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Full Length Article

Screening of Moringa Landraces for Leaf Extract as Biostimulant in Wheat

Shahbaz Khan[1,*], Shahzad Maqsood Ahmed Basra[1], Irfan Afzal[1] and Abdul Wahid[2]
[1]*Department of Agronomy, University of Agriculture, Faisalabad (38040), Pakistan*
[2]*Department of Botany, University of Agriculture, Faisalabad (38040), Pakistan*
[*]For correspondence: shahbaz2255@gmail.com

Abstract

Moringa leaf aqueous extract recently has got attraction as biostimulant. However, the comparative effectiveness of different landraces has not been yet explored. A pot study was conducted to compare biostimulant potential of two prominent local moringa landraces [White seeded moringa from Faisalabad origin (M_{FSD}) and black seeded moringa from Multan origin (M_{MUL})] and moringa PKM1 (Indian cultivated variety) alone and in combination with hydrogen peroxide (2 µM), ascorbic acid (ASA; 50 mg L^{-1}) and salicylic acid (SA; 50 mg L^{-1}) on wheat cultivar i.e. Galaxy during 2014. The integrated application of alone and blended moringa leaf extract (MLE), used as priming agent or foliar spray at seedling age of 10 and 20 days after emergence, improved significantly speed and spread of emergence (time to start emergence, time to 50% emergence, mean emergence time and emergence index) and seedling vigor (shoot and root length and fresh and dry weight), and final emergence count as compared to control, hydropriming and water spray. Exogenously application of leaf extract from M_{FSD} as priming agent reduced time to 50% emergence (14.7% alone and 20% blended MLE), mean emergence time (5%) and hence improved emergence index (25.6% alone and 29.8% blended) as compared to control. Furthermore higher root length (40% priming application) and dry weight (47% foliar application) were obtained as compared to control. Maximum biostimulant potential of leaf extract of Faisalabad origin moringa was observed regarding seedling vigor alone and in combination with growth promoting substances. © 2017 Friends Science Publishers

Keywords: Moringa landraces; MLE biostimulant; Priming; Foliar; Wheat; Vigor

Introduction

Biostimulants are natural crop growth enhancer. They not only enhance the yield and crop quality but also induce tolerance to abiotic stresses (European Biostimulant Industry Council (EBIC), 2012). Natural sources like seaweed extracts, protein hydrolysates and amino acids, humic acid, fulvic acid, complex organic materials, chitin and chitosan derivatives, microbial inoculants and plant extracts are most commonly used biostimulants in agriculture (Fuglie, 2001; EBIC, 2012; Jardin, 2012; Calvo *et al*., 2014). *Moringa oleifera* leaf extract, sorghum water extract and mulberry water extract are commonly used as crop growth enhancer when applied as seed priming agent and/or foliar spray because they positively modify plant growth and production with alterations in metabolic processes (EBIC, 2012; Rady *et al*., 2013; Yasmeen *et al*., 2013b; Semida and Rady, 2014) under normal or stress conditions. Moringa, among all the naturally occurring plant growth stimulants, has got attraction of scientific community because of a rich source of growth hormones, antioxidants, vitamins and mineral nutrients in its leaves (Shinano and Kasase, 2009; Yasmeen *et al*., 2013a; Bakhtavar *et al*., 2015). Seed priming, with MLE blend with salicylic acid, hydrogen peroxide, ascorbic acid and sorghum water extract, enhances germination parameters and seedling performance in maize (Imran *et al*., 2013). Foliar application of MLE along with benzyl amino purine and hydrogen peroxide at critical stages of wheat enhances its growth and development ultimately yield (Yasmeen *et al*., 2013b). Effectiveness of MLE as biostimulant is equal or more than synthetic crop growth enhancers. On the other hand, MLE is cost effective and environment friendly.

Moringa oleifera is native to sub Hamaylian ecological zone of Indo-Pak (Shahzad *et al*., 2013) with different landraces. There are at least three different types of *M. oleifera* in Pakistan i.e. moringa having different seed cover (e.g., white seeded moringa from Faisalabad origin (M_{FSD}) and black seeded moringa from Multan origin (M_{MUL}) and PKM1; cultivated moringa variety in India (Coppin *et al*., 2013). This diversity opened a new avenue to identify most effective biostimulant source of moringa leaf extract.

Seed priming and foliar application of MLE alone and blend with kinetin solution significantly improved the stand establishment parameters, increased chlorophyll contents,

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crop growth rate, photosynthetic rate, leaf area index and yield of normal and late sown maize crop (Bakhtavar *et al*., 2015). Seed priming with synthetic substances not only promotes germination and emergence but may also enhance subsequence growth and metabolic processes and boosts final crop yield (Sallam, 1999). Moringa leaf extract, exogenously applied either through seed or plant foliage, is considered to boost emergence, seedling growth and growth in various field crops under normal as well as unfavorable conditions (Basra *et al*., 2011). Its application as seed priming reduced emergence time, enhanced germination rate, synchronize germination and crop establishment in wheat and rice (Afzal *et al*., 2002; Farooq *et al*., 2008). Such enhancements in crop growth and development are associated with different physiological and biochemical and agronomics attributes like greater leaf area, alteration in source sink relationship, delayed senescence, increased antioxidant activities, chlorophyll, ascorbic acid and total phenolics contents which play their roles in improving crop resistant against unfavorable conditions and ultimately increase the economic outcome (Yasmeen *et al*., 2013a, b).

Furthermore, blended moringa leaf extract with other plant growth promoters (i.e., salicylic acid, hydrogen peroxide and ascorbic acid) has also enhanced growth and showed synergetic effects on germination and seedling vigor (Imran *et al*., 2013). Salicylic acid (SA), is one out of a variety of PGRs, has been publicized to effect various plant developmental processes like photosynthesis, stomatal regulation, growth and a diversity of metabolic processes (Khan *et al*., 2003; Arfan *et al*., 2007; Arena and Radice, 2016; Batool *et al.*, 2016). Foliar application of SA also increases the enzymatic activities of nitrate reductase and carbonic anhydrase which are very helpful in nutrient uptake (Aftab *et al*., 2010). Ascorbic acid (ASA), plant growth enhancer, is an organic compound needed in very low quantity to retain optimum growth and development in the higher plants (Podh, 1990). ASA is a key antioxidant molecule and acts like a primary scavenger singlet oxygen and superoxide radicals in the Halliwel-Asada pathway. It is involved in phytohormone-mediated signaling mechanisms in alteration from vegetative to reproductive growth phase in addition to the vital stage of senescence and development (Barth *et al*., 2006). It is widely accepted that hydrogen peroxide (H_2O_2) has numerous roles as a plant signaling molecule, secondary messenger, mediating the acquisition of tolerance to abiotic stress, acquired resistance, stomatal responses, and programmed cell death (Chen and Gallie, 2004; Bhattachargee, 2005; Slesak *et al*., 2007). Wahid *et al*. (2007) reported that pre-treatment of seeds with H_2O_2 signaled antioxidants induction in the seeds, which enable young seedlings to compensate ion-induced oxidative damage.

Therefore, a wire house experiment was designed to investigate efficacy of leaf extract from different moringa sources as biostimulant in combination with growth promoting substances in wheat. It is also important to highlight that previously no study conducted so far have explored the beneficial effects of hydrogen peroxide, salicylic acid and ascorbic acid in combination with MLE on wheat plants.

Materials and Methods

Experimental Particulars

The present study was designed to assess the comparative efficacy of different moringa landraces on germination and seedling vigor of wheat (*Triticum aestivum* L. cv. Galaxy) in wire house of Department of Crop Physiology, University of Agriculture, Faisalabad during the fall 2014. This experiment was laid down in completely randomized design with three replications. In the priming treatments seeds were soaked in aerated solution (8 h) of 3% moringa leaf extract of three landraces (PKM1, Indian variety), Faisalabad origin moringa (M_{FSD}) and Multan origin moringa (M_{MUL}) alone and in combination with hydrogen peroxide (H_2O_2) (2 μM), salicylic acid (SA) (50 mg L^{-1}) and ascorbic acid (ASA) (50 mg L^{-1}) (Imran *et al*., 2013). For seed priming, the ratio of seed weight to soaking solution was 1 to 5 (w/v) (Farooq *et al*., 2006; Yasmeen *et al*., 2013b). All the treatment combinations are presented in Table 1. Foliar treatment combinations were applied at the age of 10 and 20 days of seedling emergence.

Experimental Material

Plastic pots (30 cm height and 22 cm diameter) were filled with loamy soil taken from the research area of department of Crop Physiology, University of Agriculture Faisalabad. Wheat seed (Galaxy) was purchased from the Punjab seed corporation, Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. Ten seeds were sown in each pot. During the course of experiment seedlings were irrigated regularly.

Extracts Preparation

Fresh moringa leaves were collected from already established moringa trees of three landraces at research farm area of Department of Crop Physiology, University of Agriculture, Faisalabad. Before extraction process, mature, disease free and healthy leaves were rinsed with water and kept in freezer overnight. Extraction was done with a locally assembled machine. The extracts were sieved and diluted 30 times with distilled water to prepare a 3% solution (Afzal *et al*., 2012).

Seedling Emergence and Vigor Evaluation

Emergence was counted on daily basis until a constant count achieved. Data regarding emergence and seedling vigor parameters were collected by following the ISTA protocols (ISTA, 2010). Mean emergence time (MET) was calculated according to Ellis and Roberts (1981) (MET = Σ Dn/Σn). Emergence index (EI) was calculated by the following formula [EI = (number of emerged seedling(s)/days of first count) +…..+ (number of emerged seedlings/days of final count)] (AOSA, 1990). Time to 50% emergence (E50) was calculated according to Farooq *et al*. (2005) [E50 = ti + {(N/2 – ni)/(nj – ni)} × (tj - ti)]

After 30 days of emergence, five uniform seedlings were harvested from each pot and were analyzed for seedling vigor. Shoot and root length of selected seedlings were measured and averaged. Fresh and dry weight of shoots and roots of 5 plants was recorded and averaged. The seedling samples were oven dried up to constant weight to record dry weight of seedling.

Statistical Analysis

Data collected regarding emergence and seedling vigor were analyzed statistically using Statistix 8.1 version, a computer package for statistical analysis and difference among treatments' means were compared by employing least significant difference (LSD) test at 5% probability level (Steel *et al*., 1997). Microsoft Excel was used for the graphical presentation and calculations.

Results

Emergence Parameters

In the present study, efficacy of leaf extracts from different landraces of moringa was analyzed as biostimulant on wheat emergence and seedling vigor. Seed priming with MLE alone and in combination with different plant growth promoting substances significantly ($P < 0.05$) affected the emergence parameters. All the priming treatment induced early emergence. Less time was observed to start emergence in all priming treatments except hydro priming and control (no priming). All priming treatment combinations (except hydropriming) of MLE from different moringa landraces alone and in combination with different plant growth promoting substances were statistically at par (Table 2) regarding time to start emergence. Time to 50% emergence (E_{50}) was also improved in M_{FSD}+H_2O_2+SA+ASA treatment as depicted by earliness in emergence as compared to no priming and hydropriming (Fig. 1a). Mean emergence time (MET) was improved in seeds treated with leaf extract of M_{FSD} and M_{FSD}+H_2O_2+SA+ASA as depicted by early emergence (Fig. 1b), and were statistically at par with all other priming treatment combinations except control. Higher EI was recorded in seeds primed with M_{FSD}+H_2O_2+SA+ASA (Fig. 1c), which was statistically at par with alone M_{MUL}, PKM1+H_2O_2+SA+ASA and alone M_{FSD} respectively. While minimum value was recorded in control which was statistically at par with hydropriming. All priming treatment combinations improved the final emergence percentage (FEP) except control (Table 2), which was also statistically at par with hydropriming.

Table 1: Treatments combinations

Application mode	Treatments	Combinations
Priming		
1	T_1	Control
2	T_2	Hydropriming
3	T_3	PKM1 (Indian variety)
4	T_4	M_{FSD} (Faisalabad origin moringa)
5	T_5	M_{MUL} (Multan origin moringa)
6	T_6	PKM1 + H_2O_2
7	T_7	M_{FSD} + H_2O_2
8	T_8	M_{MUL} + H_2O_2
9	T_9	PKM1 + H_2O_2 + SA + ASA
10	T_{10}	M_{FSD} + H_2O_2 + SA + ASA
11	T_{11}	M_{MUL} + H_2O_2 + SA + ASA
Foliar Application		
12	T_{12}	Water Spray
13	T_{13}	PKM1
14	T_{14}	M_{FSD}
15	T_{15}	M_{MUL}
16	T_{16}	PKM1 + H_2O_2
17	T_{17}	M_{FSD} + H_2O_2
18	T_{18}	M_{MUL} + H_2O_2
19	T_{19}	PKM1 + H_2O_2 + SA + ASA
20	T_{20}	M_{FSD} + H_2O_2 + SA + ASA
21	T_{21}	M_{MUL} + H_2O_2 + SA + ASA

MLE = Moringa leaf extract; PKM1 = Indian variety; M_{FSD} = Faisalabad origin moringa; M_{MUL} = Multan origin moringa; H_2O_2 = Hydrogen peroxide; SA = Salicylic acid; ASA = Ascorbic acid

Table 2: Effect of moringa leaf extract of different landraces alone and in combinations with plant growth promoting substances on emergence parameters of wheat under wire house condition

Treatments	Combinations	Time to Start Emergence (Days)	Final Emergence %age
T_1	Control	6.00 a	90.00 b
T_2	Hydro priming	5.67 a	96.67 ab
T_3	PKM1	5.00 b	96.67 ab
T_4	M_{FSD}	5.00 b	96.67 ab
T_5	M_{MUL}	5.00 b	100.00 a
T_6	PKM1 + H_2O_2	5.00 b	100.00 a
T_7	M_{FSD} + H_2O_2	5.00 b	100.00 a
T_8	M_{MUL} + H_2O_2	5.00 b	93.33 ab
T_9	PKM1 + H_2O_2 + SA + ASA	5.00 b	100.00 a
T_{10}	M_{FSD} + H_2O_2 + SA + ASA	5.00 b	100.00 a
T_{11}	M_{MUL} + H_2O_2 + SA + ASA	5.00 b	100.00 a
	LSD	0.5080	9.3214

Different lettering shows the statistically significant difference among the performance of treatments ($P < 0.05$)
MLE = Moringa leaf extract; PKM1 = Indian variety; M_{FSD} = Faisalabad origin moringa; M_{MUL} = Multan origin moringa; H_2O_2 = Hydrogen peroxide; SA = Salicylic acid; ASA = Ascorbic acid

Vigor Parameters

Seedling vigor was also significantly ($P < 0.05$) affected with foliar and priming treatment combinations of MLE in different landraces and plant growth promoting substances. Maximum shoot length was recorded in seedlings from seeds primed with M_{MUL}+H_2O_2+SA+ASA (Fig. 2a), which was statistically at par with M_{FSD}+H_2O_2 priming and M_{MUL}+H_2O_2 foliar respectively while minimum shoot length was

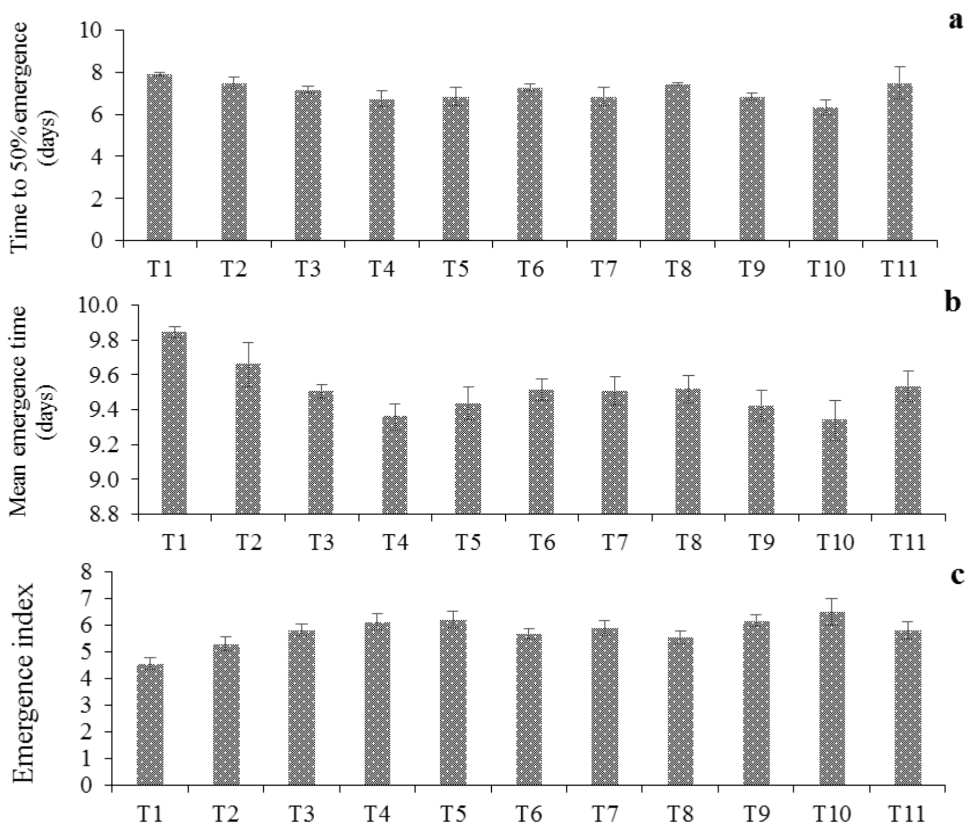

Fig. 1: Priming effect of moringa leaf extract of different landraces alone and in combinations with plant growth promoting substances on time to 50% emergence (a) mean emergence time (b) and emergence index (c) wheat under wire house condition

T_1 = Control, T_2 = Hydropriming, T_3 = PKM1 priming, T_4 = M_{FSD} priming, T_5 = M_{MUL} priming, T_6 = PKM1+H_2O_2 priming, T_7 = M_{FSD}+H_2O_2 priming, T_8 = M_{MUL}+H_2O_2 priming, T_9 = PKM1+H_2O_2 + SA+ASA priming, T_{10} = M_{FSD}+H_2O_2 + SA+ASA priming, T_{11} = M_{MUL}+H_2O_2 + SA+ASA priming

observed in control. Longest root was recorded in M_{FSD} priming (Fig. 2b), which is statistically at par with M_{MUL} priming, M_{MUL}+H_2O_2 priming and M_{FSD}+H_2O_2 priming treatments while minimum root length in control. Maximum fresh weight of seedling was observed where foliar application of M_{FSD}+H_2O_2 was done (Fig. 3a) which was statistically at par with M_{MUL}+H_2O_2 and M_{FSD} foliar application while minimum in control. Maximum seedling dry weight was recorded with foliar treatment of M_{FSD}+H_2O_2 (Fig. 3b), and was statistically at par with M_{MUL}+H_2O_2 and M_{FSD} foliar application, respectively but minimum in control.

Discussion

Emergence rate, time to 50% emergence, emergence index, root shoot lengths and seedling biomass are imperative signs of emergence, uniformity, synchronization of emergence and seedling vigor (Ellis and Robert, 1981; Bewley and Black, 1994; Basra *et al*., 2011; Lara *et al*., 2014) and seeds are regarded vigorous if finish emergence within minimum period of time (Mahboob *et al*., 2015). Good emergence rate is one of important factors which helps in achieving high seedling vigor (Basra *et al*., 2011).

The results of present study depict that all priming and foliar treatments improved the speed and spread of seedling as compared to control (no exogenous application), hydropriming and water spray. Maximum potential of leaf extract of Faisalabad origin moringa seed was observed for emergence parameters (Table 2; Fig. 1a, b and c) and wheat seedling vigor (Fig. 2 and 3) alone and in combination with H_2O_2, SA and ASA as a priming agent and foliar spray. An improvement in emergence index and noteworthy fall in emergence time may be due realness that priming of seeds encourages a cascade of biochemical alterations like enzyme activation, hydrolysis and breaking of seed dormancy (Aziza *et al*., 2004; Farooq *et al*., 2010), which are the essential elements to initiate the emergence process. Priming treatments not only enhanced the emergence and stand establishment but also growth and development as well yield attributes in wheat (Yasmeen *et al*., 2013a). Priming of seeds with MLE from different landrace alone and with H_2O_2, SA and ASA proved effective regarding

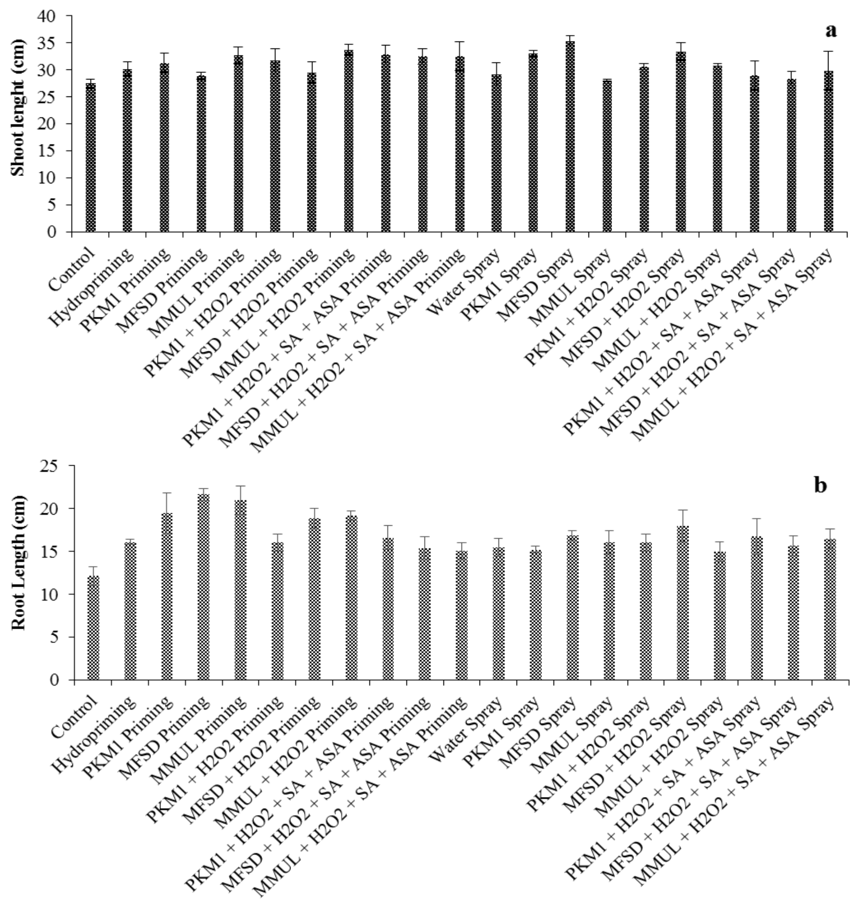

Fig. 2: Foliar and priming effect of moringa leaf extract of different landraces alone and in combinations with plant growth promoting substances on seedling shoot and root length of wheat under wire house condition

time to start emergence and final emergence percentage (Table 2), mean emergence time, time to 50% emergence and emergence index (Fig. 1a, b and c). *M. oleifera* leaves are rich in K, Ca and vitamin C and it is a rich source of growth promoting substances like zeatin (Foidle *et al*., 2001). Thus, the application of MLE either priming agent or foliar spray and their combination may enhance the performance of wheat seedling. Most of the mineral nutrients of MLE, during seed priming, seemed to be partitioned to embryo of seed, which boosted emergence of seedling and ultimately growth and development of plants (Farooq *et al*., 2010). Although most of the priming agents significantly improved the emergence of wheat but maximum enhancement (less values of time to start emergence, MET and E_{50} and highest EI) was recorded in seedling raised from seeds primed with leaf extract of M_{FSD} blended with H_2O_2+SA+ASA, followed by integration of only H_2O_2 and alone leaf extracts of M_{FSD} but also improved the seedling vigor as depicted by longer root length (Fig. 2) while foliar application of blended leaf extract of M_{FSD} with H_2O_2 increased the maximum fresh and dry weights of wheat seedlings (Fig. 3). Extract of M_{FSD} leaves may contain higher concentration of growth hormones and mineral nutrients which trigger the emergence process and may have synergetic effect with H_2O_2+SA+ASA on emergence and growth of wheat seedlings as compared to other landraces of moringa. Ahmad *et al*. (2014) reported that exogenous application of salicylic acid, ascorbic acid and hydrogen peroxide improved the growth of maize seedling at early stages of growth. They further reported that priming and foliar application of ASA, SA and H_2O_2 improved chlorophyll and leaf relative contents, seedling growth and enzymatic antioxidants activities in maize. Similar outcomes were observed in maize seedlings in response to priming with H_2O_2 (Imran *et al*., 2013). The prominent effect of H_2O_2 was observed due to signaling impact that triggers antioxidants in seeds and prevents the seedling from oxidative damage.

In current study, higher performance of wheat seedling raised from seeds primed with blended leaf extracts of M_{FSD} with growth promoting substances might be due to the maintenance of tissue water contents, rise in the activities of antioxidants and carbohydrate metabolism (Farooq *et al*., 2008). Seed priming and foliar application of MLE was

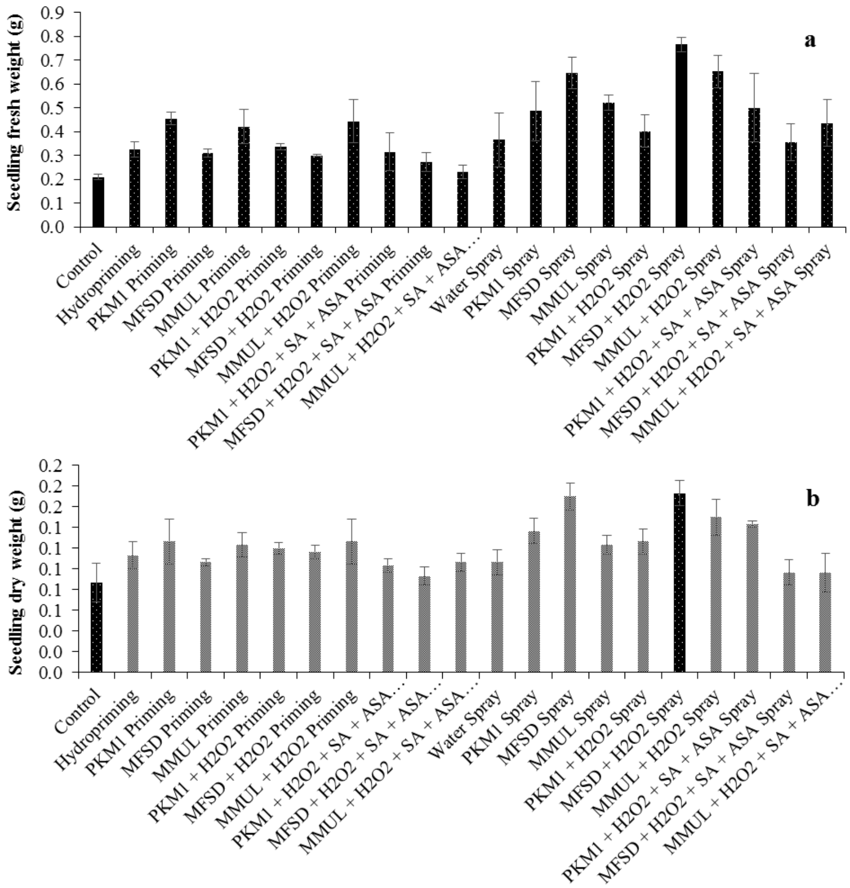

Fig. 3: Foliar and priming effect of moringa leaf extract of different landraces alone and in combinations with plant growth promoting substances on seedling fresh and dry weight of wheat under wire house condition

proved effective in improving the seedling vigor of wheat which might be the results of increased carbohydrates metabolism and hydrolytic enzyme activities (Fig. 2 and 3). Seed priming in combination of MLE+H_2O_2, sorghab water extract (SWE) + SA, SWE + H_2O_2 + ASA + SA and ASA + SA + H_2O_2 are also very effective in performance of maize seedling (Imran *et al*., 2013). According to Afzal *et al*. (2012) seed priming with MLE+H_2O_2 increased maximum α-amylase activity. Similar results were observed in common bean where plants were treated with salicylic acid and MLE as priming and foliar spray, showed significant enhancement in plant growth parameters (Rady *et al*., 2015). Wheat seed primed with optimum concentration of kinetin solution resulted in enhanced seedling growth, increased fresh and dry weight under normal and unfavorable conditions (Afzal *et al*., 2005). Application of growth promoting substances i.e. salicylic acid, ascorbic acid through foliar spray has been reported to maintain membrane integrity (Kaya *et al*., 2010) and increase photosynthetic rate (Khan *et al*., 2003). Plant hormones i.e. cytokinins have dire role for the promotion of cell division, cell elongation, chlorophyll biosynthesis and modification in apical dominance in plants (Taiz and Zeiger, 2010). Although plant development and growth is stimulated by proper exogenous application of plant hormones alone and in combination with mineral nutrient, antioxidants, organic and synthetic chemicals but are very expensive and out of access of poor farmers' resources. Leaf extract of white seeded moringa may be good alternate source of plant hormones because moringa leaves are rich source of cytokinin (zeatin) (Culver *et al*., 2012), proteins (Foidle *et al*., 2001), antioxidants (ascorbate and phenolics), vitamins and various mineral nutrients (Rady *et al*., 2015).

Conclusion

Priming and foliar application of leaf extract of Faisalabad origin moringa performed better regarding wheat seedling emergence and vigor as compared to leaf extracts of Multan origin moringa and PKM1. Leaf extract of M_{FSD} showed higher biostimulant potential that might be due to presence of higher concentration of biostimulant elements, plant growth promoting substances, mineral nutrients and antioxidants.

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