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Research Article

Protein glycosylation as a long-distance signaling cue in sunflower seedlings under NaCl stress

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Abstract

Sunflower is an important oil seed crop cultivated world-wide. The early phase of seed germination and seedling growth is important to establish the mechanisms of physiological and molecular pathways associated with salinity tolerance in the crop. The cotyledons in sunflower are important lipid reserve and, therefore, represent unique system to study the intersection of protein and lipid metabolism under NaCl stress. The present work provides information on the role of NaCl stress (120 mM) in differential regulation of protein glycosylation at the early stage of seedling development in sunflower. We analyzed cytosolic proteins for the detection of protein glycosylation from roots and cotyledons of two-day old (dark grown) seedlings. Furthermore, cotyledons were used for the extraction of oil-body membrane proteins which were also analzyed as potential markers of protein glycosylation. Thus, the present work provides preliminary information on the role of NaCl stress and subsequent protein glycosylation as a long distance signaling in sunflower.

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Introduction

Soil salinity is one of the major abiotic stress factors which is known to affect plant growth and crop productivity. Currently, salinity stress affects approximately 1.12 billion hectares of agricultural land worldwide, of which about 76 million hectares are impacted by anthropogenic activities (Basak et al., 2025). Salinity stress annually leads to infertility to 2-3 Mha of agricultural land world over (Sekmen et al., 2010). Sodium chloride (NaCl) is a predominant salt in soils which causes soil salinity and hyperosmotic stress in plants. Several sources, like wind, rock weathering, sea water flooding, low rain fall and desiccation lead to accumulation of salts in soil. Soils having electrical conductivity (EC_e) values $\geq$ 4 dSm^{-1} are classified as 'saline soils', which approximates to a concentration of 40 mM NaCl which generates an osmotic pressure of 0.2 MPa. Salt-tolerant plants (halophytes) comprise of 0.25% of known angiosperms and include 350 species among Caryophyllales, Alismatales, Malpighiales, Poales and Lamiales (Flowers et al., 2010). Glycophytes range in between salt-susceptible to salt-sensitive types, depending upon Na^+ sequestration or exclusion ability. Glycophytes and halophytes have strong differences in terms of their evolutionary and adaptational backgrounds. Sunflower (Helianthus annuus) is one of the four (other than soyabean, pea and canola) most important annual edible oilseed crops cultivated worldwide (Putt, 1997; Basak et al., 2025). It is native to North America and is now cultivated in more than 70 countries, with United States and Europe contributing to 60% of the total production. Russia and Australia have extensive cultivation of sunflower. In India, sunflower is an important oilseed crop having major consumption of sunflower oil. Parts of southern peninsula consisting of Tamil

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Nadu, Northern Karnataka, Andhra Pradesh and Maharashtra cultivate sunflower as a major rainfed crop in the late Kharif-Rabi season. Sunflower cultivation, however, faces a systemic weather risk as most of the areas are rainfed. India is a tropical country with several areas receiving heavy to moderate or scanty rainfall. Reports suggest that sunflower cultivars vary from being sensitive to semi tolerant to salt stress (Francois, 1996). Sunflower crop is highly sensitive to water stress in its critical stage of late vegetative growth and also during seed filling stage. Soil salinity has posed a major hurdle to agricultural productivity of many crops, including sunflower. Ground water irrigation in India causes salinity in > 32% of the agricultural land. Fertile soils in Central India and Indo-gangetic plains in the east-southern coastal parts of India are affected by severe to moderate salinity and comprise of 17.7% of total agricultural land of the country. Investigations on salt tolerance, stress acclimatization and productivity of sunflower are also of prime importance in context of oilseed crop research.

Oil yielding crops store lipids in their seeds as the major source of energy, facilitating seed germination and seedling growth. Lipid storing organelles, termed as oil bodies (OBs) or oleosomes, comprise of a phospholipid (PL) monolayer. The PL monolayer contains extrinsic and intrinsic proteins. Triacylglycerols (TAGs) are the major lipids stored in the OB matrix. OB membranes contain phosphatidylcholine as the major constituent (50-60%), followed by phosphatidylserine (20-30%), and phosphatidylethanolamine, phosphatidylinositol and phosphatidic acid as minor constituents, respectively (David, 2012). Three major groups of proteins, namely oleosins, caleosins and steroleosins, are specifically associated with plant OBs. Oleosins and caleosins stabilize OBs by providing them integrity. OLEOSIN 1 and OLEOSIN 2 proteins have been reported in Arabidopsis seeds (Shimada and Nishimura, 2010). Steroleosins are a kind of sterol dehydrogenase enzymes facilitating lipid mobilization in germinating seeds (Purkrtova et al., 2008). Size of oil bodies ranges from 0.5-2.5 µm in diameter (Frandsen et al., 2001; Purkrtova et al., 2008; Roberts et al., 2008). Oil boides associated with non-seed tissues differ in their structure and composition. Oil bodies present in mesocarp cells of olive (Oleaeuropaea sp.), palm (Elaeisguineensis sp.) and avocado (Persea americana) are, however, very large (10-20 µm in diameter) and are devoid of oleosins (Capuano et al., 2007; Shimada and Nishimura, 2010). Oleosins are of primary importance in the context of seed germination and seedling growth. They stabilize OBs by maintaining their small size and high surface to volume ratio. This feature renders OBs highly accessible to lipases, thus facilitating mobilization of TAGs (Dmitriev et al., 1999).

In eukaryotic cells, protein glycosylation is a highly conserved post-translational modification prevalent in different developmental and environmental changes. Glycosylation occurs in the Golgi apparatus and endoplasmic reticulum of cells where a sugar moiety or glycan is covalently joined to a mature protein. This protein modification is essential for protein folding, maintaining enzymatic activity, structural stability, intermolecular interactions, and subcellular targeting of proteins (Mustafa and Komatsu, 2014; Eichler, 2019; Sun et al. 2021). Three main basic forms of protein glycosylation are N-glycosylation, O-glycosylation, and glycosylphosphatidylinositol respectively (Liu et al., 2021). N-linked glycans in plants play pivotal roles, in protection against proteolytic degradation, facilitate correct folding, regulate protein activity, and protect root growth from osmotic stress (von Schaewen et al., 2008; Kang et al. 2008). These processes predominantly occur in the luminal space of the endoplasmic reticulum, as the entry point into the secretory system (von Schaewen et al., 2008; Kang et al. 2008). In salt-stressed conditions, N-glycosylation is essential for plant growth and adequate cell-wall formation (Kang et al. 2008; Chen et al., 2022). N-glycosylation is common among secreted and membrane-associated proteins, which participate in various cellular processes including cell wall biosynthesis, pollination, biotic stress response, and intercellular communication (Chen and Cheng, 2024). In plants, protein N-glycosylation accompanying fruit ripening can also regulate postharvest longevity of fruit (Mendez-Yañez, 2021). N-glycosylation of glycoside hydrolases in the starch and sucrose metabolism pathway suggests its role in regulating carbohydrate metabolism during embryo germination (Ying et al. 2017). Most biological processes, particularly metabolism, gene regulation, signaling, cell cycle control, protein mobility, protein-protein interactions, and cell shape, are mediated by O-linked glycosylation proteins in different sub-cellular compartments (Ma et al., 2022; Chen and Cheng, 2024). In A. thaliana, arabinosylation-based O-glycosylation of extensins (EXTs) is crucial for proper root hair elongation (Velasquez et al. 2011).

The present work provides information on the role of NaCl stress (120 mM) in differential regulation of protein glycosylation at the early stage of seedling development in sunflower. We analyzed cytosolic proteins for the detection of protein glycosylation from roots and cotyledons of two-day old (dark grown) seedlings. Furthermore, cotyledons were used for the extraction of oil-body membrane proteins which were also analzyed as potential markers of protein glycosylation. Thus, the present work provides preliminary information on the role of NaCl stress and subsequent protein glycosylation as a long distance signaling in sunflower.

Material and Methods

Seed germination and NaCl treatment

Sunflower seeds (*Helianthus annuus* L., cv. KBSH-44) were procured from University of Agricultural Sciences, Bangalore, India. Seeds were surface sterilized with 0.005% mercuric chloride (2 min) followed by washing the seeds 1 h for running tap water. Seeds were imbibed for 4 h and sowing on germination sheet pre-soaked with half-strength Hoagland nutrient solution. Hogland nutrient solution (without NaCl) were added in control set and Hoagland supplemented with 120 mM NaCl solution added in salt treatment set. Trays containing seeds were incubated in dark at 25±2 °C for 2 d. After the growth period, seedlings with uniform growth patterns were chosen for further analysis.

Sample preparation for extraction of buffer soluble proteins

Roots (2 g) and cotyledons (500 mg) obtained from 2d old seedlings grown in the absence or presence of 120 mM NaCl were ground to powder in liquid nitrogen and the powder was transferred into Eppendorf tubes and incubated in the homogenization buffer (25 mM Tris-HCl, 400 mM sucrose, 10 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA-disodium salt, 1 mM PMSF, 0.2% β-mercaptoethanol, pH 7.5) at a proportion of 1.2 ml g^{-1} FW for 30 min at 4 °C in a vortex shaker. The homogenates were centrifuged at 12,000 g for 20 min at 4 °C and supernatant collected was further centrifuged at 10,000 g for 20 min at 4 °C. Supernatants obtained from cotyledons were stored in -20 °C for further use. Root supernatants obtained were concentrated using Vivaspin 2 kDa MWCO columns (GE Health Care, UK) at 10,000 g. Prior to concentrating, the columns were saturated with buffer for 15 min at 10,000 g to avoid drying of membrane. Concentrated root homogenates obtained were proceeded for delipidation steps by acetone washings. Homogenates were incubated with 100% acetone (1:3 v/v) overnight at -20 °C followed by gentle vortex and centrifugation at 10,000 g at 4 °C for 20 min. Supernatants containing all organic contaminants mixed with acetone were discarded and the protein pellets obtained were suspended in 90% acetone followed by incubation at -20 °C for 20 min. After incubation pellets were collected by centrifugation (10,000 g for 20 min at 4 °C) followed by two more acetone (90%) washings. Pellets obtained after acetone washings were then air dried to remove all traces of acetone vapours and suspended in 200 µl homogenisation buffer. Protein suspensions were sonicated for 5 min and centrifuged at 10,000 g for 20 min at 4 °C. Root supernatants (obtained from acetone washing) and cotyledon (stored priorly) were subjected to protein estimation according to Bradford (1976). Protein aliquots equivalent to 10 µg of protein were boiled with Laemmli buffer (1:1, v/v) at 90 °C for 2 min followed by boiling in 0.2% β-mercaptoethanol at 90 °C for 2 min.

Sample preparation for extraction of oil-body membrane proteins

Oil body membrane proteins were isolated according to Millichip et al. (1996) Cotyledons obtained from seedlings grown in the absence or presence of 120 mM NaCl were homogenized in Tris-urea buffer (50 mM Tris, pH 7.5, containing 9 M urea). The homogenates were filtered through 4 layers of muslin cloth. The filtrate thus obtained was centrifuged at 10,000 g at 4 °C for 20 min. The oil body pads collected was subsequently resuspended in 1.25 mL $NaHCO_3$ (0.1 M) per gram of tissue and incubated at 4 °C for 30 min and again centrifuged at 10,000 g for 20 min at 4 °C. The oil body pads were then resuspended in excess Tris-sucrose buffer (20 mM tris, 0.2 M sucrose, pH 7.5) to wash out excess bicarbonate. After centrifugation at 10,000 g for 20 min at 4 °C, the oil body pads were resuspended in the same buffer. In order to solubilize oil body membrane proteins, oil body suspension washed in Tris-sucrose buffer was extracted four times with five volumes of diethyl ether. Residual diethyl ether was evaporated from each sample at room temperature while vortexing continuously. The residues containing oil body membrane proteins were suspended in Tris-sucrose buffer. 160 µl of this suspension was mixed with 40 µl of 10% SDS solution. The samples were heated at 90 °C in a water bath for 30 min in order to solubilise membrane proteins, and were centrifuged for 15 min at 7,000 g and 4 °C. Oil body membrane proteins in the supernatant were quantified according to Markwell et al. (1981). Protein extract containing 10 µg of protein was mixed with reducing laemmli sample buffer and heated in boiling water for 5 min prior to SDS-PAGE analysis (Laemmli, 1970). Samples were separated in 16 cm 10-20% gradient gel at 75 V (30 min) followed by 200 V for the rest of the run.

Electrophoretic analysis

Preparation of resolving gradient SDS-PAGE gel

Resolving gradient gel (10 to 20%) was prepared by mixing the resolving gel mixtures (10% and 20%) using a gradient mixer. The composition of resolving gel mixtures is shown below:

Components	Concentrations of resolving gel mixture (%)	
	10	20
Acrylamide stock (40%)	3.8 mL	7.6 mL
Resolving gel buffer (3 M Tris, pH 8.8)	1.9 mL	1.9 mL
SDS solution (10%; w/v)	0.15 mL	0.15 mL
Urea [a], solid	5.41 g	5.41 g
TEMED	10 µL	10 µL
Ammonium persulphate [b] (10%; w/v)	0.1 mL	0.1 mL
Water [c]	as required	as required

[a] Used only for making urea containing gel for the analysis of oil body membrane protein

[b] Freshly prepared and added at the end to start polymerization.

[c] As required to bring the final volume to 15 ml

Preparation of stacking gel

Stacking gel is prepared after polymerization of the resolving gel. Composition of stacking gel is as follows:

Components	Quantity
Acrylamide stock (40% T)	1.9 mL
Stacking gel buffer (0.5 M Tris, pH 6.8)	1.9 mL
SDS solution (10%; w/v)	0.15 mL
Urea [a], solid	5.41 g
TEMED	10 µL
Ammonium persulphate [b] (10%; w/v)	0.1 mL
Water [c]	as required

[a] Used only for making urea containing gel

[b] Freshly prepared and added to start polymerization

[c] As required to bring the final volume to 15 ml

Electrophoretic analysis of glycosylation in buffer proteins from seedling roots and cotyledons

Preparation of 12.5 % SDS-gel (Laemmli (1970)

- Acrylamide stock (30%) was prepared by dissolving 29.2 g of acrylamide and 0.8 g bisacrylamide in water to make a final volume of 100 mL.
- Resolving gel buffer (3 M Tris buffer, pH 8.8) was prepared by dissolving 36.35 g of Tris base in water. The pH of solution was set at 8.8 with HCl and final volume was made to 100 mL using distilled water.
- Stacking gel buffer (0.5 M Tris buffer, pH 6.8) was prepared by dissolving 6.06 g of Tris base in water. The pH of solution is set at 6.8 with HCl and final volume was made to 100 mL using distilled water.
- Sodium dodecyl sulphate (SDS) solution (10%; w/v) was prepared by dissolving 10 g of SDS in water to make a final volume of 100 mL.
- Ammonium persulphate (APS) solution (10%; w/v) was prepared by dissolving 100 mg APS in water to make a final volume of 1 mL.
- TEMED (N,N,N’,N’- tetramethylethylenediamine) was used undiluted.

- Electrode buffer [0.025 M Tris, 0.192 M glycine, 0.1% (w/v) SDS] was prepared by dissolving 15.15 g Tris base, 72 g glycine and 5 g SDS in water to make a final volume of 5 L.

All stock solutions were stored at 4°C for long term usage and ammonium persulphate (APS) was dissolved fresh in water prior to use. SDS solution was stored at room temperature.

Preparation of resolving SDS gel (12.5%)

Resolving gel was casted using the mixture of following components aliquoted from their respective stock solutions and mixed at room temperature. The composition of the resolving gel mixture is as follows:

Components	**Quantity (12.5% gel)**
Acrylamide stock (30%)	2.5 mL
Resolving gel buffer (3 M Tris, pH 8.8)	1.5 mL
SDS solution (10%; w/v)	120 µL
TEMED	6 µL
Ammonium persulphate* (10%; w/v)	120 µL
Water	2.62 mL

*Freshly prepared and added at last to start polymerization

Preparation of stacking SDS gel (5%)

Stacking gel was casted using the mixture of following components aliquoted from their respective stock solutions and mixed at room temperature. The composition of the stacking gel mixture is as follows:

Components	**Quantity (5% gel)**
Acrylamide stock (30%)	400 µL
Stacking gel buffer (0.5 M Tris, pH 6.8)	0.5 mL
SDS solution (10%; w/v)	20 µL
TEMED	4 µL
Ammonium persulphate* (10%; w/v)	13 µL
Water	1.08 mL

*Freshly prepared and added at last to start polymerization

Separation of proteins using SDS-PAGE

20 µg protein from each sample were loaded for single dimension separation on a 12.5 % linear SDS-PAGE in Mini-protean Tetra Cell (BioRad, U.S.A). The gel was run with step wise increasing voltage (25V, 35V, 55V and 75 V for 10 min each) followed by a constant voltage of 200V for 100 min.

Staining for detection of glycosylated proteins (buffer soluble and oil body membrane protein) using Pro-Q® Emerald 300 protein gel stain

Preparation of stock solutions

Pro-Q® Emerald 300 stock solution: Vial containing Pro-Q® Emerald stain (Invitrogen Bioservices Pvt. Ltd, U.S.A) was dissolved in 6 ml DMSO and stored at -20 °C for further use

Fix solution: Fixation solution was freshly prepared containing 50% methanol and 5% acetic acid in distilled water

Wash solution: Washing solution was freshly prepared containing 3% glacial acetic acid in distilled water

Oxidizing solution: 250 ml of 3% acetic acid solution was added to bottle containing periodic acid (provided in staining kit) and dissolved thoroughly. Solution was stored at 4 °C for further use

Procedure for Pro-Q® Emerald 300 staining

Gels obtained after electrophoresis were immersed in fixative solution (80 ml) for 30 min with gentle agitation in a rocker shaker at room temperature with two changes to ensure complete washing of SDS out of the gel. Followed by fixation, gels were washed in 100 ml washing solution for 20 min with gentle agitation in a rocker shaker at room temperature with single change. Gels were then immersed in 80 ml of oxidizing solution with gentle agitation in a rocker shaker at room temperature for 30 min. This was followed by washing in wash solution for 15 min. Staining was performed by diluting the stock solution of Pro-Q® Emerald by adding 500 µl of the stain in staining buffer (ready to use, provided in the staining kit). Gels were incubated in 25 ml of stain solution for 120 min in dark at room temperature. This was followed by washing in wash solution for 15 min in dark at room temperature with two changes (Steinberg, 2009).

Visualization of gels for proteins (total protein profile, glycosylated proteins)

Gels stained with SYPRO® Ruby and Pro-Q® Emerald 300 were visualized in a UV transilluminator (Gel Doc-It imaging system UVP, USA) at an excitation of 280 nm. Gels stained with SYPRO® Ruby were also visualized in Typhoon fluorescent scanner (GE Health care) at an excitation of 450 nm to view total protein profile. Proteins bands varying in their expressions in the samples (control and 120 mM NaCl treated) were quantified by software analysis (Launch Vision software, UVP, USA and Image Quant TL software, GE Health Care, UK) to highlight the differences in glycosylation and phosphorylation of proteins in response to 120 mM NaCl. Differential protein expression was graphically represented in terms of band volume (arbitrary units).

Results

NaCl stress slows down sunflower seedling growth

In the present investigations, dark-grown sunflower seedlings raised in the absence or presence of 120 mM NaCl (dissolved in Hoagland's nutrient solution) were analyzed for changes in growth patterns 2days after seed germination. Seed germination was not affected by NaCl stress but it induced changes in seedling growth by a reduction in primary root length and inhibition of hypocotyl elongation, as was evident as early as after 48 h (2d) of seed germination (Fig. 1).

Protein glycosylation in sunflower seedling roots and cotyledons in response to 120 mM NaCl stress

Cotyledons obtained from 2 d old sunflower seedlings lead to some enhancement of glycosylation of cytosoilc proteins (10,000 g supernatant) in the presence of 120 mM NaCl (Fig 2-4.). Buffer soluble proteins from cotyledons (Fig 2.) exhibited greater intensity of glycosylated proteins in comparison with roots (Fig 2-3.). Major glycoproteins expressed in cotyledons represented four bands having quantitative differences and ranged from high (> 180 kDa) to low (< 29 kDa) molecular mass. Oil body membrane proteins from cotyledons exhibited a single band of 75 kDa with increased glycoprotein expression in the presence of 120 mM NaCl stress (Fig 4.). Roots exhibited weaker expression of glycoproteins and were of high molecular weight (> 66 kDa; Fig 3.).

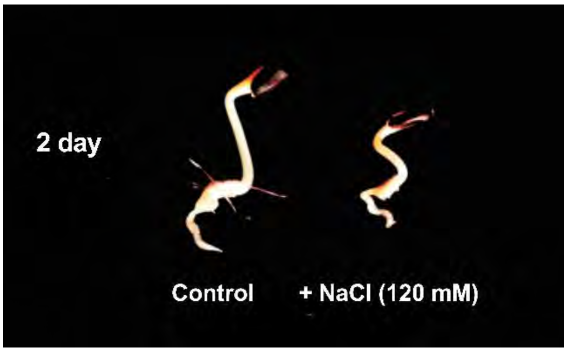

Figure 1. Effect of 120 mM NaCl stress on sunflower seedling growth in dark, 2 days after germination

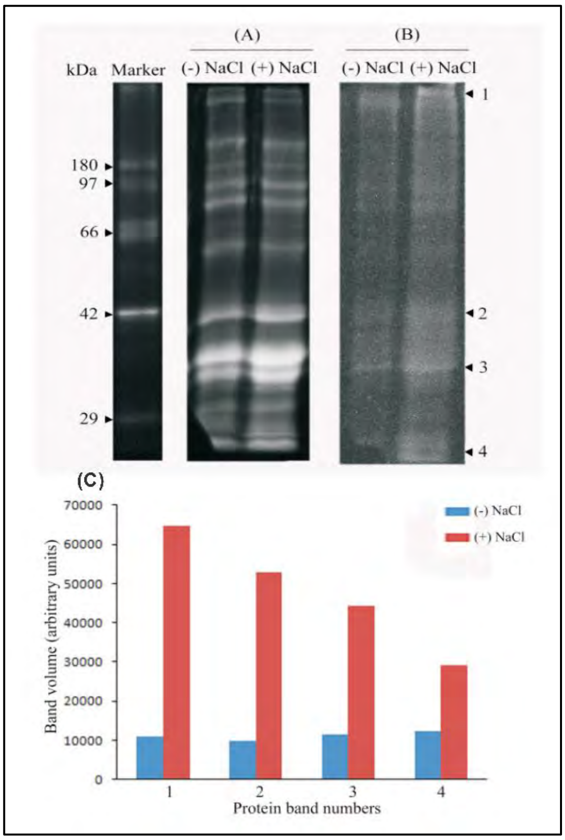

Figure 2. Electrophoretic analysis of glycoproteins from total soluble proteins (10,000 g supernatant) in 2d old sunflower seedling cotyledons grown in the absence or presence of 120 mM NaCl. (A) Total protein profile as visualized after staining with SYPRO Ruby (B) Glycoprotein profile as visualized after staining with Pro-Q Emerald stain. Differential expression of glycoproteins from total soluable proteins (10,000 g supernatant) in 2d old sunflower seedling cotyledons grown in the absence or presence of 120 mM NaCl. (C) Histograms depicting differential expression of glycoproteins represented in terms of band volume calculated using Vision Launch software (UVP, USA)

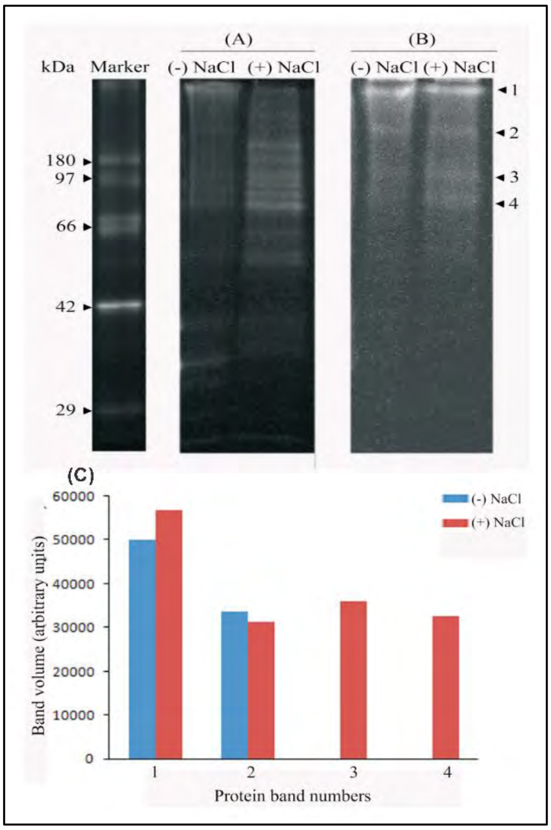

Figure 3. Electrophoretic analysis of glycoproteins from total soluble proteins (10,000 g supernatant) in 2d old sunflower seedling roots grown in the absence or presence of 120 mM NaCl. (A) Total protein profile as visualized after staining with SYPRO Ruby (B) Glycoprotein profile as visualized after staining with Pro-Q Emerald stain. Differential expression of glycoproteins from total soluable proteins (10,000 g supernatant) in 2d old sunflower seedling roots grown in the absence or presence of 120 mM NaCl. (C) Histograms depict differential expression patterns of glycoproteins in different protein bands represented in terms of band volume calculated using Vision Launch software (UVP, USA)

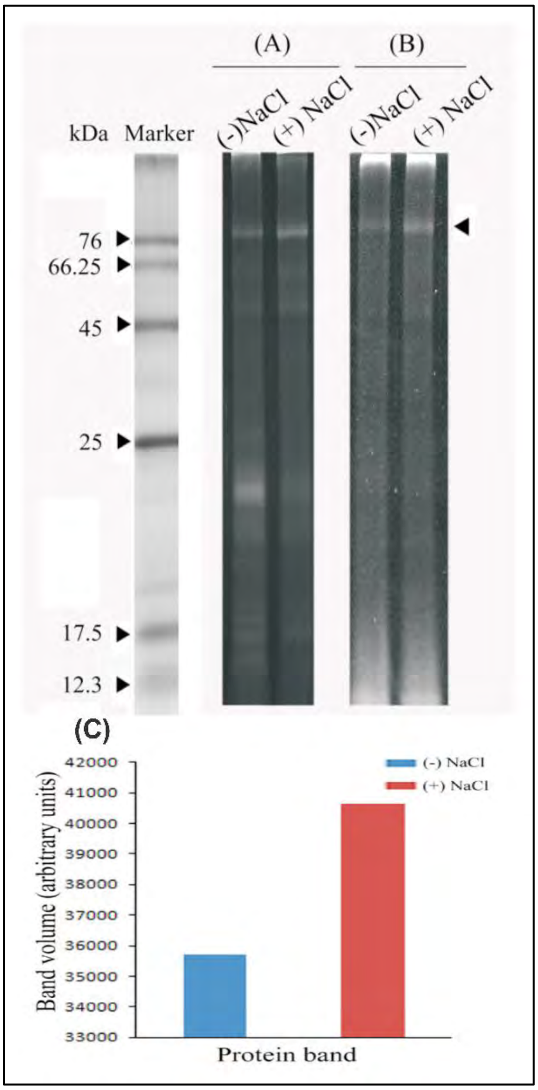

Figure 4. Electrophoretic analysis of glycoproteins from oil body membranes. OBs isolated from 2d old sunflower seedling cotyledons grown in the absence or presence of 120 mM NaCl were washed with 0.1 M sodium bicarbonate. (A) Total protein profile as visualized after staining with SYPRO Ruby (B) Phosphoprotein profile as visualized after staining with Pro-Q Emerald stain. (C) Differential expression of glycoproteins in the oil body membranes. Oil body preparations from 2d old sunflower seedling cotyledons grown in the absence or presence of 120 mM NaCl were subjected to 0.1 M sodium bicarbonate washing. Histograms depict differential expression of glycoproteins in the protein band represented in terms of band volume calculated using Vision Launch software (UVP, USA)

4. Discussion

Present observations on the effects of 120 mM NaCl stress on the growth pattern of sunflower seedlings are in congruence with earlier reports (David et al., 2010). NaCl (120 mM) stress imparts significant, although not lethal, effects on the growth and morphology of sunflower seedlings. Sunflower cultivars vary from being very sensitive to semi-tolerant to salt stress (Ashraf and Tufail, 1995; Flagella et al., 2004). Optimization for tolerable NaCl concentrations and analysis of growth pattern of NaCl-stressed sunflower seedlings has also been performed earlier by David et al., (2010). Growth pattern of sunflower seedlings (hypocotyl extension and root proliferation) exhibits concentration-dependent response in the presence of 40, 80 and 120 mM NaCl (David et al., 2010). Effect of 120 mM NaCl on seedling growth is, however, better evident in terms of inhibition of hypocotyl elongation and reduced proliferation of roots (David et al., 2010). Elemental analyses of seedling roots and cotyledons shows increased Na^+/K^+ ratio in roots and cotyledons in response to 120 mM NaCl stress (David et al., 2010). Roots and cotyledons of seedlings raised in the absence or presence of 120 mM NaCl stress also show variations in their water content (David et al., 2010). These observations highlight the adaptive features of NaCl-stressed sunflower seedlings which seem possibly to be regulated by partial Na^+ exclusion mechanisms within the cells of roots or long-distance mobilization of Na^+ to the cotyledons.

Protein N-glycosylation is an essential post-translational modification occuring in the lumen of endoplasmic reticulum (ER) and is catalyzed by oligosaccharyltransferase (OST; a major multi subunit enzyme complex) which transfers the oligosaccharide groups to asparagines, serine or threonine residues of proteins (Silberstein and Gilmore, 1996; Helenius and Aebi, 2001; Schoberer et al., 2017) This phenomenon is essential for proper protein folding and maintenance of its functionality. Defects in ER N-glycosylation in plants have been related to loss of protein functions, thus causing impaired growth and development, leading to lethality. Mutation in ER-glucosidase I (*knopf/gcsi)* gene leads to deficiency in cellulose biosynthesis caused by improper functioning of the protein N-glycosylation pathway (Boisson et al., 2001; Gillmor et al., 2002). Protein glycosylation involves the addition of glycans at specific amino acid sites of proteins. Glycans are homo-or-heteropolymers of monosaccharides connected by glycosidic linkage to the nitro- or hydroxyl groups of proteins. A majority of proteins undergo glycosylation during their secretion from ER and cisternae of golgi bodies. Glycosylation is site-specific and may involve various types of glycans (N-linked, O-linked or phospho-glycans). Glycans are attached to nitrogen or hydroxyl oxygen of specific amino acids, principally asparagine, serine, threonine or tyrosine (Helenius and Aebi, 2004).

Altered expression of glycoproteins in response to abiotic stress has been investigated in several plant species (Hurkmann et al., 1991; Showalter, 1993; Silberstein and Gilmore, 1996; Ahn et al., 1998; Gu et al., 2004; Helenius and Aebi, 2001; Mareri et al., 2019). Major pools of glycoproteins are involved in signaling events and they also act as membrane receptors thus helping in the recognition of signals from external stimuli. Protein glycosylation has been reported to be a necessary phenomenon to confer salt-tolerance to plants. NaCl stress (450 mM) in *Populus* sp. has been reported to result in an increase in hydroxyproline-rich glycoprotein (HRGP) transcripts (Gu et al., 2004). This transcript translates a major cell wall protein-extensin. HRGPs have been reported to have several functions related to root maturation, pathogen defence and mechanical wounding (Showalter, 1993; Ahn et al., 1998; Mareri et al., 2019). Barley roots exhibit an overexpression of germin proteins in response to NaCl stress (Hurkmann et al., 1991). Germins are essential proteins with oxalate oxidase activity, leading to H_2O_2 generation by the degradation of oxalate. This further helps in several defense mechanisms associated with biotic and abiotic stress (Patnaik and Khurana, 2001). Wheat embryos subjected to salt stress exhibit differential distribution of germins in coleoptiles unlike to that of control (- NaCl) where it was abundant in coleorhizae. Maturation of N-glycans in the golgi bodies has been stated to be an essential phenomenon for providing tolerance to salt stress and maintenance of proper root growth and morphology (Kang et al., 2008). Mutants of glycan maturation gene complex glycan 1 (*cgl1*) have been reported to exhibit aberrant root morphology, followed by intense callose accumulation in the primary roots of Arabidopsis in response to NaCl stress (Kang et al., 2008). Thus, Na^+ sensing leads to specific events associated with glycoprotein expression necessary for NaCl tolerance.

Present work, thus, highlights differences in the expression of glycoproteins in roots and cotyledons in the absence or presence of 120 mM NaCl stress. Cotyledons exhibit greater increase in glycoprotein expression due to salt stress, as represented in terms of protein volume. Glycoproteins comprise of major cell wall-associated arabinogalactan proteins (AGPs), chitinases or expansins (Tucker et al., 2018; Hromadová et al., 2021). These proteins vary in expression in response to abiotic stresses (Wu et al., 2020; Hromadová et al., 2021). Earlier reports have suggested the expression of chitinases to alter root architecture in presence of varying environmental conditions (Narula et al., 2020). Investigations in *Jatropha* seedling roots have recently reported upregulation of chitinase and expansin genes in the later stages of growth under salt-stress (Zhang et al., 2014). These were attributed to the changes in cell wall matrix necessary for physical barrier against salt-stress. Sunflower has been reported to accumulate hydroxylproline rich glycoproteins (HRGPs) under biotic stress (Mouly et al., 1992). Transient expression of extensin (a type of HRGP) seems to be involved in root maturation (Ahn et al., 1998). Showalter (1993) has reported the role of this glycoprotein in defense against biotic and abiotic stress. In situ hybridization of germin RNA in wheat embryo has revealed spatial differences in the expression of glycoprotein in response to NaCl stress.

Oil bodies are dynamic lipid storage bodies undergoing interaction of various biomolecules and enzymes. Seed germination involves a precise regulation of lipid metabolism accompanying oil body mobilization from cotyledons. Salt-stress induced changes in oil body mobilization will be correlated with identification of glycoproteins expressed in sunflower seedlings. Possible mechanism of maintenance of oil body membrane stability for their longer retention in the presence of 120 mM NaCl is expected to be associated with enhanced glycosylation of the membrane proteins as one of the mechanisms providing longevity to seedling growth under salt stress.

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Credit authorship contribution statement

SM: Data analysis, Data compilation, Methodology, Visualisation, Writing – original draft. **SCB**: Conceptualization, Supervision. **GB:** Formal analysis, Software, Writing – original draft. **CB:** Formal analysis, Review and editing. All authors reviewed the manuscript and approved the final version for submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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