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# Modulation of Salt Stress Induced Responses in Pea (*Pisum sativum* L.) Through Salicylic Acid and *Trichoderma* Application

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**A B S T R A C T**

Salinity Stress is one of the most important abiotic stresses that cause adverse effects on crop productivity and agricultural sustainability. The present experiment was carried out as a pot-culture in the poly-house on pea genotype HUP-2 under salinity stress, and identified various biochemical attributes which were progressively reduced with increase in salinity level due to formation of reactive oxygen species i.e. hydrogen peroxide ($H_2O_2$) and superoxide radical ($O_2^{\cdot-}$). Treatment of seeds with *Trichoderma asperellum* (T42) and exogenous application of salicylic acid, solitary or in combination, ameliorated salt stress induced responses as reflected by detoxification of both reactive oxygen species, $H_2O_2$ and $O_2^{\cdot-}$ and also observed reduction in lipid peroxidation by enhancement of osmoprotectants i.e. proline content and activation of antioxidative enzymes activity like superoxide dismutase and ascorbate peroxidase as compared to control of respective salinity levels (4, 8 and 12 $dSm^{-1}$). Exogenous foliar application of SA (0.25 mM), singly and in combination of *Trichoderma*, ameliorated the hostile effects of salinity up to the level of 8 $dSm^{-1}$ which showed a significant expansion of plant phenotype as compared to the untreated stressed plants.

## Introduction

Pulse production is greatly reduced due to the sensitivity of the plants to biotic and abiotic stresses. Pea (*Pisum sativum* L.) is the most important and globally known cool season legume vegetable crop belonging to Fabaceae family having important nutritional value i.e. high protein, carbohydrates, phosphorus, iron, calcium and vitamins content (Nutrition Facts: Peas, 2015). It is grown in many soil types, but performs better in fertile, light-textured, well-drained sandy loam soil (Hartmann *et al.*, 1988; Elzebroek and Wind, 2008). It is very sensitive to soil salinity which affects the plant growth and development leading to a pronounced decrease of biomass production and overall detrimental impact on pea productivity. Soil salinization is a serious threat to crop productivity and predicted to increase in the face of global climate change (FAO, 2011). Salinity is a major environmental factor adversely affecting crop production and agricultural sustainability in many regions of the world as it reduces the value and productivity of the affected land

(Tejera *et al.,* 2007; Qadir *et al.,* 2008; Flowers *et al.,* 2010).

Salicylic acid (SA) is a crucial endogenous naturally occurring plant produced phenolic compound that modulates plant responses to various biotic and abiotic stresses. As a signalling molecule, several researches have well investigated the significant role of SA in the regulation of defense mechanism of plants to many abiotic stresses (Gautam and Singh 2009; Simaei *et al.,* 2011; Ying *et al.,* 2013). SA accumulated in the plant tissues under unfavourable environmental conditions and contributes to increase of plant's tolerance to salinization (Syeed *et al.,* 2011; Liu *et al.,* 2014). Exogenous application of SA has been reported to influence a widespread of morpho-physiological and biochemical parameters of plants under salinity stress, including plant growth and development, mineral uptake and transport, ion balance, stomatal conductance, transpiration, photosynthetic parameters, lipid peroxidation, anti-oxidative enzyme activities, metabolite accumulation, etc. (Gautam and Singh 2009; Syeed *et al.,* 2011; Sharma *et al.,* 2012; Bastam *et al.,* 2013; Liu *et al.,* 2014).

*Trichoderma* is a fungal genus found in many ecosystems and plays an important role in biological control of soil borne pathogens. It can reduce the severity of plant diseases by inhibiting plant pathogens in the soil through their highly potent antagonistic and myco-parasitic activity (Harman and Kubicek, 1998).

There are evidences from the experimental findings that some strains of *Trichoderma* interact with roots, enhancing plant growth potentials and resistance to biotic and tolerance to abiotic stresses (Howell, 2003; Hermosa *et al.,* 2012). *Trichoderma* rhizosphere-competent strains have been shown to have direct effects on plants, increasing their growth potential, nutrient uptake, fertilizer use efficiency, rate of seed germination and stimulation of plant defensee against biotic and abiotic damages (Shoresh *et al.,* 2010). Keeping this in mind, the present study on the effect of salinity on various morphological and biochemical attributes, along with ameliorating effects of salicylic acid and *Trichoderma* over salinity stress was investigated.

## Materials and Methods

### Experimental details

The present experiment was carried out as pot culture in the poly house and the Laboratory of Stress Physiology in the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India. Disease free and healthy seeds of pea (*Pisum sativum* L.) genotype (HUP-2) and fungal bio-control agent *Trichoderma asperellum* (T42) were obtained from the Department of Genetics and Plant Breeding and Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, respectively. The experiment was laid out in Complete Randomized Design (CRD) which consisted of 13 treatments, 3 replications for each treatment. The data were obtained at different growth periods of 40, 60 and 80 days after sowing (DAS).

### Treatment details

Thirteen treatments included T0 = Control, T1 = NaCl (4 $dSm^{-1}$), T2 = NaCl (4 $dSm^{-1}$) +SA (0.25 mM), T3 = NaCl (4 $dSm^{-1}$) + *T. asperellum* T42, T4 = NaCl (4 $dSm^{-1}$) + SA (0.25 mM) + *T. asperellum* T42, T5 = NaCl (8 $dSm^{-1}$), T6 = NaCl (8 $dSm^{-1}$) +SA (0.25 mM), T7 = NaCl (8 $dSm^{-1}$) + *T. asperellum* T42, T8 = NaCl (8 $dSm^{-1}$) + SA (0.25 mM) + *T. asperellum* T42, T9 = NaCl (12 $dSm^{-1}$), T10 = NaCl (12 $dSm^{-1}$) +SA (0.25 mM), T11 = NaCl

(12 $dSm^{-1}$) + *T. asperellum* T42, T12 = NaCl (12 $dSm^{-1}$) + SA (0.25 mM) + *T. asperellum* T42.

## Seed treatment

Good, healthy looking and uniform seeds of pea variety 'HUP-2' were treated with the spore suspension of *Trichoderma asperellum* (T42) (1x $10^6$ spores $ml^{-1}$) for 4 to 5 h and then used for sowing in pots. After germination, a population of five plants per pot was maintained. After 20 days of sowing, twelve pots were imposed with 40, 80 and 120 mM NaCl treatment, which produced 4, 8 and 12 $dSm^{-1}$, respectively as measured by Electrical conductivity (EC) in order to maintain the required salinity levels in the pots at weekly intervals. In each salinity level, three pots were treated with SA (0.25 mM as foliar application), three with *Trichoderma* and three with both SA and *Trichoderma* combination, and similar number of pots were not given any salinity treatment and they served as control.

## Hydrogen peroxide

Hydrogen peroxide was determined in first fully expanded leaf from the normal and stressed plants. The estimation was done as per the protocol of Mukherjee and Choudhary (1983).

## Histochemical determination of $H_2O_2$ and $O_2^-$ in pea leaves

The histochemical staining of $H_2O_2$ and $O_2^-$ was performed as previously described by Jabs *et al.,* (1996) and Thordal-Christensen *et al.,* (1997), respectively, with some modification. In case of $H_2O_2$, different treated pea leaves were dipped in Diamino-benzidine (1mg $ml^{-1}$, pH 3) and incubate for 6-8 h in dark at 25$^o$C. Dechlorophylization was performed by transferring leaf samples in bleaching solution [ethanol/acetic acid/glycerol (3:1:1; v/v)] and boiled in a water bath for 10-15 min at 90$^o$ C. After that, leaves were briefly rinsed in distilled water twice.

However, in case of $O_2^-$, leaf samples were dipped in 0.2 mg $ml^{-1}$ NBT in 25 mM HEPES buffer (pH 7.8) and incubated at 25$^o$C in the dark for 3 h. Leaves were rinsed in 80% (v/v) ethanol for 15 min at 80$^o$C and mounted in lactic acid/phenol/water (1:1:1; v/v), the developed staining on leaves were observed through microscope.

## Malondialdehyde (MDA) content

MDA content was determined in fully expanded leaf from the top of the plants using 0.5% Thiobarbituric acid (TBA) in 20% TCA. The level of lipid peroxidation was measured in terms of MDA content, a product of lipid peroxidation, according to the methods proposed by Hodges *et al.,* (1999) and absorbance was recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The MDA equivalent was calculated as follows:

MDA [nmol / (mL fresh weight)] = [($A_{532}$– $A_{600}$)/155000] x $10^6$

## Proline content

Free Proline content in the leaves was determined by the method of Bates *et al.,* (1973) using acid ninhydrin reagent. The reaction mixture containing (1:1:1) ratio of supernatant, glacial acetic acid and ninhydrin reagent was boiled at 100$^0$C for 30 min till brick red colour developed. After cooling, proline content which indicate brick red colour was dissolved in toluene, then mixed well and the absorbance was read at 520 nm using spectrophotometer (Spectra Max M2 USA) against toluene blank.

### Superoxide dismutase activity (SOD)

SOD activity was estimated by the method as proposed by Dhindsa *et al.,* (1981). Three mL of the reaction mixture containing 0.1 mL of 1.5 M sodium carbonate, 0.2 mL of 200 mM methionine, 0.1 mL of 2.25 mM NBT, 0.1mL of 3 mM EDTA, 1.4 mL of 0.1M phosphate buffer, 1 mL of DW and 0.1 mL of enzyme extract were taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 mL riboflavin (60 µM) and placing the tubes below a light source of two 15 W florescent lamps for 15 min. Reaction was stopped by switching off the light and covering the tubes by black cloth. Tubes without enzyme extract developed maximum colour. Absorbance was recorded at 560 nm and SOD activity calculated as follows:

$$\text{Enzyme Unit (EU)} = \frac{\text{Enzyme}^{*}_{light} - (\text{Enzyme}^{\#}_{light} - \text{Enzyme}^{*}_{dark})}{\text{Enzyme}^{*}_{light} / 2}$$

* = Without Enzyme.
\# = With Enzyme.

### Ascorbate peroxidase activity

Ascorbate peroxidase activity was estimated in the first fully expanded leaf according to the method as proposed by Nakano and Asada (1980).

### Plant cell death assay

Cell death was estimated as described by Levine *et al.,* (1994). For each sample, a 400-µL aliquot of cells was incubated with 0.05% Evans blue for 30 min and then washed extensively 4-5 times with distilled water. The dye bound to dead cells was solubilized in 50% methanol with 1% SDS for 30 min at 50°C and absorbance was recorded at 600 nm.

### Data analysis

All data were presented as mean values of three replicates and analyzed using a statistical package, SPSS (Version 16.0). One-way ANOVA (analysis of variance) was employed followed by Duncan's multiple range tests to determine the significant difference among means of the treatment at $P \leq 0.05$.

## Results and Discussion

### Hydrogen peroxide content ($H_2O_2$, µmol $g^{-1}$ fresh weight)

There was a significant increment in $H_2O_2$ content with increasing level of salinity (Fig. 1A). Among the salinity level, maximum 186.04% $H_2O_2$ content was observed in T9 (86.29 µmol $g^{-1}$ fresh weight) followed by 127.30% in T5 (68.57 µmol $g^{-1}$ fresh weight) as compared to control T0 (30.17 µmol $g^{-1}$ fresh weight) at 80 DAS. SA and *Trichoderma*, alone or in combination, ameliorated salt stress induced responses at each salinity level as compared to respective salinity control. Among treatments, maximum 24.04% reduction in $H_2O_2$ content was recorded in T2 (17.0 µmol $g^{-1}$ fresh weight) followed by 21.80% in T4 (17.50 µmol $g^{-1}$ fresh weight) as compared to respective salinity control, T1 (22.38 µmol $g^{-1}$ fresh weight) at 60 DAS under 4 $dSm^{-1}$ salinity level.

### Malondialdehyde (MDA) content (nM $g^{-1}$ fresh weight)

MDA content is the key indicator of salinity induced oxidative stress responses on plant cell membrane as a lipid peroxidation. There was a significant increase in MDA content with the increasing salinity levels (4, 8 and 12 $dSm^{-1}$; Fig. 1B). Among the salinity level, maximum 206.28% MDA content was recorded in T9 (4.10 nM $g^{-1}$ fresh weigh)

followed by 146.85% in T5 (3.31 nM $g^{-1}$ fresh weigh) as compared to control T0 (1.34 nM $g^{-1}$ fresh weigh) at 40 DAS. Among treatments, SA and *Trichoderma,* applied alone or in combination, showed ameliorating effect on all the salinity levels by decreasing MDA content.

Among treatments, maximum 26.87% reduction in MDA content was observed in T2 (0.96 nM $g^{-1}$ fresh weigh) followed by 22.14% in T6 (1.28 nM $g^{-1}$ fresh weigh) as compared to respective salinity controls T1 (1.31 nM $g^{-1}$ fresh weigh) and T5 (1.64 nM $g^{-1}$ fresh weigh) respectively, at 60 DAS.

**Proline content (mg $g^{-1}$ fresh weight)**

Plants increase osmoprotectant like proline, glycine-betaine under stressful environment significantly for maintaining their homeostasis. Among salinity level, maximum 802.77% proline content was recorded in T9 (5.04 mg $g^{-1}$ fresh weight) followed by 579.28% in T5 (3.79 mg $g^{-1}$ fresh weight) as compared to control T0 (0.56 mg $g^{-1}$ fresh weight) at 80 DAS (Fig. 1C). Among treatments, maximum 42.94% reduction was observed in proline content in T10 (2.87 mg $g^{-1}$ fresh weight) followed by 37.42% in T6 (2.37 mg $g^{-1}$ fresh weight) as compared to respective salinity controls T9 (5.04 mg $g^{-1}$ fresh weight) and T5 (3.79 mg $g^{-1}$ fresh weight), respectively at 80 DAS, although the maximum proline content (6.63 mg $g^{-1}$ fresh weight) was recorded at 60 DAS in 12 $dSm^{-1}$ level of salinity.

**Superoxide dismutase activity (SOD, U $g^{-1}$ fresh weight $min^{-1}$)**

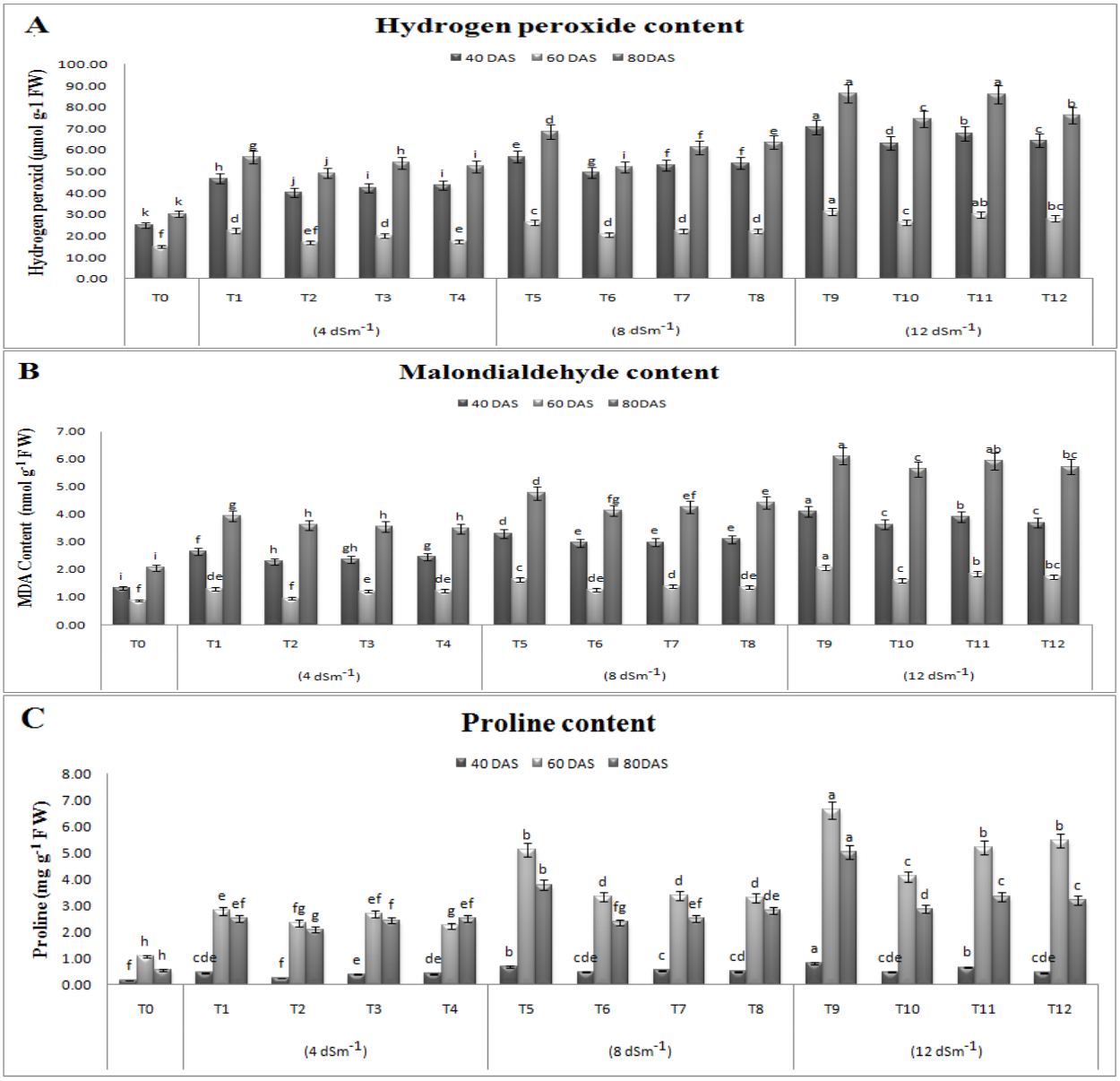
The data on superoxide dismutase (SOD) activity at different treatments under salinity stress showed a significant decrease in SOD activity with the increasing salinity levels (Fig. 1D). Maximum 94.83% SOD activity was recorded in T5 (1.13 U $g^{-1}$ fresh weight $min^{-1}$) followed by 85.05% in T1 (1.07 U $g^{-1}$ fresh weight $min^{-1}$) as compared to control T0 (0.58 U $g^{-1}$ fresh weight $min^{-1}$) at 40 DAS. Among treatments, salicylic acid and *Trichoderma* showed ameliorating effect on all the salinity levels through activation of antioxidative system. The treatment with *Trichoderma* recorded maximum 128.94% SOD activity in T11 (0.87 U $g^{-1}$ fresh weight $min^{-1}$) followed by 126.31% in combined treatment of salicylic acid and *Trichoderma* T12 (0.86 U $g^{-1}$ fresh weight $min^{-1}$) as compared to respective salinity control T9 (0.38 U $g^{-1}$ fresh weight $min^{-1}$) at 60 DAS. Maximum SOD activity was recorded in T4 (1.42 U $g^{-1}$ fresh weight $min^{-1}$) at 4 $dSm^{-1}$ salinity level.

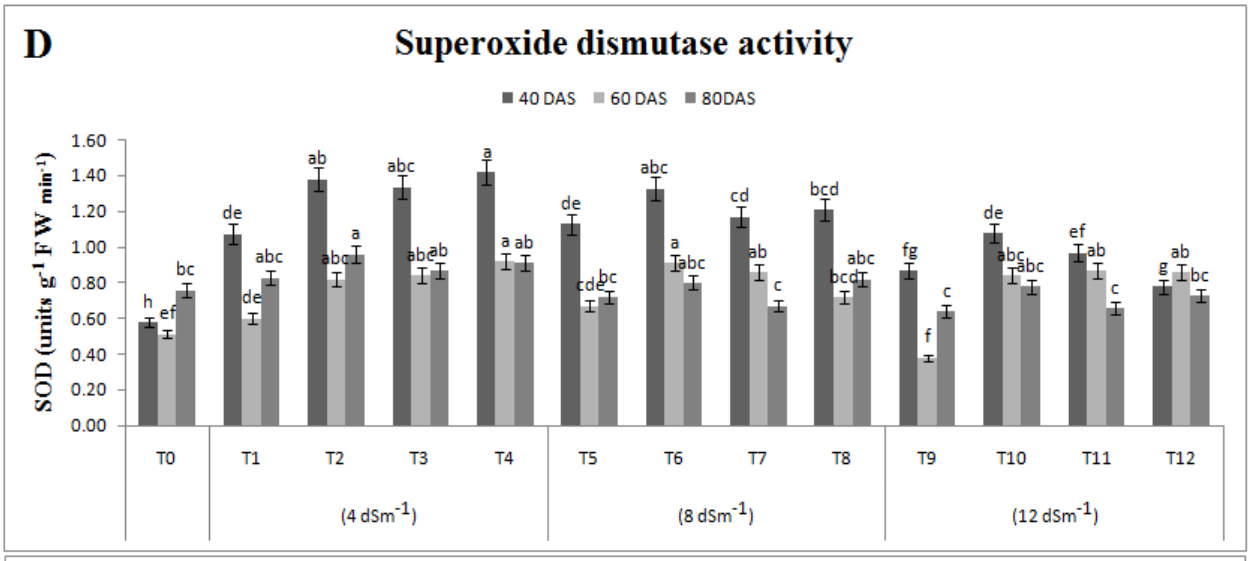
**Ascorbate Peroxidase Activity (APX, U $g^{-1}$ fresh weight $min^{-1}$)**

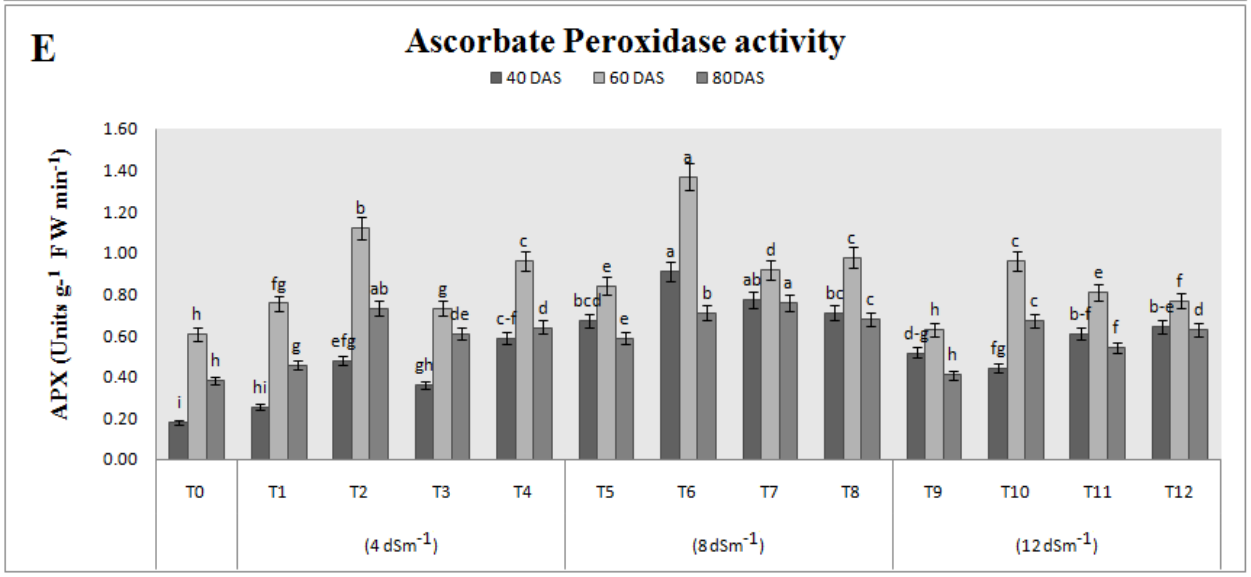
Data pertaining to APX activity are presented in Figure 1E under different salinity levels (4, 8 and 12 $dSm^{-1}$), which elucidate that there was a significant increment on APX activity with increasing level of salinity upto 8 $dSm^{-1}$ but thereafter it declined. Among salinity levels, maximum 272.2% APX activity was recorded in T5 (0.67 U $g^{-1}$ fresh weight $min^{-1}$) followed by 187.03% in T9 (0.52 U $g^{-1}$ fresh weight $min^{-1}$) as compared to control T0 (0.18 U $g^{-1}$ fresh weight $min^{-1}$) at 40 DAS. SA and *Trichoderma*, applied alone or in combination, showed increasing APX activity with increased salinity level i.e. 4, 8 and 12 $dSm^{-1}$ as compared to control of respective salinity level.

Among treatments, maximum 63.41% APX activity was recorded with treatment SA in T10 (0.67 U $g^{-1}$ fresh weight $min^{-1}$) followed by 53.65% in combined treatment of SA and *Trichoderma,* in T12 (0.63 U $g^{-1}$ fresh weight $min^{-1}$) as compared to respective salinity control T9 (0.41 U $g^{-1}$ fresh weight $min^{-1}$) at 40 DAS in 12 $dSm^{-1}$ salinity level.

**Fig.1 A-F** Effect of salicylic acid and *Trichoderma asperellum* T42 on hydrogen peroxide content (A), malondialdehyde content (B), Proline content (C), Superoxide dismutase activity (D), ascorbate peroxidase activity (E) and Plant cell death (F) on pea (*Pisum sativum* L.) genotype HUP-2 under different concentrations of salinity stress (4, 8 and 12 $dSm^{-1}$). T0 = Control, T1 = NaCl (4 $dSm^{-1}$), T2 = NaCl (4 $dSm^{-1}$) +SA (0.25 mM), T3 = NaCl (4 $dSm^{-1}$) + *T. asperellum* T42, T4 = NaCl (4 $dSm^{-1}$) + SA (0.25 mM) + *T. asperellum* T42, T5 = NaCl (8 $dSm^{-1}$), T6 = NaCl (8 $dSm^{-1}$) +SA (0.25 mM), T7 = NaCl (8 $dSm^{-1}$) + *T. asperellum* T42, T8 = NaCl (8 $dSm^{-1}$) + SA (0.25 mM) + *T. asperellum* T42, T9 = NaCl (12 $dSm^{-1}$), T10 = NaCl (12 $dSm^{-1}$) +SA (0.25 mM), T11 = NaCl (12 $dSm^{-1}$) + *T. asperellum* T42, T12 = NaCl (12 $dSm^{-1}$) + SA (0.25 mM) + *T. asperellum* T42. Data (T0 to T12) are in the form of mean ± SEM, and means followed by the same letters within the columns are not significantly different at $P \leq 0.05$ using Duncan's multiple range test




D
Superoxide dismutase activity
40 DAS
60 DAS
80DAS
SOD (units g-1 F W min-1)
T0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12
(4 dSm-1)
(8 dSm-1)
(12 dSm-1)


E
Ascorbate Peroxidase activity
40 DAS
60 DAS
80DAS
APX (Units g-1 F W min-1)
T0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12
(4 dSm-1)
(8dSm-1)
(12dSm-1)

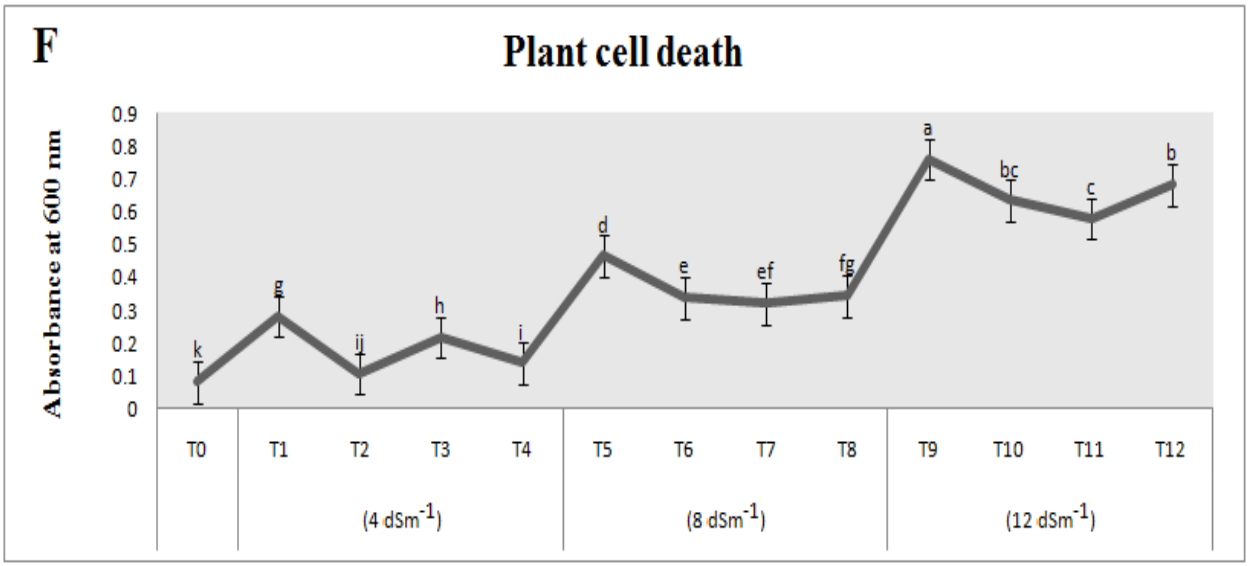
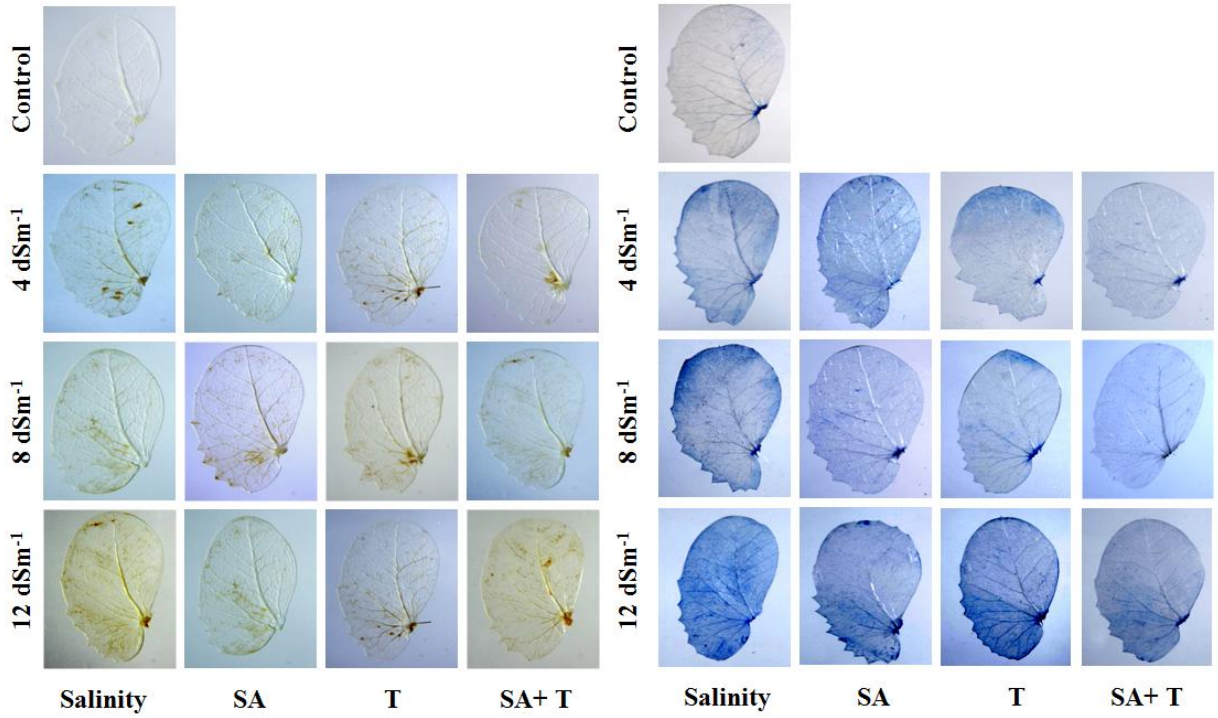

F
Plant cell death
Absorbance at 600 nm
T0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12
(4 dSm-1)
(8 dSm-1)
(12 dSm-1)

**Fig.2** Effect of salicylic acid (SA) and *Trichoderma asperellum* T42 (T) in pea under different concentrations of salinity stress (4, 8 and 12 $dSm^{-1}$). Detection of $H_2O_2$ (Left), in different treatments of pea leaves was performed using a solution of 3,3-diaminobenzidine (DAB) which binds with $H_2O_2$ and visualized as a reddish-brown in colour. Prior to imaging, chlorophyll was removed from leaves with 70 % (v/v) ethanol. However, in case of superoxide radical (Right) leaves were floated in NBT in 25 mM HEPES buffer (pH 7.8), 0.2 mg $ml^{-1}$ nitroblue tetrazolium (NBT) in which superoxide radical ($O_2$•-) produced more in high salinity level as compared to control and these $O_2$•- radicals react with NBT to produce a dark blue insoluble formazan compound on leaf surface. In both the cases, observed histochemically that formation of $H_2O_2$ and $O_2$•- increases with increasing the concentrations of salinity stress as compared to control (without impose salinity) but these responses were ameliorated by application of SA and *Trichoderma asperellum* T42, either alone or in combination, as compared to respective salinity controls at each salinity level




## Plant cell death

Observations related to plant cell death are presented in Figure 1F and expressed in absorbance ($A_{600}$). The responses of SA and *Trichoderma* used either alone or in combination were determined in pea under different concentrations of salt stresses (4, 8 and 12 $dSm^{-1}$). Data among salinity levels, maximum 825.61% absorbance was recorded in T9 (0.759 at $A_{600}$) followed by 467.07% in T5 (0.465 at $A_{600}$) as compared to control T0 (0.082 at $A_{600}$) which indicated for increased plant cell death with increasing absorbance ($A_{600}$). SA and *Trichoderma* showed ameliorating effect at each salinity level. Among treatments, maximum 62.19% reduction in plant cell death was observed in T2 (0.107 at $A_{600}$) followed by 51.23% in T4 (0.138 at $A_{600}$) as compared to respective salinity control T1 (0.283 at $A_{600}$) under 4 $dSm^{-1}$ salinity level.

Salinity stress is an important and serious environmental threat that is highly associated with drought stress. It is uniform and continues process that can be developed by both natural and human intervention. Soil salinization occurs naturally either where parent material is rich in soluble salts or in arid and semiarid regions where rainfall is insufficient due to climate change. In these areas, there is leaching out of the salts in upper most layer of fertile soil by the process of evapo-transpiration resulting in formation of salt affected soil. Salinization also occurs by salty irrigation or faulty irrigation practices where proper drainage does not exist for leaching and removal of salts making soil salty and unproductive (Azarmi *et al.,* 2016, Mazhar *et al.,* 2016 and Pourbabaee *et al.,* 2016).

Plant cell membrane is damaged due to increase in ion concentration which results in increase in reactive oxygen species (ROS) like superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide ($H_2O_2$) that lead to reduction in chlorophyll content and increase in lipid peroxidation i.e. MDA content and plant cell death, that disturb the cell integrity and negatively affect plant growth and development. Similar finding was reported by Khan *et al.,* (2013) in cucumber, Hashem *et al.,* (2016) in *Acacia gerrardii*, Habib *et al.,* (2016) in okara and Sharifi *et al.,* (2017) in wheat. Plants treated with SA and *Trichoderma* showed significant decrease in $O_2^{\cdot-}$, $H_2O_2$, MDA content, plant cell death and increase in chlorophyll content as compared to respective salinity controls (4, 8 and 12 $dSm^{-1}$). This is supported by other studies (Kumara *et al.,* 2010; Rawat *et al.,* 2011; Enteshari and Sharifian, 2012; Weizhen and Lei, 2013; Zhang *et al.,* 2016).

Reactive oxygen species such as superoxide radical and $H_2O_2$ content were significantly increased with increase in NaCl concentration in the present study (Singh and Jha, 2016; Hashem *et al.,* 2016). These were found to decrease with application of SA and *Trichoderma* under salt stress situation by activation of different antioxidative enzymes i.e., SOD and APX under salinity stress. Lipid peroxidation in plant was assessed by MDA content which is a more reliable indicator of salt stress tolerance. In the present study, there was an increase in MDA content under salinity stress as compared to control. This finding is supported by Khan *et al.,* (2013), Ahmad *et al.,* (2016) and Azarmi *et al.,* (2016). The MDA content is produced by the decomposition of polyunsaturated fatty acids during peroxidation of membrane lipids under salinity stress. It is used as biomarker for lipid peroxidation. The MDA content was significantly decreased with application of SA and *Trichoderma*, when used singly or in combination, under salinity stress.

Water is an important component in cellular structure and has a significant role in metabolic processes in plant. Salinity stress is not a problem of water scarcity but ion concentration that damages plant cell membrane. Plants have inherent capacity to protect their physiological function through production of osmoprotectants i.e. proline content under stress environment. It is an important component which contributes to reduction of injurious effects of stress factors and accelerates the restoration processes (Manchanda and Garg, 2011; Saghafi *et al.,* 2013; Koc *et al.,* 2016; Abo-Kora, 2016). It increased with application of SA and *Trichoderma*, when applied singly or in combination, as compared to respective salinity stress in the present study. SOD and APX are important antioxidative enzymes which reduce the responses of salt-induced ROS. Their activity increased significantly with increase in salinity, at low level. The SOD and APX activity were increased with application of SA and *Trichoderma*; SOD

converts reactive oxygen species superoxide radical ($O_2^{\cdot-}$) to $H_2O_2$, and the conversion of $H_2O_2$ to $H_2O$ and $O_2$ is felicitated by APX in the presence of ascorbic acid (Lee *et al.,* 2001; Mandhania *et al.,* 2006; Chutipaijit *et al.,* 2009; Kumara *et al.,* 2010; Barakat, 2011; Weizhen and Lei, 2013).

Salinity is considered a significant factor affecting crop production and agricultural sustainability. It significantly increases most of the parameters confirmed as $O_2^{\cdot-}$, $H_2O_2$, MDA content, plant cell death and decreases SOD and APX activity which directly influences the plant growth and development. Exogenous applications of SA (0.25 mM) and *Trichoderma asperellum* T42 in single and combined treatments, ameliorated the effect of salt stress by improving salt tolerance capacity of plant in various parameters studied. The application of SA in treatments (T2, T6 and T10) and *Trichoderma asperellum* T42 (T3, T7 and T11), singly or in combination (T4, T8 and T12) showed best results under each salinity level as compared to respective salinity controls (T1, T5 and T9) and mitigated salt induced oxidative stress in pea plants by modulating anti-oxidant defense system. *Trichoderma* showed positive response up to 8 $dSm^{-1}$ level of salinity stress.

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