5 Proteomic Insights of Date Palm Embryogenesis and Responses to Environmental Stress

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Abstract

Date palm (*Phoenix dactylifera* L.), a dioecious species, represents a major agricultural component of arid and semiarid regions. It plays a fundamental role in the socioeconomic balance. Dates, indeed, represent a major income and food source for local populations in the Middle East and North Africa. A better understanding of the plant's response to biotic and abiotic stress may help in improving date production, especially of elite cultivars. For this reason, molecular tools including genomics, transcriptomics and proteomics were developed to realize this response. Recently, proteomics has become widely used in biological research to explain several unknown biological functions. In the date palm production field, it is being used to elucidate the mechanism of plant resistance to environmental stress. The present chapter describes proteomic approaches used to compare the proteome of zygotic and somatic date palm embryos. Additionally, an explanation is given about the effect of the addition of different supplements to medium culture on the protein content and profile of somatic embryos. Furthermore, the response of date palm to abiotic stress including brittle leaf disease, salinity and drought at proteomic levels is described and discussed.

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5.1 Introduction

Date palm is an important plant that grown in several subtropical and tropical regions of the world, especially the Middle East. It has traditional and socioeconomic importance (El Hadrami and Al-Khayri 2012). Besides being a major income source for the producing country, date consumption is known to be beneficial for medicinal purposes because of its richness in tannins that relieve intestinal disorders. Roots are also used for toothache treatment, and pollen is rich in estrogenic compound such as estrone.

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Furthermore, leaves and roots are rich in nanocellulose and natural fiber (Alotaibi et al. 2019).

Date palm reproduction can be performed using either one of these three methods: (a) offshoot separation and transplanting, (b) seed propagation and (c) micropropagation. Micropropagation represents the fastest and the most reliable method to generate large numbers of plants (Hadrami et al. 2011), especially through somatic embryogenesis (Fki et al. 2011, 2017).

In order to estimate the quality of the regenerated seedlings from somatic embryogenesis and to evaluate the property of this biological system, powerful tools like genomic, transcriptomic and proteomics need to be used. Recently, protein signatures experimentally determined by proteomics are used to identify the phenotype, characteristics and properties of biological systems and processes. The coupling of gel electrophoresis with mass spectrometry represents a powerful method to study the somatic embryogenesis, seed maturation and germination, and plant response to biotic or abiotic stress (Jorrín-Novo 2020; Rey et al. 2019; Sghaier-Hammami et al. 2020). Advances in proteomics and other omics have and continue to contribute to crop and plant breeding programs through the identification of new gene products linked to desired agronomic traits and productivity (Jorrín-Novo 2020).

The present chapter describes studies of the proteome of zygotic and somatic embryos at different developmental stages. Proteins were extracted using the TCA phenol methods (Maldonado et al. 2008) and then separated by one and two electrophoresis gels. Differential proteins were subjected to matrix-assisted laser *desorption* ionization/time of flight (MALDI-TOF-TOF). The identification of proteins involved in the maturation and germination showed that somatic embryos lack those that are involved in the dormancy process and the storage proteins. The effect of the addition of different supplements (ABA, sucrose, arginine) to medium culture of somatic embryos showed an improvement of the protein content and especially the induction of storage proteins in treated somatic embryos. Proteomic approaches are also being used to study the response of the date palm seedlings to drought and salinity stress.

5.1.1 Proteomics Importance for Biological Research

The term *proteomics* was first given during the 1994 Siena meeting by Marc Wilkins, derived from *PROTein complement of a genOME* (Wilkins et al. 1996). The proteome should be understood as the total set of proteins or gene products present in a biological unit at a specific developmental stage and under determined external biotic and abiotic conditions. Proteomics allow the study of the proteome present in a biological assembly along with its description, quantification, genotype dependent variations, its implication in developmental and environmental related changes, post-translational modifications (PTMs), as well as its interaction with other proteins and molecular assemblies (Jorrín-Novo 2020).

Proteomics represents an important approach and a fundamental discipline in the postgenomic era, and it could answer the questions concerning production and function of proteins, such as regulation, mechanism of action, location and interaction with other proteins or molecules. This is of great relevance, taking into account that proteins are the molecules that exert the most relevant biological functions. Like genomic and transcriptomics approaches, proteomics incorporates highly developed techniques and protocols that made the analysis of a large number of proteins easier and faster (Wolters et al. 2001). The first generation of proteomics research used the two-dimensional (DE) protein separation coupled with mass spectrometry (MS) analysis of spots, and then, the second generation was based on the liquid chromatography (LC)-based shotgun strategies, and finally, the third generation was based on quantitative approaches including label and label-free variants (Jorrín-Novo et al. 2019). Therefore, it is advisable for novice proteomics users to start with the simplest

methodology using one-dimension (1D) gel electrophoresis (shown to be very important in the analysis of simple proteomes) and then move to the most sophisticated methodology such as 2-DE and gel-free. Traditional 2-DE coupled to MS is still the most frequently used platform (Heinemeyer et al. 2009).

All or most of the following steps may be included in a standard proteomic experiment, including the experimental design, sampling, preparation of tissue, cell or organelle, extraction and fractionation/or purification of proteins, labeling or modification, separation, MS analysis, identification of proteins and, finally, statistical analysis of data and validation (Jorrín-Novo 2014). Each biological system (i.e., plant species, organ, tissue, cells) has its characteristics, and each research subject has its objectives. Therefore, the most appropriate protocol should be used accordingly. In fact, a good experimental design is essential for the success of any proteomic experiment. For any differential expression proteomics design, a sufficient number of replicates are a prerequisite. It should be set up according to the dynamic nature of the proteome. Hence, a correct interpretation of the results could be reached and therefore a confident assignment of any protein as variable. This is very useful in case of the identification of the proteins as disease markers or as markers to develop plant breeding programs (Valledor et al. 2014).

5.1.2 Proteomics for Embryogenesis Studies

Somatic embryogenesis represents a successful technique for monocotyledon and dicotyledon multiplication. It is a good alternative for clonal propagation and breeding of elite plants that have limited multiplication using zygotic embryogenesis (Fki et al. 2003, 2017; Othmani et al. 2009). Several reports have described embryogenesis at the molecular level using proteomic and transcriptomic approaches (Aguilar-Hernández and Loyola-Vargas 2018; Gallardo et al. 2007; Imin et al. 2004; Roja Rani et al. 2005; Stasolla et al. 2004). A deeper knowledge of proteins involved in zygotic and somatic embryogenesis could be very useful for the improvement of the latter since the resulted seedlings are less vigorous than those from zygotic embryogenesis (Sghaier et al. 2008). Proteomics studies may also help in improving the quality of somatic embryo derived seedlings and in developing new strategies for plant multiplication using in vitro culture. In conclusion, proteins can be considered as biomarkers for embryo maturation, seed acquisition of desiccation tolerance and seedling vigor.

5.1.3 Proteomics for Plant Stress Research

The concept of *stress* is used by many biologists, but it is very elusive since it can be put in different contexts and in many ways in the scientific literature. It can further be attributed to the stressor (the environmental component) as well as to the stressed (the biological component). Environmental stresses can represent a physical response to a changing environment. The most deleterious environmental stresses, for example, are those that result from naturally occurring or man-made changes in abiotic factors including temperature, other climatic factors and chemical components. Biotic agents, like bacterial, fungal, algal and viral diseases, may also cause biotic stress in plants. Hence, plants have developed several adaptive mechanisms to confront these stresses. It is now becoming widely known that proteins are the main mediators of this resistance by playing a major role in the activation of genes and thus the control of the genome leading to physical features (such as in xerophytes) or by directly defending the plants from the stressors (i.e., antioxidant enzymes and chapronins) or indirectly (like key enzymes in osmolyte synthesis) (Bona et al. 2007; Pandey et al. 2008; Sghaier-Hammami et al. 2013; Xiong et al. 2017).

5.2 Importance of Date Palm and Methods of Multiplication

Date palm (*Phoenix dactylifera* L.), mostly cultivated in both Old World (Middle East and North Africa) and New World (American continent), constitutes the major crop in arid and semiarid areas, mainly in the regions of Southwest Asia and North Africa. It is a very important species that belongs to the palm family (Arecaceae) and has about 200 genera and more than 2500 species (El Hadrami and Al-Khayri 2012).

In addition, to the impractical multiplication of date palm via seed propagation (sexual propagation), offshoot propagation also (asexual or vegetative propagation) produces a limited number of plantlets (20–30 at most) depending on the variety, fertilization treatment, irrigation and earthing-up around the trunks (Zaid and De Wet 2002). Therefore, in vitro culture represents an effective potential alternative for date palm multiplication (Fki et al. 2003, 2017); especially somatic embryogenesis which is well-established in date palm (Al-Khayri 2003). However, somatic embryos seedlings are less vigorous than those raised from natural seed. The poor vigor of somatic embryo (SE) derived seedlings seems to be related to their incomplete maturation process (Roberts et al. 1990). Moreover, date palm SE differs from the zygotic embryos (ZE), as it lacks a seed integument and endosperm (Fig. 5.1), which are critical for seed survival and germination (Brownfield et al. 2007).

A more thorough investigation of the proteins that are involved in zygotic embryogenesis would be very useful for the improvement of micropropagation techniques of elite genotypes that represent a challenge for date palm and other woody plant species (Cairney and Pullman 2007; Chin and Tan 2018).

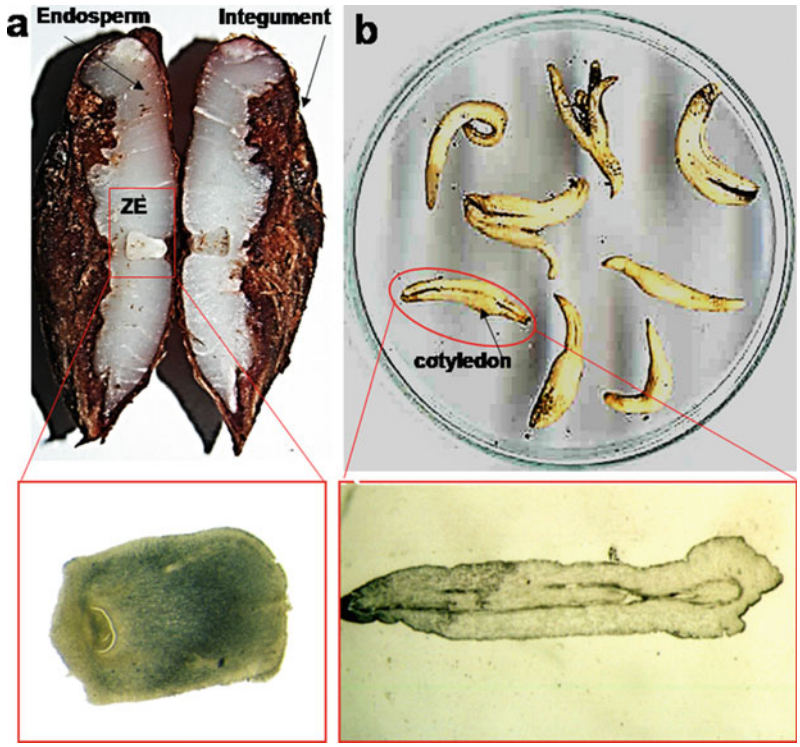

Fig. 5.1 Date palm embryos. **a** Zygotic embryo (ZE) in the middle of the seed and below it histological section, **b** Somatic embryo (SE) resulted from somatic embryogenesis and its histological section (Photos by B. Sghaier-Hammami)

5.3 Proteomics Study of Date Palm Zygotic Embryo During Development, Maturation and Germination

In order to define specific markers that characterize the different phases of zygotic embryogenesis, the accumulation of total proteins during ZE development (12–17) weeks after pollination (WAP) until maturation stage (23 WAP) and during germination (9–15 days of germination (DG) were monitored (Sghaier-Hammami et al. 2009a). Proteins (500 mg) were separated by 2-DE with IEF carried out at the 5–8 pH range (Fig. 5.2).

Following a traditional univariate analysis made with 2-DE data, 194 spots, differentially expressed in the different developmental stages, were determined. They were either qualitative or quantitative. Sixty-five variable spots, including those that showed qualitative and quantitative changes underwent through MALDI-TOF analysis, using the nonredundant National Center for Biotechnology Information (NCBI).

Seed development and germination showed that storage proteins (glutelin, prolamins) were accumulated during embryogenesis and then consumed during germination. Protein content declined during germination, especially from 9 DG–12DG (Fig. 5.2), which corresponds with the emergence of the cotyledonary leaf (Sghaier-Hammami et al. 2009b). These findings agree with those reported by Lai and McKersie (1994) and Sghaier-Hammami (2020), which revealed that starch reserves and storage proteins were quickly hydrolyzed following the germination of embryos.

Starch reserves were accumulated at the early embryogenic stages which indicate an active starch synthesis during embryonic development. After germination, changes in energy metabolism

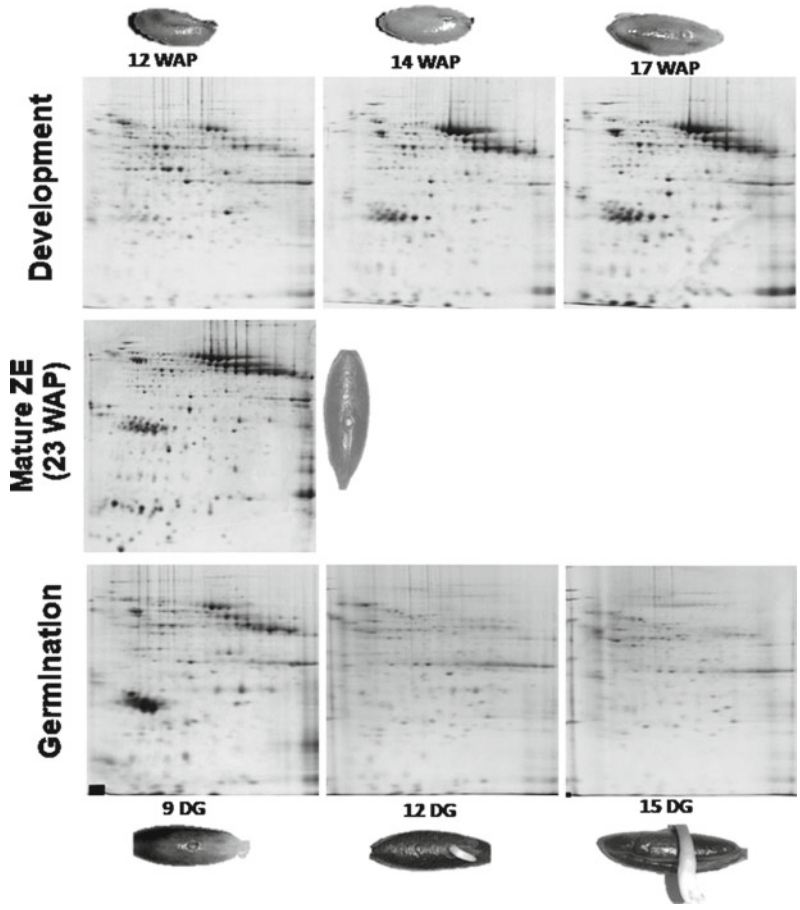

Fig. 5.2 Real gel 2-DE map between zygotic embryo at different stages development, once mature and during germination; 500 μg of protein was separated in the first dimension on an immobilized, linear 5–8 pH, gradient then separated in the second dimension on a 12% acrylamide-SDS gel. Gels were stained with Coomassie (Photos by B. Sghaier-Hammami)

(from fermentation to respiration) were observed during zygotic embryogenesis. The evolution of glycolytic and tricarboxylic acid cycle enzymes, during seed development, indicates active energy metabolism during embryogenesis.

Stress-related proteins (HSP family) are highly expressed during the early developmental stages and then decreased at germination stages. These members of the HSP family are highly expressed during zygotic embryogenesis (DeRocher and Vierling 1994).

To conclude, dramatic changes in the ZE proteome during embryogenesis and germination levels were observed. ZE continuously accumulates proteins from early to late embryogenesis stages, and these values are maximal at mature or close-to-mature embryos. A comparative study between protein content of date palm ZE and SE will be of great interest to explain the weakness of SE seedlings.

5.4 Comparison Between Protein Content of Zygotic and Somatic Mature Embryos

Somatic embryogenesis could be a good alternative for clonal multiplication, crop improvement and breeding of recalcitrant plant species (Merkle et al. 1995). However, SE seedlings are less vigorous than those raised from true seed. The quality of SE seedlings could be improved using proteomics, and a new in vitro culture for plants propagation and manipulation could be developed. A comparative proteomic study between ZE and SE protein contents was carried out.

Using SDS-PAGE and 2-DE (Sghaier et al. 2008, 2009), different proteins from ZE and SE were analyzed and identified in both embryo types (Fig. 5.3).

SDS-PAGE analysis showed a poor protein profile for SE, compared to that of ZE. Data analyzed using 2-DE electrophoresis revealed that ~60% of the spots were differently expressed in SE and ZE (335 spots out of the total 559). The variable spots were either absent in ZE or SE (qualitative variable spots; in number of 263) or differentially accumulated between the two types of embryos (quantitative variable spots; 72 spots). The differential proteins (63 variable spots) were subjected to MALDI-TOF-TOF mass spectrometry analysis combined with the nonredundant NCBI.

Most of the identified somatic embryo specific proteins belong to glycolysis pathways and amino acids metabolism, whereas those of the zygotic embryos belong to a mixture of proteins

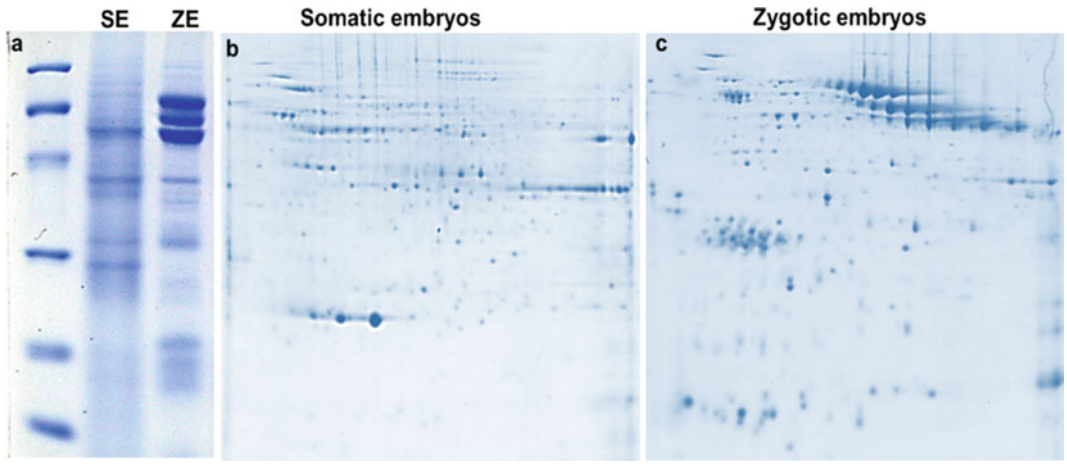

Fig. 5.3 Analysis of total proteins from mature somatic embryos (SE) and zygotic embryos (ZE) using SDS-PAGE (**a**), and 2DE (**b**, **c** for SE and ZE, respectively), 500 μg of protein was loaded on strips (5–8 pH gradient) and then separated on a 12% acrylamide-SDS gel. Gels were Coomassie-stained (Photos by B. Sghaier-Hammami)

families (storage and stress-related proteins, carbohydrate biosynthesis) (Sghaier et al. 2009). Hence, the involvement of more proteins in energy metabolism and ATP demand is being required by the SE compared to the ZE, which may facilitate rapid germination in the former without undergoing the dormancy phase.

Glutelin proteins are abundant in ZE and absent in SE, which indicates that the SE lacks reserve proteins compared to the ZE. Stress-related proteins (HSP family) are more abundant in ZE than in SE. Their accumulation was most likely induced as a consequence of seed dehydration and mediated desiccation tolerance acquisition (Karuna Sree et al. 2000). According to the previously mentioned results, HSP can be used as a maturation marker of ZE. In contrast, in SE, the degree of maturation is different which may cause desiccation sensitivity. This may allow the SE to enter rapidly in germination without undergoing the dormancy phase. For that, plantlets that are derived from SE are less vigorous than those coming from ZE (Roberts et al. 1990). Overall, a low rate of protein accumulation observed in somatic embryos was due to a lack of precursors in the medium (Komamine et al. 1992; Misra et al. 1993). It may also be the result of the absence of inducing signals (e.g., hormones and/or desiccation), which are required to stimulate the synthesis of specific molecules.

5.5 Effect of Abscisic Acid, Sucrose and Arginine on Somatic Embryo Protein Content

Maturation of somatic embryos may be induced by the application of exogenous abscisic acid (ABA), which promotes embryonic maturation and supports the accumulation of storage proteins (Corredoira et al. 2003). In addition, previous studies showed that the addition of a high concentration of sucrose to the culture medium of cucumber (Lou et al. 1996) and melon (Nakagawa et al. 2001) can enhance the induction of somatic embryos. Sghaier et al. (2009) and Sghaier-Hammami et al. (2010) described the effects of additives (ABA, arginine, sucrose) on the induction and accumulation of proteins of date palm somatic embryos reported in the current chapter. Indeed, ABA, arginine and sucrose were used separately as previously described by Morcillo (1998). There was no difference in length and width between all the treated embryos whatever the treatment used except for the 20 µM ABA treatment, which caused a significant width increase by around 1.5 times more than the untreated ones (Fig. 5.4).

Proteomics analysis showed that ABA arginine and sucrose have an important effect on date palm protein content. The amount of total proteins increased significantly after almost all treatments, and especially in those treated with 20 µM of abscisic acid (ABA), where a double amount of proteins was observed. Although, the different treatments significantly enhanced protein synthesis, still the reached levels were much lower than those found in the date palm ZE (120 mg g^{-1} FW) as previously described (Sghaier et al. 2009).

The 1-D and 2-DE protein profiles showed qualitative and quantitative differences between the untreated and treated SE (Fig. 5.5).

All 34 variable spots were subjected to MALDI-TOF-TOF mass spectrometry analysis, including those that showed qualitative and quantitative changes with the highest maximum/minimum value ratios. Identified proteins belong to the following functional categories: energy metabolism, protein translation, folding and degradation, redox maintenance, cytoskeleton and storage proteins. The major proteins implicated in energy metabolism (glycolysis, citrate cycle), protein translation, folding and degradation, redox maintenance and cytoskeleton were downregulated in SE treated by ABA or sucrose. Most proteins that belong to the translation and degradation categories were suppressed under the influence of sucrose. In contrast, storage proteins were more abundant in SE treated by ABA than the untreated ones. Arginine treatment (10 mM) led to the appearance of new proteins homologous to peroxyredoxine and 7S globulin. In fact, addition of nitrogen sources into the culture medium

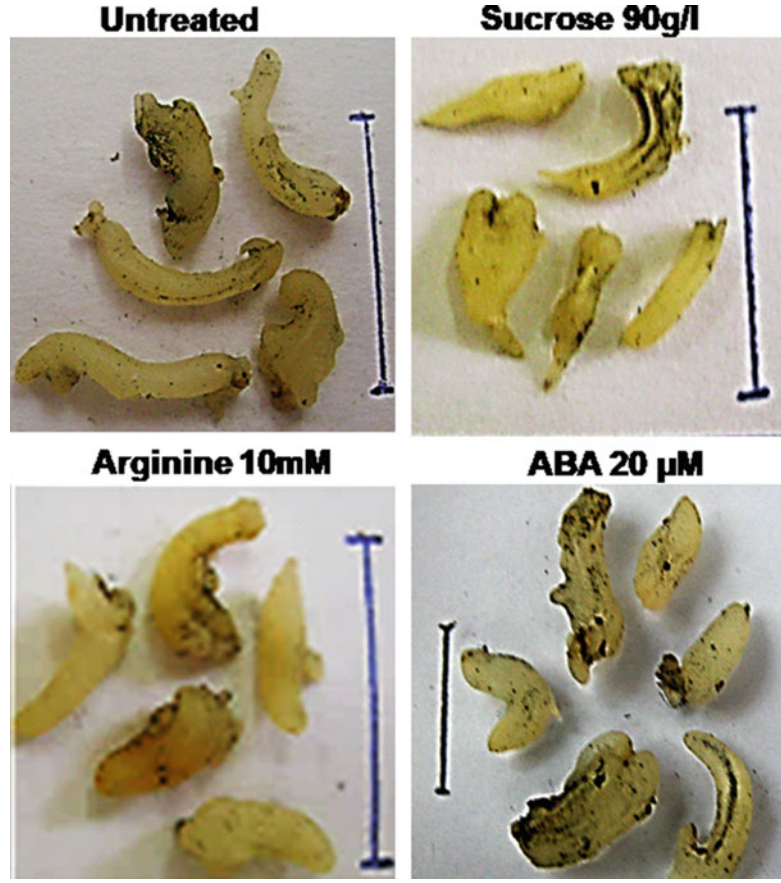

Fig. 5.4 Photos of somatic embryos matured in culture medium supplemented with sucrose, arginine and ABA (bars = 1 cm) (Photos by B. Sghaier-Hammami)

enhances storage protein accumulation and has positive effects on somatic embryogenesis in oil palm and other species (Lai and McKersie 1994; Morcillo et al. 1999).

To conclude, the application of additives contributed to the activation of storage and defense protein synthesis as well as the inhibition of other metabolic pathways that can be involved with the seed. Therefore, this would allow the preservation of the embryo in an anabolic phase. This can be confirmed by the number of down-regulated proteins which were more important than the upregulated ones for all the treated SE. Although additive treatments did not reach maximal efficiency to increase SE quality, a higher germination capacity could be acquired, and better conversion rates into a vigorous plantlet similar to those derived from the ZE was reached.

5.6 Proteomics Studies of Date Palm Response to Brittle Leaf Disease

As do most crops, date palm culture suffers from several destructive diseases. Brittle leaf disease is classified as one of the most serious. Indeed, since its appearance in Tunisia in 1980, it became more serious and epidemic, spreading to nearby regions such as Eastern Algeria (Saadi et al. 2006; Triki et al. 2003). According to previous studies, three stages of brittle leaf disease (BLD) were defined: a) Stage S1: characterized by chlorosis of a few fronds, b) Stage S2: leaflets become brittle, twisted and frizzled and c) Stage S3: the entire plant stops growing and finally dies. The timeline of the development of the disease could last 4–6 years from the appearance

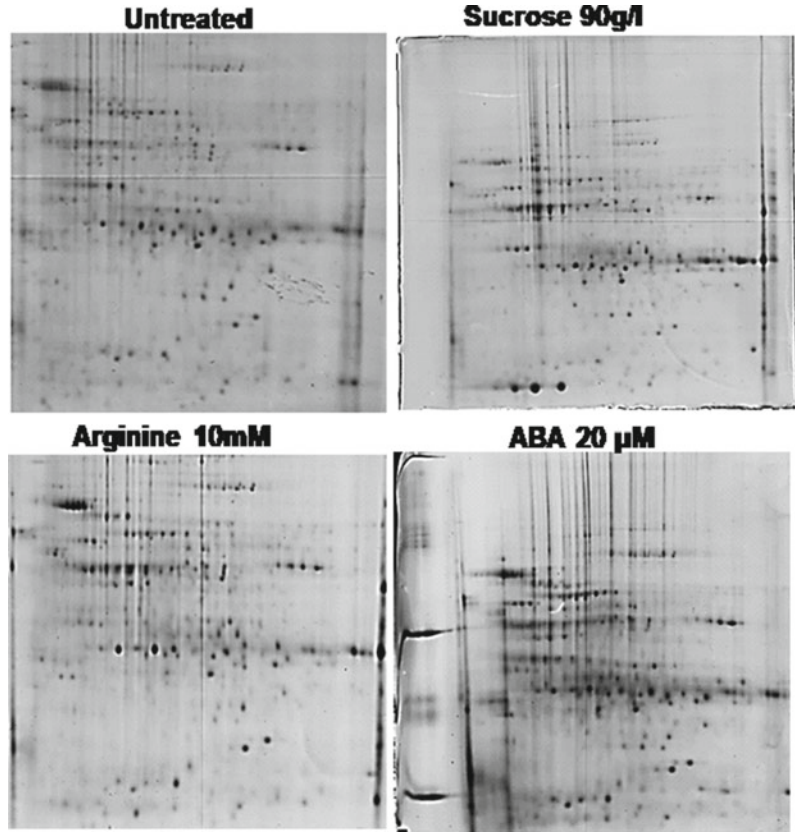

Fig. 5.5 Areal gel 2-DE map of untreated and treated SE by sucrose, arginine and ABA. 400 µg of protein was separated using the first dimension gel on an immobilized, linear, 5–8 pH gradient and in the second dimension on a 12% acrylamide-SDS gel. Gels were Coomassie-stained (Figure constructed by B. Sghaier-Hammami)

of the first symptoms (S1) to the death of the tree (S3).

Although, the BLD epidemiology could be caused by a pathogen, no biotic agent has yet been identified as the main causal agent (Triki et al. 2003). Leaflets of affected palms have been shown to contain significantly lower manganese concentration than those of healthy controls (HC). Furthermore, soils where BLD develops contain about one-half the manganese content compared to normal soils (Namsi et al. 2006).

Proteome of BLM-affected palms leaflets was reported in other studies (Marqués et al. 2011; Sghaier-Hammami et al. 2012). Different proteins were separated by 2-DE at the three stages, with IEF carried out at the 3–10 pH range (Marqués et al. 2011) and at 5–8 pH range (Sghaier-Hammami et al. 2012). The separation of proteins at the 5–8 pH range seems to be more efficient than the 3–10 pH range since the use of this latter resulted in obtaining protein spots that are concentrated in the 5–8 pH regions (Gómez-Vidal et al. 2009).

The 2-DE protein profiles of the HC and BLD-affected palm leaflets showed 364 resolved spots in the master gel (Fig. 5.6), and most of them (297 spots) were variable between samples (Sghaier-Hammami et al. 2012). The 2-DE protein profile of the HC seems to have less protein spots than BLD-affected palm leaflets at S1, S2 and S3 stages, which may be explained by an easier extraction of proteins from the affected leaves by BLD. The number of proteins in BLD-affected palm leaflets at S2 were higher than those affected at S1 and S3 (Fig. 5.6).

The major group of identified proteins corresponded to chloroplastic metabolism (60%). They belong to photosynthesis and the Calvin cycle, carbohydrate metabolism, amino acid biosynthesis, protein fate (synthesis and turnover), stress-related proteins and cytosqueleton. Actually, proteins belonging to photosynthesis

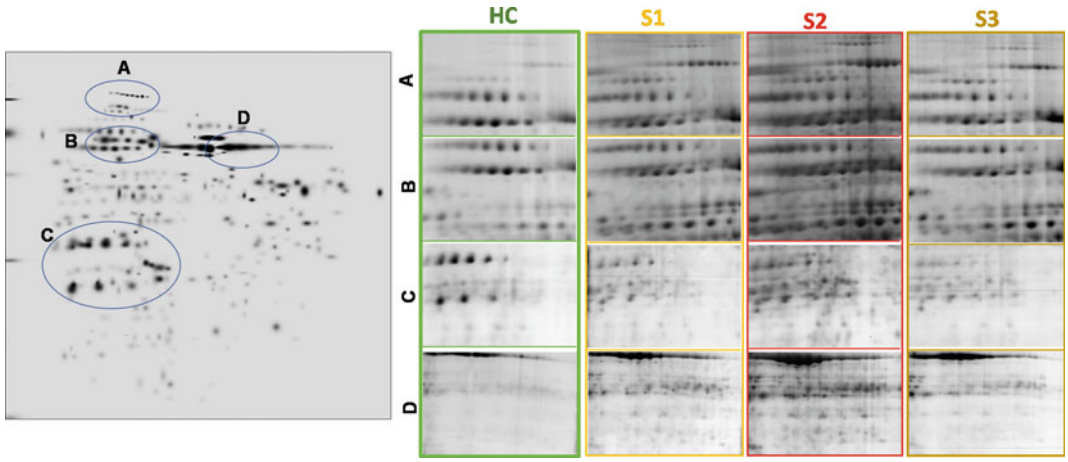

Fig. 5.6 Representative 2-DE master gel of healthy control (HC) and diseased leaves representing different disease stages (S1, S2 and S3). 500 μg of proteins were separated by 2-DE, with IEF carried out in the 5–8 pH range. Gels areas (**A**–**D**) are magnified to visualize different protein spots between samples (Figure constructed by B. Sghaier-Hammami)

electronic chain had a low intensity in the different BLD-affected palm leaflets at the three stages of disease. On the other hand, proteins belonging to proteolysis group and stress-related proteins increased in BLD-affected palm leaflets and especially at stages S2.

Seven RubisCO protein species were identified, where six of them seem to be a product of protein degradation processes (Sghaier-Hammami et al. 2012). The observed leaf chlorosis during the S2 disease stage was most likely related to the RubisCO degradation. Previous studies (Saidi et al. 2012) showed that BLD led to a decrease in the photosynthetic activity and the reduction of chlorophyll content. This may be tightly related to the increase of protease proteins observed in the BLD-affected palm leaflets (Sghaier-Hammami et al. 2012). Similarly, Adam et al. (2006) demonstrated that chloroplast proteases may participate in chloroplast biogenesis during adaptation to environmental condition changes through the degradation of some proteins.

Other proteins (MSP-33 kDa subunit) that belong to photosynthetic chains decreased in BLD-affected palm leaflets. The manganese-stabilizing protein (MSP-33 kDa) subunit has a fundamental role in the maintenance of the integrity and the activity of the manganese cluster (Seidler 1996). Furthermore, as was reported previously the decrease in the MSP-33 kDa protein subunits and the luminal oxygen-evolving system (OEC) proteins (PSBO2, PSB, PSBP, PSBO1) corroborates the hypothesis of the relation between the MFC and manganese deficiency (Marqués et al. 2011). We thus suggest here, that the decrease in MSP-33 kDa protein subunits and the degradation of RubisCO proteins can be used as biomarkers of manganese deficiency BLD-affected palm leaflets.

The induction of stress-related proteins is an immediate response of the plant to stress. The chloroplastic heat shock proteins (Hsp70 kDa), the chaperonin 60 subunit beta1 and the peroxiredoxin proteins were reported to increase in the BLD-affected palm leaflets. Additionally, transcriptomic analysis demonstrated that Hsp70kDa-related cDNA was upregulated in BLD-affected palm leaflets (Saidi et al. 2010).

The chaperonin 60 subunit beta 1 has been reported to be implicated in cell death and systemic acquired resistance and to play a role in the acclimation of photosynthesis to heat stress, possibly by protecting Ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit (RubisCO) activase from thermal

denaturation (Salvucci 2008). In addition, plant 2-Cys peroxiredoxins are targeted to chloroplasts following post-translational modifications in which they have a fundamental role in protecting the photosynthetic membrane against photooxidative damage (Baier and Dietz 1997, 1999).

To summarize, in the present proteomics, the BLD disease work was thoroughly investigated during disease development from the earliest to the latest stages (demise of the date palm tree). Changes in the 2-DE protein profile start at early disease stage (S1), in which a decrease in MSP-33 kDa subunit proteins was observed. At the S2 stage, a degradation of the RubisCO proteins was found when leaflets became chlorotic. This showed that this disease is not only specific to manganese deficiency, but it confirms that BLD is an abiotic stress.

5.7 Proteome Analysis of Response to Salinity and Drought

Salinity and drought represent the two major environmental stresses that adversely affect crop production in the world. Date palm responds differently to both types of stresses. The palm is endowed with great tolerance to extreme drought, and relatively high levels of soil salinity (Yaish and Kumar 2015). Proteome analysis are a convenient tool for testing the response of these plants to abiotic stress (Fercha et al. 2014; Mostek et al. 2015). Indeed, this technique was used previously by other investigators in 18-month-old date palm seedlings under severe salt (48 g/L NaCl) and drought (without irrigation or 82.5 g/L PEG) stresses during 1 month (El Rabey et al. 2016).

Drought stress induces the accumulation of photosynthesis-related proteins (RubisCO) and glycolysis pathways, whereas salt stress (NaCl) induces the accumulation of stress-related proteins (chaperonin proteins) and inhibits eight proteins involved in photosynthesis. Under the above conditions, the levels of ATP synthase CF1 alpha chain were significantly changed. Salt stress and severe drought stress (without irrigation) caused changes in the abundance of RubisCO activase and one of RubisCO's fragments in the same spots. A high concentration of NaCl had an inhibitory effect on the date palm biosynthesis.

In conclusion, following proteomic analysis, drought and salt stress were found to cause differential expression of genes that resulted in high or low protein abundance of the chosen protein spots. In addition, drought stress under lack of irrigation caused inhibition of the expression of all genes controlling drought tolerance.

5.8 Conclusions and Prospects

Proteomics, like other omics technologies, including transcriptomics and metabolomics, is currently playing a very important role in plant research. The combined use and integration of all of them, in the systems biology direction, will allow a deeper understanding of the different biological processes, to identify key genes linked to and relevant in plant productivity, fitness and resilience. In fact, ~80% of the researches in the plant field use this approach frequently and with successful results.

This chapter highlights the importance of the use of the proteomic approach in dissecting the proteome of the date palm somatic or zygotic embryo and the characterization of the proteins involved in stress response. Using proteomic approaches, we have shown the following:

(a) During zygotic embryo development and germination, storage proteins and stress-related proteins were most abundant during the early developmental stages and then their presence decreased during germination.
(b) Somatic embryo contains lower proteins content than zygotic embryo and lacks proteins involved in the maturation process like storage proteins and proteins related to stress tolerance which may be due to the lack of precursors in its medium culture.
(c) The application of exogenous abscisic acid (ABA), sucrose and arginine to medium culture of somatic embryos improves their content in proteins, especially those related to storage and stress.
(d) Upon abiotic stress, date palm leaves and roots showed a reduction in proteins related

to energy metabolic pathways, especially of photosynthesis and the induction of proteins relation to stress and defense.

Future research on date palm somatic embryogenesis should be based on other proteomics platforms, such as shotgun, to increase the proteome coverage as well as its integration with other omics and classic approaches in the system biology direction. The comparison between zygotic and somatic embryos will reveal which genes and gene products determine the difference and could be considered as markers of viability and vigor. In addition, new chemicals should be tested and incorporated into the in vitro culture medium at the different developmental stages, getting the somatic embryo proteome closer to that of zygotic, and ensured the success in the propagation program.

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