The new xantha-variegata-viredescens mutation in pea *xavs*

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Leaf chlorophyll variegation is occasionally observed in many plants. Usually it looks like white, yellow or dark green spots or irregular regions on leaves. Mutations in both nuclear and organelle genomes reportedly may cause this leaf variegation (1). It raises a question why and how two neighboring sectors containing different cell types, a green cell with normal-appearing chloroplasts and a white cell with abnormal plastids, can be formed in one leaf tissue when the genome of all cells is identical. The precise mechanism leading to such pseudochimeric chloroplast development in the same leaf tissues remains poorly understood. One possible explanation for generation of non-identical variegated sectors in each leaf is a threshold level of factors that arrest proplastid differentiation into chloroplasts (2).

Variegated-type mutants are more rarely observed in plants than the ordinary chlorophyll mutants. In *Arabidopsis thaliana,* only four loci are known to cause the variegated phenotype (3). During experimental mutagenesis, pea mutants with *varicgata* or *maculata* phenotypes appear two orders of magnitude less frequently than the other chlorophyll mutants (4, 5). Only four to six pea variegated mutants have been previously reported and almost all of them appeared to be extinct (6). For the above reasons, isolation of a new pea *variegata* mutant is of great interest in the course of studying the mechanisms of variegated plant appearance and chloroplast differentiation and survival.

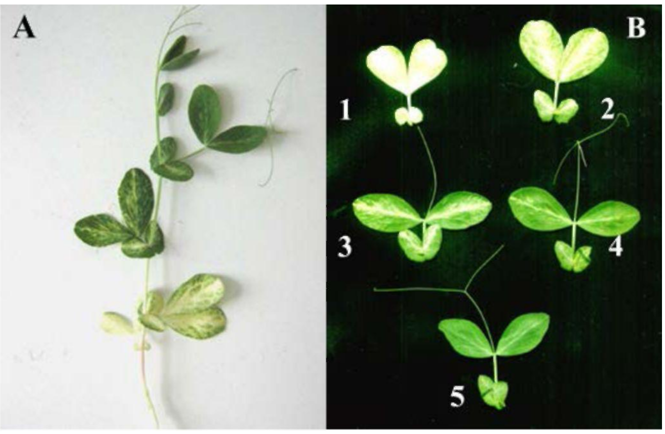

Figure 1. SGE0802 (xavs) seedling (A) andproggressive greenish gradient

Screening M_2 progeny of the EMS-treated pea line SGE revealed a new mutant SGE0802 characterized with the strong *xantha* effect on shoots and leaflets of the first expanded leaf (Figure 1).These structures are brilliant yellow, without any green spots even grown in the sunlight. Leaflets at the next three nodes (2-4) of SGE0802 sequentially and progressively become green — yellow sectors become pale or even white and green sectors arise in geometric progression from one node to the next (Figure 2). The 5th node of the mutant plants becomes completely green without any allusion of yellow or other colored sectors. It is notable, that among nearly 5000 plants analyzed, none become fully green at the 4th or 6th node — it was always the 5th node in all cases. The upper part of the SGE0802 plant is completely normal and fertility is not affected.

Figure2. The 3rd node of a SGE0802 (xavs) seedling. Pale yellow, green and dark green sectors are visible.

To determine the inheritance type of SGE0802 mutation, we crossed it with the parental line SGE in both directions, using the mutant as either the male or female parent. In both cases, all F_1 progeny were normal, without any variegation on leaves and all shoots were green. Segregation results in the F_2 for these crosses are shown in Table 1. In both crosses, the ratio of normal and variegated seedlings segregated did not differ significantly from 3:1 ($p > 0.5$). The mutation appears to be inherited as a single recessive nuclear gene. We designated the observed SGE0802 phenotype as *xavs* — *xantha-variegata-viridescens* seedlings.

Table 1. Segregation** of F_2 hybrids **from crosses of line** SGE00802 **(xavs) with parental line SGE.

Hybrid	**F_2 segregation phenotype**		**Segregation 3:1**	**Probability**
	Xavs	***xavs***		**P**
SGE0802 x SGE	184	64	0.086	0.7-0.8
SGE x SGE0802	152	56	0.410	0.5-0.6

We tried to localize *xavs* on the pea genetic map. Line SGE0802 was crossed with a number of tester lines, but we failed to find linkage of *xavs* with any of the genetic markers involved. *Xavs* is not positioned near genes i, r, d, *wb, k, st, b, a, le, gp, wlo,* oh, *p, pl.* The question concerning its location on pea gene map remains unanswered.

In *Arabidopsis thaliana,* two genes, *var1* and *var2* produce the variegation pattern, very similar to the pattern observed in *xavs* pea plants, but the Arabidopsis phenotype is constitutive during all plant ontogenesis (7). Arabidopsis variegated mutations also form three sector types: white, pale green and dark green, again as was observed in the *xavs* mutant. In Arabidopsis mutants, dark green sectors contain normal chloroplasts whereas plastids in the white sectors are vacuolated with obscure inner membrane structures. Cells of pale green sectors contain plastids of both types. Proplastids in shoot apical meristems in *var* Arabidopsis mutants are normal, and abnormal plastids lacking inner membrane structures begin to form at later stages (3). *Var* genes belong to a small gene family of 12 nuclear genes coding for special chloroplast-localized FtsH metalloproteases, homologous to bacterial metalloproteases and responsible for degradation of photodamaged proteins within the photosynthetic machinery. Without this protease activity photosynthetic complexes become corrupted and that leads to irreversible chloroplast degradation (8).

As was shown by scanning electron microscopy, the pea shoot apical meristematic region involves well differentiated primordium for 4-5 nodes (9). Pea seeds possess a well-developed embryo, so we can suggest that it also contains well-expanded primordium for the 4-5 first leaves of the future seedling. In Arabidopsis FtsH genes are stage-specific and only two loci from 12 can produce variegated phenotypes similar to *xavs.* We can suggest, that *xavs* is a homolog of one of the FtsH-family representatives and is expressed specifically during seed development and maturation, at the stage of initiation of the 1st lateral meristem primordium formation. The mutant allele *xavs* produces a non-functional protease product, but this product can migrate into proplastids and bind with inner thylakoid membranes where normal metalloproteases are usually located. A little bit later, during formation of the 4th and 5th node meristems, expression of the other FtsH genes begin and producing two opposite FtsH gradients along primordium 1-5. At primordium 5 the concentration of functional FtsH metalloproteases becomes enough to result in normal plastid development. The concentration during initiation of the 2nd - 4th primordiums is at a threshold level and that leads to a variegated phenotype appearance where some plastids may or may not develop normally. certainly, pea gene *xavs* may produce some factor other than FtsH metalloprotease, but the general mechanism of variegated phenotype origin must be the same.

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