

CHAPTER- V
CONCLUSIONS

- Present study shows how differently a wild (*I. campanulata*) and cultivated (*J. pentantha*) Convolvulaceae species alter their phenotypic characteristics, physiological and biochemical parameters, and miRNA expression profile in order to cope up with *in situ* and *ex situ* water deficit conditions.
- Under *in situ* water stress, the two species showed reduction in leaf perimeter, area, length, width and SLA, which was conspicuous in *J. pentantha*. The prominent reduction in these traits may suggest relatively higher sensitivity of *J. pentantha* towards water deficit. Contrarily, the increase in LDMC was relatively higher in *I. campanulata* than *J. pentantha*, which can be considered for its better tolerance to water stress. In response to *ex situ* water deficit, both the species showed leaf wilting which was relatively prominent in *J. pentantha*. Other than physical symptoms, the two species showed reduction in RWC and SLA under water scarcity. In these measurements, changes were less conspicuous in *I. campanulata* than *J. pentantha* under stress, which is indicative of its tolerance to drought.
- Chlorophyll content declined in the two Convolvulaceae species under both water stress conditions; wherein the level of reduction was relatively higher in *J. pentantha*. Chlorophyll is an important pigment for plant photosynthesis and its higher level of degradation renders drought sensitivity to *J. pentantha*. While relatively lesser decline in chlorophyll content can be associated with the ability of *I. campanulata* to tolerate drought stress. Amongst the two water stress conditions, the overall degree of chlorophyll reduction in both the species was comparatively higher under *in situ* conditions than *ex situ*. This could be due to variability in the duration of drought stress.
- Anthocyanin content was found to accumulate in both the species in response to *in situ* and *ex situ* water deficit, wherein the degree of accumulation was relatively higher in *J. pentantha*. As excessive accumulation of anthocyanin can trigger formation of ROS resulting in photoinhibition, its elevated accumulation in *J. pentantha* reveals its higher sensitivity towards water stress. Degree of anthocyanin accumulation was higher under *in situ* water stress than *ex situ* stress.
- Lipid peroxidation measured in terms of MDA concentration, increased in the two species under both *in situ* and *ex situ* water stress. The accretion of MDA was

higher in *J. pentantha* than *I. campanulata*. It suggests higher level of oxidative stress experienced by *J. pentantha*. On the contrary, stress endurance of *I. campanulata* can be related to lower levels of lipid peroxidation.

- Eight SOD isoforms were identified from both the species, which consist of two MnSODs, two FeSODs and four CuZnSODs. All the SOD isoforms showed induction in their activity under *in situ* and *ex situ* stress, which was relatively higher in *I. campanulata* than *J. pentantha*. Induction of CuZnSOD isoforms was most significant in the two species under both stress conditions. As SOD isoforms are actively involved in controlling the increasing levels of ROS under stress, their higher accretion in *I. campanulata* reveals its better tolerance to both *in situ* and *ex situ* stress. On the contrary, transient/less induction of *J. pentantha* SODs may demonstrate its sensitivity to stress. Amongst the two water stress conditions, activity of MnSOD and FeSOD isoforms show relatively higher activity under *in situ* stress while CuZnSODs show higher activity under *ex situ* stress in these species.
- High throughput sequencing uncovered four to eight million total small RNA reads from *ex situ* control and drought stressed libraries of *I. campanulata* and *J. pentantha*. Amongst these, one to four million total unique sequence reads were identified from the four libraries. The two species showed similar size distribution of these reads; wherein highest reads was observed for 24-nt size class, followed by second highest peak at 23-nt in control libraries and 21-nt in drought stress libraries. Amongst the unique miRNA reads, the abundance of 21-nt size class was highest in the two libraries of both the species.
- From control and drought stressed libraries of *I. campanulata*, 213 and 177 miRNAs belonging to 41 conserved miRNA families were identified respectively. While from *J. pentantha*, 150 and 176 miRNAs belonging to 35 conserved miRNA families were identified from control and drought stressed libraries respectively. Amongst these miRNA families, 34 families were analogous and remaining 8 were alien. Most of the analogous miRNA families showed high expression indicating their crucial role in normal development of both the species.
- Although most of the miRNA families were conserved between the two species, their innate expression was very different. miR156, miR160, miR164, miR167,

miR172, miR319 and miR403 were more abundantly expressed in *I. campanulata*, while miR166, miR159, miR168 and miR408 were more abundantly expressed in *J. pentantha*. Most striking differences were shown by miR403, miR858, miR390 and miR398, indicative of their distinguishing role in the two species.

- Drought responsiveness of miRNAs varied between the two species under both the water stress conditions. Under *ex situ* water stress, several miRNAs (miR156, miR159, miR160, miR164, miR167, miR171, miR172, miR393, miR394, miR395, miR396 and miR399) showed opposite regulation; wherein they were downregulated in *I. campanulata* and upregulated in *J. pentantha*. This shows differential mechanism of miRNA regulation in *I. campanulata* and *J. pentantha* in order to combat stress.
- In response to *in situ* water deficit, many of the analysed miRNAs (miR156, miR159, miR160, miR397, miR168, miR171, miR169, miR396, miR393 and miR167) were downregulated in both the species. This showed that drought responsiveness of highly conserved miRNAs remained similar under *in situ* and *ex situ* stress in tolerant-*I. campanulata*, which was not the case in sensitive-*J. pentantha*. Amidst these miRNAs, drought responsiveness of miR172, miR160, miR167, miR393, miR159, miR858 and miR169 were considered as the major drivers of drought tolerance in *I. campanulata*.