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Chapter

Salinity Tolerance in Canola: Insights from Proteomic Studies

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Abstract

Salinity considerably lowers crop yield worldwide. Production of salt stress-tolerant species will be essential to maintain the food supply in the coming decades. Brassicas, including various members of the family Brassicaceae, are very necessary sources of human food. Importantly, the key crop species that are members of the Brassicaceae family are genetically diverse and therefore their response reaction and adaptation to salinity varies greatly. Canola (*Brassica napus* L.) is commonly grown for edible oils and other uses such as biodiesel fuel production. Although most types of canola are identified as salt-resistant, plant yield and development are reduced significantly by rising salinity levels. In saline situations, the plant's genome supports a range of physiological changes in some plant characteristics. Since the function of genes cannot indicate the exact condition of cells, proteomic approaches are emerged as methods to investigate the plant's responses to stresses in the molecular levels. Exploring the proteome complements research at the genome and transcriptome level and helps elucidate the mechanism of salt tolerance in plants. Proteins are reliable indicators of salinity responses, as they are directly involved in forming the new phenotype providing adaptation to salinity. In this chapter, we review the response of the rapeseed proteome to salinity stress.

Keywords: canola, plant proteomics, molecular markers, ROS, salinity

1. Introduction

Plants developing under field conditions are exposed to many ecological factors, which define their macro and micro environment. Every deviance in these environmental variables from the optimal levels may be detrimental to the plant and cause stress. Stress is provided by abiotic factors such as elevated drought, salinity, temperature, or biotic factors such as bacteria, insects, nematodes, fungi, and viruses. Plants may also have to cope with multiple stresses. Among the abiotic stresses, salinity has emerged as one of the most important extreme agents which limit agricultural crop yield as well as making large areas unsuitable for cultivation. About 7 percent of the world regions, 20 percent of all the world's agricultural lands, and nearly half of the irrigated lands are impacted by soil salinity [1]. In addition, the areas impacted by salt increase about 10 percent annually and if the problem is not solved, about 50 percent of the arable lands will be salinized by the year 2050 [2]. Soil salinity has substantial negative effects on the growth and productivity of

plants. Reduction of plant growth and productivity can result from a disproportionate supply of photosynthetic assimilates or hormones to growing tissues [3].

The lack of cultivable areas due to salinization is a direct challenge to providing the burgeoning population with adequate supplies of food. Therefore, there is a need to grow species that, are not only able to endure high levels of salt but can also retain optimum yields levels in the presence of salt. Nonetheless, due to the multigenic and quantitative nature of salt tolerance, endeavors to improve crop production under salinity have been generally ineffective. This has motivated researchers to follow a combination of approaches utilizing both traditional and novel strategies to improve salt tolerance.

Members of the Brassicaceae are major contributors to the daily needs of humans and are used as vegetables, oils, condiments and more. Brassica members occupy the third position in vegetable oil production between various oilseed species [3, 4]. *Brassica napus* L. or as we know it, Canola, is commonly grown for edible oils and biological-based fuel production. Notwithstanding many types of canola being regarded as salt-resistant, plant development and yield decrease in response to rising salinity stress. Morphological characteristics such as shoot and root development during stress; physiological factors such as water of leaf content, chlorophyll amount, photosynthetic amount, and membrane integrity; biochemical agents such as osmolytes accumulation, the activity of antioxidant enzymes and molecular responses such as modifications of expression of salt-resistant genes are key traits for detecting and characterizing differences in resistance within and between members of the Brassicaceae family [5].

Acclimatization to stress is mediated by deep modifications in the expression of genes that lead to variations in the plant transcriptome, metabolome, and proteome. Many investigations have shown that changes in the transcript-level expression of genes do not always lead to changes in protein levels [6, 7]. Hence it is also necessary to examine changes in the proteome because proteins are direct agents of plant response to stress. Furthermore, proteins include enzymes that catalyze modifications in the amounts of metabolites, and also regulatory proteins, for example they may control the plant response to stress at the transcription and protein translation levels.

Proteins contribute to stress-acclimation mechanisms which are directed to modifications in the cell cytoplasm, cytoskeleton, plasma membrane, and combination of the intracellular compartment that include alteration in their effects, for example, cell cytoplasm affinity to water [6, 7]. Modifications in protein accumulation under stress are firmly linked to the phenotypic reaction of the plant resisting the stress. Studies of plant reactions to stress at the protein level may contribute significantly to our understanding of the processes of plant tolerance or resistance.

2. Salinity

Saline soils are among the main environmental constraints that limit plant growth owing to the high salt concentration and the process of incremental increase in salt content is known as salinization [1]. Soil salinization is a global issue and often affects the littoral zone by extending soil salinity [8]. Almost 7% of the whole global land area, 20% of the cultivated area, and about half of the irrigated area is affected by soil salinity [1]. Furthermore, salt-affected area is increasing by 10% yearly per year and more than 50% of arable land will be salinized by the year 2050 if the problem is not addressed [9].

2.1 The salinity types and causes

2.1.1 Primary or natural salinity

Salinity stress occurs as a result of salt deposition by natural processes in the soils or groundwater over a long time period. Two normal mechanisms are involved. The first one is the weathering of parental substrates containing dissolved salts. The processes of weathering decompose rock and release dissolved salts of different forms, primarily sodium chloride (NaCl), with sulfates, magnesium, calcium, and carbonates, in lower concentrations. NaCl is the most abundant form of dissolved salt. The second mechanism is the deposition of maritime salt sediments which is transported by rain and wind. "Cyclic salts" are maritime salts which are transported by wind and sedimented by precipitation.

2.1.2 Human-induced or secondary salinity

Secondary salinization is the consequence of human actions that alter the soil's hydrological equilibrium between water used (irrigation or precipitation) and water used by crop [10]. The most important factors are (i) field clearance and the replacement of perennial vegetation annuals and (ii) irrigation, with salt-rich water and without adequate drainage.

2.2 Salinity stress effects on canola development and productivity

Soil salinity, similar to drought stress, is as a major abiotic stress which causes reduced crop production globally [11]. Growth and development of many plant species, including Brassica species, are adversely affected when subjected to salinity stress, due to the restriction of essential physiological, biochemical and metabolic processes with the consequence of toxicity of ions, osmotic stress, and decreased supply of water and minerals [12]. Salinity decreases nutritional ions like Fe, Zn, and Mn levels in plant organs including leaves, stems, and roots and pods at the flowering phases in Brassica spp. [13]. Plant height is reduced due to salinity stress and caused by decreased osmotic, leaf water potential, and enhancing electrolyte leakage [14].

Salinity stress adversely affects germination of canola seeds [15], reduces the length of radicles and plumes and seedling fresh weight, decreases biomass [16–18], impairs seed filling stage and the number of pods on plants, decreases the number of seeds in each pod and pod length [19], reduces the number of leaves, flowers, branches and siliques, leads to shorter siliques, less seed per silique and 1000-weight of seed [15], decreases leaf size, leaf nutrient attraction levels, hypocotyl and root development in seedlings with a rise in IAA oxidase and activity of peroxidase enzyme [17, 20], decreases chlorophyll a, chlorophyll b and total chlorophyll [21] and also reduces total fatty acids by 25% [18].

The decline in chlorophyll concentration as salt increases causes lower dry weight and decreases average leaf weight but in salt-resistant canola cultivars, this drop in the leaf weight and height of plant does not happen [22]. Proline is accumulated in the roots of salt-resistant canola cultivars and the shoots of salt-sensitive cultivars [23].

In canola genotypes, it has been reported that the potassium (K^+) ion concentration diminish as salinity increased, while calcium (Ca^{2+}) and sodium (Na^{2+}) ion concentrations increased, decreasing the photosynthetic rate [24]. An incrementing Na^+ and the ratio of Na^+ to K^+ in shoot and root is found under salinity stress [25].

The aggregation of ions of Na^+ and chlorine (Cl^-) raises the osmotic potential and reduces the supply of water and the plant roots' nutrient absorption [26]. Toxic metabolic Na^+ ions compete with K^+ in many major physiological processes in cells [27]. Electrolyte leakage, which rises in salinity responses, is thus attributed to a rise in metabolites and ion concentrations and is correlated with an increase in the input of Cl^- and Na^+ and the omission of K^+ [28]. This leads to a considerable decrease in the shoot and root dry weight, leaf number, and shoot height under stress [29].

When subjected to biotic and abiotic stress, plants generate an abundance of antioxidant enzymes. Islam et al. [30], found that by affecting water and nutrient equilibrium, high salt concentrations in the root region, impair canola and mustard growth and yield. Lower stomatal conduction, nutrient absorption, more ion toxicity, and a misbalance in nutrient accessibility are the main reasons for decline in seed yield in Brassica spp. under salinity stress [3]. ROS-inhibitors, SOD or Superoxide dismutase, Catalase (CAT), Monodehydroascorbate reductase (MDHAR), Glutathione reductase (GR), and decreased glutathione (GSH) accumulation were greater in leaves of five salinity stress ed. canola varieties than in non-stressed plants [31]. While salt increased levels of MDA, hydrogen peroxide (H_2O_2), and phenolics were also seen in canola which was sensitive to salt. High cellular H_2O_2 concentrations were accumulated with lower MDA levels in salt-resistant canola plants [32]. There were increased amounts of chlorophyll, carotenoids, flavonoids, proline, and dissolved protein in the *Brassica napus* L. lab plantlets developed in the presence of SA and NaCl [33]. Oilseed brassicas have broad salinity stress resistance, amid these adverse effects, helping them to reconcile to a wide range of biological and environmental conditions [3].

3. Proteomic approaches

Proteomic technology exploits advances in protein isolation and protein recognition relying on mass spectrometry. This technology supports the study of tolerance mechanisms and plant reactions to abiotic stresses including salinity [34]. In 1996, Marc Wilkins [35] introduced the phrase 'proteome' for the first time, a term which is at present associated with 133,606 publications in the proteomics field as presently listed in the NCBI database, of which 15,642 publications are associated with proteome/proteomics with stress studies and only 543 publications report proteome/proteomics studies associated with plant salinity stress [36]. Proteomics alludes to the large-scale and expansive study of all the proteins (the protein equivalent of the genome) to discover cellular processes [37]. Systematic proteome studies provide information on protein abundances, protein changes, and modifications, as well as interacting protein partners and protein networks [38].

In genotypes that are susceptible to salinity stress, the plant proteome is differentially expressed. Proteomics has a wide range of applications in protein profiling analysis under stress conditions. It has a direct role in the discovery of genes and proteins involved in plant salinity stress response and tolerance processes [39]. The introduction of genes encoding proteins, for the synthesis of the osmolytes, receptors, ion channels, and salt-responsive signaling factors or enzymes into salt-sensitive plants, can confer salinity-tolerant phenotypes [40]. High-throughput proteomics is the first step in characterization of salt-responsive proteins that can be used to produce salt-tolerant plants.

Another application is in comparative study of differential expression of proteomes among control (non-stressed) plants and stressed plants. Less often, the comparison of proteomes isolated from two variant genotypes or plant species with different extreme levels of salinity stress is studied. The proteomes are distinguished

focusing on both protein quality and quantity by differential-expression in proteomic studies, which aim at both protein identification and relative quantitation [41].

Many experiments relevant to the comparative analysis of proteomes among plants exposed to salinity stress and control treatments have been conducted in economic plants such as, rice [42], *Brassica napus* [43], wheat [44], barley [45], tomato [46], soybean [47], and the model plant *Arabidopsis thaliana* and medicinal plants such as, *Andrographis paniculata* [48], *Bruguiera gymnorhiza* [49].

4. The goal of proteomics

Proteomic technologies are being more commonly used in many areas of bioscience, in addition to stress-responsive proteins detection in stress-tolerant plants, such as in the discovery of cell surface markers/biomarkers, and the production of drugs [50]. The target of proteomics is to provide complete information by revealing the regulation, amount, activities and interplay of proteins existing in complex biological systems, whole organism, specific tissues or cellular compartments in certain conditions and at a particular time [51]. Proteomics has become useful in the field of plant genomics in recent years, and may be used to identify proteins extracted from tissues/cells in response to growth and specific environmental conditions and to determine the levels of expression of the proteins found [52].

Researchers can ultimately evaluate and recognize thousands of proteins in each experiment with the application of these procedures. The relative expressions of these different proteins can be accurately determined and evaluated in different situations, and the expression of individual proteins may be appraised in intricate mixes [53]. Under stress conditions, functional identification of every protein and its metabolic processes, the protein profiles or mapping of cells, tissues, organ or organisms is valuable in the recognition of genes and gene product that are resistant to various stresses [54].

5. Reactive oxygen species (ROS) role in salinity

Salinity impacts plants by causing multiple problems including, ion toxicity, osmotic stress, nutritional deficit and genotoxicity resulting in the accumulation of ROS via oxidative stress. Salinity can result in stomatal closure, that decreases the supply of CO_2 in the leaves and prevents carbon fixation, exposing chloroplasts to extreme energy, that increases ROS development including H_2O_2, superoxide (O_2^-), singlet oxygen and hydroxyl radicals (OH-) [55, 56]. Since salinity is complicated and inflicts a water deficiency due to osmotic effects on an extensive range of metabolic processes [56]. This water shortage results in the formation of ROS [57]. ROS is extremely reactive and, by oxidation of lipids, proteins, and nucleic acid can cause cell harm [58]. In several reports, ROS manufacture has been shown to increase in salinity situations. ROS mediated membrane harm has been reported as an important factor in the toxicity to cells of salinity stress in many crops, including corn, tomatoes, citrus, peas, and pepper [55, 59, 60–62]. The increased function of GSH-Px and GR reduces the amount of H_2O_2 and MDA and ameliorates the impact on the plant (*Brassica napus* L.) by preventing oxidative harm stimulated by ROS under soil salinity stress [63].

Long term treatments with salinity, with EC 5.4 and 10.6 dS m – 1, for 60 days, have been shown to induce a substantial rise in H_2O_2 and lipid peroxidation in seedlings of wheat, which has more salt-sensitive cultivars than salt-resistant cultivars [64]. Lipid peroxidation improves the permeability of H_2O_2 and increases

in these two compounds have been observed in *Brassica napus* [65] and *Triticum aestivum* [66] with increasing salinity. It has been shown that one of the key reasons for declines in crop productivity is the generation of ROS during environmental stresses such as salinity [67]. ROS control is thus a critical mechanism for preventing undesirable cellular cytotoxicity and oxidative harm [68].

Rehman et al. [69] recorded a 2.5- and a 3-fold increase in H_2O_2 generation, along with a 2- and 3-fold increase in the content of thiobarbituric acid reactive substances (TBARS) under 100 and 200 mM NaCl stress respectively, in contrast to controls demonstrating salt-induced oxidative stress. Oxidative stress differs between plant tissues. For example, root tissues have reported as suffered most from oxidative stress caused by salinity, followed by mature and young leaves.

6. Antioxidant defense system for salt tolerance

Antioxidants extirpate ROS directly or indirect and/or regulate ROS production [70]. The antioxidant defensive mechanism comprises non-enzymatic antioxidants of low molecular weight and certain enzymes acting on antioxidants [71]. In order to prevent hyper production of ROS, non-enzymatic antioxidants including ascorbate (AsA), GSH, tocopherol, phenolic combinations (PhOH), flavonoids, alkaloids, and nonprotein amino acids work in a coordinated manner with enzymes including superoxide dismutase (SOD), CAT, peroxidase (POX), (PPO) polyphenol oxidase, ascorbate peroxidase (APX), MDHAR, dehydroascorbate reductase (DHAR), GR, glutathione peroxidase (GPX), glutathione S-transferase (GST), TRX, and PRX [70].

The catalytic action of enzymes and non-enzymatic antioxidants and the locations of activity in the cellular organs is well known. The SOD is directly relevant to plants under salinity stress and begins the first phase of defense, transforming oxygen into hydrogen peroxide [72, 73]. The H_2O_2 generated may be further transformed into H_2O with the activity of enzymes; APX, CAT, GPX, or in the AsA-GSH cycle. Aggregation of SOD has been reported as a defensive approach in canola in response to salinity [43]. The cycle of AsA-GSH or the Asada-Halliwell cycle in plant cells is the main antioxidant protective pathway to detoxification of hydrogen peroxide, consisting of non-enzymatic antioxidants GSH and AsA and four major enzymes; DHAR, MDHAR, GR, and APX [74]. In the defense system of antioxidants, the main activity is executed by the AsA-GSH cycle to decrease H_2O_2 and redox homeostasis [74]. In the leaves of five canola cultivars under salinity tension, ROS-scavenging enzymes (SOD; CAT; GR; MDHAR), and in addition reduced glutathione concentration were more in unstressed leaves [75]. An increase in APX function in response to salinity stress is reported in *Brassica napus* [63].

A crucial function in the antioxidant defense mechanism is the reduction of H_2O_2 and redox homeostasis via the AsA-GSH cycle [76]. Furthermore, for detoxifying H_2O_2 and xenobiotics, GPX and GST are essential enzymes [77]. GSH and AsA are plentiful soluble antioxidants among the non-enzymatic antioxidants in higher plants, which act in a critical role as electron doners and directly remove ROS via the GSH-AsA cycle [76]. Furthermore, beta carotene reacts with ROO radicals, OH, and O_2 leading to decreased concentrations of cellular ROS [78].

It has been observed that Selenium (Se) increases the activity of these antioxidant enzymes to deal with established stresses [79]. Selenium plays an important role in various enzymatic and non-enzymatic processes for example phytochelatins and antioxidants of GSH, which helps defeat the salt-induced mass production of ROS. It has been proven that low amounts of selenite (Na_2SeO_4) protect plants from ROS-stimulated oxidative detriment, but a higher Se amount, acts as a pro-oxidant

and promotes ROS production and oxidative stress [80]. Several researchers have identified the need for Se to improve ROS scavenging activity, decreasing MDA amounts, and membrane harm [81]. Reduced production of H_2O_2 has also been reported under increased Se [82]. Reduced H_2O_2 amounts were reported - under salinity stress in Se-treated canola (*Brassica napus* L.) plants [83]. Plants subjected to Se display less MDA under salinity conditions, demonstrating that Se is important in bringing down the lipid peroxidation by modifying the antioxidant enzymes and preserving the membrane structures of, *Brassica napus* L. [63]. Moreover, it has observed that the generation of lipid peroxidation (MDA) is decreased by increasing the amount of Se under salt conditions [84]. Reducing the fluidity of the membrane to increase membrane leakage and prevent harm to membrane proteins, ion channels, and enzymes are general effect of MDA on plant cell [85].

7. Heat shock proteins due to salinity stress

Crop breeding aims to enhance tolerances to salinity and high temperature. Organisms which survive in difficult conditions should have unique mechanisms to respond to stressful environments. One of these process would involve the induction of molecular chaperones, heat shock proteins (HSP), comprising some guarded protein families including HSP90, HSP70 (DnaK), HSP100 (ClpB), HSP60 (GroEL), and small heat shock proteins [86]. Studies of HSPs [87] have indicated that that sti1 (protein) was up-regulated in tolerance to salinity tension; this protein includes two heat shock chaperon binding motifs STI1), three tetratrico peptide repeats (TPR), and two Sti1 domains [88].

HSP90 is thought to interact with TPR-containing proteins via protein–protein interplay to modulate various cellular processes. HSP 70 has been verified in macro algae and some water plant species as a stress biomarker generated by NaCl, emphasizing its function in supporting species against stresses [89]. In order to image the entire modifications in the cells protein synthesis in tolerance to osmotic stress, dual channel imaging and warping of 2-DE protein gels have also been used. Analysis reveals that in many busy cellular surroundings, various chaperones adopt different paths to prevent protein aggregation. In canola, families of HSP have been identified in the leaf [43]. The differential expression of Hsp 70 has been reported in the root [43, 90].

It has been shown that transgenic plants expressing Hsp70 modulate programmed cell death (PCD) under hyper salinity, where Hsp70 functions as an anti-apoptotic protein [91]. In addition, ClpB/Hsp100 B2, B3 and ClpD2 are expected to act as molecular chaperones, and their expressions are significantly boosted under salt conditions [92]. Increased expression of ClpD1 and sHSP has also been shown to contribute to improved adaptation to salinity stress [93].

8. Salinity stress proteins as molecular markers

Plants under salinity stress change their gene expressions significantly to adapt to unfavorable conditions, including variations in the composition of the plant transcriptome, proteome, and metabolome. A few experiments have reported showing that protein aggregation varies considerably under stress condition [40, 92].

It is suggested that in canola root, Ras-related small GTP-binding proteins interfere with signaling of salinity stress. Proteomic investigations of canola cultivars under salinity stress have identified this protein [94]. The activation of Ras-related small GTP-binding proteins is responsible for coupling ligand-bound G proteins

with GPCRs, which in turn sets the signaling pathway for Ca^{2+} mediated inositol triphosphate (IP3) in tolerance to salinity stress in canola leaves [95]. In transgenic *B. napus* plants, the probable mechanisms of activity of *ThIPK2* is an aggregation of sodium ions in the root, with differential expression of proline amount and stress-response genes [96].

The identity of a small GTP-binding protein Ras-relevant that is up-regulated in saline conditions in canola, indicates a high possibility of G-protein-couple recipients (GPCRs) being involved in intervention in detecting salinity signals [94]. This has clearly indicated that GPCRs, in combination with G-proteins, activate the small GTP-binding protein [97]. This step is accompanied by IP3 signaling pathway activation, generation of Ca^{2+}, activation of the Ca^{2+} process, and ultimately alters gene expression [98]. In connection with the function of the IP3 mechanism in the response of canola to salt conditions, it has been reported that certain components of the IP3 pathway are induced by high salinity. A *Brassica napus* transcriptomic study showed that phosphatidylinositol-specific phospholipase C2 (BnPLC2), phosphatidylinositol 3-kinase (BnVPS34), and phosphatidylinositol synthase (BnPtdIns S1) have substantially differential expression under salinity stress [99]. Annexin recognition in canola root supports this process in the detection and signaling of salinity stress in the case of the Ca^{2+} mechanism [94]. In responding to abiotic stress, the annexin mediator activities have been defined as goals of the Ca^{2+} signaling pathway [100]. The recognition of calcium-dependent protein kinase (CPK) differential expression under abiotic stress, like salinity stress, provides further verification of the active function of the above pathways in canola [101]. CPKs detect Ca2C and function as a kinase. Altogether, the sense of salinity stress is by GPCRs and Ras-related small GTP-binding proteins, transmitting the message via the IP3 signaling pathway.

9. Regulation of gene expression

In response to salinity in canola cultivar, three layers of adjustment of gene expression have been reported. The first phase of expression of gene adjustment is at the level of transcription, which is regulated by agents of transcription. In the regulatory regions of the genome, the transcription factor is an important factor that is interrelating with some other proteins, particularly RNA polymerases and also trans or cis components. Lee et al. [102], reported that fifty-six genes that encode transcription factors in canola are changed under abiotic stresses. In resistance to salinity the message is conveyed by messaging and sensing molecules, and these transcription factors are triggered. The expression of various genes is then regulated by diverse gene regulation networks composed of transcription factors and other proteins.

Another process related to gene adjustment, which has been observed in canola, is epigenetic activities. Epigenetic adjustment of stress-tolerate genes under varying situations has been found to perform a crucial task in the plant [103]. When salinity stress is added to a pre-treated plant with osmotic stress, histone changes aggregate Na^{+} ions in a concentration that is not toxic for the plants [104]. The methylation of DNA and changes of histones in reaction to salinity have been reported in canola. If the plant is subjected to salinity stress, de novo methylation and demethylation processes happen at CpCpGpG sites [105]. The main components of epigenetic adjustment are DNA methylation, histone changes, and chromatin reconstitution [106]. Most genes which have epigenetic alteration have minor identified in the plant (canola). A significant gene undergoing methylation of DNA under salinity stress in the plant is the ethylene-responsive element-binding factor (EBF) [107].

Studies on canola are very limited in this respect. It has been shown in Arabidopsis and tobacco that histone proteins are quickly up-regulated under salinity stress and are phosphorylated, due to reduced Na^+ aggregation [104]. The potential functions of DNA methylation/demethylation and chromatin (histone) modifications in adjusting the expression of salt-responsive genes are indicated by these findings.

Lu et al. [108] found that both hypermethylation and hypomethylation in the rapeseed genome were prompted by saline stress, and hypermethylation was observed more often than hypomethylation. There is a significant role of DNA methylation in plants reaction to abiotic stress [103]. Research has shown that salinity can influence the level of DNA methylation, and the shift in the status of cytosine methylation is associated with the variations in expression in rapeseed of two stress-related genes [109].

miRNA activities have been investigated in salinity treated canola at the post-transcriptional level. miRNAs are small, single-stranded, RNA molecules of 20–24 nucleotides which regulate the aggregation and expression of mRNAs. Multiple processes like organ growth [110], phase transfer [111], stress reactions [112], and many other regulative mechanisms for plants are indirectly modulated [113]. It has been reported that more than 340 miRNAs participate in the post transcription process of adjustment of the salt-responsive genes in canola [114]. The NAC transcription factor is one of the transcription factors found to be targeted by miRNAs [115]. NACs TFs are particular TFs with a strongly conserved N-terminal NAC domain and a variable CT activation domain. The function of NAC TFs in the tolerance of abiotic stress is well known [116]. Sixty NAC TFs have been reported in *B. napus* [116]. Zhong et al. [117] detected two *B. napus* NAC TFs (BnNAC2 and BnNAC5) and found that these factors act in negative adjustment of salinity and osmotic stress tolerance.

10. Dynamic variations of the genes and proteins of canola

Many transcriptomic and proteomic research conducted under salinity stress on canola suggest that differentially expressed proteins and genes can be predominantly grouped into seven functional groups in both leaves and roots [118]. The categories, with the proteins or genes characterized in each functional group, are (a) energy metabolism and carbohydrates, (b) defenses and stresses, (c) photosynthesis (in the leaves) and metabolism, (d) structure of cells, (e) transport and membrane, and (f) division of cell, fate, and differentiation [119].

The amount of protein relevant to carbohydrates and energy metabolism is higher in canola roots under stress compared to other functional protein groups. Proteins linked to the metabolism of amino acids and the composition of cells are significant in abundance. The bulk of the proteins are from the TCA cycle, the electron transport chain, and glycolysis of carbohydrates and energy metabolism [120]. In canola leaves, the highest abundance functional proteins are those that belong to photosynthesis, the degradation and synthesis of proteins, metabolism of amino acid, and damage repair and defense response [121, 122].

In the photosynthesis-related salinity-tolerant canola cultivars, differential redundancy of chlorophyll a/b binding protein, chloroplast RuBisCO activase, ribulose bisphosphate oxygenase/carboxylase (RuBisCO) both subunits, have been found [43, 90, 118]. It appears that under salinity stress, canola changes the cytoskeleton essential ingredients (actions and tubulins). The dynamic remolding of the cytoskeleton, like the K^+ channel, is related to certain of the major transmembrane transports [40, 123]. Another interesting point that is related to the functional

group of differentially altered proteins is the unknown ones that form about 1% to 20% of total diversely altered proteins in every research outcome, particularly in researches on the root [43, 122]. The discovery of the function of these proteins could offer further insight into the path ways of salt response [124].

CDPKs, which are sensor responders with the capability to self-modifying verification by the enzymatic function is the third portion of the Ca^{2+} sensing machinery in the plant [125]. This causes CPKs to be special in their calcium-sensing dual function and then respond against the stress situation signals by downstream phosphorylation activities. In the stress response of CPKs, tremendous overlapping and cross-talk have reported [126]. There are several CPKs necessary for the reaction to a particular stress stimulus against stresses like drought, heat, salt, and cold. In *B. napus*, 25 CPKs have recognized and many have studied their expression levels under different abiotic stresses [126]. A study [127] of BnCPK2 interacting partners has been reported using a mating based split ubiquitin system (mbSUS) and BiFC. To control ROS and cell death, they suggested the role of BnCPK2 and probable interactions with NADPH oxidase-like respiratory burst oxidase homolog D (RbohD). Similar results have been obtained in which most CPKs are shown to dampen ABA signals and ROS homeostasis in the plant cell [128].

11. Suspected genes/proteins responsible for canola resistance

Multiple experiments has been conducted to recognize the major gene(s)/protein(s) responsible for salt tolerance. Understanding the main components of the salt response pathways complexities is an important step towards the production salt-tolerant canola. A study [121] identified 6 genes (hub) in resistant cultivars, including malate dehydrogenase, heat shock protein 70, triose phosphate isomerase, fructose-bisphosphate aldolase, UDP-glucose dehydrogenase and methionine synthase in the produced protein–protein interaction pathway of canola salt-induced proteins. Hub genes are highly interactive components of the response network that are known to be the network's core components [129]. Some of the suspected genes or proteins for canola resistance can be derived from research on the usage of materials that boost salinity tolerance of canola. Garg and Manchanda [85] observed, in response to plant growth promoting rhizobacteria inoculation, canola roots control glyceraldehyde-3-phosphate dehydrogenase and downregulates S-adenosylmethionine synthase, aldehyde dehydrogenase, and malate dehydrogenase under 150 and 300 mM of NaCl.

This research showed, inoculated plants exhibit substantially increased root dry weight, root length, more potassium, and less sodium and chlorine amounts compared to non-inoculated plants. The research found that the greater development of the inoculated roots was the reason for the differentially abundant bacteria-responsive proteins. As suggested in other research, inoculation with bacteria grants canola greater resistance via an enhanced redundancy of proteins which are related to glycolysis, and amino acid metabolism, TCA, and succinate dehydrogenase [90].

A few reports have revealed that overexpression of certain genes contributes to changed salinity tolerance in canola. The Dehydration-Responsive Element Binding transcription Factors (DREBs) overexpression is a major example of this. Plants transformed for great expression of DREBs show a discrete increase in their salinity tolerant expression of gene like HSF3, COR14, RD20, HSP70, and PEROX, which indicate greater resistance. The plants, which are transgene, can live in the saline condition, where wild species plants are more susceptible [130].

Considering the exogenous use of 5-Aminolevulinic acid (5-ALA) resulted in salt tolerance, in treated plants, Sun et al. [131] transformed canola with the

5-ALA-encoding gene, YHem1, and studied the growth of the transgenic canola, ability to synthesize further 5-ALA, and wild-type canola under salt conditions. They observed that under both short-term and long-term salt conditions, transgenic canola demonstration more product, more chlorophyll amount, a greater amount of antioxidant enzyme, high proline content, high sugar content, and more free amino acids contrasting to wild-type canola. They also have shown improved resistance of transgenic canola may be linked to the up-regulation of the Rubisco small subunit and a substantial amount of Fe metal. In comparison to these experiments, in which improved resistance has been documented, it has proposed that expression of *Brassica napus* TTG2 induces sensitivity to salinity stress through the down-regulation of the Tryptophan Biosynthesis 5 (TRP5) and YUCCA2 (YUC2) indole-3-acetic acid (IAA) encoding genes, thus decreasing the endogenous IAA amounts. In future research in transgenic plants, the recently evolving CRISPR/Cas9 system is expected to provide more knowledge on molecular components that respond to salinity stress [132].

12. Conclusions and prospects of proteomics

Canola as a major field crop across the world is influenced by salinity stress. Despite advances in understanding molecular interactions between plant and salt, production of salinity-resistant cultivars remains challenging. Proteomics findings help greatly to identify the physiological processes based on plant tolerance to stress, and could further be used to identify the level of stress tolerance in genotypes. To date, we have substantial information gaps concerning the regulation of abiotic stress plant response, as this adjustment is at different levels of transcription, post transcription, post-translation, and epigenetic levels [133].

A variety of stress acclimation techniques have been explained in the above studies through a combination of proteomics and physiological approaches. Nevertheless, many of the findings demonstrated the previously characterized salt-induced proteins rather than offering new mechanistic insights into salinity tolerance. More knowledge on alterations in cell metabolism and also stress-responsive proteins, which are participating in the proteome of plant, are provided whereas there is a lack of knowledge about regulating proteins in stress, expression of gene regulation and signaling proteins (mainly transcription factors), membrane proteins and transferors, owing to their limited amount in the cell or difficulty in characterization.

In the near future, the advent of new improved proteomic techniques and the study of unique developmental stages/cells/tissues or subcellular organelles, in particular using LCM (Laser Capture Mediated Micro Dissection)-mediated single-cell isolation, will allow the study of cell-specific expression, protein enrichment and low-abundant protein detection to be successfully achieved [134].

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