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Impact of Soil Drench and Foliar Spray of 24-Epibrassinolide on the Growth, Yield, and Quality of Field-Grown *Moringa oleifera* in Southwest China

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Abstract The effects of soil drench (SD) (0.4 and 0.8 mg plant^{-1}) and foliar spray (FS) (0.02, 0.04, and 0.08 ppm) of 24-epibrassinolide on the growth, yield, and quality of field-grown *Moringa oleifera* were evaluated. The results showed that all of the SD and FS treatments enhanced the net photosynthetic rate, stomatal conductance, transpiration rate, total chlorophyll content, and nitrate reductase activity in *Moringa* leaves. Both SD and FS promoted vegetative and reproductive growth of *Moringa*, but FS at an excessive concentration could suppress reproductive growth. Within a certain concentration range, the effect of FS on the reproductive growth was superior to that of SD, whereas the effect of SD on vegetative growth was better than that of FS. All of the 24-epibrassinolide treatments except for FS 0.08 ppm increased the number of seeds per fruit pod, the yield of fruit pods per tree, and the yield of seeds per tree, but the yield of the plants treated with SD was lower compared with FS. The FS 0.04 ppm treatment significantly increased the contents of oleic acid and eicosenoic acid and reduced the content of stearic acid in the seeds. All of the SD and FS treatments increased crude protein content in the leaf powder and seeds of *Moringa* as well as vitamin C in the leaves. Our results showed that the optimal concentration of 24-epibrassinolide for FS was 0.04 ppm, and the optimal concentration for SD was inferred to be higher than 0.8 mg plant^{-1}.

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Introduction

Moringa oleifera Lam is a perennial plant of the genus Moringa in the family Moringaceae, which is also often referred to as the "drumstick tree" or "horseradish tree" and originated in the sub-Himalayan region of northwest India. It is a medium-sized tree (7–10 m high) with thick gray bark, fragrant white flowers, and long green pods, and can be propagated either by direct seeding or hard stem cuttings. Due to its wide application in medicine and functional foods, *Moringa oleifera* is known as a "miracle vegetable." Its leaves are consumed in many regions as a nutritional supplement due to their rich content of vitamins A, B, C, and E, essential amino acids, and mineral elements, and they contain a rare combination of rich bioactive secondary metabolites such as glucosinolates, flavonoids, and phenolic acids, which have the potential to reduce the risk of cardiovascular diseases and cancer (Anwar and others 2007). In addition, *Moringa oleifera* can serve as an oil plant, with its seeds containing up to 80% unsaturated fatty acids, including 62–75% oleic acid. Its seed oil is a strong antioxidant and is collected for use in edible products, lubricants, and cosmetics (Anwar and Bhanger 2003). Since *Moringa oleifera* was introduced in China in 2001, it has mainly been planted in the dry-hot valley regions of the southwest, where the climate is characterized by high temperatures and drought. The results of a preliminary study conducted by our group showed that the fruit setting rate in a mature *Moringa oleifera* stand was only 2.13%, with an average of 43 pods/plant (Su and others 2012), which is lower than the corresponding values of 3.04% and 220 pods/plant recorded in southern India (Ramachandran and others 1980). Such low productivity of *Moringa oleifera* does not bring good economic benefits to the region, and it is therefore important to investigate how to increase the fruit and seed yield of *Moringa oleifera*.

Brassinosteroids (BR) are natural steroid hormones of plants that are necessary for normal plant growth and development (Li and Chory 1999). They have been recognized as new plant hormones with multiple functions and efficiencies (Yokota 1997; Clouse and Sasse 1998). BR play an extensive regulatory role in the growth and development processes of plants, such as growth, seed germination, rooting, flowering, aging, defoliation, and maturation (Müssig and others 2002; Swamy and Rao 2008). BR are non-toxic and environment-friendly substances (Kang and Guo 2011). Supplementation of plants with BR can improve drought resistance (Schilling and others 1991; Fariduddin and others 2009), the activities of carbonic anhydrase, nitrate reductase and Rubisco (Braun and Wild 1984; Hayat and others 2001) and the content of soluble proteins (Yang and others 1992; Khripach and others 2003) in addition to facilitating the distribution of assimilates to various organs of the plants (Fujii and Saka 2001), thus enhancing the potential productivity of many important economic plants. In rice, BR can increase the number of ears and the grain number, weight and length per ear (Rao and others 2002; Hnilicka and others 2007; Ali and others 2008a, b). The application of BR can also increase the pod number in a single leguminous plant and the overall seed yield (Takematsu and Takeuchi 1989; Vardhini and Rao 1998; Hayat and Ahmad 2003), improve the growth and seed yield of mustard and rapeseed plants (Hayat and others 2000; Khripach and others 2000) and enhance the yield of cotton (Ramraj and others 1997). Therefore, BR show broad prospects for application to enhance crop yields and improve crop quality in modern agricultural systems. Now, little is known about BR uptake and transport in plants exogenously treated with these regulators. When [^{14}C] BR were applied to wheat leaves, it was transported only towards the apex of the leaves (Nishikawa and others 1994, 1995). However, when [^{14}C] BR were applied to the roots of wheat seedlings that were only a few days old, it was efficiently taken up and distributed throughout the seedlings (Nishikawa and others 1995) and it was probably transported through the xylem (Nishikawa and others 1994). However, according to Symons and Reid (2004), BR are not transported over long distances.

BR activity in growth responses depends not only on dosage, but also on plant cultivar, the plant organ treated and the method of BR application. However, most studies on BR have been carried out under controlled laboratory conditions, and BR treatment has generally been performed via only seed soaking or foliar application. In the present study, a *Moringa oleifera* plantation in southwest China was treated with 24-epibrassinolide via soil drench and foliar spray under field growing conditions for the first time. Our aim was to assess the differential effects of 24-epibrassinolide, used in field conditions, on *Moringa oleifera* under different modes of application and different doses. To obtain this goal, we analyzed the impact of 24-epibrassinolide on the vegetative and reproductive growth of *Moringa oleifera* including photosynthetic efficiency, activity of selected enzymes, fruit, and seed yield parameters. Further, we evaluated the impact of 24-epibrassinolide on the quality of seeds (oil content and composition, protein content) and leaves (mineral, protein content).

Materials and Methods

Experimental Site and Plant Material

The experiments were carried out in a commercial plantation in Yuanyang County, Yunnan Province, China (102°27′E, 22°55′N, 250 m altitude above sea level). This area exhibits a subtropical dry and hot valley climate, with an annual average temperature of 23.5 °C, an extreme maximum temperature of 43.5 °C, a minimum temperature of 10 °C, and average annual precipitation of 750 mm. During the experiment, the mean annual temperature was 23.1 °C, with the highest temperature of 42.3 °C occurring in May 2015 and the lowest temperature of 10.6 °C occurring in January 2015. The mean annual precipitation was 797 mm. In June 2014, *Moringa oleifera* seeds from the same family were collected from the Yuanjiang Experimental Station of Research Institute of Resource Insects (RIRI), Yuxi city, China. Our experimental plantation was planted in July 2014, at a density of 2.0 m × 2.5 m over an area of approximately 1.1 ha. Untreated seeds of *Moringa oleifera* were used for propagation. The seeds were sown at a soil depth of 2 cm with 2 seeds per hole. After 28 days of growth, only one healthy seedling was retained. Pest and disease incidence was not found during the experiment. The soil of the experimental areas was classified as xerothermic soil (Long and others 2008), with 19.4% clay, 21.7% silt, 58.9%, sand and a pH value of 6.5. The soil in the experimental plots contained 10.51 g/kg of organic matter and 0.97 g/kg of total nitrogen, with good drainage, and combined with drip irrigation, is appropriate for agriculture. Field water management was performed using a conventional method. The physicochemical properties of the soil in the experimental field are shown in Table 1.

Table 1 Background soil physical and chemical characteristics

	TN (g kg^{-1})	TP (g kg^{-1})	TK (g kg^{-1})	AHN (mg kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})	AI (mg kg^{-1})	EC (cmol kg^{-1})	EM (cmol kg^{-1})	Organic content (g kg^{-1})	pH
Upper soil layer (0–20 cm)	0.97	0.29	17.72	39.94	0.022	130.58	5.55	7.14	1.35	10.51	6.56
Middle soil layer (20–40 cm)	0.88	0.27	18.13	35.58	0.018	103.75	7.71	8.63	1.86	7.79	6.25
Lower soil layers (40–80 cm)	0.65	0.21	19.74	30.14	0.016	101.05	6.93	7.09	1.19	6.24	6.19

The soil was sampled from three soil profiles: upper layer (0–20 cm), middle layer (20–40 cm) and lower layer (40–80 cm)

TN total nitrogen, *TP* total phosphorus, *TK* total potassium, *AHN* alkali-hydrolyzable N (nitrogen), *AP* available phosphorus, *AK* available potassium, *AI* available iron, *EC* exchangeable calcium, *EM* exchangeable magnesium

Experimental Design

In this study, the application of 24-epibrassinolide [(22R, 23R, 24R)-2α, 3α, 22, 23-tetrahydroxy-24-methyl-7a-oxa-5α-cholestan-6-one] (active ingredient (ai) of 0.004%, UNIDA Co., Ltd., China) to *Moringa oleifera* was conducted using the methods of soil drenching and foliar spray. The soil drench and foliar spray treatments were established in two separate experimental zones separated by an isolation strip. Plants of uniform height and crown were selected, and the experiments were carried out in a randomized block design with two soil drench treatments (FS1: 0.4 mg plant^{-1} and FS2: 0.8 mg plant^{-1}) and three foliar spray treatments (TF1: 0.02 ppm, TF2: 0.04 ppm, and TF3: 0.08 ppm). Each treatment was repeated four times, and each replicate included six plants. Plants untreated with 24-epibrassinolide served as the control (T0). The soil drench 24-epibrassinolide treatments were applied once on February 22, 2015. Application of 24-epibrassinolide to the roots was performed together with application of fertilizer [100 g of a K_2SO_4-type NPK compound fertilizer (16N–16P_2O_5–16K_2O) with sulfur (30%)] for each experimental plant. 24-epibrassinolide and fertilizer were mixed with 5 kg water per tree, and then was poured uniformly in circular trenches in the soil (about 25-cm wide and 20-cm deep) around the base of the plant at a radial distance of 30 cm from it. The foliar spray 24-epibrassinolide treatments were implemented with the 1st spraying on February 22, 2015, followed by spraying once every 2 weeks and were completed on June 14, 2015, with a total of nine applications. The foliar spray treatments were conducted under climate conditions of no rain and little wind, and a manual pump was used to maintain full moisture in the plants. Before both treatments, the average crown width of *Moringa oleifera* was measured as 56 ± 4.3 cm, and the average height was 128 ± 6.8 cm. The plants before hormone treatments were 8 months old.

Soil Analysis

Before the application of 24-epibrassinolide, soil samples were randomly collected from the experimental field. After air-drying, grinding, and sieving through a 2-mm sieve, the organic matter content, pH, and available contents of N, P, and K as well as Ca, Mg, and Fe in the soils were analyzed. All analyses were carried out following the procedure of Chapman and Pratt (1973).

Photosynthesis Measurements

Photosynthetic parameters were determined on June 16, 2015 (48 h after the last foliar spray). Three plants were randomly selected from each treatment, and five leaves were selected from each plant, with four measurements on each leaf. The net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (mmol H_2O m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), and intercellular CO_2 concentration (μmol CO_2 mol^{-1}) were determined using an Li-6400 Portable Photosynthesis System (Li-Cor Inc., Lincoln, Nebraska, USA).

Measurement of Total Chlorophyll and Nitrate Reductase Activity

The levels of total chlorophyll and nitrate reductase were measured on June 16, 2015. After determining photosynthetic parameters, a 0.4 g sample of mixed leaves was collected from the plants for photosynthesis measurement, half of which was used to determine the total chlorophyll content, whereas the other half was used for the determination of nitrate reductase activity. The total chlorophyll content was determined according to Zheng and others (2010). The cleaned leaf disks (5 mm in diameter) were extracted with 80% acetone. Absorption was assayed at 663 and 645 nm using an ultraviolet (UV)–Visible spectrophotometer (Unico, UV-3802, Shanghai, China). The nitrate reductase activity was assessed according to Kaiser and Lewis (1984). The NO_2^- formed was colorimetrically assayed at 540 nm after azo coupling with sulfanilamide and naphthyl ethylenediamine dihydrochloride following the procedure of Hageman and Hucklesby (1971).

Measurement of Vegetative and Reproductive Growth

Three plants from each replication were randomly selected to measure the vegetative growth and reproductive growth. During the young fruit stage in April 2015, the number of branches producing fruit per tree (NBF) and the total number of fruit set per tree (NTF) were recorded. When the fruits were ripe in July 2015, the three strongest branches were selected from each plant to determine branch length (BL) and branch diameter (BD), and the mean canopy diameter (CD), the total number of branches per tree (NTB), and the total number of ripe fruit per tree (NRF) were measured at the same time.

Yield and Physical Parameters of Fruits and Seeds

After the fruits were ripe, on June 20, 2015, all fruits from each plant for each replicate were individually harvested and weighed, and the seeds were stripped to assess the yield. For each replicate, 60 fruits were selected (10 fruits per plant) to record the mean fruit pod length (FL), mean fruit pod diameter (FD), weight per fruit pod (FW), number of seeds per fruit pod (NSF), weight of seeds per fruit pod (SW), yield of fruit pods per tree (FY), and yield of seeds per tree (SY), where $FY = NRF \times FW$, and $SY = NRF \times SW$.

Quality Evaluation of Seed Oil and Leaf Powder

Mature seeds were collected from each replicate for analysis of oil content, ester composition, and crude protein content. The oil percentage was determined referring to the method of Pant and others (2006). The contents of oleic acid, linoleic acid, palmitic acid, α-linen acid, eicosenoic acid, and stearic acid were determined according to the method of Hossain and others (2003), and the content of crude protein was analyzed referring to AOAC methods (2000).

Leaf samples of every replication in each treatment were dried in a forced-draft oven at 65 °C to constant weight. The dried samples were milled to pass through a 2-mm sieve for quality evaluation. P content was determined according to the vanado-molybdate method (Murphy and Riley 1962), and the K concentration was determined by flame photometry according to Heald (1965). The concentrations of Fe, Mg, and Ca were analyzed with an atomic absorption flame emission spectrophotometer according to Pratt (1965). Vitamin C and crude protein contents were estimated following the standard procedure described by AOAC (2000).

Data Analysis

Analysis of variance was carried out using the SPSS statistical package (version *13, SPSS*, Chicago, IL, USA). Duncan's pairwise comparison was employed when the differences between the means were significant ($P < 0.05$).

Results

Photosynthetic, Physiological, and Biochemical Characteristics

Compared with the control, none of the soil drench and foliar spray treatments significantly affected Ci ($P > 0.05$), but they all increased the levels of Pn, Cond, and Tr in *Moringa oleifera* leaves, though only the TF2 and TF3 treatments led to significant differences in these three photosynthetic parameters (Fig. 1). All of the soil drench and foliar spray treatments increased the content of chlorophyll, but only the foliar spray treatments involving a high concentration (TF2 and TF3) significantly increased the total chlorophyll content, whereas none of the soil drench treatments showed a significant effect on the total chlorophyll content (Fig. 2). All of the soil drench and foliar spray treatments enhanced nitrate reductase activity in the leaves,

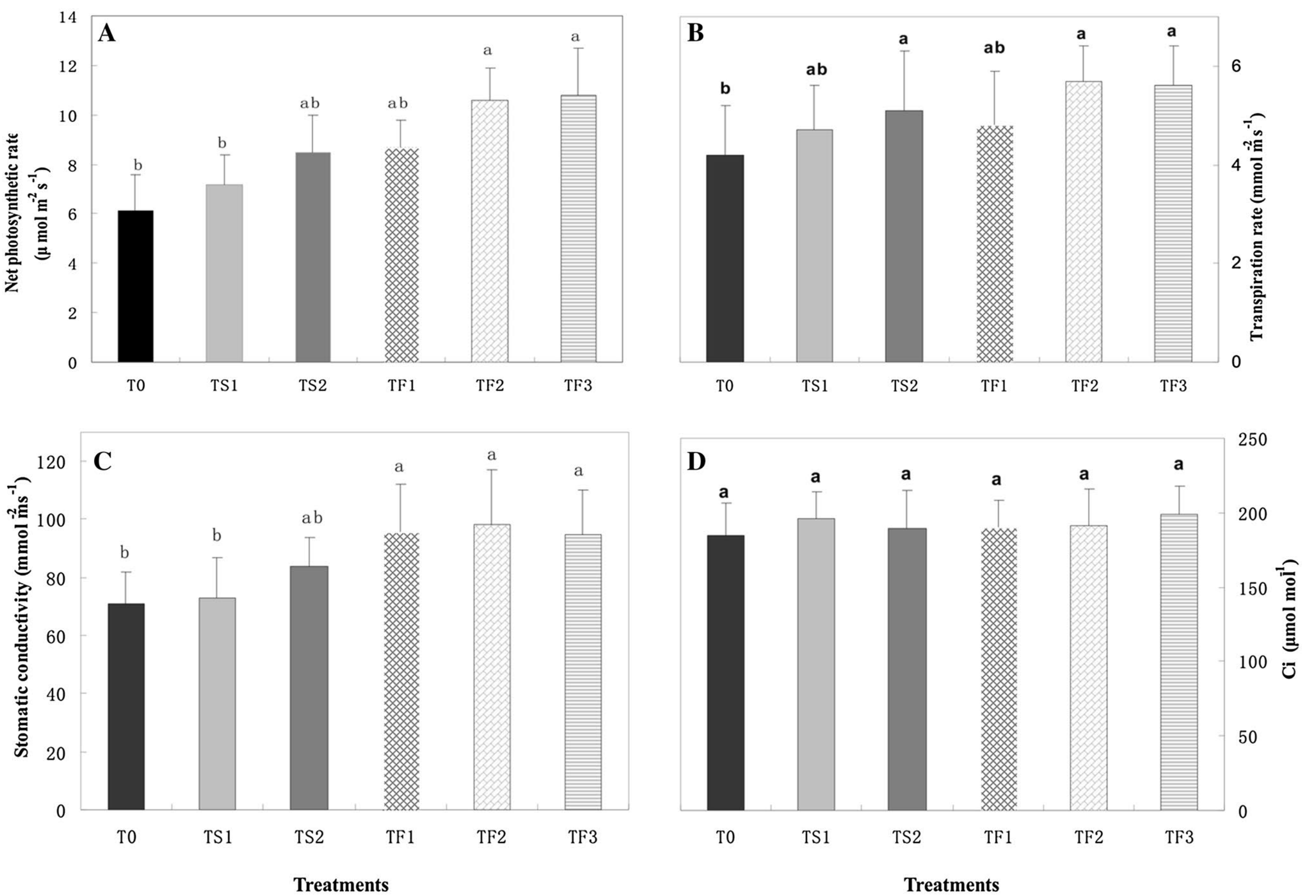

Fig. 1 Effects of soil drenching and foliar spraying of 24-brassinosteroid on the net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) (**a**), transpiration rate (mmol H_2O m^{-2} s^{-1}) (**b**), stomatic conductivity (mmol H_2O m^{-2} s^{-1}) (**c**) and intercellular CO_2 concentration (Ci) (μmol CO_2 mol^{-1}) (**d**) of *Moringa oleifera* leaves. T0 refers to the control, TS1 refers to the 0.4 mg $plant^{-1}$ soil drench treatment, TS2 refers to the 0.8 mg $plant^{-1}$ soil drench treatment, TF1 refers to the 0.02 ppm foliar spray treatment, TF2 refers to the 0.04 ppm foliar spray treatment, and TF3 refers to the 0.08 ppm foliar spray treatment. Values followed by the *same letter* showed no statistically significant differences ($P > 0.05$). Mean values ± standard error are shown

and these differences were significant compared to the control ($P < 0.05$) (Fig. 2).

Vegetative and Reproductive Growth Characteristics

With the exception of treatment FT3, vegetative growth of *Moringa* was significantly ($P < 0.05$) higher in all of the soil drench and foliar spray treatments over the control (Table 2). Although reproductive growth of *Moringa* was also higher than in the control among all of the treatments, only NBF showed a significant difference between the treatments. Foliar spray treatment involving an excessive concentration (FT3) suppressed all of the vegetative and reproductive growth parameters and significantly ($P < 0.05$) reduced the NRF/NTF ratio. With an increase in the concentration of the treatment, the various growth parameters first increased and then decreased under foliar spray, whereas the growth parameters all continuously increased under soil drenching, and the growth parameters of CD, BL and BD showed significant ($P < 0.05$) differences between the treatments at different concentrations. Regarding the reproductive growth parameters, all of the measured values obtained under the foliar spray treatments (with the exception of FT3) were higher than under the soil drench treatments, with the value measured under TF2 being highest. Regarding the vegetative growth parameters, the value observed under the TS2 soil drench treatment was highest. These results suggested that soil drench treatment was conducive to vegetative growth, whereas foliar spray treatment was conducive to reproductive growth in *Moringa oleifera*.

Fruit and Seed Growth Characteristics

With the exception of treatment FT3, all of the soil drench and foliar spray treatments significantly ($P < 0.05$) increased NSF, with no significant effect on FL, FW, SW,

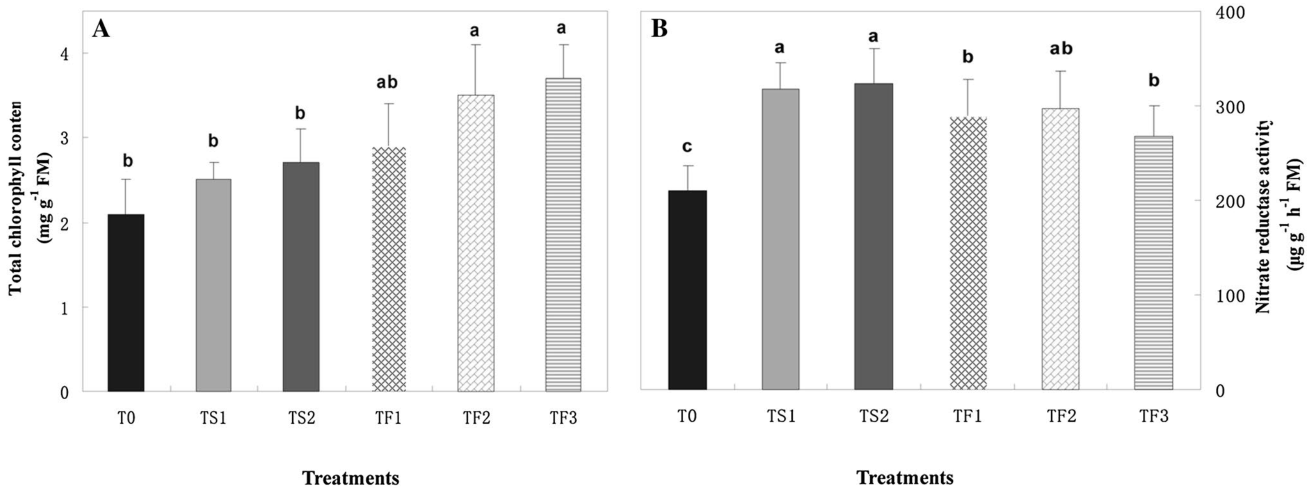

Fig. 2 Effects of soil drenching and foliar spraying of 24-brassinosteroid on the total chlorophyll content (mg g^{-1} FM) (**a**) and nitrate reductase activity (μg g^{-1} h^{-1} FM) (**b**) of *Moringa oleifera* leaves. FM refers to fresh matter; T0 refers to the control; TS1 refers to the 0.4 mg $plant^{-1}$ soil drench treatment; TS2 refers to the 0.8 mg $plant^{-1}$ soil drench treatment; TF1 refers to the 0.02 ppm foliar spray treatment; TF2 refers to the 0.04 ppm foliar spray treatment, and TF3 refers to the 0.08 ppm foliar spray treatment. Values followed by the *same letter* showed no statistically significant differences ($P > 0.05$). Mean values ± standard error are shown

Table 2 Effects of soil drenching and foliar spraying of 24-brassinosteroid on the vegetative and reproductive growth of *Moringa oleifera*

Treatments	Vegetative growth				Reproductive growth			
	CD (cm)	BL (cm)	BD (cm)	NTB	NBF	NTF	NRF	NRF/NTF
T0	127 ± 9.4 d	99 ± 5.1 c	2.5 ± 0.03 c	9.5 ± 1.5 c	4.3 ± 0.4 c	20.9 ± 1.1 a	14.3 ± 2.8 ab	68.8 ± 4.1% a
TS1	164 ± 10.7 bc	127 ± 9.1 b	3.3 ± 0.08 b	15.2 ± 1.7 ab	8.5 ± 0.5 b	21.6 ± 1.5 a	15.4 ± 1.7 a	70.4 ± 3.9% a
TS2	192 ± 8.3 a	148 ± 10.8 a	4.4 ± 0.15 a	18.2 ± 2.1 a	8.8 ± 0.7 b	21.9 ± 1.4 a	15.7 ± 2.1 a	71.5 ± 2.7% a
TF1	157 ± 6.0 c	121 ± 8.8 b	3.2 ± 0.04 b	14.7 ± 1.1 b	9.3 ± 0.9 b	22.2 ± 1.4 a	15.9 ± 1.5 a	71.9 ± 2.8% a
TF2	173 ± 6.6 b	130 ± 8.2 b	3.4 ± 0.06 b	15.9 ± 1.3 ab	11.9 ± 0.7 a	23.1 ± 1.2 a	16.1 ± 2.4 a	72.2 ± 4.7% a
TF3	121 ± 9.5 d	97 ± 4.4 c	2.3 ± 0.06 c	9.4 ± 0.8 c	3.9 ± 0.6 c	20.4 ± 2.1 a	12.2 ± 0.5 b	59.7 ± 1.2% b

T0 is the control; TS1 is the 0.4 mg $plant^{-1}$ soil drench treatment; TS2 is the 0.8 mg $plant^{-1}$ soil drench treatment; TF1 is the 0.02 ppm foliar spray treatment; TF2 is the 0.04 ppm foliar spray treatment; and TF3 is the 0.08 ppm foliar spray treatment

CD is the mean canopy diameter, BL is the length of branch, BD is the diameter of branch, NTB is the total number of branches per tree, NBF is the number of branches producing fruit per tree, NTF is the total number of fruit setting per tree, NRF is the total number of ripe fruit load per tree

Values followed by the *same letter* showed no statistically significant differences ($P > 0.05$). Mean values ± standard error

and the FD/FL ratio (Table 3). Although only the parameters of plants treated with TF2 foliar spray showed significant differences from those of the control plants, all of the soil drench and foliar spray treatments increased FD. In addition, FY and SY were increased in all plants treated with soil drench, but only FY showed a significant ($P < 0.05$) difference compared with the control, with FY being increased by 16.4–19.1% and SY being increased by 5.8–8.3%. Two foliar spray treatments, TF1 and TF2, dramatically increased FY and SY, leading to significant differences from the control ($P < 0.05$), with FY being increased by 19.4–21.4%, and SY being increased by 14.5–19.9% compared to the control. The TF3 foliar spray treatment reduced FY and significantly ($P < 0.05$) reduced SY. With an increase in the concentration, the yield parameters of the plants treated with soil drench were gradually increased, whereas those of the plants treated with foliar spray first increased and then decreased. It is worth noting that the yields under all of the soil drench treatments were lower than in the foliar spray treatments (except for TF3). These results showed that the optimal concentration of foliar spray was TF2 (0.04 ppm), whereas the optimal concentration of soil drench could not be determined, though our findings suggested that this value might be higher than 0.8 mg per plant.

Table 3 Effects of soil drenching and foliar spraying of 24-brassinosteroid on yield and physical parameters of the fruit and seed of *Moringa oleifera*

Treatments	FL (cm)	FD (cm)	FD/FL	NSF	FW (g)	SW (g)	FY (g)	SY (g)
T0	33.7 ± 3.5 a	1.51 ± 0.17 b	0.051 ± 0.003a	12.5 ± 1.3 b	11.3 ± 0.9 a	3.97 ± 0.09 a	162.6 ± 8.9 b	58.7 ± 4.9 c
TS1	32.8 ± 1.6 a	1.55 ± 0.09 b	0.046 ± 0.006a	16.1 ± 2.1 a	12.3 ± 0.8 a	4.04 ± 0.03 a	189.3 ± 7.6 a	62.1 ± 3.6 bc
TS2	36.1 ± 2.4 a	1.61 ± 0.13 b	0.055 ± 0.003a	16.9 ± 1.9 a	12.6 ± 0.4 a	4.09 ± 0.06 a	193.7 ± 10.1 a	63.6 ± 4.3 bc
TF1	32.1 ± 0.9 a	1.64 ± 0.12 b	0.045 ± 0.006a	16.7 ± 2.6 a	12.1 ± 0.4 a	4.16 ± 0.08 a	194.1 ± 9.4 a	67.2 ± 4.2 ab
TF2	36.6 ± 2.6 a	1.78 ± 0.16 a	0.043 ± 0.002a	17.2 ± 1.3 a	12.2 ± 1.1 a	4.33 ± 0.14 a	197.4 ± 9.9 a	70.4 ± 5.7 a
TF3	31.7 ± 2.1 a	1.54 ± 0.12 b	0.047 ± 0.005a	12.1 ± 2.2 b	11.8 ± 0.7 a	3.95 ± 0.02 a	155.9 ± 6.7 b	50.3 ± 5.6 d

T0 is the control; TS1 is the 0.4 mg plant^{-1} soil drench treatment; TS2 is the 0.8 mg plant^{-1} soil drench treatment; TF1 is the 0.02 ppm foliar spray treatment; TF2 is the 0.04 ppm foliar spray treatment; and TF3 is the 0.08 ppm foliar spray treatment

FL is the mean fruit pod length, FD is the mean fruit pod diameter, FD/FL is the ratio of mean diameter to mean length of fruit pod, NSF is the number of seeds per fruit pod, FW is the weight of per fruit pod (dry weight), SW is the weight of seeds per fruit pod (dry weight), SY is the yield of seeds per tree (dry weight), FY is the yield of fruit pods per tree (dry weight)

Values followed by the *same letter* showed no statistically significant differences ($P > 0.05$). Mean values ± standard error

Quality of Seed Oil and Leaf Powder

All of the soil drench and foliar spray treatments increased the content of oleic acid in the seeds, with the TF2 foliar spray treatment resulting in a significant ($P < 0.05$) difference (Table 4). In contrast, none of the soil drench or foliar spray treatments significantly affected the seed oil content or the contents of linoleic acid, palmitic acid or α-linen acid. All of the soil drench and foliar spray treatments reduced stearic acid content and increased eicosenoic acid content, but only the plants treated with foliar spray showed a significant difference compared with the control. All of the foliar spray treatments increased the crude protein content in the seeds, but not significantly, whereas all of the soil drench treatments significantly ($P < 0.05$) increased crude protein content of *Moringa* seeds. In addition, none of the soil drench or foliar spray treatments changed the contents of Ca, Mg, P, Fe, and K in the leaf powder of *Moringa*, but they did increase the content of vitamin C (Table 5). In all cases, the plants treated with TS2 soil drench and TF2 foliar spray showed higher contents of crude protein and vitamin C in the leaf powder.

Discussion

Phytohormones play an important role in optimizing the growth and reproductive development of plants. Gomes and others (2006) reported that the application of BR could enhance the growth of granadilla and stimulate the differentiation of the meristem from vegetative growth to reproductive growth in granadilla. The impact of BR on plant growth has generally been reported to show positive effects, but some negative effects have also been reported. Amzallag (2002) found that treatment with 10^{-8} BR significantly reduced the biomass and leaf length of sorghum in the early development stage. The present study showed that treatment with 24-epibrassinolide at an appropriate concentration could increase the growth of *Moringa oleifera*, but foliar spray at an excessive concentration exerted a negative effect on the vegetative and reproductive growth

Table 4 Effects of soil drenching and foliar spraying of 24-brassinosteroid on the quality of the seed oil of *Moringa oleifera*

Treatments	Oil content (%)	Oleic acid (%)	Linoleic acid (%)	Palmitic acid (%)	α-Linen acid (%)	Eicosenoic acid (%)	Stearic acid (%)	Crude protein (g/kg)
T0	27.25 ± 0.70 a	67.16 ± 1.27 b	3.74 ± 0.53 a	5.89 ± 0.38 a	1.31 ± 0.32 a	2.46 ± 0.11 b	5.95 ± 0.46 a	230.1 ± 13.7 b
TS1	29.37 ± 0.65 a	67.58 ± 1.38 b	3.80 ± 0.14 a	5.48 ± 0.22 a	1.58 ± 0.23 a	2.71 ± 0.07 b	5.55 ± 0.17 a	284.7 ± 9.9 a
TS2	26.46 ± 0.33 a	67.43 ± 1.59 b	3.43 ± 0.41 a	5.41 ± 0.14 a	1.33 ± 0.12 a	2.88 ± 0.13 ab	5.03 ± 0.28 ab	295.3 ± 15.8 a
TF1	28.72 ± 0.19 a	69.92 ± 1.72 ab	4.13 ± 0.89 a	5.39 ± 0.44 a	1.17 ± 0.13 a	3.21 ± 0.04 a	4.76 ± 0.23 b	256.8 ± 10.6 b
TF2	24.89 ± 0.17 a	74.06 ± 1.81 a	3.85 ± 0.46 a	6.09 ± 0.52 a	1.34 ± 0.17 a	3.32 ± 0.12 a	4.48 ± 0.30 b	271.2 ± 9.1 ab
TF3	28.61 ± 0.55 a	68.25 ± 1.92 ab	3.60 ± 0.08 a	5.77 ± 0.07 a	1.34 ± 0.26 a	3.06 ± 0.09 a	4.69 ± 0.18 b	258.3 ± 8.9 b

T0 is the control; TS1 is the 0.4 mg plant^{-1} soil drench treatment; TS2 is the 0.8 mg plant^{-1} soil drench treatment; TF1 is the 0.02 ppm foliar spray treatment; TF2 is the 0.04 ppm foliar spray treatment; and TF3 is the 0.08 ppm foliar spray treatment

Values followed by the *same letter* showed no statistically significant differences ($P > 0.05$). Mean values ± standard error

Table 5 Effects of soil drenching and foliar spraying of 24-brassinosteroid on the nutrition content of *Moringa oleifera* leaf powder

Treatments	Ca (mg/100 g)	Mg (mg/100 g)	P (mg/100 g)	Fe (mg/100 g)	K (mg/100 g)	Vitamin C (mg/100 g)	Crude protein (g/100 g)
T0	971 ± 29.4 a	339 ± 24.3 a	224 ± 11.2 a	11.13 ± 0.74 a	1312 ± 67.9 a	29.2 ± 2.1 b	21.9 ± 2.1 b
TS1	988 ± 20.2 a	346 ± 22.9 a	232 ± 13.6 a	10.97 ± 0.84 a	1308 ± 45.3 a	33.7 ± 2.4 b	28.1 ± 1.8 a
TS2	904 ± 32.6 a	333 ± 17.8 a	213 ± 9.7 a	12.15 ± 1.06 a	1261 ± 57.4 a	45.9 ± 3.2 a	30.3 ± 2.3 a
TF1	1023 ± 41.7 a	326 ± 13.5 a	226 ± 12.4 a	10.75 ± 0.82 a	1294 ± 49.3 a	34.3 ± 1.9 b	27.7 ± 2.6 a
TF2	962 ± 26.1 a	335 ± 18.4 a	209 ± 8.6 a	9.94 ± 0.91 a	1354 ± 51.8 a	54.7 ± 4.6 a	29.4 ± 2.3 a
TF3	991 ± 29.4 a	341 ± 10.8 a	239 ± 12.9 a	11.01 ± 0.98 a	1299 ± 39.5 a	33.1 ± 2.8 b	27.2 ± 1.6 a

T0 is the control; TS1 is the 0.4 mg plant^{-1} soil drench treatment; TS2 is the 0.8 mg plant^{-1} soil drench treatment; TF1 is the 0.02 ppm foliar spray treatment; TF2 is the 0.04 ppm foliar spray treatment; and TF3 is the 0.08 ppm foliar spray treatment

Values followed by the *same letter* showed no statistically significant differences ($P > 0.05$). Mean values ± standard error

of *Moringa oleifera*. As shown in Table 2, treatment TF3 slightly reduced CD, BL, BD, and NBF but significantly reduced the NRF/NTF ratio related to reproductive growth. Many studies have shown that excessive 24-epibrassinolide application can lead to a negative effect on the reproductive growth of plants, as reported in *Pharbitis nil* (Kesy and others 2003) and *Arabidopsis thaliana* (Janeczko and others 2003). This negative effect may be because BR are associated with the critical developmental stages of flower bud differentiation from vegetative growth to reproductive growth in plants. As described by Mandava (1988), BR mainly react in the area of meristem tissue and may cause elongated growth and cell differentiation, but excessive BR can inhibit the development of meristem tissue and the differentiation of the cells.

Our results showed that all of the 24-epibrassinolide treatments except for TF3 increased FY and SY (Table 3). This is the first time that 24-epibrassinolide treatment has been reported to increase the yield of *Moringa oleifera* in the field. The increase in the yield of *Moringa oleifera* is clearly related to the increases in NSF, FW, and SW induced by 24-epibrassinolide treatment, though these increases were not significant in these three parameters (Table 3). Pozo and others (1994) reported that the number of fruits in BR-treated grapes was increased by 66.7%, resulting in a 29.9% increment in the fruit yield. In Brazil, the yields of BR-treated soybean and common beans were shown to be increased by 22 and 83%, respectively (Ramraj and others 1997). These values were higher than the maximum yield increases of 21.4% (foliar spray treatment) and 19.1% (soil drench treatment) obtained in the present study. In contrast, a study by Serna and others (2012) on pepper showed that BR treatment only increased the number of fruits, without increasing the weight of the fruits. In this study, treatment with 24-epibrassinolide increased fruit setting (Table 2) and the weight of fruits and seeds (Table 3), but the increments were not significant. The present study also showed that foliar spray treatment at an excessive concentration (TF3) reduced FY and significantly ($P < 0.05$) reduced SY. The reason for the lower yield is shown in Tables 2 and 3. Although treatment TF3 slightly increased FD and FW, it reduced SW, NBF, and NRF. These results also imply that 24-epibrassinolide treatment at an excessive concentration may reduce the weight of seeds and increase the proportion of fruit pulp in *Moringa oleifera* plants. In this study, one possible reason for such increases in yield is that BR treatment is likely to increase the number of flowers (Papadopoulou and Grumet 2005) and fruit setting (Wubs and others 2009) in plants, leading to an increase in the number of *Moringa* fruits. Moreover, the physiological mechanism by which 24-epibrassinolide application improves the yield of *M. oleifera* is reflected by the changes in the photosynthetic parameters.

Our results showed that all of the soil drench and foliar spray treatments could enhance the levels of Pn, Cond, and Tr in *Moringa oleifera* leaves, which is consistent with the findings by Hayat and others (2011). However, the statistical analysis (not shown) showed that there is no correlation ($R^2 = 0.568$ and 0.508) between FY, SY, and Pn in *Moringa oleifera*, even though these three parameters reached their maximum values under the TF2 foliar spray treatment, exhibiting significant ($P < 0.05$) increases (Table 3; Fig. 2). In contrast, other studies have shown a strong correlation between increased yields and Pn in plants treated with BR, such as mung bean (Fariduddin and others 2008) and geranium (Swamy and Rao 2008). In addition, although foliar spray treatment at a high concentration (TF3) significantly increased the content of total chlorophyll in the leaves (Fig. 2), no correlation between the content of total chlorophyll and Pn was found (not shown, $R^2 = 0.362$). These results are not fully consistent with the findings of studies in cucumber by Yu and others (2004). The low correlation shown in our results may have been caused by the attenuation effect of BR, as Pn was measured at 48 h after 24-epibrassinolide treatment in the present work, whereas the significant increases in Pn and chlorophyll contents reported in cucumber by Yu and others (2004) were determined 3 h after BR treatment.

This study showed that the TF2 foliar spray treatment significantly increased the contents of oleic acid and eicosenoic acid in the seeds and significantly reduced the content of stearic acid (Table 4). Similarly, Maia and others (2004) found that BR treatment caused a significant increase in essential oil content of mint, whereas Swamy and Rao (2008) reported that BR at 3 μM concentration increased the geraniol content of geranium, while reducing the content of citronellol and ultimately increasing the content of aromatic oil. In the present study, BR may have activated the genetic potential of the plant itself (Swamy and Rao 2008), leading to the observed changes in *Moringa* oil components by regulating the growth and metabolism of the plant, and these changes in oil components improved the quality of *Moringa* oil. Results from this study showed that none of the soil drench and foliar spray treatments changed the mineral contents in the leaf powder of *Moringa*, but they did increase the content of vitamin C (Table 5). A similar result in mineral composition with BR treatment was observed in wheat (Shahbaz and Ashraf 2007). However, other authors found that BR treatments decreased the level of vitamin C in tomato (Ali and others 2006) and in pepper (Serna and others 2012). In addition, our results showed that 24-epibrassinolide treatment increased the crude protein content in the seeds and leaf powder of *Moringa oleifera* (Tables 4, 5), which is consistent with reported findings in beans (Kalinich and others 1985), mung bean (Fariduddin and others 2008), and groundnut (Vardhini and Rao 1998). The increased protein content observed in the present study may be associated with the increased activity of nitrate reductase (Fig. 2), as BR treatment may induce the expression of NR-mRNA (Campbell 1999), subsequently inducing the activity of functional nitrate reductase (Saroop and others 1998) and further enhancing the levels of the RNA, DNA, and proteins (Kalinich and others 1985). Therefore, this study provides an actual case in which the quality of the seed oil and leaf powder of *Moringa oleifera* was improved by treatment with 24-epibrassinolide.

Conclusion

The impact of soil drench treatment on vegetative growth was better than that of foliar spray, whereas the effect of foliar spray on reproductive growth was better than that of soil drenching. Therefore, foliar spray treatment could result in a higher yield of *Moringa oleifera*. In this study, all of the soil drench and foliar spray treatments enhanced the contents of oleic acid and eicosenoic acid in the seeds and reduced the content of stearic acid, while increasing the crude protein content in the leaf powder and seeds. These findings suggest that 24-epibrassinolide treatment can improve the functional quality of *Moringa oleifera*. Our data indicated that the optimal concentration for foliar spray was 0.04 ppm (treatment TF2), whereas the optimal concentration for soil drenching could not be determined, though it might be higher than that employed in TS2 (0.8 mg plant^{-1}) based on our data.

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