Shinohara, T., Martin, E.A. and Leskovar, D.I. (2017), *Seed Sci. & Technol.*, **45**, 167-178.
https://doi.org/10.15258/sst.2017.45.1.07

Ethylene regulators influence germination and root growth of globe artichoke seedlings exposed to heat stress conditions

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(Accepted December 2016)

Abstract

High seed germination and root vigour are important traits to improve post-transplant performance of artichoke (*Cynara cardunculus* var. *scolymus*) under heat stress conditions. The effects of exogenous applications of five ethylene regulators each at different concentrations were evaluated on germination and early root growth of artichoke at 23°C in two incubation assays. The ethylene precursors or promoters were DL-methionine (DL-MET), 1-aminocyclopropane-1-carboxylic acid (ACC) and 2-chloroethylphosphonic acid (ethephon, ETH); and ethylene inhibitors were aminoethoxyvinylglycine (AVG) and 1-methylcyclopropene (1-MCP). A subsequent study examined the effects of ETH concentrations on seeds exposed at 23 vs. 30°C (stress). Seed thermodormancy was significantly improved by the application of 30 µM L^{-1} ETH. At optimal temperature (23°C), early root growth was enhanced by ACC and ETH (range of 1-100 µM L^{-1}) with increasing root hair density, root area and lateral roots (except with ETH at 30 µM L^{-1}). Conversely, AVG induced primary root elongation but decreased root hair formation. At higher temperature (30°C), inhibition of early root growth was alleviated when seedlings were incubated at 30 µM L^{-1} ETH. Our results suggest that exogenous ethylene could be useful to alleviate heat stress on artichoke seeds and seedlings, which in turn may improve early growth during stand establishment.

Introduction

Globe artichoke (*Cynara cardunculus* L. var. *scolymus*), an allogamous member of the Asteraceae, was introduced into the USA from the Mediterranean by southern Europeans in the early 1800s (Boriss, 2005). The USA was placed ninth for total worldwide artichoke production in 2012 (FAOSTAT, 2014). Currently, the commercial production of fresh artichoke occurs in California, supplying heads to the USA market year-round with an economic impact of $55.5 million in 2014 (USDA NASS, 2015).

Artichoke is a cool-season crop that grows best at 7 to 29°C and requires adequate soil moisture during the vegetative and reproductive growth stages (Smith *et al*., 2008). Artichoke production is traditionally based on cultivars that are vegetatively propagated

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by offshoots. Due to high disease transmission, this propagation method can cause considerable losses of plants and heads, decreasing crop productivity and final profitability. To avoid these problems, new seed-propagated cultivars have been developed in the last decade (Cravero *et al*., 2005; Bonasia *et al*., 2010; López Anido *et al*., 2010). Artichoke seeds (mature achenes) germinate easily as long as soil moisture is close to field capacity and air temperature ranges from 15 to 25°C (Fernandez *et al*., 2006). However, they undergo dormancy when exposed to high temperatures (Basnizki and Mayer, 1985). After harvest, a seed pretreatment at low temperatures (approximately 4°C) for a minimum of one to two weeks is usually required to break physiological dormancy and enhance germination.

Germination and seedling growth are complex processes controlled by endogenous plant regulators (De Locke *et al*., 2000). The growth regulator ethylene is produced by most plant tissues and is involved in numerous plant developmental processes (Pirello *et al*., 2006). Exposing seeds to ethylene has stimulated germination under adverse conditions in other Asteraceae crop species such as lettuce (*Lactuca sativa* L.) and sunflower (*Helianthus annuus* L.) (Cantliffe and El Balla, 1994). The effect of low temperatures on breaking seed dormancy can also be replaced by applying ethylene precursors such as methionine (MET), 1-aminocyclopropane-1-carboxylic acid (ACC) or ethylene releasing substances such as 2-chloroethylphosphonic acid (Ethephon, ETH) to seeds as solid or liquid water-soluble formulations (Kolarova *et al*., 2010). Soil amended with Met and ACC had higher levels of ethylene and induced ethylene-specific plant responses (triple response: short, thick and apical hook) in etiolated pea (Khalid *et al*., 2006). Both Met and ACC also increased the number and length of root hairs in Brassica species such as *Arabidopsis thaliana* (L.) Heynh and *Brassica rapa* L. (Pitts *et al*., 1998; Hasegawa *et al*., 2003; Zhu *et al*., 2006). Ethylene-releasing compounds such as ethephon increased tiller production of spring cereals without changing root/shoot ratios (Rajala and Peltonen-Sainio, 2001). The most common effect of ethylene regulators is the coordinated slow down of elongation and the increase in the thickening of stems and roots, while other responses include the loss of geotropism, production of adventitious roots, shedding of leaves, flowers and fruits, and stimulation of fruit maturation and seed germination (Kolarova *et al*., 2010). However, the inhibitor of ethylene synthesis aminoethoxyvinylglycine (AVG) stimulated root elongation of barley seedlings (Locke *et al*., 2000) and increased the main root length but reduced lateral root density in common bean (Borch *et al*., 1999). In a study with canola (*Brassica napus* L.), the application of 1-methylcyclopropene (1-MCP), known as an inhibitor of ethylene action, also increased root length without affecting shoot growth (Saleh-Lakha *et al*., 2005).

In the last two decades artichoke cultivation has expanded to semi-arid and hot regions of the USA such as the Coachela Valley in California, and more recently to southwest Texas where experimental yields for fall plantings ranged from 7-12 t ha^{-1} (Leskovar *et al*., 2007). However, fields transplanted during late summer or early autumn under high temperature conditions have poor stand establishment and significant transplant losses. We hypothesised that improving early root development during the seedling stage may enhance field stress tolerance under hot and dry environments. A recent study showed that pre-conditioning artichoke seedlings to low nitrogen levels improved root length,

root surface area, and subsequent stress tolerance after field transplanting (Leskovar and Othman, 2016). Whether root development of artichoke seedlings can further be modulated by ethylene regulators is unknown. Furthermore, the effect of concentration on root growth and how these regulators affect seedling growth under high temperature stress conditions are questions still unknown. Within this context, the aim of the present work was to evaluate the effect of ethylene regulators on seed germination, seedling growth, and root development of artichoke seedlings under normal and stress temperature conditions.

Materials and methods

Seed material

Artichoke cv. Green Globe Improved (Condor Seed Production, Inc., Yuma, AZ) seeds were sterilised in 1.0% sodium hypochlorite for 15 minutes, washed with running tap water for five minutes and rinsed with distilled water three times. Cracked and/or floating seeds were discarded after soaking.

Experimental design

Study1. Effect of ethylene regulators on seed germination and root growth

A total of 16 rooting treatment combinations were evaluated: a control (distilled water) and five ethylene regulators (three promoters and two inhibitors) at three concentrations each. The ethylene regulators were: DL-MET (a.i. 99.7%, Phytochrome Inc., Tokyo, Japan) at 1, 30 and 100 µM L^{-1}, ACC (a.i. 98.0% 1-aminocyclopropane-1-carboxylic acid, CALBIOCHEM®, San Diego, CA) at 1, 30 and 100 µM L^{-1}; ETH (FLOREL®, a.i. 3.9% 2-chloroethylphosphonic acid; Lawn and Garden Products, INC., Fresno, CA) at 1, 30 and 100 µM L^{-1}; AVG (ReTain®, a.i. 15.0% aminoethoxyvinylglycine; Abbot Laboratories, Abbott Park, IL), at 1, 10 and 100 µM L^{-1}; and 1-MCP (active ingredient (a.i.) 3.8% 1-methylcyclopropene, Valent BioSciences Co., Libertyville, IL,) at 1, 10 and 100 µM L^{-1}. Prior to sowing, 5 ml of each solution was added to 90 mm-diameter Petri dishes containing a blue blotter paper. Five seeds were placed on each blotter paper and germinated in darkness at 23.0 ± 0.5°C on a thermo-gradient table controlled by thermocouples (SD 10, Shimaden Co. Ltd., Tokyo, Japan) and a cooling system (VWR 1160S, VWR®, West Chester, PA). This experiment was repeated using the same treatments plus a higher concentration, 1000 µM L^{-1}, of each ethylene regulator (total 21 treatments). During the incubation period, germination was recorded daily to calculate total percent germination and mean days to germination (MDG). MDG was calculated according to the formula $\Sigma Ti \cdot Ni / \Sigma Ni$, where Ni is the number of newly germinated seeds at day Ti. Seedlings were classified into normal (with complete morphological parts), abnormal (with broken cotyledons, less than one cotyledon, missing primary root, poorly developed, or absence of hypocotyls) and dead. At eight days after sowing, each seedling was scanned with a resolution of 600 dpi and root length (mm) as well as root scanned area (mm^2) were analysed with the software WINRHIZO LA-1600 (Regent Instruments Inc., Quebec, Canada).

Study 2: Effect of ethylene on early root development (Transfer germinated seeds to treatments)
Twenty artichoke seeds were seeded on flat water soaked paper towels (250 × 400 mm) covered on the top with an oil-paper (250 × 400 mm) to avoid evaporation and positioned into plastic containers which were placed in an incubator in darkness at 23°C for eight days to allow normal germination. After eight days, when most seeds germinated, uniform artichoke seedlings were selected and transferred five seedlings each to 90 mm-diameter Petri dishes containing a blue blotter paper with 5 ml of each concentration of ethylene regulators used in Study 1. Then, Petri dishes (three replications for each treatment) were placed in the thermo-gradient table in darkness at 23°C for an additional three days (total of 11 days after sowing). Rooted seedlings were evaluated between 8-11 days after sowing (0-3 days after treatment). Each seedling was scanned with a resolution of 600 dpi and the primary root length and hypocotyls length were analysed by WINRHIZO LA-1600. Lateral roots coming from the primary root were also counted.

Study 3: Effect of ethylene and temperature stress on seed germination and root growth
Four rooting treatments, Ethephon at 1, 30 and 100 μM L^{-1} and a control (distilled water) were evaluated at two constant temperatures, 23°C (optimum) and 30°C (heat stress). Prior to sowing, 13 ml of each solution were added to 150 mm-diameter Petri dishes containing a blue blotter paper. A total of 25 seeds were placed on each blotter paper (four replications) for the germination evaluation. Another ten seeds were placed on each blotter paper (six replications) for the root growth evaluation. All Petri-dishes were placed in germination chambers at both temperatures in darkness. Germinated seeds were classified as normal, abnormal and dead as in Study 1 and germination percentage was determined. For root growth evaluation eight days after sowing, each seedling was scanned and root length (mm) and scanned area (mm^2) were analysed with the software WINRHIZO LA-1600.

Statistical analysis
The experiments were conducted using a completely randomised design with 3-6 replications. All measurements were taken in 3-10 subsamples of 9-21 seedlings per treatment, unless otherwise indicated. All data were statistically analysed by ANOVA using SPSS (version 14.0 for Windows; SPSS Inc., Chicago, IL). Differences among treatments were performed using LSD at $P = 0.05$. If the Levene test for homogeneity of variance and/or the Shapiro-Wilk test or Kolmogorov-Smirnov test for normality of experimental error were significant, Kruskal-Wallis test was performed instead of ANOVA and multiple comparisons were performed using Mann-Whitney tests.

Results

Study 1. Effect of ethylene regulators on seed germination and root growth
Ethylene regulators DL-MET, ACC, ETH, AVG and 1-MCP (range from 1 to 1000 μM L^{-1}) did not affect the germination percentage or MDG of artichoke seeds (data not shown).

Overall the average germination was 94.2 and 95.1% and MDG was 5.0 and 4.4 days in the first and second assay, respectively. Root length for the control was 13.6 mm and increased 2-fold, to 27.9 mm with 10 µM L^{-1} AVG (figure 1). No significant difference in root length was observed with the ethylene precursors DL-MET, ACC and ETH or with the ethylene inhibitor 1-MCP. Root area for the control was 89 mm^2 and significantly increased with ETH, being the largest (164 mm^2) with 30 µM L^{-1}. There was also an increasing trend of root surface area with ACC 30 µM L^{-1} (169 mm^2). Conversely, AVG did not affect the root scanned area, rather it tended to decrease at high concentration (100 µM L^{-1}) when compared with the control. AVG induced root elongation only at 10 µM L^{-1} but decreased root hair formation at all AVG concentrations (figure 2). In contrast to AVG, the ethylene precursors ACC and ETH induced a dense root hair formation.

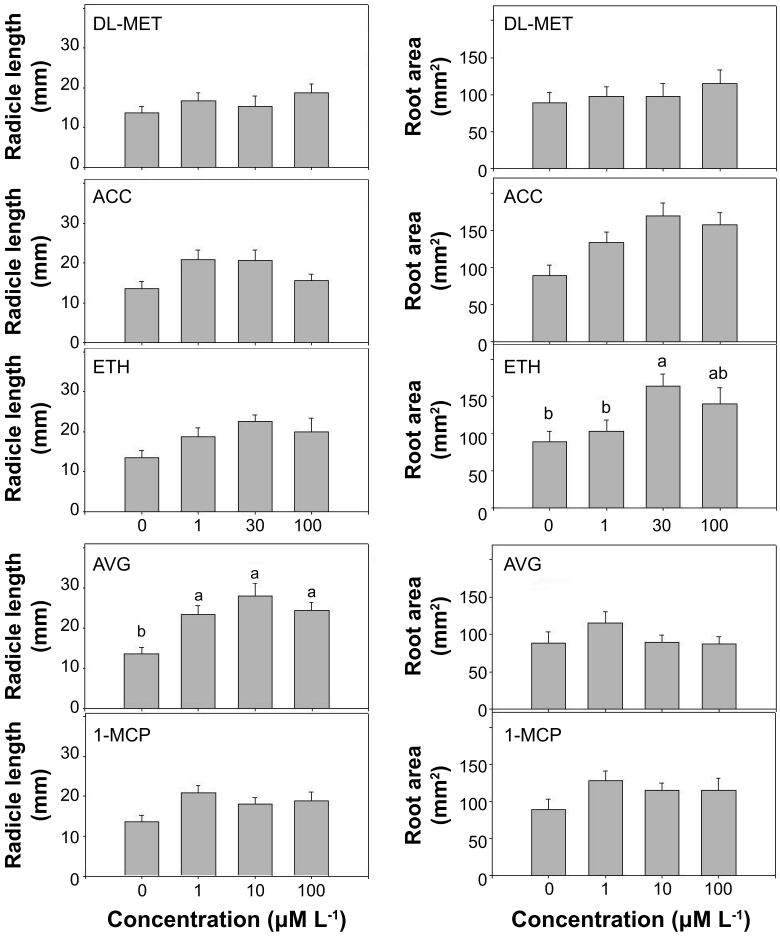

Figure 1. Root length (left) and root scanned area (right) of artichoke seedlings in response to ethylene regulators after eight days of incubation (*Study 1*). Vertical bars indicate mean ± SE (n = 10-14). Means within columns followed by different letters are significantly different (LSD or Mann-Whitney test, $P = 0.05$).

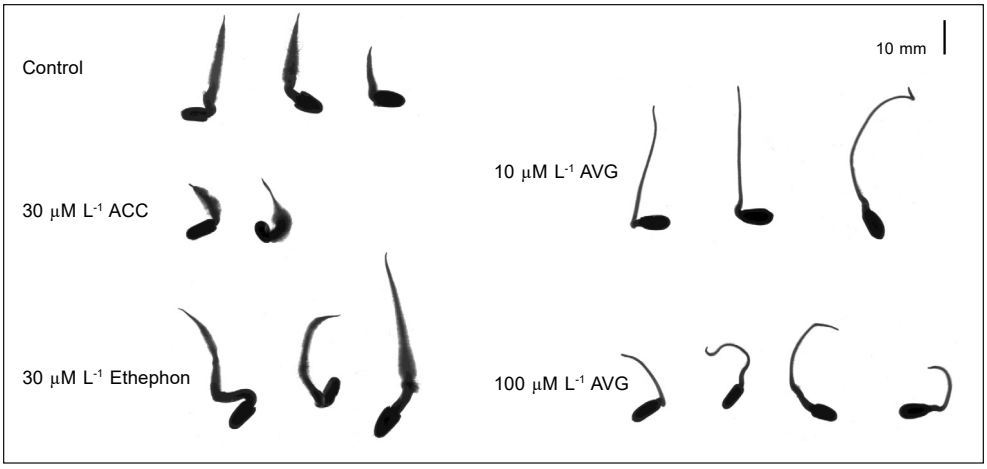

Figure 2. Artichoke root morphology in response to ethylene regulators, eight days after seeding (*Study 1*). Scale bar (10 mm) is placed in the upper right corner of the picture.

In the second assay, the overall root length and root scanned area were much larger than the first assay. This could be related to the environmental temperature fluctuation (± 0.5°C) inside the thermogradient table. Root length for the control was 24.3 mm and significantly increased to 37.2 mm with 10 μM L^{-1} AVG (figure 3), which was the same response as observed in the first assay. An inhibitory effect of ethylene regulators was observed on root elongation at the highest concentration (1000 μM L^{-1}) of ACC, ETH and AVG (11.5, 13.6 and 11.3 mm, respectively), but not with DL-MET and 1-MCP. The root scanned area for the control was 123 mm^2 and significantly increased with ethylene precursors, 1 or 30 μM L^{-1} DL-MET, 1 μM L^{-1} ACC and 30 or 100 μM L^{-1} ETH. AVG at 10 μM L^{-1} also resulted in a larger root scanned area (156 mm^2), but significantly decreased with AVG at higher concentrations (100 or 1000 μM L^{-1}).

Study 2. Effect of ethylene regulators on primary root elongation and lateral root initiation
Root measurements were taken after transferring 8-day old seedlings into the growth regulators. The primary root length measurements were variable among seedlings and treatments. Overall, all ethylene regulators (DL-MET, ACC, ETH, AVG and 1-MCP) applied during the 8-11 days after germination tended to inhibit root elongation as concentration increased from 1 to 100 μM L^{-1}, with the largest inhibition caused by 1-MCP (figure 4). Lateral root initiation was significantly promoted by 30 μM L^{-1} ETH as compared with the control or other ethylene regulators when measured three days after treatment (DAT). Other ethylene precursor (DL-MET and ACC) and inhibitors (AVG and 1-MCP) did not affect the initiation of lateral roots when applied at 10-100 μM L^{-1} (figure 5). The hypocotyl growth was significantly inhibited by ACC at 3 DAT, ETH at 2 and 3 DAT, and AVG at 3 DAT. This response was concentration dependent, with the most severe inhibition at 100 μM L^{-1} ETH or AVG (figure 6). No clear effect on hypocotyl growth was observed with either DL-MET or 1-MCP.

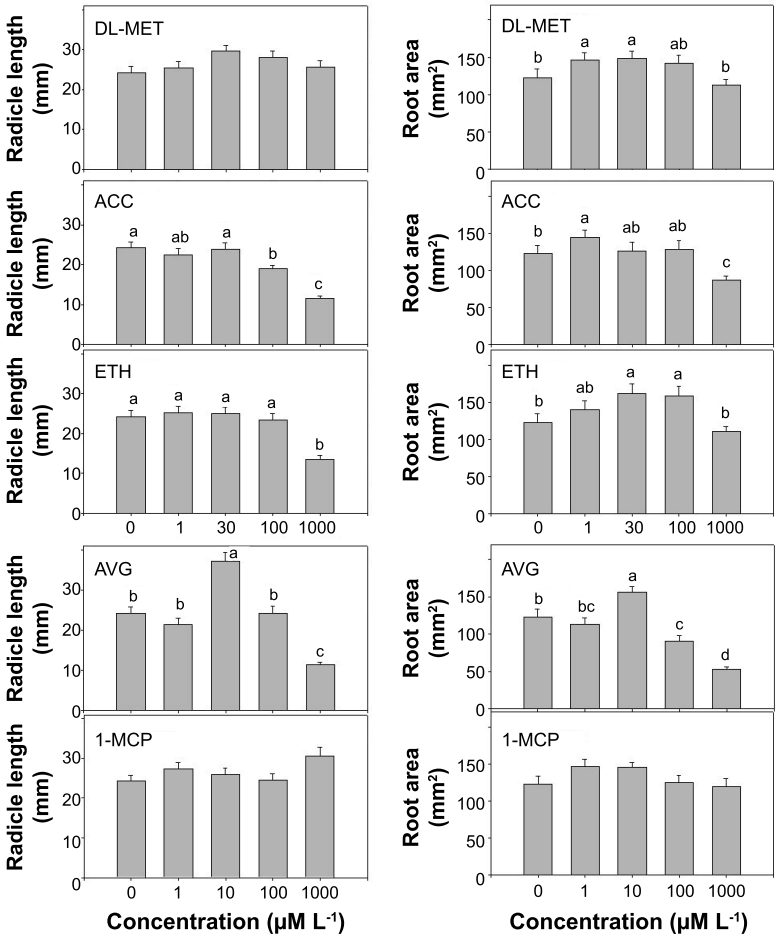

Figure 3. Root length (left) and root scanned area (right) of artichoke seedlings in response to ethylene regulators for eight days (*second assay, Study 1*). Vertical bars indicate mean ± SE (n = 20-21). Means within columns followed by different letters are significantly different (LSD or Mann-Whitney test, $P = 0.05$).

Study 3. Effect of ethylene and temperature stress on seed germination and root growth

The main temperature effect on germination was significant ($P < 0.001$), with seeds incubated at 23°C (optimum) exhibiting higher germination (85%) compared with those germinated at 30°C (56%). However, there were significant ETH concentration and temperature interactions ($P = 0.007$). The partition of this interaction showed that ETH significantly improved germination at 30°C in a concentration dependent manner, whereas it was not significantly different at 23°C. Moreover, the highest germination at 30°C was with ETH at 30 µM L^{-1} (74%) and it showed no significant differences with seeds incubated at 23°C. Similarly, MDG was not affected by ETH concentration and temperature (four days) (data not shown).

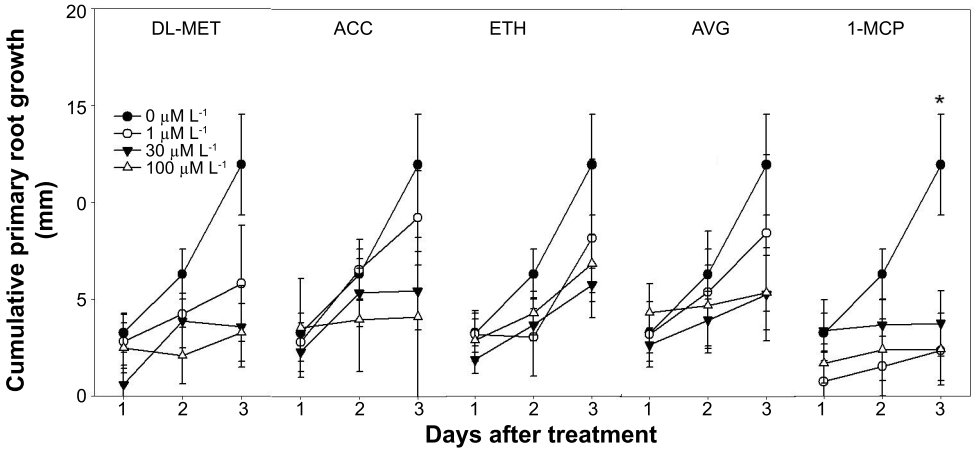

Figure 4. Primary root elongation of artichoke seedlings in response to ethylene regulators. Treatments were imposed from 8 (0 days after treatment; DAT) to 11 (3 DAT) days after seeding (*Study 2 – Transfer germinated seeds to treatments*). Vertical bars indicate mean ± SE (n = 20). Asterisk (*) indicates significant difference (Mann-Whitney test, $P < 0.05$).

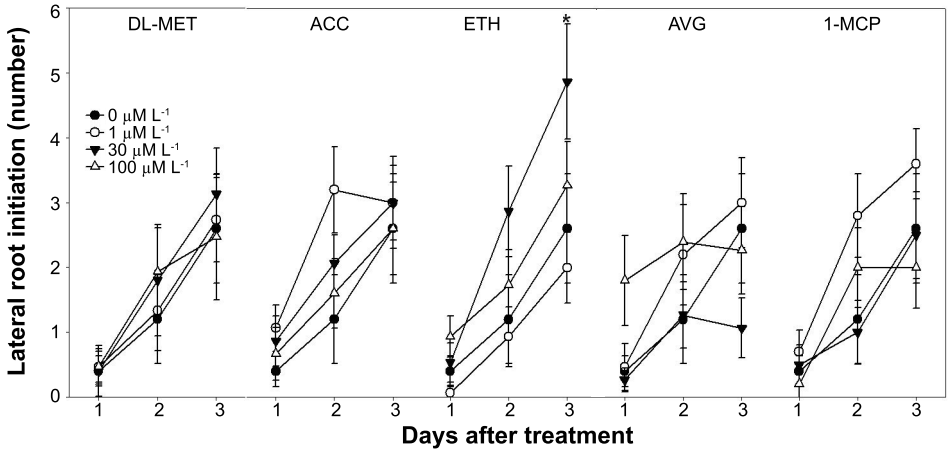

Figure 5. Lateral root initiation of artichoke seedlings in response to ethylene regulators. Treatments were imposed from 8 (0 days after treatment; DAT) to 11 (3 DAT) days after seeding (*Study 2 – Transfer germinated seeds to treatments*). Vertical bars indicate mean ± SE (n = 20). Asterisk (*) indicates significant difference (Mann-Whitney test, $P < 0.05$).

There were significant main effects and interactions of ETH concentration and temperature on root length ($P < 0.001$). Root length was significantly reduced at 30°C but significantly increased with ETH (figure 7). The longest root length was observed at 30 µM L^{-1} (53.6 mm). At 23°C, the root scanned area for the control was 264 mm^2 and significantly increased with ETH concentration, being the largest (355 mm^2) with 30 µM L^{-1}. At 30°C, the root scanned area increased with ETH application to 399 mm^2 at 30 µM L^{-1}.

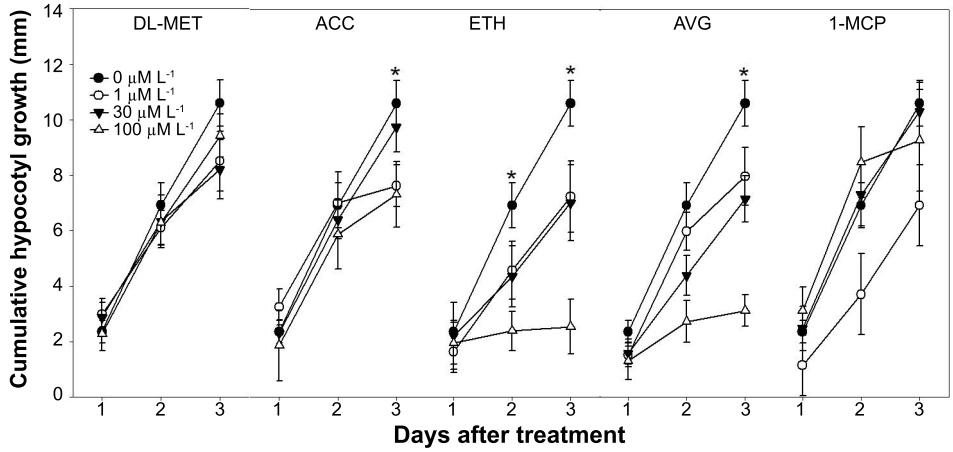

Figure 6. Hypocotyl growth of artichoke seedlings in response to ethylene regulators. Treatments were imposed from 8 (0 days after treatment; DAT) to 11 (3 DAT) days after seeding (*Study 2 – Transfer germinated seeds to treatments*). Vertical bars indicate mean ± SE (n = 20). Asterisk (*) indicates significant difference (LSD or Mann-Whitney test $P < 0.05$).

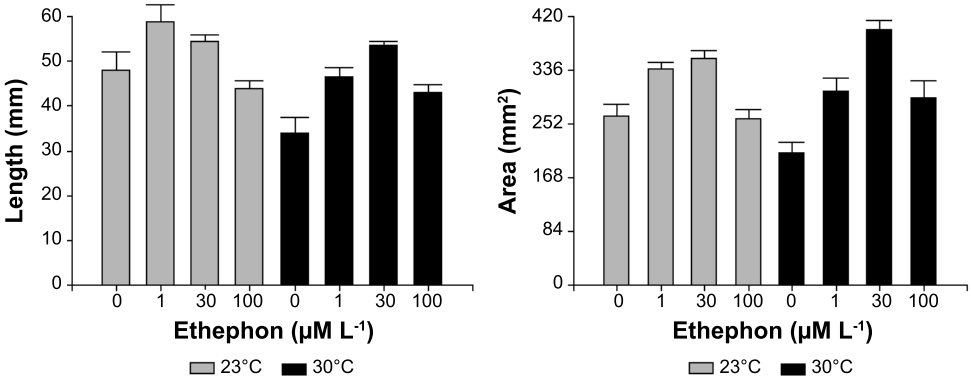

Figure 7. Root length (left) and root scanned area (right) of artichoke seedlings in response to ethephon at 23°C and 30°C (*Study 3*).

Discussion

Ethylene is a plant hormone that regulates several plant growth and developmental processes under optimal and stressful biotic or abiotic environments (Iqbal *et al*., 2013). ACC and methionine are precursors of ethylene; however, ACC (immediate precursor of ethylene) is more effective to convert to ethylene than methionine (Abeles *et al*., 1992). Overall, ethylene regulators did not affect the germination percentage or speed of germination at optimal conditions. It has been reported that ethylene, ethylene-releasing compounds (ETH) or precursors (DL-MET and ACC) stimulate seed germination in many

plant species but this response mainly occurs only when the endogenous ethylene level is low, which is particularly found in dormant seeds or when seeds are exposed to adverse environmental conditions (Matilla, 2000; Arc *et al*., 2013). AVG, an inhibitor of ethylene synthesis, had no influence on germination of lettuce seed at 25°C (Huang and Khan, 1992). The lack of response of artichoke seeds exposed to ethylene regulators under optimum temperature conditions is in agreement with previous reports in other vegetable crops such as tomato, onion and cucumber (Lalonde and Saini, 1992; Pirello *et al*., 2006). However, when artichoke seeds were incubated at 30°C in our third study, the germination percentage was significantly reduced from 85 to 56%. At this high temperature, and as previously presented, ETH at 30 µM L^{-1} promoted germination compared with the control, reaching the same level of germination to that observed at optimal conditions (23°C). Kolarova *et al*. (2010) reported that 100 g L^{-1} ethephon increased the germination capacity of beechnut seeds even after seven weeks of chilling. Ethylene, can also overcome thermodormancy in lettuce, sunflower and *Amaranthus paniculatus* (L.) (Arc *et al*., 2013). Depending on species and depth of seed dormancy, the stimulatory effect of exogenous ethylene increases with concentration in the range of 0.1 to 200 µL L^{-1} (Arc *et al*., 2013). It has been reported that the thermodormancy of artichoke seeds at 30-35°C was caused by the excretion of mucilage from vestiges of the endosperm (Basnizki and Mayer, 1985). Therefore, in this study it may possible that the breaking of thermodormancy by ethylene applications could be due to the excretion of dormancy-inducing mucilage from the endosperm; however, further anatomical investigation is required.

When we compared the effect of ethylene regulators on root growth, all studies showed a similar performance; root length was suppressed and lateral root initiation and scanned area were significantly promoted with ETH application. Furthermore, when artichoke seedlings were exposed to stress temperature (Study 3), the length and scanned area increased at 30 µM L^{-1} ETH, with a similar trend observed at the same doses at 23°C. Our results suggest that ethylene is a positive regulator of root hair formation as shown in other plant species (Tanimoto *et al*., 1995; Pitts *et al*., 1998) as well as a negative regulator of root elongation (Smith and Robertson, 1971; Locke *et al*., 2000). Qin *et al*. (2007) also reported that ethylene may change the direction of cell expansion of lettuce, from longitudinal to a mainly lateral direction. Locke *et al*. (2000) reported that a normal characteristic response of plants to high amounts of ethylene is root thickening, which may enhance root mechanical strength. In wild-type plants of Arabidopsis, Ghassemian *et al*. (2000) observed that applications of low ABA or ethylene concentrations encouraged root growth, whereas higher concentrations inhibited root development. In peach, Keshavarzi *et al*. (2014) reported that high concentration of ethylene (10 µL L^{-1}) suppressed the number of shoots whereas it increased at low concentration (0.1 µL L^{-1}).

In summary, among all ethylene regulators evaluated, the ethylene-releasing compound ETH was the most beneficial to increase germination and root growth of artichoke seedlings when incubated at high temperature. The data suggest that 30 µM L^{-1} ETH was the best to enhance root length and surface area. Improving early root growth in this species can have a positive impact to enhance field establishment of this crop when planted at extreme summer temperatures. However, and as evident in other plant systems,

ethylene responses are very variable as it can either inhibit or enhance plant developmental patterns. These responses to ethylene depend on species, genotype, tissue specificity and presence of other plant hormones, which may affect the endogenous ethylene production or its sensitivity (McManus, 2012). The interaction of ethylene with other plant growth regulators such as gibberellic acid and abscisic acid under temperature stress conditions is worthy to explore in further research especially in newly developed hybrid artichoke cultivars.

Acknowledgements

This research was partially supported by a grant given to E.A. Martin by the Fulbright Comission (USA) and CONICET (Argentina).

References

Abeles, F.B., Morgan, P.W. and Saltveit, M.E. (1992). *Ethylene in Plant Biology*, 2nd Edition, Academic Press.

Arc, E., Sechet, E., Corbineau, F., Rajjou, L. and Marion-Poll, A. (2013). ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. *Frontiers in Plant Science,* **4**, 63, 1-19. doi: 10.3389/fpls.2013.00063

Basnizki, Y. and Mayer, A.M. (1985). Germination of Cynara seeds; effect of light and temperature and function of the endosperm. *Agronomie*, **5**, 529-532.

Bonasia, A., Conversa, G., Lazzizera, C. Gambacorta, G. and Elia, A.M. (2010). Morphological and qualitative characterization of globe artichoke head from new seed-propagated cultivars. *Journal of the Science of Food and Agriculture*, **90**, 2689-2693.

Borch, K., Bouma, T.J., Lynch, J.P. and Brown, K.M. (1999). Ethylene: a regulator of root architectural responses to soil phosphorus availability. *Plant Cell and Environment*, **22**, 425-431.

Boriss, H. (2005). *Commodity Profile: Artichokes*, Agricultural Marketing Resource Center (AgMRC), Agricultural Issues Center, University of California. URL: http://aic.ucdavis.edu/profiles/artichoke2005b.pdf

Cantliffe, D.J. and El Balla, M. (1994). Improved germination of carrot at stressful high temperature by seed priming. *Proceedings of the Florida State Horticultural Society*, **107**, 121-128.

Cravero, V.P., Picardi, L.A. and Cointry, E.L. (2005). An approach for understanding the heredity of two quality traits (head color and tightness) in globe artichoke (*Cynara scolymus* L.). *Genetics and Molecular Biology*, **28**, 431-434.

Ghassemian, M., Nambara, E., Cutler, S., Kawaide, H., Kamiya, Y. and McCourt, P. (2000). Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis. *The Plant Cell*, **12**, 1117-1126.

FAOSTAT (2014). *Major Food and Agricultural Commodities and Producers – Countries By Commodity*, FAO, Rome. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Fernandez, J., Curt, M.D. and Aguado, P.L. (2006). Industrial applications of *Cynara cardunculus* L. for energy and other uses. *Industrial Crops and Products*, **24**, 222-229.

Hasegawa, N., Yamaji, Y., Minoda, M. and Kubo, M. (2003). Effects of D-methionine or L-methionine on root hair of *Brassica rapa*. *Journal of Bioscience and Bioengineerin*, **95**, 419-420.

Huang, X.-L. and Khan, A.A. (1992). Alleviation of thermoinhibition in preconditioned lettuce seeds involves ethylene, not polyamine biosynthesis. *Journal of the American Society for Horticultural Science*, **117**, 841-845.

Iqbal, N., Trivellini, A., Massod, A., Ferrante, A. and Khan, N.A. (2013). Current understanding on ethylene signaling in plants: The influence of nutrient availability. *Plant Physiology and Biochemistry*, **73**, 128-138.

Keshavarzi, M., Funnell, K., Heyes, J. and Woolley, D. (2014). Ethephon and secondary shoot induction in Gentian (Gentiana spp.) hybrids in vitro. *Scientia Horticulturae*, **179**, 170-173.

Khalid, A., Akhtar, M.J., Mahmood M.H. and Arshad, M. (2006). Effect of substrate dependent microbial ethylene production on plant growth. *Microbiology*, **75**, 231-236.

Kolarova, P., Bezdeckova, L. and Prochazkova, Z. (2010). Effect of gibberellic acid and ethephon on the germination of European beech dormant and chilled beechnuts. *Journal of Forest Science*, **56**, 389-396.

Lalonde, S. and Saini, S. (1992). Comparative requirement for endogenous ethylene during seed germination. *Annals of Botany*, **69**, 423-428.

Leskovar, D. and Othman, Y. (2016). Low nitrogen fertigation promotes root development and transplant quality in globe artichoke. *HortScience*, **51**, 567-572.

Leskovar, D.I., Goreta, S., Piccinni G. and Yoo, K.S. (2007). Strategies for globe artichoke introduction in southwest Texas. *Acta Horticulturae*, **730**, 157-163.

Locke, J.M., Bryce J.H., and 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.

López Anido, F.S., Cravero, V.P., Martin, E.A., Crippa, I. and Cointry, E.L. (2010). Differential expression of the genetic variance in F2 populations of reciprocal crosses of artichoke. *Spanish Journal of Agricultural Research*, **8**, 3, 679-685.

Matilla, A.J. (2000). Ethylene in seed formation and germination. *Seed Science Research,* **10**, 111-126.

McManus M.T. (2012). *The Plant Hormone Ethylene – Annual Plant Reviews*, Wiley-Blackwell, Hoboken, NJ. DOI: 10.1002/9781118223086

Pirello, J., Jaimes-Miranda, F., Sanchez-Ballesta, M.T., Tournier, B., Khalil-Ahmad, Q., Regad, F., Latche, A., Pech, J.C. and Bouzayen, M. (2006). SI-ERF2, a tomato ethylene response factor involved in ethylene response and seed germination. *Plant and Cell Physiology*, **47**, 1195-1205.

Pitts, R.J., Cernac, A. and Estelle, M. (1998). Auxin and ehylene promote root hair elongation in Arabidopsis. *Plant Journal*, **16**, 553-560.

Qin, L., He, J., Lee, S.K. and Dodd., I.C. (2007). An assessment of the role of ethylene in mediating lettuce (*Lactuca sativa*) root growth at high temperatures. *Journal of Experimental Botany*, **58**, 3017-3024.

Rajala, A. and Peltonen-Sainio, P. (2001). Plant growth regulator effects on spring cereal root and shoot growth. *Agronomy Journal*, **93**, 936-943.

Saleh-Lakha, S., Grichko, V.P., Sisler, E.C. and Glick, B.R. (2005). The effect of the ethylene action inhibitor 1-cyclopropenylmethyl butyl ether on early plant growth. *Journal of Plant Growth Regulatio*n, **23**, 307-312.

Smith, R., Bameur, A., Bari, M., Cahn, M., Giraud, D., Natwick, E. and Takele, E. (2008). *Artichoke Production in California*, University of California, Division of Agriculture and Natural Resources, Publication 7721, Oakland, California.

Smith, K.A. and Robertson, P.D. (1971). Effect of ethylene on root elongation of cereals. *Nature*, **234**, 148-149.

Tanimoto, M., Roberts, K. and Dolan, L. (1995). Ethylene is a positive regulator of root air development in Arabidopsis thaliana. *Plant Journal*, **8**, 943-948.

USDA NASS (2015). Vegetables 2014 Summary (January 2015). <http://usda.mannlib.cornell.edu/usda/nass VegeSumm//2010s/2015/VegeSumm-01-29-2015.pdf>

Zhu, C., Gan, L., Shen, Z. and Xia, K. (2006). Interaction between jasmonates and ethylene in the regulation of root hair development in *Arabidopsis*. *Journal of Experimental Botany*, **57**, 1299-1308.