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Comparison of seed priming techniques with regards to germination and growth of watermelon seedlings in laboratory condition

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Seeds of watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai, cv. Crimson Sweet] were used to investigate the effects of different priming techniques on seed germination and early seedling growth. The seeds were soaked in solutions of 0.2% gibberellin (50 mg L^{-1} gibberellic acid), 0.2% cytokinin (90 mg L^{-1} kinetin), 0.2% potassium nitrate (2 g L^{-1} KNO_3), 0.2% calcium nitrate [2 g L^{-1} $Ca(NO_3)_2$] or water (hydropriming) for 6 h at 25°C. After drying, five replicates of 25 seeds were distributed in plastic boxes with blotter paper and kept into a seed germinator at 26°C for 12 days. The different priming treatments significantly affect the measurements of the seed germination and growth of watermelon seedlings. The germination of watermelon seeds ranged from 0 to 100%, and was significantly greater when seeds were subjected to priming with GA_3, KNO_3, $Ca(NO_3)_2$ and water (control), and lower under cytokinin (CK) priming. The seed priming with 0.2% solution of CK resulted in 100% of abnormal seedlings, and therefore should not be used by watermelon growers. Seed priming with GA_3, KNO_3, $Ca(NO_3)_2$ and water (hydropriming) increased the shoot length, whereas GA_3, $Ca(NO_3)_2$ and water priming improved the radicle length, as well as shoot dry matter watermelon seedlings. The KNO_3 and water priming increased the root dry matter of watermelon seedlings. Seed priming with KNO_3 and $Ca(NO_3)_2$ improved the germination rate and seedling vigor index. The results of this study show that seed priming with GA_3, KNO_3, $Ca(NO_3)_2$ and water (hydropriming) may be useful tools due to their positive effects on germination percentage and growth characteristics of watermelon seedlings.

Key words: *Citrullus lanatus*, cytokinin, gibberellin, hydropriming.

INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] is an annual vine of the cucurbit family (Cucurbitaceae). It is a native species from warm and dry regions of Africa, and now widely cultivated throughout

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the world for its edible fruits (Filgueira, 2008). Among the cucurbits, watermelon is the most popular in Brazil and in the world. Optimum seed germination and seedling emergence in watermelon occur at relatively high temperatures (25-28°C). Poor seed germination is a common phenomenon at sub-optimal temperatures (Demir and Mavi, 2004), which causes a great concern for growers that growing watermelon seedlings in late winter and early spring in the southern and south eastern regions of the Brazil. These two regions were responsible for nearly 50% of Brazilian production of watermelon in the 2014/2015 season (AGRIANUAL, 2015). Delayed and reduced seedling emergence cause non-uniform stand establishment, which result in yield reductions (Singh et al., 2001) and impairs the early watermelon markets in the cool regions of Brazil.

Many treatment techniques have been developed to improve the germination of watermelon seeds, especially under improper conditions. There is no universal technique for improving seed germination. Among the methods used, pretreatment of seeds with plant growth regulators and salts are considered the most appropriate and promising because of ease of application, scale of economies, and labor-saving attributes compared with methods in which the environment must be controlled for prolonged periods of time (Demir and Mavi, 2004; Nascimento, 2005; Ghassemi-Golezani and Esmaeilpour, 2008). Indeed, seed priming treatments using salts such as potassium nitrate (KNO_3) have been effective in improving watermelon germination under improper conditions (Demir and Mavi, 2004; Nascimento, 2005). Hydropriming treatment has also be successfully applied to improve germination performance of watermelon (Huang et al., 2002) and cucumber seeds (Gurgel et al., 2009). However, few reports were documented on priming treatments using plant growth regulators (PGRs) such as gibberellin and cytokinin in watermelon seeds.

Cytokinin (CK) and gibberellin (GA_3) are key hormones controlling plant development. These plant hormones have an important role on several physiological and developmental processes, control of the cell cycle, apical dominance, including morphogenesis of shoots and roots, lateral root initiation, stem elongation, leaf and cotyledon expansion, and regulation of senescence (Al-Al-Khassawneh et al., 2006; Taiz and Zeiger, 2010; Kerbauy, 2012). Seed priming with optimal concentrations of CK and GA_3 has been shown to have beneficial effects on germination, growth and yield of a wide range of plant species (Jamil and Rha, 2007; Alonso-Ramirez et al., 2009; Nasri et al., 2012; Kandil et al., 2014).

Gibberellin at 200 mg L^{-1}enhanced the seed germination and seedling growth of papaya (Lopes and Souza, 2008). Nasri et al. (2012) reported GA_3 increased germination percentage of lettuce under salt stress conditions. Albuquerque et al. (2009) reported GA_3 increased the growth characteristic in sweet pepper. Batista et al. (2015) studied the effect of different priming techniques on germination and growth of pepper and reported that GA_3 at 200 mg L^{-1}enhanced the germination and seedling growth when compared to unprimed seeds. Iqbal et al. (2006) showed that application of CK at 100 or 200 mg L^{-1} increased the germination rate and early seedling growth of wheat when compared with hydropriming treatment. Cytokinins at 10 or 100 mg L^{-1} significantly increased the germination rate of pigeon pea seeds compared to unprimed seeds (Sneideris et al., 2015). However, seed priming with CK at 50 or 100 mg L^{-1} inhibited the primary root development of maize seedlings compared to control. These and other contradictory results seem to indicate an inherent differential response among different species or genotypes; therefore, justifying the need of conducting more research in order to investigate the effects of seed priming with CK on germination and early growth of watermelon.

This research was carried out to investigate the effects of different priming techniques on seed germination and initial growth of watermelon seedlings [*C. lanatus* (Thunb.) Matsum and Nakai].

MATERIALS AND METHODS

Plant material and priming treatments

The experiment was conducted in the Plant Propagation Laboratory of the Mato Grosso do Sul State University (UEMS), in Cassilândia, MS, Brazil (Latitude: 19°05'20'' S, Longitude: 51°48'24'' W), during the month November 2015.

Seeds of watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai, cv. Crimson Sweet] were surface sterilized with 1% (v/v) of sodium hypochlorite solution for 5 min and washed immediately with distilled water many times. The sterilized seeds were then subjected to priming by direct immersion in solutions of 0.2% gibberellin (50 mg L^{-1} gibberellic acid - GA_3), 0.2% cytokinin (90 mg L^{-1} kinetin - CK), 0.2% potassium nitrate (2 g L^{-1} KNO_3), and 0.2% calcium nitrate [2 g L^{-1} $Ca(NO_3)_2$] for 6 h at 25°C. A set of seeds subjected to direct immersion in distilled water was taken as control. After priming period, seeds were put to dry in plastic boxes (11.0 × 11.0 × 3.5 cm, type Gerbox) with germitest paper at room temperature (24-28°C) for 48 h (Eira and Marcos-Filho, 1990).

Germination and growth conditions

Five replicates of 25 seeds were evenly distributed in plastic boxes with blotter paper, properly moistened with distilled water, in a volume equivalent to 2.5 times the mass of dry paper. The boxes were then closed with lids to prevent evaporation and maintain the relative humidity close to 100%. Germination was carried out in a germination chamber under 12/12 h photoperiod (light/darkness), light fluence of 40 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), relative humidity of 90% (± 5%) and temperature of 26°C (± 2°C) for 12 days. Germinated seeds were recorded every 24 h for 12 days.

Measurements of germination and seedling growth

The germination (GC), germination rate index (GRI), early growth and vigor index of watermelon seedlings were measured. At 12 days were also measured the percentage of abnormal seedlings and dead seeds. The Equations 1 and 2 and the parameters therein were employed to express the process of seed germination:

$$G\ (\%) = S_{NG} / S_{N0} \times 100 \quad (1)$$

Where G is germination percentage, S_{NG} is the number of germinated seeds, and S_{N0} is the number of experimental seeds with viability (25 seeds):

$$GRI = \Sigma\ (n_i / t_i) \quad (2)$$

Where, GRI is the germination rate index (seed day^{-1}), n_i is the number of germinated seeds on a given day, and t_i is the time in days from the starting/sowing day (0) (Maguire, 1962).

The shoot and radicle length was measured in 15 normal seedlings randomly obtained after count of the total germination (12th day) using meter scale. The results were expressed in centimeter (cm). For the determination of dry matter of shoot and roots, all normal seedlings obtained at the end of the germination period were separated into shoot and roots, dried in a forced air circulation oven for three days at 60°C, and then weighed. The results were expressed in mg seedling^{-1}.

Vigor index of seedlings was calculated according to Zhang et al. (2007) as shown in Equation 3:

$$SVI = S_L \times \Sigma\ (n_i / t_i) \quad (3)$$

Where SVI is seedling vigor index, S_L is the shoot length in the twelfth day (cm), n_i is the number of germinated seeds on a given day, and t_i is the time in days from the starting/sowing day (0).

Statistical analyses

The normality of data was previously tested by the Kolmogorov-Smirnov test and then data were submitted to analysis of variance (ANOVA), and means of five priming treatments were compared by the Tukey test at the 0.05 level of confidence. For statistical analysis, the data expressed in percentage were previously transformed into arc sin $(x/100)^{0,5}$. The analyses were performed using Sisvar version 5.3 software for Windows (Statistical Analysis Software, UFLA, Lavras, MG, BRA).

RESULTS AND DISCUSSION

Effects of priming techniques on seed germination

The different priming treatment significantly affects the measurements of the germination process and rate of watermelon seeds (Figure 1). The germination percentage values in the control treatment (Figure 1A) were higher than the standard values (that is, 80%) for commercial watermelon seeds in Brazil (BRASIL, 2012), indicating that the seeds used in this study had high physiological quality.

The germination percentage of watermelon seeds ranged from 0 to 100%, and was significantly greater when seeds were subjected to priming with GA_3, KNO_3, $Ca(NO_3)_2$ and water (control), and lower under CK priming (Figure 1A). The high efficiency of seed priming with PGRs and salts in improving the germination and growth of the seedlings have been reported by other authors. Alonso-Ramirez et al. (2009) showed that GA_3 have strong stimulatory effect on seed germination, of which their exogenous application was repeatedly found to promote germination of *Arabidopsis* seeds even under unfavorable stress conditions. Seed priming with GA_3 caused an increase in seed germination and seedling growth of sweet pepper (Albuquerque et al., 2009), lettuce (Nasri et al., 2012) and sugar beet (Jamil and Rha, 2007; Kandil et al., 2014). Batista et al. (2015) showed that all the priming methods tested (that is, GA_3, KNO_3, $Ca(NO_3)_2$ and hydropriming) resulted in the improvement of germination rate of pepper seeds when compared to unprimed seeds (control). Huang et al. (2002) and Gurgel et al. (2009) reported that hydropriming treatment can be successfully applied on watermelon and cucumber seeds to improve germination performance, respectively.

The germination rate index ranged from 0 to 6.4 seed day^{-1}, and was significantly greater under KNO_3 and $Ca(NO_3)_2$ priming, followed by GA_3 priming and hydropriming (control), and lower under CK priming (Figure 1B). High values obtained for germination rate index indicate mean higher seed vigor of one treatment in relation to another. Marcos-Filho (2015) reported that the uniformity and speed of seedling emergence are important components of seed performance, thus directly affecting stand establishment.

The seed priming with 0.2% solution of CK resulted in 100% of abnormal seedlings (Figure 2), and was significantly greater than the other priming techniques used (Figure 1C). Although cytokinins are required for many growth and developmental processes in plants such as cell division, morphogenesis of shoots and roots, apical dominance, chloroplast maturation, leaf and cotyledon expansion, and seed germination (Hirose et al., 2008; Taiz and Zeiger, 2010; Kerbauy, 2012), the exogenous application of supra-optimal cytokinin concentrations has remarkable effect on the inhibition of cell elongation process in both shoots and roots (Taiz and Zeiger, 2010). The inhibition of internode and root elongation induced by excess cytokinin is due to the production of ethylene triggered by the enzyme 1-aminocyclopropane-1-carboxylic acid synthase (ACS) (Kerbauy, 2012). These results indicate that chances in endogenous CK concentration may negatively regulate elongation of shoots and roots, as seen in Figure 2. Aragao et al. (2001) studied the effect of seed priming with CK on germination and growth of maize and reported that benzyl aminopurine (BAP) at 50 or 100 mg L^{-1} inhibited the development of the primary root compared to control. However, seed priming with optimal concentrations of CK has been shown to have beneficial effects on germination and early growth of wheat (Iqbal et al., 2006) and pigeon pea (Sneideris et al., 2015).

Another factor that may be related to inhibiting the growth of watermelon seedlings is that the action of CK is

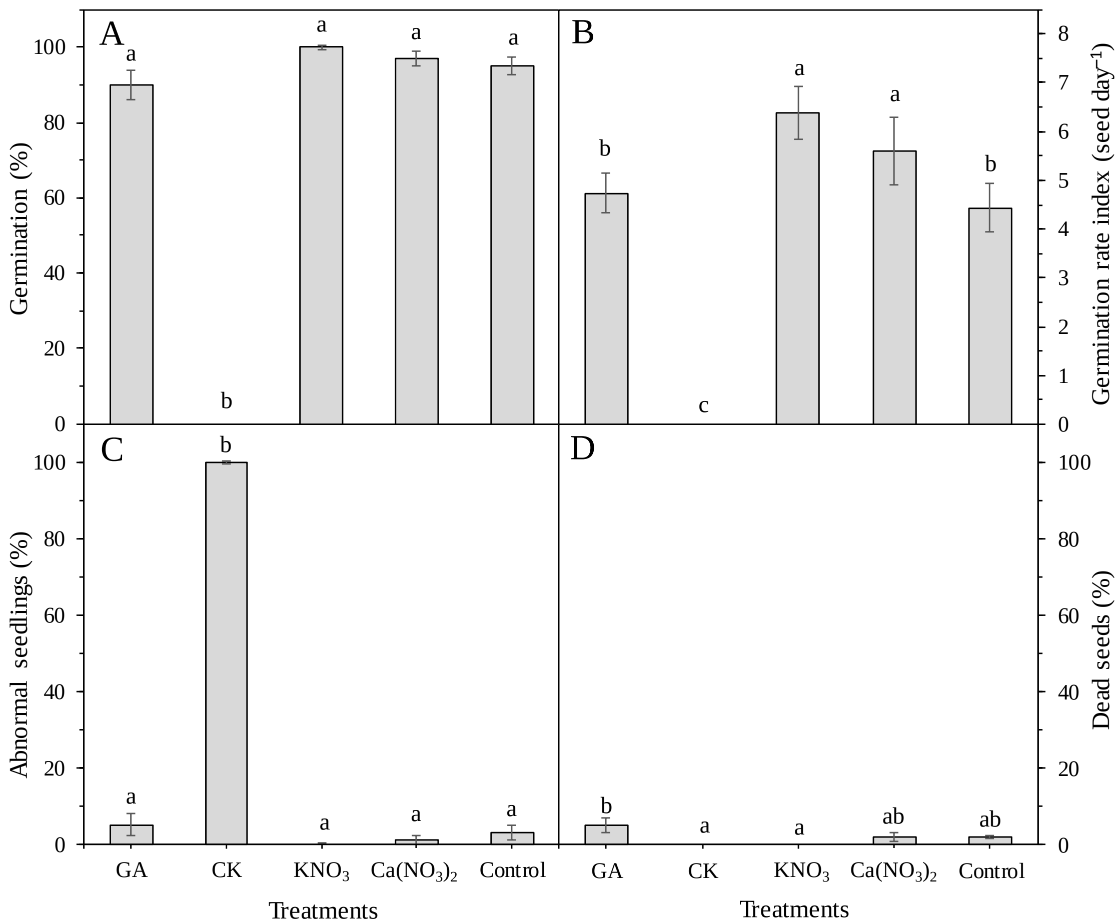

Figure 1. Effect of different priming treatments on the germination percentage ; 12 days (A), germination rate index (B), abnormal seedlings (C), and dead seeds (D) of watermelon seeds [*Citrullus lanatus* (Thunb.) Matsum and Nakai, cv. Crimson Sweet]. Bars followed by the same lower case letters are not significantly different by Tukey test at the 0.05 level of confidence. Data refer to mean values (n = 4) ± standard error.

light-dependent. Changes in fluence rate of white light were shown to have effect on the action of CK and therefore one elongation of the stem and root. Under conditions of low light fluence, as in this study (40 μmol m^{-2} s^{-1} PPFD), the cytokinin inhibits elongation of shoots and roots (Kerbauy, 2012).

The percentage dead seed varied from 0 to 5%, and was significantly greater under GA_3 priming, and lower under CK and KNO_3 priming (Figure 1D). The low percentage of dead seeds is indicative of the high initial viability of watermelon seeds used.

Effects of priming techniques on initial seedling growth

The growth of watermelon seedlings was significantly affected by different priming treatments (Figure 3). Seed priming with GA_3, KNO_3, $Ca(NO_3)_2$ and water (hydropriming) resulted in higher shoot length of watermelon seedlings (Figure 3A). These results indicate that the seed priming with gibberellic acid, salts or water were adequate to promote the shoot growth of watermelon. Batista et al. (2015) also reported the efficiency of GA_3, KNO_3, $Ca(NO_3)_2$ and water priming to enhance the shoot growth of per seedlings compared to unprimed seeds.

Radicle length of the watermelon seedlings was favored under hydropriming (3.95 cm), followed by $Ca(NO_3)_2$ and GA_3 priming, whereas the KNO_3 priming had the lowest effect (2.62 cm) (Figure 3B). Shoot dry matter of watermelon seedlings was significantly higher under GA_3, $Ca(NO_3)_2$ and water priming (Figure 3C), whereas the higher dry matter of the roots was obtained with KNO_3 and water priming (Figure 3D). Demir and Mavi (2004) and Nascimento (2005) reported that seed priming with KNO_3 enhanced the seed germination and growth of watermelon under improper conditions. The high efficiency of hydropriming treatments in improving the early seedling growth was also reported previously in

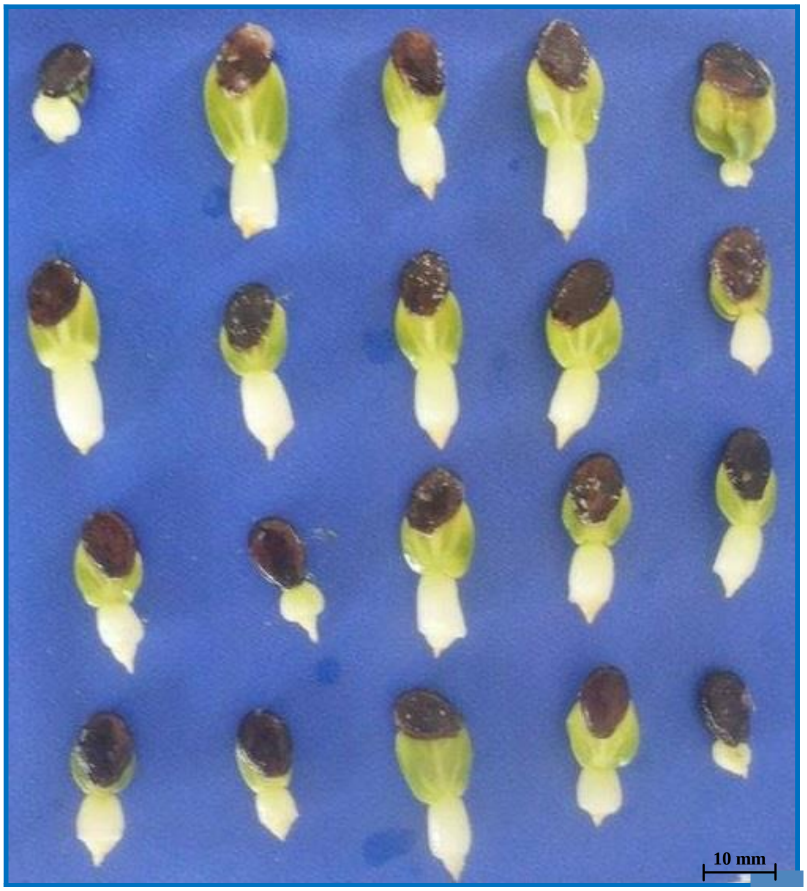

Figure 2. Abnormal seedlings proceeding from watermelon seeds of the cultivar Crimson Sweet subjected to priming by direct immersion in 0.2% cytokinin solution (90 mg L^{-1} of kinetin) at 12 days after sowing. The illustration shows seedlings with malformed roots, thickening of the hypocotyl, and without the formation of shoot.

watermelon (Huang et al., 2002), lentil (Ghassemi-Golezani et al., 2008) and cucumber (Gurgel et al., 2009). Seed priming with GA_3 has been shown to have beneficial effects on germination and growth of a wide range of plant species (Jamil and Rha, 2007; Lopes and Souza, 2008; Albuquerque et al., 2009; Alonso-Ramirez et al., 2009; Nasri et al., 2012; Kandil et al., 2014).

The seedling vigor index ranged from 0 to 72.2, and was significantly greater under KNO_3 and $Ca(NO_3)_2$ priming, followed by GA_3 priming and hydropriming (control), and lower under CK priming (Figure 4). The vigor tests allow identifying the seeds with higher or lower probability to show better performance in field conditions. Vigorous seeds more efficiently mobilize reserves from storage tissues to the embryo axis and this capacity is reflected in higher seedling growth (Marcos-Filho, 2015). Therefore, vigor tests are important tools as an aid to germination test in research on physiological conditioning of seeds.

In general, the results presented here indicate that seed priming with GA_3, KNO_3, $Ca(NO_3)_2$ and water (hydropriming) can be successfully applied to improve the germination and initial growth of watermelon seedlings. Germination and seedling emergence stages are critical for crop production; rapid and uniform field emergence is essential to achieve high yield and uniform plant stands, resulted in early maturity and reduced disease attack (Singh et al., 2001; Subedi and Ma, 2005).

Conclusions

Seed priming with GA_3, KNO_3, $Ca(NO_3)_2$ and water (hydropriming) may can be successfully applied on watermelon seeds to improve germination performance and growth characteristics of seedlings. Seed priming with KNO_3 and $Ca(NO_3)_2$ improved the germination rate and seedling vigor index. The seed priming with 0.2% solution of CK (90 mg L^{-1} kinetin) inhibited the germination and cell elongation process of the seedlings.

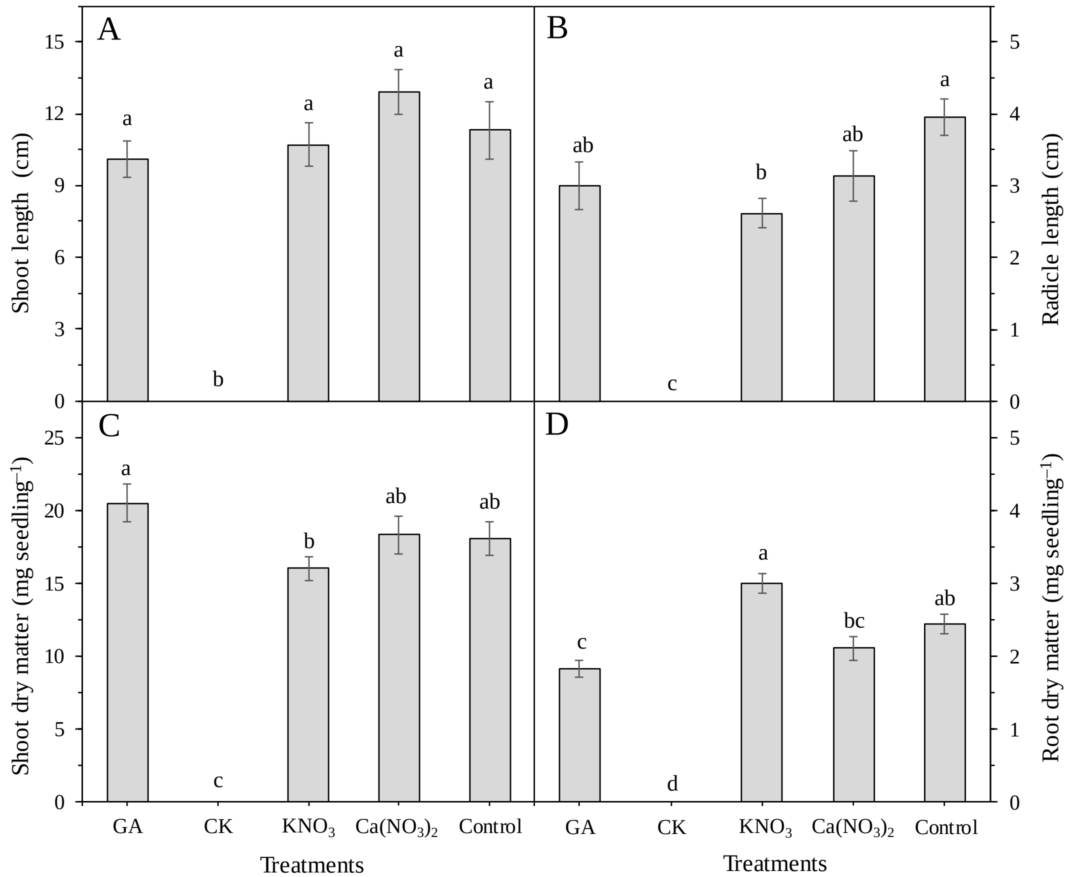

Figure 3. Effect of different priming treatments on shoot length (A), radicle length (B), shoot dry matter (C) and root dry matter (D) of watermelon seedlings [*Citrullus lanatus* (Thunb.) Matsum and Nakai, cv. Crimson Sweet]. Bars followed by the same lower case letters are not significantly different by Tukey test at the 0.05 level of confidence. Data refer to mean values (n = 4) ± standard error.

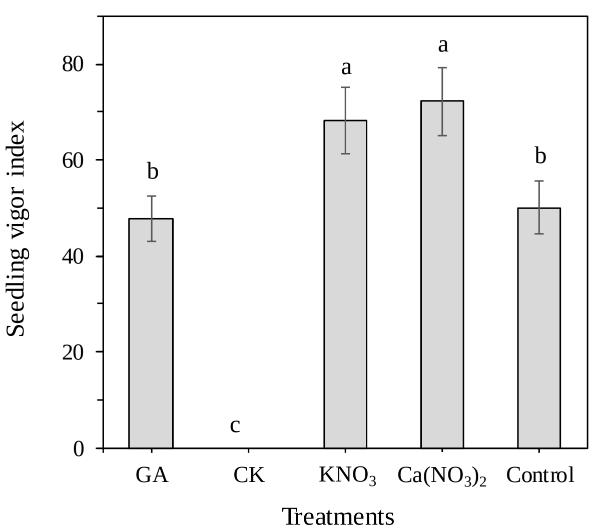

Figure 4. Effect of different priming treatments on the vigor index of watermelon seedlings [*Citrullus lanatus* (Thunb.) Matsum and Nakai, cv. Crimson Sweet]. Bars followed by the same lower case letters are not significantly different by Tukey test at the 0.05 level of confidence. Data refer to mean values (n = 4) ± standard error.

Conflict of interests

The authors have not declared any conflict of interests.

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