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Full Length Article

Moringa oleifera Leaf Extract Improves Wheat Growth and Productivity by Delaying Senescence and Source-Sink Relationship

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Abstract

The effect of exogenously-applied *Moringa oleifera* leaf extract (MLE) on delaying the leaf senescence and modifying the source-sink relationship in wheat (*Triticum aestivum* L.) was studied. MLE (3.3%) was used as seed osmopriming (SP) and/or foliar spray (F), and water as hydropriming (HP). Among seedling attributes, improved shoot and root lengths, leaf and root scores were found for SP and/or F treatments. Increase or decrease in stem dry weight simultaneously with grain dry weight might contribute for soluble stem reserves towards grain weight as showed by delayed leaf senescence in SP or combined SP+F treatment. The SP and F treatments had also earlier spike emergence and anthesis stage. Plant height, tillers number, biological and grain yields per plant were also recorded highest for SP+F treatment followed by SP. Thus, MLE being rich in zeatin, a cytokinin maintained the green photosynthetic area and enhanced grain filling that might contributed towards improved grain yield when applied as SP or in combination with foliar.

Keywords: Biostimulants; Zeatin; Grain filling; Leaf senescence; Seed priming

Introduction

Among cereals, wheat (*Triticum aestivum* L.) occupies prominent position as source of dietary protein and calories for ever burgeoning population of world (FAO, 2009). Other than abiotic or biotic stresses, several factors, which contribute to the increased production of wheat include development and timely planting of high yield wheat varieties (Sattar *et al.*, 2009), good quality seed (Farooq *et al.*, 2008) and optimal supply of fertilizer and irrigation (Kibe *et al.*, 2006). Therefore, there is always a demand to enhance its production. Nonetheless, wheat crop in rice-wheat or cotton-wheat cropping systems is often exposed to high temperature during grain filling stage and produce seed of poor quality and less vigor (Hasan *et al.*, 2013). Delayed planted crop experience poor crop stand if inadequate soil moisture or adverse soil conditions prevails under fields that usually reduces germination and seedling growth and substantially reduced crop harvest (Farooq *et al.*, 2008; Yasmeen *et al.*, 2013a). *Moringa oleifera* leaf extracts when applied for drought or salt-stressed plants modified plant phenotypic response to positively affect growth and productivity with alteration in metabolic processes (Yasmeen *et al.*, 2013a, b).

Moringa oleifera Lam., is a multipurpose tree with its origin from the sub-Hamaylian tract of India and Pakistan and belongs to Moringaceae family (Verdcourt, 1985; Olson and Carlquist, 2001). Now it is widely distributed among tropics and subtropics including Middle East and Africa, Nicaragua, Philippines, Cambodia, Central and North America and Caribbean Islands (Morton, 1991; Makkar and Becker, 1996; Fahey, 2005; Palada *et al.*, 2007). With fast growing perennial habit, moringa produces a plenty of biomass and its leaves extracts are being used as plant growth stimulant to improve crop productivity. Moringa leaf extract (MLE) when obtained from fresh leaves are rich in antioxidants, some plant secondary metabolites and osmoprotectants making it natural stimulant for growth. These leaf extracts are also source of many plant growth regulators including zeatin, a cytokinin, vitamins and several mineral elements (Yasmeen *et al.*, 2014; Rady and Mohamed, 2015; Rady *et al.*, 2015). Moringa leaf extract can be applied as seed priming or foliar spray and root zone. Several studies have shown MLE potential in improving crop resistance to drought (Yasmeen *et al.*, 2013a), salinity (Yasmeen *et al.*, 2013b; Rady and Mohamed, 2015; Rady *et al.*, 2015), heavy metal stress metal and/or salinity tolerance (Howladar, 2014) including crop productivity (Yasmeen *et*

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al., 2012, Rehman *et al.*, 2014; 2015a, b). For instance, Yasmeen *et al.* (2013a) reported improved 100-grain weight and yield with foliar applied MLE under moderate salinity but did not observe the basis of such improvement except for antioxidants and leaf ionic ratio important under salinity. In another field study, Yasmeen *et al.* (2012) found increase in wheat yield with foliar applied MLE at tillering, booting, jointing and heading stages respectively and reported extended leaf area duration as possible reason for improved yield under late sown conditions.

Nonetheless, most of above studies reported either use of MLE to improve plant performance under abiotic stress or the biochemical basis of such enhancement while response of MLE when employed for seed priming or applied to plant foliage in delaying senescence and modulation of source-sink to affect crop productivity are rarely reported. As cytokinins are considered to be regulators of leaf senescence (Davies, 2007), therefore, we hypothesized that with rich in zeatin type of cytokinin and other regulators (Rady *et al.*, 2013; Rady and Mohamed, 2013), MLE can play a role to maintain photosynthetic area by delaying senescence and affecting source-sink strength to increase yield. Further, previous researches involve MLE use in individual either as seed priming or foliar application and the effects on plant growth with combination of these strategies not studied previously. The present study, therefore, evaluated the influence of MLE applied through seed (SP) and/or plant foliage (F) individually or in combination (SP+F) on seedling growth, phenological development and the yield of wheat by delaying leaf senescence and affecting the source-sink relationship under natural greenhouse conditions.

Materials and Methods

Experimental Design and Methods

The present study was conducted during winter season 2011-2012 under natural greenhouse conditions. The study was comprised of seed hydropriming, seed osmopriming (MLE; 3.3%), seedling foliar application (MLE; 3.3%) and combination of both seed osmopriming and foliar application (MLE; 3.3%). In case of the control and foliar applications, untreated seeds were used.

Primed and control seeds (10 in each pot) of wheat cultivar Sehar-2006 were sown in plastic pots filled with 8 kg mixture of soil, sand and compost with a ratio of 1:1:1 on December 18, 2011. Pots were completely randomized using factorial arrangement and three replications. Soil used in present study was of Lyallpur soil series and according to USDA classification was of aridisolfine- silty, mixed, hyperthermic Ustalfic, Haplargid and according to FAO classification was Haplic Yermosols in nature (FAO, 2006). Crop required fertilizers were added in pots at 120-100-62.5 kg NPK ha^{-1} using urea, single super phosphate and potassium sulphate, respectively. Complete dose of required phosphorous and potash with half nitrogen was added in soil at time of sowing. The remaining dose of nitrogen was applied by splitting in half with first irrigation and tillering. Pots were irrigated to keep soil moisture near field capacity level.

Moringa Leaf Extracts Preparation and Analysis

For extraction, fully matured moringa trees located at Agronomy research area, University of Agriculture, Faisalabad were selected and fresh leaves were harvested. Collected leaves were overnight frozen (-5ºC), pressed in a locally fabricated machine and filtered twice using Whatman No. 1 filter paper. Purified extract was centrifuged at 8,000 × *g* for 15 min to get supernatant and further diluted using leaf extract to distilled water ratio of 1:30 (MLE30) (Yasmeen *et al.*, 2012). The diluted MLE was further used for seed soaking and spray the plant foliage. Using different methodologies, moringa leaf extract (MLE) was analyzed for chemical composition given in Table 1.

Total free amino acids in dried paste of moringa leaf extract were extracted and measured following Dubey and Rani (1989). Free proline content was measured according to the procedure described by Bates *et al.* (1973). Total soluble sugars determined according to Irigoyen *et al.* (1992). Total soluble phenols, total chlorophylland carotenoid contents were determined according to Spanos and Wrolstad (1990) and Arnon (1949). According to Arnon (1949), dried paste of moringa leaf extract (0.5 g) was homogenized in 50 mL of 80% (v/v) acetone and centrifuged at 3,000 × *g* for 20 min. The optical density was measured at 663 nm, 645 nm and 470 nm using UV-Visible Spectrometer (UV-160A, Shimadzu, Kyoto, Japan). For ascorbic acid (AsA) content determination, 2, 6-dichloro-indophenol method was used (Helrich, 1990). Ash content was determined according to standard method (A.S.T.M. E., 2003). Minerals in dried moringa paste such as P, K and Cam Mg, Mn, Fe, Zn and Cu contents were measured using the molybdenum-reducedmolybdophosphoric blue colour method (Jackson, 1967), flame photometer (Perkin-Elmer Model 52-A, Glenbrook, Stamford, CT, USA) (Page *et al.*, 1982) and Atomic Absorption Spectrophotometer (Perkin-Elmer Model 3300) (Chapman and Pratt, 1961). For endogenous levels of gibberellic acid (GA_3), indole-3-acetic acid (IAA) and zeatin, the extraction and purification procedures adapted were as described by Kettner and Doerffling (1995) and Pan *et al.* (2010).

Moringa Leaf Extract Application

For seed priming, wheat seeds were soaked in water (hydropriming) and MLE (3.3%; osmopriming) for 10 h using seed weight to solution volume ratio of 1:5 at room temperature. Soaking of seed was followed by washings with distilled water thrice and re-drying to original weight.

Foliar spray of MLE diluted with 1:30 ratio was done with handheld sprayer twice at two crop stages i.e. the three leaf stage and anthesis. Each experimental unit of respective treatment was sprayed with 25 mL of MLE.

Evaluation of Seedling Growth and Grain Yield and Related Traits

After one week of foliar spray (30 days after sowing), three seedlings per replicate were uprooted for vigor evaluation. For which seedling shoot and root length, leaf and root score was recorded by counting the number of leaves and roots. At maturity, plant height, numbers of tillers per plant and spike length were measured. Number of spikelets per spike, biological and seed yield per plant was determined by manual threshing of harvested wheat crop at maturity.

Crop Phenology, Number of Senescenced Leaves and Changes in Stem and Grain Dry Weight

Days to spike emergence and anthesis were observed when 50% of the plants within a pot had spike and protrusion of anthers respectively. Numbers of senescenced leaves were observed after anthesis with six days intervals until maturity. To study the source-sink relationship, main tiller from each replicate was harvested from base at six days intervals until maturity. The tiller was separated into stem by removing leaves, and grains were separated from spike by threshing and kept in oven at 70°C till the constant weight to determine the dry weight.

Statistical Analysis

Analysis of variance of collected data was carried out using statistical tool *Statix 8.1* ®. Differences among means were compared by using LSD at 5% probability level. Least significant difference test was used at 5% probability level to compare the treatments means. Graphically, data were presented by Microsoft Excel.

Results

Moringa Leaf Extract Composition and Seedling Growth

Analysis of moringa leaf extract (MLE) revealed presence of Ca (28.0 mg g^{-1}), Mg (6.70 mg g^{-1}), K (25.1 mg g^{-1}) , Na (0.75 mg g^{-1}), P (8.10 mg g^{-1}) and micro-minerals Fe (1.60 mg g^{-1}), Mn (0.84 mg g^{-1}), Cu (0.14 mg g^{-1}) and Zn (0.27 mg g^{-1}) on dry weight basis. Antioxidants such as proline (21.00 mg g^{-1} DW), soluble phenolics (6.20 mg g^{-1}), total carotenoids (3.10 mg g^{-1}) and ascorbic acid (242.40 mg 100 g^{-1} FW) along with amino acids (106.20 mg g^{-1}), soluble sugars (248.7 mg g^{-1}) and K (25.10 mg g^{-1}) as osmoprotectants were present. Plant hormones such as indole-3 acetic acid, gibberellins, zeatin a cytokinin and abscisic acid were found in high concentration (Table 1).

Table 1: Some chemical constituents of moringa leaf extract (on a dry weight basis)

Component	Value (mg g^{-1} DW)
Amino acids	106.20
Proline	21.00
Total soluble sugars	248.70
Ash	102.00
Calcium	28.00
Magnesium	6.70
Potassium	25.10
Phosphorus	8.10
Sodium	0.75
Iron	1.60
Manganese	0.84
Zinc	0.27
Copper	0.14
Soluble phenols	6.20
Total carotenoids	3.10
Total chlorophyll	3.96
Ascorbic acid (mg 100g^{-1} FW)	242.40
Phytohormones (µg g^{-1} DW):	
Indole-3-acetic acid	0.83
Gibberellins	0.74
Zeatin	0.96
Abscisic acid	0.29

Table 2: Influence of moringa leaf extracts (MLE) on seedling vigor and phenological development of wheat

Treatments	Shoot length (cm)	Root length (cm)	Leaf score	Root score	Days to spike emergence	Days to anthesis
Control	12.96 c	3.09 c	2.57 b	4.38 b	77.67 a	84.33 a
HP	12.55 c	3.93 a	2.37 b	4.33 b	76.33 bc	82.00 c
SP	15.70 a	3.97 a	2.98 a	4.60 ab	75.33 c	82.67 bc
F	14.40 b	3.40 bc	3.03 a	5.67 a	76.33 c	82.33 bc
SP + F	14.51 b	3.63 ab	2.87 a	5.23 a	76.67 ab	83.67 ab
LSD at 0.05	0.95	0.39	0.29	0.66	1.26	1.56

Means sharing the same letter in a column do not differ significantly at $p \leq 0.05$; Control = neither seed hyropriming, seed osmopriming nor seedling foliar spraying was applied; HP = seed hydropriming; SP = seed osmopriming with MLE (1:30); F = seedling foliar spraying with MLE (1:30); SP + F = seed osmopriming with MLE (1:30) in combination with seedling foliar spraying with MLE (1:30)

Moringa leaf extract (MLE) application as seed priming (SP) and/or foliar spray (F) improved seedling growth of wheat (Table 2). Maximum shoot length was recorded for SP treatment followed by the combined SP+F treatment and then foliar treatment. Maximum root length was statistically similar for seed hydropriming (HP) and the combined SP+F treatments. Highest leaf and root scores were statistically similar for all MLE treatments. However, minimum seedling growth was found for the control or HP treatments (Table 2).

Changes in Stem and Grain Dry Weight

Exogenous applied MLE affected the stem and grain weights at different days after anthesis. Increase or decrease in stem dry weight with concomitant increase in grain weight was more evident at 12 days after anthesis (Fig. 1a, b). At this stage, maximum stem dry weight was recorded for SP and was similar to HP (Fig. 1a). Decrease in stem dry weight at 12 days after anthesis with simultaneous increase in grain dry weight was found for SP or the combined SP+F treatments. However, this increase in grain weight or decrease in stem dry weight was followed by SP or combined SP+F at 18 and 24 days after anthesis (Fig. 1b).

Crop Phenology and Number of Senescence Leaves

The SP or F treatment had significant effect on number of days to spike emergence and anthesis. Earlier spike emergence and anthesis time was found for SP and F treatments, while all other treatments had similar time for spike emergence and anthesis except the control with maximum days for these traits (Table 2). Maximum number of photosynthetically active leaves was found for SP treatment at different days after anthesis. However, these numbers of photosynthetically active leaves for SP treatment were similar to HP treatment at 6 and 18 days after anthesis and combined SP+F treatment (Fig. 2).

Grain Yield and Related Traits

The MLE application, as SP and/or F, significantly affected grain yield and its traits (Table 3). Increased plant height and number of tillers per plant at maturity were recorded for the combined SP+F treatment and were statistically similar to HP treatment. On the other hand, other treatments behaved similar to the control. No significant difference was found among all treatments including the control for spike length. Nonetheless, combined (SP+F) application of MLE also expressed highest biological and grain yield per plant. This increase in biological or grain yield was followed by SP and statistically similar to F treatment (Table 3).

Discussion

Analysis of MLE (Table 1) showed diverse composition indicating the potential of leaf extract as a plant biostimulant and many studies showing their role to improve plant performance also confirmed from results of present study (Table 2, 3). Priming with MLE induced improved seedling growth had been reported in wheat (Yasmeen *et al.*, 2013c) and maize (Rehman *et al.*, 2015b). Improved seedling vigor and plant growth in treatments with seed priming (SP) of MLE, foliar spray (F) and their combination (SP+F) (Table 2) may be owed to the enhanced mobilization of germination related metabolites/inorganic solutes present in MLE (Table 1) to the growing plumule or the increase in amylase activity and reducing sugars of primed seeds (Afzal *et al.*, 2012).

Increase in stem dry weight by MLE applied as SP and/or F at anthesis (Fig. 1a, b) could be attributed to early seedling growth induced accumulation of dry matter in elongating stem and high stem weight at anthesis (Tables 2 and 3). Similarly, increase in grain weight of present study may be attributed to partitioning of assimilates to developing grains for increased grain size due to increased levels of cytokinin (Dietrich *et al.*, 1995). Thus, maintenance of green leaf area during grain filling period increased grain weight due to maximum photosynthesizing leaves or decrease in stem weight with remobilization of stem reserves in plants produced from SP or combined application (SP+F) of MLE (Figs. 1a, b and 2).

Table 3: Influence of moringa leaf extracts (MLE) on agronomic and yield components of wheat

Treatments	Plant height (cm)	No. of tillers per plant	Spike length (cm)	No. of spikelets per spike	Biological yield per plant (g)	Grain yield per plant (g)
Control	68.30 b	3.89 b	9.43 a	41.00 d	4.48 c	0.75 d
HP	67.93 b	4.23 ab	10.03 a	41.33 d	4.27 c	1.27 c
SP	72.67 a	3.78 b	10.45 a	50.83 b	5.32 b	2.05 b
F	66.83 b	3.89 b	9.43 a	45.50 c	5.37 b	1.90 b
SP + F	73.47 a	4.67 a	10.00 a	56.67 a	6.20 a	2.50 a
LSD at 0.05	1.66	0.47	1.05	2.10	0.55	0.33

Means sharing the same letter in a column do not differ significantly at $p \leq 0.05$; Control = neither seed hyropriming, seed osmopriming nor seedling foliar spraying was applied; HP = seed hydropriming; SP = seed osmopriming with MLE (1:30); F = seedling foliar spraying with MLE (1:30); SP + F = seed osmopriming with MLE (1:30) in combination with seedling foliar spraying with MLE (1:30)

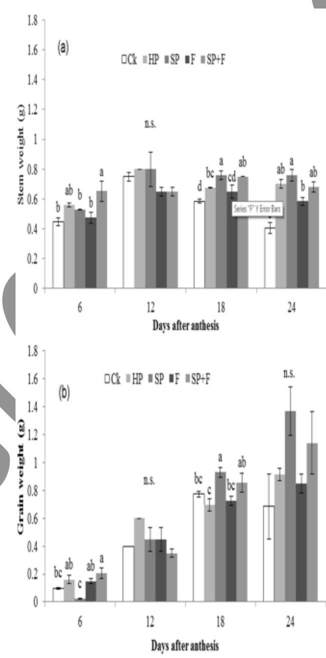

Fig. 1: Influence of moringa leaf extracts (MLE) on (a) stem weight and (b) grain weight of wheat at different periods after anthesis. Ck = control, HP = seed hydropriming, SP = seed osmopriming with MLE (1:30), F = seedling foliar spraying with MLE (1:30), SP +F = seed osmopriming in combination with seedling foliar spraying with MLE (1:30)

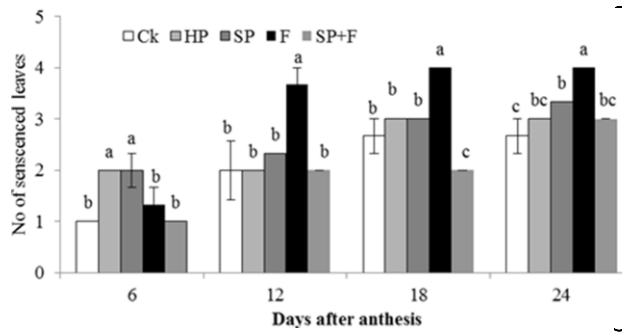

Fig. 2: Influence of moringa leaf extract (MLE) on leaf senescence rate of wheat at different periods after anthesis. Ck = control, HP = seed hydropriming, SP = seed osmopriming with MLE (1:30), F = seedling foliar spraying with MLE (1:30), SP +F = seed osmopriming in combination with seedling foliar spraying with MLE (1:30)

Application of cytokinin rich MLE at anthesis might contribute for enhanced sink capacity to be fulfilled by photo-assimilates supplied from stayed green leaves (Thomas and Howarth, 2000) or re-translocation of stem reserves of present study. High vigor levels of primed seeds or foliar applied plants provide energetic start for earlier emergence and complete other phenological events well in time (Rehman *et al.*, 2014, 2015b). Maximum number of photosynthetic active leaves observed for SP or combined application (SP+F) indicate the delayed senescence and maintaining the chlorophyll contents during anthesis (Fig. 2). Presence of zeatin-like cytokinin in MLE prevents premature leaf senescence and maintains high leaf area for photosynthetic activity during anthesis (Fig. 2; Table 1). During senescence, endogenous levels of cytokinin usually are decreased and exogenous applied cytokinin delay this process (Tetley and Thimann, 1974). This shows the possibility of cytokinin dependent activation of isopentenyl transferase (*ipt*) biosynthesis. Such type of delayed senescence with exogenously applied MLE can be important under high temperature conditions as for late sown wheat, or under drought, when leaf senescence is accelerated and root-shoot signaling is altered, due to cytokinin for carbon supply towards growing sink which need to be determined in future studies.

In addition to effects on seedling growth and phenology, maximum plant height and number of tillers per plant at maturity recorded for combined applied (SP+F) MLE (Table 3) may be due to cytokinin- or abscisic acid altered signaling from root to shoot and encouraged tillering by reducing undesired growth of unproductive tillers and improves canopy light penetration changes root-shoot ratio (Yang and Zhang, 2010). Improved emergence rate and number of tillers per plant by seed osmopriming with MLE have also been found in direct seeded rice (Rehman *et al.*, 2015a), which also contributed towards improved seed yield with combined applied (SP+F) MLE by affecting spikelet numbers per spike (Table 3) and most necessarily seed weight with enhanced sink demand fulfilled by foliar applied MLE at anthesis. This corresponds with (Yasmeen *et al.*, 2012) that foliar applied MLE at tillering, jointing, booting and heading or only heading extended seasonal leaf area duration resulting in improved wheat yield under late sown conditions. Increase in grain weight with foliar applied MLE of present study also corresponds with Warrier *et al.* (1987) that increase in grain weight of wheat was due to more assimilates supply towards developing grain with application of benzyl adenine at anthesis. Cossani and Reynolds (2012) showed a positive relationship of yield with stay green and reported it as a reliable indicator for large scale screening in wheat against high temperature. Nonetheless, reduced seedling growth, decrease in source and sink size, delay in spike and anthesis time, reduced photosynthetic area with early senescence may be reasons for a decrease in yield and attributed traits in control or water sprayed plants (Tables 2 and 3).

Conclusion

Results of this study showed that exogenously applied MLE, as seed priming and/or foliar application, improved seedling growth, had an earlier phenological development and delayed leaf senescence with extended photosynthetic active duration. This was associated with an improved translocation of biomass or stems reserves, modified source-sink contributing towards increased grain weight and ultimately seed yield of wheat. Further studies exploring physiological mechanisms such as gas exchange traits, grain filling rate and exogenously applied MLE increase in hormonal concentration particularly zeatins in different plants are suggested.

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