Plant Growth Regulator Effects on Germination and Root Traits of ‘Lambada’ and ‘Don Victor’ Onion Cultivars

Maria A. Macias-Leon and Daniel I. Leskovar¹

Department of Horticultural Sciences, Texas A&M University, College Station, TX 77834; and Texas A&M AgriLife Research, Texas A&M University, Uvalde, TX 78801

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Abstract. Onions (*Allium cepa* L.) are easily outcompeted by weeds because of slow germination and relative growth rates. Therefore, high percentage of seed germination and root vigor are important traits to improve field performance. The effects of exogenous plant growth regulators (PGRs), 2-chloroethylphosphonic acid (ethephon, Eth), indole-3-acetic acid (IAA), trans-zeatin (tZ), and 1-aminocyclopropane-1-carboxylic acid (ACC) were evaluated on the germination and root growth of ‘Don Victor’ (yellow) and ‘Lambada’ (red) onion seedlings. Seeds were soaked for 10 hours in hormonal solutions and water (hydro-priming). Seed germination improved with Eth (30 and 100 μM), Eth (100 μM) + IAA (10 μM), and IAA (3 μM) treatments. Root surface area (RSA) increased in response to Eth at 30 and 100 μM , Eth + IAA, and 3 μM IAA. Root length (RL) and root diameter (RD) were enhanced by 1 μM tZ and 100 μM ACC. Eth reduced RL and RD, whereas IAA showed no effects. A subsequent experiment evaluated synergistic effects of different PGRs. Treatment of seeds with ACC (250 μM) + tZ (0.5 μM) and ACC (250 μM) + tZ (0.5 μM) + Eth (20 μM) enhanced RL and RD. RSA was unaffected by ACC + tZ + Eth. The results suggest that exogenous PGRs could be useful to enhance germination, RL, and RSA of onion seedlings.

Onion (*Allium cepa*) is an important horticultural crop with a worldwide production of 88,475,089 tons, with China, India, the United States, Egypt, and Iran as the top five producers (FAOSTAT, 2013). In 2013, U.S. onion production was 3,166,740 tons grown on 56,454 ha (USDA, 2016). Onion represents the fifth most valuable vegetable in the United States with a fresh-market value of \$925 million (USDA, 2016).

Onion field production is subjected to a wide range of challenges. Onions are easily outcompeted by weeds because of slow germination and a relative slow growth rates, which results in bulb size reduction and less homogenous populations (Brewster, 2008). In addition, the unbranched root system with few lateral roots and absence of root hairs (Kamula et al., 1994) leads to inefficient nutrient uptake, which may result in nutrient deficiencies (Sullivan et al., 2001). Improving seed germination and root architecture may

help to overcome nutrient and water limitations and mitigate abiotic stresses.

In barley (*Hordeum vulgare*), endogenous PGRs control germination, seedling growth, and root development (Locke et al., 2000). A usual priming response is the increase in emergence rate over a range of environments and temperatures, resulting in better crop stand and higher yields (Halmer, 2004).

Auxin is known to be involved in germination and root development processes. During seed imbibition, the free natural auxin, IAA, accumulates before the initiation of root elongation (Kucera et al., 2005). In addition, auxins play an important role in promoting lateral roots by stimulating pericycle cells within elongating primary roots to undergo de novo organogenesis, leading to the establishment of new lateral root meristems (Ivanchenko et al., 2008).

Cytokinins (CTKs) are also present in developing seeds and are recognized for stimulating cell division and promoting lateral bud growth and seed germination in *Solanum andigena* (Wareing, 2016). In species such as *Orobanche* and *Striga*, CTKs appear to contribute in breaking dormancy by promoting ethylene release (Kucera et al., 2005). Substantial quantities of CTKs are sourced from root tips, which are then distributed to leaves and the rest of the plant (Wareing, 2016). Moreover, both auxins and CTKs are responsible for root gravitropism (Aloni et al., 2006).

The ethylene precursor ACC and ethylene-releasing substance 2-chloroethylphosphonic acid (Eth) are involved in breaking seed dormancy (Kolářová et al., 2010; Shinohara et al., 2017) and increasing the number and length of root hairs in *Brassica* species (Hasegawa et al., 2003) and globe artichoke (Shinohara et al., 2017).

There is a need to better understand how exogenous hormones, either individually or in combination, affect root initiation and architecture, as well as the development of primary, lateral, and adventitious roots, and root hairs of specific vegetable species such as onion. Our hypothesis was that application of PGRs such as IAA, tZ, ACC, and ethephon will enhance onion seed germination and root traits. The objective of this study was to evaluate the effect of selected PGRs applied singly or in combination on seed germination and seedling root traits of yellow and red onion seedlings.

Materials and Methods

Study 1. Effects of PGRs on seed germination and root growth at low, medium, and high concentrations. Seeds of onion (*Allium cepa*) cultivars Don Victor and Lambada (Nunhems USA Inc., Parma, ID) were used as experimental materials. ‘Don Victor’ is a gold-yellow globe-shaped cultivar with a growing season of 170–175 d, and ‘Lambada’ is an early maturing red round cultivar with a growing season of 160–165 d. Both cultivars are widely used for bulb production across the southwest of the United States and Mexico. Five grams of dry seeds of each cultivar were soaked in 30 mL of hormonal solutions for 10 h as described in Table 1 (Kakei et al., 2015); in addition, water (hydro-priming) and dried seeds were included as controls. After treatment, the seeds were washed three times with distilled water and re-dried under a laminar hood, with lights on and a continuous air flow for 48 h until the original weight was reestablished (Irfan et al., 2005).

For germination and seedling root trait evaluations, four petri dishes containing 25 seeds per treatment were placed on one layer of germination paper imbibed with 3–5 mL of distilled water. The seeds were incubated at 20 °C in the dark until no further germination was observed (9 d). Germination was recorded every 12 h to calculate mean

Table 1. Plant growth regulator treatments (study 1).

Treatments	Concn (μM)		
Control	0		
Hydro-priming	0		
IAA	1	3	10
tZ	1	3	10
ACC	10	30	100
Eth	10	30	100
IAA + Eth	10 + 100		
tZ + ACC	1 + 100		

ACC = 1-aminocyclopropane-1-carboxylic acid; Eth = Ethephon; IAA = indole-3-acetic acid; tZ = trans-zeatin.

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¹Corresponding author. E-mail: d-leskovar@tamu.edu.

germination time (MGT), using the formula $\sum Dn / \sum n$, where Dn is the number of newly germinated seeds on day D and n is the number of seeds (Ellis and Roberts, 1981). Total germination percentage (TGP) was calculated 9 d after incubation. Seedlings were classified as normal (containing a complete cotyledon and roots) and abnormal (absence of cotyledon, root, or both). RL, RSA, and RD were measured and recorded using WINRHIZO LA-1600 (Regent Instruments Inc., Quebec, Canada) with a resolution of 400 dpi. Lateral root growth was recorded if observed.

Study 2. Synergistic effects of PGRs on seed germination and root traits. Table 2 displays six hormonal priming treatments including hydro-priming (hydro) and control. Treatments were selected based on the results of study 1 and modifications were incorporated based on the literature described in Shinohara et al. (2017). Although study 1 showed that *tZ* reduced RL at higher concentrations (1–10 μM), levels were reduced by 50% compared with the lowest treatment. Ethephon treatments displayed a high RL reduction for concentrations above 30 μM ; therefore, for study 2, the concentration was reduced to 20 μM . ACC

treatments did not show any significant response in the range 10–30 μM ; therefore, the concentration tested was 250 μM . Seeds were primed and evaluated as described for study 1.

Statistical analysis. Both incubation studies were conducted using a completely randomized block design with four petri dishes per PGR treatment. Each study was replicated in the growth chambers three times (cycles) in accordance with the methodology described by Langhans and Tibbitts (1997). All treatments were analyzed by the analysis of variance using JMP 13. Differences among treatments were compared by Dunnett's test at $\alpha = 0.05$. If assumptions of normality were not met according to the Shapiro–Wilk test, $P < 0.05$, data were transformed by arcsin for analysis.

Results

Study 1. Effects of PGRs on seed germination and root growth at low, medium, and high concentrations

Total germination percentage. TGP showed significant differences among cultivars ($P = 0.002$). Averaged across all treatments, the red onion 'Lambada' had a TGP of 96% compared with 94% for the yellow 'Don Victor'. PGR treatments showed highly significant differences ($P = 0.001$) in TGP, which was enhanced with *tZ* at 3 μM by 5% compared with control seeds.

Mean germination time. Mean germination times are presented in Fig. 1. Overall, 'Don Victor' germinated 8 h faster than 'Lambada'. MGT was significantly lower for all treatments than that of the control ($P \leq 0.001$). Ethephon concentrations of 30 and 100 μM , and the combination of 10 μM IAA and 100 μM ethephon reduced MGT by

17% and 25%, respectively, compared with the control.

Root length. Root lengths are provided in Table 3. There were significant interactions for RL between the treatment and cultivar ($P = 0.005$). RL of 'Lambada' was enhanced with hydro-priming by 37%; 10 μM IAA by 26%; *tZ* at 1, 3, and 10 μM by 42%, 47%, and 32%, respectively; and ACC at 10, 30, and 100 μM by 29%, 47%, and 49%, respectively, compared to control. For 'Don Victor', RL growth was also promoted with hydro-priming by 31%; *tZ* at 1, 3, and 10 μM by 44%, 25%, and 20%, respectively; and ACC at 10, 30, and 100 μM by 17%, 20%, and 23%, respectively, compared with the control. The highest RL increase was observed at 1 μM *tZ* (3.52 cm). By contrast, both onion cultivars showed a dramatical root growth inhibition with Eth at 30 and 100 μM . 'Don Victor' RL was reduced by 53% at 100 μM Eth, whereas 'Lambada' RL was reduced by 45%.

Root diameter. In general, 'Lambada' had a higher RD than 'Don Victor' (Table 3). 'Lambada' displayed an increase in RD when exposed to hydro-priming, IAA at 1 and 10 μM , and all *tZ* and ACC concentrations. The highest increase in 'Lambada' RD was observed with *tZ* at 3 μM (0.75 mm) by 56%. RD of 'Don Victor' was also increased by hydro-priming, but only by *tZ* at 3 and 10 μM , ACC at 30 and 100 μM , and CTK + ACC. The highest RD was observed with *tZ* at 1 μM in 'Don Victor' (0.63 mm), with an increase of 40% compared with the control. RD had a dramatic reduction, by more than 50%, with Eth at 30 and 100 μM and IAA + Eth treatment in both cultivars.

Root surface area. Averaged across all treatments, 'Lambada' showed a slightly greater RSA (0.59 cm^2) than 'Don Victor' (0.56 cm^2). RSA of the different treatments is shown in Fig. 2. Ethephon at 10, 30, and 100 μM enhanced RSA by 5%, 16%, and 22%, respectively. The IAA + Eth treatment showed the greatest RSA increase (by 26%) compared with the control (0.68 cm^2 vs. 0.54 cm^2). Priming treatments with ethephon appeared to promote root hair development as ethephon concentration was increased (Fig. 3). IAA at 1 and 3 μM also improved RSA, but only by 5% and 7%, respectively.

Study 2. Synergistic effect of PGRs on seed germination and root traits

Total germination percentage. Opposite to what was observed in study 1, 'Don Victor' had a higher TGP (94.9%) than 'Lambada' (93.1%). Overall, TGP was increased for all PGR treatments. The ACC + *tZ* + Eth treatment enhanced TGP by 9% compared with the control, followed by an increase of 7% with the IAA + ACC + *tZ* + Eth treatment.

Mean germination time. 'Lambada' germinated 4 h sooner (2.97 d) than 'Don Victor' (3.16 d). No significant interaction was observed between the cultivars and treatments ($P = 0.745$). A significantly lower MGT was observed in all treatments when compared with the control. The greatest MGT

Table 2. Plant growth regulator treatments (study 2).

Treatments	Concn (μM)
Control	0
Hydro-priming	0
IAA + ACC	2 + 250
<i>tZ</i> + ACC	0.5 + 250
IAA + <i>tZ</i> + ACC	2 + 0.5 + 250
IAA + <i>tZ</i> + ACC + Eth	20 + 2 + 0.5 + 250

ACC = 1-aminocyclopropane-1-carboxylic acid; Eth = Ethephon; IAA = indole-3-acetic acid; *tZ* = *trans*-zeatin.

Fig. 1. Mean germination time of onion seedlings in response to indole-3-acetic acid (IAA), *trans*-zeatin (*tZ*), 1-aminocyclopropane-1-carboxylic acid (ACC), ethephon (Eth), Eth + IAA, and *tZ* + ACC concentrations. Vertical bars indicate mean $\pm$ SE ($n = 25$). Numbers above treatments are concentrations in μM . *, **, and *** represent significant difference at $P = 0.05$, $P = 0.01$, and $P \leq 0.001$, respectively, compared with the control (Dunnett's test). As there were no significant interaction effects between cultivars (Don Victor and Lambada) and treatments, averages of both cultivars are displayed.

Table 3. Root length (RL) and average root diameter (RD) of two onion cultivars (Don Victor and Lambada) nine days after incubation with different concentrations of IAA, *tZ*, ACC, Eth, IAA + Eth, and *tZ* + ACC.

Treatment	Concn (μM)	RL (cm)		RD (mm)	
		Don Victor	Lambada	Don Victor	Lambada
Control	0	2.44	2.61	0.45 ^{NS}	0.48
Hydro-priming	0	3.23**	3.58**	0.59**	0.69***
IAA	1	2.56 ^{NS}	3.43**	0.43 ^{NS}	0.62*
	3	2.38 ^{NS}	3.07 ^{NS}	0.39 ^{NS}	0.56 ^{NS}
	10	2.36 ^{NS}	3.31 ^{NS}	0.43 ^{NS}	0.62*
<i>tZ</i>	1	3.52***	3.74***	0.63***	0.69***
	3	3.07*	3.85***	0.55 ^{NS}	0.75***
	10	2.93 ^{NS}	3.48**	0.53 ^{NS}	0.68**
ACC	10	2.88 ^{NS}	3.39*	0.52 ^{NS}	0.65**
	30	2.93 ^{NS}	3.84***	0.53 ^{NS}	0.74***
	100	3.01 ^{NS}	3.89***	0.53 ^{NS}	0.73***
Eth	10	2.24 ^{NS}	2.54 ^{NS}	0.39 ^{NS}	0.46 ^{NS}
	30	1.95 ^{NS}	1.91 ^{NS}	0.31**	0.30**
	100	1.39***	1.46***	0.21***	0.22***
IAA + Eth	10–100	1.58**	1.64**	0.23***	0.25***
<i>tZ</i> + ACC	1–100	3.04 ^{NS}	3.83***	0.53 ^{NS}	0.71***

Shown are the mean RL and RD of three replications, consisting of four subsamples with 25 seeds each. ^{NS}, *, **, *** Nonsignificant or significantly different at $P = 0.05$, $P = 0.01$, and $P \leq 0.001$, respectively, compared with the control (determined by Dunnett's test).

ACC = 1-aminocyclopropane-1-carboxylic acid; Eth = Ethephon; IAA = indole-3-acetic acid; *tZ* = *trans*-zeatin.

Fig. 2. Surface area of onion seedlings in response to indole-3-acetic acid (IAA), *trans*-zeatin (*tZ*), 1-aminocyclopropane-1-carboxylic acid (ACC), ethephon (Eth), Eth + IAA, and *tZ* + ACC concentrations. Vertical bars indicate mean $\pm$ SE ($n = 25$). Numbers above treatments are concentrations in μM . *, **, and *** represent significant difference at $P = 0.05$, $P = 0.01$, and $P \leq 0.001$, respectively, compared with the control (Dunnett's test). As there were no significant interaction effects between cultivars (Don Victor and Lambada) and treatments, averages of both cultivars are displayed.

Fig. 3. Onion root morphology in response to 100 μM ethephon (A) and control (B), 9 d after incubation.

enhancement was obtained with the IAA + ACC + *tZ* + Eth treatment, by 13% compared with the control (Fig. 4).

Root length. A significant interaction ($P = 0.005$) was found between the cultivars and treatments for RL (Fig. 5). RL growth for 'Don Victor' was promoted by IAA + ACC + *tZ* + Eth, ACC + *tZ*, and ACC + *tZ* + Eth, by 22%, 32%, and 37%, respectively, when compared with the control. RL growth for 'Lambada' was enhanced by all PGR treatments, with IAA + ACC promoting RL growth by 70% compared with the control.

Root surface area. No significant differences in RSA were observed among the PGR treatments. In terms of cultivars, Don Victor had a higher RSA (0.53 cm^2) than Lambada (0.48 cm^2).

Root diameter. A significant interaction ($P = 0.003$) for RD was observed between the cultivars and PGR treatments (Table 4). The RD of 'Don Victor' increased with ACC + *tZ* and ACC + *tZ* + Eth, whereas other treatments showed no significant effect. The RD of 'Lambada' increased with all treatments. The highest increase was measured with IAA + ACC, with a RD of 0.70 mm compared with 0.43 mm for the control.

Discussion

Ethylene is known for inducing seed germination by promoting the rupturing of the testa and endosperm, while antagonistically interacting with inhibitory effects of ABA (Finkelstein et al., 2008). In *Arabidopsis* and *Lepidium sativum*, ethylene promotes cap and endosperm rupture by neutralizing ABA effects (Linkies et al., 2009). It is also well known that CTKs promote ethylene production; therefore, some of their effects might be mediated by ethylene (Stenlid, 1982). Nonetheless, explaining the cross talk between ethylene and CTKs is complicated by the fact that ethylene is known to reduce endogenous auxin levels, whereas exogenous applications of CTKs increase IAA levels (Saleh, 1981). In pea plants, the ethylene precursor ACC has been shown to increase ethylene production in the radicle and to promote radicle emergence (Petrucelli et al., 2003).

The results of this study were consistent with the hypothesis that PGR priming treatments might improve the time of germination and percentage of germination. IAA concentrations increased the speed of germination compared with the control, but did not affect TGP. Conversely, *tZ* enhanced TGP, but did not improve MGT. Ethephon at 30 and 100 μM improved MGT the most, but at 100 μM concentration reduced TGP. Finally, ACC priming treatments showed no significant effect in enhancing seed MGT or TGP. KeÇpczyński and KeÇpczyńska (1997) reported ACC effects on seed germination being less evident than that of ethylene, which could be attributed to the inability of seeds to convert ACC to ethylene, as observed by Satoh and Esashi (1983) in

Fig. 4. Mean days of germination in response to plant growth regulator applications. Results shown are means of two onion cultivars (Lambada and Don Victor). Vertical bars indicate mean $\pm$ SE ($n = 25$). Hormonal concentrations were indole-3-acetic acid (IAA) 3 μ M; 1-aminocyclopropane-1-carboxylic acid (ACC) 250 μ M; *trans*-zeatin (*tZ*) 0.5 μ M; and ethephon (Eth) 20 μ M. *, **, and *** represent significant difference at $P = 0.05$, $P = 0.01$, and $P \leq 0.001$, respectively, compared with the control (Dunnett's test).

Fig. 5. Root length of two onion cultivars (Don Victor, yellow; Lambada, red) in response to plant growth regulator applications. Vertical bars indicate mean $\pm$ SE ($n = 25$). Hormonal concentrations were indole-3-acetic acid (IAA) 3 μ M; 1-aminocyclopropane-1-carboxylic acid (ACC) 250 μ M; *trans*-zeatin (*tZ*) 0.5 μ M; and ethephon (Eth) 20 μ M. *, **, and *** represent significant difference at $P = 0.05$, $P = 0.01$, and $P \leq 0.001$, respectively, compared with the control (Dunnett's test).

dormant cocklebur seeds. Study 2 also demonstrated that PGR treatments increase the TGP up to 9% with the ACC + *tZ* + Eth treatment and decrease the MGT by 13% with the ACC + *tZ* treatment when compared

with the control, but only by 7% and 3% with respect to hydro-priming for TGP and MGT, respectively. In an early study by Brocklehurst and Dearman (1983), carrot, celery, and onion displayed a MGT reduction

when seeds were previously exposed to hydro-priming. Therefore, it was not possible to differentiate whether germination enhancement resulted from hydro-priming, hormonal treatments, or both for studies 1 and 2.

In *Chenopodium bonus-henricus*, PGR applications improved germination performance of seeds grown under unfavorable conditions by 68%, reducing MGT from 96 to 30 h to reach 50% total germination (Khan and Karssen, 1980). Seeds were exposed to high temperatures (29 °C), and secondary seed dormancy was broken with a combination of kinetin, ethephon, and GA₄₊₇. In our studies, PGR effects on TGP and MGT did not improve compared with that of hydro-priming; this lack of significant differences could be attributed to seeds being incubated under optimal temperature conditions. Moreover, seeds were germinated on paper as a preliminary screening of PGR effects on early onion growth. Seedling emergence in soil must be evaluated in future experiments to assess the real impact of PGRs on onion stand establishment. Emphasis should be given to treated seeds exposed to abiotic stresses, such as extreme high and low temperatures, and drought stress.

Ethylene inhibited root elongation by decreasing root cell length at 2–4 mm from the root tip in maize (Whalen and Feldman, 1988). In sunflowers and *Arabidopsis thaliana*, ACC and ethylene applications inhibited root elongation (Finlayson et al., 1996; Ivanchenko et al., 2008). In study 1, ethephon applied at 100 μ M resulted in primary root inhibition by 45% in 'Don Victor' and 53% in 'Lambada' (Table 3). By contrast, there was no statistical evidence of root growth inhibition when the ethylene precursor ACC was applied, which is contrary to what Finlayson et al. (1996) reported in sunflower. Conversely, study 2 displayed a 70% increase in root elongation with IAA + ACC in 'Lambada'. This response could be attributed to the IAA counteracting the detrimental effects of ACC. In *Arabidopsis*, Negi et al. (2008) observed a similar response of IAA at 1 μ M in a combination treatment with ACC at 1 μ M, whereas auxin overcame the negative root growth inhibition due to ACC.

Auxin has been described as a positive regulator of lateral root initiation and root development at or above 0.1 μ M (Poupart et al., 2005). IAA is known to regulate the development of primary and lateral roots (Taiz et al., 2010). In study 1, IAA applied at 10 μ M to 'Lambada' (red type) increased RL compared with the control; however, 'Don Victor' (yellow type) showed neither an increase nor a reduction of primary root growth. In onions, the auxin 1-naphthalene-acetic acid (NAA) applied at 0.01 μ M promoted lateral root development but inhibited primary root elongation (Lloret and Pulgarin, 1992). In a study by Zhang et al. (2012), indole-3-butyric acid (IBA) and NAA promoted adventitious root development. IBA induced adventitious root formation and proliferation at optimum concentrations (1–2 mg·L⁻¹, 4.9–9.8 μ M) in *Periploca sepium*. In

Table 4. Average root diameter (RD) of two onion cultivars (Don Victor and Lambada) 9 d after incubation at different priming treatments: Control, hydro-priming, IAA + ACC, IAA + ACC + *tZ* + Eth, ACC + *tZ*, and ACC + *tZ* + Eth.

Root parameter	Treatment	Onion cultivar	
		Don Victor	Lambada
Diameter (mm)	Control	0.49	0.43
	Hydro-priming	0.51 ^{NS}	0.66***
	IAA + ACC	0.49 ^{NS}	0.70***
	IAA + ACC + <i>tZ</i> + Eth	0.56 ^{NS}	0.68***
	ACC + <i>tZ</i>	0.61 ^{NS}	0.67***
	ACC + <i>tZ</i> + Eth	0.62*	0.68***

Values are means of RD of three replications, consisting of four subsamples with 25 seeds each. Hormonal concentrations were IAA 3 µM; ACC 250 µM; *tZ* 0.5 µM; and Eth 20 µM.

^{NS}, *, **, ***Nonsignificant or significantly different at $P = 0.05$, $P = 0.01$, and $P \leq 0.001$, respectively, compared with the control (determined by Dunnett's test).

ACC = 1-aminocyclopropane-1-carboxylic acid; Eth = Ethephon; IAA = indole-3-acetic acid; *tZ* = *trans*-zeatin.

a study by Zhang et al. (2012) where selected root explants were cultured on a medium supplemented with auxins (IBA and NAA), development of ≈ 10 –15 adventitious roots on every root explant was observed when IBA was added to the medium, whereas NAA addition only generated callus formation on the root explants. In our studies, it may be possible that the lack of onion root responses to IAA concentrations might be because of their low sensitivity to this type of auxin.

An early study by Ivanchenko et al. (2008) suggests that ethylene and auxin interact to suppress lateral root initiation. The cross talk between ethylene and auxin has been demonstrated, but their interactions on root branching have not yet been described. In *Arabidopsis*, application of low levels of ACC, the ethylene precursor, promotes the initiation of lateral root primordia. By contrast, higher ACC concentrations inhibit the ability of pericycle cells to start new lateral root primordia, but promote the emergence of existing lateral root primordia (Ivanchenko et al., 2008). Moreover, Tanimoto (2005) observed an increase in root hair cells when *Arabidopsis* seedlings were sprayed with ACC. Even though root hair development was not quantified in our studies, onion roots treated with Eth at 30 and 100 µM displayed a higher RSA, which could be attributed to the increase in root hair development (Fig. 3). However, it was not possible to validate if ethylene or ACC promoted lateral root development, because none of our treatments in study 1 or 2 affected lateral root initiation.

Cytokinins are negative regulators of root growth and development (Werner et al., 2001). Bertell and Eliasson (1992) showed a significant root reduction in pea roots treated with 1 µM *tZ*. Similar results were previously reported in maize roots (Bourquin and Pilet, 1990). However, our study on onions suggests an increase in primary RL at all *tZ* concentrations (1–10 µM) when compared with the control (Table 1). The largest RL was observed with *tZ* at 1 µM. As there is no literature reporting an increased RL in response to exogenous CTK application in onion, our findings open the possibility to further determine whether root responses to CTK are species specific.

There is evidence that lateral root formation is affected by the interaction between

CTKs and auxins (Aloni et al., 2006). In our study, there was no indication of lateral or adventitious root formation in any of the treatments; however, all *tZ* concentrations and combinations with other hormones showed an increase in RD in both studies (Tables 3 and 4). It has been reported that exogenous applications of CTKs increased RD by swelling the root elongation zone, which is due to the expansion of cortex cells and the formation of large intercellular spaces (Kappler and Kristen, 1986).

PGRs can positively regulate seed germination, root growth and development, or both. From the findings of this research with PGR applications in onions, the following conclusions can be drawn: 1) ethephon proved beneficial to increasing onion RSA at a moderate concentration, but reduced RL; 2) addition of IAA, *tZ*, or both could potentially control the reductions in RL and diameter in response to a single application of ethephon; 3) combining *tZ* and ACC increased RL; and 4) hydro-priming proved to be a simple and viable method to improve onion germination and root traits. There is a need for a better understanding of the hormonal pathways and molecular interactions underlying seed germination and root growth traits (Miransari and Smith, 2014). This is particularly important for onions because bulb development, uniformity, and final size are greatly affected by the time spread of seedling emergence during early establishment (Benjamin, 1990). Field experiments are necessary to validate the hypothesis that improved seed germination and root architecture after treatment with specific PGRs will translate into better stand establishment, especially under stressful abiotic stress conditions.

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