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REVIEW ARTICLE

Unlocking the mystery of plants' survival capability under waterlogging stress

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ABSTRACT

Waterlogging is a major abiotic stress affecting crop plants throughout the world, which hampers crop growth and causes yield loss. There are various types of responses in plants under this stress through the combined operation of different signaling and physiological pathways. However, the correlation between these pathways is extremely limited and not well described in the published papers. Therefore, the complex waterlogging stress-tolerance mechanisms need to be presented most coherently for a comprehensive understanding of this stress. Here, we present sequential responses in plants under oxygen-deprivation stress. The regulation of the N-end rule pathway may be treated as the initial signaling in plants after facing waterlogging stress, but still, it remains a controversial topic. All the pathways under waterlogging stress are directly or indirectly related to glycolysis, tricarboxylic acid (TCA) cycle, programmed cell death (PCD) and removal of reactive oxygen species (ROS). Scientists may consider alanine aminotransferase as the main controlling switch for surviving of plants under waterlogging stress. Triggering the genes responsible for alanine aminotransferase may act as a crucial one to develop a waterlogging tolerant plant due to its ability to control anaerobic fermentation, TCA cycle and efficient utilization of carbons.

Introduction

Plants face several abiotic stresses, such as waterlogging, salinity, drought, extreme temperature, ion toxicity or deficiency, high wind and others (1). Among these, flooding and/or waterlogging are unavoidable threats throughout the world, especially in lowland areas. Oxygen-limiting stresses, such as waterlogging, flooding and submergence, significantly affect crop growth, development and yield (2). Plants cope with waterlogging stress by different morphological, anatomical, physiological, biochemical and molecular signaling mechanisms. Among these, physiological and molecular signaling mechanisms control the other responses directly or indirectly (3). There have been a lot of reports based on morphological and anatomical changes and regulation of specific genes under waterlogging stress. The molecular physiological response is equally available but needs to be consolidated in an updated review. Different signaling and physiological pathways expressed under waterlogging stress are still uncorrelated with each other to provide a complete picture. Therefore, it is essential to present these complex mechanisms in a simple and well understandable form, and to know how plants respond under waterlogging stress.

Waterlogging stress-related signals

At the beginning of waterlogging stress, the internal oxygen concentration of the plant decreases, resulting in a declining amount of aerobic respiration. Besides, rapid accumulation of ethylene in the submerged tissues is an early and reliable waterlogging stress signal (4).

In ethylene biosynthesis pathway, methionine is converted to S-adenosyl-methionine (SAM), catalyzed by SAM synthase followed by the formation of 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS). Finally, ethylene is formed from ACC by ACC oxidase (ACO) in the presence of oxygen (5). Therefore, in the submerged root, ethylene production is restricted because of the inactivation of ACO in the

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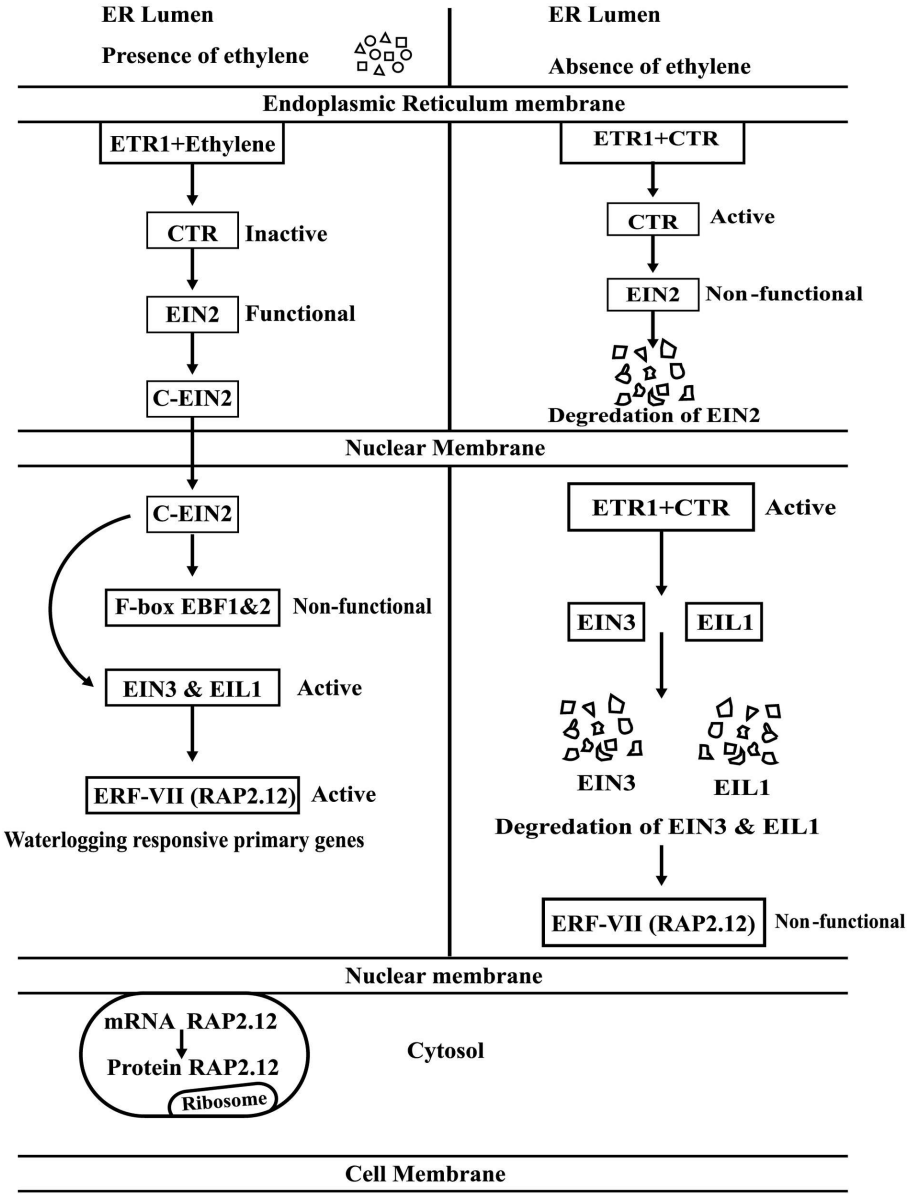

Fig. 1. Activation of ERF-VII (*RAP2.12*) through the accumulation of ethylene under waterlogged stress. Modified figure (13). CTR1: Constitutive triple response1; EBF1: EIN3 binding F-BOX protein1; EBF2: EIN3 binding F-BOX protein2; EIN2: Ethylene insensitive2; EIN3: Ethylene insensitive3; EIL1: EIN3-like1; ER: Endoplasmic reticulum; ERF-VII: Ethylene response factor-VII; ETR1: Ethylene response1; RAP2.12: Related to Apatala2

absence of oxygen. At this situation, ethylene precursor ACC is transported from anoxic (complete absence of oxygen) roots to the normaxic part (shoots) in the transpiration stream via the root-shoot vasculature (6). Initial stages of waterlogging stress, soil oxygen is partially depleted, plant root produces more ethylene due to the faster ethylene biosynthesis pathway and lowering ethylene efflux to water. But in the later stages, soil oxygen is almost completely depleted then the newly formed adventitious roots produce more ethylene by using available oxygen at the water surface region (6).

Ethylene signaling pathway in plants

Arabidopsis plant has already provided a complete pathway of expressing ethylene-responsive genes from ethylene reception to the endoplasmic reticulum (ER) through the ethylene signaling pathway (7). Ethylene is perceived by five ER membranes located ethylene receptors such as ETR1 (Ethylene response1), ETR2 (Ethylene response2), ERS1 (Ethylene response sensor1), ERS2 (Ethylene response sensor2) and EIN4 (Ethylene insensitive4) (8-10). ETR1 ethylene receptor also locates in the Golgi apparatus in *Arabidopsis* (11). Whereas, tobacco ethylene receptor NTHK1 (*Nicotiana tabacum* Histidine kinase1) is located at the cell membrane (12). However, the ER membrane is generally considered as the primary site of ethylene receptors.

Ethylene receptor activates cytosol located CTR1 (Constitutive triple response1) serine/threonine protein kinase through direct physical interaction under the absence of ethylene or low level of ethylene (Fig. 1). CTR1 suppresses the function of ER membrane located Nramp-like protein EIN2 (Ethylene insensitive2) (13). At this time, two key

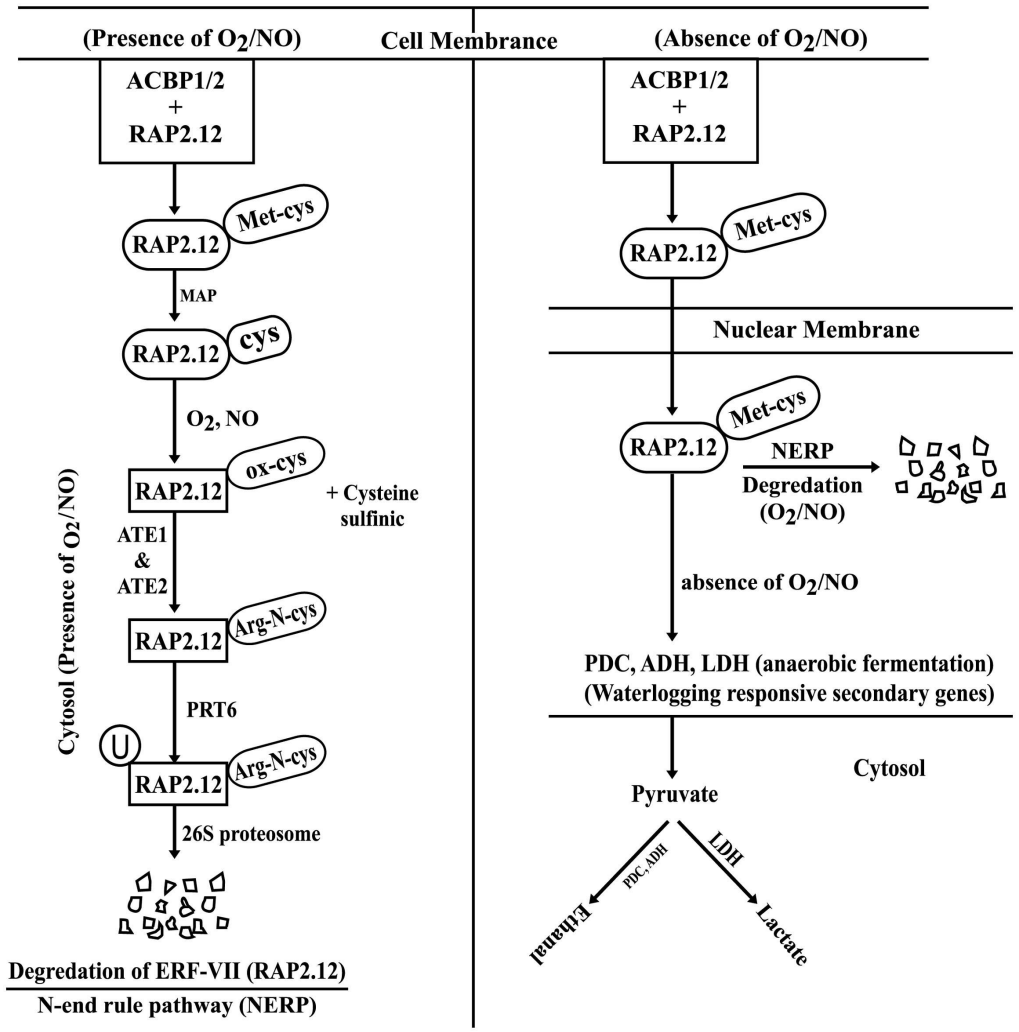

Fig. 2. Activation of fermentation genes through ERF-VII (RAP2.12) signaling during absence of O2/NO. Modified figure (28). ACBP1/2: Acyl-CoA binding protein1/2; ADH: Alcohol dehydrogenase; ATE: Arginine-tRNA protein transferases; cys: cysteine; ERF-VII: Ethylene response factor-VII; LDH: Lactate dehydrogenase. MAP: Methionine aminopeptidase; ox-cys; oxidation of the N-terminal cysteine; NO: Nitric oxide; PDC: Pyruvate decarboxylase; PRT6: Proteolysis6; RAP2.12: Related to Apatala 2

transcription factor EIN3 (Ethylene insensitive3) and EIL1(EIN3-like1) are degraded into the nucleus by the F-box proteins EBF1 (EIN3 binding F-box protein1) and EBF2 (EIN3 binding F-box protein2) through a 26S proteasome-mediated degradation pathway (14, 15). Ethylene binds with the ethylene receptor and disrupts the direct physical interaction between ethylene receptors and CTR1 in the presence of a high level of ethylene. Thus, CTR1 loses its function to suppress EIN2. Then the C-terminal fragment of EIN2 i.e. EIN-C' shuttles into the nucleus and enhances the levels of two key transcription factor named EIN3 and EIL1. These two EIN3 and EIL1 activate different primary responsive genes or transcription factors such as *ERF* (Ethylene response factor), *CHIB* (ChitinaseB), *PORA* (Protochlorophyllide oxidoreductase A) and *FLS2* (Flagellin sensitive2). Finally, these primary genes activate secondary responsive genes that allow plants to respond to specific stress through the ethylene signaling pathway (14, 15).

It would involve *ERF* group *VII* in *Arabidopsis* in the direct oxygen-sensing mechanisms. *ERF-VII* comprises *HRE1* (*ERF73*), *HRE2* (*ERF71*), *RAP2.2* (*ERF75*), *RAP2.12* (*ERF74*), *RAP2.3* (*ERF72/EBP*), *SUB1A* (*Submergence1A*), *SUB1B* (*Submergence1B*), *SUB1C* (*Submergence1C*), *SK1* (*Snorkel1*) and *SK2* (*Snorkel2*) transcription factors (16, 17). *ERF-VII* coordinated waterlogging tolerance in wheat (18), maize (19), *Arabidopsis* (20-22), *Taxodium* (23) and *Petunia* (24).

N-end rule pathway (NERP) in plants

N-end rule pathway plays a significant role in plants to survive under waterlogging stress (25-27). In this pathway, *RAP2.12* (*ERF-VII*) protein is attached with plasma membrane localized acyl-CoA binding protein1 and 2 (ACBP1 and ACBP2) (28) (Fig. 2). *RAP2.12* protein also interacts with cell membrane and cytosol located ACBPs (29). The terminal Met of *RAP2.12* is removed by the enzyme methionine aminopeptidase (MetAP) when the second amino acid of this protein cysteine (cys) residue remains exposed (30). Cysteine sulfinic or sulfonic acid is formed by the oxidation of the N-terminal cysteine (ox-cys) residue with the presence of oxygen or nitric oxide (31). An arginyl residue (Arg) is added at the N-terminus of the Oxidized cysteine by the enzyme arginine-tRNA protein transferases (ATEs) via a peptide bond (32). Arg signals a selective class of ubiquitin ligases proteolysis6 (PRT6) to polyubiquitinate for degradation of the polypeptide

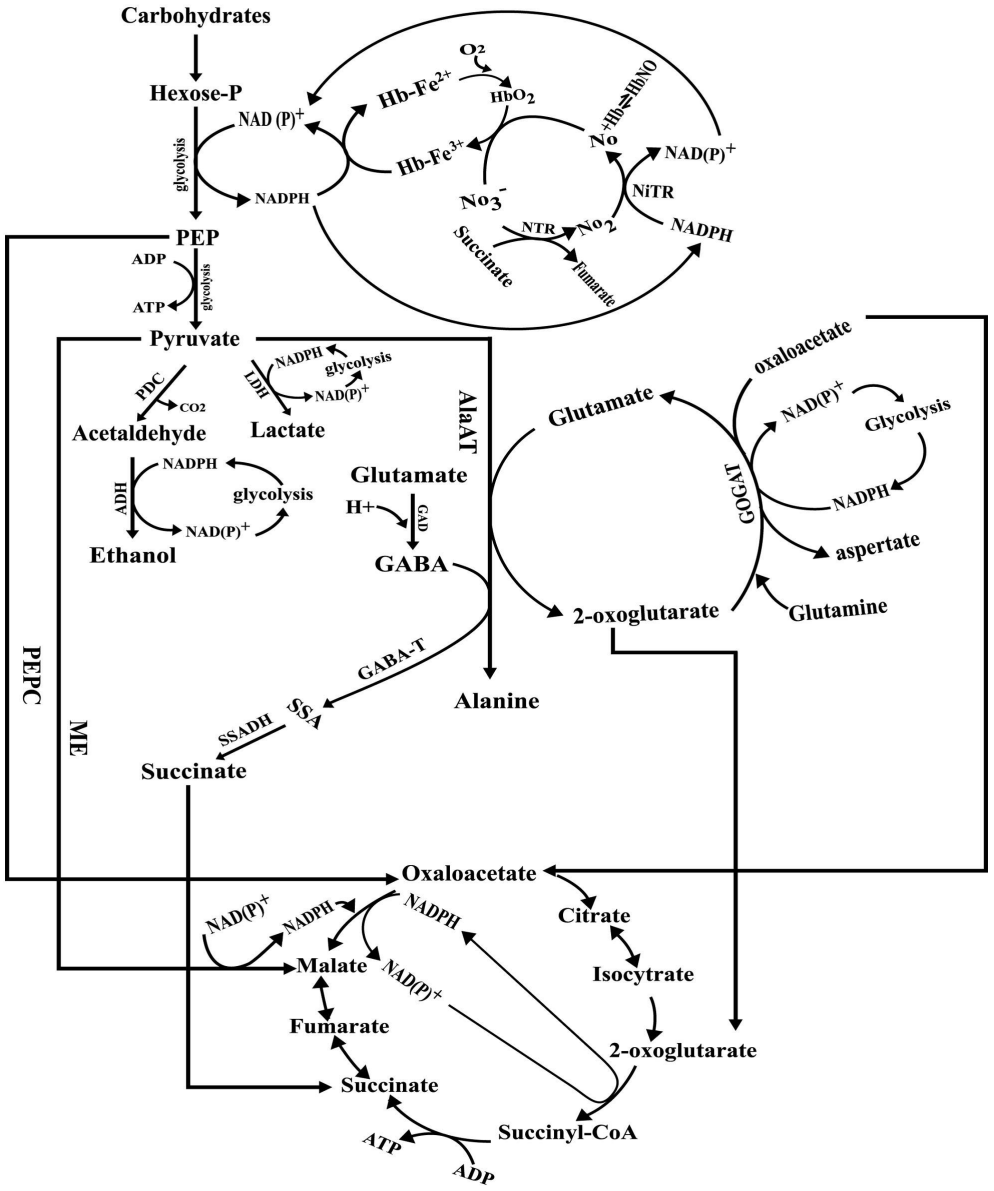

Fig. 3. Continuation of glycolysis and TCA cycle in plants using different biochemical and physiological pathways under waterlogged stress. Modified figure (16, 53, 62). ADH: Alcohol dehydrogenase; AlaAT: Alanine aminotransferase; GABA: Gama-aminobutyric acid; GABA-T: Gama-aminobutyric acid transaminase; GOGAT: Glutamine oxoglutarate aminotransferase; Hb-Fe^{+2}: haemoglobins; Hb-Fe^{+3}: Methaemoglobin; HbNO: Nitroso-haemoglobin; HbO_2: Oxyhaemoglobin; LDH: Lactate dehydrogenase; ME: Malic enzyme; NO_2^-: Nitrite; NO_3^-: Nitrate; PDC: Pyruvate decarboxylase; PEP: Phosphoenolpyruvate; PEPC: Phosphoenolpyruvate carboxylase; SSA: Succinic semialdehyde; SSADH: Succinic semialdehyde dehydrogenase.

through the 26S proteasome (33, 34). This happened in both the cytosol and the nucleus (35). Thus, *ERF-VII* proteins are degraded following the N-end rule pathway during aerobic conditions. On the other hand, plant cell cannot degrade *ERF-VII* during waterlogging or hypoxic stress due to the lack of oxygen and/or nitric oxide. Under hypoxic condition, non-degraded *ERF-VII* moves into the nucleus and takes part in the response of plant anaerobiosis (36). Though plant produces nitric oxide (NO) during the hypoxic stress, but the very rapid conversion of NO makes it nonfunctional to degrade *ERF-VII* protein. We will discuss the conversion of NO at the later stage of this paper under the heading of "Nonsymbiotic hemoglobin-nitric oxide homeostasis". Pre-adapted *Arabidopsis* plant to survive under waterlogging stress by producing ethylene-mediated NO-scavenger "Phytoglobin1", which reacts with NO and form NO-Phytoglobin1 complex (37). Thus NO temporarily loss their ability to degrade *ERF-VII* proteins under aerobic (no waterlogging stress) condition. In this mechanism, *Arabidopsis* produces a certain amount of *ERF-VII* before facing waterlogging stress, and produces fermentation genes just after the starting of waterlogging stress.

Importance of the stoppage of NERP

The *ERF-VII* transcription factor remains non-degraded during hypoxia for the lack of oxygen or nitric oxide (NO). This active *ERF-VII* enhances the activity of the first adaptive metabolic pathway in plants that is the anaerobic fermentation (22, 24). Overexpression of the *ERF-VII* transcription factors, *HRE1*, *HRE2*, *RAP2.2* and *RAP2.12* enhanced the alcohol dehydrogenase (ADH) activity only in

hypoxia but not in normoxia condition (38). *ERF-VII* not only induces the alcohol dehydrogenase (ADH) genes but also induce pyruvate decarboxylase (PDC) (29). The function of both the PDC and ADH ultimately completes the ethanolic fermentation in tobacco under low oxygen stress (39).

Though most reports explained the activation of ethanolic fermentation by the *ERF-VII* transcription factor, its effect on lactate fermentation is not well described except in *Arabidopsis* (40). Thus, *ERF-VII* activates both the ethanolic and lactate fermentation during waterlogging stress, though there is a contradictory which one is activated earlier.

Under hypoxic stress, lactate dehydrogenase (LDH) and its product lactic acid are produced earlier than ethanol. This leads to acidification of cytosol and ethanol is produced to neutralize the cytosol pH, which is first described (41) and later confirmed (42). We know this sequence of events as the Davies-Roberts hypothesis. Therefore, we may conclude that NERP activates LDH first than the PDC. Though, ethanol and lactic acid can be produced simultaneously in plants under hypoxic stress, depending on the species and the degree of O_2 deficiency (43). Over expression of enzymes for alcohol and lactate enhances waterlogging tolerance in tobacco (44) and *Brassica napus* (45) respectively.

Importance of lactic acid and ethanol

Mitochondrial aerobic respiration stops by blocking the TCA (Tricarboxylic acid) cycle due to the lack of a terminal electron acceptor during the hypoxic condition. This leads to the generation of ATP dropped from 36 to 2 moles per mole of glucose metabolized by using glycolysis and fermentation (anaerobic respiration) (46). To meet up the shortage of this energy, plant accelerates the glycolysis for its oxygen independency that leads to the reduction of carbohydrate reserve (47). In the lactate fermentation, pyruvate is converted to lactate through LDH, where NADPH is converted to $NAD(P)^+$ (Fig. 3). On the other hand, pyruvate is converted to acetaldehyde through the PDC without conversion of NADPH to $NAD(P)^+$ in ethanolic fermentation. In the next step, acetaldehyde is converted to ethanol by the enzyme ADH and converts NADPH to $NAD(P)^+$. Therefore, plants under hypoxia, continue oxygen-independent glycolysis and both the lactate and ethanolic fermentation continuously regenerate $NAD(P)^+$ and produces only 2 moles ATP instead of 38 moles (48).

Demerits of lactic acid and ethanol

Lactate fermentation is associated with cytosolic acidification, and most of the lactate is stored in the vacuole or released to the surrounding medium (42). The presence of unabated lactate might well cause cell death (49). If pH drops too much, the enzymes of the cell are denatured or precipitate, and the reactions may stop (50). In addition, proton accumulation (H^+) is increased under the low level of cytosolic pH, associated with NADH and NADPH or the hydrolysis of ATP (51). This happened for the lack of enough energy for the proper functioning of the H^+ATPase enzyme under hypoxic condition (52). In general plant switch from lactate fermentation to ethanolic fermentation to save the cell as ethanol helps to increase the cellular pH (41). Unfortunately, ethanolic fermentation may also increase the cytosolic pH, if the CO_2 (release from the reaction of pyruvate to acetaldehyde) is not extrusion to the surrounding medium (42).

Ethanol generally diffuses to the extracellular medium, which leads to a considerable loss of carbon during hypoxia and poses no major problem (53). But excess ethanol hampers ion transport system in plant which is detrimental to plant cell (54). It also has detrimental effects on somatic cell division, protoplasts growth, and embryogenesis (55, 56).

Nonsymbiotic hemoglobin-nitric oxide homeostasis

The plant contains both symbiotic and nonsymbiotic hemoglobins. Symbiotic hemoglobins or leghemoglobins are found only in nodules for N_2 fixation and nonsymbiotic hemoglobins are expressed in different parts and tissues by hypoxic stress for an oversupply of nutrients and are referred to as stress-induced hemoglobins. Nonsymbiotic hemoglobins are divided into two classes. Class 2 hemoglobins have similar properties with symbiotic hemoglobins to bind with oxygen but class 1 hemoglobins (presently known as phytoglobins) have distinct oxygen binding properties (57, 58). Overexpression of nonsymbiotic hemoglobin increases waterlogging tolerance in *Arabidopsis* (59, 60), cabbage (61) and maize (60).

There are two different nitrate reductase (NR) in the root, one in the cytosol (cNR) and the other attached to the plasma membrane and facing the apoplast (PM-NR). Plant cells produce nitric oxide (NO) by the cytosolic nitrate reductase (cNR) under hypoxic condition. Plasma membrane nitrate reductase (PM-NR) carry out the activities of cytosolic nitrate reductase (cNR), particularly during the night (62).

Methemoglobin ($Hb\text{-}Fe^{+3}$) can be reduced to hemoglobins ($Hb\text{-}Fe^{+2}$) via NADH-dependent reductases (63) (Fig. 3) to form $NAD(P)^+$ that will be used in glycolysis. NO reacts rapidly with oxyhemoglobin (HbO_2) forming nitrate (NO_3^-) and methemoglobin [$Hb(Fe^{+3})$]. Nitrate (NO_3^-) is reduced to nitrite (NO_2^-) to form fumarate from succinate by using nitrate reductase. NO_2^- is reduced NO by using nitrite reductase and the byproduct $NAD(P)^+$ is used in glycolysis (64). Nitroso-hemoglobin (NO-Hb) is formed in a plant cell to reduce the amount of the detrimental effect of NO by hemoglobins (65). As the formation of NO-Hb is a reversible reaction, the single NO again reacts with HbO_2 to form [$Hb(Fe^{+3})$] and NO_3^-.

Importance of nsHb/NO cycle

This cycle provides $NAD(P)^+$ from two different steps. The first one is MetHb to Hb, and the second is nitrite to nitric oxide. Therefore, the main advantage of this cycle is the alternative way to supply $NAD(P)^+$ for glycolysis rather than detrimental lactate and alcoholic fermentation. As this cycle contains more steps than the anaerobic fermentation, provides enough time for the plant to develop adventitious

roots, which is necessary for long-term hypoxia survival (66). The half-life of NO in biological tissues is estimated to be <6s (67). This makes it highly reactive, directly with metal complexes and other radicals and indirectly as a reactive nitrogen oxide species with DNA, proteins, and lipids (68). Thus, nsHb/NO indirectly helps to express *ERF-VII* genes in plants by reducing the amount of NO under hypoxic stress to run anaerobic fermentation. Under hypoxic condition, proton (H^+) extrusion is limited because of the shortage of ATP, as a result, proton concentration becomes high in the cytosol. nsHb/NO uptakes the excess proton and used to produce $NAD(P)^+$ from NADPH, that may help to reduce the cytosolic acidification.

The production of oxyhemoglobin (HbO_2) requires O_2 to bind with hemoglobin. This nsHb/NO cycle may not provide enough oxyhemoglobin (HbO_2) to react with NO to recycle Methemoglobin ($Hb\text{-}Fe^{+3}$) due to the limited supply of O_2 under hypoxic stress. Thus, this cycle may not continuously supply enough $NAD(P)^+$ to run glycolysis.

Alanine Aminotransferase pathway and TCA cycle

Alanine aminotransferase (AlaAT) is a complex pathway, act as a survival mechanism in *Lotus*, *Prunus*, grapevine and *Arabidopsis* against waterlogging stress (53, 69, 70, 71). In this pathway, previously produced pyruvate from glycolysis reacts with glutamate (Glu) to form 2-oxoglutarate by alanine aminotransferase (AlaAT) (Fig. 3) (53). 2-oxoglutarate reacts with glutamine to recycle glutamate by Glutamine Oxoglutarate Aminotransferase (GOGAT), where NADPH is converted to $NAD(P)^+$. This $NAD(P)^+$ recycled at glycolysis for a continuous supply of pyruvate to convert alanine. The NADPH produced in glycolysis may be used again to convert glutamine to glutamate (72). Glutamate is converted to gamma-aminobutyric acid (GABA) by using cytosolic proton (H^+) with the presence of glutamate decarboxylase (GAD) (Fig. 3). GABA reacts with pyruvate to form alanine and Succinic semialdehyde (SSA) by gamma-aminobutyric acid transaminase (GABA-T). SSA is converted to succinate by succinic semialdehyde dehydrogenase (SSADH), which is used in the TCA cycle. On the other hand, glutamate may reversibly react with oxoglutarate to form 2-oxoglutarate and aspartate by aspartate aminotransferase (AspAT) (Fig. 3). This 2-oxoglutarate is used to produce succinyl-CoA by the enzyme 2-oxoglutarate dehydrogenase (OGDH) by using $NAD(P)^+$ to produce NADPH. Succinyl-CoA converts to succinate by using ADP to produce ATP under long term hypoxic stress. Phosphoenolpyruvate (PEP) from glycolysis is converted to oxaloacetate via phosphoenolpyruvate carboxylase (PEPC). This oxaloacetate is produced in the plants by using two enzymes named phosphoenolpyruvate carboxylase (PEPC) and aspartate aminotransferase (AspAT) from two different pathways. Oxaloacetate is converted to malate by malate dehydrogenase (MDH), where NADPH is recycled to $NAD(P)^+$, which is used to the continuous biosynthesis of succinyl-CoA from 2-oxoglutarate by OGDH. Then, malate is converted to fumarate and fumarate is converted to succinate for oxidative phosphorylation to produce ATP. Therefore, the presence of more pyruvate in the hypoxic plant cell, more ATP will be produced from the TCA cycle. Another source of pyruvate in a hypoxic plant cell is the conversion of malate to pyruvate by the malic enzyme (ME). Where NADPH is produced from $NAD(P)^+$, this may be again used in the conversion of oxaloacetate to malate (53).

Importance of alanine aminotransferase pathway

Rapid and continuous conversion of pyruvate to alanine helps to continue the glycolysis pathway necessary for hypoxic ATP production (53). Excess accumulation of alanine is not harmful to plant cell as alanine production may balance the cytosolic pH under anoxic stress (73). Alanine aminotransferase pathway along with glutamate 2-oxoglutarate cycle provides $NAD(P)^+$ for glycolysis. Excess cellular proton (H^+) is indirectly consumed by this pathway during the conversion of glutamate to GABA. Alanine aminotransferase pathway indirectly produces the 2-oxoglutarate and oxaloacetate required for the TCA cycle. Thus, conversion of pyruvate to alanine produces not a single harmful product in plants under oxygen limiting stress, and it contributes to the reduction of cellular pH, provides $NAD(P)^+$ for glycolysis, consumes excess proton (H^+) and provides necessary products for the continuation of TCA cycle. Therefore, triggering the gene responsible for alanine aminotransferase may account for the ability to survive under flooding stress.

Modified TCA cycle or glyoxylate cycle

The tricarboxylic acid (TCA) cycle provides necessary energy (ATP) for growth and development in plants (74). Waterlogging stress noticeably hampers this necessary pathway as direct conversion of pyruvate to acetyl-CoA is restricted due to the non-functional activity of the pyruvate dehydrogenase enzymatic complex (75). In this situation, plants search for an alternative source of energy along with glycolysis and eventually starts the modified TCA cycle or glyoxylate cycle. The conversion of pyruvate to acetyl-CoA occurs in mitochondria and glyoxysome with three enzymes (76). Pyruvate decarboxylase (PDC) and mitochondrial aldehyde dehydrogenase (mALDH) used in mitochondria and acetyl-CoA synthetase (ACS) used in glyoxysome (Fig. 4). Pyruvate is transported to mitochondria from the cytosol and converted to acetaldehyde by PDC. Acetaldehyde is further converted to acetate by mALDH. Finally, acetate is transported from mitochondria to glyoxysome and converted to acetyl-CoA by ACS (77). Acetyl-CoA reacts with glyoxylate to form malate in the presence of malate synthase. Malate is converted to oxaloacetate that reacts with acetyl-CoA to form citrate. Citrate is converted to isocitrate and finally, succinate is produced from isocitrate by using isocitrate lyase. Finally, succinate reaches to mitochondria from glyoxysome for the production of ATP through oxidative phosphorylation. Generally, isocitrate is converted to succinate through mitochondrial TCA cycle by three consecutive steps (Fig. 3) but in the glyoxylate cycle needs only one step. That reduces the carbon uses at low oxygen stress in plants like rice (78).

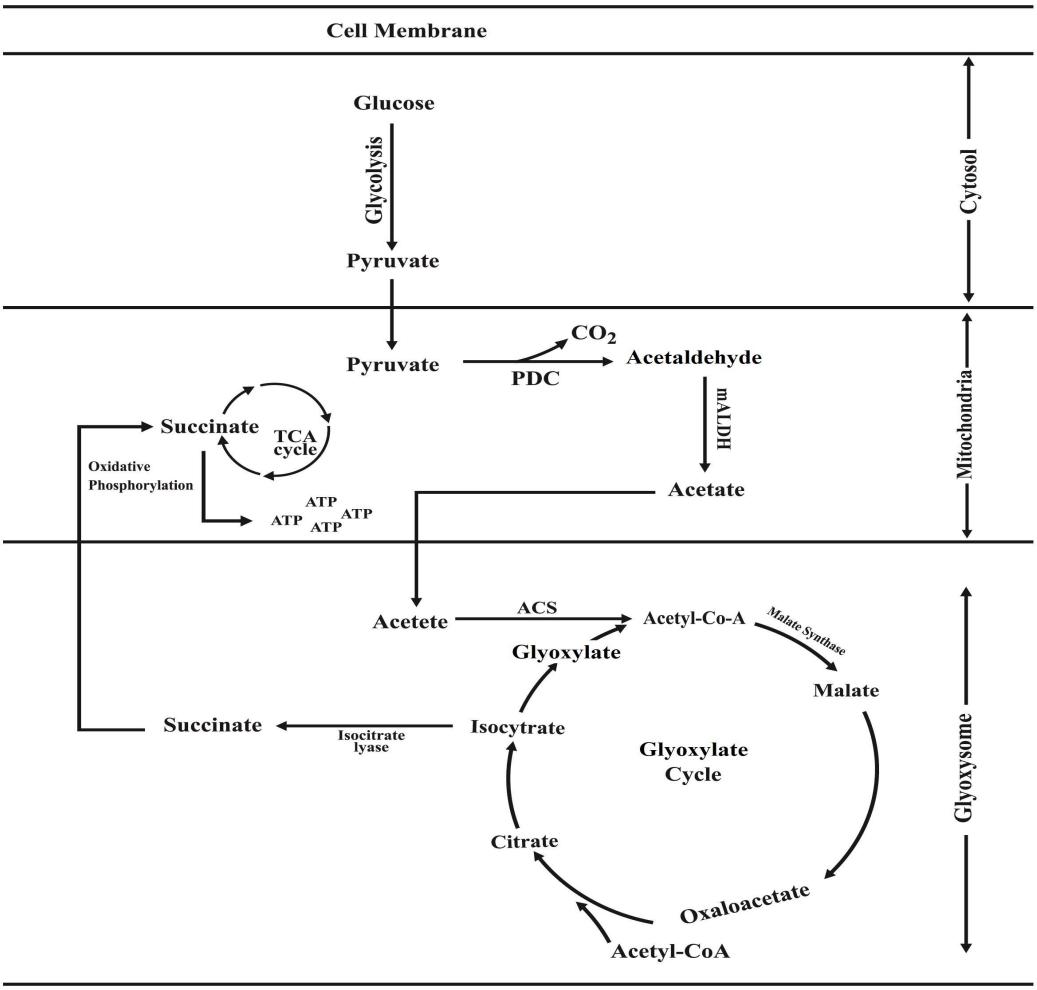

Fig. 4. Alternate TCA cycle (Glyoxylate cycle) in plants under low oxygen stress. Modified figure (77). ACS: ACC synthase; PDC: Pyruvate decarboxylase.

Calcium signaling and functioning in plants under hypoxic stress

Hypoxia increases cytoplasmic Ca^{2+} concentration in plants (79-81). The increased hypoxic Ca^{2+} concentration significantly influences different metabolic pathways, enzymatic functions, and ROS homeostasisplants. For example, cucumber plant maintains higher Ca^{2+} concentration under low oxygen stress, which prompts antioxidant enzymes, decreases ROS production, increases the number of mitochondria, plasma membrane proton ATPase (PM H^+-ATPase), tonoplast H^+-ATPase, tonoplast proton pyrophosphatase (tonoplast H^+-Ppase), different glycolytic and TCA cycle enzymes that helps ATP homeostasis (82). Modulation of glutamate decarboxylase (GAD) for the conversion of glutamate to GABA in *Arabidopsis thaliana* is also controlled by cytoplasmic Ca^{2+} concentration (83). Anoxic condition in plants quickly raises Ca^{2+} concentration and induces ADH activity (84). It also involved in antioxidant production and nitrogen metabolism in muskmelon (85). Formation of ammonium from nitrate enhances hypoxia tolerance in *Cucumis sativus* by incorporation of ammonium into amino acids and proteins (86). Plants can reduce ammonium toxicity by converting it into amino acids or by reserving into the vacuole (87).

Aerenchyma formation through the ethylene-calcium interaction

Ethylene induced calcium release mechanism under hypoxic stress has been described (88) and shown in Fig. 5. Ethylene activates plasma membrane G protein and produces plasma membrane phospholipase C (PLC). PLC hydrolyzes plasma membrane phosphatidyl inositol 4,5 biphosphate (PIP2) and produces 1,2-diacylglycerol (DAG) and cytosolic Inositol 1,4,5 triphosphate (IP3). IP3 activates the Ca^{2+} channel in the endoplasmic reticulum (ER) or tonoplast, which allows the release of Ca^{2+} to the cytosol and increases cytosolic Ca^{2+} concentration within plants' cell. In combination with DAG, IP3 activates cytosolic Protein Kinase C (PKC), which adds phosphates to the cellulases, xylanases etc., enzymes to make it active. Both DAG and Ca^{2+} can directly activate PKC independently. Therefore, this protein activation procedure through ethylene-calcium interaction may be considered as the basis of cellular homeostasis under hypoxic stress. These specific enzymes activate hypoxia-related genes, which allows programmed cell death (PCD) for aerenchyma formation. For example, protein phosphorylation activates the cell wall degrading cellulases, xylanases, xyloglucan endotransglucosylase hydrolases (XTHs) etc., for

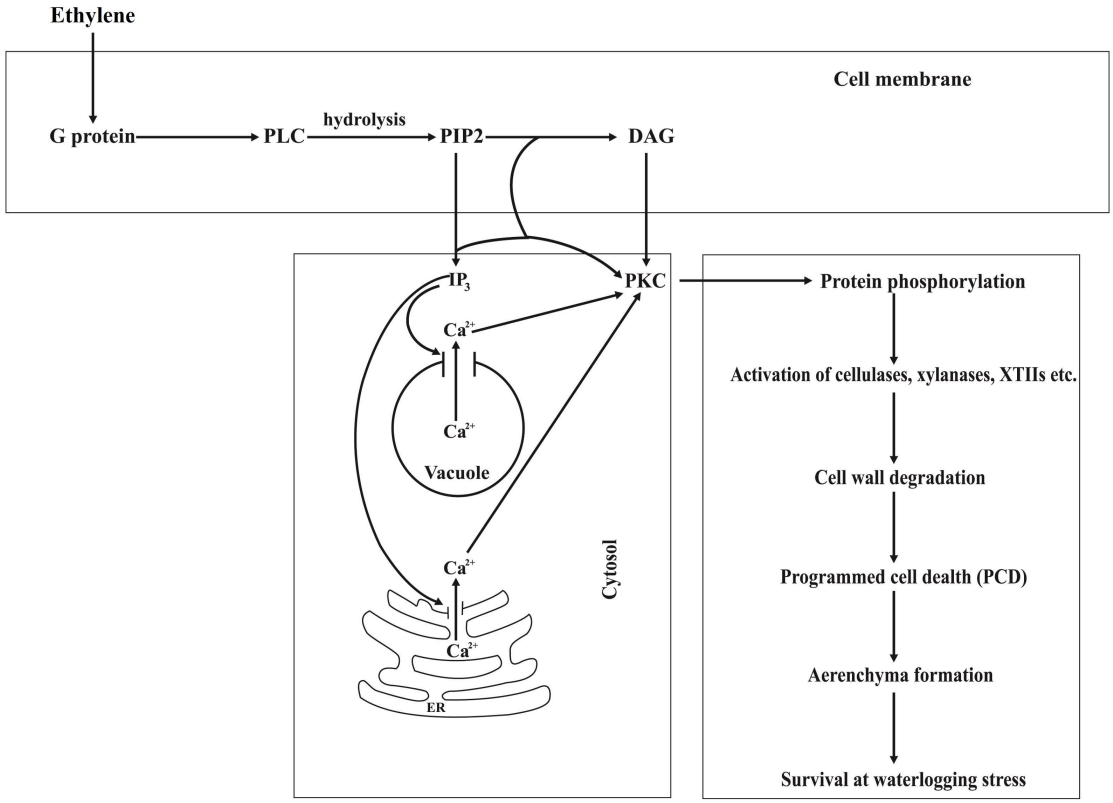

Fig. 5. Ethylene-mediated aerenchyma formation in plants under waterlogged stress. ER: Endoplasmic reticulum; DAG: 1,2-diacylglycerol; IP3: Inositol 1,4,5 triphosphate; PKC: Protein kinase C; PIP2: Phosphatidyl inositol 4,5 biphosphate; PLC: Plasmamembrane Phospholipase C; XTHs: Xyloglucan endotransglucosylase hydrolases

Table 1. Different signaling and physiological pathways in plants under waterlogging stress

Sl no.	Name of pathways	objectives	References
1	Ethylene biosynthesis	Produce ethylene signaling in plants	(7)
2	Ethylene signaling	Expression of *ERF-VII* genes	(4)
3	Disruption of N-end rule pathway	Stops the breakdown of *ERF-VII*, thus helping to express waterlogging specific lactate and ethanolic genes	(29, 38, 40)
4	Lactate and ethanolic fermentation	Continuous supply of $NAD(P)^+$ to run glycolysis	(48)
5	Nonsymbiotic haemoglobin-nitric oxide homeostasis	Alternate source of $NAD(P)^+$ to run glycolysis due to detrimental effects of lactate and ethanolic fermentation	(66)
6	Alanine aminotransferase pathway	1. Indirectly helps to supply $NAD(P)^+$ necessary for glycolysis 2. indirectly produces 2-oxoglutarate and oxaloacetate that are required for TCA cycle	(72)
7	TCA cycle	ATP production	(53)
8	Glyoxylate cycle	Skipping of two steps comparing with mitochondrial TCA cycle to avoid the loss of two carbon molecules, which indirectly helps to store ATP under hyoxic stress	(77)
9	Calcium signaling	Increases number of mitochondria, prompts antioxidant enzymes, decreases ROS production, increases plasma membrane proton ATPase (PM H^+-ATPase), tonoplast H^+-ATPase, tonoplast proton pyrophosphatase (tonoplast H^+-Ppase), increases different glycolytic and TCA cycle enzymes which ultimately helps ATP homeostasis	(82, 85)
10	Ascorbate-glutathione cycle	Remove ROS from plant cells	(104)

programmed cell death (PCD) in plants under hypoxic stress (4, 89).

RHO-like small G protein (ROP) signaling pathway for activation of anoxic ADH

Oxygen deprivation starts the ROP GTPase signal transduction pathway necessary for activating DPI (diphenylene iodonium chloride)-sensitive NADPH oxidase. This ROP GTPase signaling enhances defense responses and plants developmental processes, whereas abscisic acid-induced closure of leaf stomata is the effect of inactivation of ROP signaling (90). Free cytoplasmic Ca^{2+} binds with plasma membrane DPI-sensitive NADPH oxidase or inner mitochondrial membrane DPI-sensitive NADPH oxidase (91). Both the ROP signal transduction pathway and Ca^{2+} binding with NADPH oxidase finally activate NADPH oxidase. As a result, concentration of H_2O_2 (hydrogen peroxide) increases, that's acts as a secondary messenger for transmitting the information of

oxygen deprivation by expressing ADH (92). RopGAP4 (Rop (RHO-like small G protein of plants) guanosine triphosphatase activating protein 4) is a negative regulator of a RopGTPase, which promotes hydrolysis of GTP. Enhanced RopGAP4 expression down regulates RopGTPase and consequently down regulates H_2O_2 production. RopGAP4 expression is also controlled by the activation of NADPH oxidase (93). Perhaps the expression of RopGAP4 inside the plant cell shows the excess presence of ROS like H_2O_2 and toxic alcohol. Therefore, RopGAP4 plays a key role in maintaining the balanced volume of H_2O_2 and ADH to adjust the oxygen-deprived tissue inside the plant cell.

ROS and RNS production during low oxygen stress

Oxygen deprived plant tissues change several internal cellular processes like lipid content and composition, membrane fluidity, cytoplasmic acidosis, changes in the patterns of protein synthesis, and a decrease in the adenylate energy charge (94-96). Most of these changes are responsible for ROS production, which favors lipid degradation by peroxidation of lipids (94).

Different enzymes like plant homolog of the mammalian respiratory burst NADPH oxidase (RBOH), are responsible for systemic signaling under various environmental stress in plants by generating and amplifying ROS (97). There are different plant cellular organelles, but some maintain highly oxidizing metabolic activity. Chloroplasts, mitochondria and microbodies maintain their metabolism by transporting electron, which is also responsible for ROS production in plants to contribute an oxidative burst during abiotic stress (98). Plants stimulate H_2O_2 production as a key regulator for different responses under hypoxic stress (99). There are two main mechanisms available for H_2O_2 production under hypoxic stress. These are the consequence of the breakdown of the mitochondrial electron transport chain (m-ETC) and DPI-sensitive activation of NADPH oxidase through Rop-signaling. Under hypoxic stress, complex III of m-ETC produces superoxide (O_2^-) anion by increasing the abundance of the partially reduced ubisemiquinone anion. This O_2^- is rapidly converted to H_2O_2 through spontaneous dismutation or by a reaction with superoxide dismutase (SOD) (100). H_2O_2 may also be produced by potassium cyanide (KCN) and sodium nitroprusside (SNP) which are considered as the inhibitors of respiration (101). These are evidences for the increased formation of antioxidants, superoxide dismutase, peroxidase and catalase in pepper plants under waterlogging stress (102).

Plants Produce NO under oxygen deprivation stress by non-enzymatically from exogenous nitrite and enzymatically via cytosolic nitrate reductase and plasma membrane-bound nitrite-NO reductase (67). NO can react either with O_2 or O_2^- to produce NO_2 (nitrogen dioxide), N_2O_2 (nitrous oxide), and $ONOO^-$ (peroxynitrite). These have harmful consequences and signaling roles in biological systems (68). NO can easily be transported within the cell because of its capability to freely penetrate into the lipid bilayer. Its chemical properties make this gas as a good candidate for a signaling molecule. NO has a short half-life (in order of seconds) because of its free radical nature (one unpaired electron) (103).

Removal of hydrogen peroxide through ascorbate-glutathione cycle

A standard model for the H_2O_2 removal mechanism in the plant cell had been described (104). Ascorbate peroxidase (APX) uses two molecules of ascorbate (AA) to reduce H_2O_2 to water, with the concomitant production of two molecules of monodehydroascorbate (MDHA) (104). Monodehydroascorbate (MDHA) is enzymatically converted to ascorbate (AA) by monodehydroascorbate reductase (MDHAR) and non-enzymatically to dehydroascorbate (DHA). Dehydroascorbate is also reduced to ascorbate by the enzyme dehydroascorbate reductase (DHAR), with the concomitant reduction of glutathione disulfide (GSSG) to glutathione (GSH). We know these series of sequential steps as the ascorbate-glutathione cycle, which is responsible for the removal of harmful H_2O_2 from the plant cell.

Conclusion

Survival ability of plants under waterlogging stress depends on several consecutive signaling and physiological pathways that are involved in waterlogging stress tolerance (Table 1). All these pathways make a complex tolerance mechanism in plants at the oxygen deprivation condition. This study presents a simple illustration of interconnection with various signaling and physiological pathways under waterlogging stress condition, through which the plant tries to survive. Though increased survival ability against waterlogging stress was found in plants by manipulating the specific gene but still complete satisfactory tolerance was not found yet. Therefore, the correlation between the specific gene and pathway with other genes or pathways is to be considered efficiently to get a desirable waterlogging tolerant crop plant. Plants' responses to waterlogging stress have been progressively discovered from the fermentation to current findings. But, still we are very far away to control the highly multigenic waterlogging stress in plants. The future exciting and thrilling discoveries certainly help plant scientists at mitigating the harmful effects of waterlogging stress in the economy of farmers.

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Author Contributions

Conceptualization by KKB; Writing - original draft preparation by KKB and MZT; Writing - review and editing by MSI.

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