J. Genet. Vol. 68, No. 1, April 1989, pp. 9–21. © Printed in India.

Isolation and characterization of *gsv* mutants of grain pea (*Pisum sativum*) having white stem and green leaflets

SUSHIL KUMAR[1]*†, RAGHUVEER POLISETTY[2], NAM PRAKASH[3] and T. V. R. NAIR[3]
[1]Biotechnology Centre and Division of Genetics, [2]Division of Plant Physiology and [3]Nuclear Research Laboratory, Indian Agricultural Research Institute, New Delhi 110 012, India
†Present address: Council of Scientific and Industrial Research, Rafi Marg, New Delhi 110 001, India

MS received 17 February 1989

Abstract. Two Gsv^- mutants have been isolated in mutagenized grain pea (*Pisum sativum*). A nuclear gene is affected in each mutant. The Gsv^- plants are recessive homozygotes (*gsv/gsv*). They bear white stems and green leaves; the petiole, rachis, and veins on stipules and leaflets are also white. In the Gsv^- plants, the stem is devoid of chlorophylls, although normal amounts of chlorophyll *a* and *b* are present in leaflets. Mature chloroplasts and CO_2 reduction ability are present in mesophyll tissue of leaves but absent in stem. The characteristics of Gsv^- mutants allow the conclusion that the genes involved in chloroplast development and photosynthetic functions are regulated by different nuclear genes in mesophyll leaf cells on the one hand and green cells of stem and related tissues in functionally different segments of shoot on the other. It is proposed that segment-wise genetic control of photosynthesis in shoot is a rule among higher plants.

Keywords. *Pisum sativum* (pea) *gsv* mutants; photosynthetic nuclear gene *gsv*; regulation of photosynthesis; tissue-specific chloroplast expression.

1. Introduction

Observations made on the green photosynthetic cells of the shoots of several angiosperms, which relate maintenance, structural and photosynthetic functions of chloroplasts to nuclear and plastid genomes, have indicated that development of chloroplasts requires coordinated expression of nuclear and plastid genes (Bogorad 1967; Kirk and Tilney-Basset 1978; Sundquist *et al.* 1980; Bradbeer 1981; Schmidt *et al.* 1985). Nuclear genome products that have been shown to function in chloroplasts include the small subunit of ribulose biphosphate carboxylase (RUBISCO; RbcS), chlorophyll *a/b* binding protein (Cab), NADH-protochlorophyllide reductase and ferredoxin-I (Fed I) (Thorber and Markwell 1981; Akazeva *et al.* 1984; Dobres *et al.* 1987). The complementary plastid genome products identified in chloroplasts are the large subunit of RUBISCO (RbcL), photosystem I reaction centre protein (PsaA), photosystem II reaction centre protein (PsbA), ATP synthetase (Atp) and cytochrome F (Thorber and Markwell 1981; Fluhr *et al.* 1983; Dyer 1985; Bookjans *et al.* 1986). Experimental evidence shows that specific proteins produced from nuclear and plastid genes form oligomeric complexes for their function in chloroplasts. Analysis of major chloroplast proteins has revealed that their genes are under transcriptional and post-transcriptional controls (Smith and Ellis 1981; Thompson *et al.* 1983; Sasaki *et al.* 1984; Silverthorne and Tobin 1984; Berry *et al.* 1985; Dobres *et al.* 1987). The expression of the genes *rbcS*, *rbcL*

*For correspondence

and *cab* in shoot has been shown to depend on both light and phytochrome (Gallagher and Ellis 1982; Nelson *et al.* 1984; Silverthorne and Tobin 1984; Berry *et al.* 1985; Thompson *et al.* 1985; Tobin *et al.* 1985; Dobres *et al.* 1987). Characterization of cloned *rbcS* and *cab* nuclear genes of several plants has revealed that they occur in families (Broglie *et al.* 1983; Cashmore 1984; Dean *et al.* 1985; Lamppa *et al.* 1985; Leutwiler *et al.* 1986). Although proteins encoded by multigene-family members appear to be functionally as well as structurally similar (Corruzi *et al.* 1983; Dean *et al.* 1985; Dunsmuir 1985; Pichersky *et al.* 1985, 1987; Kohorn *et al.* 1986), there is evidence for tissue-specific expression of different members of gene families (Corruzi *et al.* 1984). Analysis of promoter sequences of the *rbcS*-family genes of pea has revealed both variable and conserved regions (Morrelli *et al.* 1985; Grandbastien *et al.* 1986). These observations imply the existence of genetic control elements that mediate expression from specific members of nuclear gene families determining chloroplast functions in specific shoot segments and tissues of segments (the green shoot segments in dicotyledons are leaves, stem, pedicel, calyx, ovary and young fruit, and in monocotyledons they are shank, husk, glumes and pericarp of caryopsis besides leaves and stem). Thus regulation of plastid gene expression has been observed at the level of gene selection, and transcriptional, translational and post-translational levels (VanGrinsven and Kool 1988).

Several nuclear gene photosynthetic mutants, of both dicots and monocots, possessing pigment-deficient phenotype have been investigated for the morphology of chloroplasts and quantities of major chloroplast proteins present in their shoot tissues (von Wettstein *et al.* 1971; Weidner *et al.* 1976; Simpson *et al.* 1977; Smellie *et al.* 1977; Mascia and Robertson 1978, 1980; Walbot and Coe 1979; Bellemare *et al.* 1982; Miles 1982; Schwarz and Kloppstech 1982; Harpster *et al.* 1984; Leto *et al.* 1985). The analyses of the mutants have not provided any insight into shoot-segment specific developmental regulation of chloroplasts.

Tissue-specific morphological and functional differences between chloroplasts have been noted in C4 plants (Edwards and Walker 1983). In their leaves, mesophyll (M) cells and bundle sheath (B) cell chloroplasts function as complementary compartments. The M cells with chloroplasts possessing relatively well-developed thylakoid membranes carry out CO_2 fixation and transport the product to B cells where carbon reduction occurs in the chloroplasts. There is evidence for differential regulation of chloroplast proteins in B and M cells. It has been shown that RbcS and RbcL are present only in B cells and Cab is much more abundant in M cells than in B cells (Broglie *et al.* 1984; Sheen and Bogorad 1987). No other developmental, morphological, biochemical or functional differences of significance have been noted between the chloroplasts of various tissues of growing shoots in C4 and C3 plants.

Thus it emerges that differences are expected between chloroplasts of various shoot segments, in terms of genetic determinants of their components, but have not been observed. The question can be simply approached genetically by reasoning that in plants viable mutants should occur in which, say, the mesophyll tissue of the leaves is photosynthetically active but one or more of the other shoot segments are non-green and inactive in photosynthesis. Experiments were therefore done to screen mutagenized populations for isolating such mutants in pea.

Pea (*Pisum sativum*) is emerging as a useful system for molecular genetics of plant biosynthetic and developmental processes. It is proving complementary to the

Arabidopsis thaliana system. Useful attributes of the pea system are: large flowers, self-pollination as sexual mechanism, a wide array of characters that are easily observable at different stages of plant life, and a large body of data existing from breeding, genetic, physiological, biochemical and molecular biology experiments (Blixt 1974; Makasheva 1983; Kumar and Sharma 1986; Kumar 1988).

It was noted from the list of genetic markers reported in pea (Blixt 1974, and literature since then) that *gp/gp* plants have phenotype similar to such as those hypothesized above. The *gp/gp* plants bear green leaves and stem, but pods are yellow in colour. In the present study we recovered two induced mutants which are characterized by photosynthetically active leaves and photosynthetically inactive stem. Here we report the isolation and characteristics of these white stem mutants.

2. Materials and methods

2.1 *Isolation of mutants*

Mutants were isolated in two multimarked strains of *P. sativum*, L48-*a gp k p pl* and L49-*dbn tac* (plants homozygous for the new marker *dbn* are pleiotropically dwarf, branched and necrotic). Mutagenesis was carried out by soaking dry seeds in aqueous 0·05% ethylmethanesulphonate solution for 16 h. The treated seeds were recovered and sown in the Institute's field to yield M_2 seeds. The mutants were identified in the M_2 generation. A mutant each was recovered in the strains L48 (=*gsv-1*) and L49 (=*gsv-2*). About 500 M_2 lines of L48 and 350 M_2 lines of L49 yielded these mutants. The L48 Gsv^- mutant was called L48-M1 and the other one was designated L49-M2. For making cross-pollinations and observations, the mutant plants, F_1's and segregating progenies were grown in the Institute's fields in plots and soil-filled pots in rabi seasons (November–April).

2.2 *Measurement of CO_2 fixation rates*

Shoots for sampling were taken from plants about eight weeks old. Samples were excised from the fourth leaf from the top and internodes above and below this leaf. The stem samples were internodes. Basal leaflet pairs comprised each leaf sample. For the purposes of an assay, a sample with its basal end retained in water-moistened cotton was enclosed in a chamber connected to a moisture-free air source at 23 ± 2°C and 1100 μ E m^{-2} s^{-1} light intensity. The rate of CO_2 exchange in the chamber was measured with an infrared gas analyser (Model 225 MK3, Analytical Development Company, UK) set in differential mode.

2.3 *Estimation of chlorophylls*

Shoot-tip comprising 5 leaves was excised from each sampled plant. Internodes cut out from the shoot were treated as stem sample. Leaflets were taken as leaf sample. For each sample chlorophylls were extracted from 1 g of fresh tissue in 500 ml of 80% acetone and estimated in micrograms by the following formulae: chlorophyll $a = [12{\cdot}7(D663) - 2{\cdot}69(D645)] \times V/10^6 \cdot W$, chlorophyll $b = [22{\cdot}9(D645) - 4{\cdot}68(D663)]$

$\times V/10^6 \cdot W$, total chlorophylls$=[20{\cdot}2(D645)+8{\cdot}02(D663)]\times V/10^6\cdot W$, where D663 = the optical density (OD) of extract at 663 nm wavelength, D645 = OD at 645 nm, V(volume) = 500 ml and W(weight) = 1 g (= 10^6 μg) (Sestak 1971).

2.4 *Electron microscopy of chloroplasts*

Leaflets of second and third leaf from the top and internodes above these leaves were sampled in each case. The samples were cut into 1 mm segments and fixed in 6% glutaraldehyde and 1% osmium tetroxide in cacodylate buffer (pH 7·0) for 3 h each. They were pre-stained with 1% uranyl acetate for 5 min before dehydration. The samples were embedded in spur. The blocks containing embedded tissue were polymerized at 60°C for 36 h. Sections were cut on an LKB Ultratome-1. After staining with uranyl acetate and lead citrate they were mounted on copper grids and observed in a Philips 300 transmission electron microscope at 80 kV (Hayat 1972).

3. Results

Both *gsv-1* and *gsv-2* mutations were found to be recessive. The Gsv^- plants (grown under natural light) were morphologically indistinguishable from their Gsv^+ parents until 3- to 5-leaf stage. Later they grew into plants whose stems were paper-white (figures 1 and 3) and stipules and leaflet portions of leaves were green, except for the midrib and veins, which appeared white (figures 2 and 4). The petiole and rachis of leaves were also almost white in colour (figure 2). In comparison, the analogous plants of the parent lines had entirely green leaves and stem. The homozygous *gsv-1* plants produced seeds naturally. Flowers were formed in normal numbers on *gsv-2* homozygotes but all of them were sterile (homozygous *gsv-2* plants were recovered as segregants from the progeny of heterozygous plants). Both types of Gsv^- plants were weaker in growth. The pods borne on *gsv-1/gsv-1* plants were smaller and crinkled (figure 5). The rest of the traits of the Gsv^- mutants were also typical of their parents. In the *gsv-1* homozygote and in its parent the immature pods were yellow because both carried the *gp* marker (figure 5). It was ascertained that Gsv^- phenotype is distinct from Gp^- phenotype. The pods on Gsv^- plants derived as gp^+ *gsv-1*/gp^+ *gsv-1*, from suitable crosses, had green pigmentation and were crinkled.

To characterize the *gsv-1* mutation, the mutant was reciprocally crossed with the strain L100 carrying the *wb* marker (table 1). The F_1 plants resulting from both the crosses were phenotypically Gsv^+. In each of the crosses, Gsv^+ and Gsv^- plants occurred in the F_2 generation in proportions consistent with the involvement of a

Table 1. Segregation of *gsv-1* mutation in reciprocal crosses.

Parents in the cross	Phenotype of F_1	Segregants for the *gsv-1* marker
L48-M1, *gsv-1 gp k p* + +/ L100, + + + + *pl wb*	Wild type	225 Wild 56 Gsv^-
L100/L48-M1	-do-	347 Wild 68 Gsv^-

Figure 1. Shoot tips of L48-M1 Gsv^- (left) and L48 Gsv^+ (right) plants.

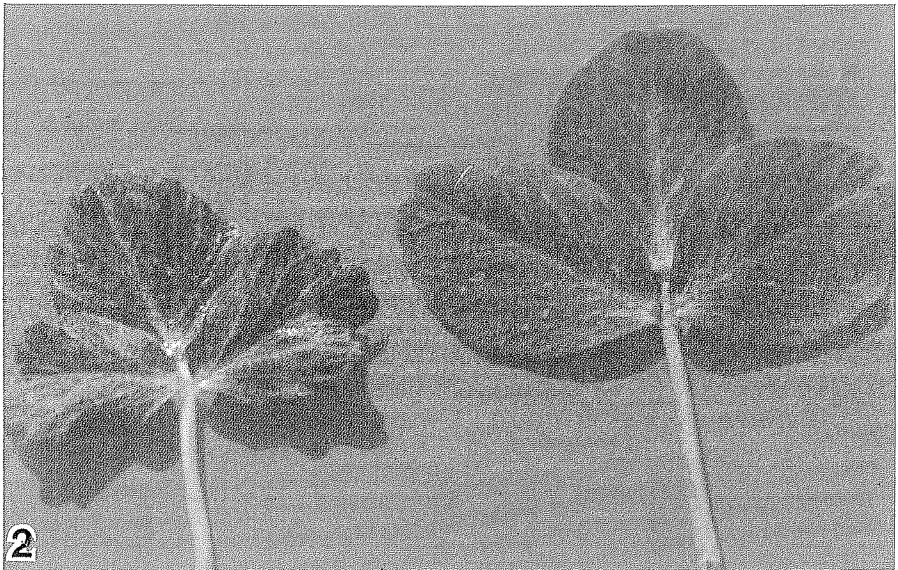
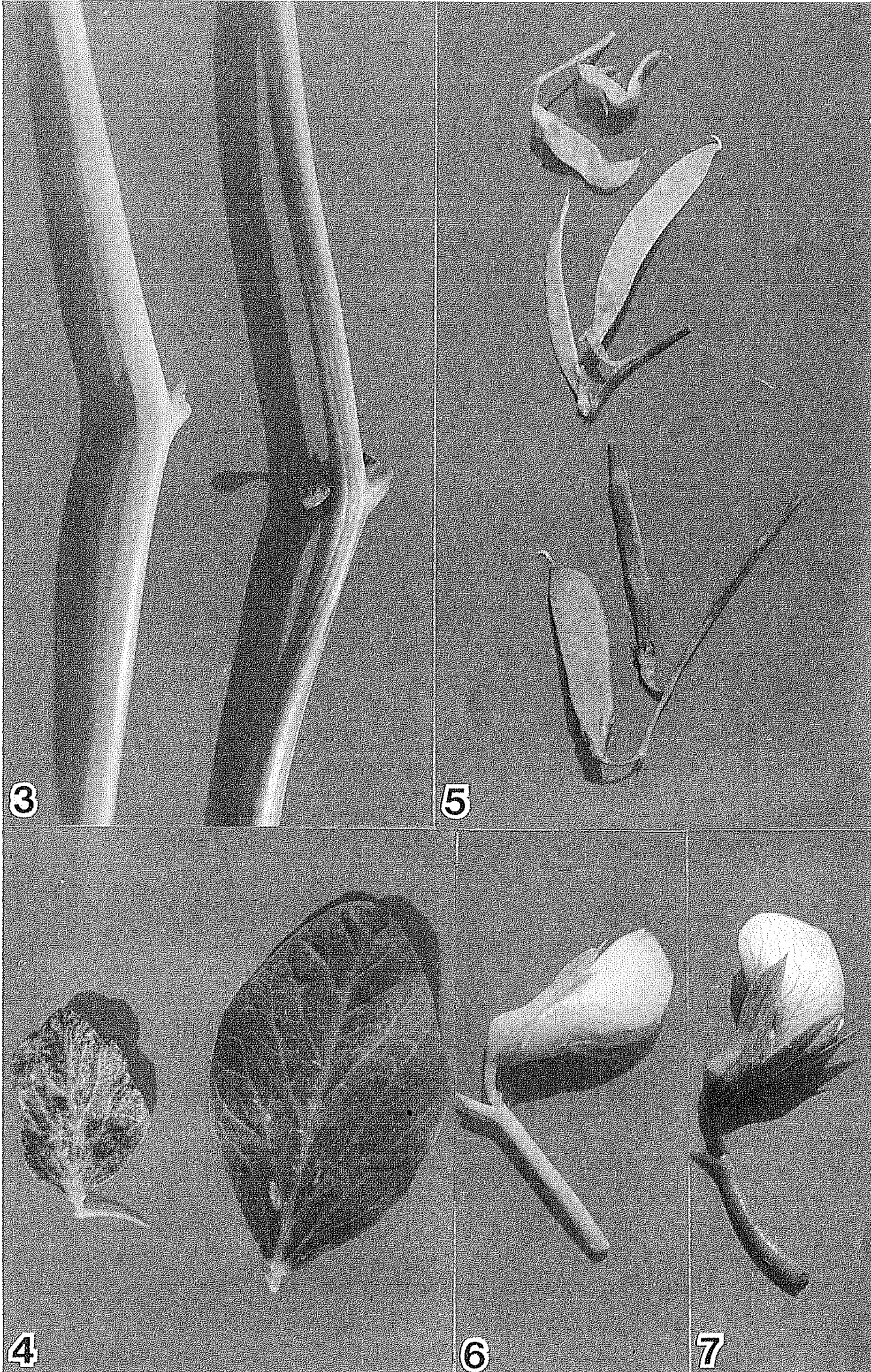


Figure 2. Leaves of L49-M2 Gsv^- (left) and L49 Gsv^+ (right) plants.



Figures 3–7. **3,** Stems of L48-M1 (left) and L48 (right) plants; **4,** leaflets of L49-M2 (left) and L49 (right) plants; **5,** pods of L48-M1 (top), L48 (middle), and L49 (bottom) plants; **6,** a flower of L48-M1 plant; **7,** a flower of L48 plant.

Table 2. Rates of CO_2 reduction in stem and leaf portions of shoots of Gsv$^+$ and Gsv$^-$ *P. sativum* plants.

P. sativum strain		mg CO_2 reduced/h/g dry weight of	
Designation	Relevant genotype	Basal pair of leaflets	Internode
L48 (wild type)	$gp\ gsv^+/gp\ gsv^+$	53·7	4·2
L48-M1 (mutant)	$gp\ gsv\text{-}1/gp\ gsv\text{-}1$	66·5	0
L49 (wild type)	$gp^+\ gsv^+/gp^+\ gsv^+$	59·6	3·7
L49-M2 (mutant)	$gp^+\ gsv\text{-}2/gp^+\ gsv\text{-}2$	15·8	0

single Mendelian gene; the observed deficiency of the Gsv$^-$ class might be indicative of selective embryogenic lethality. These observations established that Gsv$^-$ phenotype in the mutant is due to homozygosity of the mutated *gsv-1* factor (gene) of the nuclear genome.

Portions of stem and fully expanded leaves of Gsv$^-$ mutants and their Gsv$^+$ parents were assayed for their ability to take up CO_2. As shown in table 2, leaflets and stem samples of the Gsv$^+$ plants and leaflets of the Gsv$^-$ plants fixed CO_2. The stem samples of Gsv$^-$ plants failed to fix CO_2 to detectable levels. These results show that the stem segment of the shoot of Gsv$^-$ pea plants is not active in photosynthesis.

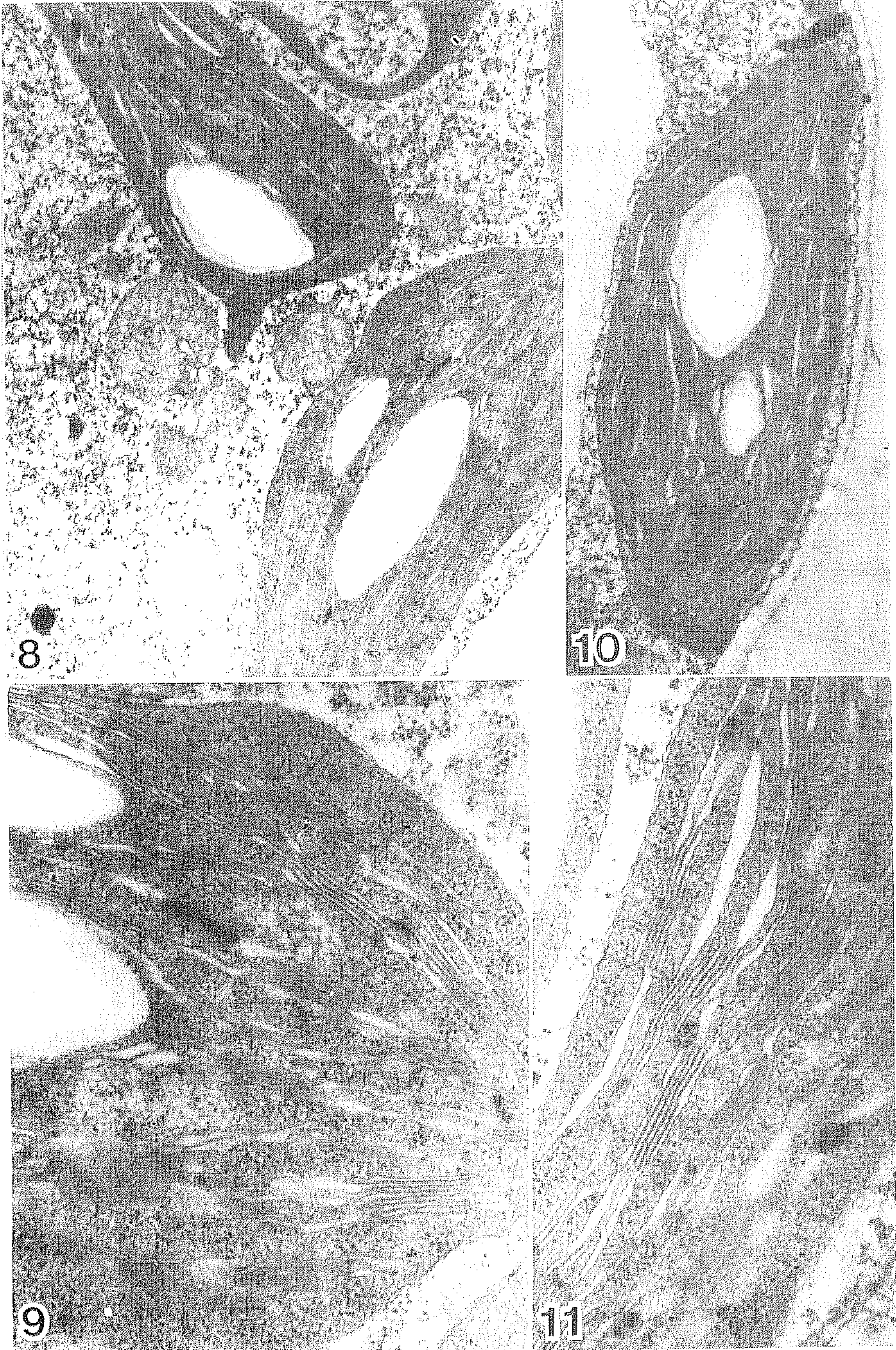
Chlorophyll contents of internodes and leaflets of fully expanded leaves of Gsv$^+$ and Gsv$^-$ plants were measured (table 3). Both chlorophyll *a* and chlorophyll *b* were present in high amounts in the leaflets of both Gsv$^+$ and Gsv$^-$ plants. In the Gp$^-$Gsv$^+$ L48 plants the total chlorophyll content of internodes was about 4% of that of leaflets; in Gp$^+$Gsv$^+$ L49 plants it was about 2% of that of leaflets. Stems of Gp$^-$Gsv$^+$ plants were relatively more deficient in chlorophyll *b*. However, chlorophyll pigments were hardly detectable in the internodes of Gsv$^-$ plants with Gp$^+$ or Gp$^-$ backgrounds. These results demonstrate that the bleached appearance of stems of Gsv$^-$ plants is due to the absence of chlorophylls from them.

Ultrastructural examination showed that the leaf mesophyll cells of Gsv$^+$ and Gsv$^-$ plants had chloroplasts possessing well-stacked lamellae as well as starch grains (figures 8–11). Normal chloroplasts were present in the stems of Gsv$^+$ plants

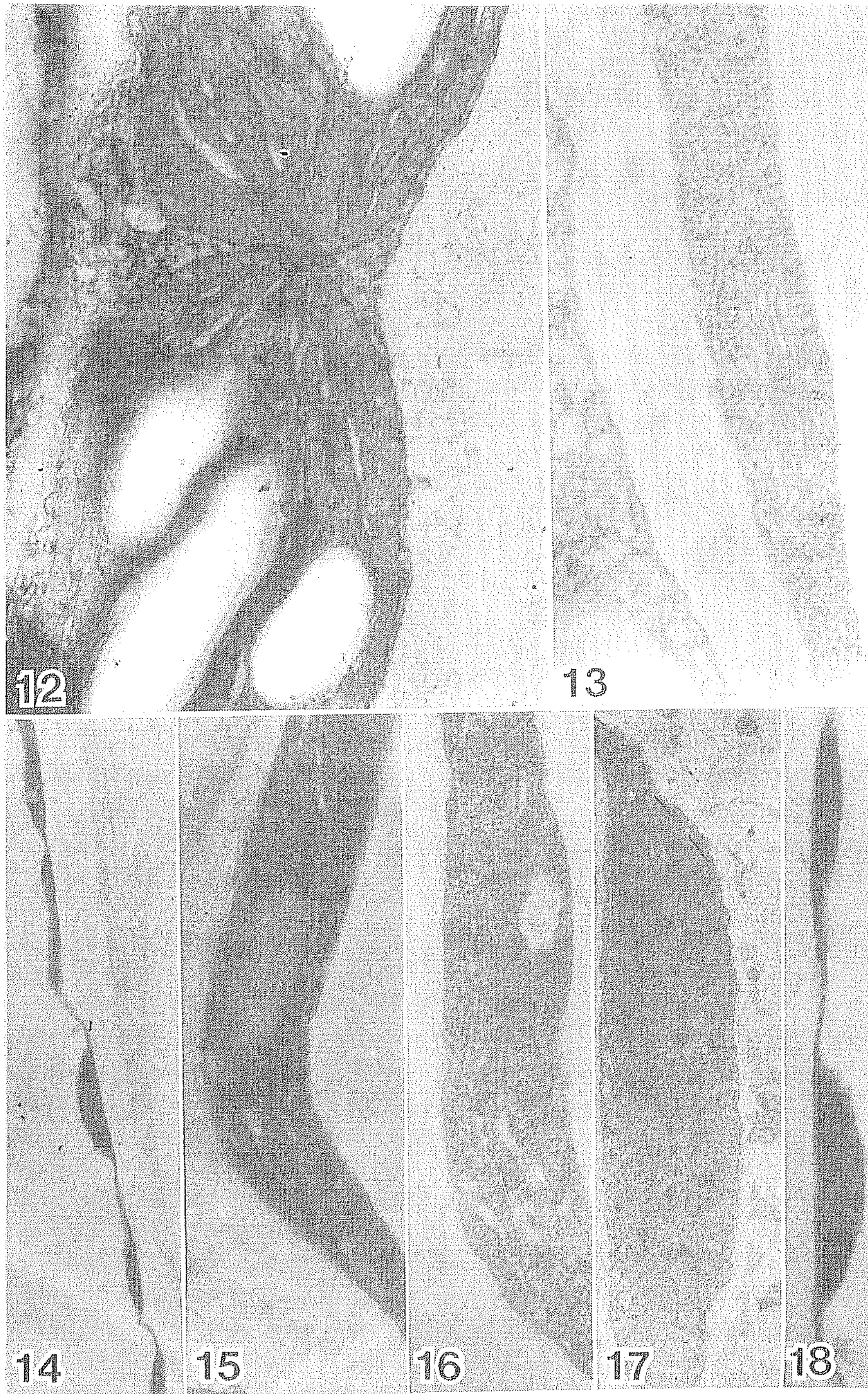
Table 3. Chlorophyll content of stem (internodes) and leaves (leaflets) of homozygous Gsv$^+$ and Gsv$^-$ *P. sativum* plants.

			Amount in μg/g fresh weight of sampled tissue		
Strain	Genotype	Tissue	Chlorophyll *a*	Chlorophyll *b*	Total
L48	$gp\ gsv^+/gp\ gsv^+$	Leaflets	1398	401	2154
		Internodes	69	1	87
L48-M1	$gp\ gsv\text{-}1/gp\ gsv\text{-}1$	Leaflets	1495	42	2301
		Internodes	< 1*	< 1*	< 1*
L49	gsv^+/gsv^+	Leaflets	1439	423	2211
		Internodes	26	6	38
L49-M2	$gsv\text{-}2/gsv\text{-}2$	Leaflets	859	400	1440
		Internodes	< 1*	< 1*	< 1*

*Pigment present in very small to non-measureable quantities.



Figures 8–11. Chloroplasts of Gsv^+ leaflet (figures 8 and 9) and Gsv^- leaflet (figures 10 and 11). The chloroplasts are normal and lens-shaped, and contain stroma in which the lamellar system consisting of grana made up of thylakoids is embedded.



Figures 12–18. **12,** Chloroplasts of Gsv^+ stem; chloroplasts have normal appearance; **13–18,** Plastids of Gsv^- stem; plastids are smaller and irregular in shape, and have little internal organization.

(figure 12). There were no mature chloroplasts in the stem cells of Gsv⁻ plants. The structures present in Gsv⁻ stem cells were much smaller than chloroplasts (figures 13–18). Their shapes were highly irregular, and they lacked the internal organization of chloroplasts; some vesicles were however present in them. These observations show that stems and related tissues of Gsv⁻ plants are white, chlorophyll-less and unable to photosynthesize because chloroplast development is blocked in them.

4. Discussion

Isolation of a mutant of new phenotype is the first step in the identification of a new biological function(s) and definition of the role of the affected gene in the determination of the concerned function. The availability of a mutant(s) for a gene opens ways to the cloning of the gene. In this work we have reported the isolation of two independent and stable nuclear gene mutants of *Pisum sativum* possessing the phenotype called Gsv^-. The Gsv^- mutants bear white stems. Their compound leaves are green except that, in each leaf, the petiole, rachis and veins of stipules and leaflets are white because of deficiency of chlorophylls. Mature chloroplasts are not formed in the stem of mutant plants, and the stem does not reduce CO_2. In the leaflets, mature chloroplasts develop and CO_2 is reduced.

The above observations on the isogenic Gsv^+ and Gsv^- plants of pea have led to the following conclusions: (i) the genes and proteins involved in chloroplast development and photosynthetic function in chloroplasts are regulated differentially, by nuclear genes, in the mesophyll tissues of leaves on the one hand and in stem and vein tissues of leaves on the other; and (ii) use of a specific gene over other analogues is the mechanism of modulation of the expression of other chloroplast-specific nuclear and plastid genes during development and differentiation of distinct shoot tissues.

A literature search revealed that the phenotype of the Gsv^- mutants might be similar to that of a mutant of peanut in which the white stem character was related to recessive mutations in two genes *ws1* and *ws2* (Branch *et al.* 1982).

Pea and peanut plants are typical dicotyledonous representatives of angiosperms. Since Gsv^- types of mutants could be isolated in pea and peanut, we propose that segment-wise genetic control of photosynthesis in shoot is a rule among higher plants.

References

Akazeva T., Takake T. and Kabayashi H. 1984 Molecular evolution of ribulose-1, 5-biphosphate carboxylase/oxygenase. *Trends Biochem. Sci.* 9: 380–383

Bellemare G., Bartlett S. G. and Chua N.-H. 1982 Biosynthesis of chlorophyll *a/b* binding polypeptides in wild type and the chlorina f2 mutant of barley. *J. Biol. Chem.* 257: 7762–7767

Berry J. O., Nikolau B. J., Carr J. P. and Klessig D. F. 1985 Transcriptional and post-transcriptional regulation of ribulose-1, 5-biphosphate carboxylase gene expression in light- and dark-grown Amaranth cotyledons. *Mol. Cell Biol.* 5: 2238–2246

Blixt S. 1974 The pea. In *Handbook of genetics* (ed.) R. C. King (New York: Plenum) vol. 2, pp. 181–221.

Bogorad L. 1967 Control mechanisms in plastid development. In *Control mechanisms in development processes* (ed.) M. Locke (New York: Academic Press) pp. 1–31

Bookjans G., Stummann B. M., Rasmussen O. F. and Henningsen K. W. 1986 Structure of a 3·2 kb region of pea chloroplast DNA containing the gene for 44 kDa photosystem II polypeptide. *Plant Mol. Biol.* 6: 359–366

Bradbeer J. W. 1981 Development of photosynthetic function during chloroplast biogenesis. In *Biochemistry of plants* (eds.) P. K. Stumpf and E. E. Conn (New York: Academic Press) pp. 424–472

Branch W. D., Hammons R. O. and Kirby J. S. 1982 Inheritance of white stem peanut. *J. Heredity* 73: 301–302

Broglie R., Corruzi G., Keith B. and Chua N.-H. 1984 Molecular biology of C4 photosynthesis in *Zea mays*: differential localization of proteins and mRNAs in two leaf cell types. *Plant Mol. Biol.* 3: 431–444

Broglie R., Corruzi G., Lamppa G., Keigh B. and Chua N.-H. 1983 Structural analysis of nuclear genes coding for the precursor to the small subunit of wheat ribulose-1, 5-biphosphate carboxylase. *Bio/Technology* 1: 55–61

Cashmore A. R. 1984 Structure and expression of pea nuclear gene encoding a chlorophyll *a/b* binding polypeptide. *Proc. Natl. Acad. Sci. USA* 81: 2960–2964

Corruzi G., Broglie R., Cashmore A. R. and Chua N.-H. 1983 Nucleotide sequences of two cDNA clones encoding the small subunit of ribulose-1, 5-biphosphate carboxylase and the major chlorophyll *a/b* binding thylakoid polypeptide. *J. Biol. Chem.* 258: 1399–1402

Corruzi G., Broglie R., Edwards C. and Chua N.-H. 1984 Tissue-specific and light-regulated expression of a pea nuclear gene encoding the small subunit of ribulose-1, 5-biphosphate carboxylase. *EMBO J.* 3: 1671–1679

Dean C., Van den Elzen P., Tanaki S., Dunsmuir P. and Bedbrook J. 1985 Linkage and homology analysis divides the eight genes for the small subunit of petunia ribulose-1, 5-biphosphate carboxylase into three gene families. *Proc. Natl. Acad. Sci. USA* 82: 4964–4968

Dobres M. S., Elliot R. C., Watson J. C. and Thompson W. F. 1987 A phytochrome-regulated pea transcript encodes ferredoxin-I. *Plant Mol. Biol.* 8: 53–59

Dunsmuir P. 1985 The petunia chlorophyll *a/b* binding protein genes: a comparison of CAB genes of different gene families. *Nucleic Acids Res.* 13: 2503–2518

Dyer T. A. 1985 The chloroplast genome and its products. In *Oxford surveys of plant molecular and cell biology* (ed.) B. J. Miflin (New York: Oxford University Press) vol. 2, pp. 147–177

Edwards G. and Walker D. 1983 *C_3, C_4: mechanisms and cellular and environmental regulation, photosynthesis* (Berkeley: University of California Press).

Fluhr R., Fromm H. and Edelman M. 1983 Clone bank of *Nicotiana tabacum* chloroplast DNA: mapping of the alpha, beta and epsilon subunits of the ATPase coupling factor, the large subunit of ribulosephosphate carboxylase and 32 kDa membrane protein. *Gene* 25: 271–280

Gallagher T. F. and Ellis J. R. 1982 Light-stimulated transcription of genes for two chloroplast peptides in isolated pea leaf nuclei. *EMBO J.* 1: 1493–1498

Grandbastien M. A., Berry-Lowe S., Shirley B. W. and Meagher R. B. 1986 Two soybean ribulose-1, 5-biphosphate carboxylase small subunit genes share extensive homology even in distant flanking sequences. *Plant Mol. Biol.* 7: 451–465

Harpster M., Mayfield S. P. and Taylor W. C. 1984 Effects of pigment-deficient mutants on the accumulation of photosynthetic proteins in maize. *Plant Mol. Biol.* 3: 59–71

Hayat M. A. 1972 *Basic microscopy techniques* (New York: Van Nostrand Reinhold)

Kirk J. T. O and Tilney–Basset R. A. E. 1978 *The plastids: their chemistry, structure, growth and inheritance* (Amsterdam: Elsevier/North-Holland Biomedical).

Kohorn B. D., Harel E., Chitnis P. R., Thornber J. P. and Tobin E. M. 1986 Functional and mutational analysis of the light-harvesting chlorophyll *a/b* protein of thylakoid membranes. *J. Cell Biol.* 102: 972–981

Kumar S. and Sharma S. B. 1986 Mutations in three of the genes determining thiamine biosynthesis in *Pisum sativum*. *Mol. Gen. Genet.* 204: 473–476

Kumar S. 1988 Recessive monogenic mutation in grain pea (*Pisum sativum*) that causes pyridoxine requirement for growth and seed production. *J. Biosci.* 13: 415–418

Lamppa G. K., Morelli G. and Chu N.-H. 1985 Structure and developmental regulation of a wheat gene encoding the major chlorophyll *a/b* binding polypeptide. *Mol. Cell Biol.* 5: 1370–1378

Leto K. J., Bell E. and McIntosh L. 1985 Assembly of photosystem II: accelerated turnover of chloroplast-encoded polypeptides in a nuclear mutant lacking photosystem II. In *Biology of photosynthetic apparatus* (eds.) K. E. Steinbeck, S. Bonitz, C. J. Arntzen and S. Bogorad (New York: Cold Spring Harbor Laboratory Press) pp. 39–45

Leutwiler L. S., Meyerowitz E. M. and Tobin E. M. 1986 Structure and expression of three light-

harvesting chlorophyll *a/b* binding protein genes in *Arabidopsis thaliana*. *Nucleic Acids Res.* 14: 4051–4076

Makasheva R. Kh. 1983 *The pea* (New Delhi: Oxonian Press)

Mascia P. N. and Robertson D. S. 1978 Studies in chloroplast development in four maize mutants defective in chlorophyll biosynthesis. *Planta* 143: 207–211

Mascia P. N. and Robertson D. S. 1980 Genetic studies of the chlorophyll biosynthesis mutants of maize. *J. Heredity* 71: 19–24

Miles D. 1982 The use of mutations to probe photosynthesis in higher plants. In *Methods in chloroplast molecular biology* (eds.) M. Edelman, R. B. Mallick and N.-H. Chua (Amsterdam: Elsevier Biomedical Press) pp. 75–107

Morrelli G., Nagy F., Fraley R. T., Rogers S. G. and Chua N.-H. 1985 A short conserved sequence is involved in the light-inducibility of a gene encoding ribulose-1, 5-biphosphate carboxylase small subunit of pea. *Nature* (*London*) 316: 200–204

Nelson T., Harpster M. H., Mayfield P. and Taylor W. C. 1984 Light-regulated gene expression during maize leaf development. *J. Cell Biol.* 98: 558–564

Pichersky E., Bernatzky R., Tanksley S. D., Breidenbach R. B., Kausch A. P. and Cashmore A. R. 1985 Molecular characterization and genetic mapping of two clusters of genes encoding chlorophyll *a/b* binding proteins in *Lycopersicon esculentum* (tomato). *Gene* 40: 247–258

Pichersky E., Hoffman N. E., Malik V. S., Bernatzky R., Tanksley S. D., Szabo L. and Cashmore A. R. 1987 The tomato *cab-4* and *cab-5* genes encode a second type of CAB polypeptides localized in photosystem II. *Plant Mol. Biol.* 9: 109–120

Sasaki Y., Tomoda Y. and Komikubo T. 1984 Light-regulated expression of ribulose-1, 5-biphosphate carboxylase at the level of transcription and gene dosage in greening pea leaves. *FEBS Lett.* 173: 31–35

Schmidt R. J., Hosler J. P., Gillham N. W. and Boynton J. E. 1985 Some chloroplast ribosomal proteins come from nuclear genome. In *Molecular biology of photosynthetic apparatus* (eds.) K. E. Steinback, S. Bonitz, C. J. Arntzen and L. Bogorad (New York: Cold Spring Harbor Laboratory Press) pp. 417–437

Schwarz H. P. and Kloppstech K. 1982 Effects of nuclear mutations on the structure and function of plastids in pea. *Planta* 155: 116–123

Sestak Z. 1971 Determination of chlorophylls *a* and *b*. In *Plant photosynthetic production, manual of methods* (eds.) Z. Sestak, J. Catsky and P. J. Jarvis (Netherlands: W. Junk NV Publishers)

Sheen J.-Y. and Bogorad L. 1987 Regulation of levels of nuclear transcripts for C4 photosynthesis in bundle sheath and mesophyll cells of maize leaves. *Plant Mol. Biol.* 8: 227–238

Silverthorne J. and Tobin E. M. 1984 Demonstration of transcriptional regulation of specific genes by phytochrome action. *Proc. Natl. Acad. Sci. USA* 81: 1112–1116

Simpson D. G., Hoyer-Hansen, Chua N.-H. and von Wettstein D. 1977 The use of gene mutants in barley to correlate thylakoid polypeptide composition with structures of photosynthetic membranes. In Proc. 4th Int. Cong. Photosynthesis (ed.) D. O. Hall, J. Coombs and J. W. Goodwin (London: The Biochemical Society) pp. 527–548

Smellie R. M., Nielson N. C., Henningsen K. W. and von Wettstein D. 1977 Development photochemical activity in chloroplast membranes. I. Studies with mutants of barley grown under a single environment. *Aust. J. Plant Physiol.* 4: 415–438

Smith S. M. and Ellis R. J. 1981 Light-stimulated accumulation of transcripts of nuclear and chloroplast genes for ribulose-1, 5-biphosphate carboxylase. *J. Mol. Appl. Genet.* 1: 127–137

Sundquist C., Bjorn L. O. and Virgin H. I. 1980 Factors in chloroplast differentiation. In *Results and problems in cell differentiation—Chloroplast* (ed.) J. Reinert (Berlin: Springer Verlag) vol. 10, pp. 201–218

Thompson W. F., Everett M., Polans N. O. and Jorgensen R. A. 1983 Phytochrome control of RNA in developing pea and mung-bean leaves. *Planta* 158: 487–500

Thorber J. P. and Markwell J. P. 1981 Photosynthetic pigment protein complexes in plant and bacterial membranes. *Trends Biochem. Sci.* 6: 122–125

Thompson W. F., Kaufman L. S. and Watson J. C. 1985 Induction of plant gene expression by light. *BioEssays* 3: 153–159

Tobin E. M., Wimpee C. F., Karlin-Neumann G. A., Silverthorne J. and Kohorn B. D. 1985 Phytochrome regulation of nuclear gene expression. In *Molecular biology of photosynthetic apparatus* (eds.) K. E. Steinbeck, S. Bonitz, C. J. Arntzen and L. Bogorad (New York: Cold Spring Harbor Laboratory Press) pp. 373–380

VanGrinsven M. Q. J. and Kool A. J. 1988 Plastid gene regulation during development: an intriguing complexity of mechanisms. *Plant Mol. Biol. Reporter* 6: 213–239

Von Wettstein D., Hennigsen K. W., Boynton J. E., Kannagara G. F. and Nielson O. F. 1971 The genetic control of chloroplast development in barley. In *Autonomy and biogenesis of mitochondria and chloroplasts* (eds.) N. K. Boardman, A. W. Linane and R. M. Smellie (Amsterdam: North-Holland) pp. 205–223

Walbot V. and Coe E. M. 1979 Nuclear gene *iojap* conditions a programmed change to ribosome-less plastids in *Zea mays. Proc. Natl. Acad. Sci. USA* 76: 2760–2764

Weidner M. Steinbiss H. and Kremer B. P. 1976 Correlations between photosynthetic enzymes, CO_2 fixation and plastid structure in an albino mutant of *Zea mays* L. *Planta* 131: 262–270