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Further Studies of Variegation and Other Leaf Pigmentation Mutants of *Collinsia heterophylla*

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ABSTRACT

Chloroplast number and appearance of orange (*vao*), blending (*vab*) and green (*vag*) variegation mutants and golden (*g*), virescent (*vr*) and yellow (*y*) leaf pigmentation mutants of *Collinsia heterophylla* Buist. (2N=14) are compared. Descriptions of phenotypes and survival potentials of the double homozygous recessives of the leaf pigmentation mutants and double homozygotes of green variegation (*vag*) and leaf pigmentation mutants are presented.

INTRODUCTION

In *Collinsia heterophylla* Buist. (Scrophulariaceae) four variegation types (all controlled by recessive nuclear genes and all transferable to offspring through male and female gametes) have been reported: (1) orange (*vao*), with pure orange or orange and green sectored leaves; (2) blending (*vab*), with leaves having yellow-green and green sectors with blending boundaries; (3) green (*vag*), with light-green and green sectored leaves and; (4) white variegation (*vaw*), with white and green sectored leaves (Gorsič and Kirby, 1996; Gorsič 1998).

The recessive alleles of the blending (*vab*) and green (*vag*) variegation loci are reversible (Delool and Tilney-Basset, 1986). The multiple allelic *vao* locus of orange variegation mutant comprises both, the reversible (vao^R and vao^{gR}) alleles, producing green sectors in orange leaves, and three non-reversible (stable) alleles: (1) *vao,* producing pure orange leaves; (2) vao^g, producing green cotyledons at emergence (instead of orange) and orange leaves and; (3) vao^a, producing orange leaves with white (alba) basal and marginal parts of the blade (Gorsič 1998).

Chlorophyll configuration of the orange variegation mutant *(vao)* has been described by Gorsič and Kirby, 1996. Chloroplast number and features of blending (*vab*) and green (*vag*) variegation mutants, and golden (*g,* Hiorth 1933), virescent (*vr)* and yellow *(y,* Gorsič 1994) leaf pigmentation mutants are reported below.

Phenotypic expression and viability of the double homozygous recessive genotypes involving *vao*, *vab* and *y* genes (Gorščič and Kirby, 1996) and the appearance and survival of double homozygotes involving *vao, vab,* and *vag* genes were previously reported (Gorščič 1998).

The appearance and survival of double homozygous recessive genotypes involving the green variegation gene (*vag*) and *g*, *vr*, and *y* leaf pigmentation genes are discussed in this report.

MATERIALS AND METHODS

Dry seeds of the 1995-97 *Collinsia* cultures, raised by the author in a greenhouse, were kept at -5º C for two days and then planted in 3 1/2 inch pots (8 seeds per pot) containing a mixture of commercial potting soil and vermiculite (3 to 1 by volume), and fertilized once every two weeks with a solution of one teaspoonful of 15-30-15 fertilizer in one gallon of water.

Stomatal guard cells of leaves' lower epidermis were examined under 440X magnification to establish the number and appearance of chloroplasts.

OBSERVATIONS

Chloroplast Number and Appearance

The most common chloroplast number in stomatal guard cells of the wild-type *C. heterophylla* plants falls between 10 and 14. The average number of chloroplasts per guard cell of green and orange sectors of leaves of the orange variegation mutants (*vao*) were reported to be 12.1 and 3.9 respectively (Gorščič and Kirby, 1996). Chloroplasts of orange sectors were smaller and lighter green than those of green sectors.

In orange leaves and in orange parts of the orange variegation (vao^g) and alba mutants (vao^a), the chloroplasts were usually slightly deformed but otherwise the same as in the orange variegation (*vao*) described above. The guard cells and mesophyll cells in all white parts of alba mutants (vao^a) were devoid of chloroplasts (occasionally, a cell showed up having 3-6 tiny, light-green chloroplasts).

The observation that some guard cells of any of the orange variegation plants had the wild type number of chloroplasts, suggests that all individuals homo- or hetero-zygous for any of the recessive *vao* alleles (*vao*/*vao*, vao^a/vao^a, *vao*/vao^a, vao^g/vao^g, *vao*/vao^g, vao^a/vao^g; Gorščič 1998) may have the same (wild type) number of proplastids in their potential chlorophyllous cells, but, in general, less than half of plastids complete the development into chloroplasts.

Chloroplasts of the guard cells in yellow-green sectors of the blending variegation mutants (*vab*) appeared normal in some cells, but in most cells they appeared pale green, broken up and shriveled, without sharp boundaries, so that a characteristic number per cell could not be ascertained. Chloroplasts seem to have been subjected to some kind of degradation process as reflected in a gradual expansion of the yellow-green areas and decrease of the green areas in leaves higher up the stem (Gorščič and Kirby, 1996).

The number and appearance of chloroplasts in the guard cells of light green areas of leaves of green variegation mutants (*vag*) were the same as in guard cells of the green sectors, that is, the wild type.

In golden (*g)* mutants (Hiorth 1933) characterized by golden coloration of young cotyledons and leaves, both turning nearly wild type green as they mature. The chloroplasts of guard cells appeared light green and slightly distorted (not oval) but wild type in number, suggesting that the golden gene (*g)* may be involved in control of synthesis of chlorophylls as well as in chloroplast formation of young cotyledons and leaves.

Chloroplasts (averaging 11 per guard cell) of pale yellow cotyledons of the virescent mutant (*vr*, Gorščič 1994) were slightly deformed and faint green turning (in 4 weeks) green (simulating the chloroplasts of leaves of virescent plants that are wild-type green at emergence).

The yellow (*y*) variant, which originated as a somatic mutation (Gorščič 1994), exhibiting yellow cotyledons and leaves throughout the plant life, had in their guard cells the wild-type number of chloroplasts of light green color and somewhat deformed shape.

Phenotypes and Survivalship of Double Homozygous Recessives

Double homozygous recessive genotypes of leaf pigmentation mutants (*g/g vr/vr*, *g/g y/y* and *vr/vr y/y*) produced albino-like seedlings that died in the cotyledonous stage.

The double homozygous recessive genotypes involving non-reversible orange variegation (*vao*, vao^{g} or vao^{a}) gene and any of the leaf pigmentation genes (*g*, *vr*, or *y*) produced albino-like cotyledon lethal seedlings, whereas the double homozygous recessives with a reverted orange variegation gene (vao^{R} or vao^{gR}) and *g*, *vr*, or *y* gene, produced viable plants: (1) *vao*/vao^{R} *g/g* (or *vao*/vao^{gR} *g/g),* had golden and light yellow sectored young leaves and green and light green sectored mature leaves; (2) *vao*/vao^{R} *vr/vr* (or *vao*/vao^{gR} *vr/vr*), had bright yellow and white sectored cotyledons and orange and green sectored leaves; and (3) *vao*/vao^{R} *y/y* (or *vao*/vao^{gR} *y/y*), had yellow and light yellow or white sectored cotyledons and leaves.

The survivalship of these double homozygotes depended on the degree (high vs. low frequency) and time of occurrence (in early vs. late embryonic stage) of *vao* or vao^{g} gene reversion. The higher the frequency and the earlier in embryonic development the *vao* or vao^{g} gene reversions occurred, the larger were the areas of leaves with photosynthetic capability and the higher was the survivalship of plants. Conversely, the lower the frequency and the later in the embryonic development the *vao* or vao^{g} gene reversions occurred, the smaller were the leaf areas with photosynthetic capability, and the lower the survivalship of seedlings.

The double homozygous recessives for the blending variegation gene (*vab*) and one of the leaf pigmentation genes (*g*, *vr*, or *y*) produced plants having mature leaves with blending sectors as follows: (1) *vab*/vab^{R} *g/g*, light golden and green, (2) *vab*/vab^{R} *vr/vr*, pale to light green and green, and (3) *vab*/vab^{R} *y/y*, pale yellow to white and yellow.

The double homozygous recessives for the green variegation gene (*vag*) and any of the leaf pigmentation genes (*g*, *vr*, or *y*) produced leaves having sharp borders between color sectors as follows: (1) *vag*/*vag*^{R} *g*/*g*, light golden and green, (2) *vag*/*vag*^{R} *vr*/*vr*, light green and dark green, and (3) *vag*/*vag*^{R} *y*/*y*, pale-yellow to white and yellow.

The survivalship of seedlings of the double homozygotes of *vab* or *vag* and one of the leaf pigmentation genes (*g*, *vr*, or *y*) depended on the frequency and the embryonic stage (early vs. late) of the occurrence of *vab* and *vag* gene reversions respectively (see above: *vao* and *vao*^{g} gene reversion vs. survivalship).

Because plants of the blending (*vab*/*vab*) and green (*vag*/*vag*) variegation mutants always carry both the nonreverted (*vab*, *vag*) and reverted (*vab*^{R}, *vag*^{R}) genes, no albino-like cotyledon lethals were observed among their double recessives involving *g*, *vr*, or *y* genes (in contrast to doubble recessives involving *vao* or *vao*^{g} and *g, vr,* or *y* - see above).

SUMMARY

The chloroplasts in the guard cells of orange sectors of leaves of orange variegations (*vao* and *vao*^{g}) and in the orange sectors of leaves of alba mutants (*vao*^{a}) are light-green, usually somewhat deformed, smaller and their number is reduced to about half of the wild-type. The guard cells of white areas of alba plants are devoid of chloroplasts.

In yellow-green sectors of leaves of the blending variegation mutants (*vab*) the pale green chloroplasts of most cells appear broken up and shriveled; therefore a typical number per guard cell could not be ascertained.

The number, shape and color of chloroplasts of the light green and green sectors of leaves of the green variegation mutants (*vag*) could not be distinguished.

Chloroplasts of guard cells of golden (*g*), virescent (*vr*, cotyledons only) and yellow (*y*) leaf pigmentation mutants appear lighter green, somewhat smaller, and slightly deformed (not uniformly oval), but their number per cell is the same as in the wild-type.

Double homozygous recessive plants of leaf pigmentation mutants (*g*/*g vr*/*vr*, *g*/*g y*/*y*, *vr*/*vr y*/*y*) were all albino-like and cotyledon lethal.

Double homozygous recessives for the <u>non-reversible</u> orange variegation (*vao* or *vao*^{g}) gene and any leaf pigmentation gene (*g*, *vr*, or *y*) were all albino-like and died in cotyledonous stage. But the double homozygous recessives of the <u>reversible</u> orange variegation (*vao*^{R} or *vao*^{gR}) gene and any of the leaf pigmentation genes (*g*, *vr*, or *y*) were viable. In these plants the green (or light green) to light green (or light yellow to white) areas of their leaves, as well as their survivalship, were directly proportional to the frequency (high vs. low) and the time of occurrence (early vs. late embryonic stage) of *vao* and *vao*^{g} gene reversions.

The double homozygous recessives involving blending (*vab*) and green (*vag*) variegation genes (both being reversible) and any of the leaf pigmentation genes (*g*, *vr*, or *y*) produced only viable plants (no cotyledon lethal albinos), having leaves with the proportion

of green to golden, virescent (cotyledons only), or yellow coloration respectively, as well as the survivalship directly proportional to the frequency and the embryonic stage (early vs. late) of occurrence of the respective *vab* and *vag* gene reversions.

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