Chapter 9

Summary and conclusions
Chapter 9

Summary and conclusions

Stomatal guard cells are popular model systems for characterizing signal transduction mechanisms and secondary messengers in plants (Fan et al., 2004; Israelsson et al., 2006). Guard cells respond to plant hormone ABA through several secondary messengers, including type 2C protein phosphatases (PP2C), G-proteins, protein kinases, sucrose non-fermenting 1 related protein kinases 2 (SnRK2s), phospholipases, besides cytosolic pH, reactive oxygen species (ROS), calcium (Ca$^{2+}$) and nitric oxide (NO) (Bright et al., 2006; Zhang et al., 2007; Neill et al., 2008).

The present work is an attempt to investigate the role of selected key signalling components in guard cells and their interaction with the other secondary messengers during ABA induced stomatal closure in epidermal strips of *Pisum sativum* and *Arabidopsis thaliana*. The initial focus was on the patterns of change in the levels of NO, cytosolic pH, ROS and their interactions with each other. Then, the sources of NO and ROS generation were assessed. Further, the experiments were extended to know the role and importance of PI3K, Ca$^{2+}$, CaM and its interactions with NO and ROS. A possible scheme of the integration of signaling components during ABA-induced stomatal closure is shown in Fig. 9.1. Finally, the bifurcation of signalling pathway if any, among the four ABA mediated responses, i.e. stomatal closure, prevention of seed germination, root growth and gene expression in protoplasts was assessed by using ABA insensitive mutants (*abi1* and *abi2*) and ABA biosynthesis mutant (*aba2*). Involvement of PP2Cs and PI3K in four ABA mediated responses, stomatal movement, seed germination, root growth and gene expression are illustrated in Fig. 9.2.
Figure 9.1 Schematic representation of the signaling components leading to the stomatal closure by ABA in epidermal strips of *P. sativum* has shown.
Figure 9.2. Schematic representation of involvement of PP2Cs and PI3K during four ABA mediated responses. Stomatal closure by ABA was WM sensitive but not other ABA mediated responses like gene expression, seed germination and root in seedlings.
Major conclusions

1. ABA induced stomatal closure was associated with an increase in not only NO, ROS but also cytosolic pH of guard cells.

2. Real time monitoring with the help of fluorescent probes indicated that (i) cytosolic alkalinization of the guard cell preceded NO production. (ii) ROS production occurs earlier than the NO production and cytosolic alkalinization by ABA.

3. As a complement, the ability of catalase or DPI to restrict the production of ROS as well as NO, and the inability of NO-modulators (scavenger of inhibitor) to prevent the rise in ROS levels in guard cells, indicated the necessity of ROS elevation for NO production during stomatal closure by ABA.

4. The prevention of ROS production by DPI and NO production by sodium tungstate indicated NADPH oxidase and nitrate reductase were the possible sources for NO and ROS, respectively during ABA induced stomatal closure.

5. Ca$^{2+}$ was necessary to sustain the rise in cytosolic pH and NO as EGTA prevented the both. As a complement, the reduction of NO, ROS and ABA-induced stomatal closure by BAPTA-AM, confirmed that the requirement of intra-cellular Ca$^{2+}$ for stomatal closure which act at upstream of NO/ROS production by ABA. In contrast, the action of BAPTA suggested, that extra-cellular Ca$^{2+}$ acted at downstream of NO/ROS elevation.

6. The restriction by W-7 of NO/ROS production as well as ABA/H$_2$O$_2$/SNP induced stomatal closure confirmed that calmodulin acted at up- and downstream of NO/ROS elevation by ABA. In contrast, the restriction by WM of NO/ROS production by ABA, but not the SNP/H$_2$O$_2$ induced
stomatal closure confirmed that PI3K acted at only upstream of NO/ROS elevation by ABA

7. The PP2Cs are the negative regulators of all the four ABA responses, included in the present study, i.e., stomatal closure, seed germination, root growth and gene expression. The sensitivity of only stomatal closure to WM indicated that the signalling pathway bifurcated at downstream of PP2C and upstream to the PI3K. The ABA promoted inhibition of seed germination, root growth and gene expression were all WM insensitive and were obviously independent of PI3K action.

Based on the above observations, generalized scheme illustrating the components of signal transduction during ABA induced stomatal closure can be drawn (Fig. 9.1).

The present work open up a few interesting leads which can be pursued in future some of these are:

(i) The regulation by upstream messengers cytosolic pH, NO and ROS production, and the mechanisms of regulation of downstream elements.

(ii) The identities of up-stream elements regulating either NR or NADPH oxidase or both.

(iii) The convergence of CaM, calcium and PI3K in regulating the stomatal closure by ABA.

(iv) The mechanism of ABA regulation bifurcation at PP2C into a PI3K-dependent and PI3K-independent pathway.