

Cyclosis in pondweed (*Elodea canadensis*): bicarbonate and NO effects using pharmacologicals

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Cyclosis is a ubiquitous phenomenon in plant cells and plays an important role in transporting organelles within a cell, cell development and differentiation. The present study examines the role of nitric oxide (NO), which influences some of the phenomena where cyclosis is known to play a role. Viagra is involved in NO production in plants. Bicarbonate is known to be a key player in photosynthetic electron transport. Effects of Viagra and bicarbonate on cyclosis were examined in the presence of various chemicals that influence calcium-related physiological effects. Viagra can stimulate or inhibit cyclosis depending on the concentration used. Both bicarbonate and Viagra behave similarly in stimulation and inhibition of diverse but evolutionarily linked processes. It is hypothesized that cyclosis evolved for the regulation of transpiration. *Elodea* being an aquatic plant has no stomata, but cyclosis, the physiological basis for the regulation of transpiration still persists as an evolutionary relict. Both calcium and bicarbonate were found to influence cyclosis positively, while lanthanum sulphate, a calcium channel blocker, had a negative effect. However, its effect was dependent on concentration, with complete blockage at 10 mM concentration. By contrast, the effect of bicarbonate was neutralized by the presence of formate, indicating a competition between the two for the same site. Cyclosis is stimulated by trifluoperazine, an antagonist of calmodulin. Another chemical, acetazolamide, an inhibitor of carbonic anhydrase which generates bicarbonate inhibits cyclosis. This supports the positive influence of bicarbonate on cyclosis. Our results point to inhibition of cyclosis by formate possibly by competing with bicarbonate for a binding site. The nature of the binding site is presently unclear. Future experiments will unravel the role of Viagra in cyclosis.

Keywords: Bicarbonate, calcium channels, cyclosis, nitric oxide, pondweed, Viagra.

CYCLOSIS is complete cytoplasmic churning or protoplasmic streaming. It is regularly observed in photosynthetic epidermal cells of the leaves of pond weed, *Elodea canadensis*, where it is made apparent by circular movement of the chloroplast. Of what use is cyclosis for a

plant cell? It is argued that diffusion is a slow process for the molecules to move¹ and it is a way to keep metabolically active cells as well-stirred compartments. Cell excretory products like ammonia are diluted to keep them below toxic levels in the cells by the churning of the cytoplasm. The process continues till the diffusion of toxic gas is complete; a slower process like diffusion is made faster by cyclosis. Cyclosis is also a way of transporting organelles within a cell to a location where their function is required. It is important in cell development and differentiation. Movement of Ca^{2+} ions creates an electric current and sets up an electric field in fertilized egg of *Fucus*². This brings about polarity for development of rhizoid-like structures in the zygote of *Fucus*. Thus the zygote is transformed to a holdfast. After differentiation of the holdfast cyclosis would abolish the polarity by destroying the established chemical gradient within the zygote. During differentiation also cyclosis is needed to establish a cell pattern, as seen in the cell culture of *Zinnia* where spiral banding appears as the cell differentiates into the xylem³. Thus cyclosis may be in the beginning of differentiation as in *Zinnia* cells or it may mark the end of the differentiation process as in *Fucus*, where it acts to abolish the concentration gradient so that the cells are ready afresh to function normally.

Hormones influence cyclosis or cytoplasmic streaming in *E. canadensis* leaf epidermal cells⁴. This plant is distributed worldwide and is a common weed. Auxins and cytokinins affect cellular processes like stomatal opening⁵. In plants nitric oxide (NO) is involved in the signal transduction pathway⁶ and Viagra is known to generate NO in plants⁷. Bicarbonate is known to be a part of the photosynthetic electron transport chain⁸. It competes with formate and nitric oxide⁸. In light of these seemingly unconnected but interesting findings, it was decided to test the effect of Viagra and bicarbonate on cyclosis in *E. canadensis*. Bicarbonate is a ubiquitous molecule in nature and living organisms could have exploited it to integrate it in signalling. Calcium channel blocker (lanthanum), formate, calmodulin antagonists and inhibitors of carbonic anhydrase having known interaction with bicarbonate were considered for their effects on cyclosis. A model for the action of Viagra in animal reproduction is now available⁹. NO signals in biology are typically characterized as either cGMP-dependent or independent¹⁰. This classification presupposes downstream targets of cGMP and an alternative cGMP-independent pathway, which involves nitrosylation of proteins. There are no experimental models to distinguish these two modes, i.e. no specific targets for NO/cGMP. The existing model suggests that Viagra is specific to the cGMP signalling cascade. Some possibilities are suggested based on the effects of formate, bicarbonate and Viagra in the present study. Signalling in plants is a subject of an intense ongoing debate, where one needs to separate the noise and false alarms from the ground realities¹¹.

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The increasing CO₂ concentration has shown to decrease the number of stomata, in a study of herbarium specimens of last 240 years¹². CO₂ concentration may further rise the level of HCO₃⁻ in aquatic ecosystems¹³. Hence the study of bicarbonate physiology and biochemistry gains importance in view of the much talked about global climate change¹⁴.

It is assumed that cyclosis is energy-dependent. The source of this energy is ATP from cyclic and non-cyclic photophosphorylations⁸, which stimulates cyclosis through stimulated ATP production. Bicarbonate could act as a non-signal molecule and stimulate ATP production through alteration of proton gradient near the protoplast surface. This surface charge could also be changed wherever calcium channels (internal) are regulated by membrane potential¹⁵. Bicarbonate might mimic hormone action by this mechanism, which is likely in view of the observed effect on cyclosis by indole-3-acetic acid (IAA) and cytokinins. Hormone-like action of bicarbonate has been observed for non-photosynthetic organs like the root, where it is able to stimulate secondary root formation in cucumber¹⁶. Similarly, Viagra has been used for induction of secondary roots by others⁷. NO has also been implicated in stomatal opening⁷, which is known to increase and stimulate transpiration in cut twigs of *Tecoma stans*¹⁷. It is also of interest to know that Viagra, a common generator of NO, is known to compete with bicarbonate in secondary root formation¹⁶. The focus of the present study has been the dynamic nature of the signal involved in cyclosis, which has been examined in the presence of interacting and counteracting agents such as formate, bicarbonate, lanthanum, trifluoperazine, EDTA and calcium ions. The study is based on simple pharmacologicals and is unique for its titration-like experiments with Viagra.

The following chemicals: lanthanum sulphate, formic acid, sodium bicarbonate, calcium chloride, HCl and solvent diethylether, were from Merck, India. Viagra (sildenafil citrate) was from Zydus Alidac, Ahmedabad, India. Trifluoperazine was from Glaxo Smith Kline Pharmaceutical, Mumbai, India. Acetazolamide was from Wyeth-Lederle. In the literature there are no reports about purification of some pharmaceuticals like Viagra. All pharmaceuticals were tablets and were purchased as such. The pharmaceuticals were analysed for calcium by EDS (electron diffraction spectroscopy). If they contained calcium, for example, Viagra, they were decalcified as follows: one tablet (50 mg) of Viagra was dissolved in 50 ml water and acidified by 6 N HCl, ether-extracted, dried and dissolved in 0.1 ml of 0.1 N NaOH, and made up to the required concentration. Trifluoperazine tablets (10 × 5 mg) were dissolved in 50 ml water, a pinch of activated charcoal was added to remove the blue colour additive, filtered and the solution made up to the required concentration by distilled H₂O and used directly. All other chemicals used were of analytical grade and were used directly.

The local ponds of Bangalore, India during rainy season (August–November) are full of *E. canadensis*. The material was collected and maintained in Botany Department, St. Joseph's College, Bangalore greenhouse aquarium. Time and temperature records were maintained.

The microscope had an objective of 45 × (NA 0.4) and an eyepiece of 10 ×. A leaf was peeled-off the stem and a small piece was cut and mounted either in distilled water or test solution under a cover slip. The leaves are only two-cell thick and permit easy observation of cyclosis. Speed calculation was done as follows. The microscope was calibrated with ocular units and micrometre scale was projected on the image. Each unit of scale was 4 × 10⁻⁶ m. By timing the movement of a single chloroplast moving past the scale, the speed of the chloroplast movement was calculated during cytoplasmic streaming process. *E. canadensis* leaf was used from an aquarium in the laboratory. The water temperature was the same as the room temperature (25°C) in ambient light conditions. Experiments were done during September, on a single day. Student's *t*-test was done with water as control; standard deviation was calculated for frequency of cells showing chloroplast movement in a microscope field.

Photosynthetic electron transport is inhibited by formate⁸. It is stimulated by bicarbonate¹⁸. The effect of these two compounds on cyclosis is antagonistic (Table 1). They must be competitively binding to the NO-binding site which modulates electron transport. NO forms a complex with heme in the proteins. It is quite likely that the complex is formed after nitrosylation of the molecule⁷. Alternatively, the heme groups in guanyl cyclase (GC) may also be a binding site for NO. Although the product of GC, i.e. cGMP is known in plants, neither the enzyme has been isolated nor the gene coding the enzyme been identified and cloned⁷. Formate inhibits cyclosis at 1 mM concentration, and it has the same effect at higher concentration (10 mM), while bicarbonate stimulates (competitive inhibitor) but does not affect the redox signalling as has been earlier suggested⁸. The alternate suggestion is that bicarbonate binds to the regulatory subunit of GC, where NO inhibits the enzyme by binding at the site (this is likely because the subunit has heme), and displaces NO when cyclosis is stimulated by GC. This mechanism is like redox signalling, but is not independent of GC. But no involvement of NO in animal model studies has been established in the presence of Viagra. In animal systems NO production is possible because nitric oxide synthase (NOS) is stimulated. Presently there are no reports of NOS in plants; instead the enzyme involved in NO production could be nitrate reductase (NR)⁷. NR in plants is regulated by a wide variety of factors, which could either be intrinsic or extrinsic¹⁹. Since the NOS gene in plants is not isolated and cloned, the only evidence for the presence of the enzyme is the detection of cross-reactivity of NOS antibodies from animals with the putative NOS of plants⁷. This is not a strong evidence for the presence

Table 1. Inhibition of cyclosis by bicarbonate and formate

Treatment (conc. in mM)	Speed (s/turn) (mean \pm SD)	FA (conc. in mM)	Speed (s/turn) (mean \pm SD)
Water	50 \pm 2 (7.8 \pm 0.46)		
Viagra (1.5)	30 \pm 2 (11.8 \pm 0.57)		
BC (1)*	35 \pm 2 (8 \pm 0.35)	FA (10)	NM
BC (1) + Viagra (1)	40 \pm 3 (9.4 \pm 0.24)	FA (1) + Viagra (1)	40 \pm 2 (11.4 \pm 0.24)
BC (10) + Ca ⁺⁺ (10)	40 \pm 2 (8.6 \pm 0.43)	FA (10) + Ca ⁺⁺ (10)	70 \pm 5 (9.4 \pm 0.16)
BC (1) + Ca ⁺⁺ (1)	30 \pm 2 (12.8 \pm 0.66)	FA (1) + Ca ⁺⁺ (1)	30 \pm 2 (10 \pm 0.21)

FA, Formic acid; NM, No movement; *, Not significant.

The results were statistically analysed with water as control and were significant ($P < 0.05$) by Student's *t*-test, which was done with the frequency of cells showing movement in a observed field of the microscope. Mean of number of cells showing chloroplast movement \pm standard deviation (SD) in parentheses.

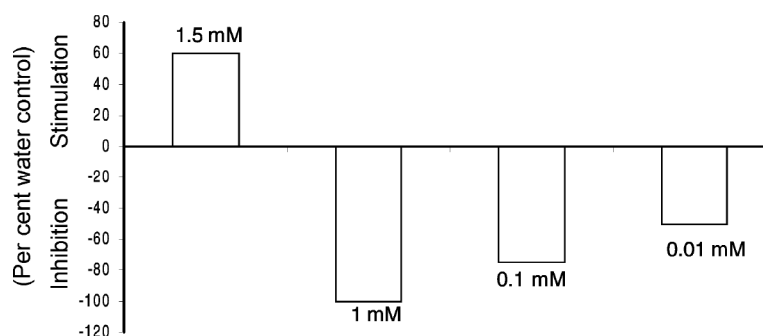


Figure 1. Inhibition of cyclosis by Viagra. Solutions were prepared as described in the text. Inhibition was recorded as per cent water control. The control speed was 50 s/turn. Statistical test was done as in Table 1. All results were significant ($P < 0.05$) with water as control, except for 1 mM Viagra test solution in which the epidermal cells did not show any movement of chloroplast. At 1.5 mM, there was stimulation of cyclosis.

of NOS in plants⁷. Conclusive evidence depends on the purification of the enzyme and cloning of the gene. An attractive but theoretical possibility is the direct involvement of photosynthetic electron transport in NO-related processes. It is yet to be tested by experiments; however earlier work on electron transport has already suggested this possibility⁸. Involvement of light and energy derived from photophosphorylation is likely because in the absence of light cyclosis is delayed till sufficient exposure to white light.

Viagra is a modulator of cyclosis and shows concentration-dependent effect; it is stimulatory at 1.5 mM (Table 1) and inhibits cyclosis at 1 mM (Figure 1). This inhibition is probably driven by the effects of Viagra on phosphodiesterase (PD5) activity, evidence for which is lacking in plant. However, it was also observed in cucumber that there is an oscillatory effect of Viagra, where the number of secondary roots and growth of hypocotyls are stimulated in a coupled manner and where Viagra had no effect or was slightly stimulatory at 10^{-3} M, while it was inhibitory at 10^{-4} M and again stimulatory at 10^{-5} M. The full range of concentration has not been tested in the present study for cyclosis which may also show the oscillation behaviour. It has also been shown in other plants

that GC inhibitors were able to suppress the expression of *PAL* genes stimulated by NO⁷. The effect of Viagra on cyclosis is unique in differing totally from the animal system cGMP-dependent effects. Further in plants as in animals, cGMP could act via cyclic ADP ribose (cADPR). The same observation has been made with respect to treachery elements differentiation²⁰. In view of the above discussion NO and bicarbonate effects are significant in the sense that both play an important role in ecosystem. Twenty years ago NO studies were made with a view to delineate the phytotoxic effect of oxides of nitrogen (NO₂, N₂O₃, NO₂⁻, NO₃⁻)²¹, when role of bicarbonate was not known.

Cyclosis is also enhanced by increasing concentrations of calcium (0.01–1 mM; Table 2). The rate almost doubles with increasing concentration of the ion. Calcium is an important component of the NO-signalling cascade. Many physiological responses of plants may be due to hormonal or environmental stimuli that are mediated directly or indirectly via the NO-signalling cascade. It has been recently suggested that NO could act simultaneously on several unrelated biochemical oxides and its redox homeostatic properties are of importance. Hence the molecule is suggested to act as a synchronizing molecule

Table 2. Reversal of Viagra-mediated inhibition of cyclosis by calcium and calmodulin antagonist trifluoperazine (TFP), effect of EDTA and lanthanum (La) ions

Treatment (conc. in mM)	Speed (s/turn) (mean \pm SD)	Change of treatment (conc. in mM)	Speed (s/turn) (mean \pm SD)
Water	50 \pm 2 (7.8 \pm 0.46)	Viagra (1)	NM
CaCl ₂ (10)	30 \pm 2 (4.2 \pm 0.26)		
CaCl ₂ (1)	15 \pm 3 (6 \pm 0.33)	CaCl ₂ (1) + Viagra (1)	60 \pm 3 (3.4 \pm 0.75)
EDTA (10)	45 \pm 3 (9.2 \pm 0.51)		
EDTA (1)	40 \pm 2 (10.2 \pm 0.19)	EDTA (1) + Viagra (1.5)	60 \pm 3 (9.4 \pm 0.1)
TFP (1)	20 \pm 2 (11.8 \pm 0.62)	TFP (1) + Viagra (1.5)	20 \pm 2 (11.2 \pm 0.71)
La ⁺⁺ (1)	40 \pm 3 (10.6 \pm 0.58)	La ⁺⁺ (1) + Viagra (1.5)	40 \pm 2 (11.6 \pm 0.92)
La ⁺⁺ (10)*	80 \pm 2 (7.6 \pm 0.29)		

NM, No movement; *, Not significant. Statistical tests were done as given under Table 1. All data were significant ($P < 0.05$).

in plants⁷. The cGMP-independent effects of NO have also been reported. It has been established in tobacco that calcium released from the internal stores, induced the expression of plants defence genes via the cGMP-independent pathway⁷. This is a clear-cut evidence of calcium being released because of nitrosylation and activation of the Ca⁺⁺ channels. The same mechanism is found in animal cardiac muscle cells⁴. Our results indicate that in *E. canadensis* cyclosis is mediated by calcium ions. The inhibition of cyclosis by Viagra is overcome by calcium (Table 2). It is possible that bicarbonate also releases calcium from internal stores within the cell by activation of the Ca⁺⁺ channels via its effect on the surface potential of organelle membranes²².

Lanthanum, a calcium-channel blocker completely blocks cyclosis in *E. canadensis* (Table 2). Lowering the concentration of lanthanum slowly reactivates the calcium channels and cyclosis begins. Our lanthanum experiments confirm the involvement of calcium in signal transduction that must occur for the cyclosis response to follow and persist. Membrane potential at the plasma membrane regulates the entry of Ca²⁺, a phenomenon known as voltage-dependent gating²². The membrane is negatively charged. Lanthanum has greater affinity to the membrane than Ca²⁺ and cannot cross membrane because of its large size. As a result, voltage-dependent regulation is abolished at the surface of the membrane.

It has been observed that hormones also activate and enhance cyclosis in *E. canadensis*⁴. Interestingly, EDTA has no effect on cyclosis. Perhaps EDTA is not able to penetrate *E. canadensis* cells and hence not able to chelate Ca⁺⁺ and inhibit cyclosis (Table 2). Thus cyclosis activation is possible in *E. canadensis* by the cGMP-independent route involving a direct action on the Ca⁺⁺ channels via protein nitrosylation. The pathway could even be NO-independent if the bicarbonate is able to influence surface potential of organelles which act as the internal storehouse of calcium. This is likely because calcium action on cyclosis is potentiated by bicarbonate. Defence (*R*) genes in tobacco are activated by cGMP-dependent kinase that increases cADPR, and this molecule can modulate Ca⁺⁺ concentration in the cell. This possibility

is ruled out because Viagra, which is supposed to increase cGMP, actively inhibits cyclosis⁷. Another possible explanation for the observed EDTA effect is the charge interaction of the COOH and ammonia groups, which are either neutral or negatively or positively charged depending on the pH. At acidic pH COOH groups are neutral and ammonia group is positively charged. So at acidic pH EDTA will not chelate calcium and its positively charged NH₃⁺ group strongly interacts with the surface negative charge of the membrane and EDTA does not enter the cell. The pH of the experiment with EDTA in the present study is that of distilled water, which hovers around near neutral but still in the acidic range.

It has been shown that auxin-induced xylogenesis requires calmodulin in lettuce (*Lactuca sativa*), a crop plant²³. The present experiments on cyclosis reveal that 1 mM concentration of trifluoperazine (TFP), a calmodulin inhibitor actually triggers and stimulates cyclosis (Table 2). In one conformation calmodulin is stimulatory as observed when TFP is added; Viagra acts independently, since TFP plus Viagra combination is stimulatory (Table 2). However, 1 mM formate slows down cyclosis. Viagra does not affect stimulation by calmodulin antagonist. In addition, there is also a possibility that calmodulin may have a negative (feedback) regulation on cyclosis which may be released by the action of TFP. This is because TFP acts further down the signal transduction pathway to release Ca²⁺ from the internal storehouse which may be stimulating cyclosis. Besides, it has been shown²⁴ that complete microtubule disassembly occurs at 2 mM concentration of Ca²⁺. In cyclosis it is likely that calcium clears microtubules and allows the chloroplast to move into the cytoplasm. But the chloroplasts in differentiating TE are caught in a three-dimensional network of actin molecules to help in cytoplasmic streaming²⁴. The hormones involved in TE differentiation are cytokinin, auxin and brassinosteroids²³. The first two hormones are responsible for TE-differentiation, induction and brassinolide is involved in the progression of differentiation. In *Zinnia* cells uniconazole is known to inhibit differentiation without affecting cell division. An exogenous addition of 0.2 nM brassinosteroid reverses inhibition²⁵. The

early calmodulin effect on differentiation is on the determination of differentiation by cytokinins. Further research should sequentially establish the order of these events, which should also be validated by genetic screens. Some regulatory aspects of Viagra/bicarbonate are summarized in Figure 2.

So far physiological studies of the effect of acetazolamide, an inhibitor of carbonic anhydrase (responsible for production of bicarbonate in C_4 plants), have not been reported for plants. It is also a well-known inhibitor of mammalian carbonic anhydrase²⁶. Since the effect of bicarbonate on cyclosis became apparent, it is of interest to know the effect of acetazolamide on the phenomenon. The effect is concentration-dependent. Inhibition or slowing down of cyclosis increased with the concentration (Table 3). This proves that bicarbonate is essential for cyclosis and its effect is independent of the photosynthetic electron transport. This is valid only if the inhibitor is not able to compete with the bicarbonate in the photosynthetic electron transport chain. Nitrosylation of the non-heme iron of the photosynthetic electron transport component has been reported. Bicarbonate availability in aquatic ecosystems plays a crucial role in ecosystem biomass production. Both CAM and C_4 photosynthesis is dependent on bicarbonate. Cyanobacteria and macro algae are known to utilize bicarbonate as carbon-concentration mechanisms¹³. These mechanisms could have developed very early in evolution. In *Hydrilla* the mode of carbon assimilation is through C_4 photosynthesis¹³. In *E. cana-*

densis also it is quite likely that photosynthesis is C_4 -type. If it is indeed the case, bicarbonate is accepted as the substrate for the first step in carbon fixation by the enzyme, PEP-carboxylase. The active site of the enzyme becoming saturated with bicarbonate is one possibility as the global CO_2 concentration increases leading to an increase in bicarbonate concentration.

The effect of bicarbonate on the photosynthetic electron transport is known⁸. Cyclosis requires energy and so the link with photosynthetic electron transport and photophosphorylation cannot be ruled out. Specialized cell components like microtubules may serve as a framework upon which streaming of cytoplasm occurs. Bicarbonate, formate and Viagra seem to compete for the same site; whether this site is the heme in the guanylyl cyclase regulatory subunit is not clear. The validation depends on the use of NO-generating substance⁷. However, it is known in cucumber plants that auxin-induced root development is dependent on NO production. In this system GC inhibitor, LY83583 reduced adventitious roots. We have observed a stimulation of secondary roots by 1.5 mM Viagra, which is a known NO generator¹⁶ (Figure 2). The observations made with cucumber seedlings and cycloses in *E. canadensis* with Viagra are identical. Interestingly shelf life of flowers can be increased by some chemicals and also by Viagra and bicarbonate²⁷.

In conclusion, our results seem to support a network of components involving calmodulin and calcium concentration that seem to influence cyclosis via cytoskeleton in *E. canadensis*. The data collected using Viagra, bicarbonate, EDTA, lanthanum formate, trifluoperazine, acetazolamide and calcium indicate their influence on the cyclosis activity network at different points. The nature of the points at which the compounds used precisely interact and produce the effects recorded has to be elucidated by further research. It is, however, clear from our studies that bicarbonate could be an important player due to not only its activity, but also regulation of cyclosis in the presence/absence of Viagra. Future research holds exciting opportunities to unravel the role of bicarbonate in the whole cyclosis network in *E. canadensis* and other systems.

Comparison of effect of Viagra and bicarbonate on other process is interesting. Both bicarbonate and Viagra behave similarly in stimulation and inhibition of diverse but evolutionary linked processes¹⁷ (Figure 2). Cyclosis may have evolved basically to regulate transpiration. During transpiration cyclosis may abolish the established concentration gradient of regulatory ligands (Ca^{2+} , bicarbonate). This could have provided selection pressure for voltage-dependent gating of Ca^{2+} channels as envisaged in the present study, but not shown in the model (Figure 2). In C_4 plant, *Amaranthus spinosus* L. (spiny amaranth) for example, bicarbonate and Viagra are stimulatory to transpiration and stimulate the opening response of the stomata¹⁷. Though there is not enough experimental

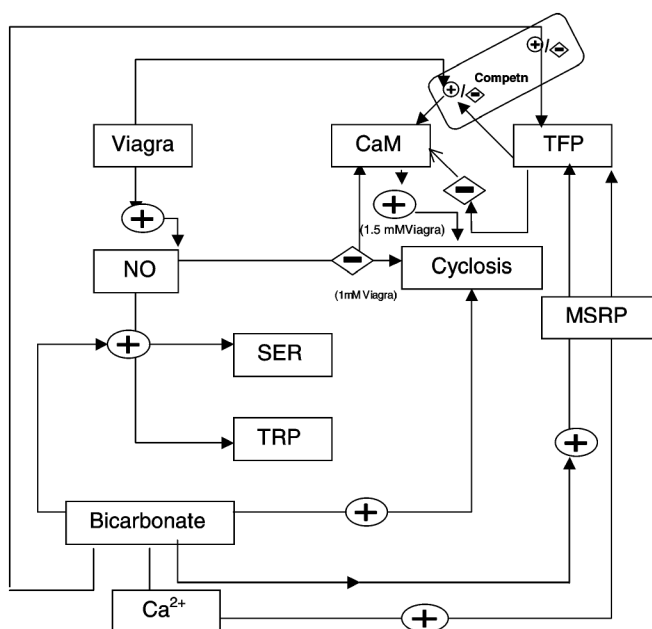


Figure 2. A model for the regulation of cyclosis and other related physiological processes. NO, Nitric oxide; SER, Secondary roots; TRP, Transpiration; CaM, Calmodulin; TFP, Trifluoperazine; MSRP, Membrane surface potential; Competn, Competition between positive and negative regulation. Evolutionary aspects of the model are given in the text.

Table 3. Inhibition of cyclosis by acetozalamide (ACZ) and its reversal by bicarbonate (BC)

Treatment (conc. in mM)	Speed (s/turn) (mean \pm SD)	Change of treatment (conc. in mM)	Speed (s/turn) (mean \pm SD)
Water	50 \pm 2 (7.8 \pm 0.46)		
ACZ (10)	80 \pm 2 (7.2 \pm 0.46)	ACZ (10) + BC (10)	30 \pm 2 (10.6 \pm 0.3)
ACZ (1)	30 \pm 2 (8.4 \pm 0.29)	ACZ (1) + BC (1)	40 \pm 2 (7.6 \pm 0.51)
Viagra (1)	NM	Viagra (1.5) + ACZ (10)	NM
		Viagra (1.5) + ACZ (1)	NM

NM, No movement; *, Not significant. Statistical tests were done as given under Table 1. All data were significant ($P < 0.05$).

evidence, in the present model, it is tempting to speculate on the evolutionary aspects of cyclosis. Diversion of ATP from photophosphorylation to cyclosis may bring about closure of the stomata. As the stomata closes, CO₂ and bicarbonate concentrations increase and the stomata opens. Thus functionally there is enough experimental evidence for bicarbonate to be an important ligand for feedback regulation of transpiration. In *Elodea* which migrated from land to aquatic habitat, and lost its stomata and transpiration, selection pressures were not strong enough to abolish the physiological basis for regulation of transpiration. In the absence of stomata in *Elodea*, ATP is not diverted for the opening of the stomata. As a result, stimulation of cyclosis by bicarbonate still persists as an evolutionary relict.

1. Nobel, P. S., In *Biophysical Plant Physiology and Ecology*, W.H. Freeman and Company, San Francisco, 1983.
2. Jaffe, L. F., Location in developing focus cell and the role of localizing current. *Adv. Morphol.*, 1978, **7**, 295–328.
3. Fukuda, H., Redifferentiation of signal mesophyll cells into tracheary elements. *Int. J. Plant Sci.*, 1994, **155**, 262–271.
4. Walker, D., 2000; www.microscopy-uk.org.uk
5. Thiel, G., Blatt, M. R., Fricker, M. D., White, I. R. and Millner, P., Modulation of K⁺ channels in *Vicia* stomatal guard cells by peptide homologous to the auxin binding protein C terminus. *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 11493–11497.
6. Hausladen, A. and Stamler, J. S., Nitric oxide in plant immunity. *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 10345–10347.
7. Lamattina, L., Carlos, G. M., Graziano, M. and Pagnussat, G., Nitric oxide: the versatility of an extensive single molecule. *Annu. Rev. Plant Biol.*, 2003, **54**, 109–136.
8. Govindjee, Weger, H. G., Van Rensen, J. J. S., Devos, O. J. and Snel, J. F. H., Formate releases carbon dioxide/bicarbonate from thylakoid membrane. *Naturwissenschaften*, 1991, **78**, 168–170.
9. Drewes, S. E., George, J. and Khan, F., Recent findings on natural products with erectile dysfunction activity. *Phytochemistry*, 2003, **62**, 1019–1025.
10. Arnold, W. P., Mittal, C. K., Katsuki, S. and Murad, F., Nitric oxide activates guanylate cyclase and increases guanosine 3',5'-cyclic monophosphate level in various tissues preparation. *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 3203–3207.
11. Cruz, M. B. T., Genoud, D., Metraux, J. P. and Genoud, J., Update in bioinformatics. Towards digital data base of plant cell signaling network: advantages, limitation and prediction aspects of digital model. *Phytochemistry*, 2005, **66**, 267–276.
12. Woodward, F. I., Stomatal numbers are sensitive to increase in CO₂ from pre-industrial level. *Nature*, 1987, **327**, 617–618.
13. Bowes, G., Facing the inevitable: plants and increasing atmospheric CO₂. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1993, **44**, 309–332.
14. Kendall, B. E., Chaos and cycles. In *Encyclopedia of Global Environmental Change* (eds Mooney, H. A. and Canadell, J. G.), John Wiley, New York, 2002, vol. 2, pp. 209–215.
15. Rubery, P. H. and Sheldrake, R. A., Effect of pH on surface charge on cell uptake of auxin. *Nature New Biol.*, 1973, **244**, 285–288.
16. Imrana, N. and Khan, N. U., Growth regulation by calcium channel in cucumber: effect of bicarbonate, Viagra, acetozalamide as Antidote for nitrophenolic toxicity. *Phytomorphology*, 2008, **58**, 15–24.
17. Imrana, N., Ph D thesis, Phytoremediation of pesticide polluted agricultural soils, Bangalore University, 2008.
18. Diner, B. A. and Petrouleas, V., Formation of NO of nitrosyl adducts of redox components of the photosystem, its reaction center, evidence that HCO₃³⁻/CO₂ bind to acceptor side on non-heme iron. *Biochim. Biophys. Acta*, 1990, **1015**, 141–149.
19. Vaucheret, H., Kronenberger, J., Lepingle, A., Vilaine, F., Boutin, J. P. and Caboche, M., Inhibition of tobacco nitrite reductase activity by expression of antisense RNA. *Plant J.*, 1992, **2**, 559–569.
20. Sugiyama, M. and Komamine, A., Effect of inhibitors of ADP-ribosyltransferase on the differentiation of tracheary elements from isolated mesophyll cells of *Zinnia elegans*. *Plant Cell Physiol.*, 1987, **28**, 541–544.
21. Martin, C. T., Morse, R. H., Kanne, R. M., Gray, H. B., Malm Strom, B. G. and Chan, S. I., Reaction of nitric oxide with tree and fungal lacasse. *Biochemistry*, 1981, **20**, 5147–5155.
22. Blatt, M. R. and Thiel, G., Hormonal control of ion channel gating. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1993, **44**, 543–567.
23. Roberts, L. W. and Baba, S., Evidence that auxins induce xylogenesis in *Lactuca* explants requires calmodulin. *Environ. Exp. Bot.*, 1987, **27**, 289–295.
24. Savidge, R. V., The tracheid-differentiation factor of conifer needles. *Int. J. Plant Sci.*, 1994, **155**, 272–290.
25. Iwasaki, T. and Shibaoka, H., Brassinosteroids act as regulators of tracheary-element differentiation in isolated *Zinnia* mesophyll cells. *Plant Cell Physiol.*, 1991, **32**, 1007–1014.
26. Kisiel, W. and Graf, G., Purification and characterization of carbonic anhydrase from *Pisum sativum*. *Phytochemistry*, 1972, **11**, 113–117.
27. www.plantea.com/cutflowers.htm

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