

Influence of 24-epibrassinolide on the vigor of lettuce seeds**Influência do 24-epibrassinolídeo no vigor de sementes de alface**

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ABSTRACT

Seeds with low vigor often have problems to establish seedlings in the field. Studies have shown beneficial effects of 24-epibrassinolide (24-EpiBL) on seed performance, including germination speed, tolerance to heat stress, and initial development of seedlings. In this study, priming with 24-EpiBL was investigated on seed lots of lettuce with different vigor levels. Two cultivars (Florence and Betty) were tested, and each cultivar was represented by three lots. Different concentrations of 24-EpiBL were studied: 10-6, 10-8, and 10-10 M. Seeds were evaluated for germination and vigor (germination first count, germination speed index, saturated salt accelerated aging [SSAA], and seedling length). Unprimed seeds and seeds primed with 24-EpiBL, in the best concentration identified during germination and vigor tests, were analyzed by the Seed Vigor Imaging System (SVIS®) software to generate vigor index and growth index values. The addition of 24-EpiBL associated with priming is efficient to improve the performance of lettuce seeds. All concentrations studied were efficient, and the best was 10-6 M.

Keywords: Brassinosteroids, *Lactuca sativa* L, Plant Regulators, Physiological Potential.

RESUMO

Sementes com baixo vigor geralmente tem problemas para estabelecer mudas em campo. Estudos demonstraram efeitos benéficos do 24-epibrassinolídeo (24-EpiBL) no desempenho das sementes, incluindo velocidade de germinação, tolerância ao estresse térmico e desenvolvimento inicial de mudas. Neste estudo, o condicionamento com 24-EpiBL foi investigado em lotes de sementes de alface com diferentes níveis de vigor. Duas cultivares (Florence e Betty) foram testadas e cada cultivar foi representada por três lotes. Diferentes concentrações de 24-EpiBL foram estudadas: 10-6, 10-8 e 10-10 M. As sementes foram avaliadas quanto à germinação e vigor (primeira contagem de germinação, índice de velocidade de germinação, envelhecimento acelerado com solução saturada [SSAA] e comprimento de plântulas). Sementes não condicionadas e condicionadas com 24-EpiBL, na melhor concentração identificada durante os testes de germinação e vigor, foram analisadas pelo software Seed Vigor Imaging System (SVIS®) para gerar índice de vigor e valores de índice de crescimento. A adição de 24-EpiBL associada ao condicionamento é eficiente para melhorar o desempenho das sementes de alface. Todas as concentrações estudadas foram eficientes, a melhor foi 10-6 M.

Palavras-chave: Brassinosteróides, *Lactuca sativa* L, Reguladores Vegetais, Potencial Fisiológico.

1 INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most commonly produced vegetable worldwide (Sala and Costa 2012). The propagation of lettuce crop is performed exclusively by seeds, with subsequent transplantation of the seedlings. Seed quality is an essential factor for development of seed-propagated crops and is related to the seedling establishment (Franzin et al., 2004). Seedlings from seeds with low vigor can be affected by suboptimal environmental conditions.

The aim of seed priming is to improve seed performance of several species. The treatment basically consists of the controlled addition of water to the seeds to activate processes that precede germination, but without allowing the protrusion of the primary root. Phases I and II of the seed imbibition process are closely monitored (Marcos-Filho 2015).

Various effects of the seed priming with 24-epibrassinolide (24-EpiBL) have been reported, including better seedling development (Silva et al., 2015), salinity resistance (Zhang et al., 2007; Ali et al., 2008; Divi et al., 2010; Dalio et al., 2011; Serna et al., 2015; Shahid et al., 2015), tolerance to high or low temperatures (Pradhan et al., 2013; Wu et al., 2014), and tolerance to heavy metals (Sharma and Bhardwaj 2007; Hayat et al., 2012; Yusuf et al., 2012; Shahzad et al., 2018), in addition to an increase in antioxidant enzyme activity (Arora et al., 2010; Semida and Rady 2014; Silva et al., 2015). Several plant organs have 24-EpiBL, but its highest concentration occurs mainly in young tissues such as pollen and immature seeds (Clouse and Sasse 1998; Rao et al., 2002; Bajguz and Tretyn 2003).

Although seed priming has been studied for some time in seeds of many cultivated species, especially onion (Caseiro et al., 2004), beet (Costa and Vilela 2006), carrot (Balbino and Lopes 2006), cauliflower (Marcos-Filho and Kikuti 2008), cucumber (Lima and Marcos-Filho 2010), and lettuce (Fessel et al., 2002; Bisognin et al., 2016), the effects of priming associated with 24 EpiBL on lettuce seeds have not been studied. Thus, this work aimed to evaluate the efficiency of the seed priming with 24-EpiBL on the physiological potential of lettuce seeds.

2 MATERIAL AND METHODS

This study was conducted at the Plant Propagation Laboratory of the Agricultural Engineering and Science Campus, Federal University of Alagoas, in Rio Largo, Alagoas State, Brazil. Two lettuce cultivars represented by three seed lots each were investigated: Lots A, B, and C (Florence cultivar) and Lots D, E, and F (Betty cultivar). All seed lots were evaluated for moisture content (fresh weight basis), which ranged from 7.6 to 9.3% for the Florence cultivar and from 6.8 to 7.9% for the Betty cultivar. All the seed lots were characterized based on germination and vigor tests (data not shown). Lots B and D were classified as high vigor and Lots A, C, E, and F with low vigor.

Seed treatment – Different concentrations of 24-EpiBL (10^{-6} , 10^{-8} , and 10^{-10} M) were compared to hydropriming (priming with water alone) and with unprimed seeds. All seed lots were submitted to preliminary evaluations to determine the water absorption rate and to identify the time of primary root protrusion (Bewley and Black 1994; Caseiro, et al., 2004), which occurred in 14 to 20 h of imbibition (data not shown). At this stage, the seed moisture content was approximately 18% and 13% for seed lots of the Florence and Betty cultivars, respectively. Therefore, we set the period of 8 h for seed priming with 24-EpiBL and hydropriming.

The treatments were performed on four repetitions of 0.5 g per lot, distributed between two layers of blotting paper (each layer consisted of three 10.3×10.3 cm sheets), moistened with 24-EpiBL solution or water alone (hydroprimed seeds) equivalent to 2.5 times the weight of the dried

substrate. The layers of blotting paper were placed on a stainless-steel screen, inside a transparent plastic box (11.0 × 11.0 × 3.5 cm), containing 40 mL of distilled water. The boxes were capped and kept at 20 °C for 8 h. Then, the seeds were maintained for 16 h at 32 °C and 38% of RH. After this period, the seeds reached the moisture content of 8.5% for the Florence cultivar and 7.0% for the Betty cultivar (data not shown). All seed lots, primed with 24-EpiBL, hydroprimed, and unprimed, were evaluated for germination and vigor:

a) Germination – Four replications of 50 seeds per lot were sown on blotter paper, previously moistened with water equivalent to 2.5 times the weight of the dried substrate and placed inside transparent plastic boxes (11.0 × 11.0 × 3.5 cm). The boxes were wrapped in plastic bags to prevent water loss and maintained at 20 °C. Evaluations were performed at four (germination first count) and seven days after sowing, and the results were showed as a percentage of normal seedlings (Brasil 2009).

b) Germination rate index (GRI) – it was obtained from the daily count of normal seedlings in the germination test, according to the formula proposed by Maguire (1962).

c) SSAA – this test was performed with 0.5 g of seeds per lot distributed in single layer on a stainless-steel screen, positioned inside a transparent plastic box (11.0 × 11.0 × 3.5 cm). Inside each box, 40 mL of saturated sodium chloride solution - NaCl (40 g of NaCl/100 mL of distilled water) was added, providing 76% of relative humidity – RH (Jianhua and McDonald 1996). The boxes were capped and kept at 41 °C for 48 h. After this period, four replications of 50 seeds per lot were sowed to germinate, as described above with evaluations performed at five days after sowing. Results were expressed as a percentage of normal seedlings (Kikuti and Marcos-Filho 2012).

d) Seedling length – Four replications of 25 seeds per lot were sown on two paper towels previously moistened with distilled water equivalent to 2.5 times the weight of dry paper. They were rolled, covered with plastic bags to prevent water loss, and kept in a germination chamber at 20 °C for seven days. After this period, the seedling length was measured with a millimeter ruler, and the measurements of each repetition were added together and divided by the number of seedlings. Results were expressed in cm seedling⁻¹ per lot.

Unprimed seeds and seeds primed with 24-EpiBL in the best concentration, identified during germination and vigor tests, were analyzed by the Seed Vigor Imaging System (SVIS[®]) software. Four repetition of 25 seeds per lot were distributed horizontally in two rows on the upper third of the surface of blotting paper (a sheet of blue overlaid a white sheet) previously moistened 2.5 times the weight of the dry substrate, and placed within transparent plastic boxes (11.0 × 11.0 × 3.5). Blue blotting paper sheets were used to provide contrast with the seedlings for better imaging.

After sowing, the boxes were wrapped in plastic bags to prevent water loss. Later, they were placed in a germination chamber in an inclined position, forming an angle of 60 to 70° with the horizontal, so that the seedling development followed the positive gravitropism. The seeds were kept at 20 °C for three days in the dark (Penaloza et al., 2005; Kikuti and Marcos-Filho 2012). After this period, the seedlings were arranged on the surface of a scanner operated by Photosmart software with a resolution of 98 dpi. The images of the seedling were analyzed by the SVIS[®] software, to generate values related to vigor index and growth index. The vigor index was generated by combining parameters of growth (70% contribution) and seedling uniformity (30% contribution).

Data analysis – The data from each test and cultivar were analyzed separately by analysis of variance in a completely randomized design and the means compared by the Tukey's test ($P < 0.05$). To identify the best concentration of 24-EpiBL for seed priming, the data were subjected to analysis of variance, in a 5×3 factorial scheme (five treatments: priming with 24 EpiBL at concentrations of 10^{-6} , 10^{-8} , and 10^{-10} M, hydropriming and unprimed seeds \times three seed lots for each cultivar). Prior to ANOVA, all datasets were checked for normality assumptions and did not require any transformation.

3 RESULTS

Seed priming with 24-EpiBL provided satisfactory results in all evaluated tests for both cultivars. In the first germination count, i.e., four days after sowing (Table I), Lot C [low vigor] (Florence cultivar) showed better performance after priming with 24-EpiBL, particularly at 10^{-6} M concentration. For the Betty cultivar, the seed lots classified with low vigor (Lots E and F) also had an increase after seed priming, including the hydropriming (water alone). At seven days after sowing (Table II), the germination of unprimed seed of Lot C was 58% and reached 89% when the seeds were primed with 24-EpiBL at a concentration of 10^{-6} M. However, in Lots E and F, the germination improved regardless of the addition of 24EpiBL.

For the GSI (Table III), in general, seed priming with 24-EpiBL achieved the best results, regardless of the cultivar, with higher values in the concentration of 10^{-6} M. In the SSSA (Table IV), the three concentrations of 24-EpiBL also benefited the percentage of normal seedlings produced for all the seed lot, but it was superior to hydropriming only in Lot A [low vigor], with remarkable results mainly at the 10^{-6} and 10^{-8} M concentrations.

Seedling length (Table V) also improved after 24-EpiBL priming for both cultivars. The three 24-EpiBL concentrations differed significantly from non-priming and hydropriming. For the Florence cultivar, all the treatments with 24-EpiBL similarly increased seedling length, but for the Betty cultivar, this happened only for Lot D.

Figure 1 presents the vigor index generated by the SVIS[®] software of the Florence (a) and Betty (b) cultivars, for unprimed seeds and after priming with 24-EpiBL in the concentration of 10^{-6} M that provided the most efficient results in the previous tests. For the seed lots of the Florence cultivar, the vigor index was not significantly different between unprimed seeds and those primed with 24-EpiBL. On the other hand, all seed lots of the Betty cultivar showed higher vigor indexes after seed priming with 24-EpiBL. The growth index (Figure 2) also did not indicate relevant differences between unprimed seeds and after priming with 24-EpiBL for the Florence cultivar. However, the seed priming with 24-EpiBL showed greater stimulus for seedling development in all seed lots of the Betty cultivar.

4 DISCUSSION

The activity of 24-EpiBL in improving the performance of lettuce seeds may be related to its inhibitory action on abscisic acid (Ekinici et al., 2012). In addition, the higher germination speed of seeds primed with 24-EpiBL could be associated with faster cell membrane reorganization, which contribute to greater seedling growth (Soares et al., 2020). Higher germination speed was also one of the main benefits achieved by Silva et al. (2015) in bell pepper seeds primed with 24-EpiBL.

Studies have demonstrated that 24-EpiBL also plays an important role in the growth and yield of different crops along with an increased resistance under various abiotic stresses (Shahzad et al., 2018). The current study found an evident increase in the tolerance to heat stress in SSAA test after seed priming with 24-EpiBL, which can be associated with chemical changes in cell membranes that improve membrane structure and permeability (Vázquez et al., 2013a). Furthermore, several studies have shown the beneficial effects of brassinosteroids at high temperatures (El-Bassiony et al., 2012; Wu et al., 2014). For brassica, seeds treated with 24-EpiBL had lower membrane lipid peroxidation (Pradhan et al., 2013). Anwar (2018) achieved several molecular modifications induced by 24-EpiBL associated with tolerance to thermal stress, including gene expression and increased protein synthesis.

Our findings indicated a greater growth of lettuce seedlings treated with 24-EpiBL. The brassinosteroids (BRs) act in synergy with auxin, and some responses mediated by auxin are favored by 24-EpiBL treatments. Previous studies also indicated a synergistic interaction among BRs and other phytohormones that may control different activities in plant metabolism (Peres et al., 2019). In *Arabidopsis*, the hypocotyl length was controlled by BRs, auxin, ethylene, and cytokinin (Gupta et al., 2012).

For lettuce, plants sprayed with 24-EpiBL had an increase in the crop yield without negative consequences on the nutritive and organoleptic attributes of the produced seeds (Serna et al., 2012). Arora et al. (2010) found that 24-EpiBL at concentrations of 10^{-10} , 10^{-8} , and 10^{-6} M benefited the

growth of *Brassica juncea* L. seedlings and increased the activity of antioxidant enzymes and protein content of leaves. Pea seeds exhibited better germination and greater root growth after seed priming with 24-EpiBL at concentrations of 5 and 10 μ M (Shahid et al., 2011). Rice seedlings of two different genotypes achieved greater growth after seed priming with 24-EpiBL, even under salt stress conditions (Vázquez et al., 2013b). Better development of rice seedlings was one of the major benefits of 24-EpiBL (Larré et al., 2014).

The use of seeds with high germinative potential is essential to obtain satisfactory results in economically important crops. Our research demonstrated various positive effects on the physiological performance of lettuce seeds primed with 24-EpiBL, especially at the concentration of 10^{-6} M, with advantages for germination speed, seedling development, and heat resistance. Thus, 24-EpiBL can be a useful alternative to improve the physiological characteristics of seed lots of this crop. This study demonstrated that 24-epibrassinolide associated with seed priming is an efficient technique to improve vigor of lettuce seeds, especially at the concentration of 10^{-6} M.

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REFERENCES

- Ali, B., S Hayat, Q. Fariduddin, and A. Ahmad. 2008. 24-Epibrassinolide protects against the stress generated by salinity and nickel in *Brassica juncea*. *Chemosphere*, 72: 1387- 1392.
- Anwar, A., L Bai, L. Miao, Y. Liu, S. Li, X. Yu and Y. Li. 2018. 24-Epibrassinolide ameliorates endogenous hormone levels to enhance low-temperature stress tolerance in cucumber seedlings. *Int. J. Mol. Sci.* 19: 2497.
- Arora, P., R. Bhardwaj and Mk Kanwar. 2010. 24-epibrassinolide regulated diminution of Cr metal toxicity in *Brassica juncea* L. plants. *Braz. J. Plant Physiol, Índia*, 22: 159-165.
- Bajguz, A., and A. Tretyn. 2003. The chemical characteristic and distribution of brassinosteroids in plants. *Phytochemistry*, 62, 1027-1046.

Balbino, E., and HM Lopes. 2006. Efeitos do condicionamento fisiológico e da secagem na germinação e no vigor de sementes de cenoura. *Rev. Bras. Sementes*, 28: 01– 08.

Bewley JD and M Black. 1994. *Seeds: physiology of development and germination*. (2nd). Ed. New York: Plenum Press.

Bisognin, MB, SM Kulczynski, M. Ferrari, R. Gaviraghi, AJ Pelegrin and VQ Souza. 2016. Desempenho fisiológico de sementes olerícolas em diferentes tempos de hidrocondicionamento. *Rev. Ciênc. Agrár.* 39: 349-359.

Brasil, Ministério da Agricultura, Pecuária e Abastecimento – Secretaria de Defesa Agropecuária. Regras para análise de sementes. Brasília: Mapa/ACS, 2009. 399p.

Caseiro, RF, MA Bennet and J. Marcos-Filho. 2004. Comparison of three priming techniques for onion seed lots differing in initial seed quality. *Seed. Sci. Technol.* 32: 365-375.

Clouse, SD and JM Sasse. 1998. Brassinosteroids: essential regulators of plant growth and development. *Annu. Rev. Plant. Biol.* 49: 427–451.

Costa, CJ and FA Villela. 2006. Condicionamento osmótico de sementes de beterraba. *Rev. Bras. Sementes*, 28: 21-29.

Dalio, RJD, HP Pinheiro, L. Sodek and CRB Haddad. 2011. The effect of 24- epibrassinolide and clotrimazole on the adaptation of *Cajanus cajan* (L.) Millsp. to salinity. *Acta Physiol. Plant.*,33: 1887-1896.

Divi, UK, T. Rahman and P. Krishna. 2010. Brassinosteroid-mediated stress tolerance in *Arabidopsis* shows interactions with abscisic acid, ethylene and salicylic acid pathways. *BMC Plant. Biol.* 10: 149-151.

Ekinci, M., E. Yildirim and A. Dursun. 2012. Mitigation of salt stress in lettuce (*Lactuca sativa* L. Var. Crispa) by seed and foliar 24-epibrassinolide treatments. *HortScience*, 47, 631-636.

El-bassiony, AM., AA Ghoname, ME El-awadi, ZF Fawzy and N. Gruda. 2012. Ameliorative effects of brassinosteroids on growth and productivity of snap beans grown under high temperature. *Gesunde Pflanz.* 64, 175-182.

Fessel, SA., RD Vieira, TJD Rodrigues and M. Fagioli. 2002. Germinação de sementes de alface submetidas a condicionamento osmótico durante o armazenamento. *Sci. Agr.* 59: 73-77.

Franzin, SM., NL Menezes, DC Garcia and CF Wrasse. 2004. Métodos para avaliação do potencial fisiológico de sementes de alface. Rev. Bras. Sementes, 26: 63-69.

Gupta, A., M. Singh, AM Jones and A. Laxmi. 2012. Hypocotyl directional growth in Arabidopsis: a complex trait. Plant physiol. 159: 1463-1476.

Hayat, S., MN Alyemeni and SA Hasan. 2012. Foliar spray of brassinosteroid enhances yield and quality of *Solanum lycopersicum* under cadmium stress. Saudi J. Biol. Sci. 19: 325-335.

Jianhua, Z., and MB McDonald. 1996. The saturated salt accelerated aging test for small-seeded crops. Seed Sci. Technol. 25: 123-131.

Kikuti, ALP and J. Marcos-filho. 2012. Testes de vigor em sementes de alface. Hortic. Bras. 30: 44-50.

Larré, CF, P. Marini, L. Moraes, L. Amarante and DM Moraes. 2014. Influência do 24-epibrassinolídeo na tolerância ao estresse salino em plântulas de arroz. Semina: Ciênc. Agrár. 35: 67-75.

Lima, LB, and J. Marcos-filho. 2010. Condicionamento fisiológico de sementes de pepino e germinação sob diferentes temperaturas. Rev. Bras. Sementes, 32: 138-147.

Maguire, JD. 1962. Speed of germination and in selection and evaluation for seedling emergence and vigor. Crop Sci. 2: 176-177.

Marcos-filho, J. 2015. Fisiologia de sementes de plantas cultivadas. (2ª ed.). Londrina: Abrates.

Marcos-filho, J. and ALP Kikuti. 2008. Condicionamento fisiológico de sementes de couve-flor e desempenho das plantas em campo. Hort. Bras. 26: 165-169.

Penaloza, P., MB Mc Donald, G. Ramirez-rosales and MA Bennett. 2005. Lettuce (*Lactuca sativa* L.) seed quality evaluation using seed physical attributes, saturated salt accelerated aging and the seed vigour imaging system. Electron. J. Biotech. 8: 1-9

Peres, ALG, JS Soares, RG Tavares, G. Righetto, MA Zullo, NB Mandava and M. Menossi. 2019. Brassinosteroids, the sixth class of phytohormones: a molecular view from the discovery to hormonal interactions in plant development and stress adaptation. Int. J. Mol. Sci. 20: 331.

Pradhan, SK, RC Gupta and M. Kumar. 2013. Effect of 24-Epibrassinolide on lipid peroxidation and proline in three Brassica species under temperature stress. *J. Stress Physiol. Biochem.* 9: 376-384.

Rao, SSR, BV Vardhini, E. Sujhata and S. Anuradha. 2002. Brassinosteroids – a new class of phytohormones. *Curr. Sci.* 82: 1239 -1245.

Sala, FC and CP Costa. 2012. Retrospectiva e tendência da alfacicultura brasileira. *Hort. Bras.* 30: 187 -194.

Semida, WM and MM Rady. 2014. Pre-soaking in 24-epibrassinolide or salicylic acid improves seed germination, seedling growth, and anti-oxidant capacity in *Phaseolus vulgaris* L. grown under NaCl stress. *J. Hortic. Sci. Biotech.* 89: 338 – 344.

Serna, M., Y. Coll, PJ Zapata, MA Botella, MT Pretel and A. Amorós. 2015. A brassinosteroid analogue prevented the effect of salt stress on ethylene synthesis and polyamines in lettuce plants. *Sci. Hort.* 185: 105-112.

Serna, M., F. Hernández, F. Coll and A. Amorós. 2012. Brassinosteroid analogues effect on yield and quality parameters of field -grown lettuce (*Lactuca sativa* L.). *Sci. Hort.* 143: 29 -37.

Silva, CB, J. Marcos-filho, P. Jourdan and MA Bennet. 2015. Performance of Bell Pepper Seeds in Response to Drum Priming with Addition of 24-Epibrassinolide. *HortScience*, 50: 873 -878.

Shahid, MA, RM Balal, MA Pervez, T. Abbas, MA Aqeel, A. Riaz and NS Mattson. 2015. Exogenous 24-epibrassinolide elevates the salt tolerance potential of pea (*Pisum sativum* L.) by improving osmotic adjustment capacity and leaf water relations. *J. Plant Nutr.* 38: 1050-1072.

Shahid, MA, MA Pervez, RM Bala, NS Mattson, A. Rashid, R. Ahmad, CM Ayyub and T. Abbas. 2011. Brassinosteroid (24-epibrassinolide) enhances growth and alleviates the deleterious effects induced by salt stress in pea (*Pisum sativum* L.). *Aust. J. Crop Sci.* 5: 500-510.

Shahzad, B., M. Tanveer, Z. Che, A. Rehman, SA Cheema, A. Sharma, H. Song, S. Rehman and Zhaorong. 2018. Role of 24-epibrassinolide (EBL) in mediating heavy metal and pesticide induced oxidative stress in plants: A review. *Ecotox. Environ. Safety*, 147: 935-944.

Sharma, P., and R. Bhardwaj. 2007. Effect of 24-epibrassinolide on seed germination, seedling growth and heavy metal uptake in *Brassica Juncea* L. *General and Applied Plant Physiology*, 33: 59-73.

Soares, TFSN, DCFDS Dias, AMS Oliveira, DM Ribeiro and LADS Dias. 2020 Brassinoesteróides exógenos aumentam a tolerância ao estresse de chumbo na germinação e crescimento de plântulas de *Brassica juncea* L. *Ecotoxicology and Environmental Safe*. 193: 110296

Vázquez, MN., YR Guerrero, LM González and WTDL Noval. 2013a. Brassinosteroids and plant responses to heavy metal stress. An overview. *Open J. Met*. 3: 34-41

Vázquez, MN, YR Guerrero, LR Ayan, L. Martínez, MCG Cepero and PDA Pieters. 2013b. Brasinosteroides y sus análogos estimulan el crecimiento de plântulas de dos genotipos de arroz (*Oryza sativa* L.) en medio salino. *Cult. Trop*. 34: 74-80.

Yusuf, M., Q. Fariduddin and A. Ahmad. 2012. 24-Epi brassinolide modulates growth, nodulation, antioxidant system, and osmolyte in tolerant and sensitive varieties of *Vigna radiata* under different levels of nickel: A shotgun approach. *Plant physiol. Bioch*. 57: 143-153.

Wu, X., X. Yao, J. Chen, Z. Zhu, H. Zhang and D. Zha. 2014. Brassinosteroids protect photosynthesis and antioxidant system of eggplant seedlings from high-temperature stress. *Acta Physiol. Plant*. 36: 251-261.

Zhang, S., J. Hu, Y. Zhang, XJ Xie and A. Knapp. 2007. Seed priming with brassinolide improves lucerne (*Medicago sativa* L.) seed germination and seedling growth in relation to physiological changes under salinity stress. *Aust. J. Agri. Res*. 58: 811-815.

Table I – Germination (%) 4 days after sowing of lettuce seeds, Florence (Lots A, B and C) and Betty cultivars (Lots D, E and F), unprimed, hydroprimed, and primed with 24-EpiBL at concentrations of 10^{-6} , 10^{-8} , and 10^{-10} M.

Cultivar	Lot	Unprimed	Hydroprimed	EpiBL 10^{-6}	EpiBL 10^{-8}	EpiBL 10^{-10}	ANOVA
Florence	A	61 ± 0.05 a*	61 ± 0.03 a	77 ± 0.03 a	69 ± 0.04 a	67 ± 0.03 a	$F_{4,45}=2.212$ $p=0.0822$
	B	96 ± 0.02 a	96 ± 0.01 a	96 ± 0.01 a	96 ± 0.00 a	98 ± 0.00 a	$F_{4,45}=0.034$ $p=0.9977$
	C	46 ± 0.03 d	56 ± 0.07 cd	81 ± 0.08 a	72 ± 0.03 ab	64 ± 0.06 bc	$F_{4,45}=9.312$ $p=0.0000$
Betty	D	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a	$F_{4,45}=0.000$ $p=1.0000$
	E	84 ± 0.01 c	99 ± 0.00 a	96 ± 0.01 ab	89 ± 0.02 bc	92 ± 0.02 abc	$F_{4,45}=5.514$ $p=0.0010$
	F	76 ± 0.07 b	91 ± 0.02 a	97 ± 0.00 a	97 ± 0.01 a	97 ± 0.01 a	$F_{4,45}=13.385$ $p=0.0000$

*Any two mean (± standard error) followed by the same letters within a row are not significantly different ($p < 0.05$) according to Tukey test.

Table II – Germination (%) 7 days after sowing of lettuce seeds, Florence (Lots A, B and C) and Betty cultivars (Lots D, E and F), unprimed, hydroprimed, and primed with 24-EpiBL at concentrations of 10^{-6} , 10^{-8} , and 10^{-10} M.

Cultivar	Lot	Unprimed	Hydroprimed	24-EpiBL 10^{-6}	24-EpiBL 10^{-8}	24-EpiBL 10^{-10}	ANOVA
Florence	A	72 ± 0.04 a*	72 ± 0.01 a	82 ± 0.02 a	77 ± 0.04 a	73 ± 0.05 a	F _{4,45} =1.523 p= 0.2009
	B	98 ± 0.01 a	98 ± 0.02 a	98 ± 0.00 a	96 ± 0.00 a	98 ± 0.00 a	F _{4,45} =0.054 p=0.9943
	C	58 ± 0.04 c	65 ± 0.07 bc	89 ± 0.02 a	88 ± 0.01 a	78 ± 0.03 ab	F _{4,45} =15.072 p=0.0000
Betty	D	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a	F _{4,45} =0.000 p= 1.0000
	E	89 ± 0.02 b	99 ± 0.00 a	96 ± 0.01 ab	96 ± 0.00 ab	92 ± 0.02 ab	F _{4,45} =4.849 p=0.0024
	F	82 ± 0.05 b	97 ± 0.01 a	97 ± 0.00 a	99 ± 0.00 a	100 ± 0.00 a	F _{4,45} =17.854 p=0.0000

*Any two mean (± standard error) followed by the same letters within a row are not significantly different ($p < 0.05$) according to Tukey test.

Table III – Germination rate index - GSI (obtained by Maguire method, 1962) of lettuce seeds, Florence (Lots A, B and C) and Betty cultivars (Lots D, E and F), unprimed, hydroprimed, and primed with 24-EpiBL at concentrations of 10^{-6} , 10^{-8} , and 10^{-10} M.

Cultivar	Lot	Unprimed	Hydroprimed	24-EpiBL 10^{-6}	24-EpiBL 10^{-8}	24-EpiBL 10^{-10}	ANOVA
Florence	A	8.1 ± 0.95 c*	10.8 ± 0.82 bc	16.4 ± 1.08 a	12.8 ± 0.78 b	11.9 ± 0.94 b	F _{4,45} =13.205 p=0.0000
	B	16.0 ± 0.24 b	23.0 ± 0.61 a	23.7 ± 0.36 a	23.3 ± 0.46 a	23.8 ± 0.33 a	F _{4,45} =15.973 p=0.0000
	C	8.2 ± 0.47 c	11.4 ± 1.51 bc	17.5 ± 1.02 a	16.2 ± 0.43 a	14.3 ± 1.24 ab	F _{4,45} =20.260 p=0.0000
Betty	D	16.6 ± 0.00 b	24.4 ± 0.24 a	25.0 ± 0.00 a	25.0 ± 0.00 a	25.0 ± 0.00 a	F _{4,45} =86.926 p=0.0000
	E	15.0 ± 0.29 c	21.7 ± 0.59 a	21.8 ± 0.48 a	15.5 ± 0.49 c	19.6 ± 0.60 b	F _{4,45} =69.328 p=0.0000
	F	13.7 ± 0.12 c	16.7 ± 0.38 b	19.3 ± 0.72 a	19.6 ± 0.38 a	16.1 ± 0.39 b	F _{4,45} =38.538 p=0.0000

*Any two mean (± standard error) followed by the same letters within a row are not significantly different ($p < 0.05$) according to Tukey test.

Table IV – Normal seedlings (%) from saturated salt accelerated aging - SSAA test of lettuce seeds, Florence cultivars (Lots A, B and C) and Betty (Lots D, E and F), unprimed, hydroprimed, and primed with 24-EpiBL at concentrations of 10^{-6} , 10^{-8} , and 10^{-10} M.

Cultivar	Lot	Unprimed	Hydroprimed	24-EpiBL 10^{-6}	24-EpiBL 10^{-8}	24-EpiBL 10^{-10}	ANOVA
Florence	A	45 ± 0.00 c*	53 ± 0.04 bc	70 ± 0.00 a	72 ± 0.01 a	64 ± 0.03 ab	F _{4,45} =9.782 p=0.0000
	B	87 ± 0.00 a	90 ± 0.00 a	84 ± 0.00 a	85 ± 0.02 a	87 ± 0.00 a	F _{4,45} =0.375 p=0.8250
	C	39 ± 0.03 b	72 ± 0.10 a	70 ± 0.00 a	77 ± 0.02 a	70 ± 0.03 a	F _{4,45} =16.406 p=0.0000
Betty	D	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a	F _{4,45} =0.000 p=1.0000
	E	80 ± 0.03 c	93 ± 0.02 ab	84 ± 0.03 ab	95 ± 0.00 a	85 ± 0.04 ab	F _{4,45} =3.617 p=0.0121
	F	78 ± 0.06 ab	90 ± 0.01 a	85 ± 0.03 a	91 ± 0.03 a	71 ± 0.05 b	F _{4,45} =6.533 p=0.0003

*Any two mean (± standard error) followed by the same letters within a row are not significantly different ($p < 0.05$) according to Tukey test.

Table V – Length (cm) of lettuce seedlings of the Florence (Lots A, B and C) and Betty cultivars (Lots D, E and F). Seeds were untreated (unprimed), hydroprimed, and primed with 24-EpiBL at concentrations of 10^{-6} , 10^{-8} , and 10^{-10} M.

Cultivar	Lot	Unprimed	Hydroprimed	24-EpiBL 10^{-6}	24-EpiBL 10^{-8}	24-EpiBL 10^{-10}	ANOVA
Florence	A	1.8 ± 0.07 b*	1.7 ± 0.03 b	2.8 ± 0.19 a	2.8 ± 0.59 a	3.0 ± 0.25 a	F _{4,45} =6.496 p=0.0003
	B	3.8 ± 0.14 c	5.9 ± 0.13 b	7.8 ± 0.21 a	7.0 ± 0.00 a	7.3 ± 0.26 a	F _{4,45} =40.745 p=0.0000
	C	2.2 ± 0.21 c	3.7 ± 0.29 b	5.5 ± 0.21 a	5.0 ± 0.00 a	4.9 ± 0.38 a	F _{4,45} =28.541 p=0.0000
Betty	D	3.8 ± 0.31 b	3.8 ± 0.14 b	6.4 ± 0.16 a	6.3 ± 0.00 a	6.4 ± 0.18 a	F _{4,45} =37.826 p=0.0000
	E	2.0 ± 0.07 c	2.0 ± 0.15 c	4.2 ± 0.37 ab	3.6 ± 0.00 b	5.0 ± 0.05 a	F _{4,45} =34.267 p=0.0000
	F	1.8 ± 0.07 c	1.9 ± 0.09 bc	3.2 ± 0.59 a	2.1 ± 0.14 bc	2.8 ± 0.15 ab	F _{4,45} =7.077 p=0.0002

*Any two mean (± standard error) followed by the same letters within a row are not significantly different ($p < 0.05$) according to Tukey test.

Figure 1- Vigor index - SVIS[®] of lettuce seed lots from unprimed and primed seed with 24-EpiBL at 10^{-6} M (24-EpiBL) of the Florence (Lots A, B and C) and Betty cultivars (Lots D, E and F). Vertical bars represent mean standard error.

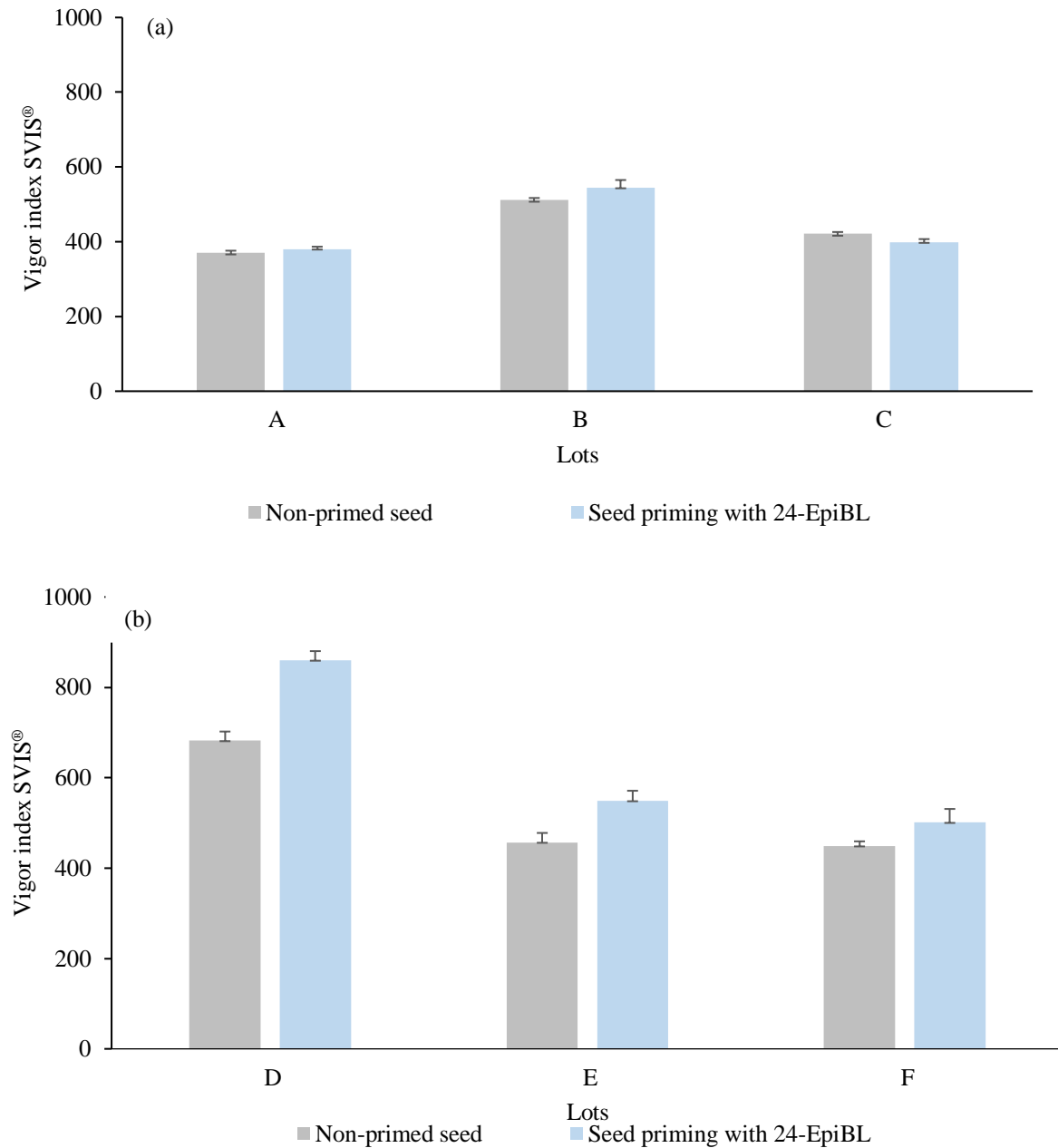


Figure 2 – Growth index - SVIS® of lettuce seedlings of the Florence (Lots A, B and C) and Betty cultivars (Lots D, E and F). Seeds were untreated (unprimed seed) and primed with 24-EpiBL at 10^{-6} M (24-EpiBL). Vertical bars represent mean standard error.

