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Genetic stability analysis associated with salt stress and salicylic acid in fenugreek (*Trigonella foenum-graecum*) plants

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Abstract. *Alhasnawi AN. 2023. Genetic stability analysis associated with salt stress and salicylic acid in fenugreek (*Trigonella foenum-graecum*) plants. Biodiversitas 24: 3601-3608.* Salt damage to plants has been attributed to a combination of many factors like the accumulation of toxic metal ions or osmotic stress. This study attempted to understand the mitigating effects of the salicylic acid (SA) treatment on the salt-stressed seed germination ability of the fenugreek plants (*Trigonella foenum-graecum* L.); SA pre-treatment along with the salt tolerance capacity have also been tested. The effects of salinity and applied SA on biochemical traits and genetic stability of plants have been estimated in this study. Respective molecular and biochemical changes occurring in plants as well as genetic analysis have been performed. The results indicated that exogenous application of SA (0.4 mmol) improved the biochemical traits in the seedlings. The evaluation of the genetic stability of the plants was also conducted. Whereas DNA marker-based genetic stability estimates are considered a reliable, accurate, and advanced technique. The Inter-Simple Sequence Repeats (ISSR) analysis has been performed with the help of 7 ISSRs primers. The 6 ISSRs primers generated 54 fragments in total, with an average of 4.5 bands for every primer. The ISSR did not reveal any polymorphic bands. Finally, it has been concluded that exogenous SA can be effectively used as a potential growth regulator for improving the biochemical traits and salinity stress tolerance of the fenugreek plants and stimulating its medicinal properties. Furthermore, ISSR markers were seen to be a good tool as potential diagnostic markers for testing the genetic stability of plants.

Keywords: Biochemical tests, DNA markers, genetic analysis, plant, salicylic acid

Abbreviations: APX: Ascorbate Peroxidases; AA: Ascorbic Acid; bp: base pair; CAT: Catalases; DNA: Deoxyribonucleic Acid; GR: Glutathione Reductase; H_2O_2: Hydrogen peroxide; $^{\bullet}OH$: Hydroxyl radicals; ISSR: Inter-Simple Sequence Repeat; MDAR: Monodehydroascorbate Reductase; POD: Peroxidases; ROS: Reactive Oxygen Species; PCR: Polymerase Chain Reaction; SA: Salicylic Acid; SOD: Superoxide Dismutase; $O_2^{\bullet-}$: Superoxide radicals

INTRODUCTION

Medicinal plants are globally valuable sources of new drugs, and additionally, it is important to conserve medicinal plants for preserving biodiversity (Ling et al. 2009). Different species of plants belonging to the genus *Trigonella* are used for functional food industries, as well as nutraceutical and pharmaceutical ones (Ling et al. 2009). Out of these species, *Trigonella foenum-graecum* L. (fenugreek) has been commonly used as a panacea in the treatment of cancer, microbial and diabetic diseases (Zandi et al. 2017). The cause of abundant medicinal properties of *T. foenum-graecum* is a result of the presence of a diverse range of important phytochemicals (Zandi et al. 2017; Sherya et al. 2019).

It has been recently shown that increasing sodium chloride (NaCl) levels decreased the germination rate of fenugreek seedlings and their growth (Saberali and Moradi 2019; El-Gebaly et al. 2022). Salinity is a vital environmental factor that limits crop productivity with adverse effects on germination, crop yield, and plant vigor (Carillo et al. 2011). Soil salinity is a major global issue owing to its adverse effect on crop productivity (Isayenkov and Maathuis 2019). Salinity affects and reduces water potential, ion toxicity (particularly, Na^+ and Cl^- ions), osmotic pressure (decreasing water uptake), causes oxidative stress and tissue damage (Ahmadi and Souri 2020; Amiripour et al. 2021), due to an uncontrolled induction of the reactive oxygen species (ROS) and hydrogen peroxide (H_2O_2) molecules (Ahmadi and Souri 2019), like hydroxyl radicals ($^{\bullet}OH$) and superoxide radicals ($O_2^{\bullet-}$) ions (Alhsanwi et al. 2016). These reactions alter the intrinsic cell membrane properties like membrane fluidity, transportation of ions across the membrane, decreased activities of various enzymes, inhibition of protein synthesis, and DNA damage, which can lead to cell death (Affenzeller et al. 2009; Sharma et al. 2012). It has also been observed that due to salinity the shoot growth is more severely affected compared to the root growth (Fernández-García et al. 2014).

Plants display many molecular and biochemical mechanisms for combating ionic and osmotic stresses (Ahmadi and Souri 2018). These include the exclusion or selective accumulation of ions, controlled ion uptake by the roots and their transportation to leaves, synthesis of compatible solute molecules, compartmentalization of ions at cellular and plant level, alternation in the photosynthesis pathway, induction of the plant hormones and antioxidant

enzymes (Muchate et al. 2016). The plants have evolved many robust ROS scavenging processes, which include the non-enzymatic and enzymatic defense pathways that counter the negative consequences of ROS production (Türkan and Demiral 2009). Some of the most important antioxidant enzymes include glutathione reductase (GR), superoxide dismutase (SOD), catalases (CAT), peroxidases (POD), ascorbate peroxidases (APX) and monodehydroascorbate reductase (MDAR) (Pessarakli 1999). The antioxidative systems have shown variable activity levels, and alleviation of oxidative damage, and an effective antioxidative system is frequently associated with increased tolerance to salt stress (Demiral and Türkan, 2005). Salicylic acid (SA) has roles in cell respiration, membrane protection, various enzyme biosynthesis, and general growth (Souri and Tohidloo 2019).

Molecular markers are an effective way of assessing the genetic variability in plants (Li et al. 2013). To date, several molecular markers were developed, used, and categorized into different groups (Monfared et al. 2018). Inter-Simple Sequence Repeat (ISSR) markers are often used in analyzing the phylogenetic and genetic diversity of plants (Ng and Tan 1994). In this technique to yield several amplification products, PCR amplification of regions of interest needs to be carried out with one primer only. Such an approach could be used as the major multilocus marker system for investigating the genetic variation among the organisms (Mohamad et al. 2017). ISSRs were used for determining the genetic stability of various plants like *Withania somnifera* (Nayak et al. 2013), *Moringa peregrina* (Forsk.) (Al Khateeb et al. 2013), and potato (Tiwari et al. 2013). ISSRs markers can be used for determining the genetic variation in the plant species, especially for studying the differentiation and population structure (Tesfaye et al. 2014; Zhao et al. 2016; Monfared et al. 2018).

Therefore, this study aimed to explore the impact of exogenously applied salicylic acid (SA) mediated amelioration of NaCl stress and determination of the genetic stability of the fenugreek plants with the help of ISSR molecular markers have also been performed and can be used to determine genetic variation in plants.

MATERIALS AND METHODS

Plant material

Fenugreek (*Trigonella foenum-graecum* L.) seeds were obtained from Samawah local markets and were of the type grown in the Al-Muthanna province area in Iraq. Fenugreek seeds having a uniform size have been used in the study. The seed surface was sterilized for 10 min using 70% ethanol, then the seeds were rinsed using sterilized distilled water, air-dried at 32°C, and stored at room temperature.

Salinity stress experiments

The experiments were conducted at the young seedling stages. Ten seeds from one batch were steeped in a solution with varying concentrations of SA (0.2, 0.4, or 0.6) mmol for 7 days; while sterile distilled water was used to immerse the control set. Then, the seeds were sown into pots (2 kg) that were filled with a mixture of sand and peat moss (1:2v/v; Sab-Germany). The test seedlings (T1 to T12) were also saturated each day for two weeks using NaCl concentrations (50 and 100 mmol), while the control seedlings "T1" were soaked in distilled water. The scheme of the experiment was as Table 1.

The biochemical, genetic, and chlorophyll content changes were analyzed in the 2 months old seedlings that were subjected to salinity stress and SA as depicted below. The plants that were subjected to no SA pretreatment prior to NaCl stress were denoted as "NaCl", and SA pretreatment prior to NaCl stress induction were denoted as "SA + NaCl".

Chlorophyll content determination

The total, a, and b chlorophyll levels [mg 100 g^{-1} (f.m.)] were determined based on the technique described by Goodwin (1977).

Biochemical tests

The activity of SOD was quantitatively analyzed [U mg^{-1} protein (f.m.)] using the technique described in Giannopolitis and Ries (1977), CAT activity [U mg^{-1} protein (f.m.)] following the protocol by Cakmak and Marschner (1992), and POD activity [U mg^{-1} protein (f.m.)] by Kar and Mishra (1976). Quantitative analysis of the proline levels [mg^{-1} g (d.m)] in the samples, was conducted following the technique outlined by Bates et al. (1973).

Extraction and analysis of plant genomic DNA

The plant genomic DNA was extracted using a small-scale DNA-isolation kit (FavorPrep™ Plant Genomic DNA-extraction, Favorgen Biotech Corp., Mini Kit, Taiwan). The quality of isolated DNA was tested using agarose gel electrophoresis. Seven ISSR markers were used in the study. The annealing temperature varied and ranged between 40 and 60°C.

Table 1. The scheme of the experiment

S. no.	NaCl (mmol)	Salicylic acid (mmol)
T1	0	0
T2	50	0
T3	100	0
T4	0	0.2
T5	50	0.2
T6	100	0.2
T7	0	0.4
T8	50	0.4
T9	100	0.4
T10	0	0.6
T11	50	0.6
T12	100	0.6

The PCR amplification has been performed using the following parameters: a 20μL reaction mixture containing 2.5μL DNA, 2μL (each) of 10 μM ISSR primers, 10 μL PCR Green 2X Master (WizPure™ PCR 2X Master, Wizbio Solutions), and the remaining sample consisted of RNase-Free water. Thirty-five cycles were performed for amplifying the DNA samples (T100™; Bio-Rad Laboratories, Hercules, CA, USA). The following were the PCR conditions: denaturation for 5 min at 95°C, 35 cycles of denaturation for the 40 sec at 95°C, annealing at 60°C for 30 sec, and elongation at 72°C for 60 sec. A final 5 min extension was granted at 72°C.

ISSR analysis

The ratio of the amplified bands and the A, T, G, C, and G+C content of the ISSR markers have been determined according to the primer designed by Alpha DNA company. The ISSR amplification products derived from pure DNA were scored as either absent (0) or present (1) for every sample to determine the variation between the accessions using 2 different treatments "The T1" control sample (Sample A) and "The T2 sample (Sample B).

Statistical analysis

The results are expressed as an average value (±SE) for every treatment (n=3). In this study three replications. The data were analyzed using the SAS software (ver. 9.1 for Windows). The significant difference was evaluated using Duncan's multiple range test at the α level of 0.05.

RESULTS AND DISCUSSION

Chlorophyll content determination

The effect of the different SA concentrations (0, 0.2, 0.4, and 0.6 mM) on the total chlorophyll, chlorophyll a, and b levels in the fenugreek seedlings, which were previously subjected to various NaCl (0, 50, or 100 mM) levels (Figure 1A, Figure 1B, Figure 1C). Salinity-stressed fenugreek seedlings at 50 and 100 mmol NaCl concentrations had significantly lower chlorophyll-a levels T2, that gave 5.38 mg 100 g^{-1} (f.m.) and T3 was 3.77 mg 100 g^{-1} (f.m.) in comparison to the control sample T1 was 8.84 mg 100 g^{-1} (f.m.). However, under the same NaCl stress levels, the exogenous application of SA (0.6 mM) increased the chlorophyll-a level in T11 that gave 6.45 mg 100 g^{-1} (f.m.) (for 50 mmol NaCl) and T12 was 3.98 mg 100 g^{-1} (f.m.) (for 100 mmol NaCl) samples (Figure 1A). No significant difference in the chlorophyll-b levels was recorded under salinity and non-salinity stress conditions. However, the use of different SA levels (i.e., 0.2, 0.4, and 0.6 mmol) on the seedlings subjected to 50 and 100 mmol NaCl stress significantly increased the chlorophyll-b levels. The maximal values were noted in the seedling samples T4 was 4.43 mg 100 g^{-1} (f.m.) and T8 was 4.66 mg 100 g^{-1} (f.m.) (Figure 1B). The NaCl stress significantly decreased the total chlorophyll levels from 11.75 to 7.59 mg 100 g^{-1} (f.m.) at 50 mmol NaCl stress and to 5.79 mg 100 g^{-1} (f.m.) at 100 mmol NaCl. The results of this experiment indicated that the SA content increased the total chlorophyll levels of the seedlings that were stressed with NaCl. Furthermore, the values noted in the majority of cases were higher than the seedling samples that were only subjected to stress (Figure 1C).

Proline content analysis

The results of proline content analysis for the seedlings subjected to NaCl stress, i.e., T2 was 13.13 mg^{-1} g (d.m) and T3 was 17.03 mg^{-1} g (d.m) samples. The results showed that the proline content significantly increased in these samples in comparison to the control sample, i.e., T1 was 11.27 mg^{-1} g (d.m). On the other hand, the plants grown in non-saline conditions that underwent the SA treatment i.e. [T4 (11.11), T7 (10.22), and T10 (12.16)] mg^{-1} g (d.m) did not display any significant effect on the proline levels compared to the T1 control sample 11.27 mg^{-1} g (d.m). The plants that were grown under the NaCl stress and subjected to an exogenous SA application [T5 (13.72), T6 (18.89), T8 (16.50), T9 (19.88), T11(17.42), and T12 (20.06)] mg^{-1} g (d.m), showed a significant increase in their proline content in comparison to the control and non-saline samples (control, T1), or the seedlings subjected to T4, T7, and T10 treatments (Figure 2A).

Activities of antioxidant enzymes

The activities of chosen antioxidant enzymes were implemented as shown in (Figure 2B, Figure C, Figure D). Results indicated that the highest CAT activity was noted in the plants grown under stressed / and SA-treated T3 was 71.36 U mg^{-1} protein (f.m.), T9 was 86.72 U mg^{-1} protein (f.m.) and T12 was 84.76 U mg^{-1} protein (f.m.) samples in comparison to the recordings T1 control sample which reached 44.60 U mg^{-1} protein (f.m.) (Figure 2B). The analysis of variance (ANOVA) was carried out for determining the effect of the salinity stress on the CAT activity. The present study showed that stress elicited either by NaCl induced marked significant increases in CAT activity.

Salinity along with an exogenous SA treatment and their interaction significantly increased the POD activities in tested samples. According to the findings, POD activity increased as saline stress levels rose. However, the application of SA and NaCl affected the POD activity levels. The highest value of POD activity belonged in T9 at 13.65 U mg^{-1} protein (f.m.), T3 at 9.21 U mg^{-1} protein (f.m.), and T2 at 6.62 U mg^{-1} protein (f.m.), whereas the lowest value belonged in T1 was 4.31 U mg^{-1} protein (f.m.) (control) (Figure 2C).

ANOVA values indicated that the seedlings exposed to NaCl stress (i.e., 50 and 100 mM; T2 at 45.30 and T3 at 56.63 U mg-1 protein (f.m.), respectively) had significantly affected SOD activities compared to the non-saline control samples T1 was 35.11 U mg^{-1} protein (f.m.). The results indicated that the exogenous of SA (0.4 mmol) to the NaCl-stressed plants displayed the highest SOD activity in T9 at 76.48 U mg^{-1} protein (f.m.) compared to T2 was 45.30 U mg^{-1} protein (f.m.) and T3 was 56.63 U mg^{-1} protein (f.m.) samples and for T4 at 33.44 U mg^{-1} protein (f.m.), T7 at 34.55 U mg^{-1} protein (f.m.) and recordings T10 at 34.06 U mg^{-1} protein (f.m.) (only SA) and recordings T1 treatment at 35.11 U mg^{-1} protein (f.m.).

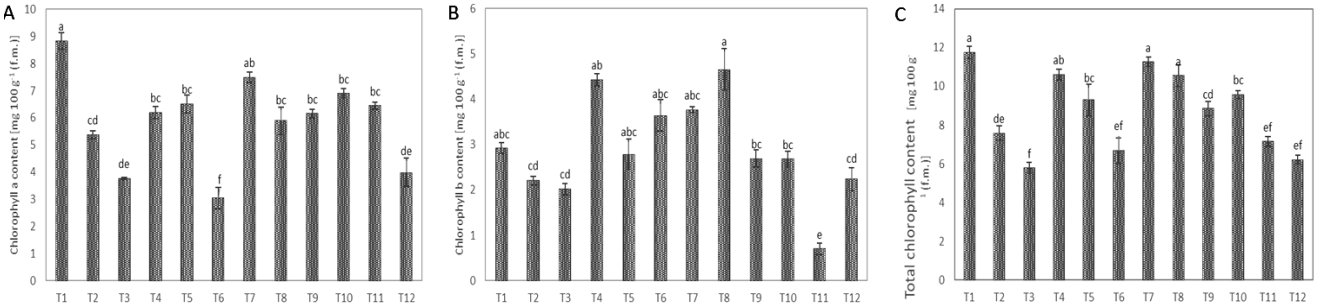

Figure 1. Influence of SA on chlorophyll-a, b, and total in fenugreek plants under salt stress. **[T1]**; NaCl (0 mmol) + SA (0 mmol) [Control], **[T2]**; NaCl (50 mmol) + SA (0 mmol), **[T3]**; NaCl (100 mmol) + SA (0 mmol), **[T4]**; NaCl (0 mmol) + SA (0.2 mmol), **[T5]**; NaCl (50 mmol) + SA (0.2 mmol), **[T6]**; NaCl (100 mM+ SA 0.2 mmol), **[T7]**; NaCl (0 mM+ SA (0.4 mmol), **[T8]**; NaCl (50 mM+ SA 0.4 mmol), **[T9]**; NaCl (100 mmol) + SA (0.4 mmol), **[T10]**; NaCl (0 mmol) + SA (0.6 mmol), **[T11]**; NaCl (50 mmol) + SA (0.6 mmol), **[T12]**; NaCl (100 mmol) + SA (0.6 mmol)

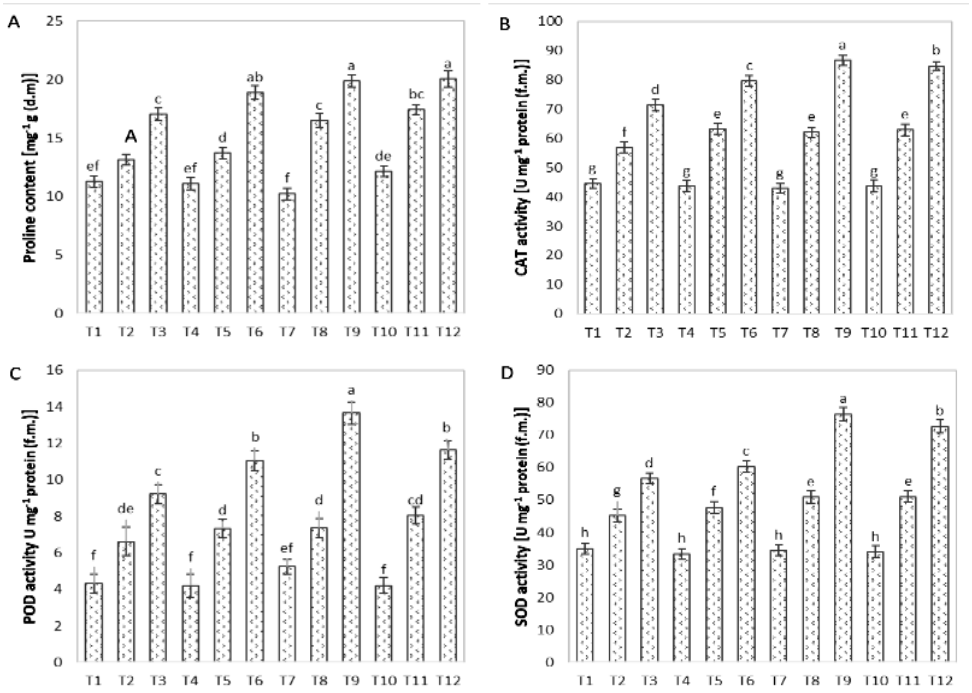

Figure 2. Influence of SA on proline content [mg-1 g (d.m)], CAT activity[U mg-1 protein (f.m.))], SOD activity [U mg-1 protein (f.m.)], and POD activity [U mg-1 protein (f.m.)] in fenugreek plants under salt stress **[T1]**; NaCl (0 mmol) + SA (0 mmol) [Control], **[T2]**; NaCl (50 mmol) + SA (0 mmol), **[T3]**; NaCl (100 mmol) + SA (0 mmol), **[T4]**; NaCl (0 mmol) + SA (0.2 mmol), **[T5]**; NaCl (50 mmol) + SA (0.2 mmol), **[T6]**; NaCl (100 mM+ SA 0.2 mmol), **[T7]**; NaCl (0 mM+ SA (0.4 mmol), **[T8]**; NaCl (50 mM+ SA 0.4 mmol), **[T9]**; NaCl(100 mmol) + SA (0.4 mmol), **[T10]**; NaCl (0 mmol) + SA (0.6 mmol), **[T11]**; NaCl (50 mmol) + SA (0.6 mmol), **[T12]**; NaCl (100 mmol) + SA (0.6 mmol)

Genetic stability analyses

Employing molecular markers to investigate the genetic stability and all correlations existing between the biochemical values and the DNA levels of the fenugreek samples, the researchers compared the values of the highest proline concentrations treatment "T9" (NaCl 100 mmol + SA 0.4 mmol), with the DNA control samples (T1). Seven ISSRs primers (consisting of 3-degenerate commercial and 4-specific primers) were chosen for the genetic analysis (Table 2).

ISSR-marker mean percentage values for the nucleotides were seen to be - A (33.36%), C (26.01%), G (24.56%), T (15.84%), Others (1.73%), C+G (50.57%) and A+T (47.70%). In this study, a higher percentage of A nucleotides was seen compared to other nucleotides (Table 3).

The 6 ISSRs primers yielded 54 products in total (3 products for $(CT)_9T$, 4 for $(AG)_9C$, $(TG)_8C$, $(GA)_8V$, and 6 for $B(CT)_8T$, $(GA)_8HC$primers), where 4.5 bands were the average per primer. The usage of one primer resulted in the amplification of no fragments (0; (GT)8C) (Table 4). The total number of the amplified bands that were produced using the ISSRs primers ranged between 3 and 6 bands; while the amplicon size varied between 300 bps and ≥1800 bps.

Table 2. Details of sequence and ISSRs primer

Primer sequence (5'-3')	Annealing temp. (°C)	Length mers	Primer sequence (5' - 3')
$(AG)_9C$	53.4	19mers	AGAGAGAGAGAGAGAGAGC
$(TG)_8C$	48.3	17mers	TGTGTGTGTGTGTGTGC
$B(CT)_8T$	50.0	18mers	BCTCTCTCTCTCTCTCTT
$(CT)_9T$	53.4	19mers	CTCTCTCTCTCTCTCTCTT
$(GT)_8C$	53.4	17mers	GTGTGTGTGTGTGTGTC
$(GA)_8V$	47.5	17mers	GAGAGAGAGAGAGAGAV
$(GA)_8HC$	48.0	18mers	GAGAGAGAGAGAGAGAHC
Total		125	
Mine		17.65	

Note: B= C, G, T; H= A, C, T; V= A, C, G

Table 3. The primer sequence and percentage of nucleotides

Primer sequence (5'-3')	Percentage						
	A%	C%	G%	T%	Others	(A+T)	(C+G)
$(AG)_9C$	47.37	5.26	47.37	0.00	0.00	47.37	52.63
$(TG)_8C$	0.00	5.88	47.06	47.06	0.00	47.06	52.94
$B(CT)_8T$	0.00	44.44	0.00	50.00	5.56	50.00	44.44
$(CT)_9T$	0.00	47.37	0.00	52.63	0.00	52.63	47.37
$(GT)_8C$	0.00	5.88	47.06	47.06	0.00	47.06	52.94
$(GA)_8V$	47.06	0.00	47.06	0.00	5.88	47.06	47.06
$(GA)_8HC$	44.44	5.56	44.44	0.00	5.56	44.44	50.00
Mine	33.36	26.01	24.56	15.84	1.73	47.70	50.57

Note: B= C, G, T; H= A, C, T; V= A, C, G

Table 4. Details of the banding pattern were provided by ISSRs primers and samples

Primer sequence (5'-3')	Samples	No. of bands	Size (bp)
$(AG)_9C$	A	4	300-700
	B	4	300-700
$(TG)_8C$	A	4	1000-1700
	B	4	1000-1700
$B(CT)_8T$	A	6	500-1800
	B	6	500-1800
$(CT)_9T$	A	3	500-1800
	B	3	500-1800
$(GT)_8C$	A	-	-
	B	-	-
$(GA)_8V$	A	4	300-1400
	B	4	300-1400
$(GA)_8HC$	A	6	250-1300
	B	6	250-1300
Total		54	

The researchers analyzed the genetic data based on the presumption that every band represented "M" a 100 bp DNA ladder (DNA Marker) and the DNA content of the sample which "T1" DNA control (A) and compared to "T9" DNA samples (B). The results of the ISSR analysis and the number of amplified DNA fragments indicated that the primers and a 100% genetic similarity amongst the "T1" DNA control (A) and compared to "T9" DNA samples (B), which indicated higher genetic stability presented in the study.

Discussion

The results indicated that the total chlorophyll, chlorophyll-a, and b levels significantly decreased under the salt stress conditions when compared to the control, which was also noted in the earlier studies in rice cultivars (Mandal et al. 2019). The salt stress (i.e., 100 mM) affected the leaf chlorophyll content as noted earlier in *Capsicum annuum* L. (Hand et al. 2017). Plants treated with salt-stress conditions (NaCl, 50 and 100 mmol) exhibited a substantial decrease in the index of chlorophyll stability compared to the NaCl, 0 mmol (control), which subsequently reduces the net photosynthetic rate. A decrease in the chlorophyll concentration was attributed to the loss of the photosynthetic capacity and the inhibitory effect of all accumulated ions on the chlorophyll synthesis in the samples (Hakim et al. 2014). The chlorophyll degradation was induced under different stresses (in addition to NaCl stress) (Hakim et al. 2014). The seed priming with SA decreased the negative effect NaCl had on plants as seen by the total chlorophyll, chlorophyll a, and b levels, which was similar to that noted in earlier studies, pumpkin seedlings growth under saline had 49, 53 and 56% less a, b and total chlorophyll, respectively, than untreated samples (Rafique et al. 2011). SA increases the chlorophyll biosynthesis-related enzyme activities, and important part of the manipulation of the photosynthetic system to increase chlorophyll levels (Ma et al. 2017). It has also been shown that SA increased the function of the photosynthetic system in maize plants under salt conditions by mobilizing the chlorophyll biosynthesis and tissue nitrate levels (Singh et al. 2015). The above observations and the results of experiments performed herein indicate that higher chlorophyll concentration in plants subjected to SA, could improve the photosynthesis mechanism and relieve the NaCl-related stress levels in the fenugreek seedlings.

Numerous studies have linked the with increasing of NaCl, the proline content increased significantly. Similar results were noted in the earlier studies *Dianthus superbus* (Caryophyllaceae) (Ma et al. 2017), and *C. annuum* (Amirinejad et al. 2017), which indicated that the proline accumulation caused an osmotic adjustment at a cellular level, protected the membrane integrity and mediated the salt-induced damage. The proline molecules act as the membrane protectors and stabiles the proteins and the membrane structures. A modification in the proline accumulation was noted in the salt-sensitive and salt-stressed *Ocimum basilicum* plants, which highlighted the importance of increasing and improving the salt tolerance of the *O. basilicum* (Alhasnawi 2019a). A positive correlation was noted between the proline accumulation and the plant growth rate, which indicated that the SA-induced proline synthesis enzymes could have improved the growth potential of the salt-stressed plants. Thus it has been concluded that the SA ameliorated the salt-stress levels by alleviating the proline metabolism system (Misra and Saxena 2009). It was presumed that the SA improved the salt tolerance of the plants as they protected the protein-

turnover machinery in response to the stress damage and the upregulation of the stress-protective enzymes based on the activity of the enzymes involved in the metabolism of proline. Additionally, it has been revealed that SA's exogenous caused oxidative stress, which protected the lentil plants from further damage (Misra and Saxena 2009).

In this study, the salt stress significantly enhanced the CAT, SOD, and POD activities in the fenugreek plants. The study's findings showed that the antioxidant enzyme system was further activated under salt stress. Similar results were noted in earlier studies *Solanum tuberosum* L. (Sajid and Aftab 2014), *Oryza sativa* L. (Alhsanwi et al. 2016, 2017). The salt-stressed *D. superbus* (Caryophyllaceae) subjected to SA showed the maximal CAT, SOD, and POD enzyme activities compared to the control or the salt-stressed plants alone, which was also noted earlier (Ma et al. 2017; Morsi et al. 2018). The antioxidant protection system that included the non-enzymatic antioxidant molecules, like glutathione and ascorbic acid; in addition to the antioxidating enzymes like SOD, APX, CAT, and POX, regulate the harmful effects of the ROS (Alhasnawi et al. 2016). For preventing ROS damage to the cellular components, numerous detoxification systems have been evolved by plants, like the synthesis of antioxidating molecules and enzymes. A reduction in oxidative damage and higher resistance to salinity was attributed to the effective antioxidant systems in *O. basilicum* (Alhasnawi 2019b). It was also seen that the SA application increased the POD, CAT, and SOD activities; and decreased the ROS levels and the oxidative damage to the membranes, hence was regarded as a vital component for improving salt tolerance in *O. basilicum* (Alhasnawi 2019b). The mutant and the transgenic plants that over-expressed the antioxidant enzymes showed higher ability to scavenge the ROS molecules, thus, increased the salt tolerance.

The genetic stability of the fenugreek plants using the ISSR markers was also assessed. The amplification profiles obtained using 7 ISSR primers indicated no polymorphism among tested plants. Results of the research indicated that the plants with the maximal antioxidative enzyme levels and proline content were genetically stable and similar to the control samples. The results showed that the specific and the degenerate primers could be amplified, which indicated genetic stability. To date, several molecular markers were used to determine the genetic stability of the plants. The researchers used the PCR-based marker system that included DNA markers for studying the genetic stability in the fenugreek plants samples. Nisa et al. (2019) investigated the stress condition which was correlated to the maintenance of gene silencing during DNA replication in plants, also discovered that to give the repair machinery access to the DNA, significant chromatin remodeling is required during DNA-repair processes. Therefore, chromatin dynamics is challenged by DNA damage and can lead to DNA damage accumulation and genome instability.

The most important feature of a DNA barcode is its universality. ISSR uses universal markers; it requires only small amounts of DNA and is quick to perform, reproducible, technically simple, and used for determining the genetic stability and variability in the plants (Yin et al. 2013; Mohamad et al. 2017). Similar results were reported by Song and Bent (2014), it has been demonstrated that SA treatment lowers the buildup of DNA damage in plants. Exogenous SA enhanced the maintains the stability of membranes, and the photosynthetic rate of the growth of barley plants will be enhanced (Muthulakshmi and Lingakumar 2017). These can be used these markers for studying the genetic stability and variability of the plants and also determining the center of diversity, evolutionary origin, genetic structure, and domestication (Abate 2017).

In conclusion, an exogenous SA enhanced the growth rate of the Fenugreek plants under the NaCl stress conditions in the present investigation. SA was able to help overcome the detrimental effects of salinity stress as it increased the endogenous levels of the proline content and activities of antioxidative enzymes and increased the chlorophyll content of the plants. An increase in the SA concentrations had no impact on the genetic stability of the fenugreek plants. The ISSR markers were used for studying genetic stability, as an effective tool for generating the genetically-stable diagnostic markers for the plants. In conclusion, this report shows that exogenously applied SA is efficient in reducing the negative consequences of sodium chloride stress. These impacts of biochemical traits and genetic stability may depend on the plant or species, and further studies are needed to confirm our results.

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