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RESEARCH

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Physiological and productivity responses of fenugreek to foliar spraying with molybdenum and cysteine

Mahmoud Ahmed Khater[1*], Mohamed Salama Abd El Wahed[1] and Dina Mohamed Salama[2]

Abstract

Background Fenugreek (*Trigonella foenum-graecum* L.) is one of the oldest cultivated medicinal plants, as well as it is rich with medicinal antioxidant components. The effect of foliar spraying with molybdenum, cysteine on growth, quality and productivity of fenugreek was evaluated in a conducted greenhouse experiment during 2019/2020 and 2020/2021 growing seasons.

Results Results showed that there were different effects of molybdenum, cysteine and their interaction on morphological characters, photosynthetic pigments, seed yield and yield components and some chemical components of fenugreek plants. However, there was a variety of polypeptide bands with different molecular weights ranging from 18.94 to 250 kDa and was detected with polymorphic ratio 61.91%. Moreover, multiple bands varied in their molecular weight were detected in all tested plants using ISSR primers. The polymorphic percentage reached 70.00, 73.33, 76.19, 72.22, 77.77 and 70.59% in all tested plants with primer IS-01, Is-02, IS-03, IS-04, Is-05 and IS-06, respectively.

Conclusions It was cleared that foliar spraying with molybdenum, cysteine and their interaction enhanced and improved growth, quality and productive characters of fenugreek. Moreover, there were molecular changes in fenugreek under study and generated bands using the six ISSR primers.

Keywords *Trigonella foenum - graecum* L., Foliar spraying, Molybdenum, Cysteine

Background

Trigonella foenum - graecum (L.) is belonging to family Fabaceae and commonly known as Fenugreek. It is one of the oldest cultivated medicinal plants, so that, it cultivated widely over all the world, i.e., Egypt, India, China, Greece, Ethiopia, Morocco, Ukraine, Turkey, etc. (Petropoulos 2002). However, seeds and leaves of fenugreek are rich with medicinal components (alkaloids, steroids, glycoside, volatile components,polyphenoland amino acids). Moreover, its seeds are rich in vitamin E (antioxidant) that protects body tissue from damage caused by substances called free radicals which can harm cells, tissue and organs (Aheret al. 2016). Diosgenin present in fenugreek prevents cell growth and induced apoptosis in the H-29 human colon cancer cell line (Raju and Bird 2006). Polyphenolic extract of fenugreek seed acts as a protective agent against ethanol-induced abnormalities in the liver (Kaviarasan and Anuradha 2007).

Molybdenum (Mol.) is a constituent of the nitrogenase enzyme and every bacterium which fixes nitrogen needs molybdenum during the fixation process. Molybdenum has a positive effect on yield quantity, quality and nodule forming in legume crops. Application of molybdenum into the soils has increased the contents of potassium, phosphorus and crude protein (Anonymous 2005). Moreover, inoculation with phosphate solubilizing

*Correspondence:
Mahmoud Ahmed Khater
mahmoudkhater2000@gmail.com
[1] Botany Department. Agriculture and Biological Institute, National Research Centre, Giza, Egypt
[2] Vegetable Research Department, Agriculture and Biological Institute, National Research Centre, Giza, Egypt

bacteria increased number of branches per plant, number of flowers per plant, number of pods per plant and dry weight/plant of cowpea (Harshini et al. 2022).

Cysteine (Cys.) is the metabolic precursor of essential biomolecules such as vitamins, cofactors, antioxidants such as glutathione which plays an important role in plant stress responses and toxic levels of heavy metals responses (Alvarez et al. 2012). Foliar application of cysteine mitigated the unfriendly effects of NaCl stress on flax plants. Moreover, cysteine enhanced the growth parameters, photosynthetic pigments, amino nitrogen, total phenols, and new polypeptides in NaCl-stressed plants. (Hebat-Allah and Shifaa, 2022). This study was undertaken to estimate and evaluate the effect of foliar spraying with molybdenum, cysteine and their interactions on growth, quality and productivity traits of fenugreek plants.

Methods

The present study was conducted during the two winter growing seasons of 2019/2020 and 2020/2021 under the normal weather conditions at the un-controlled insect free cage of botany department National Research Centre, Dokki, Cairo, Egypt. Fenugreek seeds were obtained from Legume Research Department, Field Crop Institute, Agricultural Research Center, Giza, Egypt. Fenugreek seeds were sown directly in pots, filled with a mixture of peatmoss and sand, and arranged in a split-plot design with three replications. The physical and chemical properties of studied soil were determined and presented in Table 1. Three concentrations of molybdenum (0, 50 and 100 ppm) in the main plots and four concentrations of cysteine (0, 25, 50 and 100 ppm) in the sub-plots were used in this study.

Recorded data

Plant growth parameter

Plant height (cm), number of pods/plant, plant fresh and dry weights (g), seeds number/pod, seeds weight/plant and 100-seed weight were estimated at harvest stage.

Chemical analysis

Total chlorophyll content and indoles concentrations (ppm) were determined in fenugreek leaves in both vegetative and harvest stage. Moreover, both flavonoids (mg/g) and nitrogen percentage were determined in plant and seeds.

Total nitrogen (%) was determined in dry seeds according to the methods described by Chapman and Pratt (1978) and was multiplied by 6.25 to calculate protein% (Gendy 2013).

Table 1 Physiological and chemical analysis of soil

Characteristics	Value
Physical properties	
Particle size distribution	
Coarse Sand%	73.8
Fine Sand%	15.5
Silt%	6.5
Clay%	4.2
Texture soil	Sandy
Chemical properties	
Organic matter content%	1.24
pH	7.8
EC ds/m	0.74
Cations meq/L	
Na^+	4.15
K^+	0.23
Ca^{++}	1.84
Mg^{++}	1.25
Anions meq/L	
HCO_3	0.64
CO_3	Nil
SO_4	0.93
Cl^-	5.6

Extraction of seed storage proteins and SDS-PAGE analysis

Fenugreek seeds were ground to fine powder with mortor and pestle to extract proteins and the extraction technique was according to Saraswati et al. (1993). Protein profiling of seed samples was performed using SDS-PAGE as described by Laemmli (1970). Molecular weight of the protein bands was determined by simultaneous running of standard molecular weight proteins.

DNA isolation

The genomic DNA was isolated from 1 g of young and soft leaves of plants under study using CTAB procedure (Doyle and Doyle 1987). DNA quality was checked using 1.0% agarose gel electrophoresis.

ISSR amplification

Six different ISSR primers (Table 2) were used in this study. ISSR amplification reactions were carried out in 15 µl volume containing 1 µl DNA (40 mg), 7.5 µl Master Mix, 1 µl template DNA and 1 µl primer. The amplification reaction consisted of an initial denaturation step at 94 °C for 7 Min., followed by 35 cycles of 30 Sec. at 94° C (denaturation), 45 Sec. at 52° C (annealing) and 2 Min. at 72° C (extension) followed by a final extension step at 72 °C for 5 Min. Amplification products were electrophoresed on 1.5% agarose in 1×TBE buffer. The gels

Table 2 ISSR primer sequences used for DNA fingerprinting of fenugreek plants under study

	Primer code	Primer sequence (5′ → 3′)
1	IS-01	(CT)8 GC
2	IS-02	(CT)8 TG
3	IS-03	(GT)6 GC
4	IS–04	(GT)7 TCC
5	IS–05	(GAG)3 GC
6	IS–06	(CT)8 AC

were stained with ethidium bromide and documented using gel documentation system. Each experiment was repeated twice with each primer and those primers which gave reproducible fingerprints were considered for data analysis.

Amplified fragments were scored manually for the presence (+) or absence (−) of homologous bands to develop a binary matrix of different ISSR phenotypes.

Polymorphism % was calculated according to this equation:

$$\text{Polymorphism percentage (PB\%)} = (\text{UB} + \text{PB})/\text{Total bands}$$

where UB = Number of unique bands, PB = Number of polymorphic bands.

Statistical analysis

All collected data were statistically analyzed by analysis of variance according to split-plot design, and differences among means were determined by least significant differences (LSD) according to Silva and Azevedo (2016).

Results

Effect of Mol. and Cys. on morphological criteria of fenugreek at harvest stage

Collected data recorded the effect of both Mol. and Cys. with different concentrations on morphological, yield and yield components criteria, i.e., plant height (PH), pods number/plant(PN), seed number/pod (SN), seed weight/plant (SW), 100-seed weight (100-SW), plant fresh weight (FW) and plant dry weight (DW).

Data presented in Table 3 showed that there were different effects of both Mol. and Cys. and their interaction on morphological characters of fenugreek plants, i.e., plant height (PH), it noticed that there was a significantly positive effects by increasing Mol. concentration on plant height of fenugreek, and the highest value (60.39 cm) was recorded with 100 mg/L Cys. Moreover, under all Cys. concentrations, these positive effects were subsequent according to normal curve (increasing plant height by increasing mol. conc.) up to 50 mg/L that recorded the highest value (53.54 cm) and subsequently by reduction (49.317 cm) with 100 mg/L Mol.

However, the interaction between Mol. and Cys concentrations on plant height recorded variable variants. It was cleared that the best interaction was (66.00 cm) with 50 mg/L of Mol. and 100 mg/L Cys. (Table 3).

It was cleared that the effect of both Mol. and Cys. and their interactions on plant fresh weight (FW)(g) was seemed to be in the same trend of previous character (PH) and the best fresh weights were (2.85 and 2.63 g) with 50 mg/L of both Mol. and Cys., respectively (Table 2). Moreover, the desirable effect was achieved at the interaction of 50 mg/L of both Mol. and Cys., which was the highest fresh weight (3.07 g). Other else, it noticed that plant dry weight (DW) was as previous characters (FW).

Table 3 Effect of Mol. and Cys. on morphological criteria of fenugreek at harvest stage

	Plant height (cm)					Plant fresh weight (g)					Dry weight/plant (g)				
	Cys. (mg/l)				Mean	Cys. (mg/l)				Mean	Cys. (mg/l)				Mean
Mol. (mg/l)	0	25	50	100		0	25	50	100		0	25	50	100	
0	31.33	50.0	52.67	58.17	48.04	1.63	2.33	2.80	2.03	2.20	0.44	1.13	1.07	0.83	0.87
50	39.67	48.50	60.00	66.00	53.54	1.87	2.80	3.07	2.80	2.63	0.47	1.47	1.57	0.87	1.09
100	36.67	41.33	61.67	57.00	49.17	1.53	2.62	2.67	2.57	2.34	0.40	0.73	1.47	0.67	0.82
Mean	35.89	46.61	58.11	60.39		1.68	2.58	2.85	2.47		0.44	1.11	1.37	0.79	
Mol. LSD	2.59					0.164					0.051				
Cys. LSD	2.99					0.190					0.059				
Mol. X Cys. LSD	5.18					0.328					0.103				

Table 4 Effect of Mol. and Cys. on yield and yield components of fenugreek at harvest stage

	Pods number/plant					Seeds number/pod					Seeds weight/plant (g)					100-seed weight (g)				
	Cys. (mg/l)				Mean	Cys. (mg/l)				Mean	Cys. (mg/l)				Mean	Cys. (mg/l)				Mean
Mol. (mg/l)	0	25	50	100		0	25	50	100		0	25	50	100		0	25	50	100	
0	3.67	6.00	6.00	5.33	5.25	7.33	9.67	10.33	10.00	9.33	0.75	1.60	1.30	1.30	1.24	2.23	1.99	2.01	2.64	2.22
50	4.67	5.00	6.33	8.00	6.00	8.67	9.33	10.33	10.00	9.58	0.84	1.07	1.77	1.23	1.23	2.32	1.98	1.87	1.94	2.03
100	3.33	5.33	6.00	7.87	5.63	7.67	9.33	9.67	9.67	9.08	0.75	1.10	2.71	1.27	1.46	2.17	1.98	1.99	1.95	2.02
Mean	3.89	5.45	6.11	7.07		7.88	9.45	10.11	9.89		0.78	1.26	1.93	1.27		2.24	1.99	1.95	2.18	
Mol. LSD	0.729					0.491					0.112					0.175				
Cys. LSD	0.842					0.567					0.130					0.201				
Mol. X Cys. LSD	1.458					0.981					0.225					0.349				

Effect of Mol. and Cys. on yield and yield components of fenugreek at harvest stage

Data in Table 4 study the effect of both Mol and Cys on yield and its components of fenugreek plants (number of pod/ plant (NP), number of seeds/pod, seeds weight/ plant and 100 seeds weight). Meanwhile, data illustrated that there were significant effects for using both Mol and Cys lonely and with interaction. However, the best effect of Mol. and Cys. on number of pods was (6.00 and 7.07) that achieved using the concentration of (50 mg/l) Mol. under all Cys concentrations, and (100 mg/l Cys.) under all Mol concentrations, respectively. Also, the best interaction (8.00) was achieved with 50 and 100 mg/l of both Mol. and Cys., respectively, Table 4

Moreover, the highest numbers of seeds /pod were (9.58 10.11, and 10.33) that recorded with 50 mg/l for both Mol., Cys. and its interaction, respectively. On the other hand, there was a highly significant increment in seed yield per plant under all treatments comparing with control. Whereas, the highest seed yields (1.93, 1.46, and 2.71 g) were achieved by using 50 mg/l., Cys., 100 mg/l., Mol and their interaction, respectively, Table 4**.**

Effect of Mol. and Cys. on chlorophyll contents of fenugreek at both vegetative and harvest stage

Generally, there were markedly significant increases in chlorophyll contents by increasing Mol. concentrations up to 100 mg/l (9.02 and 5.52 mg/g) in both stages (vegetative and harvest) comparing with control plants (8.22 and 4.65 mg/g). On the other hand, there were significant decreases in chlorophyll contents under all Cys. concentrations as compared with control plants. Moreover, the highest chlorophyll contents recorded (10.72 and 5.97 mg/g) were recorded using 50 mg/l Mol. interacting with 50 mg/ml Cys. in both vegetative and harvest stage, respectively, Table 5.

Effect of Mol. and Cys. on chemical constituents of fenugreek at vegetative and harvest stage

The effect of both Mol. and Cys and their interactions on chemical components of fenugreek plants and seeds are listed in Tables 6 and 7. Generally, data in Tables 5 and 6 showed that spraying plant with Mol. and Cys. increased all studied chemical components (N%, Indoles and Flavonoids) in plants and seeds. However, nitrogen percentages were increased in both plants and seeds and reached the maximum increment at 50 ppm for both Mol. and

Table 5 Effect of Mol. and Cys. on chlorophyll concentration in fenugreek at vegetative and harvest stage

	Chlorophyll Conc. (mg/g) V					Chlorophyll Conc. (mg/g) H				
	Cys. (mg/l)				Mean	Cys. (mg/l)				Mean
Mol. (mg/l)	0	25	50	100		0	25	50	100	
0	9.73	7.52	8.71	6.91	8.22	6.64	3.22	4.87	3.87	4.65
50	9.98	7.82	9.80	7.90	8.88	6.74	3.63	4.92	4.65	4.90
100	9.87	8.17	10.72	7.32	9.02	6.66	4.62	5.97	4.94	5.52
Mean	9.86	7.84	9.75	7.37		6.64	3.83	5.25	4.83	
Mol. LSD	0.0321					0.0199				
Cys. LSD	0.0371					0.0230				
Mol. X Cys. LSD	0.0642					0.0398				

Table 6 Effect of Mol. and Cys. on chemical constituents of fenugreek plants at vegetative stage

	Nitrogen %					Indoles Conc. (ppm)					Flavonoids (mg/g) ofs				
	Cys. (mg/l)				Mean	Cys. (mg/l)				Mean	Cys. (mg/l)				Mean
Mol. (mg/l)	0	25	50	100		0	25	50	100		0	25	50	100	
0	0.57	0.45	0.58	0.55	0.54	53.58	182.27	183.38	207.33	156.64	1.73	2.00	2.16	2.49	2.09
50	0.67	0.47	0.58	0.57	0.57	53.89	205.02	233.78	230.17	180.71	1.74	2.21	2.26	2.65	2.21
100	0.58	0.44	0.58	0.48	0.52	55.75	220.92	289.63	241.08	201.32	1.73	2.25	2.60	2.62	2.30
Mean	0.61	0.45	0.58	0.53		53.64	202.74	235.60	226.20		1.73	2.15	2.34	2.59	
Mol. LSD	0.017					0.473					0.0034				
Cys. LSD	0.019					0.547					0.0040				
Mol. X Cys. LSD	0.033					0.947					0.0069				

Table 7 Effect of Mol. and Cys. on chemical constituents of fenugreek seeds at harvest stage

	Nitrogen %					Indoles Conc. (ppm)					Flavonoids (mg/g)				
	Cys. (mg/l)				Mean	Cys. (mg/l)				Mean	Cys. (mg/l)				Mean
Mol. (mg/l)	0	25	50	100		0	25	50	100		0	25	50	100	
0	0.19	0.12	0.22	0.20	0.18	87.86	84.90	60.31	58.28	72.83	0.58	0.66	0.40	0.38	0.50
50	0.21	0.18	0.31	0.22	0.23	87.96	71.49	70.02	67.80	74.32	0.59	0.55	0.59	0.65	0.59
100	0.17	0.16	0.20	0.16	0.17	87.85	75.38	73.35	71.41	77.00	0.58	0.49	0.49	0.57	0.53
Mean	0.19	0.15	0.24	0.19		87.89	77.26	67.89	65.83		0.59	0.56	0.49	0.53	
Mol. LSD	0.0089					0.345					0.0020				
Cys. LSD	0.0102					0.399					0.0023				
Mol. X Cys. LSD	0.0177					0.691					0.0040				

Cys. These results may be due to the role of Mol. in nitrogenase system present in all nitrogen fixing organisms. Nitrogenase enzyme consist of two different enzyme protein and Mg-ATP complex. Larger enzyme protein unit contains Fe-Mo-S complex (Srivastava and Gupta 1996). In addition, Agarwala et al. (1978) found that Mol. deficiency lowers the activity of catalase enzyme but stimulate the activity of peroxidase. These results are in harmony with those reported by El-Mansi et al.(2000) on pea. Bhagiya et al. (2005) found that Mol. application at a rate of 4 kg/ha increased seeds N, P and K content. In addition, Gad (2012) reported that Mol. application to groundnut significantly increased seed mineral composition. Also, Gendy (2013) reported that foliar spraying

Table 8 Effect of Mol. and Cys. on protein profile of in fenugreek

No.	MW (KDa)	1 Cont	2 0/25	3 0/50	4 0/100	5 50/25	6 50/50	7 50/100	8 100/25	9 100/50	10 100/100	P
1	250.00	+	+	+	+	+	+	+	+	+	+	MB
2	170.75	+	+	+	+	+	+	+	+	+	+	MB
3	137.82	−	−	−	−	−	−	−	+	−	−	UB
4	130.84	+	−	+	−	+	+	−	−	+	+	PB
5	125.98	+	−	+	−	−	−	−	−	−	−	PB
6	115.15	−	+	−	+	+	+	+	−	+	+	PB
7	101.56	+	+	+	+	+	+	+	+	+	+	MB
8	77.28	+	+	−	−	−	−	−	−	−	+	PB
9	69.46	−	−	−	−	−	−	−	−	−	+	UB
10	64.04	−	−	+	+	+	+	+	+	+	−	PB
11	55.15	+	+	+	+	+	+	+	+	+	+	MB
12	46.46	−	−	+	−	−	−	−	−	−	−	UB
13	45.99	+	−	−	−	−	−	−	−	−	−	UB
14	44.99	−	−	−	−	−	+	−	−	−	+	PB
15	38.62	+	+	+	+	+	+	+	+	+	+	MB
16	32.54	−	−	+	−	+	−	+	−	+	+	PB
17	27.93	+	+	+	+	+	+	+	+	+	+	MB
18	23.95	−	−	−	−	−	−	+	−	+	−	PB
19	22.54	+	+	+	+	+	+	+	+	+	+	MB
20	21.20	−	+	−	−	−	−	−	−	−	−	UB
21	18.94	+	+	+	+	+	+	+	+	+	+	MB
Total		12	11	13	10	12	12	12	10	13	14	119

TB	MB	UB	PB	PB%
21	8	5	8	61.91%

TB = Total amplified fragments, MB = Monomorphic bands, UB = Unique bands PB = Polymorphic bands and PB (%) = Percentage of polymorphism

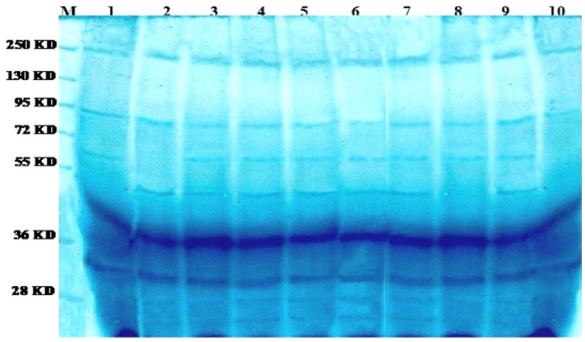

Fig. 1 Effect of Mol. and Cys. on protein profile of fenugreek

with 30 ppm Mol. increased N, P, K and protein % in fenugreek.

SDS-protein electrophoresis

Data in (Table 8) and (Fig. 1) illustrated the effect of interaction between three different concentrations of cysteine (0, 50 and 100 ppm) in a recombination with three different concentrations of molybdenum (25, 50 and 100 ppm) on protein banding patterns of *Trigonella foenum - graecum* (L.) plants by SDS–PAGE electrophoretic protein patterns. These results revealed a total of 21 polypeptide bands with different molecular weights ranging from 18.94 to 250 kDa were detected with polymorphic ratio 61.91%.

However, all bands distributed as follow: 8 bands with molecular weights (250, 170.75, 101.56, 55.15, 38.62, 27.93, 22.54 and 18.94 KDa) were monomorphic band (found in all samples), 8 bands with various molecular weights (130.84, 125.98, 115.15, 77.28, 64.04, 44.99, 32.54 and 23.95 KDa) were polymorphic bands (found in more than one sample) and there were 5 unique bands (found only in one sample) with molecular weights (137.82, 69.46, 46.46, 45.99 and 21.20 KDa) in treatments number (8, 10, 3, 1 and 2), respectively (Table 8 and Fig. 1).

Molecular markers assay

ISSR is a popular marker system, owing to its ability to detect polymorphisms without requiring the sequence information necessary for primer design. In the ISSR analysis, 6 primers were used for polymorphism screening. A total of 109 bands in the size ranged from 103.17 to 1500.00 bp were produced by examining across treated plants. The total number of bands was 109 ranged from 15 bands (Primer-IS-02) to 21 bands (Primer-IS-03) (Table 9, Fig. 2).

The scored data were analyzed and revealed that there were three types of molecular bands depending of its appearance on agarose gel. However, the total amplified fragments (TAF) that induced in the treated plants using all primers were 109 bands divided to 29 monomorphic bands (MB), 25 unique bands (UB), and the maximum part of bands was 55 polymorphic bands (PB) as shown in Table 9, Fig. 2.

Data scored in Table 9 draw the attention to molecular changes in fenugreek under study and generated bands using the six ISSR primers. However, multiple bands varied in their molecular weight were detected in all tested plants using these different primers. The polymorphic percentage reached 70.00, 73.33, 76.19, 72.22, 77.77 and 70.59% in all tested plants with primer IS-01, Is-02, IS-03, IS-04, Is-05 and IS-06, respectively. Moreover, all primers varied in inducing fragments (bands), whereas, every primer induced variable numbers of amplified bands which varied in total number, type (Monomorphic, Polymorphic and Unique) and finally in the range of molecular size of these amplified bands which varied also between primers.

Discussion

These results are in agreement with those obtained by Bakry et al. (1987), El-Mansi et al. (1994 and 2000) on pea and Kandil et al. (2013) on common bean and Gendy

Table 9 Effect of Mol. and Cys. on ISSR-markers of fenugreek plants

Primers	Marker size (bp)	Amplified bands				PB %
		TAF	MB	UB	PB	
IS-01	984.47–111.84	20	6	5	9	70.00
IS-02	998.71–103.17	15	4	3	8	73.33
IS-03	763.99–115.30	21	5	7	9	76.19
IS-04	1285.37–167.25	18	5	3	10	72.22
IS-05	1500.00–173.41	18	4	4	10	77.77
IS-06	1302.69–283.58	17	5	3	9	70.59
Total		109	29	25	55	
Average		18.17	4.383	4.17	9.17	

TAF = Total amplified fragments, MB = Monomorphic bands, UB = Unique bands PB = Polymorphic bands and PB (%) = Percentage of polymorphism

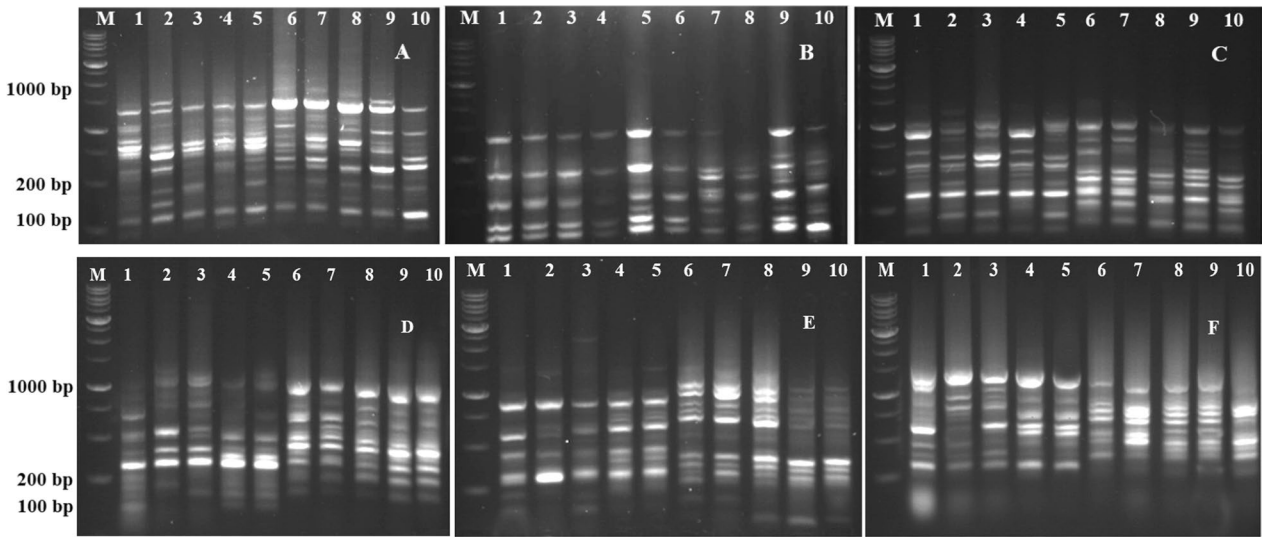

Fig. 2 PCR amplification profile generated in treated fenugreek under study. **a** IS-01, **b** IS-02, **c** IS-03, **d** IS-04, **e** IS-05, **f** IS-06. M = DNA Marker; 1 = control; 2, 3 and 4 = cys. (0 mg/l) interacting with (25, 50, and 100 mg/l mol), 5, 6 and 7 = cys (50 mg/l) interacting with (25, 50, and 100 mg/l mol), and 8, 9 and 10 = cys (100 mg/l) interacting with (25, 50, and 100 mg/l mol)

(2013) on fenugreek. They reported that spraying plants with Mo at 40 ppm significantly increased vegetative growth parameter and dry weight of different plant parts of pea and common bean.

Moreover, Gendy (2013) showed that spraying fenugreek plants with molybdenum at 30 ppm significantly affected number of pods/plant, seed yield/ plant and per hectare compared to control treatment. Spraying plants with Mo at 30 ppm was the most favorable treatment for increasing seed yield /plant and per hectare followed by spraying with 15 ppm and control treatment.

The increment in seed yield may be owe directly to the increment of vegetative growth parameter viz, plant height, number of both branches and leaves/plant, dry weight/ plant and the increment in enzymes activity. These results are in agreement with those reported by Ramadan (1997) and El-Mansi et al. (2000) on pea plants, Bhagiya et al. (2005) on groundnuts and Togay et al. (2008) on lentil plant. They reported that spraying the above-mentioned plants with Mol significantly increased number of pods/plant, pod weight, yield/ plant and total yield (Gendy 2013).

The test weight increased with the successive levels of applied molybdenum but difference could not reach the level of significance. The increase in these yield attributing characters with application of molybdenum fertilization might be due to its unique role in enhancement of nitrogen fixation, thereby, increasing availability to the plants for efficient growth and development. The increase in yield attributes was probably due to source and sink relationship. The improvement in photosynthesis and carbohydrate metabolism resulting into greater formation of photosynthates and metabolites in source and later on translocated in the newly formed sinks, i.e., reproductive structures (flowering and seed setting) which ultimately increased pods per plant and test weight. Similar findings were also reported by Johansen et al. (2007), Cvijanovic et al. (2011) and Sharma et al., (2014).

Foliar fertilization has been used as a supplemental strategy to plant nutrition especially in crops with high yield potential. Applying nutrients in small doses stimulates photosynthesis and increases yield performance. Harshini et al. (2022) stated a field experiment to evaluate the influence of molybdenum and biofertilizers on growth and yield of cowpea and reported that treatment with application of molybdenum 4 g/kg seed along with rhizobium and phosphate solubilizing bacteria (PSB) was better in growth and yield parameters and more productive and can be recommended to farmers after further trails.

Sirlene et al. (2022) evaluated the efficiency of foliar application of molybdenum (Mo) on soybean and maize and found that application of Mo increased leaf NR activity, nitrogen and protein content, rubisco activity, net photosynthesis, and grain yield. These results indicated that foliar fertilization with Mo can efficiently enhance nitrogen metabolism and the plant's response to carbon fixation, resulting in improved crop yields.

Treatment with N-acetyl-cysteine has recently been reported to improve growth in heavy metals stressed plants through the coordinated induction of antioxidant

defense and phytochelating systems coupled with the plant phenolic pool to mitigate oxidative stress under stress conditions (Colak et al. 2019). Moreover, Colak et al. (2020) reported that there is no data have been reported concerning the stress-alleviating effect of N-acetyl-cysteine in sugars and polyamines in plants exposed to heavy metals stress, so that, they studied the effects of heavy metals (Cd, Hg and Pb, 100 μM) on accumulation of soluble sugars and polyamine content in roots and shoots of wheat seedlings, the water potential and proline content in shoots and the role of N-Acetyl-cysteine in protection against heavy metal toxicity.

Foliar application of cysteine mitigated the unfriendly effects of NaCl stress on flax plants. Moreover, cysteine enhanced the growth parameters, photosynthetic pigments, amino nitrogen, total phenols, and new polypeptides in NaCl-stressed plants (Hebat-Allah and Shifaa 2022).

Protein polymorphism helps in distinguishing plant germplasm at specific levels. Polymorphisms occurring within amino acid sequences may result due to specific environmental factors in different geographical regions (Haliem and Al-Huqail 2013). Therefore, these polymorphisms may serve as genetic markers because they can be highly polymorphic and their variability is generally highly heritable (Khater et al. 2015, 2016). Additionally, protein polymorphisms resulting from insertions or deletions between mutated sites of protein bands are codominant, and these were found in agreement with Mondini et al. (2009). Moreover, appearance of new bands (unique) usually results from different DNA structural changes (e.g., breaks, transpositions, deletions), which leads to changes in amino acids, and consequently the protein formed (Humera 2006). Moreover, proteins might play a role in signal transduction, anti-oxidative defense, anti-freezing, heat shock, metal binding, anti-pathogenesis or osmolyte synthesis which were essential to a plant's function and growth (Ganapathi et al.2008).

Conclusions

It was cleared that foliar spraying with molybdenum, cysteine and their interaction enhanced and improved growth, quality and productive characters of fenugreek. Moreover, there were molecular changes in fenugreek under study and generated bands using the three ISSR primers.

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Author contributions
MSA and DMS performed the field experiments and chemical analysis. MAK performed the statistical analysis of recorded data and molecular DNA and protein analysis. All authors read and approved the final manuscript.

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All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The participants declare that the work has been consented for publication.

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