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Exploring the potential of *Moringa oleifera* leaf extract (MLE) as a seed priming agent in improving wheat performance

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Abstract: Seed priming with plant extracts and chemicals has been used as an important growth enhancement tool in crop plants; however, the mechanisms of their action are not fully explored. In this research, an attempt was made to understand the mechanism of various seed priming treatments in greenhouse-grown wheat. The seed priming treatments used were hydropriming, on-farm priming, moringa (*Moringa oleifera* Lam.) leaf extract (MLE) priming, and $CaCl_2$ priming. Results showed that all the seed priming treatments were effective in improving germination and seedling growth attributes of wheat over the control. However, MLE followed by $CaCl_2$ emerged as the most effective tools. The main mechanisms in this regard were the induction of an antioxidative system together with increased chlorophyll contents, ascorbic acid, and soluble phenolics contents. The results strongly support the view that seed priming with MLE is economical and can be effectively used to improve wheat growth under greenhouse conditions.

Key words: Antioxidant enzymes, ascorbic acid, $CaCl_2$ priming, hydropriming, phenolics, pigments

1. Introduction

Wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), rice (*Oryza sativa* L.), and cotton (*Gossypium hirsutum* L.) are economically important crops all over the world. In Pakistan, wheat serves the dual purpose of being a staple food as well as a cash crop, contributing 13.1% and 2.7% to the value added in agriculture and GDP, respectively. In arid and semiarid regions of the world like Pakistan, one potential for successful crop production is the pre-sowing seed treatments, also referred to as seed priming.

Seed priming not only improves the speed and uniformity of germination (Khan et al., 2008; Khalil et al., 2010) but also stimulates various biochemical changes in the seed, which are vital in breaking dormancy, the mobilisation or hydrolysis of seed reserves, enzyme activation, and the emergence of embryonic tissues (Asgedom & Becker, 2001; Çatav et al., 2012). It minimises emergence time with improved germination rate and percentage, and synchronises emergence (Harris et al., 2002). This may be due to the enhanced mobilisation of metabolites/inorganic solutes to germinating plumule/embryo, leading to enhanced growth (Taiz & Zeiger, 2002). Previously, various chemicals, salts, and plant growth regulators have been used in seed priming to improve the seed emergence, plant growth, and the crop yield (Ruan et al., 2002; Farooq et al., 2006) but as they were costly they could not be utilised on a large scale. Therefore, there arose a need to search for seed priming agents of natural origin, which is also a step towards organic agriculture.

The extracts obtained from some crop and tree residues have been reported to play roles in crop growth and yield (Chung & Miller, 1995; El Atta & Bashir, 1999; Ahmed & Nimer, 2002; Farooq et al., 2008). Moringa (*Moringa oleifera* Lam.) leaf extract (MLE) is a newly discovered plant growth enhancer that not only improves seedling emergence of rangeland grasses but also seedling vigour and growth as compared to other seed priming techniques (Nouman et al., 2012a, 2012b). MLE caused a 20% to 35% yield enhancement in different crops by exhibiting a large number of roots, improving the growth of young plants, increasing the leaf area duration, strengthening the plants by increasing resistance to pests and diseases, and producing more and larger fruits (Fuglie, 2000). MLE, being rich in K, Ca, Fe, amino acids, ascorbates,

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and growth regulating hormones such as zeatin, proved an ideal plant growth enhancer (Makkar & Becker, 1996; Basra et al., 2009a, 2009b). According to Phiri (2010), MLE spray improved the germination of sorghum by 29% and wheat hypocotyl length by 14.9%.

Keeping in mind these growth promoting characteristics, the present study was designed to evaluate the potential of MLE as priming agent in comparison with other existing seed priming techniques in terms of seed emergence, plant growth, yield, and antioxidant content of wheat grown in natural climatic conditions.

2. Materials and methods

Experimental site and conditions: The experiment was conducted during the winter season of 2009–10 in a wire house under natural conditions. The average value of relative humidity was 61.40%, rainfall 3.92 mm, temperature 21.82 °C, pan evaporation 2.53 mm (ET_o), and sunshine 6.88 h during the crop growing season. Twenty-five wheat seeds of cv. Sehar-2006 were sown in soil-filled (10 kg) earthen pots on 19 November 2009. The soil medium (soil + compost + sand, 1+1+1) was sandy loam with pH 7.53, ECe 4.03 dS m^{-1}, TSS 45.06 mmol L^{-1}, SAR 12.65, along with K^+ Na^+, and Cl^- contents of 0.47, 33.14, and of 22.97 mmol L^{-1}, respectively. Tap water was used for irrigation when required. Ten days after emergence, 6 uniform seedlings were maintained in each pot. Fertilisers applied were urea, single super phosphate (SSP), and potassium sulphate at 120, 100, and 62.5 kg NPK ha^{-1}, respectively. The full dose of P and K and half of the N was mixed into the soil prior to sowing. The remaining N was applied in equal portions at the first irrigation and tillering.

Plant material: Wheat seeds (Sehar-2006) were obtained from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. The germination percentage was evaluated by placing 20 seeds between 2 layers of Whatman's filter paper No. 1. Moringa leaves were collected from the nursery area of the Department of Forestry, University of Agriculture, Faisalabad, Pakistan. Analysis of the moringa leaves showed that they possessed 191.86 of superoxide dismutase, 7.09 of catalase, 21.99 of peroxidase (IU/mg protein), 8.19 mg g^{-1} total phenolics, 0.36 mmol g^{-1} ascorbic acid, and 1.40 mg g^{-1} total soluble protein. The leaves were stored overnight at freezing temperature; then the MLE was extracted using a locally fabricated machine and sieved through cheesecloth. Then the required concentrations (1:10, labelled here MLE10, and 1:30, labelled here MLE30) were prepared by mixing the MLE with distilled water.

Seed priming: Wheat seeds were primed at room temperature with distilled water (hydropriming), on-farm priming, MLE (10 and 30) (Nouman et al., 2012a), and $CaCl_2$ (–1.25 MPa) (Farooq et al., 2006) for 12 h in aerated solutions and dried in the shade to become closer to their original weight at 28 ± 2 °C (Basra et al., 2002), while 1 set was not primed.

Seed emergence: Seedling emergence was counted daily until the final emergence, and the emergence index (EI) (AOSA, 1083), mean emergence time (MET) (Ellis & Roberts, 1981), and time taken for 50% emergence (E50) (Farooq et al., 2005) were calculated with the following formulae:

$$EI = \frac{No.\ of\ emerged\ seeds}{Days\ of\ first\ count} + \text{———} + \frac{No.\ of\ emerged\ seeds}{Days\ of\ final\ count}$$

$$MET = \frac{\sum Dn}{\sum n}$$

where n was the number of seeds that emerged on day D and D was the number of days counted from the beginning of germination, and

$$E50 = ti + \frac{\left(\frac{N}{23} - ni\right)(tj - ti)}{nj - ni}$$

where N was the final number of seeds that emerged and n_i and n_j were the cumulative number of seeds that emerged by adjacent counts at times ti and tj, respectively, when $ni < N/2 < nj$.

Plant vigour evaluation: Ten days after emergence, 3 plants were maintained in each pot to find out the effect of seed priming on plant vigour and quality parameters. Shoot and root lengths, and fresh and dry weights were recorded. Leaf area was measured on a leaf area meter (CI-203 Laser leaf area meter, CID Inc., USA) 75 days after sowing (DAS), when plants were fully grown.

Yield parameters: At maturity, plants were harvested and threshed manually to record the number of grains per spike, 100 grain weight (g), and grain yield per plant (g).

Biochemical parameters: For biochemical analysis, leaf samples were harvested 75 DAS. The leaves were analysed for the following biochemical attributes:

Leaf chlorophyll content: Wheat leaves (0.5 g) were ground in 80% acetone to determine chlorophyll (*a* and *b*) content. The absorbance of filtrate was determined at 663 and 645 nm and the chlorophyll content was calculated as described by Arnon (1949).

Total soluble proteins (mg g^{-1}): Total soluble proteins (TSP) were determined by using the protocol devised by Bradford (1976). Fresh wheat leaves (0.5 g) were ground in 1 mL of phosphate buffer saline with pH 7.2 (10 mM Na_2HPO_4, 2 mM KH_2PO_4, 2.7 mM KCl, and 1.37 mM NaCl) and 1 µM of cocktail protease inhibitor. After grinding, the plant material was centrifuged at 12,000 rpm for 5 min at room temperature. The supernatant was transferred to another cuvette in order to estimate the total soluble proteins. The absorbance was recorded at 595 nm using a spectrophotometer (UV-4000, O.R.I., Germany) and the total soluble protein content was quantified by putting absorbance readings in an equation derived from the standard curve.

Enzymatic antioxidants (IU min^{-1} mg $protein^{-1}$): Wheat leaf samples (0.5 g) were ground in 5 mL of 50 mM phosphate buffer (pH 7.8). Then the ground sample was centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was stored in Eppendorf tubes for further assay to determine superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). The protocol devised by Giannopolitis and Ries (1977) was used to determine SOD activity at 560 nm by using a UV spectrophotometer (UV-4000, O.R.I. Germany). One unit of SOD was quantified by the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction. POD and CAT activities were determined by using the protocol described by Chance and Maehly (1955) by recoding absorbance at 240 and 470 nm, respectively, by using a UV spectrophotometer (UV-4000, O.R.I. Germany). CAT activity was measured as units (µmol of H_2O_2 decomposed per min) per mg of protein while POD 1 unit activity was defined as the change of 0.01 absorbance unit per min per mg of protein.

Non-enzymatic antioxidant: Total phenols of leaf samples were determined, using gallic acid as a reference standard (Ainsworth & Gillespie, 2007). The standard curve was prepared by reading the absorbance of different concentrations of gallic acid (500, 250, 150, and 100 mg L^{-1}) at 760 nm using a UV spectrophotometer (UV-4000, O.R.I., Germany). Total phenol levels in samples were determined by plotting the calibration curve prepared using gallic acid standards.

The leaf samples' ascorbic acid levels were measured according to the protocol devised by Yin et al. (2008). Wheat leaves (0.5 g) were ground in 2 mL of TCA (5%) with a little clean sand and were centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant was used for ascorbic acid determination. The absorbance was measured at 525 nm using 100 mM PBS as a control. From this absorbance value, the ascorbic acid content of samples (mmol g^{-1} fresh weight) was determined on the basis of the standard curve.

3. Results

The effects of different doses of MLE (i.e. MLE10 and MLE30) as a seed priming agent were compared with other priming agents (hydropriming, $CaCl_2$, and on-farm priming). The emergence, seedling vigour, yields, and antioxidant status of the wheat were assessed.

Emergence phase: The results showed that seed priming with different priming agents significantly increased the emergence index of wheat seeds (Figure 1). A maximum emergence index was recorded in seeds primed with MLE30 followed by $CaCl_2$ and hydropriming, while the lowest was recorded in on-farm priming (Figure 1). The analysis of the data showed that seed priming with different priming agents did not affect the mean emergence time (MET) (Figure 1). In contrast, time to 50% emergence

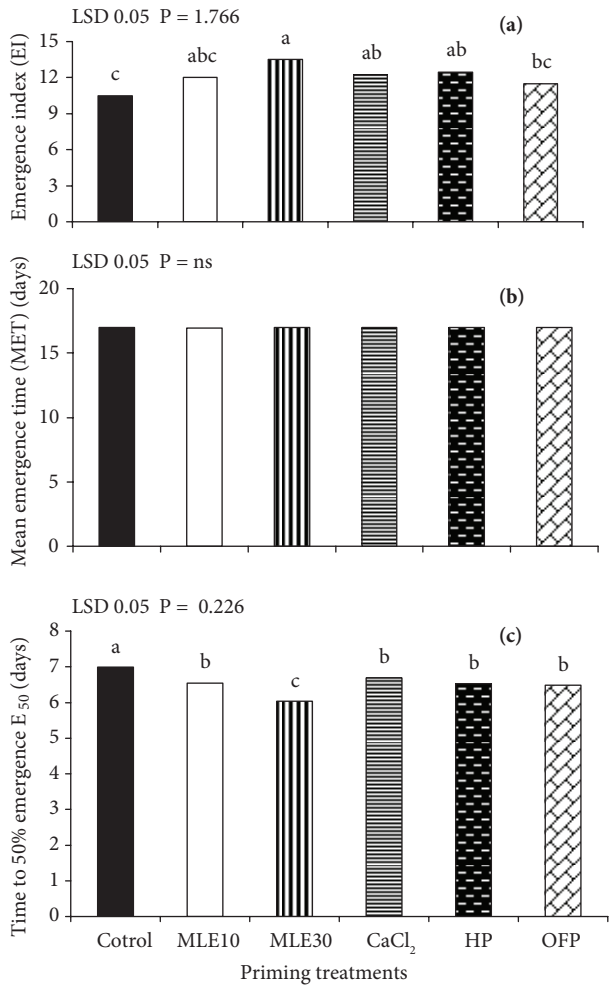

Figure 1. Effect of seed priming on emergence index (EI) (a), mean emergence time (MET) (b), and time to 50% emergence (E50) (c) of wheat cv. Sehar-2006. (MLE10 = 10 times diluted MLE, MLE30 = 30 times diluted MLE, HP = Hydropriming, OFP = On-farm priming). Treatments not showing the same letters differ significantly at a 5% probability level.

(E50) showed that seed priming significantly reduced the time to attain E_{50}. However, the maximum reduction in time to 50% emergence in wheat seeds was observed in MLE30 (Figure 1).

Seedling vigour: Seed priming improved seedling vigour attributes (Figures 2 and 3). A maximum improvement in fresh shoot weight and dry weight was observed in seedlings raised from seeds primed with MLE30 followed by MLE10, whilst minimum improvement was observed in the control. The significantly highest increment in shoot length was found with $CaCl_2$ seed priming followed by MLE30 seed priming (Figure 2). Shoot length was observed to be in a decreasing order from hydropriming, to on-farm priming, to MLE10 priming, to the control. Similarly, the highest fresh root weight and dry weight was recorded when seeds were primed with MLE30 (Figure 3) followed by hydropriming and MLE10. MLE10 seed priming ranked third regarding

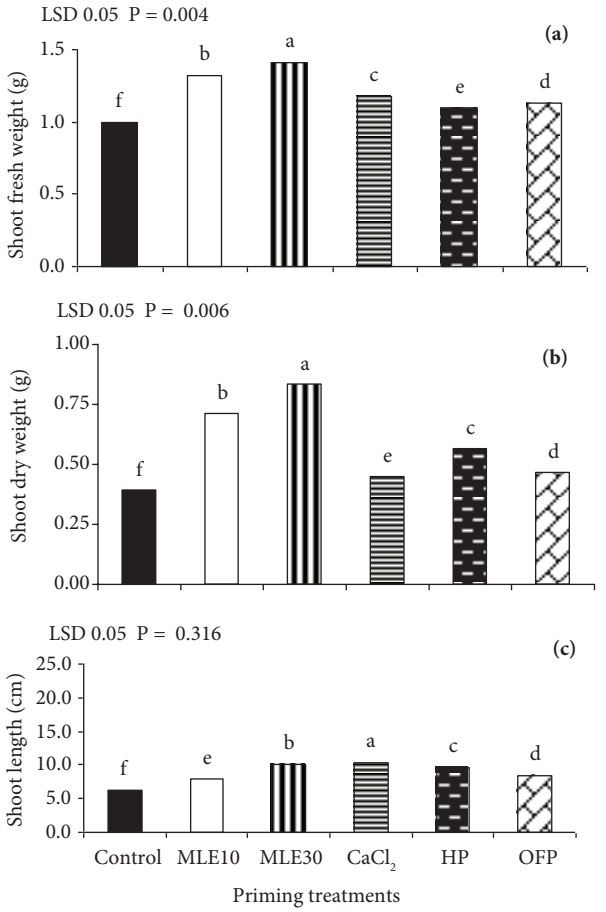

Figure 2. Effect of seed priming on fresh shoot weight (a), dry shoot weight (b), and shoot length (c) of wheat cv. Sehar-2006. (MLE10 = 10 times diluted MLE, MLE30 = 30 times diluted MLE, HP = Hydropriming, OFP = On-farm priming). Treatments not showing the same letters differ significantly at a 5% probability level.

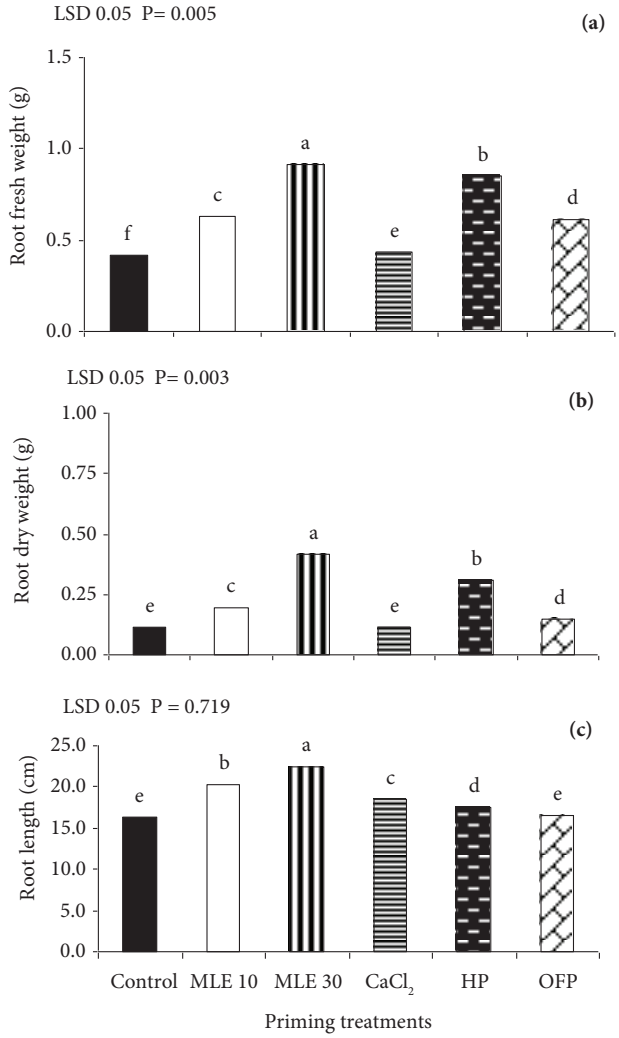

Figure 3. Effect of seed priming on fresh root weight (a), dry root weight (b), and root length (c) of wheat cv. Sehar-2006. (MLE10 = 10 times diluted MLE, MLE30 = 30 times diluted MLE, HP = Hydropriming, OFP = On-farm priming). Treatments not showing the same letters differ significantly at a 5% probability level.

fresh root weight. MLE30- and MLE10-primed seeds responded with a significant increase in root length, but MLE30 was the superior one (Figure 3). The root length observed in the case of $CaCl_2$ was shorter than that of MLE priming but longer than that of hydropriming and on-farm priming. The maximally reduced root length was found in non-primed seed.

Leaf area: The largest leaf area was produced in seedlings that emerged from MLE30 primed seeds followed by MLE10 (Figure 4). Hydropriming and on-farm priming showed similar values for leaf area. In this case, $CaCl_2$ produced the lowest value for leaf area, being less than other priming treatments but still greater than the control. Overall, MLE30 produced more vigorous seedlings.

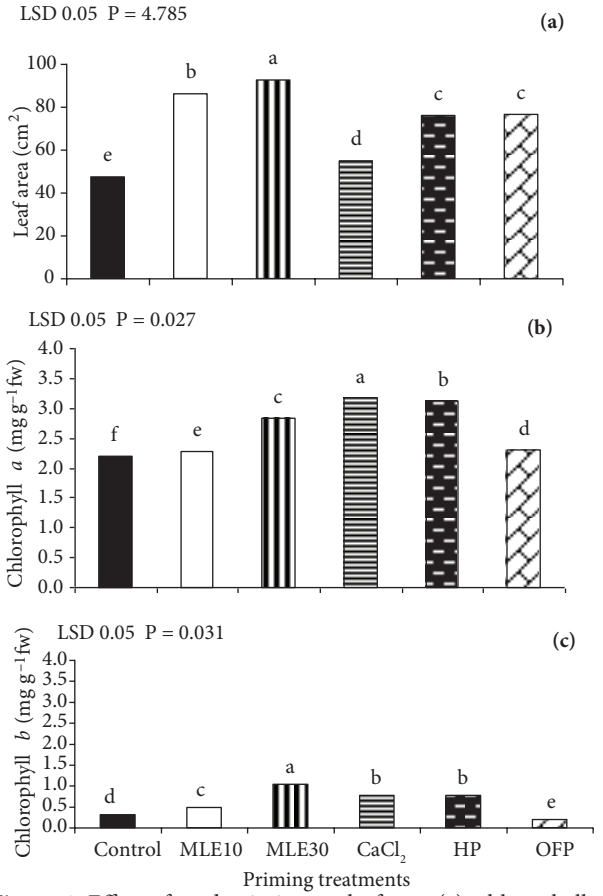

Figure 4. Effect of seed priming on leaf area (a), chlorophyll *a* (b), and chlorophyll *b* (c) of wheat cv. Sehar-2006. (MLE10 = 10 times diluted MLE, MLE30 = 30 times diluted MLE, HP = Hydropriming, OFP = On farm priming). Treatments not showing the same letters differ significantly at a 5% probability level.

Leaf chlorophyll contents: Priming also showed significant influence on leaf chlorophyll contents, which were maximally increased by MLE30 followed by $CaCl_2$ seed priming (Figure 4). The MLE10 exhibited chlorophyll *a* contents statistically less than MLE30 and $CaCl_2$, but higher than on-farm priming and hydropriming. Nevertheless, on-farm priming and hydropriming were significantly better for chlorophyll *a* as compared to the control. The lowest value of chlorophyll *a* was found in the leaves of plants raised from non-primed seeds. Likewise, the highest quantity of chlorophyll *b* was also noted in the MLE30-primed seeds (Figure 4). $CaCl_2$ and hydropriming were statistically similar (although inferior) to MLE30 but superior to other priming treatments. The performance of non-primed seeds was better than that of on-farm priming. Pronounced effects of seed priming were observed with MLE30 and $CaCl_2$ for leaf chlorophyll *a* and *b* contents.

Yield parameters: A larger number of grains with more than 100 grain weight were obtained from MLE30 primed plants (Figure 5), which resulted in a higher grain yield per plant (Figure 5). All the priming treatments showed better yield than the non-primed control.

Total soluble protein and antioxidants content of leaf: Total leaf soluble protein content showed a positive response towards seed priming treatments (Figure 6). MLE30 increased protein content, and MLE10 was significantly less than MLE30 but higher than other treatments. Hydropriming exhibited better leaf protein content than $CaCl_2$ and on-farm priming. The protein content obtained in $CaCl_2$ and on-farm priming were even less than the control.

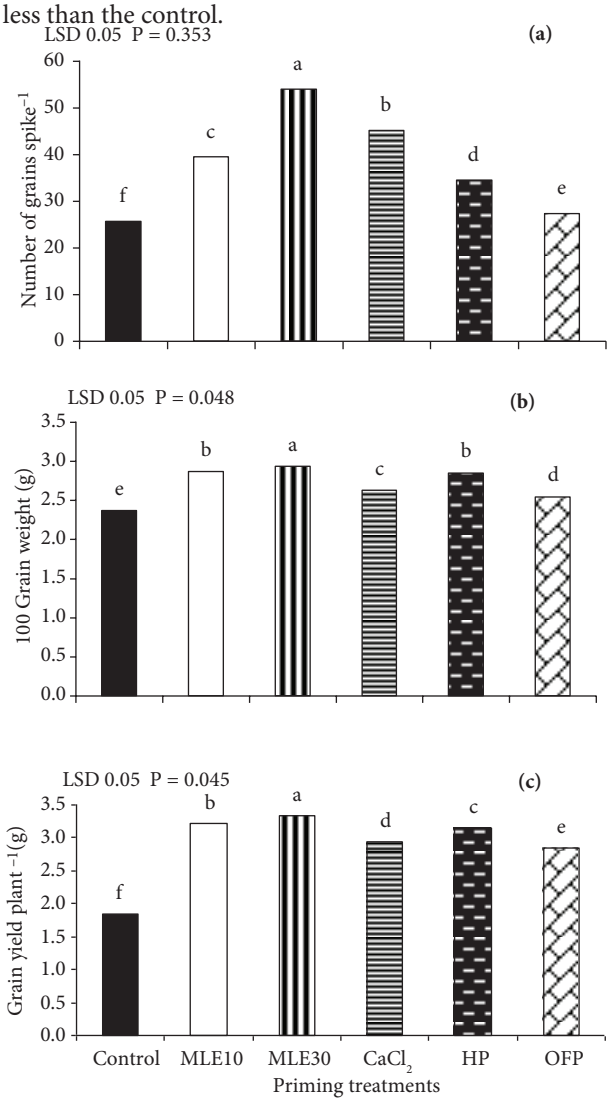

Figure 5. Effect of seed priming on number of grains per spike (a), 100 grain weight (b), and grain yield per plant (c) of wheat cv. Sehar-2006 (MLE10 = 10 times diluted MLE, MLE30 = 30 times diluted MLE, HP = Hydropriming, OFP = On-farm priming). Treatments not showing the same letters differ significantly at a 5% probability level.

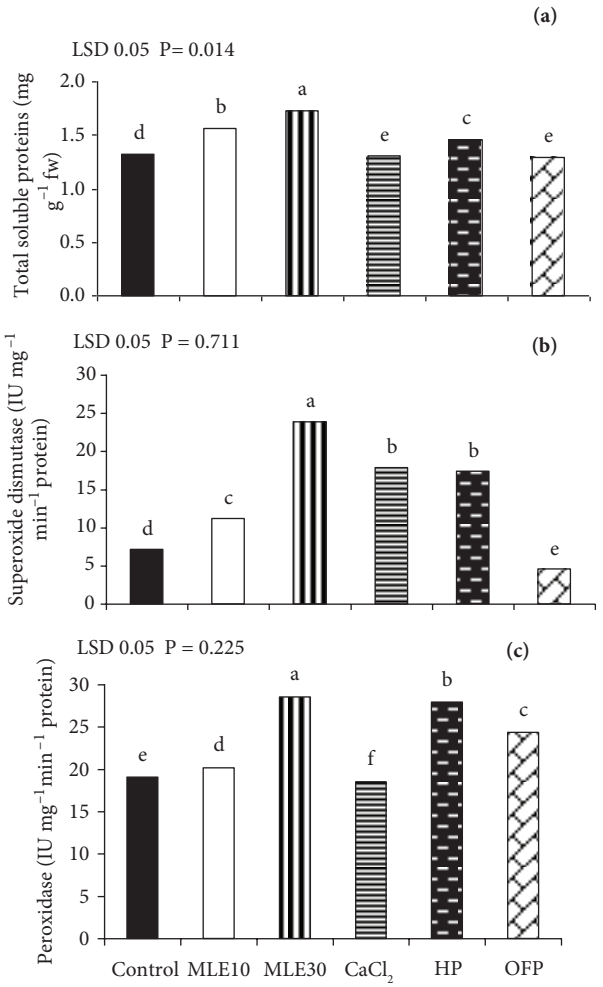

Figure 6. Effect of seed priming on total leaf soluble protein (a), superoxide dismutase (b), and peroxidase (c) of wheat cv. Sehar-2006. (MLE10 = 10 times diluted MLE, MLE30 = 30 times diluted MLE, HP = Hydropriming, OFP = On-farm priming). Treatments not showing the same letters differ significantly at a 5% probability level.

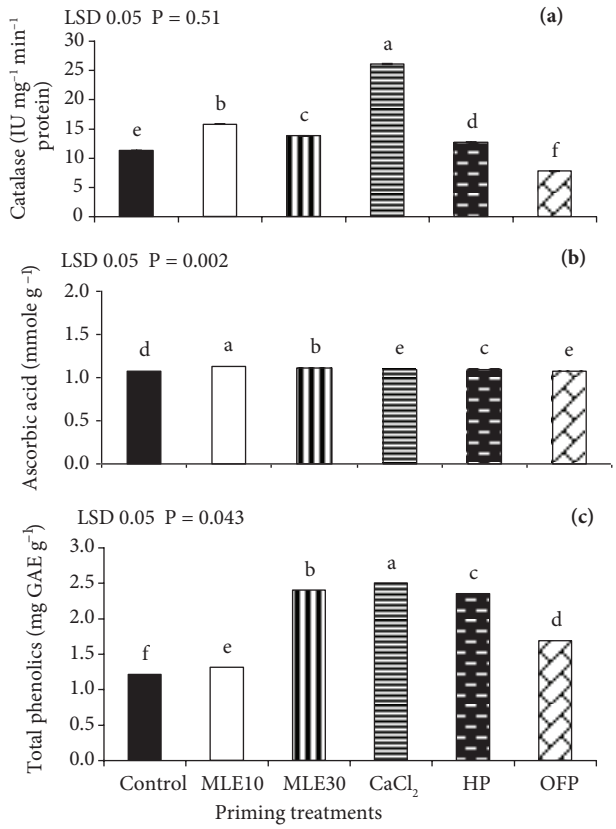

Figure 7. Effect of seed priming on leaf catalase (a), ascorbic acid (b), and total phenolics (c) of wheat cv. Sehar-2006. (MLE10 = 10 times diluted MLE, MLE30 = 30 times diluted MLE, HP = Hydropriming, OFP = On-farm priming). Treatments not showing the same letters differ significantly at a 5% probability level.

Higher activities of super oxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were recorded in the MLE30 seed priming agent as compared to other primed and non-primed seed treatments. Hydropriming was followed by MLE30 treatment in the case of SOD and POD (Figure 6), but in the case of CAT, MLE30 was followed by MLE10 seed treatment (Figure 7). The lowest activity of SOD, CAT, and POD was observed when seeds were on-farm primed and primed with $CaCl_2$. Nevertheless, seed enhancement techniques significantly affect the enzymatic antioxidants.

A significant increase in ascorbic acid content was also found in the case of MLE10 seed priming followed by MLE30 (Figure 7). The plants raised from hydroprimed seeds produced an ascorbic acid content that was similar to $CaCl_2$ priming but less than MLE30 priming. The least ascorbic acid but maximum total phenolic content were produced in $CaCl_2$ priming treatments (Figure 7).

4. Discussion

In this study, seed priming enhanced the speed and total final germination count of wheat, and the maximum increase in these attributes was observed in MLE30 treated seeds (Figure 1). It has already been reported that 30 times diluted MLE significantly increased seed and seedling vigour in wheat (Afzal et al., 2008), maize (Basra et al., 2011), and many grass species including *Cenchrus ciliaris*, *Panicum antidotale*, and *Echinochloa crusgalli* (Nouman et al., 2012a). All seed priming agents enhanced the seedling shoots, fresh root weight, and dry weight of wheat plants, particularly with MLE30. However, seed priming with $CaCl_2$ caused the maximum increase in shoot length while longer roots were observed with MLE30 priming (Figures 2 and 3). Seed priming enhances the rate of metabolism, which resulted in an increased speed of germination and

emergence (Ashraf et al., 2008). Such a marked effectiveness of moringa is due to the fact that its leaves are a rich source of zeatin, ascorbic acid, Ca, and K (Fuglie, 1999; Foidl et al., 2001), which regulates the seed germination and seedling establishment related metabolism.

In the present study, MLE30 priming improved emergence rate and wheat yield followed by $CaCl_2$ priming. Nouman et al. (2012a) reported MLE30 as a promising tool for improving the emergence rate and plant biomass of range grasses. Similarly, Yasmeen et al. (2012) observed a higher crop growth rate and seasonal leaf area duration under exogenous application of MLE30. The higher concentration of calcium and other mineral contents in *M. oleifera* leaves might be responsible for promoting seed emergence rate and plant vigour (Foidl et al., 2001; Anjorin et al., 2010). Farooq et al. (2006) reported osmohardening with $CaCl_2$ as a good priming technique in improving the emergence and plant height in rice, while its effectiveness was also confirmed in wheat in the present study. Moreover, the MLE efficacy might also be due to a higher zeatin concentration in *M. oleifera* leaves (5–200 μg g^{-1} of fresh weight) as reported by Fuglie (1999) or due to higher or enhanced mobilisations of metabolites/inorganic solutes to germinating plumule, which results in enhanced growth (Taiz & Zeiger, 2002).

Higher crop yield is the ultimate goal for cereal cultivation and a number of strategies are underway to increase this attribute. Crop yield in cereals is mainly determined by optimum plant population, number, and the size of grains. A number of reports show that seed priming enhanced the crop productivity by either increasing the emergence, number, size of grain, or a combination of the former (Khan et al., 2006; Ashraf et al., 2008; Athar et al., 2008). Farooq et al. (2006) reported that the highest value of 1000 kernel weight in rice was produced by KCl followed by $CaCl_2$ seed priming. In the present study, seed priming with different priming agents increased the number of grains per spike and 100 grain weight. Greater partitioning of photoassimilates to developing grains increases grain size (Taiz & Zeiger, 2002). The level of cytokinin positively correlates with final grain weight in maize (Dietrich et al., 1995). As moringa leaves are rich in zeatin (Foidl et al., 2001), MLE priming was effective due to other growth promoting factors, which is in line with Gupta et al. (2003). In some earlier studies it has been observed that seed priming with plant growth regulators, inorganic salts, compatible solutes, or sugar beet extract improved seed germination by providing a physiological advantage (Afzal et al., 2006; Pill & Savage, 2008). The enhanced yield by seed priming arises from the events taking place during earlier stages of crop growth such as faster production of more vigorous seedlings (Farooq et al., 2006). Likewise, Ruan et al. (2002) observed improved seedling vigour with $CaCl_2$ and $CaCl_2$ + NaCl priming in greenhouse conditions.

Seed priming with MLE30 followed by $CaCl_2$ priming improved the photosynthetic pigments. As a well-known priming tool in rice (Farooq et al., 2006) and wheat (Afzal et al., 2011), $CaCl_2$ priming was tested in the present study for wheat, which proved effective in enhancing the speed and spread of emergence and seedling vigour as well; however it was not as effective as MLE30, which increased seedling vigour and chlorophyll content in wheat leaves. The effectiveness of MLE might also be attributed to higher protein and antioxidants in addition to mineral contents (especially calcium and potassium) in moringa leaves (Foidl et al., 2001). Aslam et al. (2005) also reported potassium, calcium, and magnesium contents in moringa leaves collected from Punjab, Pakistan of 19,732–24,397, 1839–2097, and 18,950–26,349 mg kg^{-1}, respectively.

In this study, different priming agents enhanced total leaf soluble proteins, although this effect was more pronounced with MLE30 capital Working with wheat, Al-Hakimi and Hamada (2001) observed that seed priming with ascorbic acid increased leaf soluble proteins, while MLE contains ample amount of ascorbic acid (Price, 2000). Thus, increased leaf protein with MLE30 priming was one of the reasons for improved wheat growth.

It is evident that priming with antioxidant compounds such as ascorbic acid and tocopherol can increase free radical scavenging enzymes such as SOD, CAT, and POD in seeds (Chang & Sung, 1998), which also improve the capacity of a plant to maintain its growth behaviour under stress conditions (Sekmen Esen et al., 2012). Moringa leaves are a rich source of antioxidants, calcium, potassium, and iron (Barciszweski, 2000); the major ones are ascorbate, carotenoids, phenols, and flavonoid (Iqbal & Bhanger, 2006). Basra et al. (2011) reported an increase in the emergence potential and phenolic content in maize seedlings when the seeds were primed with MLE30. The increase in phenolic contents due to MLE priming might be attributed to a higher content of vitamin C in MLE as reported by Burguieres et al. (2007). In the present study, seed priming improved seedling vigour and increased the activity of scavenging enzymes in wheat leaves, as evident from increased SOD and POD by MLE30, CAT and TPC by $CaCl_2$, and ascorbic acid by MLE10 seed priming (Figures 6 and 7). Catalase, which is involved in the conversion of H_2O_2 to water and oxygen, is a major H_2O_2 scavenging enzyme in all aerobic organisms. Previously, Basra et al. (2004) reported that priming resulted in a great enhancement in CAT and SOD activities in plants. Hydropriming decreased CAT activity in the present study, which confirmed the findings of Srinivasan and Saxena (2001) who reported that CAT activity was not increased after hydropriming in radish. Therefore, it is

likely that enhanced antioxidant enzyme activity in wheat cultivars due to MLE30 and $CaCl_2$ priming was due to the high content of antioxidants found in MLE (Iqbal & Bhanger, 2006).

5. Conclusion

Although all the priming treatments performed better than the control, MLE as a priming agent effectively enhanced seed emergence, seedling vigour, leaf area, leaf total soluble protein, and yield contributing attributes as well as ascorbic acid contents. Nevertheless, $CaCl_2$ priming had more stimulatory effects on leaf chlorophyll *a* and catalase, and total phenolic contents were more pronounced as compared to MLE priming. The induction of antioxidative system is the main mechanism for better wheat performance in natural conditions.

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References

Afzal I, Basra SMA, Farooq M & Nawaz A (2006). Alleviation of salinity stress in spring wheat by hormonal priming with ABA, salicylic acid and ascorbic acid. *International Journal of Agriculture and Biology* 8: 23–28.

Afzal I, Rauf S, Basra SMA & Murtaza G (2008). Halopriming improves vigor, metabolism of reserves and ionic contents in wheat seedlings under salt stress. *Plant Soil and Environment* 54: 382–388.

Afzal I, Basra SMA, Ahmad N, Cheema MA, Haq MA, Kazmi MA & Irfan S (2011). Enhancement of antioxidant defense system induced by hormonal priming in wheat. *Cereal Research Communication* 39: 334–342.

Ahmed DM & Nimer AM (2002). Effects of *Acacia senegal* (L., Wild) on sandy soils. A case study of El Damokya Forest, Northern Kordofan State. *University of Khartoum Journal Agricultural Sciences* 10: 106–118.

Ainsworth EA & Gillespie KM (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols* 2: 875–877.

Al-Hakimi AMA & Hamada AM (2001). Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamin or sodium salicylate. *Biologia Plantarum* 44: 253–261.

Anjorin TS, Ikokoh P & Okolo S (2010). Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. *International Journal of Agriculture and Biology* 12: 431–434.

Arnon DT (1949). Copper enzyme in isolated chloroplasts polyphenols oxidase in *Beta vulgaris*. *Plant Physiology* 24: 1–15.

Asgedom H & Becker M (2001). Effect of seed priming with nutrient solutions on germination, seedling growth and weed competitiveness of cereals in Eritrea. In: *Proc. Deutscher Tropentag 2001*, p. 282. Univ. Bonn and ATSAF, Margraf Publishers Press, Weickersheim.

Ashraf M, Athar HR, Harris PJC & Kwon TR (2008). Some prospective strategies for improving crop salt tolerance. *Advances in Agronomy* 97: 45–110.

Aslam M, Anwar F, Nadeem R, Rashid U, Kazi TG & Nadeem M (2005). Mineral composition of *Moringa oleifera* leaves and pods from different regions of Punjab, Pakistan. *Asian Journal of Plant Sciences* 4: 417–421.

Athar H, Khan A & Ashraf M (2008). Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environmental and Experimental Botany* 63: 224–231.

Barciszweski J, Siboska G, Rattan SIS & Clark BFC (2000). Occurrence, biosynthesis and properties of kinetin (N6-furfuryladenine). *Plant Growth Regulation* 32: 257–265.

Basra SMA, Zia MN, Mehmood T, Afzal I & Khaliq A (2002). Comparison of different invigoration techniques in wheat (*Triticum aestivum* L.) seeds. *Pakistan Journal of Arid Agriculture* 5: 11–16.

Basra SMA, Zahar M, Rehman H, Yasmin A & Munir H (2009a). Evaluating the response of sorghum and moringa leaf water extracts on seedling growth in hybrid maize. In: *Proceedings of the International Conference on Sustainable Food Grain Production: Challenges and Opportunities,* p. 22. University of Agriculture, Faisalabad, Pakistan.

Basra SMA, Zahoor R, Rehman H, Afzal I & Farooq M (2009b). Response of root applied brassica and moringa leaf water extracts on seedling growth in sunflower. In: *Proceedings of the International Conference on Sustainable Food Grain Production: Challenges and Opportunities,* p. 23. University of Agriculture Faisalabad, Pakistan.

Basra SMA, Iftikhar MN & Afzal I (2011). Potential of moringa (*Moringa oleifera*) leaf extract as priming agent for hybrid maize seeds. *International Journal of Agriculture and Biology* 13: 1006–1010.

Bradford M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Annals of Biochemistry* 72: 248–254.

Çatav ŞS, Bekar İ, Ateş BS, Ergan G, Oymak F, Ülker ED & Tavşanoğlu C (2012). Germination response of five eastern Mediterranean woody species to smoke solutions derived from various plants. *Turkish Journal of Botany* 36: 480–487.

Chance M & Maehly AC (1955). Assay of catalases and peroxidases. *Methods in Enzymology* 2: 764.

Chang SM & Sung JM (1998). Deteriorative changes in primed shrunken-2 sweet corn seeds during storage. *Seed Science and Technology* 26: 613–626.

Chung IM & Miller DA (1995). Effect of alfalfa plant and soil extracts on germination and growth of alfalfa. *Agronomy Journal* 87: 762–767.

Dietrich JT, Kaminek V, Belvins DG, Reinbett TM & Morris RD (1995). Changes in cytokinins and cytokinin oxidase activity in developing maize kernel and the effects of exogenous cytokinin on kernel development. *Plant Physiology and Biochemistry* 33: 327–336.

El-Atta HA & Bashir IA (1999). Adverse effects of *Eucalyptus camaldulensis* (Dehn) leaf extract on germination and seedling growth of wheat. *University Khartoum Journal of Agricultural Sciences* 7: 70–79.

Farooq M, Basra SMA & Wahid A (2006). Priming of field-sown rice seed enhances germination, seedling establishment, allometry and yield. *Plant Growth Regulation* 49: 285–294.

Farooq M, Basra SMA, Hafeez K & Ahmad N (2005). Thermal hardening: a new seed vigor enhancement tool in rice. *Journal of Integrated Plant Biology* 47: 187–193.

Farooq M, Aziz T, Basra SMA, Cheema MA & Rehman H (2008). Chilling tolerance in hybrid maize induced by seed priming with salicylic acid. *Journal of Agronomy and Crop Science* 194: 161–168.

Foidl N, Makkar HPS & Becker K (2001). The potential of *Moringa oleifera* for agricultural and industrial uses. In: *Proceedings of the International Workshop "What development potential for Moringa products?"*, pp. 47–67. Dar-es-Salaam, Tanzania.

Fuglie LJ (1999). *The miracle tree: Moringa oleifera. Natural Nutrition for the Tropics*. pp. 68. Church World Service, Dakar, Senegal.

Fuglie LJ (2000). New Uses of Moringa Studied in Nicaragua. ECHO Development Notes #68, June, 2000. [online] Website: http://www.map-abcdf.com.ph/documents/submitted%20papers/NEW%20USES%20OF%20MORINGA%20STUDIED%20IN%20NICARAGUA.pdf. [accessed on 03 July 2009].

Giannopolitis CN & Ries SK (1977). Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiology* 59: 309–314.

Gupta NK, Gupta S, Shukla DS & Deshmukh PS (2003). Differential responses of BA injection on yield and specific grain growth in contrasting genotypes of wheat (*Triticum aestivum* L.). *Plant Growth Regulation* 40: 201–205.

Harris D, Tripathi RS & Joshi A (2002). On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice. In: *Direct Seeding: Research Strategies and Opportunities.* Pandey S, Mortimer M, Wade L, Tuong TP, Lopes K, Hardy B (eds.). pp. 231–240. International Rice Research Institute, Manila, Philippines.

Iqbal S & Bhanger MI (2006). Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *Journal of Food Composition and Analysis* 19: 551–554.

Khalil SK, Mexal JG, Rehman A, Khan AZ, Wahab S, Zubair M, Khalil IH & Mohammad F (2010). Soybean mother plant exposure to temperature stress and its effect on germination under osmotic stress. *Pakistan Journal of Botany* 42: 213–225.

Khan A, Ahmad MSA, Athar HR & Ashraf M (2006). Interactive effect of foliarly applied ascorbic acid and salt stress on wheat (*Triticum aestivum*) at the seedling stage. *Pakistan Journal of Botany* 38: 1407–1414.

Khan A, Khalil SK, Khan AZ, Marwat KB & Afzal A (2008). The role of seed priming in semi-arid area for mungbean phenology and yield. *Pakistan Journal of Botany* 40: 2471–2480.

Makkar HPS & Becker K (1996). Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. *Animal Feed Science and Technology* 63: 211–228.

Nouman W, Siddiqui MT & Basra SMA (2012a). *Moringa oleifera* leaf extract: An innovative priming tool for rangeland grasses. *Turkish Journal of Agriculture and Forestry* 36: 65–75.

Nouman W, Siddiqui MT, Basra SMA, Afzal I & Rehman H (2012b). Enhancement of emergence potential and stand establishment of *Moringa oleifera* Lam. by seed priming. *Turkish Journal of Agriculture and Forestry* 36: 227–235.

Pill WG & Savage WEF (2008). Effects of combining priming and plant growth regulator treatments on the synchronization of carrot seed germination. *Annals of Applied Biology* 113: 383–389.

Price LM (2000). ECHO's Technical Note. [online] Website: http://www.echonet.org [accessed on 08 June 2012].

Ruan S, Xue Q & Tylkowska K (2002). Effects of seed priming on emergence and health of rice (*Oryza sativa* L.) seeds. *Seed Science and Technology* 30: 451–458.

Sekmen Esen AH, Özgür R, Uzilday B, Tanyolaç ZO, Dinç A (2012). The response of the xerophytic plant *Gypsophila aucheri* to salt and drought stresses: the role of the antioxidant defence system. *Turkish Journal of Botany* 36: 697–706.

Srinivasan K & Saxena S (2001). Priming seeds for improved viability and storability in *Raphanus sativus* cv. Chinese pink. *Indian Journal of Plant Physiology* 6: 271–74.

Taiz L & Zeiger E (2006). *Plant Physiology*. 4th ed. Sunderland, MA, USA: Sinauer Associates.

Yasmeen A, Basra SMA, Ahmad R & Wahid A (2012). Performance of late sown wheat in response to foliar application of *moringa oleifera* lam. leaf extract. *Chilean Journal of Agricultural Research* 72: 92–97.

Yin H, Chen Q & Yi M (2008). Effects of short term heat stress on oxidative damage and responses of antioxidant system in *Lilium longiflorum*. *Plant Growth Regulation* 54: 45–54.