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Differential gene expression of salt-stressed *Peganum harmala* L.

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Abstract The response of *Peganum harmala* L. seedlings subjected to salinity was investigated through the observation of germination at the 4th, 6th and 8th days under normal and two salinity levels (150 and 200 mM NaCl). Genetic response of *P. harmala* was examined by quantitative estimation and electrophoretic separation of catalase and salt-soluble proteins. The gene expression of catalase and osmotin were investigated using RT-PCR. Final percentage of germination at the eighth day of germination was reduced from 85% in the control to 70 and 30% under the concentration of 150 and 200 mM. The catalase activity and protein content increased as the salinity increased compared to control seedlings. The electrophoretic separation of catalase and salt-soluble proteins exhibited stress-related isozymes and protein bands. RT-PCR of cat1, cat2-3 and cat3 and osmotin genes exhibited up-regulation and down-regulation of genes subsequent to salinity. The reduced germination percentage of salt stressed seedlings was attributed to oxidative damage and osmotic imbalance. The increased catalase activity and protein content were concluded as protective response of *P. harmala* seedlings to salinity induced oxidative stress and osmoregulation. The additional isozyme bands in the salt-stressed seedlings indicated modulation of CAT gene expression in response to elevated H_2O_2 subsequent to salinity. The stress specific gene expression was interpreted as molecular mechanism by which plants can tolerate salinity stress. The up-regulation of cat2-3 gene in relation to stress suggests it crucial role in salinity tolerance in *P. harmala* and further studies are needed for its sequence identification.

1. Introduction

Plants are exposed to a multitude of abiotic stresses including salinity, drought, and temperature extremes which are limiting factors of plant growth and productivity [8]. Plants suffering saline conditions generally show osmotic imbalance resulting in changes in ion concentrations [21]. The high levels of Na^+ and Cl^- were found to enhance loss of membrane integrity and toxic effects of enzyme systems [10,21,59]. As a result, secondary stresses, such as oxidative stress, linked to production of toxic reactive oxygen intermediates are observed [21,59]. At the molecular level, plants respond by complex signaling

networks involving hormones, receptors, protein kinase cascades, transcription factors and modulation of gene expression [3,44]. Besides, a number of regulatory and functional stress-related proteins are synthesized that can directly protect against environmental conditions and/or regulate gene expression and signal transduction in response to stress [69,80].

Reactive oxygen species (ROS) are produced during normal metabolism of the plants, which are equipped with antioxidant enzymatic and nonenzymatic defense systems. Salinity is among abiotic stresses that enhance the production of ROS as well as inhibition of antioxidant systems in plants [11,21]. The accumulation of ROS results in modifications of polypeptide amino acid sequences, fragmentation and aggregation of proteins in addition to lesions in DNA [64]. Several authors showed a close relationship between the efficiency of antioxidant defense system and salt tolerance [64]. Catalase, along with hydroperoxidases and superoxide dismutases, serves as an efficient scavenger of ROS preventing cellular damage [21,64].

Catalase (CAT; E.C.1.11.1.6) is an iron porphyrin enzyme that is the major scavenger of H_2O_2 removing its cellular bulk allowing modulation of H_2O_2 concentration. Plants contain monofunctional, tetrameric and heme-containing catalases that are abundant in peroxisomes or glyoxysomes [24,62,64]. The catalase genes are expressed differentially in response to genetic and developmental signals in addition to biotic and abiotic stresses emphasizing its important defensive roles against oxidative stress [30,58,71].

Several proteins have been characterized to play prominent roles in the regulation of K^+ and/or Na^+ fluxes [47]. Osmotin is a multifunctional 24 kDa basic protein belonging to PR-5 protein family [2,66] induced by salt, water and low temperature stresses [6,27]. Osmotin was found to facilitate solute compartmentation providing osmoregulation or osmotic adjustment by being involved in metabolic or structural alterations [6,13,56,66,67,85]. Also, osmotin has been shown to possess antifungal activity which is correlated with its permeabilization and dissipation of the plasma membrane potential of sensitive fungi [1,60]. These properties make it an important target gene for potential improvement of stress tolerance [7,35].

Among the approaches to overcome salinity problems is to investigate the biological mechanisms involved in salt tolerance, as well as expression profiling of the genes included in salt tolerance [9,37]. Among these genes are those responsible for oxidative protection (e.g. catalase) and for osmotic balance (e.g. osmotin and osmotin-like proteins). Since the genes induced by application of salt stress are usually regulated at the transcriptional level, expression profiling has become an important tool to investigate how an organism responds to environmental changes as well as to understand the up- and downregulating genes under salt-stress [9,15,19,31,33,37].

Peganum harmala L. (*Zygophyllaceae*) is a perennial wild herbaceous and bushy flowering plant that can reach 30–90 cm high with short creeping roots, white flowers and round seed capsules carrying more than 50 seeds [16]. It is native to arid and semiarid rangeland, widely distributed in North Africa, the Middle East, Turkey, Pakistan, India, and Iran. It is common in Egypt along the northern coastal region occupying niches in neglected areas and disturbed ground as well as along the roads that receive runoff water in addition to the recorded rainfall [14]. It has been known since ancient times as Harmal, Sadhab berry which have been used as folk medicine [52]. The most abundant alkaloids are peganine

and harmine that have been used as antiprotozoal [51,63] and non-hepatotoxic, non-nephrotoxic nature and may be considered for clinical application in humans [43].

Based on a previous study, the threshold of salinity tolerance of the Egyptian populations of *P. harmala* was found to be 150 mM NaCl while the plant suffers salinity stress when treated with 200 mM NaCl [38]. The mechanism by which *P. harmala* respond to salinity has not been so far reported at the level of gene expression. The aim of the present study was to investigate the genetic response of *P. harmala* seedlings subjected to salt stress by monitoring the activity and gene expression of catalase as oxidative protection enzyme and stress related proteins that are involved in osmotic balance. This was achieved through time-lapse observation of germination under normal and two salinity levels, quantitative estimation and electrophoretic separation of catalase and protein as well as PCR amplification of catalase and osmotin genes.

2. Material and methods

2.1. Plant material

Fresh representative specimens of *P. harmala* were collected during fruiting stage (April–May) from the Mediterranean coastal strip at Burg Elarab (Lat 30° 54′ 13″ N Long 29° 33′ 13″E). Forty mature seeds were spread over Petri dish (9 cm diameter) containing two filter papers (Whatman No.2) covered by 10 ml distilled water (control) or 10 ml of NaCl solution (150 and 200 mM). The petri dishes containing seeds were incubated in growth chamber at 25 °C, short day light conditions (12 h/12 h day/night) and 50% relative humidity. The number of germinating seeds was recorded every day until the maximum germination was attained (8 days). The percentage of germination was calculated as number of germinated seeds/total number of seeds × 100.

2.2. Preparation of extracts

Salt soluble proteins were extracted from control and salt-stressed seedlings at the 4th, 6th and 8th day of germination. The green leaves of germinated seedlings were weighed and homogenized twice with 5 ml of 0.5 M NaCl then centrifuged at 10,000 rpm for 10 min, thereafter the clear supernatant (containing salt soluble proteins) was collected in fresh Falcon tube. A part of this extract was used directly for quantitative estimation and electrophoretic separation of catalase isozymes. The salt soluble proteins were precipitated by adding 2 volumes of cold acetone to one volume of soluble protein solution then kept overnight in the refrigerator. The tubes were centrifuged for 15 min in at 5000 rpm, the supernatant was discarded carefully and the remaining pellets were retained and dried under vacuum to eliminate acetone residue. The pellets were re-dissolved in saline solution containing 0.8% NaCl and 0.2% $NaNO_3$ and were used for the quantitative estimation as well as electrophoretic separation of salt soluble proteins.

2.3. Quantitative estimation of catalase activity

Catalase activity was quantitatively assayed in salt-soluble protein fraction [39]. The reaction mixture with final volume of

10 ml containing 40 μl enzyme extract was added to 9.96 ml of H_2O_2/phosphate buffer (pH 7.0). Catalase activity was determined by measuring the rate of change of H_2O_2 absorbance in 60 s at 250 nm using spectrophotometer. The blank sample was prepared using buffer instead of the enzyme extract. One unit of enzyme activity was defined as the amount of the enzyme that reduced 50% of the H_2O_2 in 60 s at 25 °C [41].

2.4. Catalase isozyme electrophoresis

Electrophoretic separation of catalase isozymes was carried out using separating gels (8 cm × 10 cm × 0.75 mm) of 7.5% acrylamide concentration. Aliquots (20 μl) of the extract of each sample were applied onto the gels and the electrophoresis was carried out at constant current of 20 mA/gel at the room temperature for required time for the marker dye to reach the end of gel then the gels were stained for catalase isozyme [72]. The stained isozyme loci were numbered collectively in the order of increasing migration from the anode with the most anodally migrating isozyme designated 1, the next 2 and so on [74].

2.5. Quantitative estimation of proteins

Protein contents were quantitatively assayed at the 4th, 6th and 8th days of germination by measuring absorbance at 280 nm and 260 nm [73] using spectrophotometer model UV-9200 (Qualitest USA LC). The protein concentration was estimated by applying the formula: Concentration (mg/ml) = $(1.55 \times A280) - (0.76 \times A260)$.

2.6. Electrophoretic separation of proteins

SDS–polyacrylamide gel electrophoresis was applied for electrophoretic separations of salt soluble proteins using discontinuous buffer system of 4% stacking gels and 12% separating gels [42]. Electrophoresis was carried out using Tris/glycine (1 M, pH 8.3) as electrode buffer at a constant current of 10 mA/gel for the stacking gel and 15 mA/gel for the separating gel at 15 °C for approximately 1hr. Following the removal of the electrophoretic assembly, gels were stained using 0.05% Coomassie brilliant blue R250 for about 2 h, and then destained in a mixture of 500 ml methanol, 425 ml H_2O and 75 ml glacial acetic acid for the time required for bands to appear as blue in clear background. Stained gels were photographed and the relative mobility (R_f) of each band was calculated as the ratio between the distance migrated by a band to that migrated by the bromophenol blue indicator. The molecular weight of protein was estimated by applying the following formula: Log MW = the distance of a band migration (R_f)/the distance of the bromophenol blue migration.

2.7. Data analysis

The experiment which was carried out for quantitative estimation of protein and catalase activity comprised all possible combinations of 3 levels of salinity (0, 150 and 200 mM NaCl) with 3 periods of germination (4, 6, 8 days) with three replicates. The variation in salinity variable in relation to the period of treatment was assessed by applying analysis of the variance (ANOVA) using the statistical software program SPSS version 21.0 [36]. The data were subjected to analysis of variance and post hoc comparisons were done with Duncan's multiple range test.

2.8. Expression profiling of catalase and Osmotin genes

2.8.1. Extraction of DNA and RNA

The samples selected for genomic gDNA and total RNA manipulation were those of *P. harmala* seedlings at the 6th day of germination of both control and under the concentration of 200 mM NaCl. The seedlings were transferred to a sterile mortar and ground into fine powder with liquid nitrogen. Genomic DNA (gDNA) was isolated from 50 mg of seedlings using Mini Kit Qiagen (www.qiagen.com) for DNA extraction following the manufacturer's instructions. RNA was extracted from 30 mg of seedlings using BioFlux Kit for RNA extraction following the manufacturer's instructions. After gDNA and RNA quantification, 500 ng of each samples were electrophoresed to confirm its integrity, and then the samples were stored at −20 °C.

2.8.2. Primer design

Synthetic forward and reverse primers developed from catalase genes of *Arabidopsis thaliana* by Smykowski et al. [68] were designed to amplify the target sequences using PCR. Three primers (*cat*1 F: 5′ TCAT CGG GAA GGA GAA CAAC; R: 5′ ACC AAA CCG TAA GAG GAG CA (*Cat*2-3 F: 5′ CAG GTT CGT CAT GCT GAG AAG; R: 5′ TTA GAT GCT TGG TCT CAC GTT; *Cat*3 F: 5′ CAA ACA GGC TGG AGA CAG GT; R: 5′GAC GGA TTT AAC GAC CAA GC3′) were applied to amplify catalase gene [68]. Osmotin primer ATOSM34 was provided by Qiagen (www.qiagen.com) according to the published sequence of *Arabidopsis thaliana* Accession No. G72090 Gene ID 826770 (F:5′ATG GAG AAT TTC CTC AA; R: 5′TTG ATC GGC TTG CTA AG).

2.9. Semi-quantitative reverse transcription PCR (sqRT-PCR)

2.9.1. Complementary DNA (cDNA) synthesis

The RT007 AMV Reverse Transcriptase (BIOER TECHNOLOGY http://www.bioer.com.cn) was used to create cDNA from RNA template. In the first step, 7 μl of RNA was mixed with 2 μl of reverse primer and 3 μl of depec water in sterile 1.5 ml tubes, centrifuged briefly for collection and incubated in water bath at 65–70 °C for 5–10 min then chilled on ice for 2–10 min. During the second step, 4 μl of 5× Taq DNA polymerase buffer, 2 μl of 10 mM dNTP mixture, 0.6 μl of RT007 AMV reverse transcriptase and 1.5 μl of depec water were collectively added into the mixture of the first step. After incubation for 60 min. at 41–42 °C, the reaction was terminated by heating to 70 °C for 5 min. then the samples containing total cDNA were stored at −20 °C.

2.9.2. Polymerase chain reaction for genomic and cDNA (PCR)

The polymerase chain reaction (PCR) mixture contained PCR beads tablet (manufactured by Amersham Pharmacia Biotch), which containing all of the necessary reagents except the primer and the DNA which added to the tablet. Genotyping PCRs for genomic DNA (gDNA) were set up in sterile thin-walled 0.2 ml PCR tubes. The 25 μl reaction consisted of:

2 µl gDNA template, 2.5 µl 5× Taq DNA polymerase buffer, 2.5 µl MgCl$_2$ (1 mM), 10 mM KCl, 50 mM Tris–HCl, 2 µl dNTPs (10 mM), 2 µl 25 pmol forward primer, 2 µl pmol reverse primer, 0.5 µl Taq DNA polymerase (2 units/µl) and the total volume was completed to 25 µl using 11.5 µl sterile distilled water (SDW).

Genotyping PCRs for cDNA were set up in sterile thin-walled 0.2 ml PCR tubes. The polymerase chain reaction (PCR) mixture contained PCR beads tablet (manufactured by KAPA2G™Fast HotStart) The 25 µl reaction consisted of: 2 µl cDNA template, 5 µl 5× Taq DNA polymerase buffer, 0.5 µl MgCl$_2$ (25 mM), 0.5 µl dNTPs (10 mM), 1.3 µl forward primer (10 µM), 1.3 µl reverse primer (10 µM), 0.1 µl Taq DNA polymerase (5 units/µl) and the total volume was completed to 25 µl using 14.4 µl sterile distilled water (SDW).

Reactions were cycled as follows: the denaturation at 94 °C for 2 min, 30 cycles each consists of the following steps: denaturation at 94 °C for 1 min, annealing (at 48 °C for cat1 and cat2-3 and cat3, 55 °C for cat2-2, 38 °C for ATOSM34) for 2 min, extension at 72 °C for 1 min, then the final extension at 72 °C for 10 min and hold at 4 °C; then, 7 µl of 6× tracking buffer (manufactured by Qiagen Kit) was added to the amplification product.

2.9.3. Agarose gel electrophoresis

DNA and RNA were visualized using agarose gel electrophoresis (1.5% agarose in 1× TBE buffer and 0.25 µg/µl ethidium bromide) gel electrophoresis. DNA and RNA samples containing 1× loading buffer (5% glycerol, 0.05% bromophenol blue) were loaded onto gels immersed in 1× TBE buffer along with 5 µl Quick-Load™ 1 kb ladder (New England Biolabs). Samples were electrophoresed for approximately 60 min at 75 V. DNA/RNA was visualized using GDS 7500 UV transilluminator (UVP).

2.9.4. Gel analysis

The gels were scanned for band rate of flow (Rf) using gel documentation system (AAB Advanced American Biotechnology 1166 E. Valencia Dr. Unit 6 C, FullertonCA92631). The different M.W. of detected bands were determined against the DNA marker.

3. Results

3.1. The response of *P. harmala* seedlings to the levels of salinity

The final percentage of germination of *P. harmala* at the eighth day of germination was reduced from 85% (34 seeds germinated out of 40 seeds used) in the control to 70 (28 out of 40) and 30% (12 out of 40) under the concentration of 150 and 200 mM, respectively (Fig. 1). The gradient inhibitory effect of the two NaCl concentrations was clearly observed at the fourth, sixth and the eighth days. Therefore, all the experiments were carried out on the 4th, 6th, and 8th day of germination of both control and the two salinity levels.

3.2. Quantitative estimation of catalase

The highest activity of catalase was observed in 4-day old seedlings of control and the two salinity levels (32, 49 and 45 gFWT^{-1} min^{-1} for control, 150 mM and 200 mM salinity

respectively, Fig. 2). As the age of the seedlings increased up to 6 days, the activity of catalase was reduced to 17, 16 and 42 gFWT^{-1} min^{-1} for the control and the two salinity levels (Fig. 2). On the other hand, more reduction in catalase activity was observed after 8 days reaching 12 gFWT^{-1} min^{-1} in the control, 16 gFWT^{-1} min^{-1} in 150 mM treated seedlings and 22 gFWT^{-1} min^{-1} under 200 mM salinity maintaining a highly significant increase ($p \leqslant 0.005$) compared to their corresponding controls (Fig. 2).

3.3. Electrophoretic separation of catalase isozyme

The banding pattern of catalase isozyme for the studied *P. harmala* seedlings collectively exhibited 7 isozyme loci CAT1, CAT2, CAT3, CAT4, CAT5, CAT6 and CAT7 (Fig. 3). The 4-day old seedlings of control were characterized by CAT1 while the seedlings of both salinity levels were distinguished by CAT2 at the 6th and 8th days of germination. On the other hand, CAT3 and CAT4 were observed in the 6-day-old seedlings treated with 150 and 200 mM respectively. At the 4th and the 6th days of germination, CAT5 was observed in the control seedlings, while it was recorded in 8-day old seedlings treated with 150 mM. CAT6 was absent after 4 and 6 days when seedlings were treated with 200 mM. CAT7 appeared only after 4 and 6 days when treated with 150 and 200 mM NaCl respectively (Fig. 3).

3.4. Quantitative estimation of salt soluble proteins

The salt-soluble proteins content of the control decreased with increasing age of seedlings (Fig. 4). At the 4th day of germination, the protein content decreased as the severity of salinity increased compared to control (Fig. 4). However, there was a pronounced increase in the protein content of seedlings treated with 150 mM NaCl for 6 days (0.45 mg/gm compared to 0.15 mg/gm of both the corresponding control as well as 200 mM NaCl treated seedlings (Fig. 4)). At the 8th day of germination, the protein content significantly increased to 0.25 and 0.43 mg/gm under the concentration of 150 and 200 mM NaCl respectively compared to 0.15 in control (Fig. 4). Statistical analysis indicated that the increase in protein content was highly significant ($p \leqslant 0.005$) under the effect of 150 mM salinity levels at the tested days of germination. On the other hand, both control and 200 mM NaCl treated seedlings exhibited similar values at the 6th day of germination (Fig. 4). In the meantime, the interaction between the two variables was also highly significant ($p \leqslant 0.005$).
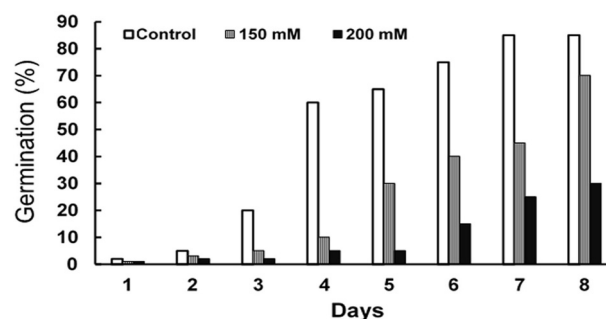

Figure 1 Percentage of germination of *Peganum harmala* under control and salinity (150 and 200 mM NaCl).
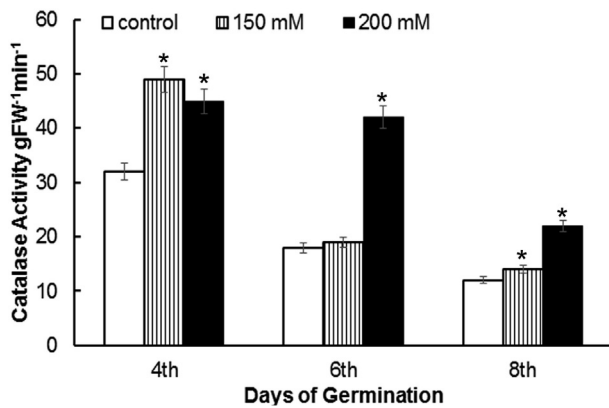
Figure 2 Activity of catalase of *Peganum harmala* seedlings under control and salinity (150 and 200 mM NaCl) at the 4th, 6th and 8th days of germination. * Differences are highly significant ($p \leqslant 0.005$) compared to corresponding control.
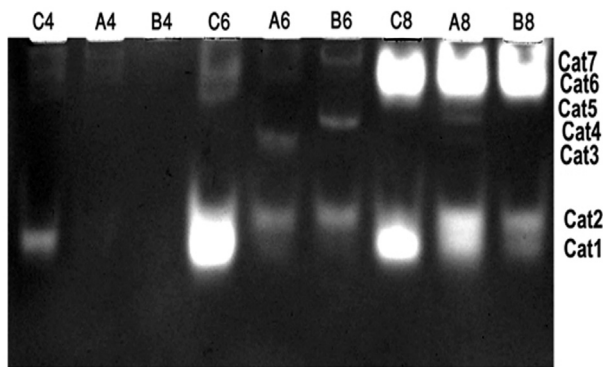

Figure 3 Electrophoretic separation of catalase isozyme of *Peganum harmala* seedlings under control and salinity (150 and 200 mM NaCl) at the 4th, 6th and 8th days of germination.
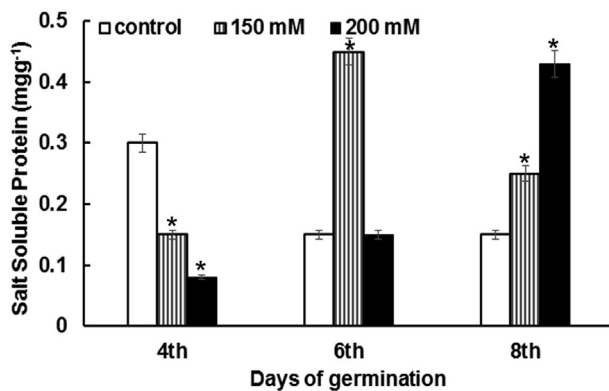

Figure 4 Salt-soluble protein content of *Peganum harmala* seedlings under control and salinity (150 and 200 mM NaCl) at the 4th, 6th and 8th days of germination. * Differences are highly significant ($p \leqslant 0.005$) compared to corresponding control.

3.5. Electrophoretic separation of salt-soluble proteins

The electrophoretic separation of salt-soluble proteins of control seedlings at 4 days exhibited four protein bands with
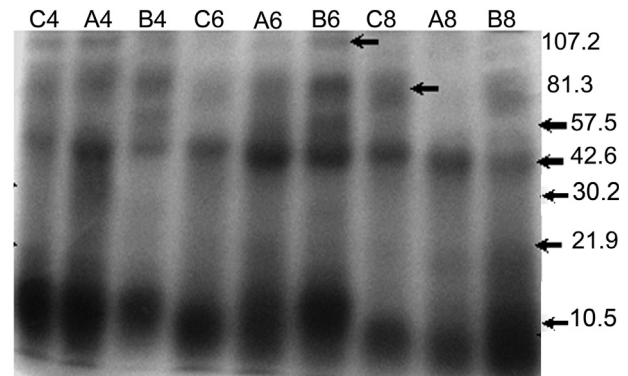

Figure 5 Electrophoretic separation of salt-soluble proteins of *Peganum harmala* seedlings under control (C) and 150 mM NaCl (A) and 200 mM NaCl (B) at the 4th, 6th and 8th days of germination.

molecular weights ≈107.1, 81.3, 42.7, 10.5 KD (Fig. 5). Additional two protein bands with molecular weight of 30.2 KD and 57.5 KD were observed in the seedlings treated with 150 and 200 mM respectively. At the 6th day of germination, the protein band with 107.15 KD of the control seedlings disappeared while the seedlings treated with150 mM exhibited an extra protein band with molecular weight of 21.9 KD. In the meantime, the band with molecular weight 30.2 KD disappeared in the seedlings treated with 150 mM while it was observed in 200 mM treated seedlings. At 8 day-old-seedlings, the band with molecular weight 21.9 KD was also observed in the seedlings treated with 200 mM (Fig. 5).

3.6. The expression of stress related genes

The samples amplified using Cat1 primer pair (F and R) revealed an amplification product (≈1500 bp; Fig. 6) only in control while no amplification product was observed under the concentration of 200 mM NaCl. When Cat2-3 primer pair was applied, amplification product was observed only under the concentration of 200 mM NaCl (≈1500 bp; Fig. 6). The amplification product was observed in both control and salt treated samples when Cat3 primer pair were applied (≈800 bp Fig. 6). The samples amplified using ATOSM34 primers exhibited an amplification product (≈550 bp) in samples treated with 200 mM NaCl while no products were observed in control samples.

4. Discussion

4.1. Effect of salinity on seed germination

The present study demonstrated that salinity adversely affected the germination of *P. harmala* seeds reaching its maximum reduction under the concentration of 200 mM NaCl (30%) compared to control (85%). Several authors also observed the reduction in germination percentages of other plant species subsequent to salinity and drought [22,50,70,82]. One of the direct effects of salt stress is that it can produce osmotic imbalance which affects seed germination by limiting water availability during germination [21,56]. Therefore, the activity and events normally associated with
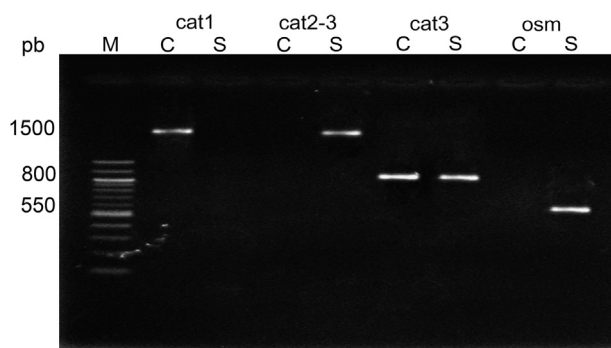
Figure 6 Electrophoretic separation of RT-PCR products of using three catalase primer pairs (cat1, cat2-3 & cat3) and osmotin (osm) of *Peganum harmala* seedlings under control (C) and 200 mM NaCl (S) at the 6th day of germination. M = 1 Kpb DNA ladder.

germination (e.g. enzyme activity, mobilization of macromolecules, mRNA accumulation) are delayed and/or proceed at a reduced rate [38,55,56,78,82].

4.2. Effect of salinity on catalase activity

In the present study, it was observed at the three tested age of seedlings that as the salinity increased, catalase activity significantly increased compared to their corresponding control seedlings (Fig. 2). Catalase has already been reported to play a major role in scavenging reactive oxygen species under abiotic stress [46,54,76,79,83]. Besides, a substantial increase in catalase activity was observed in salt-tolerant cotton [29] and barley [40] cultivars compared to salt-sensitive ones. It was also concluded that the increased activity of catalase enzyme was associated with lowering the oxidative damage upon spraying maize with H_2O_2 [28]. Salinity is among abiotic stresses that enhance the oxidative damage which can induce antioxidative defense mechanisms [11,28,46,56]. Therefore, the observed increase in catalase activity in the present study can be interpreted as a protective response of *P. harmala* seedlings to salinity induced oxidative stress.

In the present study, the increase in catalase activity under salt stress was correlated with the detection of additional isozyme bands in the salt-stressed seedlings (CAT2, CAT3, CAT4, CAT7; Fig. 3). In the meantime, the salt stressed seedlings were distinguished by the absence of CAT1 isozymes. These observations can be interpreted as modulation of CAT gene expression in response to elevated H_2O_2 subsequent to salinity [12,18,26]. There are many indications that hydrogen peroxide acts as a signal molecule that affects expression of several genes including antioxidant enzyme encoding genes [4,48]. Catalase enzyme is activated when the concentration of H_2O_2 exceeds the degrading capacity of other H_2O_2 degrading enzymes (e.g. peroxidases), thus modulation of catalase gene expression is required for maintaining cell's H_2O_2 homeostasis so as to regulating its role as a second messenger [5,24,48,57].

The present study demonstrated that the three investigated catalase genes of *P. harmala* exhibited stress-specific changes at the transcriptional level. These changes include the stress down-regulation of cat1 mRNA while cat2-3 was

up-regulated in relation to stress. Many stress up-regulation and down-regulation of genes have also been reported by several authors, for instance, the up-regulation of genes in salt stressed wheat, barley, *Suaeda salsa*, smooth cordgrass and radish [18,61,75,77,81]. On the other hand, down-regulation of genes was observed in salt stressed potato [45] and mangrove [20] which was attributed to negative feedback regulation due to enhancement of H_2O_2 levels as second messenger. The up-regulation and down-regulation of genes subsequent to salinity was interpreted as molecular mechanism by which plants can tolerate salinity stress [23]. The differential expression of catalase genes in sunflower seedlings under Cd stress have been related to the synthesis of CAT isoforms less sensitive to oxidation, which would prevent enzyme inactivation and H_2O_2 accumulation [12]. The up-regulation of cat2-3 gene in relation to stress suggests it crucial role in salinity tolerance in *P. harmala* and further studies are needed for its sequence identification.

4.3. Effect of salinity on stress related proteins

It is observed in the present study that the amount of salt-soluble proteins significantly increased reaching 0.45 and 0.43 mg/g at the 6th and 8th day-old-seedling under 150 and 200 mM salinity (Fig. 4). However, this increase was correlated with the lowest germination percentage (40 and 30%, Fig. 1) which can indicate that most of the accumulated salt-soluble proteins were devoted to osmoregulation instead of growth [38,53,65,69,84]. Besides, stress related protein bands with molecular weights of 21.9, 30.2 and 57.5 KD were observed in the protein profile of salt-soluble proteins (Fig. 5). Among symptoms of salinity stress is the inhibition of regular protein synthesis while contributing factors of salinity tolerance is *de novo* synthesis of stress related proteins [25,32,84]. Therefore, the stress related protein bands observed in the present study can be interpreted as newly synthesized proteins that are involved in osmotic adjustment and water regime regulation of salt stressed *P. harmala*.

The amplification product that was observed only in salt stressed *P. harmala* using ATOSM34 primers (Fig. 6) indicated the upregulation of osmotin gene subsequent to salinity. It was observed that upregulation and over expression of osmotin genes can enhance salinity tolerance in several plant species [6,13,15,17,34,49]. Therefore, the observed induction of osmotin genes in the present study can be interpreted as an impact for salinity tolerance in salt stressed *P. harmala* seedlings.

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