RESEARCH

PERFORMANCE OF LATE SOWN WHEAT IN RESPONSE TO FOLIAR APPLICATION OF *Moringa oleifera* Lam. LEAF EXTRACT

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A rise in temperature during early spring inducing early maturity is a key yield-reducing factor in late sown wheat (*Triticum aestivum* L.). *Moringa oleifera* Lam. leaves are rich in zeatin, a cytokinin that plays a role in delaying leaf senescence, in addition to other growth-enhancing compounds such as ascorbates, phenolics, and minerals. The objective of this study was to optimize dose and optimum growth stage for foliar-applied moringa leaf extract (MLE) and its role in delaying leaf senescence in late sown wheat. The wheat crop was sown on 16 December 2008; MLE (diluted 30 times) was applied at different growth stages from tillering to heading and heading alone and distilled water was sprayed as a control. All the MLE treatment results were better than the control. However, an increase of 10.73%, 6.00%, 10.70%, and 4.00% was evident in 1000 grain weight, biological yield, grain yield, and harvest index, respectively, with MLE spray at tillering + jointing + booting + heading. The MLE spray used only at heading gave 6.84%, 3.17%, 6.80%, and 3.51% more than the control 1000 grain weight, biological yield, grain yield, and harvest index, respectively. The MLE extended seasonal leaf area duration (Seasonal LAD) by 9.22 and 6.45 d over the control when applied at all growth stages and a single spray at heading, respectively. We conclude that it is possible that the presence of growth-promoting substances in MLE foliar spray can delay crop maturity and extend seasonal LAD and the grain-filling period, thereby leading to greater seed and biological yields in late sown wheat.

Key words: Crop maturity, seasonal leaf area duration, late sowing, *Moringa oleifera*, wheat.

Wheat (*Triticum aestivum* L.) has a prominent position among the cereals that supplement nearly one-third of the world population's diet by providing half of the dietary protein and more than half of the calories (Dhanda *et al*., 2004). As a result, there is always pressure to harvest higher wheat yields to feed the burgeoning population. Many factors contribute in increasing yield, such as early and on-time sowing (Akhtar *et al*., 2006; Sattar *et al*., 2010), seed quality (Farooq *et al*., 2008), availability of high-yielding varieties (Hussain *et al*., 1998), judicious use of inputs such as fertilizers and irrigation (Mullaa *et al*., 1992; Kibe *et al*., 2006), and effective weed management (Abouziena *et al*., 2008).

Late sowing of wheat is a major problem in the rice-wheat (Hobbs and Gupta, 2002) and cotton-wheat (Khan *et al*., 2010) areas of Asia. Hobbs and Morris (1996) observed a 1% decrease for each day that wheat sowing was postponed after the optimum sowing date (15-20 November). Regmi *et al*. (2002) also reported a yield decline in wheat when it was sown after the third week of November. A major reason for late sowing is the late harvest of the preceding crops. The inputs applied to the wheat crop were not efficiently utilized and resulted in reduced yield under late sowing (Hobbs and Gupta, 2002). In late sown wheat, all the growth stages, such as tillering, flowering, and grain filling, are adversely affected by the shortened growing period. The reduction in the optimum growth period caused by a rise in temperature leads to leaf senescence resulting in a photosynthetic rate that is too low to meet plant C economy (Hensel *et al*., 1993; Sharma-Natu *et al*., 2006). As a result, it affects two important yield parameters, i.e., the number of grains per spike and grain weight (Ugarte *et al*., 2007).

The initiation of leaf senescence is subjected to regulation by both internal and environmental factors. This reduction in growth can be compensated by cultivating short-duration varieties (Tahir *et al*., 2009) that are generally low yielding. The other effective approach is the exogenous application of plant growth regulators (PGRs) involved in promoting plant growth and development under normal and stressful conditions (Brathe *et al*., 2002). Although plants are capable of producing PGRs endogenously, they respond well to the exogenous application. Plants can store excessive amounts of exogenously supplied hormones in the form of reversible

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conjugates that are released in active forms when needed in any plant part during growth (Davies, 1987). It is known that the degree of leaf senescence is inversely proportional to cytokinin content (Xu and Huang, 2009).

Among the PGRs, cytokinins are reported to delay plant senescence (Amin, 2003; Shani *et al.*, 2006) and thus extend the stay-green period. In a field trial applying a commercial product containing cytokinin, cytogen, has been reported to increase yield in corn (*Zea mays* L., 26.3%), rice (*Oryza sativa* L., 45.8%), pepper (*Piper nigrum* L., 24.4%), cucumber (*Cucumis sativa* L., 62.9%), and cantaloupe (*Cucumis melo* L., 36.8%) (Mayeux *et al.*, 1983). Gupta *et al.* (2003) also reported that benzyl adenine, a cytokinin, could be used to improve the sink and source capacity of wheat to increase grain yield. The cytokinin level is positively correlated to final grain weight in maize (Dietrich *et al.*, 1995). Cytokinin-induced seed yield enhancement has also been observed in soybean (Nagel *et al.*, 2001).

Since commercial cytokinin sources are cost-intensive, there is a need to explore their natural sources. Various sources, such as seaweed extract, humic acid, and *Moringa oleifera* leaf extract (MLE) have been introduced; they are inexpensive, environmentally friendly, and feasible under natural soil and plant systems (Crouch *et al.*, 1990; Nabati, 1991; Yan and Schmidt, 1993; Foidle *et al.*, 2001). Since *M. oleifera* leaves are rich in zeatin, they can be used as a natural cytokinin source (Price, 2007; Basra *et al.*, 2011). Spraying the leaves of many field crops with MLE diluted with water produced some notable effects such as a longer and more vigorous life span, heavier roots, stems, and leaves, bigger fruits, and higher sugar levels (Foidle *et al.*, 2001); this substantiates its potential to be used as a foliar spray to accelerate young plant growth, especially under sub-optimal conditions. Applying MLE produced a 20% to 35% increase in the yield of peanut (*Arachis hypogaea* L.), onion (*Allium cepa* L.), tomato (*Lycopersicon esculentum* [L.] Mill.), corn (*Zea mays* L.), and sugar cane (*Saccharum officinarum* L.) (Foidle *et al.*, 2001).

In addition to zeatin, MLE is also rich in ascorbates, phenolic compounds, K, and Ca (Makkar *et al.*, 2007), which are being used exogenously as plant growth enhancers. As a natural source with a balanced mix of various growth-promoting substances, MLE may prove to be a potential source to promote plant growth. Many studies have explored its nutritional, cosmetic, and medicinal aspects (Tsaknis *et al.*, 1999; Fahey, 2005), but a few have reported it as a crop growth enhancer, although, to our knowledge, there is no published study available about normal or late sown wheat crops. Wheat is the major staple food for millions of people; however, a heat-induced yield decrease associated with leaf senescence is a major problem in late sown wheat. The objective of this study was to evaluate the potential of MLE as a foliar application on different growth stages of a late sown wheat crop to reduce high temperature stress on crop growth and yield attributes.

MATERIALS AND METHODS

Field experiment details

To apply MLE (diluted 30 times) in the field, wheat cv. Sehar was sown in the Agronomic Research Area, University of Agriculture Faisalabad (24° to 37° N, 61° to 76° E) during 2008-2009. The experiment was laid out in a net plot size of 5.0 × 2.0 m in a completely randomized block design (CRBD) with three replicates.

Soil texture was clay loam. The crop was sown in 25 cm spaced rows with a single row hand drill at a seed rate of 110 kg ha^{-1} on 16 December 2008. Nitrogen and P were applied at 120 and 100 kg ha^{-1}, respectively, with urea and single super phosphate (SSP) as a fertilizer source. Whole P and one third of the N were applied as a basal dose. The rest of the N was applied in two equal splits in the first and second irrigations. The crop was harvested on 14 April 2009.

MLE foliar application

The MLE (diluted 30 times: MLE30) previously optimized in a laboratory study (data not shown) was applied to a field-grown wheat crop with a hand sprayer. Treatment details were: i) foliar spray with water (control), ii) MLE foliar spray at tillering, iii) MLE foliar spray at tillering + jointing, iv) MLE foliar spray at tillering + jointing + booting, v) MLE foliar spray at tillering + jointing + booting + heading, and vi) MLE foliar spray only at heading.

Growth and yield traits

At physiological maturity, growth traits were examined five times during the growing season at 5-d intervals after each foliar spray; leaf area was calculated with plant samples taken from a randomly selected unit area of each plot. Seasonal leaf area duration (Seasonal LAD) = $(LAI_1 + LAI_2) \times (T_2 - T_1)/2$, net assimilation rate (NAR) = TDM/LAD g m^{-2} d^{-1}, and crop growth rate (CGR) = $(W_2 - W_1)/(T_2 - T_1)$ g m^{-2} d^{-1} were determined (Hunt, 1978) where Seasonal LAD = leaf area duration of the whole crop growing season, LAD = leaf area duration between two harvests, TDM = total DM accumulated between two harvests, W_1 = oven-dry weight of first harvest, W_2 = oven-dry weight of second harvest, and $T_2 - T_1$ = time interval between two harvests.

The crop was harvested when it fully ripened and data regarding agronomic and yield-related traits were recorded by following standard procedures. The harvest index (HI) was calculated as a ratio between grain yield and biological yield.

Statistical analysis

Data were statistically analyzed by Fisher's ANOVA and least significant difference (LSD) test at P = 0.05 and applied to compare the treatment means (Steel and Torrie, 1997).

RESULTS

Crop growth analysis

Foliar application of MLE at different growth stages revealed a significant improvement in growth and development compared with the control (water spray). The crop attained the maximum leaf area index (LAI) 75 d after sowing (DAS) and with the highest LAI value was under foliar application of MLE at tillering + jointing + booting + heading (T + J + B + H). However, MLE at other growth stages produced less LAI than T + J + B + H, but was higher than foliar application of water (Figure 1a). Nonetheless, a decreasing trend was observed in LAI after 75 DAS, but this reduction was minimal in plants with MLE applied at the T + J + B + H stages followed by foliar application at heading, while the maximum reduction was observed in unsprayed plants (Figure 1a). The effect of applying MLE on seasonal leaf area duration (Seasonal LAD) was also significant ($P < 0.05$) and plants with foliar application of MLE stayed green more than the control. However, higher Seasonal LADs were recorded in plants sprayed at the T + J + B + H stages; this was followed closely by spraying MLE at heading alone (Figure 1b). Foliar spray of MLE caused a gradual rise in crop growth rate of late sown wheat crop and showed a maximum growth rate in foliar spray at the T + J + B + H stages (Figure 1b). Afterwards, the crop growth rate decreased, but the lowest reduction was observed in the case of MLE foliar spray at the four growth stages T + J + B + H and followed by CGR produced by applying MLE at heading, while the highest reduction was in plants sprayed with water (Figure 2a). The maximum gain in the net assimilation rate was observed up to 75 DAS compared with the control under MLE foliar spray at any growth stage with a subsequent reduction; the foliar spray at the T + J + B + H stages showed the least reduction in the net assimilation rate followed by NAR produced in MLE at heading, whereas the highest reduction was exhibited by foliar spraying with water (Figure 2b).

Yield attributes

The response of MLE on yield and its related traits was substantiated. The maximum number of fertile tillers and grains per spike, 1000 grain weight, biological and economic yield, and harvest index were recorded when MLE was sprayed at the T + J + B + H growth stages compared with the control (Table 1). Nonetheless, similar numbers of fertile tillers and grains per spike were recorded when MLE was applied at the T + J and T + J + B stages with the control having the lowest values for these traits (Table 1). However, the response of 1000 grain weight, biological and economic yield, as well as harvest index varied among foliar applications at different stages and were the highest in T + J + B + H spray followed by

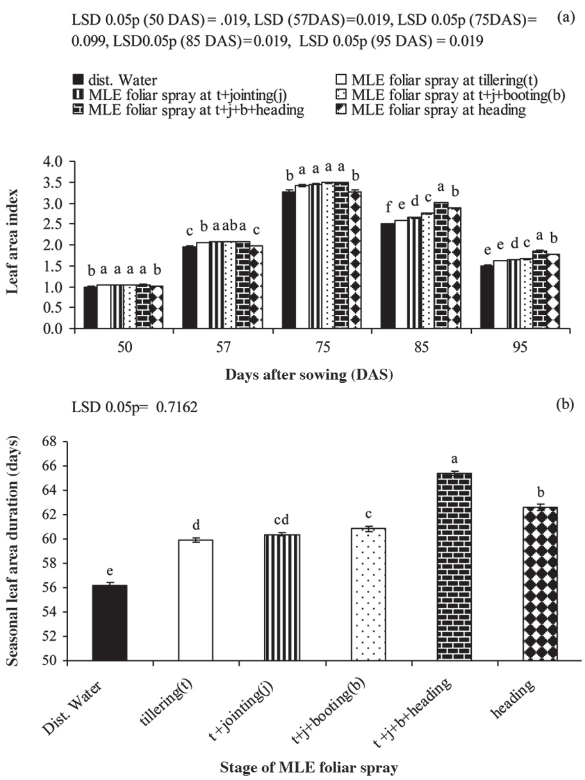

Figure 1. Effect of exogenous application of moringa leaf extract (MLE) on leaf area index (a) and seasonal leaf area duration (b) of wheat cv. Sehar-2006 under late sown conditions.

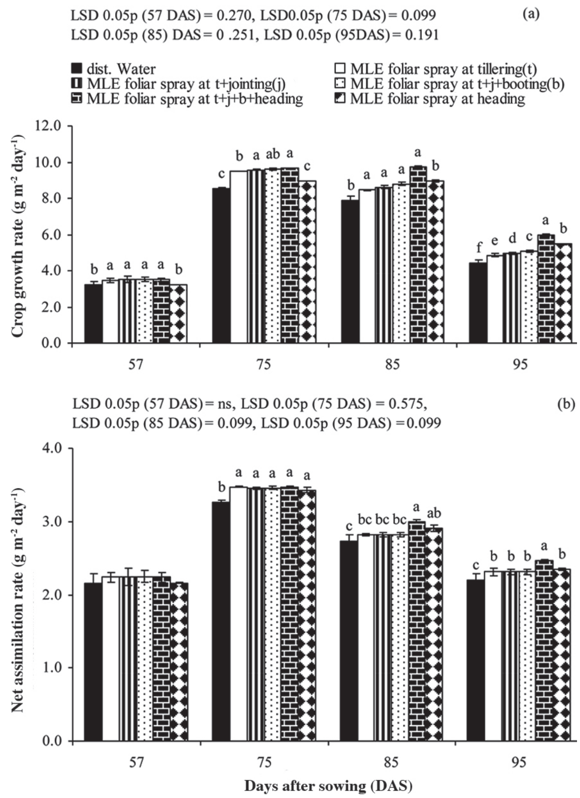

Figure 2. Effect of exogenous application of moringa leaf extract (MLE) on crop growth rate (a) and net assimilation rate (b) of wheat cv. Sehar-2006 under late sown conditions.

the heading treatment. Spraying MLE at the four critical growth stages caused the highest increase (%) in 1000 grain weight compared with the control for other yield-related traits, i.e., number of fertile tillers, grains per spike, and biological and economic yield. However, there was no significant ($P > 0.05$) difference for these traits when MLE was applied at tillering, tillering + jointing, and tillering + jointing + booting crop stages (Table 1), while minimum values for these traits were observed in plants when only water was applied. Nevertheless, the performance of MLE at any growth stage was better than the control and the pronounced effects of MLE were observed when it was sprayed at the four crop stages (T + J + B + H) and the maximum was reached when MLE was applied at heading.

DISCUSSION

Late sowing of wheat shortens the growth period, which is a prerequisite for harvesting higher yields (Farooq *et al*., 2008). Postponing wheat sowing after mid-November produces a yield reduction of 50 kg ha^{-1} d^{-1} (Khan *et al*., 2010). The rise in temperature during early spring further complicates the problem (Wardlaw and Wrigley, 1994; Mahmood *et al*., 2010). In cool-season cereal species, heat stress decreased chlorophyll contents leading to a lot of physiological damage and leaf senescence suffered the most (Xu and Huang, 2009). Spano *et al*. (2003) emphasized that when the assimilates that were supplied to the grain decreased due to accelerated senescence, as in late sown wheat, delaying leaf senescence may be an advantageous attribute. Foliar spray with MLE at T + J + B + H showed the highest value of seasonal leaf area duration (Figure 2) because of delayed leaf senescence, which resulted in a 10.70% increment in grain yield compared with the control. It is reported that foliar application of BAP soon after anthesis results in increased sink size (Hosseini *et al*., 2008). According to Gupta *et al*. (2003), exogenously applied BA enhanced wheat grain yield (cvs. Kalyansona and HD 2285) by 8.8% and 13.70%, and 5.66% and 13.33%, under normal and late sown conditions, respectively. Likewise, in the present study, MLE is rich in zeatin (Barciszweski *et al*., 2000) and other growth-enhancing substances (Nambiar *et al*., 2005), and foliar spray applied during the four critical growth stages, especially heading, appeared to enhance the plant's sink capacity during the grain filling period. However, we are not certain whether the sink demand has been met via the supply of photoassimilates from subtending leaves (Thomas and Howarth 2000) or retranslocation of reserves from stems (Watanabe *et al*., 1997) since results reported here were obtained from a single study conducted in a single location; however, these preliminary results are interesting and provide important evidence for future findings. In our study, plants not sprayed with MLE were both limited in source and sink mainly because of the reduced leaf area index (LAI), seasonal leaf area duration (Seasonal LAD), and CGR, while MLE foliar spray enhanced these attributes (Figures 1a, 1b, and 2a). The correlation of LAI, SLAD, and CGR with 1000 grain weight (0.981, 0.995, and 0.980, respectively, $n = 6$, $P < 0.01$) and number of grains per spike (0.783, 0.843, and 0.806, respectively, $n = 6$, $P < 0.01$) showed that changes in 1000 grain weight were more critical in enhancing sink size than the number of grains per spike; MLE spray at T + J + B + H growth stage, followed by MLE foliar spray at heading alone, effectively fulfilled sink demand.

Applying MLE containing cytokinins exhibits longer seasonal leaf area duration (SLAD) compared with the control. In addition, moringa leaf is also rich in ascorbates, carotenoids, phenols, K, and Ca which are similar to other plant growth enhancers (Foidle *et al*., 2001). Under combined heat and drought stress, a rise in wheat grain yield, more stable cell membranes, and chlorophyll were observed by exogenously applying cytokinin (Gupta *et al*., 2000). Thermotolerance and yield stability in maize were reported by Cheikh and Jones (1994) as a result of high cytokinin content in the maize kernel during the heat stress period. Under late planting of wheat, maintenance of photosynthetic activity due to increased temperatures during maturation (Paulsen, 1994) and efficient utilization of these photosynthates linked with a high harvest index (Gifford and Thorne, 1984; Blum *et al*., 1994) are two important determinants of grain yield. The highest recorded harvest index (Table 1) for MLE sprayed from the start to the end of wheat growth (T + T + B + H) showed that photosynthetic activity was maintained until maturity, which resulted in both longer seasonal leaf area duration and stay green period. This delayed onset of leaf senescence is reported as causing about 11% more C fixation in *Lolium temulentum* (Thomas and Howarth, 2000). An extension in the active photosynthetic period may enhance total photosynthate availability in the annual crop life cycle and higher mass per grain can be achieved if the assimilated C supply can be maintained to grain during the grain filling period (Spano *et al*., 2003).

Applying MLE at the heading stage, although statistically different, closely followed MLE at the four growth stages for values of 1000 grain weight (40.85 g), biological yield (13.65 t ha^{-1}), grain yield (3.19 t ha^{-1}), and harvest index (23.39); this can be due to the ability of maintaining green leaf area duration, "stay green", throughout the grain filling period or remobilizing soluble carbohydrates (stem reserves) during grain filling (Stoy, 1965), and significantly increasing grain weight of wheat by attracting more assimilates towards the developing grain by applying benzyl adenine at anthesis (Warrier *et al*., 1987). In later grain filling phases, leaf senescence caused a shortage of assimilates, then extended photosynthesis duration by providing more photoassimilates translocated to the grain and improving grain weight as an outcome of amplified carbohydrate content.

Table 1. Effect of foliar application of moringa leaf extracts (MLE) on yield parameters of late sown wheat.

Treatments	Number of fertile tillers	Increase or decrease over control	Number of grains spike^{-1}	Increase or decrease over control	1000 grain weight	Increase or decrease over control	Grain yield	Increase or decrease over control	Biological yield	Increase or decrease over control	Harvest index	Increase or decrease over control
	m^{-2}	%		%	g	%	m^{-2}	%	m^{-2}	%	%	%
Control	341.90d		39.67c		38.23d		13.23e		2.99d		22.60d	
MLE30 spray at T	356.70b	4.31	41.33b	4.20	39.99c	4.59	13.40d	4.46	3.12c	1.28	23.31bc	3.13
MLE30 spray at T+J	357.30b	4.51	41.67b	5.04	40.06c	4.78	13.42c	4.35	3.12c	1.46	23.24c	2.84
MLE30 spray at T+J+B	358.30b	4.80	41.33b	4.20	40.09c	4.85	13.43c	4.35	3.12c	1.49	23.24c	2.82
MLE30 spray at T+J+B+H	378.00a	10.55	43.67a	10.08	42.34a	10.73	14.02a	10.70	3.31a	6.00	23.61a	4.44
MLE30 spray at heading	348.30c	1.87	40.67bc	2.52	40.85b	6.84	13.65b	6.80	3.19b	3.17	23.39b	3.51
LSD at P = 0.05	3.03		1.08				0.02		0.02		0.13	

T:tillering growth stage; J: jointing growth stage; B: booting growth stage; H: heading growth stage; LSD: least significant difference.

In conclusion, the highest harvest index in late sown wheat occurred at heading, the most appropriate stage for foliar spray of MLE30,and any other single stage such as tillering, jointing, and booting produced statistically lower results than the heading stage but significantly better than the control. Thus, in order to improve performance of late sown wheat, MLE30 was an economical growth enhancer when foliar sprayed at four growth stages (T + J + B + H).

CONCLUSIONS

It can be concluded that the foliar spray of MLE in late sown wheat at tillering + jointing + booting + heading increased seasonal leaf area duration (Seasonal LAD) along with the grain filling period and delayed maturity resulting in 10% more yield than in the control. A 6.8% yield increase was also observed by a single foliar spray at heading under field conditions.

Rendimiento de trigo sembrado tarde en respuesta a la aplicación foliar de extracto de hojas de *Moringa oleifera* Lam. Aumento en temperatura durante inicios de primavera induciendo madurez temprana es un factor clave en la reducción de rendimiento en siembra tardía de trigo (*Triticum aestivum* L.). Las hojas de *Moringa oleifera* Lam. son ricas en zeatina, una citoquinina que tiene rol en retraso de senescencia foliar, además de otros compuestos que mejoran crecimiento como ascorbatos, fenoles, y minerales. Este estudio se planeó para optimizar dosis y estado de crecimiento óptimo para extracto foliar de moringa (MLE) aplicado foliarmente y su rol en retraso de senescencia foliar en siembra tardía de trigo. El cultivo de trigo se sembró el 16 de septiembre y MLE (diluido 30 veces) se aplicó en diferentes estados de crecimiento desde macollamiento hasta espigadura y en espigadura, mientras se asperjó agua destilada como control. En resultados todos los tratamientos MLE fueron mejores que el control. Sin embargo, un aumento de 10,73; 6,00; 10,70 y 4,00% fue evidente en peso de 1000 granos, rendimiento biológico, producción de grano e índice de cosecha, respectivamente, con aspersión de MLE en macollamiento + encañado + estado de bota + espigadura. Aspersión de MLE sólo en espigadura dio 6,84; 3,17 y 3,51% más de peso 1000 granos, rendimiento biológico, producción de grano, e índice de cosecha respectivamente, comparado con control. MLE extendió la duración del área foliar estacional (LAD estacional) en 9,22 y 6,45 d sobre el control cuando se aplicó a todos los estados de crecimiento y aspersión a espigadura, respectivamente. Concluimos que posiblemente debido a la presencia de sustancias, la aspersión foliar de MLE puede retrasar la madurez de cultivo, extender LAD estacional y período de llenado de grano conduciendo, por lo tanto, a mayores producciones de semilla y biológica en trigo sembrado tardíamente.

Palabras clave: madurez del cultivo, duración de área foliar estacional, siembra tardía, *Moringa oleifera*, trigo.

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