



Intracellular trafficking and cytoplasmic streaming under abiotic stress conditions

JESHIMA KHAN YASIN^{1*} AND ANIL KUMAR SINGH²



ARTICLE INFO

Received on : 12-02-2019
Accepted on : 21-09-2019
Published online : 03-12-2019

ABSTRACT

Cytoplasmic streaming is one among the vital activities of the living cells. In plants cytoplasmic streaming could clearly be seen in hypocotyls of growing seedlings. To observe cytoplasmic streaming and its correlated intracellular trafficking an investigation was conducted in legumes in comparison with GFP-AtRab 75 and 35S::GFP:δTIP tonoplast fusion protein expressing arabidopsis lines. These seedlings were observed under confocal microscopy with different buffer incubation treatments and under different stress conditions. GFP expressing 35S::GFP:δTIP tonoplast lines were looking similar to the control lines and differ under stress conditions. Movement of cytoplasmic invaginations within the tonoplast and cytoplasmic sub vesicle or bulb budding during cytoplasmic streaming was observed in hypocotyls of At-GFP tonoplast plants. We found the cytoplasmic bulbs/vesicles or sub vesicle formation from the plasma membrane. The streaming speed also depends on the incubation medium in which the specimen was incubated, indicating that the external stimuli as well as internal stimuli can alter the speed of streaming.

KEYWORDS

Cytoplasmic streaming, intracellular transport, legumes, stress tolerance

INTRODUCTION

Vesicles are of different types based on origin and their function. Of these vesicles, membrane bound vesicles contribute a major role in transport as well as carrying apoptotic particles or storage molecules. These membrane bound vesicles can isolate a portion of cytoplasm with specific constituents of it from the total cytosol of a plant cell. Apart from vesicles, intracellular organelles are also being transported along with streaming cytoplasm. Though these two were identified and discussed as separate events we identify both these intracellular activities as dependent ones. Migration of vesicles and organelles at a constant speed creates a pressure and pull on the cytoplasm. Vesicles isolate excess water from the cytoplasm to prevent bursting of the cell; facilitate isolating salt and other toxic minerals in protecting the cell and deposit them in vacuoles or facilitating mineralization of specialized cells containing deposits of silicon or calcium as in the case of animal cells. Myosins are actin bases motor proteins (Berg et al., 2001, Hannie et al., 2010). Vesicles act as cargo for molecules in transporting proteins for post translational modification as well as delivering proteins or other molecules at its site of requirements. Surface of the transport vesicles are with clathrin or SNARE protein mostly interacting with Rab GTPases and they connect with myosins to walk on actin and cytoskeleton tracks (Yasin and Seema, 2015). Earlier we reported that, it may not only be by the myosins, but kinesins may also act in transporting cargo with the cell as the shuttling distance between two myosin molecules will be less but it could be shuttled between myosins and kinesins when it is required to transport quick or a faster streaming is required to maintain a homeostasis within the cell (Yasin, 2015).

In crop cultivation, most of the failures and yield loss happens due to abiotic and biotic stress conditions (Yasin et al., 2014, Yasin). Most of the biotic stress conditions are controllable and abiotic stress can only be managed to certain limits, beyond which it becomes a cellular emergency and the cell signals for apoptosis leading to death of the organism. Increased stress activates cellular activity as well as ATP expenses. Intracellular and inter cellular stress response activities were independent of energy levels regulated activities but with limited roles. Once trafficking starts, the involvement of ATPs becomes crucial to maintain the balance in gene expression and intra cellular homeostasis (Yasin, 2015). As result of this present investigation, we identified cytoplasmic streaming in stress response of both legumes and arabidopsis and is explained in this manuscript.

Materials and methods

We chose to work with drought tolerant legumes developed / identified by our lab with susceptible control identified from the total gene bank pigeonpea collection, in comparison with GFP-AtRab75 and 35S::GFP:δTIP green fluorescent tonoplast intrinsic protein Arabidopsis lines with constitutive expression 35S promoter expressing GFP fused proteins kindly provided by University of Tokyo. Samples of legumes grown under field conditions as well as in controlled conditions were used for the present investigation.

Samples were prepared fresh and turgidity was maintained still observing under microscopes. Slides were prepared with phosphate buffer prepared for pH 7 (Na₂HPO₄·7H₂O (mw: 268.07 g/mol) 0.0578 M + NaH₂PO₄·H₂O (mw: 137.99 g/mol)

¹Division of Genomic Resources, ICAR-National Bureau of Plant Genetic Resources, PUSA campus, New Delhi, India

²Division of Land & Water Management, ICAR-Research Complex for Eastern Region, Patna-800 014 (Bihar) India

*Corresponding author email: labdrive.legume@gmail.com

0.0422 M) as well as in de-ionized water. Samples were observed under confocal microscope. Periodical stages as well as movies were recorded for GFP fluorescence, chloroplast autofluorescence and merged images were also recorded.

RESULTS AND DISCUSSION

AtGFP 35S::GFP:δTIP tonoplast as well as GFP-AtRab75 arabidopsis lines were normal growing seedlings under control conditions. GFP expression was good in 35S::GFP:δTIP tonoplast lines were sufficient to explain the present objectives under investigation. Movement of cytoplasmic invaginations within the tonoplast and cytoplasmic sub vesicle or bulb budding during cytoplasmic streaming was observed in hypocotyls of At-GFP tonoplast: transgenic plants (Fig. 1).

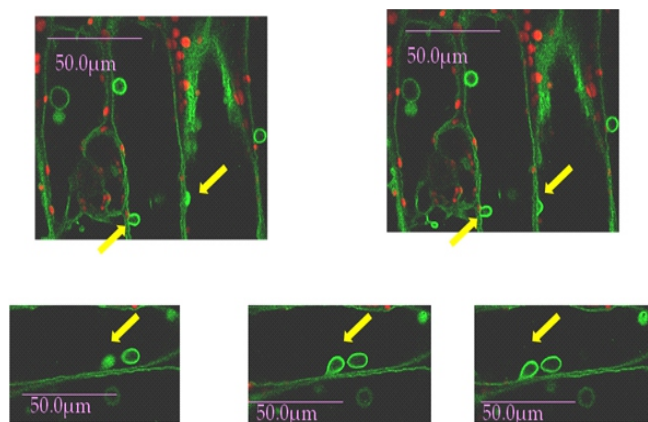


Fig. 1: Invaginations of plasma membrane to form vesicles containing part of cytoplasm to be shuttled
 Green – GFP labeled TIP fusion proteins in tonoplast / plasma membrane
 Red – Autofluorescence of chloroplast
 Yellow markings indicate the invaginations in developing vesicles

Under abiotic or biotic stress conditions after initial signals perception, the cells prepare for either of the two extreme conditions viz., to escape and survive the stress conditions or to die by killing the cells to protect the other cells or the whole organism may die. To escape from a stress they form more vesicles emerging from plasma membrane enclosing the toxic constituents of the cell and transport them to outside of the cell or to vacuoles. The change from buffer to water and reverse was sensed so critically by the young seedlings and they produced more vesicles as well as rapid movements with faster streaming.

Vesicles emerge from tonoplast membrane and from plasma membrane. They emerge, grow and enlarge to stream in cytoplasm and coalesce with the plasma membranous wall. The phenotype of the plants were normal comparable with Col-0 arabidopsis. Vesicles were earlier reported to be formed from vacuoles and vice versa apart from ER and Golgi complex. But we found the cytoplasmic bulbs / vesicles or sub vesicle formation from the plasma membrane (Fig. 2). The streaming speed also depends on the incubation medium in which the specimen was incubated, indicating that the

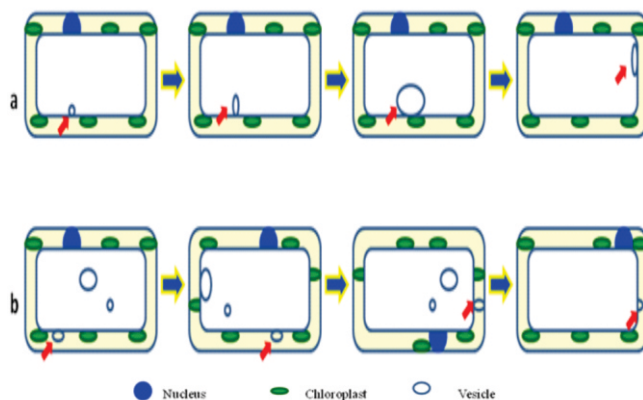


Fig. 2a: Vesicles movement in a. cytoplasm and b. through vacuoles
 Red arrows highlight the vesicle and blue arrows indicate their periodical location changes indicating their movements

external stimuli as well as internal stimuli can alter the speed of streaming (Fig. 2b). Immediately after receiving the signals the streaming is too fast compared to the neutral ambient condition.

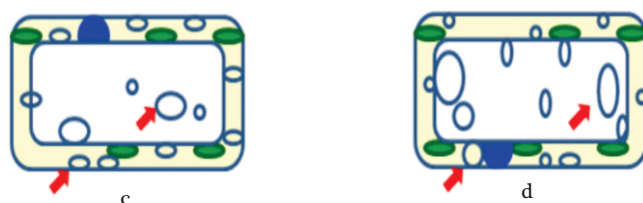


Fig. 2b : Excess vesicles traffic in cytoplasm and through vacuoles under stress conditions

Myosins have neck region which is having sensitivity are directly correlated with streaming and intra cellular traffic (Li and Nebenfuhr, 2008). Intracellular transport is dependent on myosin intensity but myosin movements depend on ATP hydrolysis which in turn correlates with stress tolerance. Kimura et al, (2003) couldn't explain the intracellular transport with myosins as the faster shuttling is due to the exchange of cargo by alternate shuttling between myosins and kinesins but it was exactly at the same speed of cytoplasmic streaming in NtXI based transport. Kinesins have a longer step size known as kinesin jump as in cin8 of kinesin-5 family member than myosins and this shuttling can explain the transport and streaming speed.

Membranes get hydrated (Lasic, 1988) to enlarge and break open a blub from lipid bilayers of plasma membrane. These bulbs either transport or take away the unwanted from the cytosol to vacuole. In other way, it takes excess water from one place and transports it to the location of its requirement. Thus it plays a role in maintaining homeostasis, turgidity and neutrality of cellular environment especially during stress conditions.

ACKNOWLEDGMENT

We thank profusely to Prof. S R Bhat, NRCPB for providing confocal microscopic facilities in conducting the research.

REFERENCES

- Berg JS, Powell BC and Cheney RE. 2001. A millennial myosin census. *Molecular Biology of the Cell*. 12: 780–794.
- Hannie SH, Ruijter NCA, Emons AMC and T Ketelaar. 2010. Actin and myosin regulate cytoplasm stiffness in plant cells: a study using optical tweezers. *New Phytologist*. 185: 90–102.
- Kimura Y, Toyoshima N, Hirakawa N, Okamoto K and Ishijima A. 2003. A kinetic mechanism for the fast movement of Chara myosin. *Journal of molecular biology*. 328(4): 939-950.
- Lasic DD. 1988. The mechanism of vesicle formation. *Biochemical Journal*. 256(1): 1-11
- Li JF and Nebenfuhr A. 2008. The tail that wags the dog: The globular tail domain defines the function of myosin V/XI. *Traffic*. 9:290-298.
- Yasin JK and Seema K M. 2015. Role of rabgtpases and its interacting molecules in stress tolerance. *Int. J Sci & Res*. 4(6): 1228-1232
- Yasin JK, Nizar MA, Rajkumar S, Verma M, Verma N, Pandey S, Tiwari SK and Radhamani J. 2014. Existence of alternate defense mechanisms for combating moisture stress in horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.]. *Legume Research: An International Journal*. 37(2): 145-154
- Yasin JK. 2015. Intra cellular pH flux and cyclosis in plant cells under abiotic stress. *Journal of AgriSearch*. 2(2):150-1.

Citation:

Yasin JK and Singh AK. 2019. Intracellular trafficking and cytoplasmic streaming under abiotic stress conditions. *Journal of AgriSearch* 6(4): 153-155