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Minireview

Energy metabolism in plants under water deficits

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Regardless of the mechanisms of stress resistance, the maintenance of functional integrity in plants imposes an energetic cost. This mini-review summarized the present knowledge on energy metabolism in plants under drought conditions with the focus on studies performed on wheat in author's laboratory.

During evolution plants have adapted to fluctuating environmental conditions, however their capacity to avoid, overcome or neutralize the effects of stress is limited. Although plants are able to avoid water deficits in their tissues, e.g. by reduced transpiration, they loose turgor and dehydrate when the tissues are not protected from dehydration or when drought is severe. Cellular dehydration causes reduction in the water potential (chemical activity of water) which subsequently may lead to metabolic disorders and membrane injuries. Higher plants vary considerably in their tolerance to dehydration: lethal leaf water potential ranged from — 1.2 MPa to — 500 MPa [1]. The biochemical basis of dehydration tolerance is not fully understood and despite a significant progress which has been achieved in the isolation of water deficit-induced genes, this knowledge is not sufficient to specify univocally the genes and/or gene products required for dehydration tolerance [2]. Adaptation to drought is certainly multifunctional and involves important modifications in gene expression [2], which lead to anatomical and morphological changes [3] as well as to adjustment of the metabolism to new cellular conditions [4]. These modifications may result in accumulation or depletion of certain metabolites, alterations in the activities of many enzymes and synthesis of certain stress-specific proteins. Preferential synthesis of some metabolites is associated with cellular osmoregulation enabling maintenance of growth or with protection of SH groups of proteins against denaturation [3]. All these processes leading to drought tolerance consume energy and this aspect reflects the costs of acquiring tolerance.

The plant response induced by environmental changes which causes phenotypic alterations without any inheritable genetic alterations is termed acclimation. Acclimative responses, e.g. predehydration, lead to physiological and biochemical adjustment which enables survival under a water deficit which is lethal to non-acclimated plants. For example, leaves of wheat subjected to dehydration during the early stages of its development were able to tolerate a significantly higher water deficit (acclimated leaves) at the mature phase than the leaves developed under optimal irrigation (non-acclimated leaves).

Abbreviations used: AEC, adenylate energy charge; ARC, anabolic reducation charge; CaM, calmodulin; CRC, catabolic reduction charge; WSD, water saturation deficit.

Successful acclimation of plants to drought conditions has been postulated to depend on high availability of NADPH [3, 5] and ATP [6]. This implies that such a response may be caused either by increasing the rate of energy regeneration or decreasing the rate of the energy-consuming pathways which are not of primary importance for cell survival. It seems therefore that in order to understand the effects of water deficits on plant metabolism it is necessary to know whether the metabolism is affected primarily at the level of ATP and NAD(P)H utilization or at the level of ATP- and NAD(P)H-regenerating pathways.

MODIFICATION OF ENERGY-REGENERATION UNDER WATER DEFICITS

ATP regeneration

Green plant cells have two separate electron transport systems coupled to ATP synthesis: photophosphorylation in chloroplasts and oxidative phosphorylation in mitochondria. ATP can be also regenerated during glycolysis, the only known mechanism of ATP regeneration in non-green cells under anoxia.

It is known that water stress reduces the level of ATP in both higher plants, e.g. hemp [7], *Xanthium strumarium* [8], sweet pepper [9], bean [10], *Digitalis lanata* [11] and wheat [12], and lower plants, e.g. mosses [13, 14]. Sensitivity of ATP level to even a slight water deficit is somewhat surprising in view of the finding that both photophosphorylation and oxidative phosphorylation are more resistant to dehydration than the photosynthetic carbon metabolism [15]. Under a mild water deficit (20% of tissue water loss; Table 1) even a transient increase in the ATP/ADP ratio is noted, which clearly indicates that, in drought, ATP consumption is more restricted than ATP production by limited CO_2 supply caused by stomatal closure and increased mesophyll resistance for CO_2 transfer to chloroplasts [15].

It is of interest that the observed changes of ATP level are negligible if desiccation of leaves is taken into consideration: the concentrations of ATP remain constant in water stressed wheat leaves [12] or even rise in dehydrated leaves of *Xanthium strumarium* [8]. Thus, one may presume that plant metabolism is not limited by insufficient ATP supply and that the rate of ATP synthesis responds to metabolic demand [16]. Similarly, practically unchanged adenylate energy charge (AEC; ATP + 0.5 ADP/ATP + ADP + AMP) until a severe water loss (60% water loss; Table 1) implies that the rate of ATP-regenerating pathways is determined by ATP demand [17].

The response of a plant expressed in terms of ATP level depends strongly on the rate of dehydration. In rapidly and severely dehydrated plants, both the decrease in ATP level and AEC has been observed [15], whereas in slow wilting plants ATP concentration and AEC values remained on the same level or even were slightly increased [8, 12]. These differences may reflect some acclimatory responses involved in protection of ATP regeneration during slow development of water deficit. However, the anabolic reduction charge (ARC; NADPH/NADP + NADPH) decreases with developing water deficit in leaves independently of the rate of water loss, whereas the catabolic reduction charge (CRC; NADH/NAD + NADH) decreases only at severe water deficit (Table 1). These observations seem to point to an important role of respiration and/or photorespiration

Table 1

Energy status of non-acclimated wheat leaves under water deficit.

Leaf water deficit is expressed as water saturation deficit (WSD) calculated as follows: [(fresh weight following 24 h rehydration — actual fresh weight)/(fresh weight following 24 h rehydration — dry weight)] × 100%.

Leaves	ATP/ADP	AEC	ARC	CRC	NAD(H)/ NADP(H)	NADH/ NADPH	ATP/ NADP
Control	5.4	0.87	0.6	0.3	0.8	0.4	16.8
20% WSD	6.0	0.87	0.5	0.3	0.8	0.5	19.4
40% WSD	4.8	0.86	0.4	0.3	1.9	1.3	23.9
60% WSD	3.9	0.84	0.3	0.2	2.0	1.1	28.0

in regulation of ATP and NAD(P)H level in plant tissues [17, 18].

Direct estimation of ATP production in plants is hardly possible since, at present, techniques for direct determination of P/O ratio in leaves *in vivo* are not available [19]. Oligomycin at a wide range of concentrations (from 0.25 $\mu g\ ml^{-1}$ to 10 $\mu g\ ml^{-1}$) decreased the rate of dark respiration in wheat leaf segments by only about 13% [17] and in barley protoplasts by about 15–20% [20]. Therefore the rate of ATP production in plant material could be calculated theoretically from the rate of oxygen consumption, assuming that the ADP : O ratio is 3 and 1 for the cytochrome and the alternative pathway, respectively [19, 21]. The respiratory capacity of wheat leaves for either pathway is almost equal [17]. This means that respiration in the presence of cyanide which represents the capacity of the alternative pathway is equal to that in the presence of SHAM which is ascribed to the capacity of the cytochrome pathway. Both respiratory capacities have been corrected for residual respiration, i.e. respiration in the presence of both cyanide and SHAM. The combined capacities of the cytochrome and the alternative respiratory pathways exceed the rate of the endogenous respiration, since the alternative pathway is operative only in about 50%. Thus, the theoretical respiratory ATP production *via* the alternative pathway did not exceed 16% of the total ATP production in the non-acclimated wheat leaves (Table 2). Water deficit increased in wheat leaves the rate of O_2 consumption and the capacity of both the cytochrome and the alternative pathway [17]. There is a lack of correlation between the capacity of alternative respiration and the actual participation of this pathway in total respiration. The physiological significance of the increased alternative pathway capacity without its increased engagement is unclear [22]. Such respiratory responses of wheat leaves to water deficits result in higher rate of ATP production, which was about 60% higher in water deficient leaves in comparison to the fully turgid leaves of non-acclimated plants (Table 2).

A reliable value of the phosphorylation potential (ATP/ADP × P_i) in plant under water deficit has not been so far obtained, as the data on the phosphorylation potential should refer to defined compartments, and the measurements in the whole tissue extracts are misleading. Adenylates are absent in vacuoles, whereas the large amount of metabolically inactive (60–80% of total pool) phosphate is located in va-

Table 2

Respiratory characteristic of non-acclimated and acclimated wheat leaves under water deficit (WSD = 50%).

Respiratory rates are expressed as O_2 uptake in nmol O_2/g dry weight per s. V_{total} is the rate of O_2 uptake measured in the absence of the inhibitors (KCN and SHAM). Cytochrome pathway capacity was determined as the SHAM-resistant respiration corrected for residual respiration. Alternative pathway activity ($\rho\ V_{alt}$) was determined in the presence of inhibitors of both pathways. In parentheses, the theoretical respiratory ATP production (nmol/g dry weight per s) calculated from the oxygen uptake of wheat leaves, assuming a P : O ratio of 3.0 and 1.0 for the cytochrome and the alternative respiration, respectively is given.

Leaves	V_{total}	Cytochrome pathway capacity	Alternative pathway activity	Residual respiration
Non-acclimated				
Control	24.7 (102)	14.3 (86)	8.2 (16)	2.1
Stressed	34.0 (162)	24.8 (149)	6.7 (13)	2.4
Acclimated				
Control	26.0 (114)	17.8 (107)	3.2 (7)	4.9
Stressed	37.8 (155)	21.9 (131)	11.6 (24)	4.1

cuoles and therefore changes in other compartments are masked [23].

NAD(P)H regeneration

NADPH and NADH are not metabolically interchangeable: NADPH is utilized for endoergonic reductive biosyntheses and NADH is involved in utilizing the free energy of metabolite oxidation to synthesize ATP. Water deficits decrease the NADPH/NADH ratio 2.5–3 fold in wheat leaves [15, 18]; this may indicate restriction in the amount of reduction equivalents available for anabolic reactions. However, the NADPH/NADH ratio lowered under water deficit may be advantageous because in that way it protects against electron donation to oxygen and hence, against formation of the potentially deleterious O_2^- (from triplet chlorophyll) and/or H_2O_2 (from the Mehler reaction). Since electron transport is considered to continue at a considerable rate even in severely stressed leaves [24, 25], NADPH regeneration would not be restricted by water stress. Increased carbon flow to such metabolites as malate, aspartate and alanine concomitantly with the delayed flow to other end products (e.g. carbohydrates) seems to support this supposition [26]. The relatively more intense incorporation of ^{14}C into metabolites of the glycolic acid pathway (photorespiration) also suggests that NADPH is removed from chloroplasts and enables the formation of sucrose on the alternative pathway when sugar synthesis in the Calvin cycle is restricted. Maintenance of the reduced glutathione content in water deficient leaves at the same level as in fully turgid ones suggests that protection against oxidative stress is sufficient enough to prevent the oxidation of glutathione and other non-protein thiol-containing compounds [27–29]. *In vivo* chlorophyll *a* fluorescence measurements provide additional evidence that water deficit does not exert a photoinhibitory effect since the F_v/F_m ratio (variable/maximal chlorophyll fluorescence) remains practically the same for dehydrated and turgid leaves [15]. In conclusion, it is evident that leaves possess mechanisms by which they can down-regulate the excess of excitation energy under water deficits.

It is interesting to note (Table 1) that water deficit increases the ratio of (NAD + NADH) to their phosphates (NADP + NADPH) in wheat leaves [15, 18]. Phosphorylation of NAD(H) to NADP(H) is catalyzed by NAD kinase (ATP : NAD^+ 2'phosphotransferase; EC 2.7.1.23). In wheat, two types of NAD kinase are present: a CaM-dependent and a CaM-independent enzymes, activated directly by Ca^{2+} [30, 31]. The former enzyme located in chloroplast stroma catalyzes the light-induced conversion of NAD to NADP using the ATP produced by photophosphorylation [30, 32, 33]. The occurrence of differentially regulated NAD kinase isoforms may permit final and more flexible control over pyridine nucleotide levels. Total NAD kinase activity remains practically unchanged in dehydrated leaves but the CaM-dependent NAD kinase activity declines rapidly in response to a mild water deficit [34].

MODIFICATION OF ENERGY-REQUIRING PATHWAYS UNDER WATER DEFICITS

The lowered ratio of reduced/oxidized pyridine nucleotides is associated with the thiol/disulfide redox potential, which governs the extent of photosynthesis, effectiveness of cytochrome and cyanide-resistant respiration [35, 36] and the activities of multiple enzymes of various metabolic pathways. These widespread effects are connected with large energy input for maintaining the thiol:disulfide potential which was estimated to be about 30% of the total respiratory energy in wheat leaves as based on the effect of NEM [37]. Drought stress lowers the energy input into regulation of the SH/SS equilibrium and concomitantly the amount of total SH groups (protein-SH and non-protein-SH, expressed per gram dry weight). Since the amount of SS groups does not increase at the expense of SH groups [38], the observed decrease in the SH/SS ratio seems to be due to the increased rate of proteolysis. Decreased protein content is a common response of plants to water deficits [39] and lowered thiol : disulfide potential may contribute to this decrease since (i) inhibition of protein synthesis may be mediated by oxidized glutathione; the potential regulation of protein synthesis initiation may be attributed to activation of a cAMP-independent protein kinase that phosphorylates the alpha subunit of eIF-2 [40] and (ii) SH oxidation increases susceptibility of proteins to hydrolysis [41]. Water deficits activate specific proteolytic pathways [42] and induce synthesis of a variety of stress-related proteins that help reverse protein denaturation [2, 42].

A quantitative evaluation of the efficiency of energy-consuming reactions was based on the measurements of the respiration rate in wheat leaves in the presence of specific inhibitors such as rifampicin, cycloheximide, orthovanadate and others [17]. This method is based on the assumption that elimination of an energy-consuming process by a selective inhibitor results in the reduction of ATP production proportional to the ATP requirement of this pathway [43, 44]. The energy requirements of the processes studied were quantify either directly, as the percentage of respiratory inhibition by specific non-respiratory inhibitors, or indirectly as the theoretical respiratory ATP expenses calculated from the experimentally derived values of decreased respiration and theoretical values of respiratory ATP production (Table 2). As shown in Fig. 1 the inhibitors applied reduced respiration to a different extent, e.g. RNA synthesis, estimated from the inhibitory effect of rifampicin, required about 20% of the total respiratory ATP production in fully grown wheat leaves and for protein synthesis, the proportion of ATP consumption calculated from the effect of cycloheximide was about 22% of the total. Water deficit increases an ATP input into all the main known synthetic processes, i.e. RNA and protein syntheses, ATP-dependent proteolysis, Ca^{2+}-dependent reactions and intracellular ion transport [17]. However, this increased ATP requirement varied and is the greatest for ATP-dependent proteolysis and for intracellular ion transport (Fig. 1). The observed increase in ATP demand for both these processes in water deficient leaves is consistent with the finding that a number of drought-induced gene products are involved in the degradation of abnormal proteins (ubiquitin) and in sequestration of ions during water loss [2]. Although water deficit decreases leaf sensitivity towards inhibitors of RNA and protein synthesis and of Ca^{2+}-mediated processes (Fig. 1), the calculated energy expenditure for these processes is higher in droughted leaves than in the control ones. This increased ATP requirement could be explained by the increased activity of the cytochrome pathway under water stress conditions (Table 2). Thus, in non-acclimated leaves, the effect of water deficiency on ATP consumption was, in decreasing order, significant for: ATP-dependent proteolysis, vanadate-inhibited ion transport, Ca^{2+}-dependent reactions, protein and RNA syntheses.

ENERGY REQUIREMENT FOR THE ACQUISITION OF HIGHER DEHYDRATION TOLERANCE

Despite a general agreement that acclimation of plants to any kind of environmental stress leading to a higher stress tolerance is associated with higher requirements for metabolic energy

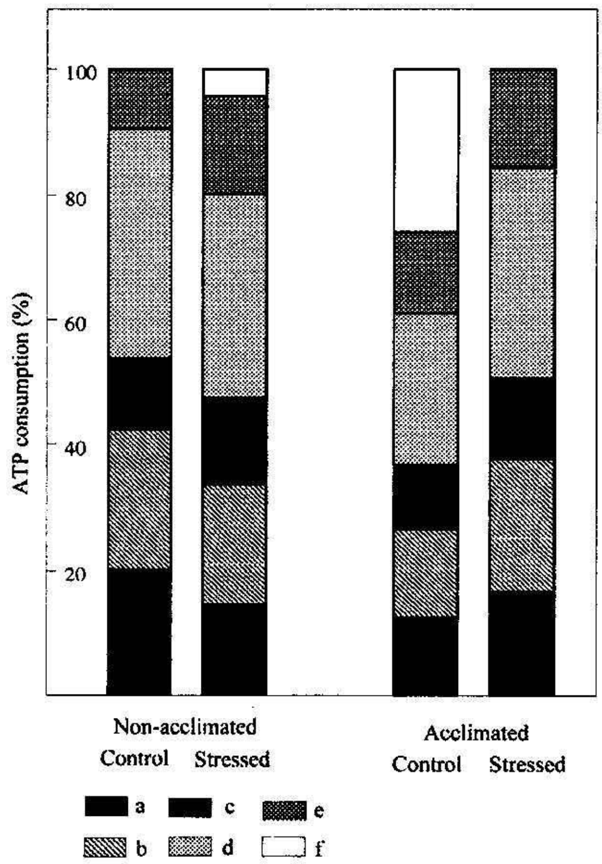

Fig. 1. Comparison of ATP consumption (in % of total respiratory production) for syntheses of RNA (a, rifampicin) and protein (b, cycloheximide), ATP dependent proteolysis (c, tosylamino-2-phenyl ethyl chloromethyl ketone), Ca^{2+}-mediated processes (d, $LaCl_3$), P-type ATPases (e, vanadate) in wheat leaves of non-acclimated control and stressed plants and of acclimated control and stressed plants.
The unfulfill bars (f) mean the amount of ATP consumed in unidentified process. ATP consumption was calculated on the basis of experimental data [17].

[3, 45], data on plant energy metabolism related to drought acclimation are practically none.

Acclimation of plants to water deficits results in higher ATP/ADP ratio (Table 3) which may indicate that ATP-regenerating pathways are potentially in excess of the ATP demand. The increased content of ATP in acclimated leaves is related to a somewhat higher production of respiratory ATP which may be attributed to increased activity of the cytochrome pathway with a simultaneous decrease of the activity of the alternative pathway from 16% to 6% of total respiration (Table 2). As a result, the NADP(H)/NAD(H) ratio increases significantly [18] and this has been linked also with the increase in CaM-dependent NAD kinase activity [34].

Acquisition of a higher dehydration tolerance is associated also with an important modification of ATP expenses: decreased energy demand for RNA and protein syntheses and for Ca^{2+}-dependent processes is accompanied by increased energy expenses for maintenance of the thiol/disulfide redox potential [17, 37]. This points to the significance of protein S-thiolation in regulation of metabolic processes by oxidation/reduction of enzyme thiols allowing more efficient protection against oxidative processes during subsequent water deficit.

Comparison of the ATP consumption for protein synthesis and ATP-dependent proteolysis reveals another difference in the response of wheat plants to water deficit. In the drought-nonacclimated leaves the ATP expense for ATP-dependent proteolysis corresponds to about a half of the requirement for protein synthesis, whereas in acclimated leaves it is increased to about 75% of the requirement for protein synthesis (Fig. 1). Since ATP-dependent proteolytic regulation in plants involves phytochrome, regulation of chromatin structure, gene transcription, DNA repair, cell-cell interaction and signal transduction [42], the increased expenses for ATP-dependent proteolysis in acclimated leaves point to the importance of these processes in acquisition of the higher dehydration tolerance.

Acclimation to drought is also associated with the increased availability of reducing equivalents for reductive biosyntheses (Table 3). The 50% decrease in the NADH/NADPH ratio could be ascribed to the lowered activity of the glycolic acid pathway [26] and alternative res-

Table 3

Energy status of acclimated wheat leaves under water deficit

Leaves	ATP/ADP	AEC	ARC	CRC	NAD(H)/ NADP(H)	NADH/ NADPH	ATP/NADPH
Control	5.9	0.87	0.7	0.4	0.3	0.2	16.1
20% WSD	6.8	0.87	0.7	0.3	0.4	0.2	17.9
40% WSD	6.8	0.87	0.5	0.2	0.6	0.2	28.3
60% WSD	6.7	0.86	0.4	0.1	0.7	0.4	40.1

Table 4

Respiratory characteristic of non-acclimated and acclimated stressed wheat leaves as a percentage of respective controls.

In parentheses, differences in respiratory ATP production are given (in nmol ATP/g dry weight per s).

Leaves	V_{total}	Cytochrome pathway capacity	Alternative pathway activity	Residual respiration
Non-acclimated Stressed	137.7 (+60)	173.4 (+63)	81.7 (−3.0)	114.3
Acclimated Stressed	145.4 (+41)	123.0 (+24)	362.5 (+17)	83.7

piratory pathway (Table 2) in the acclimated leaves. A significant shift of the ratio of non-phosphorylated to phosphorylated pyridine nucleotides, associated with the increase in the calmodulin-dependent NAD kinase activity [34], reflects the increased demand for reducing equivalents on acclimation.

In response to water deficit, acclimated leaves show a significant shift in contribution of the two respiratory pathways in ATP production (Table 4). Although the increase of the alternative respiratory pathway is relatively high, it does not exceed 30% of uninhibited respiration, so that its contribution is the same as in the non-acclimated control leaves (Table 2). Acclimation also changes the leaf sensitivity towards cycloheximide, TPCK (tosylamino--2-phenyl ethyl chloromethyl ketone) and $LaCl_3$ (Fig. 1). Thus, energy expenditure for protein turnover and for Ca^{2+}-mediated processes in acclimated leaves under water deficits returns to the initial values for non-acclimated control leaves. This puzzling result may imply that the observed changes in energy expenditure during acclimation are not related to the level of drought tolerance. On the other hand, it may be assumed that the changes in the amount and distribution of ATP during acclimation are needed for the reorganization of plant metabolism and/or cellular structures, so as to achieve higher dehydration tolerance in acclimated leaves and ensure their ability to run the basic metabolic processes at the same cost as in the susceptible non-acclimated leaves.

CONCLUSIONS

Water deficit seems to limit to a greater extent energy-utilizing than energy-regenerating pathways. The need for removing unused reducing power from chloroplasts resulted in increased activity of the glycolic acid pathway and the alternative respiratory pathway. As a result, the availability of reduced equivalents for anabolic reactions decreased more than the availability of ATP, and catabolic reduction charge remained unaltered. Dehydration of leaves resulted in greater expenses for the intracellular ion transport and ATP-dependent proteolysis.

Acclimation to drought seems to be associated with decreased expenses for RNA and protein syntheses and for Ca^{2+}-dependent processes despite a slight increase in total ATP production. Since the observed restriction in ATP-utilizing pathways is associated with a higher ATP/ADP ratio, the higher ATP pool found in the acclimated leaves seems to result from a decreased ATP demand. Acquisition of a higher stress tolerance seems to be related to a decrease in ATP demand, at least for the above-mentioned processes simultaneously with higher ATP demand for maintenance of the thiol/disulfide redox potential. As judged from the increased NADPH/NADH ratio, availability of NADPH for reductive biosyntheses is also higher in acclimated than in non-acclimated plants. Lowered activity of such pathways as glycolic acid pathway and alternative respiratory pathway (with maintenance of its greater capacity) seems to indicate that acclimation is also related to a restriction in wasteful oxidation of different metabolites.

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