Molecular basis of two traits studied by Mendel

Genes encoding two traits studied by Mendel have been cloned.

(a) Seed Shape (the *r* locus of chromosome 7)).
(b) Stem Length (the *le* locus of chromosome 4).

Seed Shape (the *r* locus of chromosome 7)).

Bhattacharyya et al. (1990) have cloned the gene that encodes the starch branching enzyme isoform I (SBEI) and showed that the gene is interrupted by the insertion of a transposon-like element in the coding sequences.

- SBEI was found in the developing pea embryos from round seeded lines, not from the wrinkled seeded lines.
- Antibody raised against SBEI was used to screen a cDNA expression library.
- Northern blot analysis showed that the transcript levels in the developing embryos of wrinkled seeded lines are larger than that of the round seeded lines. The level of transcripts was much reduced in the embryos of the wrinkled seeded lines than that of the round seeded lines.
- The cDNA ypon hybridization to a DNA blot showed restriction length polymorphisms (RFLP) that co-segregated to the *R* locus studied by Mendel.
- An insertion in the mutant allele (*r*) caused the polymorphisms. It is transposon-like sequence with similarity to Ac/Ds family of transposable elements from maize.

Development of the wrinkled phenotype (Bhattacharyya et al. 1993)

- Two isoforms of SBE are expressed in the developing pea embryos.
- SBEI starts to express highly at early stages, while SBEII towards the later stages.
- No detectable activity of SBEI is found in the developing embryos of the wrinkled seeded plants.
- As a result of this the amylose is not branched and starch yield is reduced; sucrose content goes up, and therefore, sweet peas.
- Increases sucrose also increased the osmotic potential and higher water uptake during embryo development resulting increased cell size.
- During maturation cells shrink due to dehydration, and winkled seeds result.

- Membrane lipid content increases due to increased cell size in the mutant.

Stem Length (the *le* locus of chromosome 4).

Earlier it was shown that conversion of GA_{20} to the biologically active compound GA_1 is greatly reduced in *le* plants (dwarf) as compared to that in the *Le* plants (tall) (Ingram et al. 1984).

Two groups independently cloned the stem length gene (Le) that encodes a gibberellin 3B-hydroxylase (Lester et al. 1997; Martin et al. 1997).

Both groups applied a very similar approach in cloning the *Le* gene. Based on biochemical analysis data it was assumed that GA 3 β-hydroxylase is mutated in the *le* plants, and *Le* encodes this enzyme of the gibberellin (GA_1) synthesis involved in stem elongation.

- A putative gene (*GA4*) encoding *Arabidopsis* GA 3 β-hydroxylase was cloned by transposon tagging in 1995 (Chiang et al. 1995).
- Lester et al. (1997) used the part of the *Arabidopsis GA4* cDNA to screen a cDNA library to clone a partial cDNA. On the other hand, Martin et al. (1997) applied PCR approach in cloning the gene.
- Both groups characterized the gene from the *le* mutant and *Le* wild type plants by sequencing, and showed that amino acid alanine at residue 229 is changed to threonine in this gene.
- Martin et al. sequenced the gene in two additional *le* mutants, le^d and *le-3* and showed that in both mutants the gene is mutated. le^dcarries a frame shift mutation due to deletion of base G^{376} in addition to alanine to threonine convertion in the position 229. le^d is a null mutant and result severe dwarf phenotype.
- Both groups showed that the activity of the *E. coli* expressed mutant enzyme(s) was greatly reduced as compared to that of the wild type enzyme.
- Lester et al. also showed that the gene they have cloned mapped to the le locus.
- Therefore, *Le* encodes GA 3 β-hydroxylase to promote the conversion of GA_{20} to the biologically active compound GA_1 in tall pea plants.