Apoplast proteomic analysis reveals drought stress-responsive protein datasets in chilli (Capsicum annuum L.)

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Abstract

Drought is one of the major environmental constrains that limit plant performance worldwide. Plant apoplast which acts as connecting link between environment and plant protoplast carries multiple functions in plant metabolism and signalling. To investigate the drought induced changes in apoplast, proteome analysis in conjunction with antioxidant enzyme activity changes were studied in chilli (Capsicum annuum L). Drought induced apoplast proteome revealed augmented phenyl alanine ammonia lyase, peroxidase activities and reduced catalase activity. LC-MS analysis of apoplast proteome revealed differential expression of proteins under water stress conditions. Up-regulation of 43 protein species which encompass stress related proteins such as defensins, peroxidases, polygalaturonase inhibitor proteins, superoxide dismutase proteins were observed. Unlike control, twenty unique protein species were identified to be present in proteome of drought treated plants. Qualitative and quantitative changes in apoplast proteome emphasize the dynamics of plant apoplast and its role in drought stress. This work would provide insights into drought induced proteomic changes in apoplast and also would prove to be useful for protein phenotyping.

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1. Data

We present proteome data and enzyme activity data of leaf apoplast subjected to drought conditions. Elite chilli genotype (S-10) was subjected to two water regimes - 100% Field Capacity (Control) and 40% Field Capacity (Drought Treated). Malate Dehydrogenase (MDH) activity was found to be 1% in treated and 0.4% in control apoplast samples. Malate Dehydrogenase activity is widely used as a specific marker to identify degree of cell membrane integrity and level of cytosolic contamination [1].

In the present data, Fig. 1 represents effect of drought on contents of apoplast. Phenolic content in control (21.4 mg/g) and treated (48.02 mg/g) was represented in Fig. 1A. There observed an increase in phenolic content by 1.24 folds under drought. In Vitis vinifera there was an increased production of phenolics under abiotic stress conditions [2]. Phenylalanine Ammonia Lyase (PAL) activity was also increased in drought by 0.56 folds (Fig. 1B). Our results were in accordance with Gholizadeh [3]. The peroxidase activity in control and stressed samples were 0.16 and 0.30 units/mg protein respectively (Fig. 1C). Increased peroxidase activity in apoplast under stress conditions was also reported in wheat root cells [4], chilli leaves [5]. Data revealed a decrease by 17.9% in treated (0.73 units/mg protein) when compared to control (0.89 units/mg protein) (Fig. 1D). Drought induced decline in catalase activity was observed in wheat [6]. Statistical analysis was provided in supplementary File S1.

1.1. LC-MS analysis.

A total 208 protein species were identified from the LC-MS analysis of control and treated proteomes. Among 208 protein species, eight were of different origins such as, cytoplasm (5), ribosomal (2) and mitochondrial (1) origin due to cytoplasmic contamination of apoplast fluid. LC-MS proteome data of 208 protein species was provided in Supplementary Table S2.
Fig. 1. Changes in Phenol content (A), PAL activity (B), Peroxidase activity (C) and Catalase activity (D) under 100% and 40% FC. Values are represented as mean ± SD. * represents significant difference at $P = 0.05$.

Fig. 2. Functional Annotation of 106 proteins identified in chilli leaf apoplast.
Among the 208, 106 protein species were considered for further analysis, as they contain at least 2 unique peptides. Trentin et al. [7], also studied proteins with at least 2 unique peptides in Arabidopsis thaliana apoplast proteome. Based on their role in Biological process, 106 proteins were categorised into six groups (Fig. 2) viz., metabolic process (Table 1), cell organization and biogenesis (Table 2), regulation of biological process (Table 3), defense response and transport functions (Table 4) and undefined proteins (Table 5).

In the present study, differential expression of proteins was observed upon drought stress. Among 106 protein species identified, 43 proteins were up-regulated and 43 proteins were down-regulated in treated sample in comparison with control (Fig. 3). Twenty protein species were found to be uniquely identified in drought treated sample.

Drought induced apoplast proteome exhibited increased abundance in 10 proteins and decreased abundance in 19 proteins which were involved in diverse metabolic processes. This shows the negative effect of drought on various metabolic processes. Decreased expression levels of proteins involved in cell organisation can be implicated to depletion in cell organisation ability of plant cell under drought. Coping with a variety of abiotic stresses is highly dependent on up and down-regulation of proteins resulted from altered gene expression. Though most of the proteins were expressed under normal conditions, differential expression is often seen under stress conditions [8]. Imbalance in cellular redox metabolism under drought results in increased oxidative damage. To counterattack, plants produce several ROS scavenging enzymes. In our present study, among four peroxidases that were identified, one peroxidase (B9VRK9) was up-regulation where as three were down-regulated and there is a non-significant increase in the abundance of superoxide dimutase in treated sample. Kosova et al. [9], also reported increased abundance of ROS scavenging enzymes under cold in wheat. Drought induced chilli apoplast proteome revealed up-regulation of cell wall reprogramming proteins. Cell wall reprogramming was one of the important strategies of plant to withstand deleterious effects of stress [10].

Among 20 unique proteins identified in drought, seven proteins were related to metabolic processes, while two proteins were recognised to have role in regulation of biological process, two proteins were identified to take part in defence mechanism, one protein is known to play role in cell organisation and biogenesis and undefined (Fig. 4).

2. Experimental design, materials and methods

2.1. Plant material

Elite chilli genotype (S-10) seeds were procured from Horticultural Research Station, Lamfarm, Guntur, Andhra Pradesh. Seeds of S10 genotype were grown in black trays containing a mixture of peat and vermiculite (2:1 v/v) for 45 days followed by transplantation into pots (one plant/pot) and allowed for acclimatization for one week. Plants were grown in greenhouse under control conditions- 16 h light/8 h dark photoperiod at 27 °C during the day and 21 °C at night, and watered regularly.

2.2. Imposition of stress

Drought stress was imposed to plants using gravimetric method [11]. This method involves weighing pots twice a day followed by replenishing the water lost by evapotranspiration to maintain required field capacity (FC). Chilli plants were subjected to two water regimes viz., 100% FC (control), 40% FC (drought stress) for one week.

2.3. Apoplast protein extraction

Apoplastic proteins were extracted using the infiltration method described by (O’leary et al., [12]). All fresh green leaves were excised from plants and were washed in distilled water to remove cellular proteins from the cut ends. Leaves were dried and infiltrated using extraction buffer (0.1 M potassium phosphate buffer pH-7). Leaves were blotted gently, rolled carefully and loaded into 20ml syringe barrel. The syringe barrel was placed into centrifuge tubes. Apoplastic fluid was obtained at bottom of
### Table 1
Abundance change in protein species involved in metabolic process during the drought stress in chilli.

| S.NO | Accession | Description | MW [kDa]/ calc.pI | Abundance Ratio: (T/C) | S.NO | Accession | Description | MW [kDa]/ calc.pI | Abundance Ratio: (T/C) |
|------|-----------|-------------|-------------------|------------------------|------|-----------|-------------|-------------------|------------------------|
| 1    | A0A1U8E5V7 | beta-xylosidase | 85.6/8.06         | 0.684                  | 17   | K4FXE7    | Triose phosphate isomerase | 27.1/5.99 | 100                    |
| 2    | A0A1U8G957 | Peroxidase   | 34.4/8.19         | 2.095                  | 18   | A0A1U8HJ07 | Malate dehydrogenase aspartyl protease | 36.1/8.9 | 0.059                  |
| 3    | A0A1U8FME6 | Acidic endochitinase Q | 27.6/7.12 | 1.304                  | 19   | A0A1U8GWV9 | Subtilisin | 46.3/8 | 100                    |
| 4    | A0A1U8GQ7  | Subtilisin-like protease | 79/6.48  | 0.794                  | 20   | A0A1U8EX6  | Subtilisin | 82.9/6.35 | 0.245                  |
| 5    | A0A075VXE8 | Uncharacterized protein | 53.7/7.05 | 1.232                  | 21   | A0A1U8FZZ3 | alpha-1,4-fucosidase | 55.8/8.41 | 100                    |
| 6    | A0A1U8F4N5 | Glucanendo-1,3-beta-glucosidase | 38.4/7.52 | 0.548                  | 22   | A0A1U8H921 | Acidic mammalian chitinase | 42.4/8.81 | 0.99                   |
| 7    | A0A1U8FXF2 | Acidic endochitinase pcht28 | 27.3/4.98 | 100                    | 23   | A0A1U8FRY3 | Uncharacterized protein | 27.4/5.96 | 0.613                  |
| 8    | A0A1U8EC57 | Early nodulin | 35.7/8.78         | 0.731                  | 24   | J1KTS6    | ATP synthase subunit beta | 54.1/5.06 | 0.424                  |
| 9    | A0A1U8HET9 | Ribonuclease MC | 34.5/7.11         | 1.073                  | 25   | A0A1U8E6R9 | Basic 30 kDa endochitinase | 36.4/6.81 | 100                    |
| 10   | A0A1U8HBB0 | Ribonuclease MC | 35.7/7.23         | 1.377                  | 26   | A0A1U8FT89 | Zingipain-2-like | 38.4/6.23 | 1.505                  |
| 11   | A0A1U8E494 | Acetylajmalan esterase | 41.2/8.98 | 1.368                  | 27   | A0A1U8FAG3 | Aspartic proteinase | 46.4/7.91 | 0.088                  |
| 12   | B9VRKK7   | Peroxidase   | 34.9/9.2          | 0.674                  | 28   | A0A1U8FTT0 | Uncharacterized protein | 82.2/8.48 | 0.493                  |
| 13   | A0A1U8GZB5 | Peroxidase   | 35/4.79           | 0.321                  | 29   | A0A1U8GJ92 | Subtilisin | 81.8/5.9 | 0.694                  |
| 14   | A0A1U8GAI0 | CO(2)-responsesecreted protease | 81.2/5.94 | 0.321                  | 30   | A0A1U8GNT7 | Alpha-amylase | 48.1/6.16 | 0.284                  |
| 15   | A0A1U8DSU4 | Subtilisin   | 83.7/7.59         | 3.355                  | 31   | A0A1U8FYA1 | Peroxidase reticuline oxidase | 36.3/9.2 | 0.448                  |
| 16   | A0A1U8H5T7 | Somatic embryogenesis receptor kinase | 21.3/7.85 | 0.202                  | 32   | A0A1U8H994 | Beta-xylosidase | 62.8/8.95 | 0.3                    |
| 33   | A0A1U8EYS7 | Peptidylprolyl isomerase | 23.6/8.41 | 100                    | 35   | A0A1U8GCP7 | Alpha-xylosidase | 104.7/6.9 | 0.126                  |
| 34   | A0A1U8E46G | Elongation factor 1-alpha | 49.3/9.13 | 0.445                  | 36   | A0A1U8G5D0 | Glucan endo-1,3-beta-glucosidase | 52.9/5.97 | 100                    |
the tube after leaves were centrifuged at 1000×g for 15 mins at 4°C. The protein sample was immediately stored at −20°C until further analysis.

2.4. Cytoplasmic contamination assay

Apoplastic fluid was tested for the presence of cytosolic contamination using Malate Dehydrogenase (MDH, EC 1.1.1.37) assay by comparing with whole leaf protein as a control according to method described by Alves et al., [13]. Apoplast protein extract was mixed with 50mM NADH, 0.2mM Tris-Hcl (pH 7.5) and 0.4mM oxaloacetate. Change in the absorbance at 340 nm was monitored over 3 min using UV/Visible Spectrophotometer (Eppendorf Biospectrometer Kinetic). To assess cytoplasmic contamination, total soluble proteins were extracted by using potassium phosphate buffer (pH-7). Leaves were homogenized in buffer and were centrifuged at 700×g for 10 mins at 4°C (18), the supernatant was used for MDH enzyme assay. Cytoplasmic contamination was calculated as the percentage of MDH activity in the apoplast protein extract compared with activity in total leaf soluble protein extract.

2.5. Estimation of total phenolics (TP)

For the estimation of total phenolics, to 1ml of apoplastic extract 0.5ml of Folin-Ciocalteau reagent, 7.5ml ddH2O was added and incubated for 10 min at room temperature, and then 1.5ml of 20% sodium carbonate was added and incubated for 20 min at 400C. Solution was cooled and absorbance was recorded at 755 nm. Estimation of total phenolics (mg/g) was measured as described by Tohma et al., [14].
| S.NO | Accession | Description                     | MW [kDa]/ calc.pI | Abundance Ratio: (T/C) | S.NO | Accession | Description                     | MW [kDa]/ calc.pI | Abundance Ratio: (T/C) |
|------|-----------|---------------------------------|-------------------|-----------------------|------|-----------|---------------------------------|-------------------|-----------------------|
| 1    | A0A2G2XYJ9 | probable carbohydrate esterase | 29.5/8.72         | 0.719                 | 17   | A0A2G3AL78 | Globulin                        | 46.8/8.35         | 0.385                 |
| 2    | A0A2G2YR88 | Beta-galactosidase              | 92.5/7.66         | 0.674                 | 18   | A0A2G2ZSR6 | - ascorbate oxidase homolog     | 59.8/9.03         | 0.971                 |
| 3    | A0A2G2YXJ9 | aspartyl protease              | 52.4/8.54         | 0.623                 | 19   | A0A2G3A116 | Antimicrobial protein            | 12.8/8.95         | 5.846                 |
| 4    | A0A1U8G2S5 | Polygalacturonase inhibitor 1  | 36.7/8.27         | 0.659                 | 20   | A0A2G2YK18 | Non-specific lipid-transfer protein | 13.7/8.95         | 1.976                 |
| 5    | A0A2G2YW0 | putative amidase               | 54.2/9.09         | 5.69                  | 21   | A0A1U8CHD2 | neutral ceramidase              | 85.7/8.09         | 100                   |
| 6    | A0A2G2ZC6U | Miraculin                      | 23.5/8.95         | 0.419                 | 22   | A0A2G2Z114 | Transketolase, chloroplastic    | 80.9/6.04         | 100                   |
| 7    | A0A2G2YGS4 | Uncharacterized protein        | 53.7/8.63         | 100                   | 23   | A0A2G2YVL9 | proline-rich protein            | 25.8/9.33         | 1.664                 |
| 8    | A0A1U8HA07 | Auxin-binding protein          | 22.6/7.77         | 1.004                 | 24   | A0A2G2ZBQ7 | Superoxide dismutase            | 28.2/8.28         | 0.609                 |
| 9    | A0A1U85C4 | pathogenesis-related leaf protein | 17.4/8.32    | 0.217                 | 25   | A0A2G2YH5Q5 | Ripening-related protein        | 28.8/5.69         | 0.275                 |
| 10   | A0A2G2Y9E7 | aspartyl protease              | 47.9/8.34         | 1.212                 | 26   | A0A2G22D03 | Miraculin                       | 22.9/8.21         | 100                   |
| 11   | A0A2G3AY5J | Uncharacterized protein        | 56.5/6.05         | 0.78                  | 27   | A0A1U8EU88 | uncharacterized protein         | 25.3/7.9          | 0.221                 |
| 12   | A0A2G2Y8V4 | pathogenesis-related protein   | 28.5/7.94         | 100                   | 28   | E9JEC2   | Epidemis-specific secreted glycoprotein | 33.7/9.36         | 100                   |
| 13   | A0A1U8G7K7 | thaumatin-like protein         | 24.2/8.18         | 1.138                 | 29   | A0A2G3AM91 | Nucleoside-diphosphate kinase   | 16.3/6.79         | 100                   |
| 14   | A0A1U8EB49 | basic secretory protein        | 25.3/8.53         | 1.022                 | 30   | A0A2G2Y4E8 | Cysteine proteinase inhibitor   | 12.9/8.94         | 3.47                  |
| 15   | A0A1U8EB43 | desiccation-related protein    | 37.8/8.31         | 1.239                 | 31   | A0A2G2Y95 | Uncharacterized protein         | 81.6/6.04         | 0.064                 |
| 16   | A0A1U8EBK0 | protein trichome birefringence | 46.3/9.04         | 3.586                 | 32   | A0A2G2Y52 | Uncharacterized protein         | 26.9/9.79         | 0.335                 |
| 33   | A0A2G2ZG61 | alpha-glucosidase              | 100.8/6.74        | 100                   | 45   | A0A2G2YKB1 | Non-specific lipid-transfer protein | 15.5/8.24         | 1.398                 |
| 34   | A0A2G2YYV2 | Uncharacterized protein        | 48.7/9.25         | 1.455                 | 46   | A0A2G2Z69 | Non-specific lipid-transfer protein | 12.8/8.66         | 0.425                 |
| 35   | A0A2G2XLYL | Expansin                       | 28.2/7.99         | 1.126                 | 47   | A0A2G2V7A7 | Uncharacterized protein         | 9.2/8.13          | 3.238                 |
| 36   | A0A2G2ZD2 | protein P21                    | 25.1/6.81         | 0.527                 | 48   | A0A2G3AB80 | Subtilisin                       | 81.3/6.6          | 0.652                 |
| 37   | A0A2G2ZAL7 | Non-specific lipid-transfer protein | 15.5/8.94   | 1.177                 | 49   | A0A2G3AY0 | probably LRR receptor           | 51.8/8.81         | 0.975                 |
| 38   | D91C46 | Polygalacturonase-inhibiting proteins | 29.8/9.13 | 1.856                 | 50   | A0A2G2ZC11 | Alpha-mannosidase              | 116.9/6.58        | 0.479                 |
| 39   | A0A2G3A9X4 | Endochitinase                  | 37/9.26           | 0.641                 | 51   | A0A2G3AUX5 | Endochitinase B                 | 32.8/5.03         | 2.717                 |
| 40   | A0A2G3AA26 | acidic endochitinase           | 27.8/9.14         | 0.56                  | 52   | A0A1U8FSR6 | Ribulose bisphosphate carboxylase | 20.5/8.13         | 0.825                 |
| 41   | A0A2G2YHGC | Carboxypeptidase               | 57.1/5.6          | 1.231                 | 53   | A0A1U8FR64 | uncharacterized protein         | 54.9/8.1          | 0.637                 |
| 42   | A0A2G2YN0 | alpha-L-arabinofuranosidase    | 74.9/8.33         | 0.404                 | 54   | A0A2G2YU11 | cysteine-rich repeat protein    | 26.8/7.01         | 0.325                 |
| 43   | A0A2G2Y3P2 | Carboxypeptidase               | 55.4/7.08         | 0.375                 | 55   | A0A1U8DS5A1 | uncharacterized protein         | 40.6/7.14         | 2.025                 |
| 44   | A0A059P572 | Polygalacturonase inhibiting protein | 38.9/8.87 | 0.382                 | 56   | A0A2G2YNT9 | Glucan-endo-1,3-beta-glucosidase | 37.9/9.03         | 0.609                 |
2.6. Estimation of phenylalanine ammonia lyase (PAL)

For the estimation of phenylalanine ammonia lyase content, to 0.3ml of apoplastic extract, 1.2ml of Tris buffer (25mM, pH-8.8) and 1.5 ml of L-phenylalanine (12mM) was added. The rate of conversion of L-phenylalanine to trans-cinnamic acid was determined at 290nm as described by Sri deepthi et al., [15].

2.7. Estimation of peroxidise activity

For the estimation of peroxidise activity, for 0.5 ml of apoplastic extract, 1.5 ml of pyrogallol solution (0.05 M) and 0.5ml of H2O2 was added. The change in absorbance was recorded at 430 nm for 3 min. POD activity was quantified according to the method described by Abhayashree et al. [16].

2.8. Estimation of catalase activity

For the estimation of catalase activity, to 40μl of apoplastic extract, 2.5ml of potassium phosphate buffer (50mM, pH-7) and 0.5ml of H2O2 were added. The rate of decomposition of H2O2 was determined at 240nm for 3 min. Catalase activity was quantified according to the method described by Huseynova et al. [17].
2.9. LC-MS analysis

2.9.1. Sample preparation

Protein samples (50 μg) were reduced with 50 mM DTT at 60 °C for 1 h and the cysteine-groups were blocked using a 50 mM IAA solution at room temperature for 30 min. The protein samples were then subjected to trypsin digestion by adding trypsin in 1:30 ratio (Trypsin: Protein) at 37 °C in a dry bath for 16 hours. After trypsinization, samples were dried in speed vac and reconstituted in 20 μl of Milli-Q water with 0.1% formic acid and desalting was performed and then subjected to LC-MS.

2.9.2. Proteome analysis

LC-MS analysis is performed in 1290 Infinity UHPLC system, 1260 infinity Nano HPLC with Chip cube, 6550 iFunnel Q-TOFs (Agilent technologies, USA) at Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay. Samples were loaded in an analytical C18 column (PepMap RSLC C18 2 μm, 100 A × 50 cm). Mobile phase consists of solvent A: 0.1% FA in Milli-Q water, solvent B: 80:20 (ACN: Milli-Q water) + 0.1% FS. The raw LC-MS data was analyzed using Thermo Proteome Discoverer 2.2 software with Sequest-HT Uniport, capsicum annuum and plants databases.

2.9.3. Statistical analysis

All the samples (for both assays and LC-MS analysis) were collected triplicate and data were analysed with One-Way ANOVA at 5% probability. Data were represented as Mean ± SD.

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Transparency document

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.104041.

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