration / Reaction time zero starch concentration) × 100.

Determination of starch content

Starch was extracted and determined using a modified method of Knee (1973). Briefly, 0.05 g of the sample powder per replicate (3 replicates per treatment) was mixed with 10 ml distilled water, boiled for 60 min in a water bath, cooled to room temperature and centrifuged for 5 min at full speed in a bench top centrifuge (Optolabor, Johannesburg, South Africa). The pellet was discarded, and the volume of the supernatant was adjusted to 100 ml with distilled water. One millilitre (per replicate per treatment) of the resultant sample extract was mixed with 7 ml distilled water and 2 ml iodine solution (300 mg KI plus 30 mg I_2 per litre). The starch content of the mixture was determined by measuring light absorption at 620 nm using a spectrophotometer (Shimadzu, Milton Keynes, UK). Starch concentrations were determined by means of a standard graph.

Extraction and determination of total reducing sugars

The total reducing sugars was determined based on the method of Başkan et al. (2016). Briefly, the total reducing sugars were extracted by mixing 1 g of the sample powder per

replicate per treatment with 30 ml 95% ethanol and boiling for 15 min on a boiling water bath. The sample was then centrifuged for 10 min at full speed in a bench top centrifuge (Optolabor, Johannesburg, South Africa). The pellet was discarded, and the supernatant was transferred to a round bottom flask. The solvent (ethanol) was evaporated under a vacuum and the remaining extract was transferred to a test tube mixed with 1 ml distilled water and washed three times with 1 ml hexane and again three times with 1 ml chloroform to remove lipids. Residues of hexane and chloroform were evaporated under nitrogen gas and the sample extract was cleaned using solid phase extraction clean up method. The total reducing sugars were determined by measuring light absorbance at 450 nm using a spectrophotometer (Shimadzu, Milton Keynes, UK) and the total reducing sugars concentrations were determined by means of a standard glucose calibration curve.

Data analysis

Analysis of variance (ANOVA) for two factor factorial data, i.e. incubation period and treatment as factors, was performed using Statistical Package for the Social Sciences (SPSS) (SPSS for Windows, Version 16.0. Chicago, SPSS Inc). Means were separated using least squares difference (LSD) at 5% level of significance. Graphs were drawn using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA 98052-7329, USA).

Results

Amylase activity

The effects of ethylene, 1-MCP, GA_3, B-nine, GA_3 + 1-MCP, ethylene + B-nine treatments and the control on amylase activity of the germinating 'Puma' barley seeds after 24, 48 and 72 h from soaking are shown in Fig. 1. There were no significant amylase activity differences between treatments after 24 h, but after 48 and 72 h respectively. After 48 h, the amylase activity of the seeds treated with ethylene, GA_3, 1-MCP, GA_3 + 1-MCP and the control were not significantly different. However, the amylase activity of the seeds treated with ethylene + B-nine was significantly lower compared to that of the seeds treated with GA_3, 1-MCP and the control, but not significantly different from that of the seeds treated with ethylene and GA_3 + 1-MCP. The seeds treated with B-nine had significantly lower amylase activity compared to ethylene, 1-MCP, GA_3, GA_3 + 1-MCP, ethylene + B-nine and the control, respectively. After 72 h, the amylase activity of the seeds treated with ethylene, 1-MCP, GA_3, GA_3 + 1-MCP, ethylene + B-nine and the control were not significantly different, but the amylase activity of the seeds treated with B-nine was significantly lower compared to the seeds treated with ethylene, 1-MCP, GA_3, GA_3 + 1-MCP, ethylene + B-nine and the control, respectively.

Starch content

The effects of ethylene, 1-MCP, GA_3, B-nine, GA_3 + 1-MCP, ethylene + B-nine treatments and the control on starch content of the germinating 'Puma' barley seeds after 24,

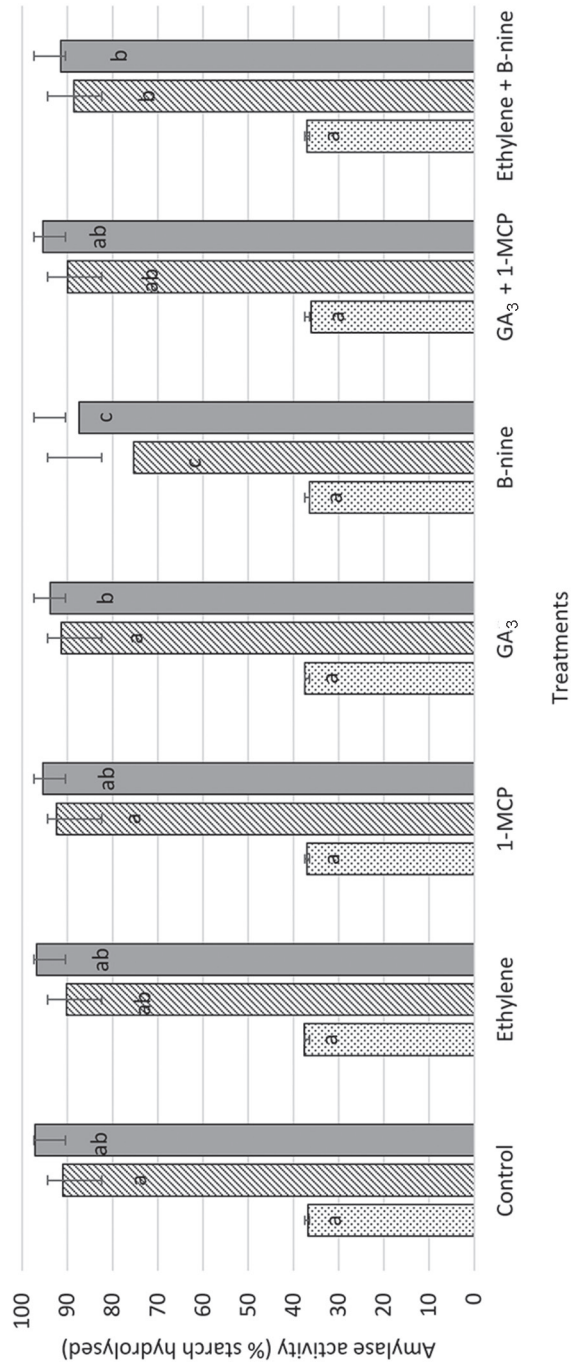

Figure 1. The effects of ethylene, 1-MCP, GA_3, B-nine, GA_3 + 1-MCP, ethylene + B-nine treatments and the control on amylase activity of the germinating 'Puma' barley seeds after 24, 48 and 72 h of incubation, respectively. Error bars represents the standard deviation

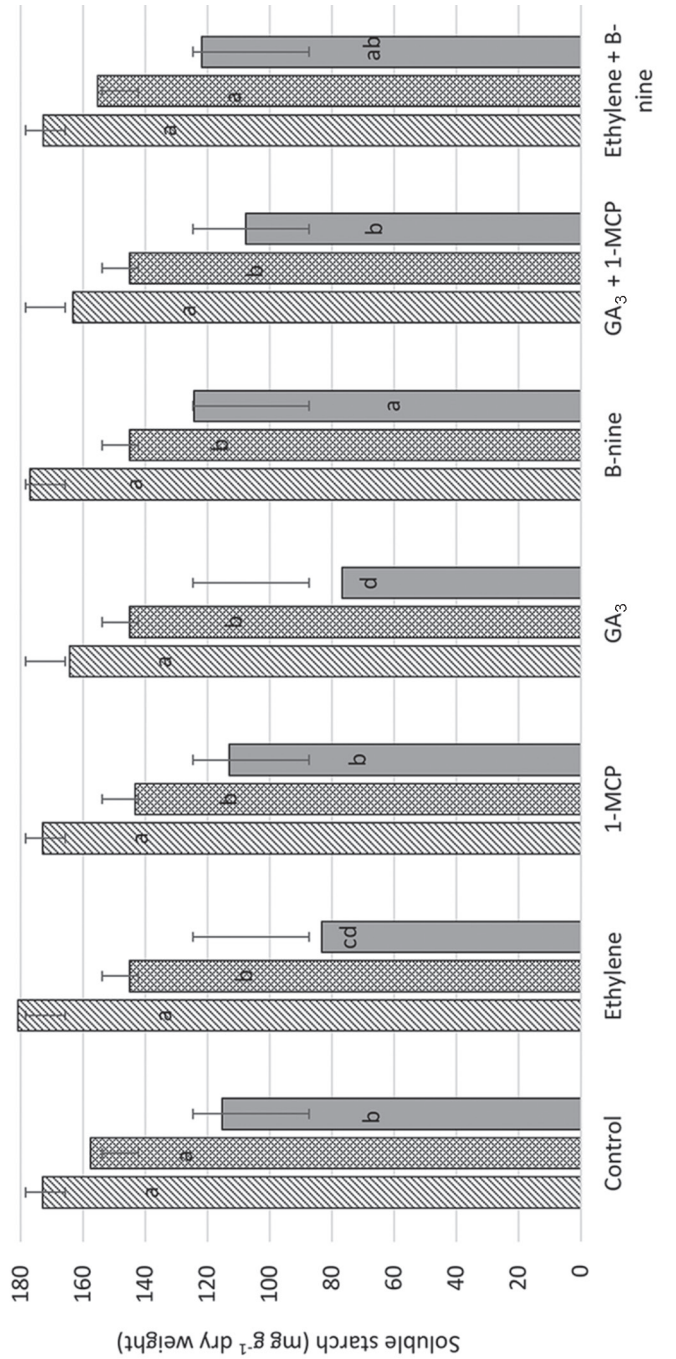

Figure 2. The effects of ethylene, 1-MCP, GA_3, B-nine, GA_3+1-MCP, ethylene+B-nine, treatments and the control on starch content of the germinating 'Puma' barley seeds after 24, 48 and 72 h from soaking, respectively. Error bars represents the standard deviation

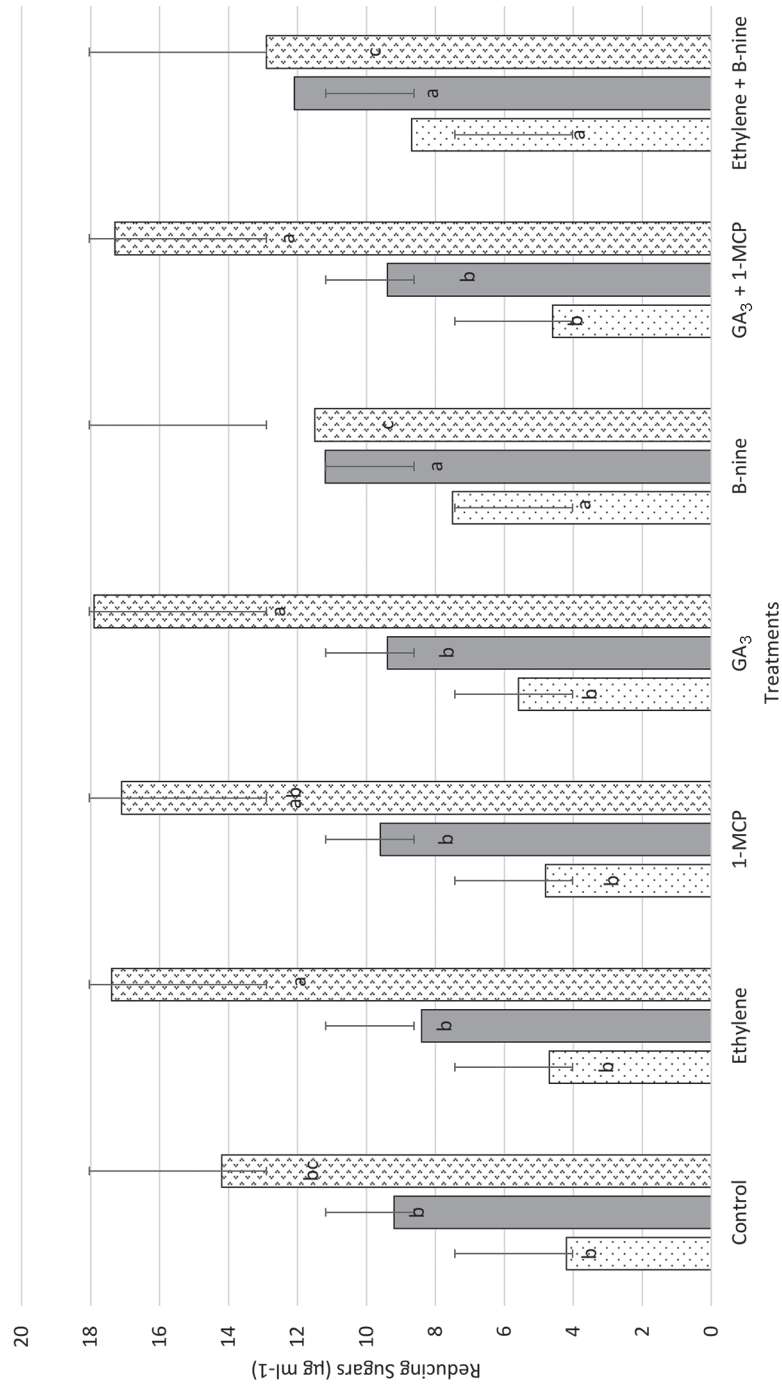

Figure 3. The effects of ethylene, 1-MCP, GA_3, B-nine, GA_3 + ethylene, GA_3 + 1-MCP, ethylene + B-nine treatments and the control on total reducing sugars content of the germinating 'Puma' barley seeds after 24, 48 and 72 h from soaking, respectively. Error bars represents the standard deviation

48 and 72 h from soaking is shown in Fig. 2. There were no significant starch content differences between treatments after 24 h, but after 48 and 72 h, respectively. After 48 h, the starch content of the seeds treated with ethylene, GA_3, 1-MCP, B-nine and GA_3 + 1-MCP were not significantly different, but, were significantly lower compared to that of the seeds treated with ethylene + B-nine and the control. The starch content of ethylene + B-nine and the control seeds were not significantly different. After 72 h, the seeds treated with GA_3 and ethylene had significantly lower starch content and the seeds treated with B-nine had significantly higher starch content compared to the control. The starch content of the seeds treated with ethylene and GA_3 were not significantly different. The starch content of the seeds treated with 1-MCP, GA_3 + 1-MCP and ethylene + B-nine were not significantly different from the control, but, significantly higher compared to that of the seeds treated with ethylene and GA_3. The starch content of the seeds treated with ethylene + B-nine and B-nine were not significantly different.

Total reducing sugars

The effects of ethylene, 1-MCP, GA_3, B-nine, GA_3 + 1-MCP, ethylene + B-nine treatments and the control on total reducing sugars of the germinating 'Puma' barley seeds after 24, 48 and 72 h from soaking are shown in Fig. 3. The total reducing sugars content differ significantly between treatments after 24, 48 and 72 h, respectively. After 24 and 48 h, the total reducing sugars content of the seeds treated with ethylene, 1-MCP, GA_3, GA_3 + 1-MCP and the control were not significantly different, but significantly lower compared to that of the seeds treated with B-nine and ethylene + B-nine. The total reducing sugars content of the seeds treated with B-nine and ethylene + B-nine were not significantly different, but, were significantly higher than that of the control. After 72 h, the total reducing sugars content of the seeds treated with ethylene, GA_3 and GA_3 + 1-MCP were significantly higher compared to that of the seeds treated with B-nine, ethylene + B-nine and the control. The total reducing sugars content of the seeds treated with B-nine and ethylene + B-nine were not significantly different from the control but were significantly lower compared to that of the seeds treated with 1-MCP. However, the total reducing sugars content of the seeds treated with 1-MCP was not significantly different from that of the seeds treated with ethylene, GA_3, GA_3 + 1-MCP and the control.

Discussion

In barley malting, GA_3 is routinely used to enhance germination and grain modification (Briggs 1998). Notwithstanding, GA_3 also causes undesirable levels of proteolysis, rate of respiration and rootlets growth (Yamada 1985; Briggs 1998). Ethylene, a plant hormone with germination stimulation and root growth restriction effects (desirable effects in malting) on barley, was thought to be a potential alternative to GA_3 treatment (Locke et al. 2000; Briggs et al. 2004). However, the effects of ethylene on storage reserve mobilisation relative to GA_3 treatment has not been clearly established. Changes that occur to storage reserves during the germination stage of malting have profound influence on bio-

chemical attributes of malt (Briggs 1998). This study, therefore, compared the effects of ethylene and GA_3 treatments on reserve starch mobilisation during germination of 'Puma' barley seeds. This was achieved by comparing amylase activity, total starch and total reducing sugars contents of 'Puma' barley seeds treated with ethylene, 1-MCP, GA_3, B-nine, GA_3 + 1-MCP, ethylene + B-nine and the control after 24, 48 and 72 h of incubation at 20 ± 1 °C in the dark.

Inhibiting GA synthesis (B-nine treatment) significantly reduced amylase activity after 48 and 72 h, respectively (Fig. 1). These results indicate that endogenous GA synthesis is important for amylase activity. GA_3 treatment is generally known to stimulate amylase activity in poor malting barley seeds (Kobayashi et al. 1995). However, in this study, GA_3 treatment had no effect on amylase activity. Failure by GA_3 treatment to stimulate increased amylase activity may suggest that after-ripened 'Puma' barley seeds produces enough endogenous GA hence increasing GA level by exogenous GA_3 treatment had no effect on amylase activity. In a study with different doses, GA_3 was found to enhance reserve mobilisation up to a certain concentration level, above which, it was less effective (Subedi and Bhattarai 2003).

The amylase activity of both ethylene and 1-MCP treated seeds did not differ significantly from the control (Fig. 1). This suggests that ethylene has no effect on amylase activity of the germinating 'Puma' barley seeds. However, ethylene in ethylene + B-nine treatment rescued 'Puma' barley seeds from B-nine induced reduction of amylase activity. The amylase activity of ethylene + B-nine treated seeds was significantly higher compared to seeds treated with B-nine alone, after 48 and 72 h, respectively (Fig. 1). These results show that ethylene is important for amylase activity where endogenous GA synthesis is inhibited.

GA_3 and ethylene treated seeds had significantly lower starch content compared to the control after 72 h (Fig. 2). Again, there were no statistical starch content differences between ethylene and GA_3 treated seeds after 72 h (Fig. 2). These results suggest that both ethylene and GA_3 treatments increases starch mobilisation during germination of 'Puma' barley seeds. This could be probably because both ethylene and GA_3 stimulates synthesis and release of enzymes by the aleurone layer (Jacobsen 1973; Hartman et al. 2010).

Seeds treated with GA_3 + 1-MCP had significantly higher starch content compared to GA_3 alone treated seeds after 72 h (Fig. 2). This shows that GA_3 treatment need ethylene action to enhance starch hydrolysis after 72 h. 1-MCP inhibits ethylene perception by competing with ethylene for binding sites on ethylene receptors (Taiz and Zeiger 2010). The results also showed that ethylene treatment needed GA synthesis to enhance starch mobilisation after 72 h. Inhibiting GA synthesis in ethylene + B-nine treated seeds had significantly high starch content compared to ethylene alone treatment after 72 h (Fig. 2). B-nine inhibits GA synthesis by inhibiting important enzymes in the synthesis of bioactive GAs (Rademacher 2016). It can be noted that there is a positive interaction between ethylene treatment and GA synthesis, as well as between GA_3 treatment and ethylene action, on starch mobilisation during germination of 'Puma' barley seeds.

The total reducing sugars for GA_3 treated seeds were not significantly different from the control seeds after 24 h (Fig. 3). However, B-nine treated seeds had significantly

higher total reducing sugars compared to both the control and GA_3 treated seeds after 24 and 48 h (Fig. 3). Moreover, the total reducing sugars for the seeds that were treated with treatments where GA synthesis was not inhibited (ethylene, 1-MCP, GA_3, GA_3 + 1-MCP) were significantly lower after 24 and 48 h, and significantly higher after 72 h compared to the seeds that were treated with treatments where GA synthesis was inhibited (B-nine and ethylene + B-nine) (Fig. 3). These results suggest that endogenous GA synthesis is important for utilisation of the reducing sugars by the germinating 'Puma' barley seeds. Free sugars are used for respiration and synthesis of new structural material in the plumule and radical of the germinating grain (Subedi and Bhattarai 2003). In a study to compare the effects of GA_3 on dry matter content of the embryo of germinating maize seeds, Subedi and Bhattarai (2003) found that, the dry matter content of the embryos of GA_3 treated seeds increased while that of the control decreased after 24 h. However, the dry matter contents of both GA_3 treated and the control seeds increased continuously after 48 h (Subedi and Bhattarai 2003). Generally, the dry matter content of the embryo increases because of assimilation of the products of hydrolysis of reserve storage. Since there was no significant total reducing sugars difference between GA_3 treated and the control seeds after 24 h, it could be assumed that after-ripened 'Puma' barley seeds produce enough GA to promote early utilisation of reducing sugars. Generally, after-ripening stimulates barley seeds to produce GA (Chen and Chang 1972), and in this study seeds were stored long enough to after-ripen.

GA_3 treated seeds had significantly high total reducing sugars compared to both B-nine treated and the control seeds after 72 h (Fig. 3). This could be probably due to increased synthesis and release of enzymes by the aleurone layer hence increased endosperm starch hydrolysis (Jacobsen 1973; Briggs et al. 2004). Generally, sugars decrease at the beginning of imbibition due to the respiration rate that is higher than the rate of release of sugars from reserve materials (Harris and MacWilliam 1954; Kunze 2004). However, sugars increase at late stages of germination due to mobilisation of the endosperm storage reserves (Harris and MacWilliam 1954; Kunze 2004). It could, therefore, be noted that GA_3 treatment enhances sugar accumulation during the late stages of germination in 'Puma' barley.

Ethylene treatment had no effect on total reducing sugars of the germinating 'Puma' barley seeds after 24 h, but, significantly increased the total reducing sugars after 72 h (Fig. 3). Since sugars decreases during early stages of incubation and increases during late stages of germination, these results may suggest that ethylene treatment has no effect on early reduction of sugars (24 h), but, enhances accumulation of total reducing sugars during the late stages of germination (after 72 h). This enhanced sugar accumulation could be probably through stimulation of synthesis and release of enzymes from aleurone layer (Jacobsen 1973; Hartman et al. 2010) hence increased reserve starch hydrolysis. It could, therefore, be noted that, both GA_3 and ethylene treatments enhance sugar accumulation during germination of 'Puma' barley.

It could therefore be concluded that like GA_3, ethylene promotes starch degradation during germination of 'Puma' barley seeds. It is therefore, possible to replace GA_3 treatment with ethylene treatment without interfering with starch changing processes during

barley malting. However, the effectiveness of ethylene treatment requires active GA synthesis.

References

Başkan, K.S., Tütem, E., Akyüz, E., Özen, S., Apak, R. 2016. Spectrophotometric total reducing sugars assay based on cupric reduction. Talanta. **147**:162–168.

Bewley, J.D. 2001. Seed Germination and Reserve Mobilization. Encyclopedia of life sciences, Nature Publishing Group, pp. 1–7.

Briggs, D.E. 1998. Malts and malting. Springer Science & Business Media.

Briggs, D.E., Brookes, P.A., Stevens, R., Boulton, C.A. 2004. Brewing: science and practice. Elsevier.

Chen, S.S., Chang, J.L. 1972. Does gibberellic acid stimulate seed germination via amylase synthesis? Plant physiol. **49(3)**:441–442.

Corbineau, F., Xia, Q., Bailly, C., El-Maarouf-Bouteau, H. 2014. Ethylene, a key factor in the regulation of seed dormancy. Frontiers Plant. Sci. **5(539)**:1–13.

Frank, T., Scholz, B., Peter, S., Engel, K.H. 2011. Metabolite profiling of barley: Influence of the malting process. Food chem. **124(3)**:948–957.

Harris, G., MacWilliam, I.C. 1954. Chemical aspects of malting IV. Changes in the carbohydrates of barley during malting. J. I. Brewing **60(2)**:149–157.

Hartman, I., Sladarsky, V.U.P., Prokes, J., Sladarsky, V.U.P., Helanova, A., Sladarsky, V.U.P., Hartmann, J. 2010. The relationship between barley starch content and malt extract. Kvasny Prum. **56**:423–427.

Jacobsen, J.V. 1973. Interactions between gibberellic acid, ethylene, and abscisic acid in control of amylase synthesis in barley aleurone layers. Plant Physiol. **51(1)**:198–202.

Johnson, R. 2007. Measuring amylase activity in cereal grains. Colby Journal of Research Methods **9**:11–13.

Kleinwächter, M., Meyer, A.K., Selmar, D. 2012. Malting revisited: Germination of barley (*Hordeum vulgare* L.) is inhibited by both oxygen deficiency and high carbon dioxide concentrations. Food chem. **132(1)**:476–481.

Knee, M. 1973. Polysaccharide changes in cell walls of ripening apples. Phytochemistry **12(7)**:1543–1549.

Kunze, W. 2004. Technology brewing and malting. 3rd Edition. VLB Berlin. Germany.

Kobayashi, M., Gomi, M., Agematsu, J., Asami, T., Yoshida, S., Sakurai, A. 1995. Fluctuation of endogenous gibberellin and abscisic acid levels in germinating seeds of barley. Bioscience, biotechnology, and biochemistry. **59(10)**:1969–1970.

Locke, J.M., Bryce, J.H., Morris, P.C. 2000. Contrasting effects of ethylene perception and biosynthesis inhibitors on germination and seedling growth of barley (*Hordeum vulgare* L.). Journal of experimental botany. **51(352)**:1843–1849.

MacGregor, A.W., Matsuo, R.R. 1982. Starch degradation in endosperms of barley and wheat kernels during initial stages of germination. American Association of Cereal Chemists. **59(3)**:210–216.

Palmer, G.H. 2018. Barley and malt. In: Stewart, G.H., Russel, I., Anstruther, A. (eds). Handbook of brewing. 3rd Edition. CRC Press, Taylor & Francis Group, Boca Raton. Chap 5: pp. 107–126.

Perveen, A., Naqvi, I.M., Shah, R., Hasnain, A. 2008. Comparative germination of barley seeds (*Hordeum vulgare*) soaked in alkaline media and effects on starch and soluble protein. Journal of Applied Sciences and Environmental Management. **12(3)**:5–9.

Rademacher, W. 2016. Growth retardants: Effects on gibberellin biosynthesis and other metabolic pathways. Annual review in plant biology. **51(1)**:501–531.

Roux, E. 2011. Near infrared (NIR) spectroscopy for selection of malting barley in South African breeding programmes (MSc Thesis, Stellenbosch: University of Stellenbosch).

Subedi, C.K., Bhattarai, T. 2003. Effect of gibberellic acid on reserve food mobilization of maize (*Zea mays* L. var Arun-2) endosperm during germination. Himalayan Journal of Sciences. **1(2)**:99–102.

Taiz, L., Zeiger, E. 2010. Plant physiology (5th edn). Sinauer Associates Inc. Sunderland, Massachusetts.

Woonton, B.W., Sherkat, F., Maharjan, P. 2005. The influence of barley storage on respiration and glucose-6-phosphate dehydrogenase during malting. Journal of the Institute of Brewing. **111(4)**:388–395.

Yamada, K. 1985. Endogenous abscisic acid in barley and use of abscisic acid in malting. Agricultural and Biological Chemistry **49(2)**:429–434.