

The *Coch* gene controls the subsequent differentiation of pea axial meristems into lateral structures

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The *coch* mutant is well known from Wellensiek (1) and Marx (2) initial studies and for many years it was considered to affect only stipules and flowers of pea plants. The *coch* phenotype is characterized with strongly reduced, sometimes filimorpha stipules. In some cases, particularly in the middle nodes of the plant, stipules can transform into small additional, adventitious leaves with leaflets and tendrils. The flowers of the *coch* mutants have numerous abnormalities, such as additional vexillum and wings instead of keel petals, an irregular number of stamens and low fertility due to abnormal opening of flowers (1, 2, 3).

In the last ten years it has been established that the compound leaf of pea forms as a result of interactions within a pool of homeiotic genes including *Uni*, *Coch*, *Tl* and *Af*. Interactions of genes *Uni* and *Coch* determine stipule formation, whereas interactions of *Uni*, *Tl*, and *Af* determine the formation of leaflets and tendrils (4). In addition, *Coch* is strongly involved in flower morphogenesis (unlike genes *Tl* and *Af*), affecting the shape and number of its elements (4, 5). All these data indicate that *Coch* could be one of the key factors in the formation of meristems of numerous organs and tissues of the plant, maybe not only pea, but in other Fabaceae as well. In addition, we found that *coch* had strictly visible effects on the pea root nodule meristem development and differentiation (6). The effects of the *coch* mutation on root nodule development have been confirmed by Ferguson and Reid (7).

In earlier research we found three new EMS and γ -ray induced mutants. Two of them showed a phenotype similar to *coch* — SGE239 (EMS) and SGR856 (γ -ray) (6,8,9), and one displayed a phenotype resembling *het* (heterophyllus) — SGE624 (9). The SGE624 mutant phenotype was characterized by stipules that were less strongly narrowed than in *coch*, but varied in shape and nearly normal flower formation. A number of allelic tests performed between these mutants and classic *coch*-lines NGB1743 (Nordic Gene Bank) and Wt11745, Wt11303 (Wiatrowo) revealed that all three new mutants are alleles of *coch* (9). SGE624 appeared to carry a weak *coch* allele — *coch*^{het} (9).

Root systems of the above mutants and standard *coch* lines were in general visually normal. All mutants formed active nitrogen-fixing nodules evidenced by pink coloration due to leghemoglobin synthesis. In contrast to the wild-type, small well-developed roots were formed at the tips of the mutant nodules. Only SGE624 (*coch*^{het}) did not produce this root-like structure, or produced only a few of them.

EMS-induced *coch* mutant line SGE239 was selected for further study because it had relatively good fertility and decreased plant size. A portion of the root system and an individual nodule of both SGE239 and ancestor line SGE is shown in Figure 1. It seems clear, that the *coch* mutant nodules produce additional rootlets. These rootlets develop from the nodular lateral meristem that forms a heavy root-tissue layer around the nodule. It was presumed that these abnormal nodules were able to fix nitrogen normally because the mutant plants did not show any signs of nitrogen starvation.

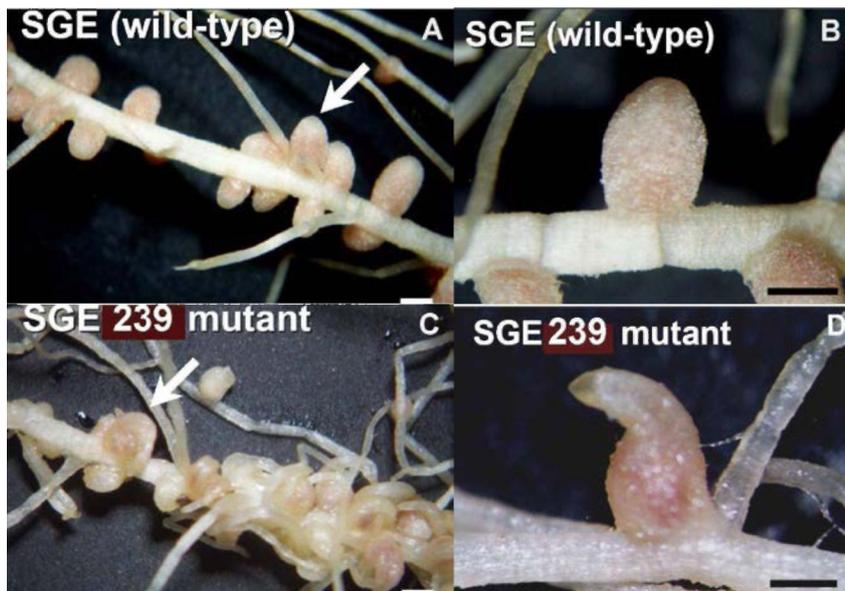


Figure 1. The root nitrogen-fixing nodules of mutant coch line SGE239 (C,D) and SGE ancestor line (A, B).

The stage at which nodules in the SGE239 mutant were transformed to a rootlet was identified by microscopic analysis. It was found that early stages of root nodule development in the mutant were similar to the parental line SGE.

Both wild type and mutant plants show the normal process of root hair curling, infection thread growth, nodule induction and primordium formation. Then similarly in both lines the infection thread penetrates nodule primordium, bacteria endocytosed into plant cell cytoplasm and immature nodular circular meristem is differentiated at the distal end of the nodule primordium. The meristem of mature indeterminate nodules is formed from one part of the circular meristem at the next stage of wild-type nodule development. But at the same time in SGE239 nodules a root meristem is developed from the part of nodular immature circle meristem at the distal end of young nodules. At a later stage, root growth in the mutant is observed at the tip of the nodule and the vascular system of this rootlet is connected to one of the vascular bundles of the nodule (Fig. 2). Thus, the pea gene *Coch* acts at the stage of mature indeterminate nodule meristem development, specifying nodule or root meristem identity.

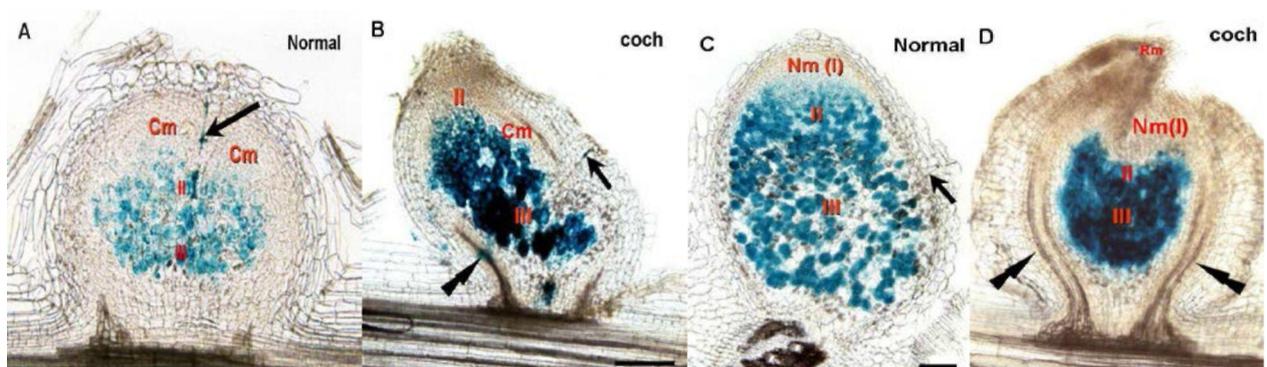


Figure 2. Two late stages of pea root nodule formation. A,C — ancestor line SGE; B,D — coch mutant line SGE239. Arrows

SGE239 mutant plants formed elliptical or spatulate stipules (Fig. 3A). These stipules resembled simple leaves with petiole and one leaf blade. Mismatched stipule pairs were also observed. For example, stipules of the same pair could be different in size or only one stipule was developed. Nevertheless, it is well known that some mutant alleles of *coch* can transform stipules into additional lateral compound leaves (1, 2, 4), as shown on Fig. 3C (4).

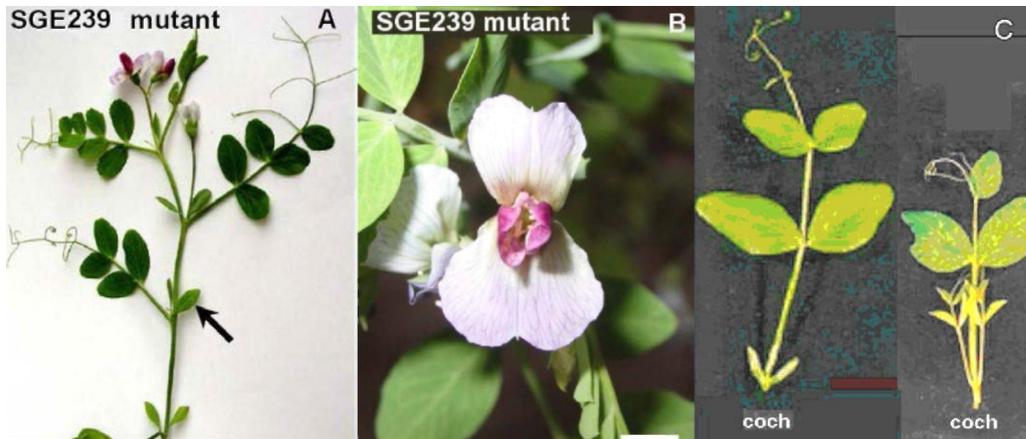


Figure 3. Stipules and flower of *coch* mutants. A — SGE239 plant. Arrow indicates reduced stipules. B — Double-vexillum

SGE239 mutant flowers are open and the pistil and stamens are not hidden by the two fused keel petals which are free and frequently transformed into extra vexillum or wing structures. The general number of petals is often increased and they are of variable shape. The double-vexillum opened flower of SGE-239 is shown on Figure 3B. Stamen number is also frequently increased and their bases are not fused into a tube. Usually one carpel is observed in mutant flowers but bicarpellum flowers were also found. Flowers are partially sterile due to the opened-flower type and mutant plants have more lateral branches in comparison with the wild type.

Thus, it was found that development of stipules, flowers and nodules are impaired in the SGE239 mutant line which is typical for all *coch* mutants.

According to Gourlay et al. (10), *Coch* acts in the course of compound pea leaf development at the stage of stipule meristem formation determining its fate to be a stipule meristem. This apparently gives evidence of a common mechanism of the *Coch* gene action to determine the fate of meristems during formation of various pea organs.

The current model of the formation of compound pea leaf (11) considers the gene *Uni* as a key gene in determination of meristems of lateral structures of a compound leaf. According to this model *Uni*, alone or in interaction with other genetic factors, "tries" to transform any indeterminate meristem of the leaf primordium into the rachis meristem, i.e. converting the leaflet meristem to the one producing a shoot-like structure. Expression of the genes *Tl* and *Af* in the corresponding lateral meristems of the leaf leads to inhibition of *Uni* action and determines their fate to be meristems of leaflets and tendrils. *Coch* also inhibits the effect of *Uni* at the base of the leaf that leads to the isolation of the future stipule meristems from partly undifferentiated cells of rachis meristems and to its differentiation into the stipule meristem.

In *coch* mutants a prolonged expression of *Uni* in the stipule primordia leads to the situation where the stipule meristem does not differentiate at a time and retains its original predetermination to be a leaf

rachis. As a result, depending on the duration of elevated expression of *Uni*, either a simple leaf (short action) or a lateral compound leaf (long action) are formed instead of a stipule. Thus, the *coch* mutation deprives the corresponding meristem of the ability to further differentiate and its development progresses toward basic axial differentiation.

During compound leaf formation in *coch* mutants the lateral meristem at the base of future leaves loses the ability to differentiate into stipules, but maintains basic differentiation of the compound leaf rachis, and as a result an adventitious lateral compound leaf is formed (Fig. 4).

We do not know what meristematic factor is inhibited by *Coch* during the flower corolla and root nodule

formation, but taking into account that the meristem-determination mechanism is highly conserved (12), we may conclude that if this is not *Uni*, it is a close homolog. Obviously, one of the external circles of the flower, the corolla, can be considered as a separate shoot. If the lateral meristem located at the base of the axis of this shoot loses its ability to differentiate into the keel petals in *coch* mutants it differentiates in a basic shoot-corolla way and as a result the *coch* plants fail to develop true keel petals, but have additional slightly differentiated wing-like petals and sometimes a second additional vexillum (Fig. 4).

During symbiotic root nodule formation in *coch* mutants the lateral part of its circular meristem apparently loses the ability to differentiate into nodule tissues leading to its reversion to root type. As a result, the lateral (cortical) part of the nodule gets thicker, total nitrogen fixation zone is reduced and a part of nodule meristem produces roots instead of nodule tissues (Fig. 4).

It is assumed that mechanisms of formation of various plant meristems are highly conserved (12). The genes controlling meristem formation and functioning could be recruited in evolution to control pea compound leaf and root nodule meristem formation and functioning. Therefore, genetic studies of legume compound leaf and nodule formation can be important not only for understanding the morphogenesis of organs specific to the Fabaceae family, but also for understanding the basic mechanisms of plant development.

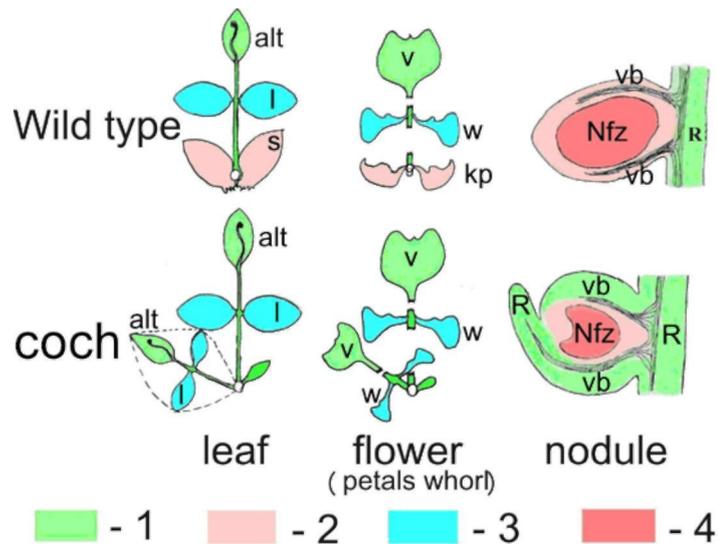


Figure 4. Effects of mutation in the *Coch* locus on various lateral meristem determination. 1 — organs with basic axial *Coch*-independent meristem determination; 2 — lateral organs with *Coch* meristem determination; 3 — lateral organs with meristem determination by other homeotic genes; 4 — nodule nitrogen-fixation zone. alt — apical leaflet of tendril; l — leaflet; s — stipule; v — vexillum; w — wings; kp — keel petals; R — rootlet; vb — vascular bundles; Nfz — nitrogen-fixation zone.

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