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β-Glucan-Mediated Alleviation of NaCl Stress in *Ocimum basilicum L.* in Relation to the Response of Antioxidant Enzymes and Assessment DNA Marker

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ABSTRACT
Salinity is one of the most important abiotic stresses which can negatively affect the plant metabolic processes in the world. This can impact the plant production, either for economic or sustenance benefits. The salinity stress can cause many physiological and biochemical changes in the plants. β-glucans are important polysaccharides, which are present in the cell walls of various cereal grains. They protect the plant responses and occur in plant suspensions. In this study, the researchers attempted to investigate various physiological mechanisms and determine the role of the β-glucans in the NaCl-mediated stress conditions on the *Ocimum basilicum* L. seedlings. For this purpose, they carried out an experiment for assessing various shoot and root parameters along with the antioxidant enzyme activities, proline levels and the ISSR markers. When the seedlings were exposed to the NaCl stress conditions, they showed a significant decrease in the growth parameters and an increase in the antioxidant and proline levels compared to the control seedlings grown under normal saline conditions. On the other hand, the β-glucan-treated seeds, when grown under the saline stress conditions, showed better growth parameters as well as high antioxidant enzyme activities and proline levels, compared to the control and NaCl-treated plants. Furthermore, a PCR analysis was carried out using the ISSR-marker technology, which could help in evaluating the DNA fingerprints and genetic variations in the plants. The results indicated that the exogenous application of the β-glucans could protect the antioxidant enzyme activities and protect the plants against the salinity stresses, without affecting the DNA-markers without affecting the genetic variations and could be a better choice for use in DNA-markers.

Keywords: NaCl-stress, β-glucan, antioxidants, molecular markers, DNA variations, genetic stability.

INTRODUCTION

Many traditional medicines make use of the medicinal plants, owing to their biological properties. In the past few years, the researchers investigated the biological properties of various plants, like the antiviral, antibacterial, anti-inflammatory and antioxidation properties of the compounds extracted from medicinal plants (Baldim et al., 2018). Common basil (*Ocimum basilicum* L.), from the *Lamiaceae* family, is a cosmopolitan and aromatic herb, which grows in many regions in the world. It is a multipurpose plant, which has many applications in the perfume, pharmaceutical and food industries. This plant, along with its essential oil, has been used for treating dysentery, rhinitis, mental fatigue colds, and nausea (Hassanpouraghdam et al., 2011).

It is native to Asia, South America and Central Africa, but is cultivated in several other regions, like the Mediterranean region. Basil has a wide genetic diversity with ≈50–150 species existing in the world. It has glossy, fragrant and lanceolate leaves, and is able to grow up to a height of 90 cm, based on the different morphological characteristics like aromatic composition, shape, size and colour of the flowers and leaves. Due to its aroma, it is known as the "king of the herbs" (Filip, 2017).

Many abiotic stresses like the chemical toxicity, oxidative stress, salinity, drought or extreme temperatures are the major parameters which

affect the agricultural productivity and harm the environment (Kordi et al., 2013). Salinity is a major abiotic stress that affects a large proportion of arable land, worldwide, and can hinder the ability of the basil plant to uptake water in its root zone, by decreasing the soil's water potential. This severely affects the productivity of the plant (Elhindi et al., 2017).

The plant's antioxidant protection system that consists of many non-enzymatic antioxidants like glutathione and ascorbic acid, along with numerous enzymatic antioxidants, like PerOXidase (POX), SuperOxide Dismutase (SOD), CATalase (CAT) and Ascorbate PeroXidase (APX), can regulate the negative effects of the Reactive Oxygen Species (ROS), which are accumulated under the stress conditions. Despite the presence of this antioxidation system in the plant, if the plant becomes exposed to salinity stress, oxidative damage occurs, causing an imbalance between the ROS accumulation and detoxification (Alhasnawi et al., 2016).

β-glucans are glucose polymers, which exist in many fungi, bacteria and plant cell walls. When the β-glucan receptor, present on the neutrophilic granulocytes, binds to the macrophages with β-glucan, it induces the production of antioxidating enzymes and ROS species, which, in turn, increase the anti-stress and anti-microbial activities (Kim et al., 2009).

β-Glucans are divided into 2 major subgroups, based on their monosaccharide units, i.e., β (1–3) glucans, which consist of linear molecules with ≈1500 glucose units, and the β (1–6) glucans, which include short chains consisting of ≈140 glucose units, with around 15% of the internal links that connect the points present in the 3,6-disubstituted molecules, giving rise to branched polymers. The β (1–3) and β (1–6) glucans display differing biological activities. β (1–3) offers a "ladder" structure to the cell walls, whereas β (1–6) helps in the binding and stabilisation of the cell walls. The chains in the glucans act as an anchor for the mannoproteins and covalently link chitin and the β (1–3) glucans (Gonzaga et al., 2013).

Several DNA markers have been used for investigating the genetic diversity of the various plant species as they help in assessing the genetic variability (Dos Santos et al., 2011). One such marker includes the ISSR (Inter-simple sequence repeat) marker, which is a Polymerase Chain Reaction (PCR)-based technique that helps in assessing the DNA fingerprints and uses short primers from the microsatellite regions. The use of this method is helpful, as the molecular markers assist in classifying the population genotypes, and provide baseline information for managing the gene pool and implementing plant breeding strategies (Alhasnawi et al., 2019). ISSRs are very polymorphic and can be used in many studies related to phylogeny, genome mapping, evolutionary biology, genetic diversity and tagging (Reddy et al., 2002).

In this study, the researchers aimed at investigating whether the varying concentrations of β-glucans could effectively decrease the negative effects of the NaCl stress on the growth of the *Ocimum basilicum* L plant. The researchers investigated the growth and various biochemical parameters for determining the effect of NaCl stress and the β-glucan treatment on the DNA marker levels of the seedlings. This information could offer a strong theoretical basis for increasing the production and plantation of this plant in the saline zones.

MATERIALS AND METHODS

Plant material and culture conditions

All experiments were conducted in the laboratory at the Biology Department, College of Education for Pure Sciences, Al Muthanna University, and the laboratory at the Kufa University, Iraq.

Experiment 1: Initially, the seeds were soaked in 5 different β-glucan concentrations (1, 2, 3, 4, 5 mgL^{-1}) for 5 min, while the control seeds were soaked in distilled water. Thereafter, all the seeds were transplanted into small plastic pots (10.5 cm diam. × 5 cm height) which contained a mixture of sterilised Peat Moss [90.0% organic matter; 52.0% carbon; 0.3% nitrogen; pH (H_2O) 3.5–4.5] and clay-loam soil, which were mixed in a 2:1 (% v/v) ratio. The seeds (500 mg/ pot) were planted and grown under laboratory conditions. After 3 weeks, the saplings were investigated for their morphological characteristics in order to determine the optimal β-glucan concentration. On the basis of all the results, the researchers selected an optimal β-glucan concentration of 4 mgL^{-1} and used it for the subsequent experiments.

Experiment 2: This experiment was carried out during the seed germination and seedling stage. Solutions containing an optimal 4 mg

L^{-1} β-glucan concentration and 150 mM NaCl (Khaliq et al., 2014) were used for seed germination, while distilled water was used as the negative control. For preventing osmotic shock, the salt solution was added slowly to the soil, over a period of 3 days. The germination potential was determined after 3 weeks; the seedlings were imaged and investigated for their morphological characteristics. They were categorised with regards to their shoot and root length, fresh or dry weight, etc.

Growth parameters

The Fresh Weight (FW) of the shoot was determined after harvesting the saplings, whereas the Dry Weight (DW) was measured after placing the samples in the forced-draft oven (65°C) for 48 h or till a constant dry weight was noted.

Biochemical analysis

The researchers determined the PerOxiDase (POX) activity (Giannopolitis and Ries, 1977), with a few modifications (Qiu et al., 2014). Additionally, the CATalase (CAT) activity (Cakmak and Marschner, 1992) was determined after modifying the assay (Li et al., 2011). SuperOxide Dismutase (SOD) activity (Giannopolitis and Ries, 1977) was also measured after modifying the assay (Li et al., 2011). Finally, the researchers determined the proline (Bates et al., 1973) levels after a few modifications (Alhasnawi, 2017)

Statistical analysis

The data collected from the above-mentioned experiments were statistically analysed using a factorial Completely Randomised Design (CRD). All analyses were conducted after 3 weeks. The factors in the CRD included the 3 different treatments, i.e., T1 control (with distilled water); T2 containing 150 mM NaCl; and T3 (150 mM NaCl + 4 mgL^{-1} β-glucan). This data was also subjected to a normality test before being analysed by the ANalysis Of Variance (ANOVA) using the SAS software (Rel. 9.1 for Windows, Ver. 6.1.7600, Software SAS 9.1.3 Ser. Pack 4 XP-PRO platform, SAS Institute Inc., Cary, NC, USA). The significant differences between the mean treatment values were noted using the Duncan's Multiple Range Test (DMRT), wherein different letters represented the statistical difference at the α=0.05 level. The researchers expressed the spread of all values as the mean ± SD (n=3), for representing the standard errors.

ISSR-PCR analyses of the genomic DNA

The researchers extracted the genomic DNA from the Control (C) samples [i.e., samples subjected to 0 mgL^{-1} β-glucan + 0 mM NaCl] and from the seedlings that were subjected to various Treatments (T) [i.e., 4 mg L^{-1} β-glucan + 150 mM NaCl]. All DNA was also extracted from the lyophilised leaves with the help of a plant genomic DNA extraction kit (Favorgen Biotech Corp.). The researchers determined the ISSR sequences of the design primers having restricted sites on the primer sequences (5' to 3') for designing primers using the alpha DNA. Table 1 presents the details of these ISSR primer sequences. The PCRs were carried out for a total volume of 20 µl (with 10µl of PCR Green 2X-Master, 2µl of 10 µM ISSR Primer, 2µl of Template DNA and the remaining volume was made of RNase-Free water). The PCR was set up using one primer from the set of 7 ISSR primers, wherein 30 PCR cycles were carried out in the thermal cycler (T100™; Bio-Rad Laboratories, Hercules, CA, USA) using PCR program as follows: 5 min of Initial Denaturation at 95°C, followed by 30 cycles of 3 steps:

Table 1. Nucleotide sequences of the ISSR markers and annealing temperatures used for PCR amplification of *Ocimum basilicum L.*

ISSR Primer		Primer Sequence 5' – 3'	Length mers	T (°C)
IS1	$(AG)_8T$	AG AG AG AG AG AG AG AG T	17	47.7
IS2	$A(CAG)_5$	A CAG CAG CAG CAG CAG	16	49.0
IS3	$(CAC)_7G$	CAC CAC CAC CAC CAC CAC CAC G	16	-
IS4	$(CTC)_6G$	CTC CTC CTC CTC CTC CTC G	19	58.0
IS5	$(GAA)_7$	GAA GAA GAA GAA GAA GAA GAA	21	50.6
IS6	$(GA)_8G$	GA GA GA GA GA GA GA GA G	17	51.4
IS7	$(AG)_9C$	AG AG AG AG AG AG AG AG AG C	19	53.4
Total			125	

50 s of DNA denaturation at 95°C, 50 s of annealing at 60°C, 60 s of extension at 72°C, and lastly, 5 min of final extension at 72°C. The PCR amplification reactions were thereafter analysed by agarose gel electrophoresis, using 2% (w/v) of agarose gel dissolved in 1 × TBE buffer (pH 8.0). Electrophoresis was carried out at 60V and a 1500 bp molecular size ladder was used as the standard marker. The ISSR-PCR bands were observed as either marker, absent (0), or present (1) at every band position for the 4 species. Finally, the researchers determined the total no. of bands, approximate fragment size (bp), mean frequency of the alleles that were present and absent for every ISSR primer for the 2 treatments.

RESULTS AND DISCUSSION

Table 2 presents the ANOVA results, which showed that the *Ocimum basilicum* L. plant was significantly affected by the salinity stress. There was a marked decrease in the fresh biomass of the seedlings which were exposed to a 150 mM NaCl-induced salinity stress. When the researchers analysed the 3-week old seedlings, they noted that the shoot length, FW and DW of the shoots and roots were severely affected by the salinity treatment, in comparison to the control (distilled water) seedlings. The differing salinity stresses led to a decrease in the FW and DW of the shoots and the root samples, compared to the control samples. In this study, the decrease in the growth of the seedlings could be attributed to the decrease in the soil water content, which further increased the osmotic pressure. This was generally noted under the salinity stress conditions. It was stated that NaCl could reduce the rate of the *Ocimum basilicum* L germination because of a decrease in the water potential, which reduced the imbibition rate (Delavari et al., 2014). Hence, the differing NaCl salt stresses affected the growth of basil seedlings, such that the control and non-saline seedlings showed a better FW than the treated samples. Suppression of the plant growth under the salt-induced conditions was attributed to 2 main reasons. First, the soil salinity reduced the ability of the plants to take up the moisture from the soil, which decreased their growth rate. The osmotic effect was a result of salt stress. Secondly, if the photosynthetic leaves accumulated a large salt concentration, it could affect the leaf cells, which, in turn, might influence the growth and photosynthetic rate of the plants (Khaliq et al., 2014).

A higher NaCl concentration in the plant growth medium could cause many primary and secondary effects which might hinder the growth and development of the plants. The primary effects include osmotic stress and ionic toxicity. Ionic toxicity takes place when the high Na^+ and Cl^- concentrations present in the cell cytoplasm affect the physiological and biochemical processes in the plants; whereas osmotic stress was induced when the decreasing water potential reduced the turgor pressure, which led to the loss of cellular water (Jampeetong and Brix, 2009). On the other hand, the secondary effects of the NaCl stress included inhibition of the K^+ uptake, generation of the reactive oxygen species and membrane dysfunction in the cells (Mühling, 2003). This decreased the photosynthetic area that was available for the continuous growth of the plants. Furthermore, a decreased leaf surface area also reduced the light attraction, which further lowered the total photosynthetic capacity. As a result, the plant growth and performance was hampered and the dry matter of the plants was reduced as well (Khalil et al., 2010).

In this study, the decrease in the plant growth was attributed to a low soil water content which further increased the osmotic pressure that was noted under the salt stress conditions. Hence, it

Table 2. Length of shoot (cm plant^{-1}), FW of shoot (mg plant^{-1}), DW of shoot (mg plant^{-1}), Length of root (cm plant^{-1}), FW of root (mg plant^{-1}), and DW of root (mg plant^{-1}), in seedlings of *Ocimum basilicum* L. under control, 150 mM NaCl salinity and 150 mM NaCl + 4 mg L^{-1} β-glucan.

Specification	Control	150 mM NaCl	150 mM NaCl + 4 mg L^{-1} β-glucan
Length of shoot (cm plant^{-1})	6.10±0.21^a	3.37±0.23^c	4.27±0.12^b
FW of shoot (mg plant^{-1})	106.67±8.82^a	38.03±1.20^c	49.33±2.10^b
DW of shoot (mg plant^{-1})	17.76±1.37^a	6.37±0.29^c	9.24±0.44^b
Length of root (cm plant^{-1})	2.70±0.06^a	1.43±0.07^c	1.83±0.09^b
FW of root (mg plant^{-1})	26.33±2.03^a	13.00±0.58^b	16.67±0.88^b
DW of root (mg plant^{-1})	3.97±0.35^a	2.00±0.12^b	2.57±0.15^b
The values (mean ± SE) (n=3) with different letter within columns are statistically different α=0.05 according to Duncan's test			

was seen that varying NaCl levels affected the basil plant growth rate, while the plants grown under non-saline conditions showed a higher FW value compared to the treated plants. Table (2) indicated that the exogenous application of 4 mg L^{-1} β-glucan could increase and improve the shoot length, FW and DW of the shoots as well as the root length, even under the saline conditions. The data also indicated that the FW and the DW of the roots were similar to the control values when the basil plants were treated with β-glucan and grown under saline stress conditions.

Earlier studies showed that β-glucans represent many vital polysaccharides (Laroche and Michaud, 2007). This polysaccharide layer could create a microenvironment around the cell walls, which could protect the cells from the osmotic disequilibrium that took place across the cell membranes. This protected the cells from dehydration under the hypersaline condition (Xu et al., 2013). The (1→3,1→6) β-D glucans (will be referred to as β-glucans) form a significant structural component of the cell walls (Williams et al., 2013), and if their content changed, it could alter the mechanical cell wall properties (causing a loosening of the cell walls) and lead to cell elongation. These results indicated that β-D-glucans could play a vital role in the growth and elongation of the plants (Hoson and Nevins, 1989). β-glucans were seen to increase the resistance response of the plants and along with laminarihexaose (a beta-(1→3)-glucan oligosaccharide) could stimulate the protection reactions in the cell suspensions. They were recognised by a particular receptor, which indicated that this molecule could activate the plant's defence mechanism (Inui et al., 1997).

The biochemical analyses also indicated that the POX, SOD, CAT enzyme activities in the *Ocimum basilicum* L. plant were affected by the salt stress, with or without the application of β-glucans (Fig. 1, A, B, C). Salinity stress caused a significant increase in POX activities, i.e., 0.161 Units g^{-1} (FW) min^{-1}, while the control plants showed the least value of 0.111 Units g^{-1} (FW) min^{-1}. On the other hand, the highest POX activity of 0.200 Units g^{-1} (FW) min^{-1} was noted in the salt-stressed seedlings which were supplemented with β-glucan, in comparison to the control or even the salt-stressed seedlings without any β-glucan (Fig. 1A).

The researchers also noted that the control showed the lowest SOD values of 15.37 Units g^{-1} (FW) min^{-1}. Furthermore, the salt stress significantly increased the SOD activity, i.e., to 59.24, while the plants grown in the presence of β-glucan and salt, exhibited the highest SOD value of 86.21 Units g^{-1} (FW) min^{-1} (Fig. 1B). The control yielded the lowest CAT value of 59.11 Units g^{-1} (FW) min^{-1}; while it increased to 294.70 Units g^{-1} (FW) $min^{-1}$when the plants were grown under saline stress conditions. Lastly, the seedlings that were grown under saline conditions and were supplemented with β-glucan, showed the maximal CAT activity of 294.70 (Fig. 1C).

Earlier studies indicated that under medium salt stress conditions, the various antioxidant activities (like SOD, CAT and POX) showed a significant variation (Letters, 2012). An increase in these antioxidative enzyme activities under the salt stress could be attributed to the increasing ROS production and a build-up of the plant protective mechanism for reducing the oxidative damage which was triggered by the stress in the plants. The POX enzyme in the chloroplast and cytosol helped in scavenging H_2O_2 (Hand et al., 2017). It was noted that the salinity stress increased the production of the ROS species (e.g., $OH^{\bullet}$, H_2O_2 and O^{2-}). ROS was seen to significantly damage the various biomolecules like proteins, DNA and lipids, causing cell death (Mittler, 2002). The plants possessed an antioxidant defence system for scavenging the ROS. The various antioxidant enzymes like CAT and Ascorbate PeroXidase (APX) could effectively scavenge the hydrogen peroxide (H_2O_2) molecules, and inhibit the membrane lipid peroxidation, which could be induced by a high H_2O_2 concentration. This was one of the major factors which could reduce the salinity stress-related effects (Abdelaziz et al., 2018).

The results of this study showed that an exogenous application of β-glucans significantly improved the antioxidant activities of the plants. Similar results were reported earlier (Alhsanwi et al., 2016), wherein the SOD, CAT and POX activities increased in the NaCl-stressed seedlings that were treated using different polysaccharide (β-glucan) concentrations. The β-glucans existing in the plants played a vital role in shielding the plants against the biotic stresses. Different β-glucan molecules from the polysaccharides, which were extracted and isolated from the natural sources, showed many applications and helpful properties. The conducted research indicated the effect and interrelationship between the lipids, phenolic compounds and β-glucans with their antioxidative enzyme activities in 9 oat varieties.

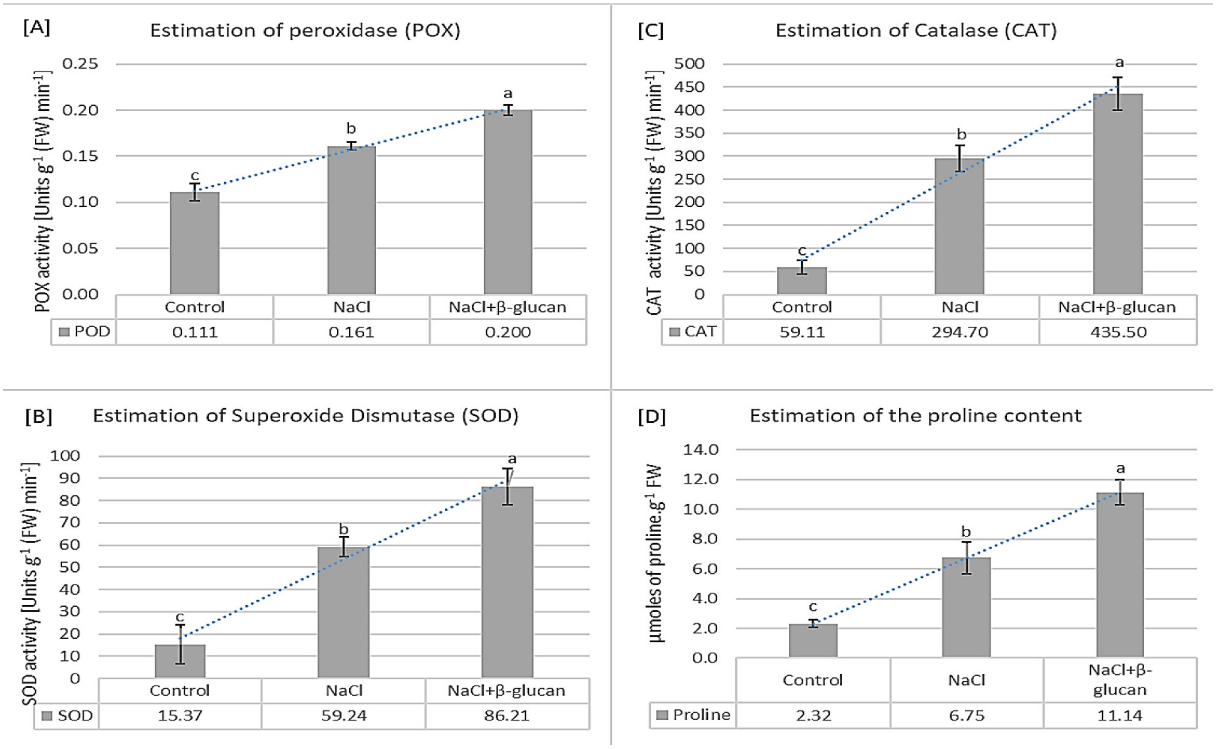

Fig. 1. Specific activity of POX activity [Units g^{-1} (FW) min^{-1}], SOD activity [Units g^{-1} (FW) min^{-1}], CAT activity [Units g^{-1} (FW) min^{-1}], and µmoles of proline. g^{-1} FW, in seedlings of *Ocimum basilicum* L. under control, 150 mM NaCl salinity and 150 mM NaCl + 4 mg L^{-1} β-glucan. Values are the mean ± SD (n=3). Bars indicated by the same letters are not significantly different α=0.05.

Furthermore, after comparing the mean values, it was noted that the different concentrations of the exogenously applied polysaccharides (β-glucan) could significantly increase the SOD, CAT and POX activities in the callus, in comparison to the control and the NaCl-grown seedlings. The maximal SOD, CAT and SOD levels were seen when the plants were treated with 1 mg/ml of the exogenous polysaccharide (β-glucan), under saline conditions. SOD, CAT and POX were antioxidant enzymes and scavengers of the ROS (Alhasnawi et al., 2017).

One study showed that the β-glucan concentrations in oats were significantly affected by the genotypic and environmental factors, and could also affect the antioxidative enzyme activities in the plants (Brindzová et al., 2008). Furthermore, it was seen that the β-glucans extracted from the barley plant significantly affected its antioxidant activities. The various concentrations of the β-glucan and the different biological activities were influenced by the physiological conditions (like molecular size and structure) of the β-glucan, which were further based on the source and extraction techniques that were applied (Kofuji et al., 2012). The various polysaccharide solutions could display an enzymatic protection activity and could stop or reduce the ROS formation, which led to the β-glucan degradation (Faure et al., 2012). Fig. 1D highlighted the significant difference in the proline levels for the various treatments. The control seedlings showed the lowest proline level of 2.32 µmoles of proline. g^{-1} FW, while the seedlings grown under the saline stress showed a higher proline level of 6.75 µmoles of proline. g^{-1} FW. The maximal proline level was noted in the seedlings which were subjected to saline stress and supplemented with β-glucan, i.e., 11.4 µmoles of proline. g^{-1} FW.

Proline accumulated in the cytosol under the saline conditions and was related to the osmotic adjustment, which helped the plant to adapt to the saline stress (Alhasnawi, 2019). An increased proline level under the stress conditions was attributed to the breakdown of the proline-rich proteins or the de novo proline synthesis. It could have also resulted from a lack of feedback inhibition of the biosynthetic enzymes, which occurred due to the proline sequestration away from its synthesis site, due to the relaxed feedback inhibition of all regulatory step enzymes, or by the low activity of the enzymes which were involved in the proline

degradation like proline oxidase or proline dehydrogenase (Summart et al., 2010).

Under the salt stress conditions, the exogenous polysaccharides (β-glucan) increased the proline production. The resulting proline accumulation showed a protective activity and helped in ROS scavenging. This increased the adaption and growth of the seedlings under saline stress conditions. β-glucan could effectively decrease the harmful effects of the oxidative agents on the callus cells. Accumulation of proline or enzymatic antioxidants could help in the ROS regulation and decreasing the harmful effects of ROS, which improves the salt tolerance of the rice callus cells. The study showed that the exogenous β-glucan could elevate the salinity resistance of the rice callus cells. This system could be used for investigating the role played by the β-glucan in increasing plant tolerance (Alhsanwi et al., 2016).

Here, the researchers used 7 ISSR primers for screening the β-glucan-induced genetic variation, if present, in the *Ocimum basilicum* L. seedlings. They implemented the ISSR-PCR assay for determining the DNA fingerprints and investigating the presence of any genetic variability. The ISSR primers ranged between 16–21 bp (Table 1). The results of this study were similar to those noted earlier (Alhasnawi et al., 2015). The mean percent values of A (13.87), C (8.17), G (11.09), T (3.37), A+T (15.75), and C+G (19.28) were noted for each of the 7 ISSR Primers (Table 3). Here, the primers amplified the scorable bands with a molecular size ranging between 300–1800 bp. The IS2 Primer was seen to amplify the least number of bands per sample, i.e., 3; while the IS5 primer amplified the maximal number of 11 bands (Table 4).

The samples that were analysed in the study represented the 2 types of treatments, i.e., the Control (C) and the β-glucan treatment (T) (Fig 2). The seedlings subjected to these treatments were selected randomly. In total, 7 primers were screened at different temperatures in this study. Since one of the primers, i.e., IS3, did not generate any PCR amplification product, it was not included in this study. The remaining 6 primers generated 68 amplification products. Hence, every primer generated an average of 11.3 products from the 2 samples. 68 marker bands were analysed. The DNA analysis indicated that there was no DNA variability between the T and the C seedling samples (Table 4). Another study reported the presence of genetic diversity and a different DNA fingerprint for the control and the treated

Table 3. The percentage of A, C, G, T, A+T, and C+G per ISSR Primer

ISSR Primer		*A %*	*C %*	*G %*	*T %*	A+T	C+G
IS1	$(AG)_8T$	47.06	0.00	47.06	5.88	52.94	47.06
IS2	$A(CAG)_5$	37.50	31.25	31.25	0.00	37.50	62.50
IS3	$(CAC)_7G$	31.82	63.64	4.55	0.00	31.82	68.18
IS4	$(CTC)_6G$	0.00	63.16	5.26	61.58	31.58	68.42
IS5	$(GAA)_7$	66.67	0.00	33.33	0.00	66.67	33.33
IS6	$(GA)_8G$	47.06	0.00	52.94	0.00	47.06	52.94
IS7	$(AG)_9C$	47.37	5.26	47.37	0.00	47.37	52.63
Total		277.5	163.3	221.8	67.5	314.9	385.1
Average		13.87	8.17	11.09	3.37	15.75	19.25

Table 4. ISSR primers tested for number of amplified fragment and fragment size (bp) for control and treatment

ISSR Primer		No. of bands		Total No. of bands	Fragment size (bp)	
		C	T		C	T
IS1	$(AG)_8T$	4	4	8	300–700	300–700
IS2	$A(CAG)_5$	3	3	6	600–1500	600–1500
IS3	$(CAC)_7G$	-	-	-	-	-
IS4	$(CTC)_6G$	3	3	6	400–1100	400–1100
IS5	$(GAA)_7$	11	11	22	300–1800	300–1800
IS6	$(GA)_8G$	5	5	10	350–1000	350–1000
IS7	$(AG)_9C$	8	8	16	300–1600	300–1600
Total		34	34	68		
Average				11.3		

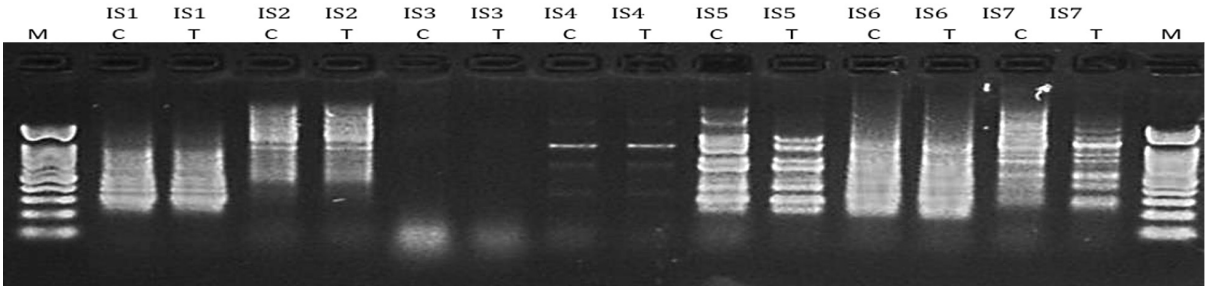

Fig. 2. 7 ISSR primers tested for Control (C) and Treatments (T) samples for detecting genetic differences

plants. In the past, the ISSR analysis was used for determining the randomly-distributed genes through the genome, especially, those that were dominant and showed a higher variation between the various taxa. This technique was also used for determining the inter- and the intra-genetic diversity in the plants (Alhasnawi et al., 2015; Haritha et al., 2016; Mohamad et al., 2017). In the past few years, this technique was also used for detecting the sequence restructuring within the genomes of *Hordeum chilense* which consisted of retrotransposons or SSR-rich regions (Cabo et al., 2013). Many molecular markers have been used for acquiring important information related to genetic polymorphism. This knowledge was useful for developing and improving plant populations (Souza et al., 2013). This was the first study which used the ISSR markers for characterising and evaluating the DNA variation in the seedlings which were subjected to NaCl-stress and supplemented with β-glucan. However, the ISSR-PCR results indicated that there was no significant DNA variation between the 2 treatments.

CONCLUSIONS

This study showed that NaCl stress significantly decreased the root and shoot development of the *Ocimum basilicum* L plants. However, when β-glucans were added to the saline-stressed plants, the polysaccharide reduced the negative effects of the salt stress on the growth and defence mechanisms, by increasing the SOD, CAT, POX and the proline levels in plants. These antioxidant molecules further reduced the membrane oxidative damage caused by the NaCl stress and improved the plant growth. The researchers concluded that the β-glucans could increase the NaCl tolerance of the *Ocimum basilicum* L. plants as they scavenged and neutralised the ROS levels, specifically under the saline stress conditions. They further evaluated the DNA fingerprints to determine whether the application of the exogenous β-glucan under the NaCl stress conditions led to any significant genetic variations in the plants after germination. However, no variations were noted. These results could help in understanding the defence mechanism of the plants under the NaCl stress conditions. Furthermore, they could be used for large-scale conservation and plantation of the *Ocimum basilicum* L plants.

Acknowledgments

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