6. Summary

Though cotyledon number has been a major criterion to classify the flowering plants into monocot and dicot, only during last decade the information about genetic regulation of cotyledon development being obtained. The application of molecular and genetic techniques to study embryo development has provided a wealth of information about the role of different genes in embryo formation in *Arabidopsis.* However, in other plants such as tomato the information about embryo development and regulation of cotyledon number is limited.

In this study, we isolated and characterized two groups of cotyledon development mutants of tomato (*Lycopersicon esculentum* L.). The first group of mutant showed multiple cotyledons in the seedling and were named as *po*lycotyledon (*poc*) mutants. The genetic analysis of *poc* mutant revealed that *poc* is a monogenic recessive nuclear gene. In this group, nine mutant lines were isolated, but all these lines belonged to same complementation group, designated as *poc]-]* through *poc 1-9.* The *poc* mutation has a strong pleiotropic effect at all the stages of plant development right from embryogenesis till fruit setting. The studies on tomato embryogenesis revealed that the primary effect of *poc* mutation on cotyledon number is likely at the transition from globular to heart stage. In *poc* embryo this transition is accompanied with the thickening of radial axis of the embryo, consequently, *poc* embryo is 1.8 times wider than the wild type embryo. The increase in diameter of the embryo in lateral direction is followed by simultaneous initiation of 3-4 cotyledons on shoot end of the embryo.

The seedlings of homozygous *poc* mutants on germination showed cotyledon number ranging from 2-4. Among these tetracotyledons accounted for nearly 50% of seedlings followed by 35% tricots, others were with splitted cotyledons and only 1.5% of seedlings were dicots. All cotyledons in *poc* mutant arose from the same node on the

seedling hypocotyls ruling out the possibility of transformation of leaf in to cotyledons. The *poc* mutation affected the dark phenotype of the seedlings and etiolated seedlings showed shorter hypocotyls than the wild-type control. The roots of *poc* mutant grown in vermiculate were also shorter in length compared to the wild type. The role of phytochrome and/or component of the signal transduction chain was ruled out as *poc* double mutants with either of the phytochrome deficient mutant *au* or *hp* retained characteristic features of *poc* at all the stage of development.

The most prominent feature of *poc* mutant was alteration of vegetative and reproductive developments compared to wild type. The mutant showed altered phyllotaxy with a frequency of about 30% resulting in whorl phyllotaxy at the first node instead of normal spiral phyllotaxy. On the basis of variable expression of leaf morphology, the *poc* mutants were classified in to three major classes.

A. The class A mutant were bushy, leafy and showed formation of epiphyllous leaves with almost 100% frequency. The leaves of this class were strongly curled and had reduced leaf area. The fully mature plant was shorter than the wild type.

B. In class B, the plants were slightly shorter than the wild type. The plants were sparingly branched and have slightly reduced leaf area as compared to the wild type. In this group formation of epiphyllous leaves was rarely seen.

C. The class C mutant showed the extreme changes in leaf morphology with leaves ranging from simple lanceolate to compound leaf with six leaflets and one terminal leaflet. However, in all these leaves, the symmetry of organization was altered and small and minor leaflets were totally absent. The lobbing of leaf margin was also absent in this class.

Similarly, the reproductive development of *poc* mutant was also drastically altered. While the plants of A and B classes flowered at the same time as the wild type,

the onset of flowering was delayed in class C. In *poc* mutant, the reproductive development was severely affected and in most plants the inflorescence shows altered phyllotaxy. Moreover, the number of flowers per inflorescence was much higher than the wild type and all the flowers bloomed at the same time.

The floral organs in the *poc* mutant showed increase in number but a decrease in length of organs. The number of stamens was reduced in the class C of *poc* mutant. In A and B class the sepals showed some homeotic changes to petals at a frequency of 7.5%. The petals showed proliferation of tissue resulting in out growth on the inner side of flower, which invaded the fused stamen tube leading to the separation of stamens. In all the three classes the stamens showed absence of fusion and in class C the stamens were fused to carpels. Interesting, from the fully mature flowers of class C new inflorescence originated leading to formation of many flowers and in some instances big leaves.

In tomato, the expansion of the cotyledons is stimulated by light. We have also isolated a mutant, which showed defect in the cotyledon expansion named Narrow petioleless cotyledons *(npc)*. Interestingly, the expression of the mutant phenotype was age dependent. The freshly harvested seeds of *npc* mutant were phenotypically similar to wild type in all aspects of development; germination, hypocotyl elongation in light and dark, and cotyledon expansion. However, after storage of seeds for one year the *npc* mutant seedlings showed delayed germination, reduction in hypocotyl elongation and narrow petioleless cotyledons. This age dependent effect on these processes appeared only after three months of storage from harvest. The cotyledon area in *npc* mutant was reduced by more than 50% and the petiole was totally absent. In comparison to *poc*, the *npc* mutant showed less pleiotropic effect on vegetative and reproductive development, however plant height was slightly reduced than the wild type.

Interestingly, the one year old *npc* mutant shows partial constitutive photomorphogenesis in dark manifested by short hypocotyls, unfolded cotyledons and open hook. The double mutant *npc au* shows some inhibition of hypocotyl elongation and reduction of the cotyledon area similar to *npc*. In contrast, the *npc poc* double mutant shows the narrow cotyledons of *npc* mutant and all the other features of *poc*.

Finally, it is evident that these two mutants are very unique, in comparison with the mutants of *Arabidopsis*. The highest frequency of polycot seedlings in *poc* mutant and the age dependency of *npc* mutant show the necessity to investigate the plant development in other systems too.