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POSSIBLE ROLE OF ALTERNATIVE RESPIRATION IN PLANTS

K. ANNAMALAINATHAN AND JAMES JACOB

INTRODUCTION

Mitochondrial electron transport (MET) in plants may proceed through either the cytochrome pathway (CP) or the alternative pathway (AP). The cyanide resistant alternative respiratory pathway in plant mitochondria is one of the special features of plant respiration. Transfer of electrons from NADH through the CP is coupled at three sites with the production of ATP at the rate of three molecules per NADH. In contrast to the CP, the AP does not contribute to the generation of proton motive force beyond that point in MET where the two pathways bifurcate. The AP shares electron from the ubiquinone pool with the CP and bypasses two out of the total three phosphorylating sites (Lambers 1980). Therefore, 65% energy of the electron remains uncoupled in the AP (Moore and Siedow 1991). The unutilised energy is dissipated as heat in the AP. The key enzyme responsible for this pathway of plant respiration, uncoupled from ATP synthesis, is alternative oxidase (AOX). The AOX protein is found in every examined plant species (Vanlerberghe and McIntosh 1997).

The role of this enzyme has been the subject of a great deal of speculation in many plant species. The only well known function for the AP is the thermogenesis in aroids and other plant species where heat is produced during anthesis. The generated heat volatilises aromatic compounds to attract pollinating insects (Raskin *et al.*, 1987). Thus AP serves a vital biological function in these species. But why AP is present in all forms of plants is an intriguing question. What role does it have ?

The enzyme AOX appears to lower the production of reactive oxygen species (ROS)

in tobacco cells (Maxwell *et al.*,1999; Yip and Vanlerberghe 2001). It is reported that AP, being an overflow pathway for energy rich electrons derived from metabolic oxidation, may prevent over-reduction of the MET (Ribas-Carbo *et al.*, 2000). However, the functional role of AOX in cellular metabolism is yet to be established in many plants. The scope of the present review is to understand the recent developments in the science of AP and its physiological significance in plants.

ENERGY TRANSDUCTION

The AP has been generally considered as a wasteful process, because it is not involved in the production of ATP. However, some are of the opinion that this pathway is linked to energy transduction (Wilson 1980). The first hint that the low yield of ATP in cyanide resistant mitochondria was not due to inefficient process of oxidative phosphorylation itself but to the occurrence of a non-phosphorylating electron transport pathway was reported by Hackett *et al.* (1960). The non-phosphorylating nature of this pathway was demonstrated by using specific inhibitors namely salicylhydroxamic acid (SHAM) and potassium cyanide (KCN) or antimycin A. The addition of CN or antimycin A causes diversion of electrons through the AP resulting in marked decline in the efficiency of phosphorylation and ATP production. On the other hand, the addition of an inhibitor of AP considerably increases the efficiency of oxidative phosphorylation by diverting the electrons to the phosphorylating CP (Passam 1974). Early studies like these demonstrated that production of ATP associated with MET was not the main function of AP.

THE FUNCTIONAL ORGANIZATION OF THE ALTERNATIVE PATHWAY

It has constantly been assumed that like the CP, the AP also should react with oxygen through a terminal oxidase that is now well proved and known as alternative oxidase (AOX) (Elthon *et al.*, 1989). AP has been considered to be extremely short, consisting of very few elements like ubiquinone (UQ) and the terminal oxidase (Fig. 1). However, some proposals also have been made about the existence of some intermediary components in the AP. The rate of alternative respiration is the same with cyanide or antimycin. This fact showed that the electron must diverge to the AP at a point located before the site of antimycin in other words before the complex III in CP. The UQ, a small hydrophobic molecule has been recognised as a branch point of three pathways namely the CP, the AP and the flavoprotein pathway. It has been demonstrated that UQ is reoxidized if a pulse of oxygen is given in anaerobic mitochondria sample. Further the reoxidation is strongly inhibited by SHAM indicating UQ was a component of the AP (Storey, 1976). Reoxidation of UQ could have also been observed upon giving a pulse of oxygen to anaerobic mitochondria of potato in which AP activity was inhibited and this clearly demonstrates that UQ is an electron carrier shared by both the CP and the AP (Fig. 1). Many early observations clearly point to the fact that UQ plays a central role in both MET pathways and the flavoprotein pathway and it represents the branching point of these three pathways (Huq and Palmer 1978; Siedow *et al* 1978). The UQ is a small molecule containing 10 isoprene units (50 C) and have enough length of 56 Å to stretch across the inner mitochondrial membrane (Trumpower 1981). The AOX is located on the outer surface of the inner mitochondrial membrane.

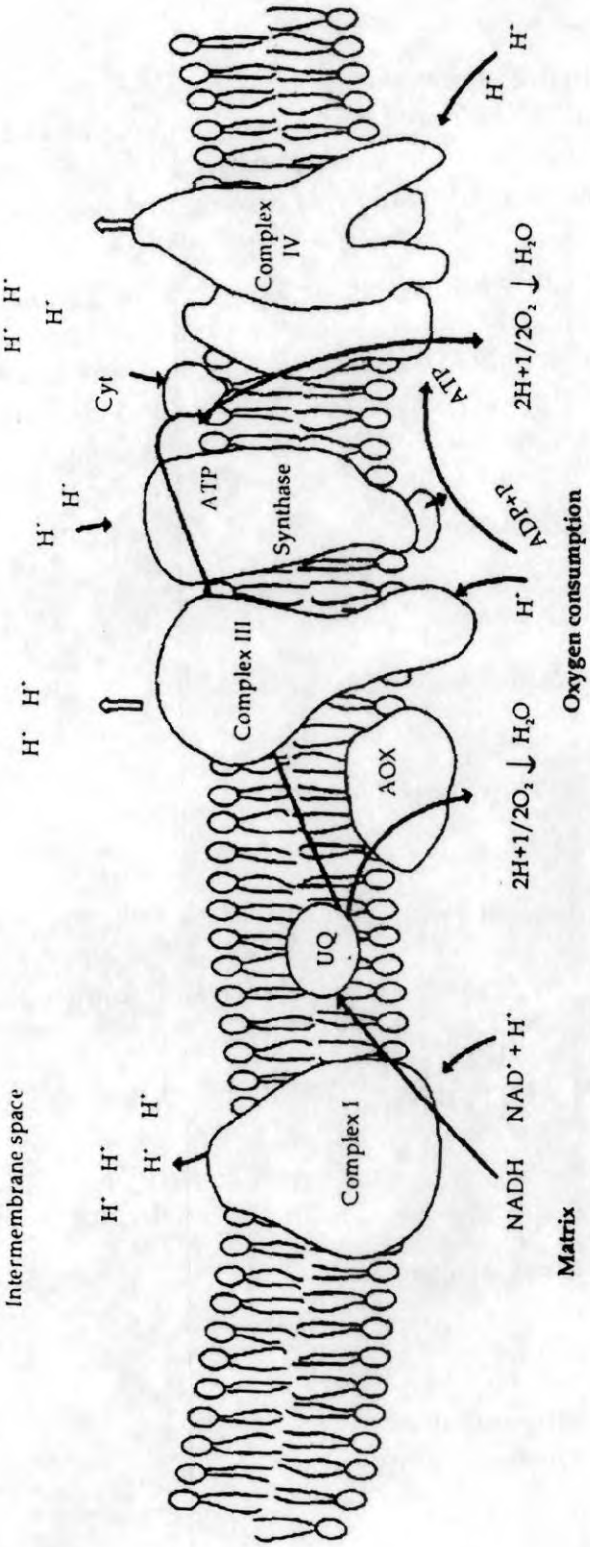

Fig. 1 : Diagramatic representation of cytochrome-C and alternative pathays of respiration in mitochondria

Regulation of electron partitioning between cytochrome and alternative oxidase

Experimental data suggest that electron partitioning between the CP and AP is modulated by the reduction state of the UQ pool and other components of MET. Tissue maturity, biosynthetic regulation of AOX protein components and redox status trigger activation of the CP via reduction or modification of sensor proteins (Svensson and Rasmusson 2001).

Published data indicate that CP is less active in light than in darkness, whereas AOX is upregulated by light (Atkin *et al.*, 2000; Millenaar and Lambers 2003). Upon illumination the activities of AOX and NADH dehydrogenase dramatically increase, which is in part due to enhanced protein synthesis (Svensson and Rasmusson 2001). The increase in AOX activity in light is not only due to the result of enhanced biosynthesis but also from the activation of the enzyme (Fig. 2) that occurs via the reduction of the

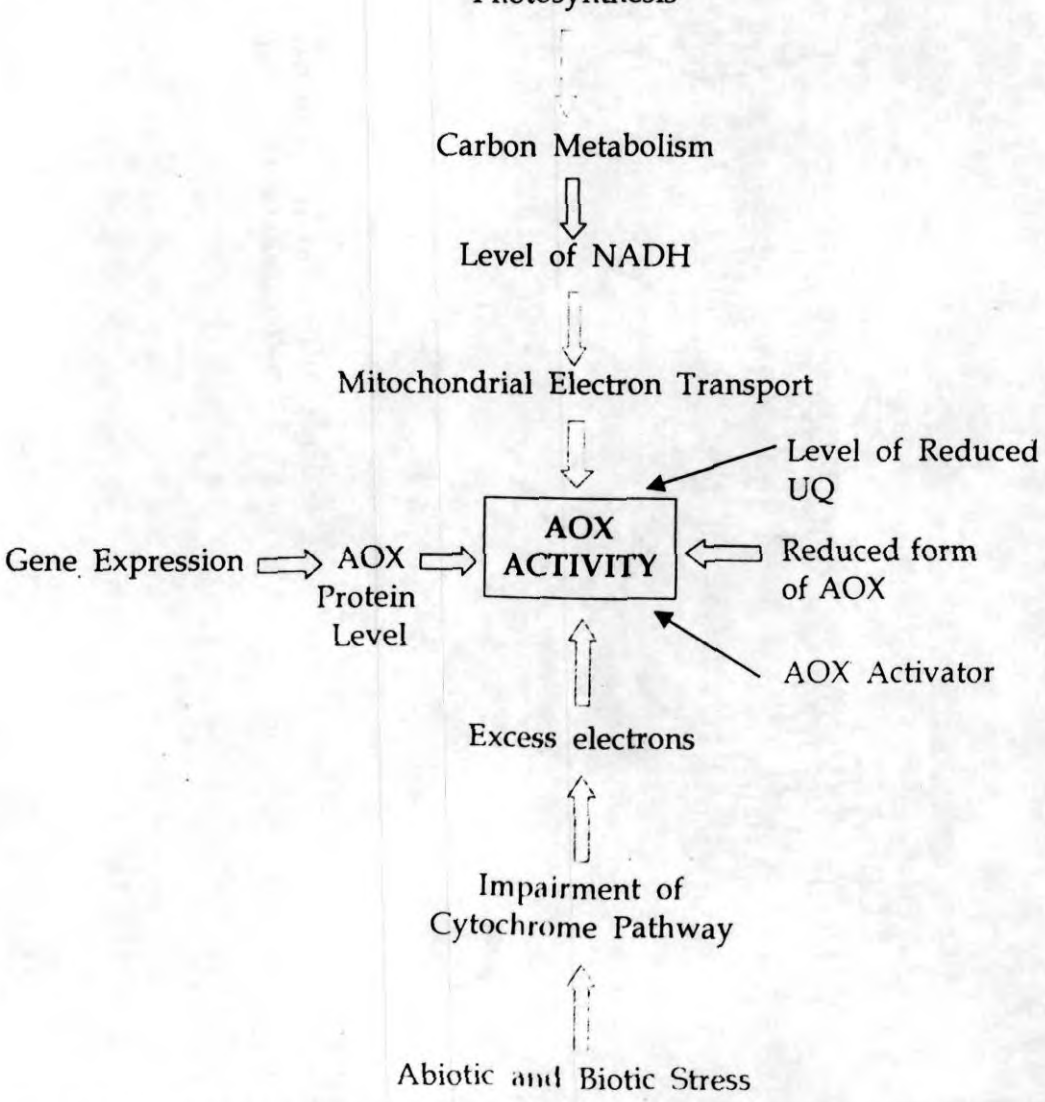

Fig. 2 : Schematic diagram showing various factors which control the flow of respiratory electrons to the alternative pathway (AP)

disulfide bond present in the AOX homodimer (Umbach and Siedow 2000). The increased expression of AOX in the light could result partly from the production of H_2O_2 of chloroplastic origin (Wagner 1995). Additionally, it has been reported that the accumulation of carbohydrates during active photosynthesis promotes the engagement of AP (Fig. 2) (Azcon-Bieto 1992).

Regarding the activity of AOX, AOX capacity and AOX engagement have to be distinguished (McDonald *et al.*, 2002). The AOX capacity in plant cells or tissue can be measured by the addition of a CP inhibitor (such as CN or antimycin A) followed by the addition of an AOX inhibitor (such as SHAM). The capacity is generally defined as the CN-resistant (but SHAM sensitive) O_2 uptake. Thus the capacity of AOX is a measure of maximum possible flux of electrons to AOX. The engagement of AOX is a measure of the actual flow of electrons through AP, in the absence of any inhibitors. It can be measured by using the oxygen isotope discrimination technique (Guy and Vanlerberghe 2005). An online ^{18}O isotope discrimination study demonstrated that the metabolic conditions inherent to a particular growth condition and biochemical regulatory properties of AOX are critical factors that control the electron partitioning between CP and AP (Guy and Vanlerberghe 2005). Interestingly, they have found that temperature and water status are parameters that have influence over AOX engagement.

BIOCHEMISTRY OF ALTERNATIVE OXIDASE

Plant AOX exists in the outer surface of the inner mitochondrial membrane as a less active homodimer that is covalently linked via disulfide bridges or as a more active non-covalently linked reduced homodimer (Umbach *et al.*, 1994). It has been proposed that NADPH arising from malate or isocitrate dehydrogenase activity contributes to the reduction state of the AOX. Additionally, AOX activity was shown to be induced by increasing the UQ pool size (Dry *et al.*, 1989). Two AOX protein bands have been identified in immunoblots of mitochondria from various organs of soybean with apparent molecular masses of 34 and 36 kDa (Kearns *et al.*, 1992). Tanudji *et al.* (1999) have reported multiple AOX bands in various soybean tissues. The enzymes are denoted as AOX1, AOX2 and AOX3. The mature forms of AOX1, AOX2 and AOX3 proteins have 280, 277 and 277 aminoacid residues with predicted molecular masses of 32.2, 31.8 and 31.7 kDa, respectively (Finnegan *et al.*, 1997). All sequencing data showed highly similar polypeptides in many plants. The general conserved sequences include two possible alpha-helical membrane spanning region, a surface exposed alpha-helix and N and C-terminal hydrophilic regions (Vanlerberghe and McIntosh 1997). It appears that increase in AOX protein generally parallels with an increase in AP activity. The AOX protein and mRNA all increased during the ripening process in many fruits (Cruz-Hernandez and Gomez-Lim 1995).

ALTERNATIVE OXIDASE GENE EXPRESSION

Monoclonal antibodies raised against the AOX of *Sauromatum guttatum* were used to isolate a cDNA clone known as Aox 1, encoding a 42 kDa protein (Elthon *et al.*, 1989). In *Hansenula anomala* an Aox 1 clone was isolated on the basis of transcript abundance after addition of KCN or antimycin A (Sakajo *et al.*,1991). The Aox 1 DNA clones have been isolated from a number of plants including tobacco, soybean and mango

(Vanlerberghe and McIntosh 1997). All Aox genes sequenced to date encode a highly similar polypeptides. Aox gene expression can specifically be altered through genetic transformation in tobacco (Vanlerberghe *et al.*, 1994), potato (Hiser *et al.*, 1996) and soybean (Finnegan *et al.*, 1997). The Aox genes were introduced in both sense and antisense orientations under CaMV 35S control. Sense plants with overexpression of AOX protein and mRNA abundance were observed in tobacco tubers and leaves but antisense plants with drastically lowered AOX were not isolated (Hiser 1996). However, a complete silencing of AOX expression by an antisense transgene was achieved in tobacco cells (Vanlerberghe *et al.*, 1994).

AOX has proved to be a useful protein with which to study the regulation of respiratory gene expression because it functions as a single gene product and also responds readily to many environmental conditions. The AOX expression appears to be regulated by catabolite repression and ROS (McIntosh *et al.*, 1998). The message for gene expression could involve ROS that originate from high reduction levels of the UQ pool in the light, probably induced by photorespiratory NADH and accumulation of oxidative substrates like pyruvate and succinate. Both the PQ in chloroplast and UQ pool in mitochondria make good sensors of the redox state of the MET due to their central location and function.

In soybean, AOX is encoded by a family of three genes (Whelan *et al.*,1996; Tanudji *et al.*, 1999). Multigenic encoding was also reported in rice (Ito *et al.*, 1997) and *Arabidopsis* (Saisho *et al.*, 1997). The expression of individual gene in soybean is regulated by tissue specific factors and environmental stimuli. AOX 1 has been detected in appreciable amounts in cells treated with antimycin A. AOX 2 is reported in photosynthetic tissues and AOX 3 seems to be constitutively expressed in all tissues except in root nodules (McCabe *et al.*, 1998; Tanudji *et al.*, 1999; Djajanegara *et al.*, 2002). It has been reported that multiple pathways exist in soybean to regulate expression of Aox genes and Aox 1 specifically induced by a variety of stress and metabolic conditions like low temperature, oxidative stress, incubation with antimycin A, citrate or salicylic acid etc. Altering the electron flow by the addition of antimycin A to tobacco suspension cells increased the levels of AOX 1 mRNA (Vanlerberghe and McIntosh 1994). H_2O_2, an ROS inducer also had a positive effect on the expression of the AOX gene in *Petunia hybrida* cells (Wagner 1995).

POSSIBLE PHYSIOLOGICAL ROLE OF ALTERNATIVE PATHWAY OF RESPIRATION

(a) Modulate the reduction state of MET components and ATP production

Any metabolic condition that leads to accumulation of either reduced UQ, mitochondrial NADH, cytosolic NADPH or pyruvate has the potential to increase electron flow to AP. Increased electron flow to AP is expected when there is an imbalance between the respiratory carbon metabolism and downstream electron transport through CP. Therefore, the most general function of AOX may be to balance carbon metabolism and regulate the electron transport (Vanlerberghe and McIntosh 1997). The MET rate is adjusted rapidly via activation of AOX. This may be a mechanism to prevent over-reduction of respiratory chain components (Wagner and Krab 1995). AOX may act to

shunt electrons from reduced UQ when CP is saturated with electrons or restricted by the availability of ADP. When cytosolic ATP demand and ADP availability are low, AOX activity is likely to play a role in which the reducing equivalents can be oxidized without ATP production (Lambers 1985). The engagement of AOX is mainly in order to regulate the redox state and avoid too high levels of reducing equivalents in the cells (Day and Wiskich 1995).

Pyruvate accumulation also could activate AOX. A transgenic plant lacking AOX showed production of large quantities of ethanol in cell culture (Vanlerberghe *et al.*, 1994). This aerobic fermentation is the result of a large accumulation of pyruvate in the cells. Therefore, activity of AOX seems to be essential for the maintenance of a balance between carbon metabolism and electron transport.

An important function of AP is to stabilize the reduction state of the UQ pool (Qr/Qt, *ie* ratio of reduced UQ to total UQ). If the AP is blocked with inhibitor SHAM, then Qr/Qt ratio will be less stable (Millenaar *et al.*, 1998). By stabilizing Qr/Qt ratio an increase in the production of free radicals and fermentation products can be prevented. In this way potential cell damage is prevented (Juszczuk *et al.*, 2001). The increase of AP rate can dampen to some extent the generation of ROS, which accompanies high rate of respiration.

(b) Nutrient uptake

Inorganic phosphate (Pi) uptake across the plasma membrane is an energy dependent process operated by a proton/Pi symport mechanism. Plants which have been grown under P limiting conditions displayed an enhanced capacity for Pi uptake (Muchhal and Raghothama 1999) and this rapid uptake was accompanied by a dramatic increase of respiratory O_2 consumption indicating the additional energy demand required to support increased H^+ ATPase activity (Weger 1996). The increase in O_2 uptake rate is due to enhanced activity of both CP and AP (Sakano 1998).

Given the non-phosphorylating nature of AOX respiration it has been suggested that elimination of AOX might increase crop yield (Gifford *et al.*, 1984). The argument is similar to the one about photorespiration (Osmond *et al.*, 1997). But studies by Sakano (1998) and Yip and Vanlerberghe (2001) have presented hypothetical functions of AOX as a part of a pH-stat during periods of rapid cytosolic acidification. The role of AOX is more relevant during periods of rapid changes in total dark respiration rate that occurs to support rapid nutrient uptake. The activity of AOX allowed high rates of respiration during phosphate uptake without large increase in UQ-pool reduction thereby dampen to some extent the generation of ROS (Millar *et al.*, 1998).

(c) Drought and oxidative stress

In nature plants are subjected to many forms of environmental stresses. The abiotic stresses such as extremes of temperature, drought, pollutions, etc and biotic stresses such as pathogen invasion, herbivory, parasitism etc are common in various agro-climatic regions. At the cellular and molecular levels a common feature of stress is the formation of free radicals and ROS - strong oxidants that can cause significant damage to membranes and vital molecules like DNA and proteins. It is possible to understand

the influence of environmental stress from respiratory metabolism before the plant shows visible symptoms (Smith *et al.*, 2000).

Aox 1 gene expression is reported to be altered by reactive oxygen species (ROS) such as superoxide, H_2O_2, hydroxyl radical. There is a hypothesis that ROS can enhance the expression of Aox genes (Vanlerberghe and McIntosh 1996). The generation of harmful ROS is a natural consequence of metabolism in an aerobic environment. They act as signals regulating the expression of a range of genes (Schreck *et al.*, 1991).

Kumar and Sinha (1994) have explained the possible role of AP in temperature rise in water stressed sorghum plants. Ribas-Carbo *et al.*, (2005) have recently studied the effect of three different levels of water stress on the activity of AOX. Unlike many other stresses, water stress did not affect the levels of mitochondrial AOX protein. They have shown that severe water stress caused a significant shift of electrons from the CP to the AP. The electron partitioning through the AP increased from 12% under well-watered conditions to nearly 40% under severe water stress. Consequently, the calculated rate of mitochondrial ATP synthesis decreased by 32% under severe water stress. It has been reported that addition of 25 µM antimycin to soybean cell suspension caused a dramatic increase of intracellular ROS (Djajanegara *et al.*, 2002). Clear over-expression of AOX in soybean wild-type plants experiencing water stress has been reported (Ribas Carbo *et al.*, 2005; Annamalainathan *et al.*, 2006) and it was likely that drought mediated impairment of electron transport through CP lead to diversion of more electrons to AP. Further the cyt-c activity was inhibited by antimycin A and this led to diversion of more electrons through AP as an 'overflow' mechanism to oxidise the excess NADH. This can lead to stabilization of the UQ pool and prevention of excess ROS generation in mitochondria (Ribas-Carbo *et al.*, 2000).

The level of AOX protein in a tissue can change in response to different growth conditions. Increased levels of AOX may be a response to abiotic stresses (Bartoli *et al.*, 2005; Noguchi *et al.*, 2005). Higher AOX biosynthesis by up-regulation of Aox genes enhances photosynthetic electron transport (PET) rate under drought conditions (Bartoli *et al.*, 2005). To demonstrate the expression pattern of AOX protein under drought stress

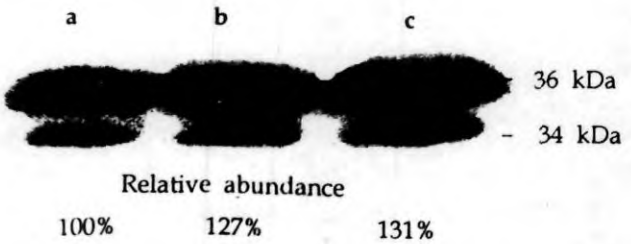

Fig. 3 : The alternative oxidase protein (AOX) of mitochondrial preparations detected by the AOX monoclonal antibody. The two isoforms of AOX (34 and 36 kDa) and their relative abundance are indicated. Soybean plants were subjected to various levels of drought stress. Lane a: well irrigated control plants, lanes b and c: partial and severe drought imposed plants, respectively. Mitochondrial protein of 40 µg was loaded uniformly in each lane. The relative abundance of the protein was calculated based on the value for control plant as 100%

condition, an experiment was conducted with well irrigated and drought stressed soybean plants. The level of water stress (by withholding irrigation) was characterized in terms of light-saturated stomatal conductance (g_s) : well irrigated (g_s > 0.2 mol H_2O m^{-2} s^{-1}), mildly or 50% water stressed (g_s between 0.1 and 0.2 mol H_2O m^{-2} s^{-1}) and severely water stressed (g_s < 0.1 mol H_2O m^{-2} s^{-1}). Mitochondria were isolated from 0, 50 and 100% water stressed plants and subjected to immunoblot with AOX antibodies (Fig. 3). Compared to the unstressed plants the 50 and 100% water stressed plants recorded 27 and 31% increase of AOX protein abundance, respectively (Annamalainathan *et al.*, 2006). This particular study demonstrates the probable role AOX over-expression under drought condition. The AP plays an important role of bypassing the electron transport when the normal activity of the CP is restricted or impaired by any stress or other cellular parameters which are not conducive for the normal functioning of CP (Wagner and Krab, 1995; Yip and Vanlerberghe 2001; Annamalainathan *et al.*, 2001).

Transgenic plants with different levels of AOX expression is a very convenient system to test the functional role of alternative respiration. Transgenic soybean plants with reduced AOX expression were used to test the hypothesis that AP functions as a protective mechanism during the leaf tissue experiencing oxidative stress (Annamalainathan *et al.*, 2006). This pathway is probably preventing the over-reduction of electron transport components in mitochondria and thereby avoids the production of excess ROS and thus eventually protects the membranes from oxidative damages during environmental stress conditions.

(d) Chilling stress

Some studies show that low temperature increases the amount and activity of the AOX in plants (Stewart *et al.*, 1990; Gonzalez-Meler *et al.*, 1999). The potential for AP to ameliorate chilling stress has been explained based on the dissipation of excess energy as heat when this pathway is active (Purvis and Shewfelt 1993; Ribas-Carbo *et al.*, 2000). The levels of AOX and rates of AP increase when exposed to low temperature in tobacco cultures, maize seedlings, mature leaves of mung beans and pea (Vanlerberghe and McIntosh 1992, Gonzalez-Meler *et al.*, 1999). Chilling stress causes photo-oxidative damage under illuminated conditions by increasing the formation of harmful ROS (Wise 1995). It has been postulated that the AP can stabilize the reduction state of the UQ pool when the cytochrome pathway is impaired during chilling stress and dampen the generation of ROS (Millenaar *et al.*, 1998; Ribas-Carbo *et al.*, 2000).

(e) Pathogenesis

CN-resistant AP was studied in *Arabidopsis* during infection with *Pseudomonas syringae* (Simons *et al.*, 1999). Total leaf respiration increased as the leaves became necrotic. The pathogen rapidly induced AOX mRNA. Further, the increase in mRNA has been correlated with the increase in AOX protein and increased expression of AOX was confined to the infected leaves. It has been established that the rapid induction of AOX was associated with necrosis and production of ethylene. There was increased pyruvate level also in the infected leaves which suggested that increased substrate levels were respired through AP. Salicylic acid (SA) is known to induce AP by activating

expression of the Aox gene (Xie and Chen 1999). It has been reported that salicylic acid analogues which are capable of inducing pathogenesis-related genes confer enhanced disease resistance also. Given the recently demonstrated roles of mitochondria in plant disease resistance, the involvement of AOX, at least in part through the metabolic alteration in plant defence responses can not be ruled out. Additionally, the involvement of mitochondrial functions and modulations of the rates of CP and AP during programmed cell death in plants suggest the possible role of AOX in plant disease responses (Robson and Vanlerberghe 2002).

(f) Photosynthesis

In photosynthetic cells, the capture of light by the photosystems in the chloroplast leads to the transport of electrons through an array of redox components and results in the production of ATP and NADPH in the chloroplast. The predominant electron transport pathway inside the chloroplast is non-cyclic and results in the reduction of NADP by ferredoxin-NADP oxido-reductase to NADPH. Light exerts a control on the photosynthetic process and enzyme activities including a number of enzymes of the Calvin cycle. Light also has a major role in the regulation of gene expression (Pfannschmidt *et al.*, 2003).

In green cells mitochondrial respiration is of major importance both in the light and dark. Oxidative phosphorylation in mitochondria is necessary to optimise photosynthetic metabolism in chloroplast as the former is needed to balance cellular energy and redox status. Any inhibition of mitochondrial ATP synthase or oxidative phosphorylation results in partial inhibition of photosynthesis in pea (Kromer *et al* 1988). Evidence has been also provided to show the mitochondrial response in light through the dissipation of excess photo-reductants (Padmasree and Raghavendra 1999; Igamberdiev *et al.*, 2001; Lis and Atteia 2004). The redox state has an important role in the metabolism of both chloroplasts and mitochondria. Light generates redox signals in the chloroplasts that can be exported to cytosol and mitochondria through redox shuttles. The ATP/NADPH ratio can also be regulated and balanced by export of NADPH from the stroma to the cytosol. NADPH cannot cross the membranes directly, it must be transported via shuttle systems.

Photosynthetic efficiency decreases when plants are exposed to light intensities higher than what is necessary for normal metabolism. This phenomenon known as photoinhibition is due to the over-reduction of the photosynthetic electron transport components (PETC) causing the inactivation of the PS II reaction centre. Photorespiration is believed to be a redox sink regulating the ATP/NADPH ratio. Therefore, photorespiration appear as an energy sink to avoid the over-reduction of the PETC and contributes to the prevention of photoinhibition. In *Chlamydomonas* photoinhibition was increased when the cells were incubated in the presence of cyt-c inhibitor (KCN or antimycin A). The photoinhibitory recovery rate was also slower in these cells (Singh *et al.*, 1996). The AOX pathway is likely to play a role in the prevention of photoinhibition of photosynthetic apparatus by oxidizing the reducing equivalents without ATP production (Lambers 1985). Export of chloroplastic NADPH through the malate valve and DHAP via the phosphate translocator on the chloroplast membrane produce more

NADPH in the cytosol. It has been proposed that oxidation of cytosolic NADPH and utilization of excess mitochondrial NADH through AOX pathway may protect green cell from photoinhibition (Lis and Atteia 2004).

Oxygen consumption in leaves of various plants was found to be up to 3.5 fold higher in the light than in the dark (Padmasree *et al.*, 2002). It has been explained that this in part is due to the oxidation of photorespiratory NADH via the AP. In agreement with this, it has been reported that AOX increased substantially in mature leaves under photoinhibitory conditions (Lennon *et al.*, 1995). In addition, by immunoblot analysis, AOX was detected in illuminated mature and senescent potato leaves but could not be detected in dark treated leaves (Svensson and Rasmusson 2001). In soybean, significant increase in Aox 2 mRNA was reported when the etiolated leaves were transferred to light. This explains a possible role of light in the transcriptional regulation of AOX protein synthesis (Finnegan *et al.*, 1997).

SIGNIFICANCE OF ALTERNATIVE RESPIRATION IN RUBBER PLANTS

Natural rubber (*Hevea brasiliensis*) or para rubber tree is a perennial tree crop. It is the source of 95-98 percent of the natural rubber produced throughout the world. *Hevea* is planted in over 20 countries stretching from countries in South East Asia to Ivory Coast in Africa and some parts of Central and South America, with an estimated cultivated area of 9 million hectare (ISRG 2006).

Natural rubber latex is the cytoplasm of specialized tissues called laticifers, which are oriented in the bark tissues of the tree. Tapping is a systematic wounding process of thin shaving of the soft bark tissue. Upon tapping laticifers are cut opened and they expel latex. Latex contains 25-35% rubber as small particles suspended in a serum together with 5-6% non-rubber substances like proteins, acids, salts, sugars, oils, resins etc. The remaining major component is water (d'Auzac and Jacob 1989). In tapped trees there is a partition of carbon assimilates between two important physiological process, namely, growth of the tree and rubber biosynthesis. The percentage of allocation for these two processes is a clonal character (Templeton, 1968). Such a partitioning process is not warranted in an untapped tree (except for a maintenance factor) with small amount of latex in their latex vessels which is not harvested. Therefore the biomass of an untapped tree is significantly larger than that of a tapped tree of similar age. Additionally, tapping causes loss of photosynthates through increased maintenance respiration which can also have a bearing on the biomass of the trees. It has been reported that maintenance respiration was generally high in tissues with high metabolic activity (Szaniawski, 1981).

Tapping resulted in enhanced respiratory activity (Annamalainathan *et al.*,1998). The soft bark tissue respiration, including CP and AP rates were higher in tapped trees than untapped trees (Annamalainathan *et al.*, 2001). The AP mediated oxygen uptake significantly increased due to tapping. The increased rate of AOX recorded in the tapped tree could be explained by wound-induced stimulation of AOX. In another study, the respiratory rates were measured in isolated mitochondria from soft-bark tissue of tapped and untapped trees. NADH was used as a substrate for mitochondrial electron transport reactions. The NADH dependant total respiration rate was significantly higher in tapped

trees. The AP mediated oxygen uptake also significantly increased due to tapping (Fig 4). The potential or maximum capacities of CP and AP were measured with the addition of ADP in the presence of appropriate inhibitors of MET chain. The potential rate of AP was recorded in which the CP activity was impaired (Fig 4). The capacity is generally defined as the oxygen uptake resistant to the cyt inhibitor and sensitive to the AOX inhibitor.

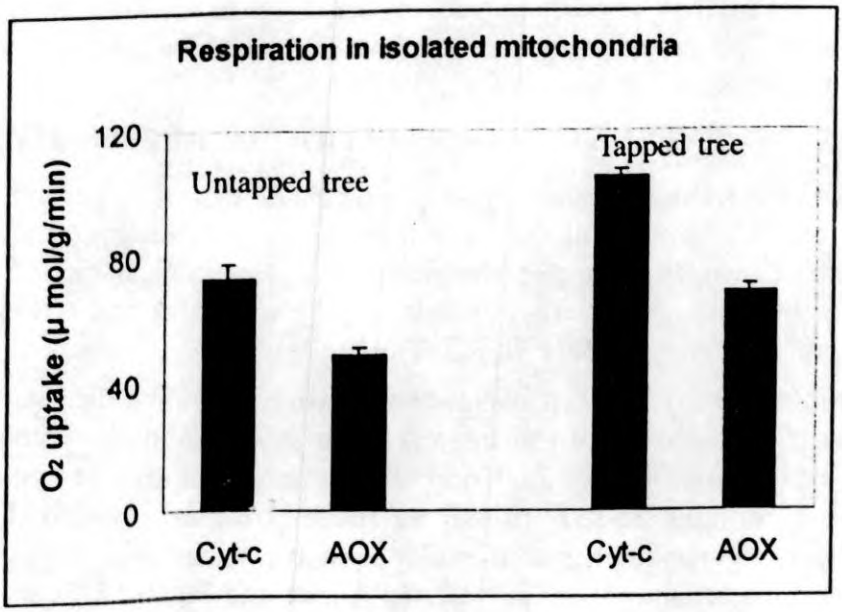

Fig. 4 : Rates of respiration in mitochondria isolated from the soft-bark tissue of untapped and tapped trees of *Hevea brasiliensis*. The capacity of AP (AOX) and CP (Cyt-c) were assayed by the addition of a CP inhibitor (such as CN or antimycin A) followed by the addition of an AP inhibitor (such as SHAM) and *vice versa*, respectively

It has been reported that *Hevea* clone RRII 105, the most popular and high yielding rubber clone in India, has the highest percentage of loss of biomass in a study in which 10 clones were included (Annamalainathan *et al.*, 1998). There was a direct positive relationship between the yield and shoot biomass loss (Sethuraj, 1992). The high rubber yielding clones with proportionately more tapping-induced biomass reduction also had increased rates of AOX activity (unpublished data). It could be explained that the occurrence of more AP may be one of the reasons for the unaccountable biomass loss upon tapping. Tapped trees recorded higher CP possibly to supply adequate amounts of ATP for the enhanced metabolism, including rubber biosynthesis.

The enhanced respiration found in tapped trees was related to the extremely high concentration of ATP reported in latex (Annamalinathan *et al.*, 2001, Sreelatha *et al.*, 2004). Significant quantities of ATP are lost through the latex and that may have a bearing on the loss of biomass in a tapped tree (Annamalainathan *et al.*, 2001). Respiration studies were carried out in trees affected by tapping panel dryness (TPD), a physiological disorder affecting rubber trees. Compared to healthy trees TPD affected trees recorded significantly higher rates of respiration and lower ATP content (Krishnakumar *et al.*,

2001). This was mainly due to the increase in the non-phosphorylating AP in the TPD affected trees. By this way the TPD syndrome in the tapped bark tissue seems to be analogous to the senescence process of other plant organs.

CONCLUDING REMARKS

The AP in algae and higher plants has been generally considered as a wasteful process but many studies show that AP has certain vital physiological significance. It is considered to be a protective pathway in mitochondria akin to photorespiration in chloroplast. AP bypasses two out of the three phosphorylating sites in the MET and hence this may be an unsuitable trait for the overall energy metabolism of the cell. However, AOX may have a protective role during environmental and biotic stresses as reported in many studies. Various findings indicate that the functional role of AOX is tissue and organ specific. If we eliminate the expression of AOX by antisense technique, the plants succumb to drought or other abiotic factors mediated oxidative stress. Thus, alternative respiration, like photorespiration may be a necessary evil.

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