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# EFFECT OF APPLICATION OF GROWTH ELICITORS AND *IN SILICO* ANALYSIS OF REGULATORY PROTEINS IN SWEET PEA (*PISUM SATIVUM* L.) AGAINST DROUGHT STRESS

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**Abstract**

Drought stress has an impact on the growth and yield of sweet pea (Pisum sativum). Drought stress has a negative impact on morphological and physiological characteristics including as height, shoot and root length, leaf area, fresh and dry weight, anthocyanin, chlorophyll a and b, soluble protein, and $H_2O_2$. The salicylic acid (SA) and ascorbic acid (AA) are used to modulate plant response against dry conditions and provide protection against oxidative damage. To overcome this loss SA and AA (0.5, 1 and 1.5 mM) were used by foliar application. Lab work was performed to illustrate the harmful effects of drought stress and beneficial role of SA and AA on *P. sativum.* These effects of drought stress were analysed by different computational analyses. The drought stress is very much effective at proteomic level and some proteins like PIP2-2, DREB2A and 14-3-3 like proteins are activated which prevent the plant from such dangerous effects. The activation of these proteins is up-regulated by SA application. So in dehydrated conditions it would be better choice to use SA and AA to compensate the shortage of water for plant. Because these compounds provide resistance against drought stress and restore the damages caused by drought stress and enable the plant to survive in such harmful conditions.

**Key words:** *Pisum sativum*, Drought Stress, Salicylic acid, Ascorbic acid, Protein-Protein Interaction, Refined and Docking.

## Introduction

Drought is the major abiotic stress that badly affects major crops yield that will be reduced by >50% in 2050 and almost 90% in 2100 (Yinpeng *et al*., 2009). Its effect was observed in a vast variety of plants which varied for interspecific and intraspecific interaction (Jaleel *et al*., 2009). It causes the decrease in cell elongation and cell turgor which ultimately reduces the plant growth. It has a cruel impact on the enzymes of nodules, and it induces stomatal closure, gas exchange limitation, damages of cell structure and ceases the reactions catalysed by enzymes (Jaleel *et al*., 2007d).

*Pisum sativum* is an important member of the second most important legume family and it is producing 27% of crop cultivation in the overall world (Farooq *et al*., 2008). It is easily available inexpensive source of protein, complex carbohydrates, vitamins, and minerals (Smykal *et al*., 2012). Salicylic acid (SA) has piqued scientists' interest in the last 20 years due to its capacity to elicit systemic acquired resistance in plants against a variety of pathogen attacks. SA protects and modulates the plant response against drought stress and herbicides (Ananieva *et al*., 2004). It controls physiological processes such as seed germination, fruit yield, glycolysis, flowering, photosynthesis rate, stomatal conductance, and plant transpiration rate (Khan *et al*., 2003). Ascorbic acid (AA) is an antioxidant molecule due to its dietary significance. It is used for the cleaning of ROS in plants photosynthesis, photo-protection and provides defence against ozone. It is applied against oxidative stress i.e., in cell expansion and cell division (Smirnoff, 2000) and used for the modulation of gene expression in plant that required for defence response against drought stress (Pastori *et al*., 2003).

Abiotic stress-related genes activate DREB2A, a key transcription factor involved in dehydration signalling pathways (Agarwal *et al*., 2006). DREB2A expression is dependent on SA (Chini *et al*., 2004), as well as its activation and expression improve plant survival against drought stress in VrDREB2A (Chen *et al*., 2016a), and GmDREB2 (Chen *et al*., 2016b). Drought stress induced ZmDREB2A in maize seedlings (Mizoi *et al*., 2013). Aquaporins (PIP2-2) are channel proteins that regulate water flow in plants. These proteins in plants are responsible for transporting water and other tiny neutral molecules through biological membranes (Kapilan *et al*., 2018). Drought conditions trigger PIP2-2 regulation, which can restrict water transport quickly (Ranganathan *et al*., 2016). As a result, plants need to have a variety of adaptive responses to deal with it (Ranganathan *et al*., 2017). In plants AQPs play a key role in cell differentiation, cell expansion, leaf function, nitrogen remobilization, plant reproduction, heavy metal toxicity, signal transduction, and ROS detoxification (Kayum *et al*., 2017). SA is a unique regulator of aquaporin activity, suggesting new connections between abiotic stressors, plant defence, and water transport regulation. (Boursiac *et al*., 2008).

14-3-3 protein interacts with a variety of different molecules and plays several roles in stress reactions. Rice plants harbouring the ZmGF14-6 gene, which encodes the maize 14-3-3 protein, have been found to be more drought tolerant (Campo *et al*., 2012). Drought stress tolerance in plants is aided by a 14-3-3-like protein that regulates nutrition management, and metabolic stresses (Liu *et al*., 2016). Based on our findings we hypothesised that changes in 14-3-3 proteins control a number of pathways implicated in SA-induced abiotic stress tolerance. When the Arabidopsis GF14 gene expresses a

14-3-3 protein, it was discovered that when this protein was introduced into cotton, it improved drought stress resistance (Yan *et al*., 2004). Keeping in view the aim of this present lab work was to analyse the morphological and physiological changes in *P. sativum* against drought stress and restoring these parameters back using different concentration of SA and AA by foliar application. Different *in-silico* approaches were applied to check regulation of DREB2A, PIP2-2 and 14-3-3 like protein under drought stress. Then restoring their balance by using SA, which provides the protection to withstand the plant against drought stressed conditions.

## Materials and Methods

**Fieldwork procedure:** The field experiment was conducted in the experimental area of University of Education, Township, Lahore. Healthy seed of *Pisum sativum* (variety Sarsabz) were collected from Four Brothers Seeds Corporation and grown to check the growth performance of plants against drought stress condition. Its effects in *P. Sativum* were studied by foliar application of variable concentrations of salicylic acid (SA) and ascorbic acid (AA) to minimise this extreme effect. The healthy seed were sown in the month of November under the control conditions (temp. 16.4-23.6$^{o}$C humid. 84%, dew point 24$^{o}$C and pressure 996 millibar) (Deskoy *et al*., 2017). The soil was gathered from the field and included a suitable amount of manure for the greatest plant growth and output. Plastic pots of 8-inch diameter and 10-inch height were used.

**Experimental layout:** Seed were sown in about 1-inch depth from the soil surface and distance between the seeds was about 3 inches. Six seeds were planted in each pot and allowed to grow in a controlled environment. The pots were arranged in five groups to perform the experiment in complete randomized design and every group of pots consisted of six replicates for further treatment. One group was kept as control (grown in normal condition) and other as negative control (with drought stress). Three groups of pots were treated at a time with SA and AA (0.5, 1.0 and 1.5 mM) respectively.

**Germination, thinning and application of drought stress:** The complete germination of seeds took place after one week and thinning of seedling was performed after one week of germination. After 15 days of seed's germination drought stress was applied for one week (Alexieve *et al*., 2001). The first treatment of SA and AA by foliar application was given after six days of drought stress at 0.5 mM while second and third treatments were applied with a gap of one week at 1.0 mM and 1.5 mM respectively (Sajid *et al*., 2016).

**Morphological traits of vegetative growth:** The plants were harvested one week after the last treatment and morphological characters such as plant height, shoot and root length, dry and fresh weight, leaf area, number of leaves, number of branches and pods per plant, and number of seeds per pod were studied to compare the damage caused by drought stress with normally grown plants. The SA and AA application was recorded to determine the compensation of damages caused by drought stress in *P. sativum*.

**Physiological attributes:** The data of some physiological characters like relative water content and cell membrane permeability, chlorophyll a, and b, malondialdehyde, anthocyanin, soluble protein and hydrogen peroxide content was recorded. The leaf area was recorded using the centimetre graph paper method. The relative water contents and membrane permeability was detected by (F. Wt. - D. Wt.) / (T. Wt. - D. Wt.) x 100 and Yang *et al*., (1996) method respectively. Chlorophyll content was determined by Arnon (1949) method. Anthocyanin and soluble protein contents were determined through Bradford method (1976), hydrogen peroxide by Velikova *et al*., (2000).

**Statistical analysis**

All of the observed plant characters were statistically analysed using the CoStat software. Microsoft excel was used to plot the graphs of all studied morphological and physiological parameters. The data in the complete randomised design (CRD) was subjected to a one-way analysis of variance (ANOVA) approach, and the least significant difference (LSD) test (P= 0.05) was used to compare the means and evaluate whether there were any significant differences for the measured parameters.

**Prediction of PPIs among PIP2-2 and DREAB2A, PIP2-2 and 14-3-3-like protein:** UniProt (https://www.uniprot.org/uniprot/) was used to download the P. sativum aquaporin (PIP2-2), drought response element binding transcription factor 2A (DREB2A), and 14-3-3-like protein sequences in FASTA format. With the help of a Swiss model, homology modelling was carried out. Comparative modelling led to the creation of 3D models of target sequences. To investigate the SMTL for protein structures related to evolution, sequential data was provided. The Swiss model performed this function using blast (Camacho *et al*., 2009), HHblits (Remmert *et al.,* 2012), and the modelling tool ProMod3. GMQE (Biasini *et al*., 2014) and QSQE were used to choose the models that resulted (Biasini *et al*., 2014; Bertoni *et al*., 2017). The scoring method QMEAN is used in the Swiss model (https://swissmodel.expasy.org), which uses mean force statistical potentials to obtain per-residue estimates (Waterhouse *et al*., 2018).

For molecular structure analysis, visualisation of 3D structures of proteins, and structural editing, UCSF Chimera (https://www.cgl.ucsf.edu/chimera/) (Pettersen *et al*., 2004) and PyMOL (https://pymol.org/2/) (DeLano, 2002) were used. Finally, a PDB file containing an energy-minimized structure was created and validated using PROCHECK (Laskowski *et al*., 2006), ERRAT (Colovos & Yeates, 1993), and VERIFY3D (Eisenberg *et al*., 1997). The HawkDock server (http://cadd.zju.edu.cn/hawdock) (Weng *et al*., 2019) was used to predict and analyse PPI structures using the docking algorithm 'ATTRACT' (Zacharias, 2003) function of scoring and the free energy decomposition analysis MM /GBSA (Chen *et*

*al*., 2016). The refined structure of PIP2-2 was taken as receptor and DREB2A and 14-3-3 protein was taken as ligand. Low score and low energy, according to this server, yield better outcomes (Feng *et al.,* 2017). Furthermore, numerous docked complicated models were generated and sorted in increasing order by allocation scores using the HawkDock service.

**Prediction of protein-protein interaction sites:** The BIPSPI (http://bipspi.cnb.csic.es/xgbPredApp/) server was used to anticipate PPIs sites. This web server uses a machine-learning algorithm to predict binding sites and residue interaction (Dona *et al.,* 2017). It was predicted using the 'PREDICT from structural data' option for input 3D structures of PIP2-2 and DREB2A, as well as 14-3-3-like proteins.

## Results

**Effects of SA and AA on plant height shoot, root length, leaf area, fresh and dry weight:** The growth of *P. sativum* was severely affected by drought stress and the use of SA and AA helped to regain these reduced characters. The height, shoot and root length of control plants were maximum but decreased in drought-stressed condition and remained less than half. 1st, 2nd, and 3rd group were treated with 0.5, 1.0, 1.5 mM of SA and AA respectively. By using different concentration of SA and AA the values of these parameters of drought, stressed plants were increased 4-fold as in 1st and 2nd group while in 3rd group these values also increased (Table 1, Fig. 1). Leaf area, fresh and dry weight was minimum in drought stressed plants and maximum in control group. Leaf area was regained gradually in 1st, 2nd and 3rd group. Fresh and dry weight was increased gradually in all treated groups but in 3rd group this increase was more than four time as compared to drought stressed plants (Table 1, Fig. 2).

**Effects of SA and AA on total number of branches, leaves and pods per plant, seeds per pod:** When compared to control plants, drought-stressed plants had fewer overall branches, leaves, and pods, as well as fewer seeds per pod. The number of leaves in 1st, 2nd and 3rd group of plants became two, one and three times increased, respectively. The number of branches in 1st group was increased &its growth rate was doubled as compared to the 2nd group and in 3rd group it became triple as compared to 2nd group. The number of pods in drought stressed were reduced and remained one third as compared to control plants. In first, second and third group the number of pods were increased in gradual way (Table 1, Fig. 3).

**Effects of SA and AA on relative water, membrane permeability and chlorophyll a, b content:** The relative water content of drought stressed plant was decreased four hundred times less than control group. RWC and membrane permeability content increased by foliar applications of both SA and AA on drought stressed plant (Table 1). In 1st and 2nd group this increase in RWC was more than eighty times but in 3rd group it was more than one hundred times as compared to drought stressed plant. The membrane permeability was minimum in control group, but it increased in drought stressed as in 1st and 3rd group membrane permeability content increased more than ten times but in 2nd group four times with respect to drought stressed (Fig. 4).

Chlorophyll a and b contents in drought stressed plant were minimum and maximum in control plants and increased by using of both SA and AA on drought stressed plant. In 1st, 2nd group chl a content was increased more than three times but in 3rd group this increment was less than ten times as compared to drought stressed plant. In 1st, 2nd and 3rd group of plants chl b content was increased more than three, one and four times as compared to drought stressed plants respectively (Table 1) (Fig. 5).

**Table 1. Effect of foliar applications of different concentrations of salicylic acid and ascorbic acid on drought stressed pea plant.**

| Morphological characters | Control | Drought stressed | SA+AA (0.5mM) | SA+AA (1.0 mM) | SA+AA (1.5 mM) |
|---|---|---|---|---|---|
| Plant height (cm) | 85.66 | 37.66 | 42.66 | 47.00 | 50.33 |
| Shoot length (cm) | 55.66 | 26.66 | 30.16 | 34.33 | 35.33 |
| Root length (cm) | 31.00 | 11.00 | 13.16 | 14.83 | 17.33 |
| Fresh weight (mg) | 20.33 | 05.26 | 06.80 | 08.73 | 12.86 |
| Dry weight (mg) | 10.36 | 02.31 | 03.41 | 04.31 | 06.63 |
| Leaf area ($cm^2$) | 09.33 | 05.36 | 06.33 | 07.16 | 08.66 |
| No of leaves | 23.00 | 06.00 | 11.66 | 15.66 | 18.66 |
| No of branches | 15.66 | 05.00 | 07.66 | 09.66 | 12.33 |
| No of pods per plant | 13.00 | 03.66 | 05.33 | 07.16 | 09.33 |
| No of seed per pod | 08.33 | 03.00 | 04.66 | 05.77 | 06.66 |
| **Physiological attributes** | | | | | |
| RWC | 470 | 22.5 | 109.3 | 112.6 | 133.3 |
| RMP | 38.75 | 44.46 | 57.22 | 72.29 | 77.3 |
| Chl. A | 0.67 | 0.12 | 0.27 | 0.43 | 0.53 |
| Chl. B | 0.10 | 0.01 | 0.04 | 0.06 | 0.06 |
| Anthocyanin | 0.44 | 0.66 | 0.59 | 0.49 | 0.42 |
| Soluble protein | 1.10 | 1.47 | 1.28 | 1.21 | 1.17 |
| $H_2O_2$ | 0.21 | 0.47 | 0.34 | 0.26 | 0.21 |

The values are expressed as mean value of all characters

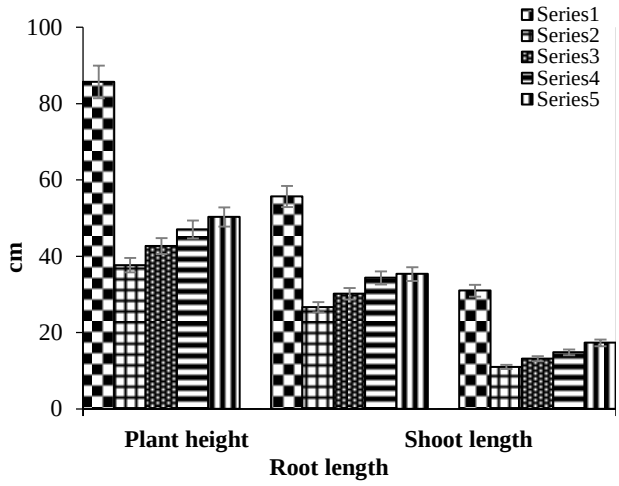



Fig 1. Series 1, 2, 3, 4 & 5 showed control, drought stressed, 1st, 2nd & 3rd groups, respectively. Plant height, shoot & root length were increased from drought stressed to1st, 2nd & 3rd group respectively.

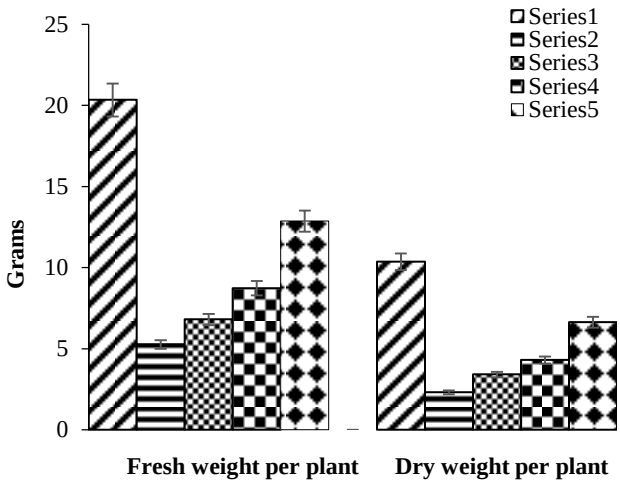



Fig. 2. Series 1, 2, 3, 4 & 5 showed control, drought stressed, 1st, 2nd & 3rd groups, respectively. Fresh & dry weight per plant was increased from drought stressed to 1st, 2nd & 3rd group respectively.

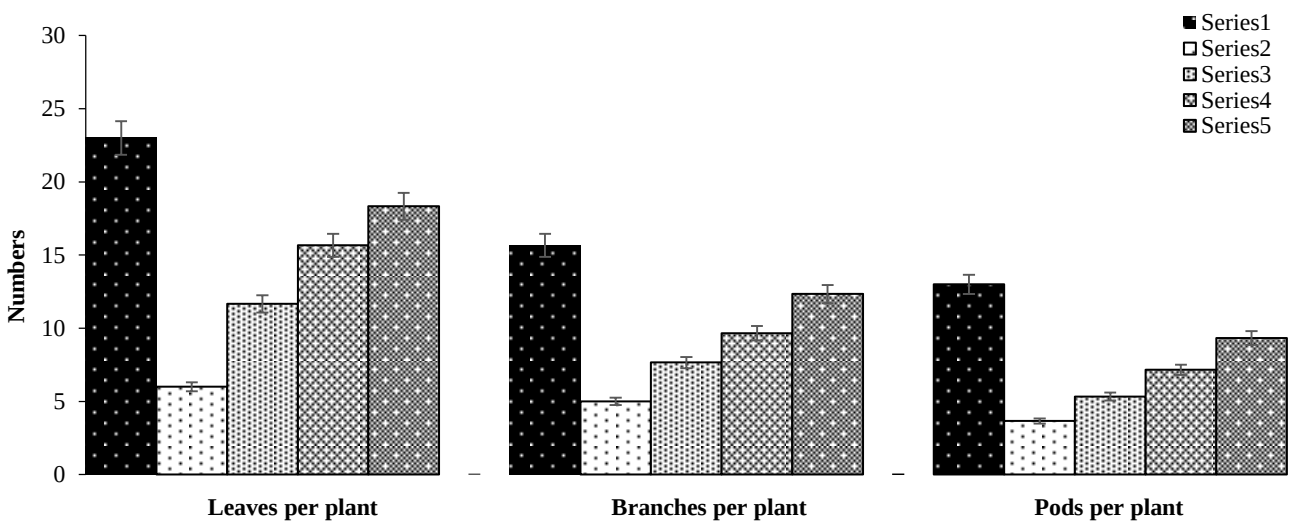



Fig. 3. Series 1, 2, 3, 4 & 5 showed control, drought stressed, 1st, 2nd & 3rd groups respectively. Leaves, branches & pods per plant were increased from drought stressed to1st, 2nd & 3rd group respectively.

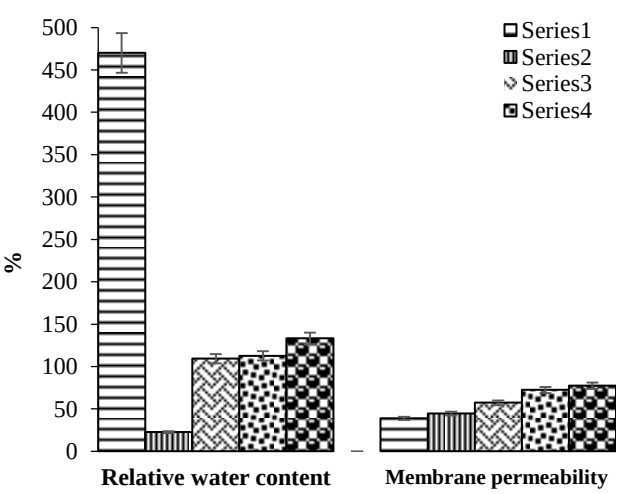



Fig. 4. Series 1, 2, 3, 4 & 5 showed control, drought stressed, 1st, 2nd & 3rd groups, respectively. Relative water content & membrane permeability were increased from drought stressed to1st, 2nd & 3rd group respectively.

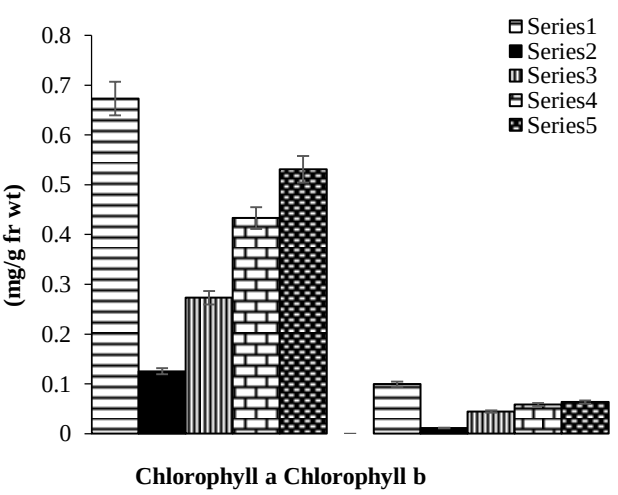



Fig. 5. Series 1, 2, 3, 4 & 5 showed control, drought stressed, 1st, 2nd & 3rd groups respectively. Chlorophyll a & Chlorophyll b were increased from drought stressed to1st, 2nd& 3rd group respectively.

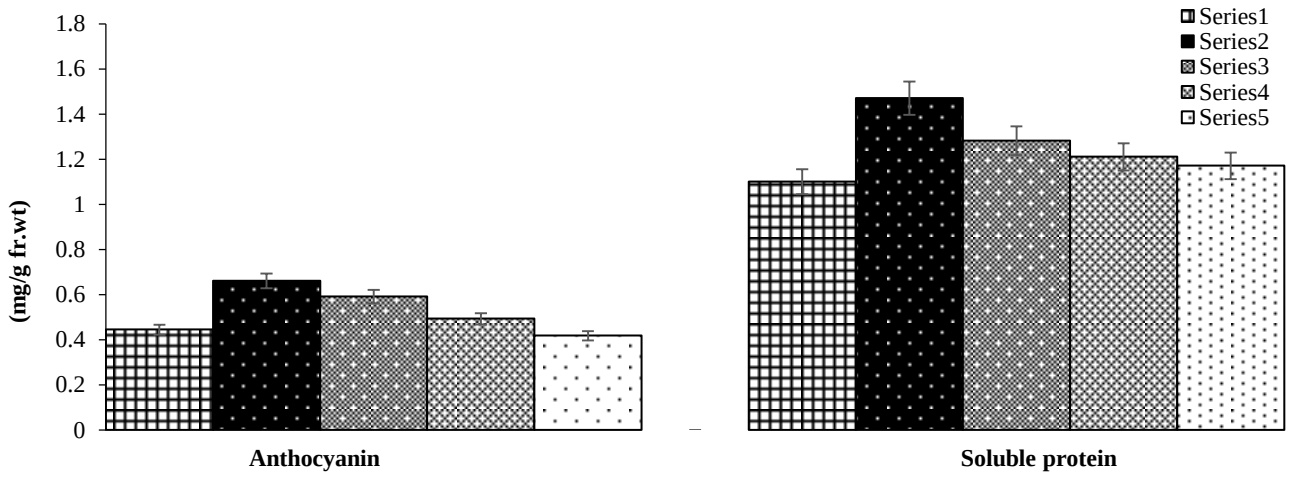



Fig. 6. Series 1, 2, 3, 4 & 5 showed control, drought stressed, 1st, 2nd& 3rd groups respectively. Anthocyanin & soluble protein were decreased in drought stressed to1st, 2nd & 3rd group respectively.

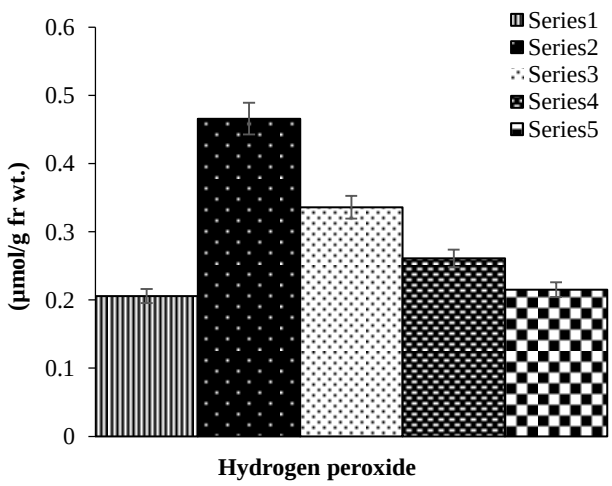



Fig. 7. Series 1, 2, 3, 4 & 5 showed control, drought stressed, 1st, 2nd & 3rd groups respectively. Hydrogen per oxide was decreased in drought stressed to1st, 2nd & 3rd group respectively.

**Effects of SA and AA on anthocyanin, soluble protein and hydrogen peroxide content:** Drought-stressed plants had the highest levels of anthocyanin, soluble protein, and hydrogen peroxide, while control plants had the lowest. By foliar application of both SA and AA on drought stressed plants the values of these parameters were decreased one, two and three-fold in 1st, 2nd and 3rd groups respectively (Table 1). Anthocyanin contents in 1st and 2nd group were decreased three and four-fold respectively while in 3rd group decreased almost five-fold and became equal to control group of plant. The soluble protein contents in 1st, 2nd and 3rd group were decreased three, four and four & half times as compared to control group of plants (Fig. 6). Hydrogen peroxide contents decreased three times in control group of plants as compared to drought stressed plants. It decreased in 1st group more than one-fold, in 2nd group less than one-fold and in 3rd group half fold as compared to drought stressed plants, respectively (Fig. 7).

**Retrieval and identification of aquaporin, DREAB2A, 14-3-3-like protein sequences and model building:** The UniProt database was used to get the sequences of P. sativum aquaporin (PIP2-2), DREB2A, and 14-3-3-like proteins in FASTA format (Table 2). The 'Swiss model' with (ProMod3 3.2.0) was used to obtain the 3D structure of these proteins. For all of the proteins included in the Swiss model, the results are produced in the form of Global Model Quality Estimation (GMQE), oligo-state, and QMEAN Z-score, which yields the following results. Query coverage of PIP2-2 is 0.97, Oligo-State is Homo-tetramer with 0.81GMQE and -2.11 QMEAN Z-score. While Query coverage of DREAB2A is 0.54, Oligo-State of is Monomer with 0.16GMQE and -3.30 QMEAN Z-score but Query coverage of 14-3-3 like protein is 0.89, Oligo-State of is homo dimer with 0.85 GMQE and-1.04 QMEAN Z-score. When 3D structures of query proteins are visualised by the UCSF Chimera software it showed the followings results. Negatively charged residues were highlighted in red, while hydrophobic residues were highlighted in green in the 3D structure. The binding sites for mercury and cadmium ions were indicated in yellow and blue accordingly in their structures. However, interactions between residues and Hg and Cd ions were found in the binding sites (Figs. 8, 9).

**3D protein structures evaluation and validation:** Ramachandran plots, which were utilised to validate the 3D models, were generated using the web programme 'PROCHECK.' For a high-quality model with great stereo-chemical properties, the percentage in the Ramchandran plot's core region should be at least 90%. The percentage of amino acids in the most favoured region, additional allowed region, and usually allowed region were calculated for all PIP2-2, DREB2A, and 14-3-3 related proteins. In PIP2-2, 91.7 percent of amino acids residues were discovered in the most favoured region, 7.7% in the most allowed region, and 0.7 percent in the generously allowed zone. 91.8 percent of amino acids residues are found in the most favoured area of DREB2A, 6.6 percent in the most permissive region, and 1.6 percent in the liberally permissive region. There are 96.30 percent amino acids residues in the most favoured portion of 14-3-3 related protein, 3.70 percent amino acids residues in the most allowed region, and 0.0 percent amino acids residues in the freely allowed sector. The residues in the most favoured region were shown in red

(A, B, L), additional permitted region residues were shown in yellow (a, b, l, p), generously permitted region residues were shown in light yellow (a, b, l, p), disallowed region residues were shown in white, non-glycine and non-proline residues were shown in white, and overall residue numbers were shown in white (Fig. 10).

The online web tools ERRAT and VERIFY3D were utilised for additional investigation of selected models. The overall quality factor of the PIP2-2, DREB2A, and 14-3-3-like protein models was 98.656%, 65.517%, and 100%, respectively, according to ERRAT. If at least 80% of the amino acids in the 3D/1D profile have a score of > = 0.2, the protein structure is displayed in 3D. (pass). With an average 3D/1D score of 81.94 percent, the 14-3-3 comparable protein model had the largest proportion of residues, whereas the graph scores of PIP2-2, DREB2A, and 14-3-3 like protein models were 0.70 percent, 0.73 percent, and 0.68 percent respectively (Table 3).

**Docking results:** When PIP2-2 docked with 14-3-3 like protein it showed the -5763.95 score and -21.99 binding energy complex (Table 4). In PIP2-2 the residue number 105, 275, 15, 93, 282, 220, 94, 151, 274, 104, 204, 236, 106, 101, 150, 147, 229, 232, 230, 231 were docked with the residue numbers 212, 213, 204, 216, 214, 211, 220, 221, 224, 227, 48, 222, in 14-3-3 like protein (Table 5). While when PIP2-2docked with DREB2A it showed the -4329.20 score and -42.49 binding energy complex (Table 4).In PIP2-2 the residue numbers 12, 105, 15, 267, 9, 101, 220, 264, 282, 204, 229, 232, 230, 228, 231 were docked with the residue numbers 28, 31, 27, 23, 22, 30, 32, 33, 29, 161, 120 in DREB2A (Table 6) (Fig. 11).

**Putative docking sites identification for PIP2-2 on 14-3-3 Protein and DREB2A in *P. sativum:*** To predict docking sites the online server 'BIPSPI' was employed. The appearance and position of docking sites, as well as sequence characteristics of 14-3-3 Protein and DREB2A were used to explore the link between docking sites for PIP2-2. The interaction of 14-3-3 Protein and DREB2A with PIP2-2 was demonstrated using a docking technique which yielded a list of possible novel docking sites. BIPSPI server predicted potential interacting sites from sequences of docking proteins. PIP2-2, 14-3-3 Protein and DREB2A possessed maximum docking sites. However, when PIP2-2 docked with 14-3-3 protein then it shown 20 docking site residues while 14-3-3 shown 12 docking site residues respectively (Table 5). However, when PIP2-2 interacted with DREB2A, it revealed 15 docking site residues, whereas DREB2A revealed 11 docking site residues (Table 6). The predicted binding sites in the structures of PIP2-2, 14-3-3 Protein and DREB2A were highlighted in green colour throughout the interactive display (Figs. 12, 13)

**Table 2 List of *Pisum sativum* L. PIPs and their basic characterizations.**

| UniProt ID | Protein name | Gene name | Size (aa) | Molecular function |
|---|---|---|---|---|
| W0M6C5 | PIP2-2 | PIP2-2 | 287 | Channel activity |
| E0A164 | DRE-binding transcription factor 2A | DREB2A | 164 | DNA-binding transcription factor activity |
| Q9XG89 | 14-3-3-like protein | 14-3-3 | 261 | Regulation of hormonal induction in response to stress stimuli |

**Table 3. Results of verify3D analysis of PIP2-2, DREB2A and 14-3-3 like protein.**

| Model | % of residues having average 3D/1D score | Graph value of model (Score) |
|---|---|---|
| PIP2-2 | 76.53% | 0.70% |
| DREB2A | 76.52% | 0.73% |
| 14-3-3 like protein | 81.94% | 0.68% |

**Table 4. List of PPI interaction pairs between PIP2-2 and DREB2A, a 14-3-3-like protein, with their score and binding energy.**

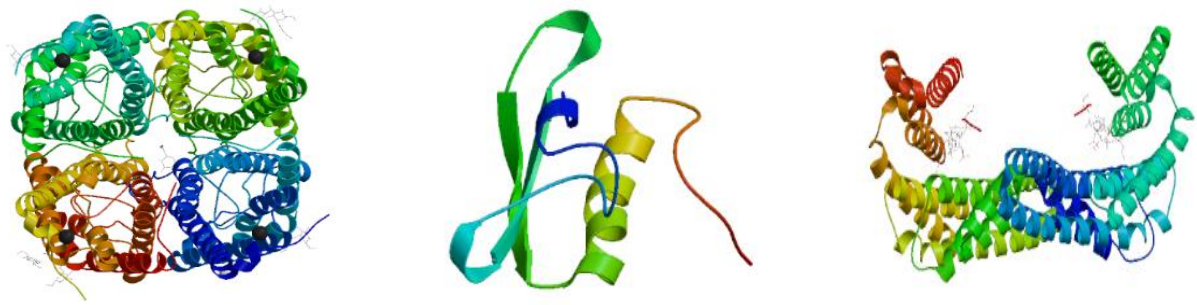
| PPIs | Score | Binding Energy of Complex (Kcal/mol) |
|---|---|---|
| PIP2-2-14-3-3 | -5763.95 | -21.99 |
| PIP2-2-DREB2A | -4329.20 | -42.49 |

**Table 5. Prediction of docking sites of potentially interacting PIP2-2, 14-3-3 protein in *Pisum sativum* L.**

| Docking protein | Docking sites | Residues |
|---|---|---|
| PIP2-2 | GAAFFTIYSGLPHGSFINPR | 105, 275, 15, 93, 282, 220, 94, 151, 274, 104, 204, 236, 106, 101, 150, 147, 229, 232, 230, 231 |
| 14-3-3 | FTFELLKNDSLQ | 212, 213, 204, 216, 214, 211, 220, 221, 224, 227, 48, 222 |

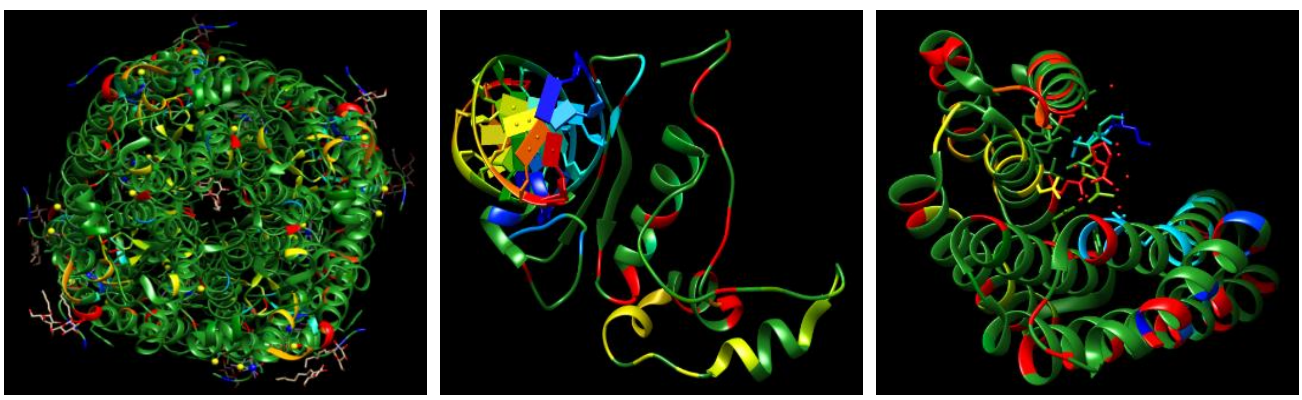
**Table 6. Prediction of docking sites of potentially interacting PIP2-2, DREB2A in *Pisum sativum.***

| Docking proteins | Docking sites | Residues |
|---|---|---|
| PIP2-2 | EGAHQGTAFLINPAR | 12, 105, 15, 267, 9, 101, 220, 264, 282, 204, 229, 232, 230, 228, 231 |
| DREB2A | QRKWVAEIRPI | 28, 31, 27, 23, 22, 30, 32, 33, 29, 161, 120 |



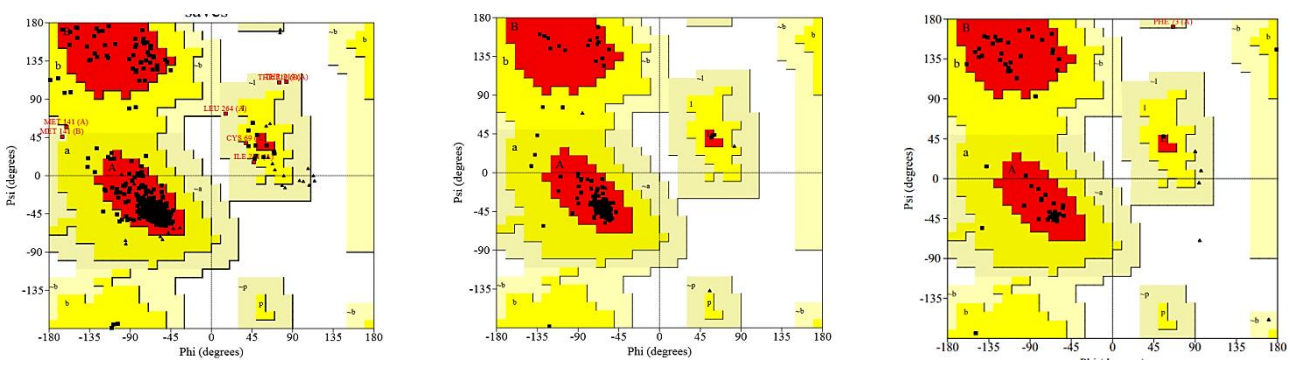
**PIP2-2** **DREB2A** **14-3-3 like protein**

Fig. 8. 3D structures of PIP2-2, DREB2A and 14-3-3 like protein obtained by homology modeling server 'Swiss model.



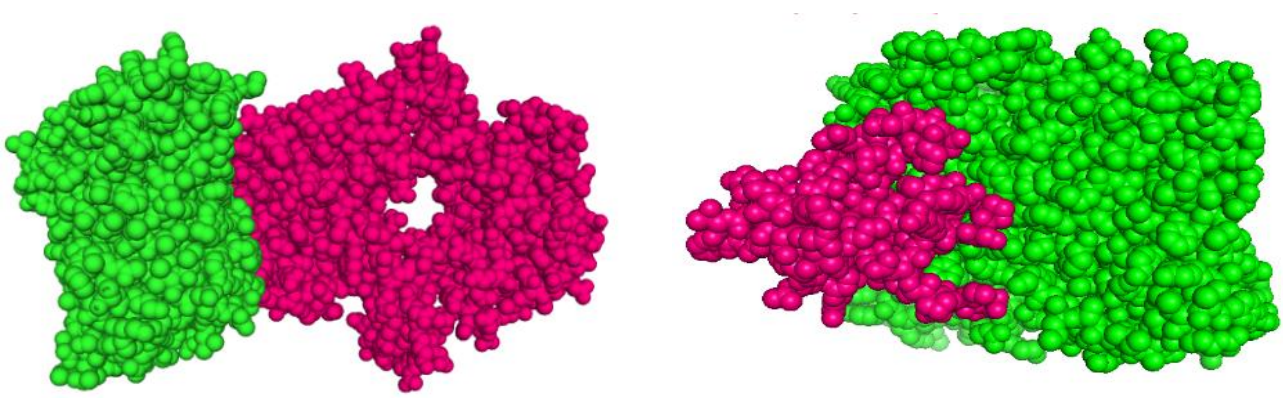
**PIP2-2** **DREB2A** **14-3-3 like protein**

Fig. 9. UCSF Chimera software was used to visualise 3D structures of PIP2-2, DREB2A, and a 14-3-3-like protein. Green was used to highlight hydrophobic residues, whereas red was used to highlight negatively charged residues.




**PIP2-2** **14-3-3 like protein** **DREB2A**

Fig. 10. PROCHECK developed Ramachandran plots to validate the PIP2-2, DREB2A, and 14-3-3 similar proteins.



**(PIP2-2) -(14-3-3)** **(PIP2-2)-DREB2A**

Fig. 11. Docking of *Pisum sativum* L. PIP2-2 shown in green colour and 14-3-3, DREB2A shown in hot pink colour. The docking results were visualized through Pymol tool.

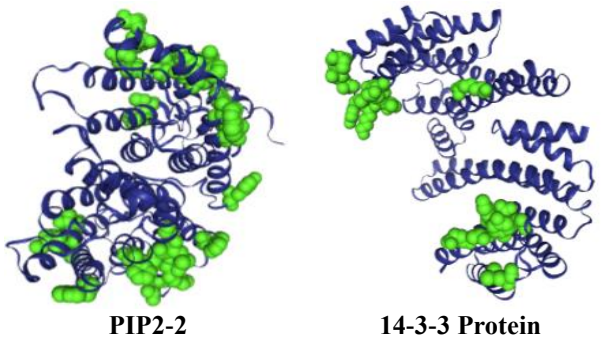



Fig. 12. Interactive visualization of predicted potential residues (binding sites) of PIP2-2 and 14-3-3 protein was highlighted in green colour.

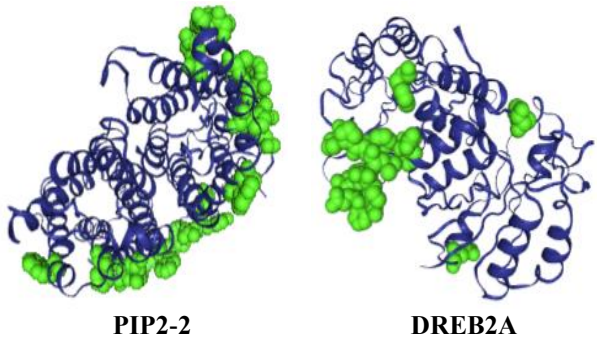



Fig. 13. PIP2-2 and DREB2A anticipated possible residues (binding sites) were highlighted in green colour in an interactive graphic.

## Discussion

To promote plant growth and yield, many chemicals and substances are used all over the world. The impact of these chemicals is determined by their concentration, mechanism of action, and plant developmental stage. In present study, the growth parameters of *P. sativum* and its morphological and physiological characters and DREB2A, PIP2-2 and 14-3-3 like protein their up regulation by SA were noticed under the influence of drought stress conditions. SA and AA were applied to induce the drought stress tolerance in pea plant. SA provides protection and monitors the plant reaction against drought stress (Senaratna *et al*., 2002).

The application of SA to a plant delays the onset of stomatal closure caused by drought stress (Saruhan *et al*., 2012). AA caused the regulation of developmental senescence and plant defence mechanisms. It affects the biosynthesis and hormone level by signalling under drought-stressed situations. It is used for cell growth; cell division and all the phases of the cell cycle are affected. It functions as a cofactor and controls numerous enzymes regulated mechanisms of the plant (El-Mashad & Mohamed, 2011). Drought stress caused a considerable reduction in the values of growth parameters of the pea plant. But all these values of all parameters were increased by the application of SA and AA. Plant height decreased in *P. sativum* due to drought stress and the same result shown in drought-stressed pea and corn plant (Khan *et al*., 2015). Root and shoot length were reduced in drought-stressed pea plant and the same result was shown in drought-stressed pea plant (Munns & Tester, 2008). Root and shoot growth in drought-stressed soybeans was increased by SA application. Leaf area was decreased in drought-stressed pea plant and the same result was shown (Lazaridou & Koutroubas, 2004) in drought-stressed clover plant (*Talex andrinum*). The number of branches per plant was increased through SA in drought stressed pea plant and same results were described in drought-stressed pea plant. In drought-stressed pea plants the total number of leaves per plant decreased but the outcomes were comparable (Anjum *et al*., 2008).

Fresh and dry weight was reduced in drought stress pea plant and the same result was shown in drought-stressed pea plant (Zeid & Shedeed, 2006). Numbers of pods and seeds per pod were increased in drought stressed pea plant due to SA application and the same result was shown in drought-stressed pea plant (Zhang *et al*., 2003). Relative water content of leaf was decreased in pea plant bearing drought stress, and the same result was shown in drought-stressed pea plant (Nayyar & Gupta, 2006). Chlorophyll a and b content were decreased in drought-stressed pea plant, and the same result was shown in drought-stressed pea plant (Kiani *et al*., 2008), while in drought-stressed Vicia faba plant AA boosted chlorophyll content (Reiahi & Farahbakhsh, 2013).

Malondialdehyde content was increased in drought-stressed pea plant and the same result was shown (Escuredo *et al*., 1998). Membrane permeability content was decreased in drought-stressed pea plant and AA induced the decrease in membrane permeability content in drought-stressed cauliflower plant (Latif *et al*., 2016). Anthocyanin content was increased in drought-stressed pea plant and the same result was shown in drought-stressed sunflower (Ebrahimian & Bybordi, 2012). In drought-stressed pea plant the soluble proteins content was enhanced, and the same result was showed in drought stressed pea plant (Larmure *et al*., 2005). AA induced the increase in soluble protein content in drought-stressed pea plant and the same result was shown in drought-stressed *Vicia faba* plant (Reiahi & Farahbakhsh, 2013). Hydrogen peroxide content was increased in drought-stressed pea plant and SA induced the accumulation of hydrogen peroxide content in drought stressed tomato plant (Peter *et al*., 2012) and AA induced the decrease in hydrogen peroxide content in drought-stressed cauliflower plant (Latif *et al*., 2016).

A proteome analysis of SA-induced drought tolerance in plants has recently been published, with over 150 abiotic responsive proteins discovered (Kang *et al*., 2012a, b). SA controls stress-responsive proteins in plants that govern energy metabolism, signal transduction, ROS scavenging, photosynthesis, and ion homeostasis, among other functions. The expression of DREB2A is salicylic acid (SA) dependent (Chini *et al*., 2004), and its activation and expression promote plants survival against drought stress (Sakuma *et al*., 2006a, 2006b). SA is unique regulator of aquaporin activity (PIP2-2), implying new connections between abiotic stressors and water transport regulation (Boursiac *et al*., 2008). In terms of aquaporin relocalization mode of action of SA was investigated more specifically at the level of root

epidermal cells. SA also shifted PIP aquaporin's subcellular position in plant roots (Lebrun-Garcia *et al*., 2002). The 14-3-3 family of phosphoserine binding proteins interacts with a wide range of signalling molecules and is involved in stress responses. Rice plants with the ZmGF14-6 gene, which encodes the maize 14-3-3 protein, have been discovered to be more drought resistant (Campo *et al*., 2012). A 14-3-3-like protein that affects nutrient management and metabolic stresses aids drought stress tolerance in plants (Liu *et al*., 2016). Wheat seedlings with SA treatments had more 14-3-3 proteins (Kang *et al*., 2012b).

This experimental data is useful for confirming previously published physiological conclusions on SA improved abiotic stress tolerance (Hayat *et al*., 2010). The proteomic findings will aid future study on SA tolerance networks and the many pathways in plants with higher abiotic stress tolerance (Rajjou *et al*., 2006). In R. intraradices, 14-3-3-like proteins activate aquaporins, resulting in drought tolerance in Zea Mays (Tao *et al*., 2016).

## Conclusion

Drought stress significantly inhibited the growth of *P sativum* variety Sarsabz and influenced on many morphological, physiological, and biochemical characteristics and proteomic level. But foliar application of SA and AA showed good result to overcome the damages caused by drought stress in pea plant. So due to decreased water availability for plants growth it would be better choice to use SA which cause up regulation of DREB2A, PIP2-2 and 14-3-3 like protein which enhanced the potential of plant to overcome the damaging effects of drought stress, and AA through foliar application to compensate the shortage of plant water content. In the results of different approaches of *in-silico* study it is proved that the PIP2-2 docked with DREB2A and 14-3-3 like protein which indicated that these proteins are playing a big role in pea plant to enable it to withstand the damaging effects of drought stress. These proteins are up-regulated using SA applications. So, it is obvious that both SA and AA provide resistance against drought stress and enable the plants to survive in such drought stressed conditions.

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