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The influence of He-Ne laser on agro-morphological criteria, ISSR marker and SDS-PAGE of *Moringa oleifera*

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Abstract

Background: *Moringa oleifera* L. (moringa) is a promising plant for nutritionally and medicinally uses. The influence of laser radiation on moringa seeds (dry and wetted) was investigated, using helium–neon (He–Ne) laser source at λ = 632.8 nm wavelength and 5 cm beam diameter for 25, 45, and 90 min. Agro-morphological criteria, SDS-PAGE protein banding patterns, and ISSR markers were investigated to elucidate the influence of He–Ne laser on moringa.

Results: Most agro-morphological criteria increased especially with wetted seed group pre-laser treatments (25 min). SDS-PAGE and ISSR profiles showed changes which include the absence of some bands and the appearance of few novel bands. ISSR markers showed a change in band number in all investigated samples with a total number of 172 bands. The polymorphic bands were 91 bands with 19 unique bands. The average mean percentage of polymorphism was recorded 52.91% at samples treated with a laser at different times. Wetted seed treatment for 25 min recorded 40 bands as new bands. Genetic template stability (GTS) values were recorded in all treatments. The lowest GTS % was recorded 76.74 at 25 min (wetted seeds) while highest GTS % was recorded 83.72 at 25 min (dry seeds). According to the data of GTS, produced by laser treatment for 25 and 45 min for wetted and dry seeds, respectively, it is more effective on genome stability than other treatments.

Conclusion: He–Ne laser treatments of wetted seeds achieve biostimulation in many aspects of agro-morphological criteria. ISSR analysis is a highly sensitive method for the detection of DNA alteration induced by laser treatments. The exposure of dry and wetted seeds to He–Ne laser at different times affected the genomic template stability percentage (GTS %). Also, the laser treatments have a great effect on gene expression by switching on or off some genes that may lead to the presence or absence of certain proteins, respectively.

Keywords: He–Ne laser, Agro-morphological criteria, SDS-PAGE, ISSR marker, Genomic template stability (GTS), *Moringa oleifera*

Introduction

Genus *Moringa* belongs to an angiosperm family (*Moringaceae*); it consists of 13 species such as *Moringa oleifera*. *M. oleifera* is widely cultivated over the world for its nutritional and medicinal values (Leone et al. 2015). *M. oleifera* is used as human food, medicinal plant, animal fodder, fertilizer, antimicrobial substance, bio-fuel, and ornamental plant (Gopalakrishnan et al. 2016).

Moringa tree has a wide range of medicinal and therapeutic properties, for example, anti-fibrotic, anti-inflammatory, anti-microbial, anti-hyperglycemic, anti-oxidant, anti-tumor, and anti-cancer properties. Moringa leaves have a large amount of vitamin C, calcium, β carotene, and potassium, and also, leaves are considered an essential source of different types of antioxidant compounds as flavonoids, ascorbic acid, carotenoids, and phenolics (Siddhuraju and Becker 2003). So, its leaves possess an antioxidant activity against free radicals and give significant protection against oxidative damage (Sreelatha and Padma 2009).

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Laser rays belong to unionizing radiation. The laser is an abbreviation of "light amplification by stimulation of radiation." Nowadays, the application of laser gained more attention in the field of agriculture (Pandey et al. 2015 and Swathy et al. 2016). Many studies have been reported the effect of laser on pre-sowing seeds in different crops and vegetables (Thatoi et al. 2016, Asghar et al. 2016, and Asghar et al. 2017). Laser radiation on seeds stimulates a series of positive effects such as enhancement of plant growth, reduction of germination time, increase in the number of flowers per plant, and increase of yield (Niculita et al. 2008).

Molecular markers are very useful in genetic analysis. The most commonly used marker systems are restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), random amplified polymorphic DNAs (RAPDs), microsatellites or simple sequence repeats (SSRs), and inter-simple sequence repeats (ISSRs). The ISSR marker is used for amplification of the regions between microsatellite loci. This type of marker does not need any information about the amplified sequences and shows a large value of polymorphism in the samples. Similarly, ISSR is considered as an important marker in studies of genetic relationships (Joshi et al. 2000, Reddy et al. 2002, and Adawy et al. 2004).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a biochemical technique used to analyze the effect of different treatments on genes expression. Protein profiling is important in studies related to abiotic and biotic stress (Kiegle et al. 2000 and Masoje et al. 2001). Previous studies showed that He–Ne lasers influenced plant growth and metabolism, Govil et al. (1991) and Cai et al. (2000) investigated the enhancement of soluble protein content in the irradiated drying corn seedling. Similarly, the concentration of soluble proteins and the activities of functional proteins were increased significantly by the laser pretreatment (Chen et al. 2005).

Gao et al. (2016) reported that He–Ne laser preillumination enhanced salt tolerance in tall fescue seedlings via upregulation expression level of some antioxidant enzyme genes and phytochrome B gene. They studied the effect of He–Ne laser irradiation on cell wall reconstruction mediating by cell wall polysaccharides and DNA fragmentation in tall fescue seedlings and evaluated the role of cell wall reconstruction and DNA damage repair in the induction of enhanced adaption capacity to saline conditions by the laser irradiation and further explored the physicochemical mechanism of the protective effects of He–Ne laser illumination on plants under unfavorable growth conditions.

This study aims to examine the comparative effect of He–Ne laser irradiated seeds of moringa from Egypt on agro-morphological criteria, genomic DNA stability using ISSR markers and protein profiling using SDS-PAGE.

Materials and methods

Plant materials

Moringa oleifera L. seeds were collected from the farm of the Egyptian Scientific Society of Moringa, National Research Centre, Giza, Egypt.

Laser parameters

Experimental seeds were divided equally into two groups (dry and wetted). Twelve seeds were subjected to a helium–neon (He–Ne) laser (model of the devise) with an average power density of 10 mW cm^2 (wave length of 632.8 nm and beam diameter = 5 cm) used to apply a dose of 300 mJ cm^2 for 25, 45, and 90 min.

Agro-morphological criteria

The dry and wetted seeds irradiated with He–Ne laser were planted in pots (30 × 25 cm) under complete randomized design. Nine healthy seeds were sown in each pot at a depth of ~ 2.5 cm. The agro-morphological criteria (shoot length (cm), shoot weight (g), root length (cm), root weight (g), number of leaves/plant and leaves weight/plant (g)) were recorded after 50 days from planting (seedling stage) to compare short-term laser effect with respect to the untreated control. The data of agro-morphological criteria were statistically analyzed using the Co-State statistical program.

Molecular analysis

ISSR-PCR

The plant genomic DNA was isolated from the young leaves (15 days old seedling) using Gene Jet Plant Genomic DNA purification Mini Kits (Thermo Scientific K0791). The extracted genomic DNA was quantitated using a NanoDrop 1000 spectrophotometer (Thermo Scientific) and diluted to 50 ng/μl to use it as template for PCR.

ISSR-PCR was performed by using 12 ISSR primers (Table 1). PCR amplification for isolated DNA was performed in 0.2 ml PCR Eppendorf (25 μl) which consisted 12.5 μl Dream Taq green PCR Master Mix 2X (Thermo Scientific K1081), 1 μl primer (10 pmol) (Metabion, German), and 1 μl Template DNA (50 ng/μl) and was completed to 25 μl by water (nuclease-free). Thermocycler (Bio-Rad) was programmed as follows: 94 °C for 5 min (one cycle) then 94 °C for 1 min, 45 °C for 1 min, 72 °C for 1.5 min (35 cycles), and finally, 72 °C for 7 min (one cycle) and hold at 4 °C. Then, 100 bp DNA Ladder and 20 μl of DNA amplified PCR product were loaded in

Table 1 ISSR-PCR primers used for the genotyping of Moringa

Name primer	Sequence (5′-3′)	
ISSR-6	CGCGATAGATAGATAGATA	$CGC(GATA)_4$
ISSR-7	GACGATAGATAGATAGATA	$GAC(GATA)_4$
ISSR-8	AGACAGACAGACAGACGC	$(AGAC)_4GC$
ISSR-9	GATAGATAGATAGATAGC	$(GATA)_4GC$
ISSR-10	GACAGACAGACAGACAAT	$(GACA)_4AT$
ISSR-11	ACACACACACACACACYA	$(AC)_8YA$
ISSR-12	ACACACACACACACACYC	$(AC)_8YC$
ISSR-13	AGAGAGAGAGAGAGAGT	$(AG)_8T$
ISSR-16	TCTCTCTCTCTCTCTCTA	$(TC)_8TA$
ISSR-HB09	GTGTGTGTGTGTGC	$(GT)_6GC$
ISSR-HB-13	GAGGAGGAGGC	(GAG)3GC
ISSR-HB14	GTGGTGGTGGC	(GTG)3GC

A adenine, *T* thymine, *G* guanine, *C* cytosine

each well of agarose (1.7%), which was placed in 1X TAE buffer and ran at 100 V for about 2 h. The gel was photographed by gel documentation (Bio-Rad) and was analyzed by Total Lab program to find out the molecular size of each band.

Genomic template stability (GTS %) was calculated according to the equation described by Liu et al. (2007), GTS = $(1 - a/n) \times 100$, where a is the average number of polymorphic bands detected in each treated sample and n is the number of total bands in the control .It was suggested that alternations in DNA profiles due to genotoxic exposure could be regarded as changes in genomic template stability (GTS, a qualitative measure of genotoxic effects).

Protein profiling using SDS-PAGE

SDS-PAGE was performed according to Laemmli (1970) and described by Tsugama et al. (2011). Soluble proteins were extracted by grinding 1 g leaves

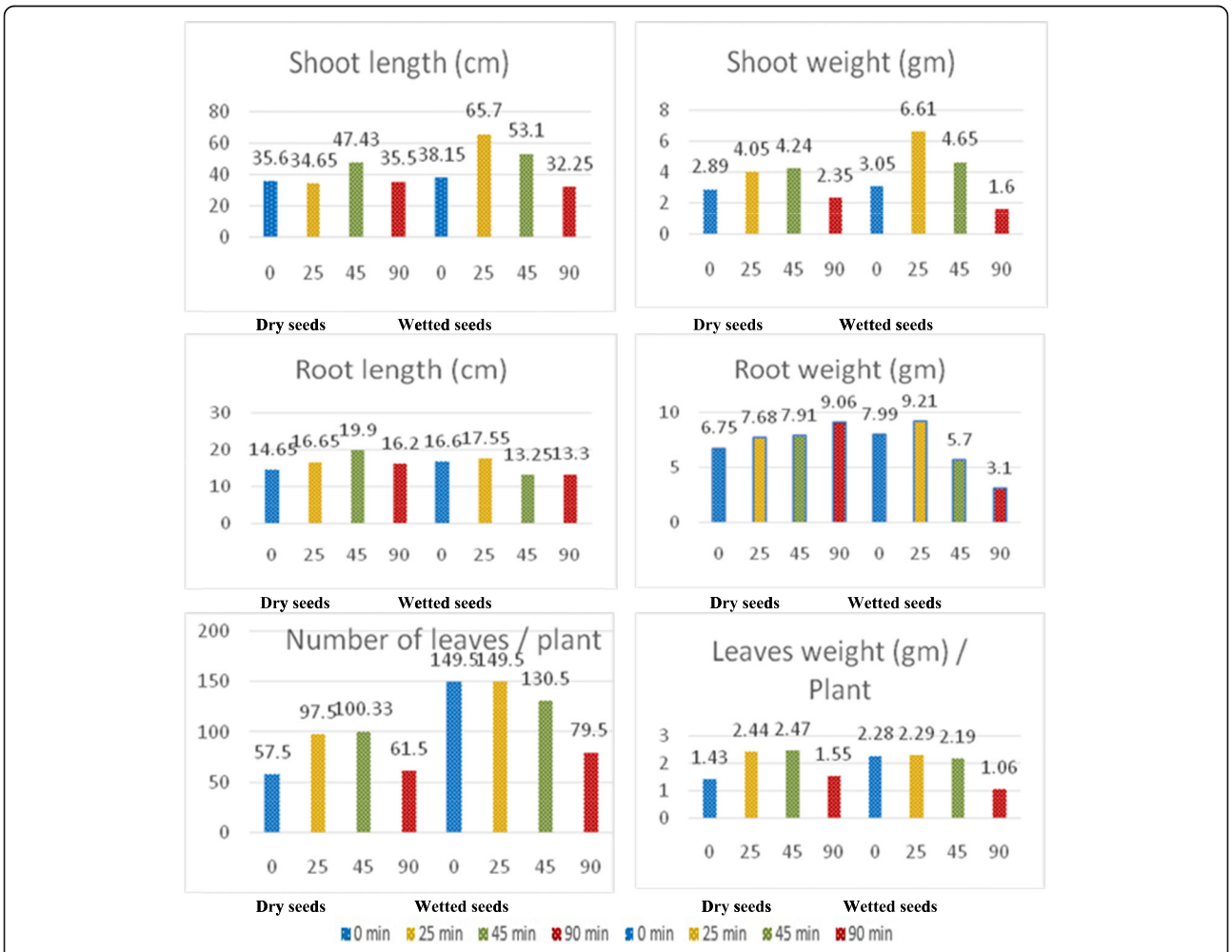

Fig. 1 Effect of He–Ne laser irradiation on agro-morphological criteria of moringa

Table 2 Mean squares (MS) of analysis of moringa under the effect of He–Ne laser irradiation

Source	df	Mean square (MS)					
		Shoot length	Root length	No. of leaves	Shoot weight	Root weight	Leaves weight
Replicates	2	0.2722^{ns}	0.5930^{ns}	0.55^{ns}	0.313^{ns}	0.0935^{ns}	0.9276^{ns}
Times	7	0.000^{***}	0.000^{***}	0.000^{***}	0.000^{***}	0.000^{***}	0.000^{***}
Error	14	0.0166	0.237	1.323	0.048	0.0766	0.0387

df degrees of freedom, *ns* non-significant
***Highly significant at 0.005 level of probability

from each treatment, added to 1 ml extraction buffer (10% SDS, Glycerol, 1 M Tris base, pH 8.8, 0.25 M EDTA) in Eppendorf tube (1.5 ml), left in the refrigerator overnight, then vortexed for 15 s and centrifuged at 12,000 rpm at 4 °C for 20 min. The supernatants which contain soluble proteins were transferred to new Eppendorf tubes and kept at deep-freeze until use for electrophoretic analysis, and the protein was then run in acrylamide gel 10% concentration. The marker of used protein is BLUltra Prestained Protein Ladder (GeneDirex, Cat No. PM001-0500). Protein bands were visualized by staining the gel using 0.1% Coomassie brilliant blue (R-250), and after the bands become clear, the gels were photographed by a digital camera (Sony, made in Japan) and transferred directly to the computer, and then the protein bands were analyzed by Total Lab program to find out the molecular mass of each band and scored depending on the presence (+) and absence (–) of bands.

Results

Effect of laser irradiation on agro-morphological criteria:

The effect of He–Ne laser on agro-morphological criteria such as root and shoot (lengths and weights) and leaves (number and weight) for dry and wetted seeds at different exposure times (0, 25, 45, and 90 min) were depicted in Fig. 1 and Tables 2 and 3. The seedling from wetted seeds that were irradiated for 25 and 45 min showed higher shoot length of 65.7 and 53.1 cm respectively, followed by dry seed irradiated for 45 min which had a shoot length of 47.43 cm. The root length also was recorded to be higher in seedlings that were raised from laser-treated dry seeds for 45 min (19.9 cm), followed by wetted seeds for 25 min (17.55 cm). The highest shoot fresh weight was recorded to be 6.61 g and 4.65 g in seedlings from wetted seeds that were irradiated for 25 and 45 min, respectively. Similarly, the higher root fresh weight value was recorded to be 9.21 g in seedlings from wetted seeds that were irradiated for 25 min and 9.06 g in seedlings from dry seeds that were irradiated for 90 min. The highest number of leaves were recorded 149.5 in seedlings from wetted seeds that were irradiated for 0 (control) and 25 min, then 130.5 in seedlings from wetted seeds that were irradiated for 45 min, while the number of leaves were recorded to be 97.5 and 10.33 in seedlings from dry seeds that were irradiated for 25 and 45 min, respectively. The highest leaf weights were recorded to be 2.44 g and 2.47 g in seedlings from dry seeds that were irradiated for 25 and 45 min, respectively. The leaf weights were recorded to be 2.28, 2.29, and 2.19 g in seedlings from wetted seeds that were irradiated for 0, 25, and 45 min, respectively.

Effect of laser irradiation on moringa genome using ISSR marker

Twelve primers were able to form amplified bands from control and treated seeds. A total of 172 amplified loci

Table 3 Mean performance values of moringa under the effect of He–Ne laser irradiation

Seeds	Time (min)	Shoot length	± SD	Root length	± SD	No. of leaves	± SD	Shoot weight	± SD	Root weight	± SD	Leaves weight	± SD
Dry	0	35.60^{e}	0.2	14.65^{d}	1.15	57.50^{g}	0.5	2.89^{d}	0.03	6.75^{c}	0.17	1.43^{b}	0.09
	25	34.65^{f}	0.15	16.65^{c}	0.35	97.50^{d}	0.5	4.05^{c}	0.39	7.68^{b}	0.21	2.44^{a}	0.36
	45	47.43^{c}	0.05	19.90^{a}	0.00	100.33^{c}	1.53	4.24^{c}	0.05	7.91^{b}	0.72	2.47^{a}	0.11
	90	35.50^{e}	0.11	16.20^{c}	0.00	61.50^{f}	0.5	2.35^{e}	0.22	9.06^{a}	0.12	1.55^{b}	0.25
Wetted	0	38.15^{d}	0.15	16.60^{c}	0.40	149.50^{a}	0.5	3.05^{d}	0.19	7.99^{b}	0.04	2.28^{a}	0.04
	25	65.70^{a}	0.00	17.55^{b}	0.35	149.50^{a}	1.50	6.61^{a}	0.35	9.21^{a}	0.06	2.29^{a}	0.02
	45	53.10^{b}	0.2	13.25^{e}	0.15	130.50^{b}	1.53	4.65^{b}	0.19	5.70^{d}	0.15	2.19^{a}	0.22
	90	32.25^{g}	0.05	13.30^{e}	0.20	79.50^{e}	1.50	1.60^{f}	0.01	3.10^{e}	0.35	1.06^{c}	0.13
LSD 0.05		0.226		0.853		2.014		0.384		0.485		0.345	

For all value in each column with the same letter, the difference between the means is not statistically significant
LSD least significant difference at 0.05

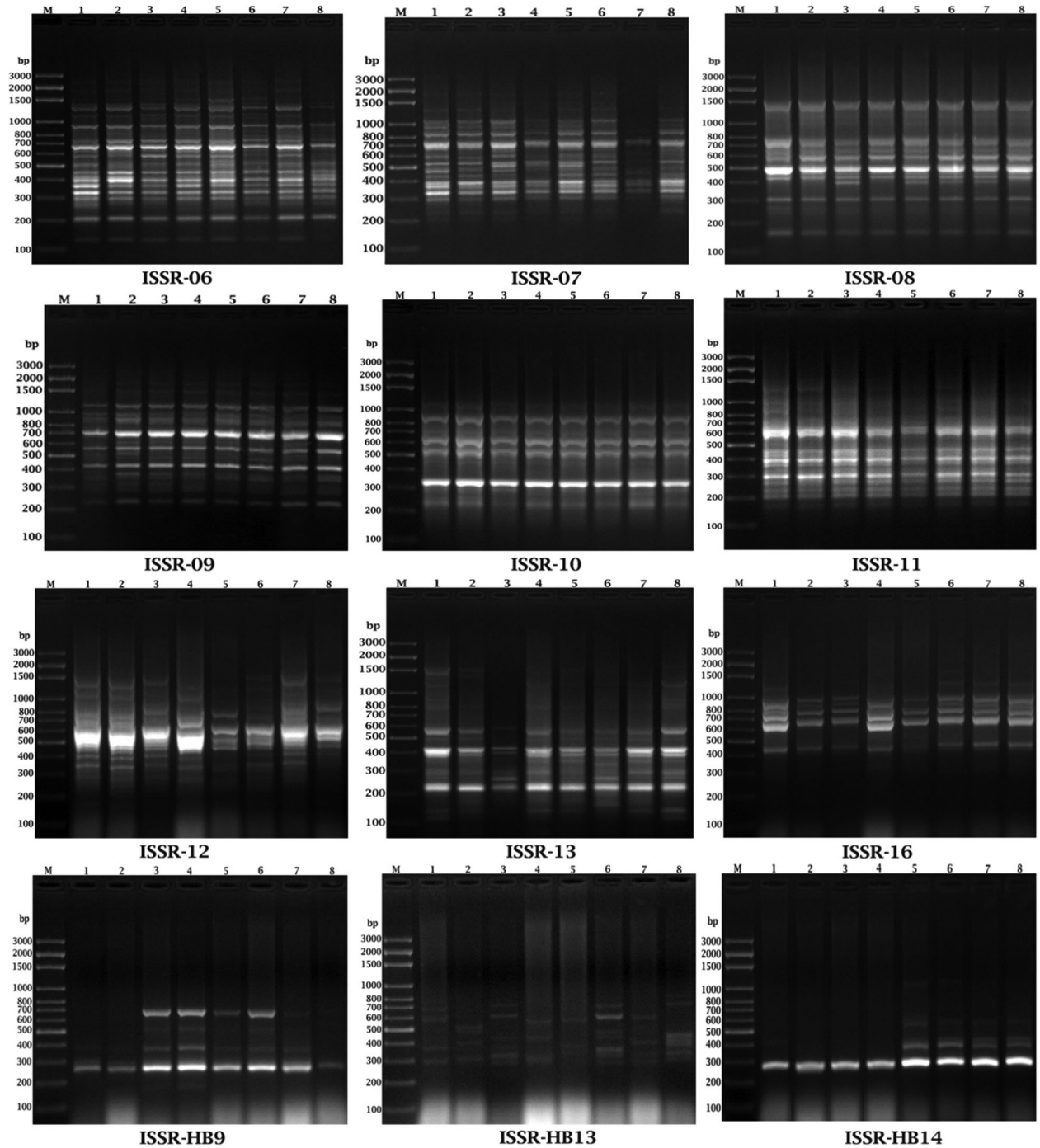

Fig. 2 The effect of different time exposure of He–Ne laser irradiation on genomic DNA for dry and wet moringa seeds using ISSR analysis. Lane1 = dry seed control, lanes 2, 3, and 4 = dry seeds treated at (25, 25, and 90 min, respectively), and lane 5 = wetted seed control, lanes 6, 7, and 8 wetted seeds treated at (25, 25, and 90 min, respectively), M is the molecular weight DNA marker

(bands) from the twelve primers were identified in the samples with molecular size ranging from 148 to 1618 bp (Fig. 2 and Tables 4 and 5). A total of 172 amplified bands produced 91 polymorphic bands with 19 unique bands mostly from wetted seeds (10 bands). The average of polymorphism in the studied samples was 52.91% (Table 4).

The maximum ISSR polymorphic bands compared to control were recorded 40 and 39 at exposure time 25 min in wetted seeds and 45 min of dry seeds, respectively (Table 5).

Table 4 The effect of different time exposure of He–Ne laser irradiation on genomic DNA of dry and wetted moringa seeds using ISSR analysis

Ser. no.	Primer	Allele size range (bp)	Total number of bands	Monomorphic bands	Polymorphic bands	Unique bands	Polymorphism percentage
1	ISSR-6	148–1579 bp	24	14	10	(232 bp) at 90 min (dry) (546 bp) at 0 min (wetted)	41.6
2	ISSR-7	202–1413 bp	21	12	9	(673 bp) at90 min (wetted) (446 bp) at 45 min (wetted) (204 bp) at 0 min (dry)	42.9
3	ISSR-8	168–1441 bp	12	11	1	0	8.33
4	ISSR-9	206–1609 bp	17	13	4	0	23.5
5	ISSR-10	225–1078 bp	10	9	1	(251 bp) at 45 min (wetted)	10
6	ISSR-11	188–1264 bp	13	10	3	(867 bp) at 0 min (dry)	23.1
7	ISSR-12	254–1491 bp	17	3	14	0	82.4
8	ISSR-13	152–1471 bp	21	5	16	(1333 bp) at 25 min (dry) (916 bp) at 0 min (dry) (573 bp) at 45 min (dry) (310, 164 bp) at 25 min (wetted) (285 bp) at 45 min (wetted)	76.2
9	ISSR-16	438–1618 bp	11	1	10	(1681 bp) at 25 min (wetted)	90.91
10	ISSR-HB9	201–674 bp	7	1	6	(201 bp) at 90 min (dry)	85.7
11	ISSR-HB13	288–871 bp	12	-	12	(524, 418 bp) at 25 min (dry)	100
12	ISSR-HB14	305–1189 bp	7	2	5	(1189 bp) at 25 min (wetted) (1092 bp) at 0 min (wetted)	71.4
Total number of bands		–	172	81	91	19	52.91
Average		–	14.33	6.75	7.58	1.6	–

The percentage of genomic template stability (GTS %) values were calculated in Table 5. It recorded a maximum value 83.72% at 25 min (dry seeds) followed by 79.65% at 45 min (wetted seeds) and 90 min (dry and wetted seeds) and 77.32% at 45 min (dry seeds), while the minimum value was recorded 76.74% at 25 min (wetted seeds).

Effect of laser irradiation on protein profile (SDS-PAGE)

The application of laser irradiation (He–Ne) for 25, 45, and 90 min caused a significant effect on the expression of some genes that lead to changes in protein banding patterns of moringa as shown in (Fig. 3 and Table 6). The total number of 32 protein bands was recorded. The main polypeptide bands were located between 27 and 185 kilodaltons (KDa). Most changes were represented in the appearance of a few new bands with molecular weight (Mw) of 155 and 76 KDa in both dry and wetted seeds from all treatments and disappearance of some bands with Mw 120, 59, and 57 KDa from some treatments of dry and wetted seeds (Table 6).

Also, from Fig. 3 and Table 6, it was concluded that at 25 min and 90 min treatments, the maximum number of protein bands were recorded 31 bands at 25 and 90 min in dry and wetted seeds, respectively. While the minimum number of protein bands were recorded 29 bands at 90 min, 45 min (dry seeds), and 25 min (wetted seeds) treatments. The percentage of polymorphic bands (polymorphism) was recorded 15.6% in both dry and wetted seeds.

Discussion

Effect of laser irradiation on agro-morphological criteria

The present investigation revealed that He–Ne laser treatments on dry and wetted seeds at different exposure times (0, 25, 45, and 90 min) had a significant effect on root and shoot (lengths and weights) and leaves (number and weight) as shown in Fig. 1 and Tables 2 and 3. It was observed from agro-morphological criteria values that laser exposure enhances the values of agro-morphological criteria of wetted seeds compared with dry seeds. The recommended exposure time of He–Ne laser was recorded 25 min in wetted seeds while in dry seeds, it was recorded 45 min; these exposure times gave the best agro-morphological values in most agro-morphological criteria.

Recently, it was observed that the physical methods had a biostimulatory role in a majority of agricultural crop plants. He–Ne laser was considered the most significant biophysical methods for enhancing the plant seed germination, different growth parameters, and plant development (rendering them safe and

Table 5 Number of new appeared (+) and disappeared (–) bands as compared to control and genomic template stability (GTS) percentage of moringa seedlings pre-treated with laser light He–Ne using twelve ISSR primers

Primer		Dry seeds			Wetted seeds			Cont
		25 min	45 min	90 min	25 min	45 min	90 min	
ISSR-6	+	491	1107, 266	1579, 266, 232	1107, 266	657	657	24
	–	0	657, 257	257	546, 491, 417, 257	1579, 546, 417	1579, 600, 546, 491	
ISSR-7	+	0	509, 202	0	0	446	673	21
	–	204	204	1413, 473, 431, 204	509	1413, 998, 473, 431, 509, 202	509, 202	
ISSR-8	+	0	398	0	0	0	0	12
	–	0	0	0	0	0	398	
ISSR-9	+	366	366	366	463	507, 463	366	17
	–	463	0	507	612	0	612	
ISSR-10	+	0	0	0	0	251	0	10
	–	0	0	0	0	0	0	
ISSR-11	+	0	0	0	1264, 428	0	0	13
	–	867, 428	1264, 867	1264, 867	0	0	0	
ISSR-12	+	390, 323	1341, 744	323	1491, 1251, 744, 390, 354	1341, 744, 684, 496, 402, 345, 254	1491, 684, 402	17
	–	402, 345	1251, 1972, 684	1251, 818, 402, 345, 307	818	0	307	
ISSR-13	+	1333, 177	573, 251	329, 177	251, 310, 164	1158, 285, 152	1185, 801, 251, 152	21
	–	1158, 916, 801	1435, 1158, 916, 827, 801, 471, 152	1435, 916, 827, 471	329, 177	280	177	
ISSR-16	+	987, 675	987, 675	0	1618, 1270, 900, 479, 438	1270, 900, 479, 438	1270, 900, 479, 438	11
	–	1270, 703, 607	703, 607	1270, 807	0	0	0	
ISSR-HB9	+	0	674, 653, 504	674, 653, 504, 201	504	0	0	7
	–	380	0	0	0	653	674, 653, 380, 300	
ISSR-HB13	+	524, 476, 418	632, 440, 349, 288	440, 321	733, 632, 476, 440, 349, 321, 288	632, 288	871, 733, 476, 321	12
	–	871, 733, 595	871, 595	871, 733	595, 398	595	595	
ISSR-HB14	+	0	0	0	1189, 423	423	423	7
	–	423	423	423	1092	1092	1092	
Total number of bands		28	39	35	40	35	35	172
GTS%		83.72%	77.32%	79.65%	76.74%	79.65%	79.65%	100%

(+) appearance of new bands, (–) disappearance of normal bands

friendly to the environment) (Asghar et al. 2017). Similar results were investigated by Urva et al. (2017), who stated that seedling growth parameters for 10-day-old seedlings were enhanced under the effect of 25, 50, and 75 mJ low-power continuous-wave laser light in *Moringa oleifera*, and optimum laser energy level has more acceleratory effect since at three laser energy levels, the responses were significant.

He–Ne laser irradiation caused a significant effect on seed germination, growth parameters, enzyme activity, and thermodynamic properties of the *Triticum aestivum* plant (Jamil et al. 2013). Similarly, continuous laser He–Ne wave with low power had an effect on the thermodynamics of seed, the rate of germination, and the activity of enzymes during germination process in sunflower (Perveen et al. 2010).

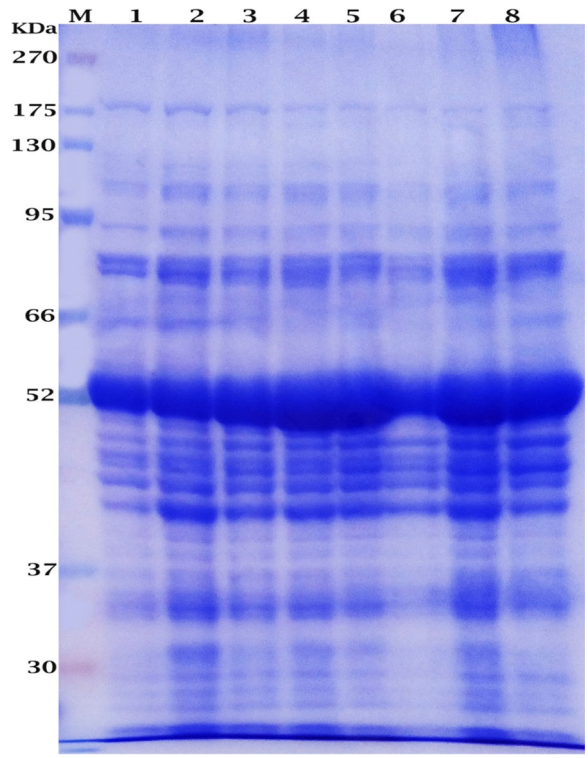

Fig. 3 SDS-PAGE soluble protein of moringa seedlings pre-treated with laser light. Lane1: control (untreated with laser), pre-treated dry seeds plants for: lane 2, 25 min; lane 3, 40 min; and lane 4, 90 min. Lane 5: control (untreated with laser), pre-treated wetted seeds plants for: lane 6, 25 min, lane 7, 40 min; and lane 8, 90 min. M is the molecular weight protein marker

Effect of laser irradiation on moringa genome using ISSR marker

ISSR profiles evaluated remarkable differences between the control and He–Ne laser-treated seedlings (dry and wetted) with different primers (Fig. 2 and Tables 4 and 5). Twelve primers were used to compare the control with dried and wetted moringa seeds treated with He–Ne laser at different times. Atienzar and Jha (2006) and Ozturk et al. (2010) stated that the difference in DNA profile reflected the nucleotide substitution or alterations (point mutations) to complex chromosomal rearrangements. The results indicated disappearance (–) of normal bands and appearance of new bands (+) in comparison with the control. A total of 172 amplified bands produced 91 polymorphic bands, and 19 bands of them were unique bands mostly from wetted seeds (10 bands) with 52.91% polymorphic average.

The disappearance of bands may be referred to the presence of DNA photoproducts (e.g., pyrimidine dimers), which can reduce amplification of DNA in the PCR reaction (Donahue et al. 1994 and Atienzar et al. 2000). The disappearance of the amplified bands mainly affected the high molecular weight bands because the odds of obtaining DNA photoproducts increase with the length of the amplified fragment (Atienzar et al. 2000). On the other hand, mutations (new annealing events) can only be responsible for the appearance of new bands if they occur at the same locus. A minimum of 10% of mutations may be required to get a new PCR product (Atienzar et al. 2000). Thus, the new loci could be referred to mutations while DNA damage leads to the disappearance of loci (bands).

GTS % is related to the level of DNA damage, the efficiency of DNA repair, and replication. DNA polymorphism detected by the ISSR technique in different treatments as compared with the control could be used as an investigation tool for further sequencing studies and as a beneficial technique. Changes in the ISSR profile induced by laser treatments can be regarded as changes in genomic DNA template stability, and these genetics effects can be directly compared with alteration in other parameters.

It was found that the minimum value of GTS % was 76.74%, and this value was recorded at laser exposure time 25 min on wetted seeds. There is an inverse relationship between GTS % and polymorphism value; this means that the wetted seeds' exposure to laser radiation for 25 min recorded minimum GTS % and maximum polymorphism values. This may be related to the improvement some of agro-morphological criteria such as shoot length and weight, root weight, and number of leaves.

GTS % indicated changes in ISSR profiles to compare their changes with different growth parameters (root and shoot length) in moringa plants. DNA analysis considered a more sensitive test, since it is able to detect temporary DNA changes that may not finally appear as mutations (Labra et al. 2003).

Effect of laser irradiation on proteins profile (SDS-PAGE)

The alterations in the electrophoretic patterns of leaf proteins are indicative of the ability of both dry and wetted seed treatments to alter the gene expression of plant. Protein profile changes were represented in the appearance of few new bands with molecular weight (Mw) 155 and 76 KDa in both dry and wetted seeds at all treatments and disappearance of some bands such that having Mw 120, 59, and 57 KDa for some treatments of dry and wetted seeds (Fig. 3 and Table 6). Some electrophoretic bands disappeared due to the deletion of their corresponding genes (El-Khallal and Mohamed 2004). On the other hand, the appearance of new characteristic bands could be explained on the basis of the mutational event at the regulatory system of the unexpected gene(s) that activate it (Abdelsalam et al. 1993 and 1997 and El-Nahas

Table 6 SDS-PAGE of soluble proteins of two weeks old of moringa seedlings pre-treated with laser light

Number of bands	MW (KDa)	Dry seeds					Wetted seeds				
		Control	25 min	45 min	90 min	Polymorphism	Control	25 min	45 min	90 min	Polymorphism
1	185	+	+	+	+	M	+	+	+	+	M
2	155	–	+	+	+	P	-	+	+	+	P
3	120	+	–	–	–	P	+	-	–	–	P
4	115	+	+	+	+	M	+	+	+	+	M
5	112	+	+	+	+	M	+	+	+	+	M
6	106	+	+	+	+	M	+	+	+	+	M
7	102	+	+	+	+	M	+	+	+	+	M
8	93	+	+	+	+	M	+	+	+	+	M
9	82	++	++	++	++	M	++	++	++	+	M
10	79	++	++	++	++	M	++	++	++	+	M
11	76	–	+	+	+	P	-	+	+	+	P
12	70	+	+	+	+	M	+	+	+	+	M
13	65	+	+	+	+	M	+	+	+	+	M
14	59	+	+	-	-	P	+	-	–	+	P
15	57	+	+	-	-	P	+	-	+	+	P
16	52	+++	+++	+++	+++	M	+++	+++	+++	+++	M
17	49	+	+	+	+	M	+	+	+	+	M
18	47	++	++	++	++	M	++	++	++	++	M
19	45	+	+	+	+	M	+	+	+	+	M
20	44	++	++	++	++	M	++	++	++	++	M
21	43	++	++	++	++	M	++	++	++	++	M
22	41	++	++	++	++	M	++	++	++	++	M
23	39	+	+	+	+	M	+	+	+	+	M
24	38	+	+	+	+	M	+	+	+	+	M
25	37	+	+	+	+	M	+	+	+	+	M
26	36	+	+	+	+	M	+	+	+	+	M
27	35	++	++	++	++	M	++	++	++	++	M
28	34	++	++	++	++	M	++	++	++	++	M
29	31	++	++	++	++	M	++	++	++	++	M
30	29	+	+	+	+	M	+	+	+	+	M
31	28	+	+	+	+	M	+	+	+	+	M
32	27	+	+	+	+	M	+	+	+	+	M
Total number of bands		30	31	29	29	% of poly-morphism = 15.6	30	29	30	31	% of poly-morphism = 15.6
Mean of polymorphism		15.6									

P polymorphic bands, *M* monomorphic bands

2000). Also, Telma et al. (2008) documented that the treatment with a short term of oxidative stress lead to overexpression of different genes encoding heat shock proteins. Changes in the band intensity may be resulted by certain mutational events that would have occurred in the regulatory genes, which would lead to inhibition, attenuation, or constitutive gene expression. Therefore, the corresponding bands become faint or become more intense. This conclusion is in accordance with Abdelsalam et al. (1993) and Gamal El-Din et al. (1988), as they stated that increased band intensity is due to duplication of chromosomal complement in treated plants. These variations included appearance of new bands, disappearance of some bands, and changes in band intensity in comparison with control plants. The same results were

reported by the other authors (Fayez 2000 and Telma et al. 2008).

Conclusions

It was conducted from the present research that He–Ne laser treatment of wetted seeds achieves biostimulation in many aspects of agro-morphological criteria. In general, most agro-morphological criteria increased especially with wetted seed group pre-laser treatments (25 min) in comparison with the dry seed groups. ISSR analysis is a highly sensitive method for the detection of DNA alteration induced by laser treatments. The exposure of dry and wetted seeds to He–Ne laser at different times affects the genomic template stability percentage (GTS %); GTS % considers a highly sensitive parameter compared with the traditional methods such as root and shoot lengths. Also, the laser treatments have a great effect on gene expression by switch on or off for some genes that lead to presence or absence of certain proteins, respectively. Finally, the SDS-PAGE, ISSR analysis, and other growth parameters are considered an important tool for identifying DNA profile change induced by laser treatments.

Abbreviations

A: Adenine; AFLPs: Amplified fragment length polymorphisms; C: Cytosine; df: Degrees of freedom; DNA: Deoxy ribonucleic acid; EDTA: Ethylenediaminetetraacetic acid; G: Guanine; GTS%: Genomic template stability percentage; He–Ne: Helium–neon; ISSRs: Inter-simple sequence repeats; LSD: Least significant difference at 0.05; M: Monomorphic band; MS: Mean square; ns: Non-significant; P: Polymorphic band; RAPDs: Random amplified polymorphic DNAs; RFLPs: Restriction fragment length polymorphisms; SD: Standard deviation; SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis; SSRs: Simple sequence repeats (microsatellites); T: Thymine

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Authors' contributions

SAO and WAR preformed the laboratory analysis. SAO and WAR wrote the paper, performed the data, and coordinated the data collection. So, this work was carried out in collaboration between the two authors. Both authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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