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REVIEW

WATERLOGGING STRESS IN WHITE (*TRIFOLIUM REPENS* L.) AND RED (*TRIFOLIUM PRATENSE* L.) CLOVER AND THE ROLE OF PROTEOLYSIS

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Summary: Waterlogging has a range of negative effects on plant growth and productivity. The development of hypoxia in flooded soils disturbes plant metabolism since roots normally require oxygen for optimal production of adenosine triphosphate. Within the plant, anaerobic respiration leads to the production of ethanol or lactate, causing cellular and membrane damage, impairing nutrient uptake and facilitating pathogen entry. Long-term waterlogging worsens soil mechanical and chemical properties decreasing its agricultural quality. This review focuses on waterlogging stress in *Trifolium* spp – important fodder legumes with high nutritional value for grazing animals, and its impact on plant proteolysis. The biochemical and functional characteristics of plant proteases are discussed, focusing on the specific proteolytic events observed under excessive water stress. The current knowledge about plant protease inhibitors and their role in control over endogenous proteolysis is briefly reviewed.

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Key words: Clover (*Trifolium spp.*); waterlogging; proteolysis; plant proteases and protease inhibitors.

Abbreviations: ATP – Adenosine triphosphate; BBIs – Bowman-Birk inhibitors; Clp – Caseinolytic protease; FtsH – Filamentous temperature sensitive H; IUBMB – *International Union of Biochemistry and Molecular Biology;* NADPH – Nicotinamide adenine dinucleotide phosphate; PCD – Programmed cell death; PI – Protease inhibitor; PS II – Photo system II; STI – Soybean trypsin inhibitor.

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Contents

INTRODUCTION

The predicted increase of earth population, which at the end of the 21st century will reach 12 milliard people, will result in significant shortage of the basic food resources. Further complication is the climate change which will additionally challenge the agricultural sector. The industrial pollutants and urbanization have caused the so-called "greenhouse effect" which induces higher water evaporation rates and subsequently torrential rainfalls leading to flooding of large areas of farmlands. As a result water excess stress is imposed to plants with different scale – from soil over-saturation with water (waterlogging) to partial or complete submergence of plants in water. The negative effect of excessive water on soil fertility is particularly harmful for the quality and productivity of crops. Waterlogged soil lacks oxygen, which obstructs the normal plant growth and development. The chemical composition of waterlogged soils changes, the growth of anaerobic bacteria is favourised.

Plants vary in their adaptation to excess water stress - some vascular plants can live underwater, others develop adaptations upon repeated flooding as those on riverbanks. Generally, plants use "escape" strategies (development of aerenchyma, shoot outgrowth) as well as "quiescent" strategies (no further biomass accumulation, change in the pattern of protein synthesis, "stress-surviving" metabolic regime with degradation of reserve proteins) to fight the flooding stress. During the period of recovery plants restore their biochemical and physiological parameters to the pre-stress levels. Generally waterlogging has negative consequences like poor plant survival and productivity. The total protein content of waterlogged plants is severely reduced which lessens the nutritional value of the forage.

Elucidation of protein changes observed in different varieties of fodder plants subjected to excessive water and their subsequent recovery will contribute to understanding of the protective mechanisms against this kind of stress as well as to introducing the acquired knowledge in the agricultural practice in farmlands which are at risk of flooding.

1. Climate change and threats of flooding

Flooding and excessive soil water could be due to natural disasters but also to local anthropogenic factors. Waterlogging occurs at continuous soil water saturation resulting from heavy long-lasting rainfalls combined with poor vertical draining. The more often observed climatic abnormalities could originate from the global effect of the anthropogenic activities (Rosenzweig et al., 2008). Often soil structure, climate, topographic and hydrological factors have cumulative effect. The natural annual rainfall pattern, terrain draining and soil structural characteristics, as well as above- and underground rivers and large-scale deforestation are among the major reasons for the large number of lands predisposed to waterlogging in Bulgaria (Aleksandrov and Kazandjiev, 2010).

Prolonged waterlogging endangers the natural biodiversity and could lead to the extinction of certain species, as well as to deforestation of the affected areas. In addition to the negative ecological aspects flooding stress dramatically affects growth, development and productivity of crop plants (Voesenek et al., 2006, Irfan et al., 2010) resulting in significant financial losses in the agricultural sector. Waterlogging has a long lasting effect on soil quality, even after withdrawal of water bodies, since the water soluble substances of humus are redistributed in the lower soil layers. Thus the root system is deprived not only from oxygen but also from valuable nutrient compounds. The adverse effect of excessive water is particularly harmful for grass and short stalked plants whose root system occupies exclusively the waterlogged soil layers such as cereals, vegetables and legumes.

1.1. Human activity for alleviation and prevention of excessive water adversities

European Union has approved and introduced a directive 2007/60/EC (Directive 2007/60/EC of the European parliament and of the council of 23 October 2007 on the assessment and management of flood risks) which regulates the needed steps and measures for prevention of flooding events. The Directive required Member States to first carry out a preliminary assessment by 2011 to identify the river basins and associated coastal areas at risk of flooding. For such zones flood risk maps should be prepared by 2013 and the Member States must establish flood risk management plans focused on prevention, protection and preparedness by 2015. The Directive applies to inland waters as well as all coastal waters across the whole territory of the European Union.

The preventive measures alone are often not effective enough and require subsequent restoration activities. Besides the mechanical removal of debris, rubble and pumping of the excessive water out of the affected farmland areas, necessary steps for remediation of the damaged soils should be taken in order to restore its fertility. Such methods include aeration and drying via deep plowing the soil, application of coal additives capable of capturing the CO_2 derived from anaerobic fermentation in soils, the construction of drainage ditches, the leveling of the adjacent terrain and de novo deposition of undamaged humus layer.

1.2. Response and adaptation of plants to flooding

Adaptation of plants to stress is acquired via changes in biochemical processes, as well as new anatomical and morphological structures. Ceasing of O_2-supply to the root system in waterlogged plants has deleterious effect on physiological processes. In plants, oxygen is involved in more than 200 different biochemical reactions, and 95% of its content is consumed during cellular respiration which secures the energy supply (Rawyler et al., 2002). Oxygen has poor solubility in water - 33 times less than in gasous phase, and its diffusion in water is 10 000 times slower than in the air (Sairam et al., 2009). Under hypoxia (insufficient oxygen supply) limited mitochondrial respiration occurs, while at anoxia (complete lack of oxygen) the only ATP source remains the anaerobic glycolysis (Fukao and Bailey-Serres, 2004). In such conditions cell metabolism enters in economic regime which ensures only the organism survival. Each plant cell could sustain at least an hour of anoxia via switching form aerobic metabolism to anaerobic fermentation. Low fermentation efficiency is the main cause for reduced metabolism and arrested biomass accumulation under hypoxia (Beha et al., 2002).

The products from anaerobic respiration are additional stressors for the plant cell as they have a potential to harm cellular structures and metabolism. Therefore the physiological background and the actual conditions of flooding are extremely important i.e. stagnant water is much more damaging since it is enriched in products of anaerobic respiration. Prolonged hypoxia is also accompagnied by increased production of reactive oxygen species which have deleterious effect on cellular structures. Lipid membranes, proteins, carbohydrates and nucleic acids are the major targets of free radicals (Blokhina et al., 2003).

Plant species and cultivars may differ significantly in their capacity to tolerate excess water. The complexity of the protective reactions depends on the duration and intensity of the applied stress. Additionally the sensitivity towards flooding may vary depending on the developmental stage (Jackson and Ram, 2003). The decrease in O_2 intracellular concentration negatively influences root growth and respiration long before the critical stages of hypoxia are reached. This reduces the permeability and the active absorption of water and soluble nutrients by the root system (De Rijke et al., 2005). Flooded plants exhibit retarded growth and thicker stems. They begin to wilt and loose chlorophyll. Apoptosis and formation of aerenchyma tissue are often observed in roots (Irfan et al., 2010). This special tissue provides a network of intracellular gas-filled spaces that permits an internal diffusion of oxygen from the aboveground plant parts toward the roots (Fukao and Bailey-Serres, 2004; Gibberd et al., 2001). Legumes tend to respond to flooding with dying off the major root and formation of new adventive roots close to the surface (Gibberd et al., 2001).

Flooding leads to nitrogen depletion which negatively affects various physiological and biochemical processes. Stagnant water severely reduce nitrogen availability in soil due to accumulation of phenolic compounds which react with soil nitrogen (Unger et al., 2009). It is suggested that nitrogen limitation induces an early, reversible stage of senescence which is characterized with specific set of protease activities, distinct from those involved in natural senescence. They facilitate the degradation of non-essential proteins into amino acids in order to increase the chances of plant survival or seed set (Kingston-Smith et al., 2005).

2. Clovers as fodder plants – ecophysiological and biochemical characteristics

Flooded agricultural areas usually are recovered by application of suitable fertilizers or construction of drainage systems but the negative impact of excessive water on wildlife may result in persistent changes in biodiversity and proportion of cohabiting species. This requires the development of environmentally friendly methods for bioremediation applicable in wild meadow areas which will not interfere with the natural flora and fauna. Planting of slowly growing perennial legumes such as clovers and alfalfa, capable to fix nitrogen, represents a good approach for bioremediation of areas affected by floods (Sairam et al., 2009). Clovers are particularly preferred as bioremediators due to comparatively high leaf protein content and tender texture, which make them also an excellent fodder crop (De Rijke et al., 2005). Clovers have lower polyphenol content compared to alfalfa which gives them the advantage to retain nitrogen in its organic form after harvesting (Sullivan and Hatfield, 2006).

Genus *Trifolium* (clovers) contains approximately 300 species which belong to legume family (*Fabaceae*). Clovers have a cosmopolitan distribution in temperate climatic regions, they are also found at high altitude in the mountains, as well as in the tropics. Clovers could be annual or perennial plants with highly manifested geno- and phenotype diversity. The different *Trifolium* species differ significantly in tolerance against waterlogging and the increased survival of resistant varieties is due to the bigger capacity to form adventitious roots (Gibberd et al., 2001). Under optimal growth conditions, one gram of fresh leaves contains approximately 80 mg of digestible protein which characterize clovers (both white and red one) as excellent fodder crops (Programme for the development of alternative agriculture in Rhodopa Mountain – 2001, http://www.mzh.government.bg/odz-haskovo/Libraries/). Clovers are comparable to alfalfa, which is one of the most productive legumes (Milić et al., 2011), in regard to the content of assimilative amino acid (assimilation of 91.41% and 47.12% for proline and glycine respectively). Protein content of white and red clover varies, even among the different genotypes – in white clover it is in the range from 22.92 % to 24.86 % of the dry weight, while in red clover it is between 14.63% and 15.71% (Penkov et al., 2003). According to the “Programme for the development of alternative agriculture in Rhodopa Mountain – 2001” (in Bulg.), highly productive grass mixtures containing white and red clover (10% and 20% respectively) have been recommended for areas with altitude of 200-600 m, while for areas with higher altitude (500-800 m) have been suggested grass mixtures containing only red clover (25%).

2.1. Red clover (Trifolium pratense)

Red clover (*Trifolium pratense* L.) is a major leguminous fodder in moderately moist areas around the world (Isobe et al., 2003). It is naturally well-adapted to wet and cooler climates, which determines the area of its cultivation – mainly mountain regions. Red clover is characterized with good yield and high levels of nutrients and additives. Under optimal growth conditions it can produce 1000-1200 kg quality hay or 4-5 t of fresh mass per hectare. Red clover tolerates acidic soils (pH ~ 4.5) where the cultivation of alfalfa is not possible. It is characterized with particularly high content of phytoestrogens (Coon et al 2007) which makes it a valuable medicinal plant.

2.2. White clover (Trifolium repens)

White clover (*Trifolium repens*) is another high quality forage legume (Dolanska and Čurn, 2004) widely distributed from the arctic and temperate regions of Europe and North America to the subtropical Mediterranean latitude. It is the preferred legume component in grass seed mixtures and could be used also for erosion control cover on moist fertile soils. Being a low growing perennial which preserves its nutritional quality throughout all vegetation seasons, white clover is the preferred crop sown for grazing (Finne et al., 2000). When cultivated properly according to the agrophysiological prescriptions white clover may double the fodder protein yield thus contributing to the increase in animal protein production. *T. repens* is an allotetraploid species with high levels of genetic variation both within and between populations (Casey et al., 2010). Regardless the great agricultural importance of white clover, published data on its genetic and molecular characteristics is scarce compared to available information regarding molecular and biochemical characteristics of other legumes such as *Pisum sativum*, *Medicago sativa*, *Lotus japonicus* and *Glycine max* (Asp et al., 2004).

3. Regulation of protein metabolism under stress and recovery

Protein homeostasis is maintained constantly via various biological pathways within cells that control their biosynthesis, folding, trafficking and degradation (Gottesman et al., 1997; Hegde end Ploegh, 2010). The intensity and fine tuning of these processes are dependent on developmental stage, nitrogen and nutrient supply, light intensity and environmental factors (Vaseva et al., 2012). Protein breakdown has been recognized as essential for the adaptation of plants to environmental conditions (Kidrič et al., 2014). Proteases have to be tightly regulated at several levels since they are essential players in many plant physiological processes and peptide bond hydrolysis is irreversible. All the cellular structures with peptide bonds could be potential protease targets (Sakamoto, 2006). The expression of protease-coding genes is regulated at transcriptional and post-transcriptional levels. Often proteases are initially synthesized as inactive zymogens (pre-pro-polypeptides) and their activity is bocked by endogenous inhibitory proteins. Proteases are subjected to spatial and temporal regulation via compartmentalization, post-translational modification (glycosylation, phosphorylation, co-factor binding), limited proteolysis and degradation. Protease inhibitors constitute a specific very important mechanism for regulation of proteolytic activity (Van der Hoorn, 2008).

4. Plant proteases

The occurrence of proteases in all living organisms indicates their important metabolic and regulatory functions. Among processes requiring participation of proteases are: protein homeostasis and repair; amino acid recycling, zymogene processing, regulation of the activity of eukaryotic polypeptide hormones, programmed cell death (Vierstra, 1996, Feller et al., 2008). *Arabidopsis* genome contains over 800 protease coding genes, which are distributed into 60 families belonging to 30 clans and amount to almost 3% of the proteome (Rawlings et al., 2012). However, most of the studies on proteolytic degradation remains limited and the specific biological functions of great amount of plant proteases are still unknown (Tripathi and Sowdhamini, 2006).

4.1. Classification and functions of plant proteases

The IUBMB Enzyme Nomenclature system classifies proteases as hydrolases acting on peptide bonds with the number EC 3.4 with further sub-classification based on the catalytic type. Most of the plant proteases belong to the following five classes: cysteine- (EC 3.4.22), serine- (EC 3.4.21), aspartic- (EC 3.4.23), threonine- (EC 3.4.25) and metalloproteases (EC 3.4.24).

Serine proteases (ser-proteases) constitute a large class of proteolytic enzymes in plants with size between 19 to 110 kDa (the majority having a molecular weight of 60-80 kDa) and optimal pH for most of them within the region pH 7-11 (Antão and Malcata, 2005). The largest family comprises the subtilisin-like proteases or subtilases (Tripathi and Sowdhamini, 2006). Another family – that of caseinolytic proteases (Clp) comprises chloroplast located ser-proteases (Sakamoto et al., 2008). Since the main *in vitro* Clp substrates are unstructured or weakly folded proteins, like casein, they could play a very important role in the recycling of defective or damaged molecules (Kress et al., 2009). Other ser-type plant proteases are the ATP-independent DEG/HtrA proteases; the tylakoide bound ATP-independent SppA, and the ATP-dependent Lon protease (Adam et al., 2006).

Cysteine proteases are characterized with neutral to acidic pH optimum (Watanabe et al., 2009) and are activated by thiol substances; a major proportion of them is localized in vacuoles or they are secreted in extracellular compartments (Forsthoefel et al., 1998). Most plant cys-proteases belong to the papain-like, legumain-like, caspase and Ca^{2+}-dependent calpain families (Shindo and Van der Hoorn, 2008; Grudkowska and Zagdańska, 2004). Plant ubiqutin C-terminal hydrolases and ubiquitin specific proteinases also are cys-proteases. Cysteine proteases play a key role in remobilisation of storage proteins during germination (Feller, 2004), during differentiation of vasculature and the onset of senescence including PCD (Asp et al., 2004). They also participate in many processes related to responses to biotic and abiotic stresses. In mature green tissue cys-proteases comprise 30% of the total protease activity, reaching 90% under stress conditions (Grudkowska and Zagdańska, 2004).

Threonine proteases participate in the complex oligomeric structure of the 26S proteasome and related compound peptidases (Rawlings et al., 2012).

Metalloproteases are rare in plants and less studied than those in animals and bacteria. Among the most investigated plant metalloproteases is the FtsH protease, which is an integral ~70 kDa protein with ATP- and proteolytic domains (Sakamoto et al., 2008, Yu et al., 2005). The major FtsH substrates in chloroplasts are D1 of PS II and Rieske protein (Fe-S protein) – both with relatively short half-life (Watanabe et al., 2009). Metalloproteases are involved in meiosis, regulation of root and shoot meristem size, sensitivity to auxin conjugates, plastid differentiation, nodulation and thermo-tolerance.

Aspartic proteases, which in plants have additional saposin-like plant-specific insert, are optimally active at low pH (below pH 5) (Feller, 2004). Families of this catalytic type are so far known to contain only endopeptidases, with no exopeptidases (Rawlings et al., 2012).

4.2. Subcellular localization and regulation of plant proteases

Most of the proteolytic activity in crude plant extracts originates from vacuoles. Vacuoles contain various classes of proteases optimally active at acidic pH (pH 3-6) (Müntz, 2007; Carter et al., 2004). Cysteine proteases, such as legumains and papain-like proteases, and aspartic proteases are the most common, but other catalytic types are also represented (Feller, 2004). Vacuolar ATP-dependent proteases have not been identified (Vierstra, 1996).

Mitochondria are cellular organelles with relative autonomy; they maintain their own set of endogenous proteases (Feller, 2004). Mitochondria proteolytic system includes ATP-dependent proteases which are in close relation with chaperones. Mitochondrial proteases isolated in *Arabidopsis* belong to FtsH, Lon and Clp families.

Each of the major chloroplast compartments contains defined proteases, some involved in non-selective degradation, and several of them are probably functioning as highly specific processing peptidases. The ATP-dependent Clp (serine-type) proteases occur in stroma. DegP proteases (serine-type), are found within the thylakoid lumen and on both sides of thylakoid membranes, and the SppA protease (serine-type) on the stromal side of the thylakoid (Adam et al., 2006).

Non-functional proteins of the endoplasmic reticulum are degraded in the cytosol (Leidhold and Voos, 2007). The main proteolytic system in the cytosol and nucleus is the ubiquitin/26S proteasome pathway, which eliminates misfolded, damaged and/or regulatory proteins (Smalle and Vierstra 2004, Kurepa et al., 2009). The ubiquitine-dependent proteolysis in plants is involved in hormonal signaling (Graciet and Wellmer, 2010).

Proteases are also present in peroxisomes (Palma et al., 2002). About 70% of the total proteolytic activity in these organelles can be assigned to serine endopeptidases. It is suggested that they participate not only in the turnover of peroxisomal proteins but also in the breakdown of proteins from other cell compartments in advanced stages of senescence (Distefano et al., 1997).

The apoplast is the first site of proteolytic defense against pathogens. Extracellular proteases, which hydrolyse proteins into amino acids for subsequent absorption into the cell, constitute a very important step in nitrogen metabolism (Vierstra, 1996). A Ca^{2+}-independent metalloprotease with slightly acidic pH optimum (pH 6), which has been related to leaf growth and expansion was identified in the extracellular matrix (Messdaghi and Dietz, 2000). Carnivorous plants use extracellular proteases to digest their victims (Vierstra, 1996).

Little is known about the complex regulation of proteases in plants. Adaptation to stress conditions and the alterations in protein profile could be an outcome of enhanced protease activity (Kidrič et al., 2014). The change in proton gradient is a prerequisite for activation of certain proteases. For example during germination the lowering of the pH in the vacuole is a signal inducing activation of an aspartate protease (phytepsin) located in the vacuoles of the storage tissue. The activated aspartate protease on its turn specifically activates cysteine proteases (by limited proteolysis), which degrade the grain storage proteins (Müntz, 2007). Proteolysis may be also part of light-regulated processes. For example, under limited nitrate availability and in the absence of light nitrate reductase gene expression is suppressed and the available enzymes are inactivated by reversible phosphorylation. This phosphorylation is a signal for its rapid degradation by proteases (Vierstra, 1996).

4.3. Protease inhibitors

Protease inhibitors (PIs) are ubiquitously distributed in animals, plants and microorganisms and constitute an important mechanism of regulating proteolytic activity. PIs are often classified according to the class of protease they inhibit (for example: cysteine or serine protease inhibitors) (Kidrič et al., 2014). Some PIs are composed of multiple inhibitor units and the so-called 'pan-inhibitors' (such as α2-macroglobulin) that target proteases of different catalytic classes (Délano-Frier et al., 2008).

Large quantity of PI is localized in vacuoles and in seeds (Rawlings et al., 2012). PIs are important part of the plant defense system against parasites and pathogens. These inhibitors act against digestive herbivorous proteases of insects and mites (Habib and Fazili, 2007). The expression of PI coding genes is induced by different chemical signals such as polygalacturonate, abscisic acid, auxins, systemins, jasmonates, salicylic acid and ethylene (Kang et al., 2002). Recent studies point at the involvement of PIs in the response to abiotic stress and control over endogenous proteolysis (Kidrič et al., 2014). In plants, the most abundant classes of PIs are cystatins and serine PIs.

Cystatins inhibit mainly cysteine type proteases from papain and legumain families. These peptidases play key roles in normal intracellular protein degradation, as well as defense against biotic and abiotic stress (Shindo and Van der Hoorn, 2008). It is suggested that the regulation of cystatin expression is under hormonal control since promoter sequences of cys-PIs contain gibberellin- and ABA-binding domains (Benchabane et al., 2010).

The two largest families of serine protease inhibitors are Kunitz- and Bowman-Birk PIs. Kunitz-type PIs are activated by mechanical stress and by abiotic factors such as water shortage (Kang et al., 2002). The Bowman-Birk inhibitors (BBIs) are well-studied serine protease inhibitors that are relatively abundant in plants. Until now, all identified BBIs have been found in *Fabaceae* and *Poaceae*. Their synthesis may be induced by infection or wounding, particularly in vegetative tissues.

5. Proteolysis under waterlogging

Plant adaptation to stress and the alterations in protein profile could be an outcome of enhanced protease activity (Feller et al., 2008). The role of proteolytic degradation under waterlogging comprises the following major points: rearrangement of metabolism through selective degradation of key enzymes; degradation of short-lived proteins involved in cell signaling; removal of oxidatively damaged, improperly folded or irreversibly denatured proteins; recycling of carbon-starvation-related amino acids and hastening of senescence under source-sink regulation; protection against potential biotic stress.

The studies on proteolytic degradation in waterlogged legumes are quite limited. It was found that the roots of two clover species (*Trifolium fragiferum* and *Trifolium repens*) were characterized with higher porosity under waterlogging associated with lysogenous breakdown of cortical cells, thus proving morphological changes caused by stress-induced protease activity (Aschi-Smiti et al., 2003). Recent studies on *Arabidopsis* show that the endo-type Deg protease and the exo-type FtsH proteases co-operatively degrade D1 during the PSII repair *in vivo* (Kato et al., 2012). The same authors report up-regulation in concert of other chloroplastic proteases, such as Clp and thylakoid membrane-bound protease SppA.

In a recent study on protein changes in red and white clover subjected to water excess and subsequent recovery we have tried to provide some insights into regulatory mechanisms of proteolytic processes in leguminous forage plants under stress (Stoychev et al., 2013). *T. repens* tolerated excessive water better than *T. pratense*. A substantial transient increase of ClpP levels in most waterlogged samples was detected by immunoblotting. Red clover plants exhibited also a prominent increase of ClpA under stress conditions. The detected changes in the plastidial Clp system subunits in waterlogged red clover suggested alterations in plastid ATP-dependent proteolysis (Stoychev et al., 2013). Two-dimensional electrophoretic analyses revealed that FtsH protease diminished under waterlogging (Stoychev et al, unpublished data). FtsH is located exclusively in the stroma-exposed regions of the thylakoid membrane (Lindahl et al., 1996). It has been implicated in the degradation of soluble and membrane-associated forms of unassembled Rieske Fe–S protein (Lindahl et al., 2000). Clp can partially substitute for FtsH given the functional similarity between Clp and FtsH. This assumption correlates with the increase in Clp protease subunits in waterlogged clover leaves (Stoychev et al., 2013).

After recovery, FtsH protease showed over-restoration of abundance (data not published), possibly in an adaptive manner for future stress protection (Bruce et al., 2007).

6. Concluding remarks

Waterlogging has turned into a phenomenon with disastrous consequences for agriculture and ecological balance. Floods contribute to loss of soil quality and agricultural production. Proteolysis under excessive watering reduces digestible proteins in fodder crops. The study of the behavior of plant proteases under waterlogging can expand our knowledge about their physiological functions and regulation. The acquired knowledge on the physiological role of plant proteases and protease inhibitors could be introduced in agricultural practice for establishment of novel protective strategies in plant breeding, as well as for development of innovative biotech products. The studies on tolerance of different clover species to waterlogging could have great environmental and economic impact and should be regarded as a priority research topic for agriculture in areas with temperate climate.

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