Performance of Bell Pepper Seeds in Response to Drum Priming with Addition of 24-Epibrassinolide

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Abstract. Drum priming systems are among the most common methods of seed priming for commercial treatment. The supply of water to the seeds is controlled by physical means, and seeds are able to reach a desired moisture content without the use of osmotic solutions for hydration control. The brassinosteroid (BR), 24-epibrassinolide (24-EpiBL), has been shown previously to have a significant effect on seed performance. Therefore, the aim of this study was to verify the feasibility of drum priming associated with added 24-EpiBL on enhancement of bell pepper seed performance. Two bell pepper cultivars (AF-6 and AF-7) were tested, and each cultivar represented, respectively, by three and four seed lots with different initial physiological potentials. Seed performance was determined by evaluating standard germination, initial seedling growth, and changes on the antioxidant activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX). Seed Vigor Imaging System (SVIS®) was used to assess the initial seedling growth. The nondenaturing polyacrylamide gel electrophoresis (PAGE) technique was used to evaluate possible changes in the enzymatic antioxidant system. Several advantages were verified in the drum priming technique with added 24-EpiBL compared with the traditional procedure (water alone). Germination time was reduced followed by a seedling growth increase. Concomitantly, seed enzymatic activity was improved. However, results showed different response for each enzyme. Drum priming with 24-EpiBL demonstrates viability for commercial treatment and enhancement of bell pepper seeds.

Seed vigor is an important attribute of quality for successful food production (Rajjou et al., 2012). Bell pepper (Capsicum annuum L.) multiplication is performed by seeds, which are characterized by slow germination and low uniformity of seedling development (Bosland et al., 2012). As a consequence, direct sowing in the field is not considered as a viable alternative to the more costly plug transplants that are required for uniform field stands. A promising strategy that may permit direct field sowing is required for uniform field stands. A promising alternative to the more costly plug transplants that are currently used is the use of seed priming, which is defined as a procedure that prepares seeds for faster and more uniform development (Bosland et al., 2012). Seed priming treatments increase the rate of germination and improves the uniformity of seedling development (Bosland et al., 2012). As a consequence, direct sowing becomes feasible (Bosland, 2012). Seed priming is an efficient procedure to enhance germination of bell pepper seeds, a study was carried out on seed lots obtained with different vigor levels (Parera and Cantliffe, 1994). 24-EpiBL was tested for priming seeds (10 g) in parcels of six cycles of 4 h (total 24 h) with the drum injection (El-Maarouf-Bouteau et al., 2011).

The effect of 24-EpiBL on the activity of antioxidant enzymes might contribute to the tolerance of seeds to stress conditions such as high or low temperatures, salinity, organic pollutants, and pathogen attack (Agami, 2013; Ahammad et al., 2013a, 2013b; Bajguz and Hayat, 2009; Divi and Krishna, 2010; Pradhan et al., 2013; Rady, 2011; Wu et al., 2014; Zheng et al., 2007, 2014). Brassica juncea seeds treated with 24-EpiBL under salt stress showed similar length to those observed under favorable environmental conditions (Ali et al., 2008).

Materials and Methods

Seed treatment. Two bell pepper cultivars (AF-6 and AF-7), represented, respectively, by three and four hybrid seed lots were obtained with different physiological potentials. Previs-

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and drum priming with 24-EpiBL solution. The data for each cultivar were analyzed separately.

Fridovich, 1971), CAT (Woodbury et al., 1971), and POX (Hamill and Brewbaker, 1969).

Antioxidant enzyme system. Three grams of frozen seeds were homogenized in a chilled mortar with 9 mL buffer consisting of 100 mM potassium phosphate buffer (pH 7.5), 1 mM ethylenediaminetetraacetic acid, and 3 mM DL-Dithiothreitol. The homogenate was centrifuged at 12,000 rpm for 10 min at 4 °C, and the supernatant was subaliquoted and stored at -80 °C for further analysis. The concentration of protein in the sample was determined with the method of Bradford (1976) using bovine serum albumin as the standard. Enzyme activity was visualized by staining after running the extracts with 40 μg protein and electrophoresed at constant current of 20 mA per gel at 4 °C. The staining protocols used for specific enzymes were as described: SOD (Beauchamp and Fridovich, 1971), CAT (Woodbury et al., 1971), and POX (Hamill and Brewbaker, 1969).

Experimental design and statistical analysis. The data for each cultivar were analyzed separately by analysis of variance in a completely randomized design and factorial scheme of three treatments: control, drum priming with water, and drum priming with 24-EpiBL solution.

Seed germination. Germination tests were conducted with four replications of 50 seeds. Seeds were distributed on blotter paper moistened with water equivalent to 2.5 times the paper weight, and then placed at bottom of plastic boxes (28.5 x 18.5 x 10.0 cm) under alternating temperature of 20–30 °C. The number of normal seedlings was recorded daily to obtain speed of germination indexes (Maguire, 1962). Germination percentages were calculated at seven (first germination count) and 14 d (final germination count) after sowing.

Initial seedling growth. Seed Vigor Imaging System software was used to verify the initial seedling growth (Hoffmaster et al., 2005; Kikuti and Marcos-Filho, 2013). Four replications of 25 seeds were used for each seed lot. Seeds were placed in two horizontal rows on the upper third of the surface of previously moistened paper towels. The towels were rolled up and transferred to a germination chamber at 25 °C and kept for 6 d. Seedlings were then arranged on the surface of a scanner operated by Photosmart software and scanned at 98 dpi resolution. The whole seedling images were analyzed by the SVIS® software, which calculated data on seed vigor index, seedling length, and seedling uniformity index. The seed vigor index was generated by a combination of growth parameters (70% contribution) and seedling uniformity (30% contribution), both based on the maximum possible length of bell pepper seedling 6 d after sowing. Results are expressed as mean values obtained for each seed lot.

Overall germination of the seven seed lots was relatively high at 14 d, ranging from 95% to

Table 1. Germination (%) at 14 d in bell pepper seed lots of cultivars AF-6 and AF-7. Control seeds (Cont) were untreated whereas drum-primed seeds with water (Trad) are compared with those primed with 24-epibrassinolide (EpiBL).

| Cultivar | Seed lot | Cont | Trad | EpiBL | Seed lot | Cont | Trad | EpiBL |
|----------|----------|------|------|-------|----------|------|------|-------|
| AF-6     | 1        | 97   | 98   | 100   | 4        | 98   | 99   | Ac    |
|          | 2        | 98   | 99   | 100   | 5        | 98   | 99   | Ab    |
|          | 3        | 98   | 99   | 100   | 6        | 95   | 99   | Aa    |
|          |          |      |      |       |          |      |      | 1.0   |
| AF-7     |          |      |      |       |          |      |      | 1.0   |

Any two means followed by the same uppercase letters within a column and lowercase letters within a row indicate no statistically significant difference (Tukey’s test; P ≤ 0.05).

Table 2. First germination count (%) at 7 d in bell pepper seed lots of cultivars AF-6 and AF-7. Control seeds (Cont) were untreated whereas drum-primed seeds with water (Trad) are compared with those primed with 24-epibrassinolide (EpiBL).

| Cultivar | Seed lot | Cont | Trad | EpiBL | Seed lot | Cont | Trad | EpiBL |
|----------|----------|------|------|-------|----------|------|------|-------|
| AF-6     | 1        | 95   | 97   | 99    | 4        | 77   | BCb  | 95    |
|          | 2        | 97   | 99   | 99    | 5        | 87   | Aa   | 90    |
|          | 3        | 98   | 99   | 100   | 6        | 72   | Cc   | 90    |
|          |          |      |      |       |          |      |      | Aa    |
| AF-7     |          |      |      |       |          |      |      | 6.1   |

Any two means followed by the same uppercase letters within a column and lowercase letters within a row indicate no statistically significant difference (Tukey’s test; P ≤ 0.05).

Table 3. Speed of germination index (%) at 7 d in bell pepper seed lots of cultivars AF-6 and AF-7. Control seeds (Cont) were untreated whereas drum-primed seeds with water (Trad) are compared with those primed with 24-epibrassinolide (EpiBL).

| Cultivar | Seed lot | Cont | Trad | EpiBL | Seed lot | Cont | Trad | EpiBL |
|----------|----------|------|------|-------|----------|------|------|-------|
| AF-6     | 1        | 8.1  | 9.7  | 9.9   | 4        | 6.7  | B  | 7.7   |
|          | 2        | 8.0  | 9.8  | 9.9   | 5        | 7.1  |    | 7.7   |
|          | 3        | 8.1  | 9.9  | 9.9   | 6        | 6.8  | B  | 7.5   |
|          |          |      |      |       |          |      |      | 9.4   |
| AF-7     |          |      |      |       |          |      |      | 3.0   |

Any two means followed by the same uppercase letters within a column and lowercase letters within a row indicate no statistically significant difference (Tukey’s test; P ≤ 0.05).

Table 4. Vigor index (%) obtained by Seed Vigor Imaging System software in bell pepper seed lots of cultivars AF-6 and AF-7. Control seeds (Cont) were untreated whereas drum-primed seeds with water (Trad) are compared with those primed with 24-epibrassinolide (EpiBL).

| Cultivar | Seed lot | Cont | Trad | EpiBL | Seed lot | Cont | Trad | EpiBL |
|----------|----------|------|------|-------|----------|------|------|-------|
| AF-6     | 1        | 395  | 447  | 510   | 4        | 414  | B   | 430   |
|          | 2        | 329  | 419  | 506   | 5        | 464  | A   | 476   |
|          | 3        | 375  | 434  | 507   | 6        | 428  | B   | 411   |
|          |          |      |      |       |          |      |      | 438   |
| AF-7     |          |      |      |       |          |      |      | 4.2   |

Any two means followed by the same uppercase letters within a column and lowercase letters within a row indicate no statistically significant difference (Tukey’s test; P ≤ 0.05).

Table 5. Length (cm) of bell pepper seedlings obtained by Seed Vigor Imaging System software of cultivars AF-6 and AF-7. Control seeds (Cont) were untreated whereas drum-primed seeds with water (Trad) are compared with those primed with 24-epibrassinolide (EpiBL).

| Cultivar | Seed lot | Cont | Trad | EpiBL | Seed lot | Cont | Trad | EpiBL |
|----------|----------|------|------|-------|----------|------|------|-------|
| AF-6     | 1        | 6.6  | 7.6  | 9.6   | 4        | 6.6  | B   | 7.1   |
|          | 2        | 3.8  | 5.8  | 9.0   | 5        | 7.5  | A   | 7.8   |
|          | 3        | 6.2  | 8.6  | 10.1  | 6        | 5.9  | Ab  | 7.5   |
|          |          |      |      |       |          |      |      | 7.8   |
| AF-7     |          |      |      |       |          |      |      | 9.2   |

Any two means followed by the same uppercase letters within a column and lowercase letters within a row indicate no statistically significant difference (Tukey’s test; P ≤ 0.05).
100% regardless of the treatment (Table 1). For cultivar AF-6, there was a small, but statistically significant difference between the germination of drum-primed seeds with 24-EpiBL and water alone for lot 1. For cultivar AF-7, no differences were noted among the treatments in the absence or presence of 24-EpiBL.

During the first germination count at 7 d (Table 2), seeds from lots 1, 3, and 6 showed faster germination after drum priming with 24-EpiBL. For example, lot 6 seeds (lowest initial physiological potential in cultivar AF-7) showed mean germination percentages of 72% for unprimed seed, 90% for seeds primed with water, but 96% for seeds primed with 24-EpiBL.

Speed of germination indexes (Maguire, 1962) were also improved after priming (Table 3). Drum priming with 24-EpiBL increased the speed of germination indices of seeds from lots 4, 5, and 7 of cultivar AF-7. However, although increases in the speed of germination indexes of cultivar AF-6 were also verified after priming with 24-EpiBL, these were not significant when compared with traditional drum priming (water alone).

A consistently positive effect of drum priming with 24-EpiBL was reflected in the seed vigor indexes, seedling length, and uniformity of seedling development (Tables 4–6, respectively). The increases were more pronounced for seed lots of cultivar AF-6 than for those of AF-7, especially for lot 2, seed lot with the lowest initial physiological potential in cultivar AF-6, where seedlings from 24-EpiBL-primed seeds were significantly taller than seedlings from drum priming in absence of this bioregulator (Fig. 1).

Patterns of enzyme activities varied in response to drum priming with 24-EpiBL. Five of the seed lots (1, 2, and 3—cultivar AF-6; 4 and 7—cultivar AF-7) showed higher POX activity after drum priming with 24-EpiBL than either the control or drum priming with water alone (Fig. 2). The intensity of SOD band also increased after drum priming with 24-EpiBL (Fig. 3), but except for lot 5, the level of activity did not differ between 24-EpiBL and water alone (Fig. 3). CAT activity also showed increases after traditional drum priming, but treatment with 24-EpiBL revealed higher band intensity for most seed lots (Fig. 4).

Discussion

This study showed that the germination of bell pepper seeds is enhanced by priming with 24-EpiBL and that the drum priming method can be used effectively to achieve the benefits of early germination and uniform seedling development. Drum priming with 24-EpiBL resulted in significantly greater seedling development when compared with drum priming using water alone, especially in seed lots with lower initial physiological potential. Furthermore, 24-EpiBL treatment is associated with increases in antioxidant enzyme activity, which may account for the improved germination performance and seedling development.

Table 6. Uniformity index* of bell pepper seedling development (obtained by Seed Vigor Imaging System software) of cultivars AF-6 and AF-7. Control seeds (Cont) were untreated whereas drum-primed seeds with water (Trad) are compared with those primed with 24-epibrassinolide (EpiBL).

| Cultivar AF-6 | Cultivar AF-7 |
|--------------|--------------|
| Seed lot     | Cont | Trad | EpiBL | Seed lot | Cont | Trad | 24-EpiBL |
| 1            | 707 Ab | 797 Aab | 820 Aa | 4        | 725 Ab | 789 Aa | 790 Aa   |
| 2            | 742 Ab | 862 Aa | 859 Aa | 5        | 790 Ab | 743 Ab | 866 Aa   |
| 3            | 686 Bb | 658 Bb | 759 Ba | 6        | 735 Ab | 738 Ab | 820 Aa   |
| —            | —     | —     | —     | 7        | 788 Ab | 751 Ab | 841 Aa   |
| C.V. (%)     | 8.2   | 6.1   |        |          |        |        |          |

*Any two means followed by the same uppercase letters within a column and lowercase letters within a row indicate no statistically significant difference (Tukey’s test; \( P \neq 0.05 \)).
For a long time, gibberellins (GA3) and abscisic acid (ABA) had been considered as the two key phytohormones of the seed germination process, with antagonistic roles in the regulation of these events. However, researchers have shown that BRs, in parallel with GA3, can also have an antagonistic effect on ABA, and BRs signaling is essential for successful germination (Divi and Krishna, 2010; Xi and Yu, 2010; Zhang et al., 2009). According to Leubner-Metzger (2001), BRs interact with GA3 to induce the activity of β-1,3-glucanase class I (βGLU I) and accelerate the rupture of the micropylar endosperm during seed germination. The cell wall, particularly in bell pepper seeds, exerts a high mechanical resistance to growth of the embryonic axis (Watkins and Cantliffe, 1983). Therefore, it seems reasonable to assume that treating bell pepper seeds with BRs may have weakened the cell wall over the embryonic axis and favored germination. Wang et al. (2011) also found an increase in cucumber germination from seeds treated with BRs, and also reported that the beneficial effect of BR can be verified even under salt stress.

In addition to enhanced early germination of bell pepper, this study showed that drum priming with 24-EpiBL causes a significant increase in the subsequent growth of seedlings. Yusuf et al. (2012) also found enhanced growth of mung bean seedlings after seed treatment with 24-EpiBL. Fridman and Savaldi-Goldstein (2013) demonstrated that the growth response of seedlings treated with 24-EpiBL is partly due to the influence of BR in cell expansion. BRs affect the activity of xyloglucan endotransglucosylase/hydrolase (XTH), an enzyme responsible for cell wall loosening that acts on xyloglucan, the most important hemicellulosic polysaccharide in cell wall. Activity of XTH reduces the resistance between xyloglucan chains and allows for cell expansion (Sun et al., 2005).

Exposure of seeds to BRs may also improve seedlings growth due its important role in cell division. In this context, Fathkhistinova et al. (2002) found an increase in the mitotic rate of meristematic cells of wheat roots treated with 24-EpiBL, showing similar effects to the roots treated with cytokinins. For Arabidopsis cell suspension culture, the effect of 24-EpiBL was even more remarkable because cell division was verified even in the absence of cytokinins (Hu et al., 2000).

The antioxidant activity of CAT, SOD, and POX in bell pepper seeds after drum priming with 24-EpiBL was also improved. Antioxidant activity during the germination process and the development of embryonic axis is crucial for the decrease of oxides and peroxides, and inhibits possible chemical lesions in the cell. The enzymes CAT, SOD, and POX are part of this antioxidant system. SOD is the first line of defense against superoxide radicals (O2−), converting it to oxygen (O2) and hydrogen peroxide (H2O2) in mitochondria and the cytosol (Apel and Hirt, 2004). H2O2 is toxic at high concentrations; CAT converts it to water and oxygen in peroxisomes, and POXs do so in the cytosol (Ashraf, 2009). O2− e H2O2 can lead to formation of hydroxyl radical (OH•) via the Haber–Weiss reaction, which is the most aggressive radical species that can cause extensive cellular damage (Bailly, 2004). Therefore, the balance among SOD, CAT, and POX activities is essential, since, OH• radical is not eliminated enzymatically.

Levels of oxides and peroxides in the cell are directly proportional to seed respiratory rate, because the mitochondrial respiratory chain complex is one of the most important source of ROS (Noctor et al., 2007); electron leak from transport chains generates O2−, and subsequently H2O2 by O2 dismutation. Germination is closely associated to the increase in the respiration rate and there is a continued increase of ROS during this process, which may cause damage to nucleic acids, lipids, and proteins (Beckman and Ames, 1997; Bray and West, 2005). As a result, the completion of seed germination may be affected by damage on DNA and important seed storage compounds (Kramer and Colville, 2011). The response of SOD, CAT, and POX observed in this study suggests the increase in activity of these ROS scavenging enzymes may provide a potential mechanism that leads seedlings to faster establishment. Seeds with higher detoxification activity at the beginning of imbibition tend to favor the development of the embryonic axis (Bailly, 2004).

Application of 24-EpiBL on bell pepper seeds during drum priming led to enhanced germination and seedlings length in parallel with higher activity of SOD, CAT, and POX. Possibly, one of the reasons for improvement of bell pepper seedling development is induction of antioxidant enzymes activity, which reduces potential damage caused by O2− and H2O2 during imbibition and reactivation of the seed metabolism, and favors early seedling growth. A study on the dynamics of the antioxidant system and seedling establishment has linked antioxidant enzymatic activity as well as concentration of ROS to the protrusion of the primary root. Chen and Arora (2011) demonstrated that primary root protrusion occurred when the concentration of ROS reached a stationary level, suggesting

Fig. 3. Activity staining for superoxide dismutase in bell pepper seeds of two cultivars, AF-6 and AF-7, following nondenaturing polyacrylamide gel electrophoresis. Each cultivar was represented by three and four seed lots, respectively. (a) Unprimed seeds; (b) drum-primed seeds using water alone (traditional); (c) drum priming with the addition of 24-epibrassinolide.
efficient antioxidant enzyme activity is essential for successful germination.

In conclusion, this study showed that drum priming with 24-EpiBL can be used for commercial priming treatment of bell pepper seeds. The advantages achieved by inclusion of 24-EpiBL in the priming treatment can be verified on germination performance, seedling development, and antioxidant enzyme activity. However, it is still necessary to assess whether these beneficial effects on early development will enhance the field performance of seedlings as well as the effect of such priming on seed longevity and persistence during seed storage. Investigation of these important components is currently being performed.

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