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Green Alba Variant of the Orange Variegation Mutant of *Collinsia heterophylla*

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ABSTRACT

A new green alba allele (*vao*^{A}) of the orange variegation (*vao*) locus of *Collinsia heterophylla* Buist. (2N=14) has been identified. The homozygous *vao*^{A}/*vao*^{A} plants are characterized by having green or green and orange sectored cotyledons and leaves with white or diluted basal and marginal parts. The green alba allele (*vao*^{A} = *vao*^{aR}) is dominant over the orange alba allele (*vao*^{a}), and recessive, regarding its alba dilution aspect, to the orange variegation (*vao*), revertant orange variegation (*vao*^{R}), and the wild-type (*Vao*) alleles.

INTRODUCTION

The orange variegation locus (*vao*) of *Collinsia heterophylla* Buist. (Scrophulariaceae), first reported by Goršič and Kerby (1996), appears to be the most mutable of the four variegation loci identified to date (Goršič 1998, 2000). Besides the dominant wild-type green allele (*Vao*) five recessive forms are known: (1) *vao*, producing pure orange cotyledons and leaves; (2) *vao*^{R} (reverted *vao*), producing green sectors in orange cotyledons and leaves (Delool and Tilney-Basset, 1986); (3) *vao*^{g}, producing green cotyledons at emergence (turning orange in 2 weeks) and orange leaves; (4) *vao*^{gR} (reverted *vao*^{g}), producing green cotyledons (turning orange and green in 2 weeks) and green and orange sectored leaves and; (5) *vao*^{a} (orange alba), producing orange cotyledons and leaves having white or diluted basal and marginal parts.

Among progenies of some self-fertilized and intercrossed orange alba homozygotes (*vao*^{a}/*vao*^{a}) and orange (*vao*/*vao*^{a}) and wild-type heterozygotes (*Vao*/*vao*^{a}) a new mutant - green alba, *vao*^{A} - appeared having green cotyledons and leaves with typical alba pattern of basal and marginal dilution. The relationship of this new *vao*^{A} allele to the other alleles of the *vao* locus has been investigated, and the results are reported below.

MATERIALS AND METHODS

Cultures of this investigation were grown from seeds obtained from 1996-1998 greenhouse plantings of *Collinsia*. Dry seeds were kept at -5°C for 48 hours before sowing into a 3:1 mixture of commercial potting soil and vermiculite (8 seeds per 9 cm pot). Seedlings were fertilized once every 2 weeks with a solution of one teaspoonful of 15-30-15 fertilizer per gallon of water. All plants were grown under the same greenhouse conditions; no analysis of temperature and light intensity influence on variegation was attempted (Martinez-Zapater 1993).

Methods of hybridization, used in this investigation, have been recently described (Gorsic 1994). The fitness of phenotypic segregation ratios has been tested by calculating Chi-square, using the Yates' term for sample size below 50.

ORIGIN OF ALBA MUTANT AND HYBRIDIZATION RESULTS

Among eight offspring of a self-fertilized orange alba plant (h96207-12, culture h9777), 5 plants were typical orange alba plants (vao^a) having orange cotyledons and leaves with white or diluted basal and marginal parts (Fig. 1A), and 3 were green alba plants (vao^A) having green cotyledons and leaves with white or diluted basal and marginal parts of the blade (Fig. 1B). A cross between two orange alba plants (vao^a/vao^a *x* vao^a/vao^a, culture h9778) produced 11 offspring: 10 plants were orange alba (vao^a), and one plant was green alba (vao^A). Another cross between two orange variegated heterozygotes (vao/vao^a *x* vao/vao^a, culture h97246) produced 14 orange plants (*vao*) and two alba plants - one orange alba (vao^a) and the other green alba (vao^A). In all these cases the green alba allele (vao^A) apparently originated by mutation of the recessive orange alba allele (vao^a).

Among 35 offspring of a self-fertilized wild-type plant heterozygous for orange alba (Vao/vao^a, culture h97119) 24 plants were wild-type (*Vao*/--), 11 were orange alba (vao^a/vao^a) and one plant was green alba (vao^A). Eight self-fertilized wild-type siblings of h97119-14 green alba plant (vao^A) produced a total of 114 offspring: 90 were wild-type (*Vao*/--), 21 were orange alba (vao^a/vao^a), and 3 were green alba (vao^A). Since the total number of the recessive offspring (21 + 3 = 24) is less than 1/4 (28.5) of total progeny, and because the 3 green alba plants came from different individuals, the conclusion may be made that, in these cultures too, the vao^A alleles originated by mutation of the recessive vao^a alleles rather than the dominant *Vao* alleles of the respective Vao/vao^a hybrids.

These original (6) alba plants (vao^A) produced flowers and were used in crosses, but only one of them (h97119-14) was vigorous enough to produce, upon self-fertilization, viable seed. Thirteen plants grown from these seeds had green cotyledons and leaves with alba dilution pattern, but 3 of them had nearly white cotyledons and died in the cotyledonous stage. The first generation green alba mutants (vao^A) were used in crosses: (1) with the wild-type plants (*Vao*/*Vao*), producing wild-type offspring; (2) with orange plants (*vao*/*vao*), producing green sectored orange (non-alba) offspring (vao^R); (3) with orange alba plants (vao^a/vao^a), producing either green alba (vao^A) only, or orange alba (vao^a) and green alba (vao^A) in a 1:1 ratio and; (4) with green alba plants (vao^A), producing green alba (vao^A) only, or green alba (vao^A) and orange alba (vao^a) offspring in a 3:1 ratio (Table 1).

These hybridization results established the green alba (*vao*^{A}) as a dominant trait over orange alba (*vao*^{a}), but recessive, regarding its alba dilution pattern, to orange variegation mutant (*vao*) and the wild-type (*Vao*).

By close examination of the cotyledons and leaves of the green alba plants, two different phenotypes were identified: (1) individuals exhibiting only green and pale-green to white diluted areas and; (2) plants with green and orange sectored cotyledons and leaves with pale-green to white basal and marginal parts (Fig. 1B).

Crosses between the wild-type (*Vao*/*Vao*) and green alba plants (*vao*^{A}) produced two types of hybrids: (1) hybrids that produced, upon self-fertilization, wild-type and green alba, or wild-type and green and orange sectored alba plants in a 3:1 ratio, and; (2) hybrids producing, upon self-fertilization, wild-type and orange alba in 3:1 ratios (Table 1). The former were *Vao*/*vao*^{A} heterozygotes, and the latter were *Vao*/*vao*^{a} heterozygotes. These hybridization results proved that the original green alba plants, used in these crosses, were indeed *vao*^{A}/*vao*^{a} heterozygous.

The heterozygosity (*vao*^{A}/*vao*^{a}) of the first generation green alba plants was confirmed as well by the 3:1 ratio of green alba vs. orange alba (*vao*^{a}/*vao*^{a}) obtained upon self-fertilization (Table 1). The green alba plants of these progenies were either all green alba plants (without any orange sectors), or they were green alba (*vao*^{A}/*vao*^{A}) and green and orange sectored alba plants (*vao*^{A}/*vao*^{a}) in a 1:2 ratio (Table 1). Because the extent of the basal and marginal dilution of cotyledons and leaves of both the orange (*vao*^{a}) and the green alba (*vao*^{A}) plants varied greatly, it was not always possible to distinguish with certainty between the *vao*^{A}/*vao*^{A} and *vao*^{A}/*vao*^{a} individuals.

The revertant orange and green sectored orange variegation plants (*vao*^{R}/*vao*, Goršič 1998) and the green and orange sectored alba plants (*vao*^{A}/*vao*^{a}) appear very similar (Fig. 1C). Progenies of the self-fertilized orange and green sectored orange variegation heterozygotes, *vao*^{R}/*vao*^{A}, segregated into orange and green sectored plants without dilution (*vao*^{R}/*vao*^{R}, *vao*^{R}/*vao*^{A}) and green alba plants with dilution (*vao*^{A}/*vao*^{A}) in a 3:1 ratio (Table 1). These crosses established the recessive relationship of *vao*^{A} to *vao*^{R} allele.

Since both the orange (*vao*^{a}) and green alba (*vao*^{A}) plants exhibited a parallel pattern of dilution in their cotyledons and leaves, it is likely that the diluting process, be it genetic or epigenetic, is the same in both mutants. In several progenies of selfed alba plants the extremely diluted (nearly white) cotyledon lethal seedlings equaled in number the seedlings with barely detectable dilution (together making up half of the total progeny) indicating that both the *vao*^{a} and *vao*^{A} alleles vary in their capacity to control the deleterious dilutions, bringing about a 1:2:1 phenotypic ratio for nearly white, moderately diluted, and mildly diluted seedlings.

The microscopic examination (under 440X magnification) of stomatal guard cells of leaves' lower epidermis revealed that the white areas carried no apparent chloroplasts, the diluted areas carried a mixture of small and normal sized chloroplasts, and the green areas carried 10-14 wild-type chloroplasts. In most alba plants the basal and marginal dilution was observed in all young vegetative leaves and bracts of inflorescence. In some green alba plants (*vao*^{A}/*vao*^{A}) the cotyledons were pure green and the leaves exhibited no apparent dilution, appearing wild-type. These fully green "cryptic recessive" alba plants, when crossed with orange alba plants (*vao*^{a}/*vao*^{a}), produced green or green and orange sectored alba plants (*vao*^{A}/*vao*^{a}).

To explain the expression of orange in the *vao*^{A}/*vao*^{a} heterozygotes, one has to assume either an incomplete dominance of *vao*^{A}, an inactivation of dominant *vao*^{A} allele, or the back mutation or reversion of the *vao*^{A} to *vao*^{a} allele. The observation that orange and green sectored alba plants are produced by some self-fertilized *vao*^{A}/*vao*^{A} plants as well, compounds the mystery of formation of orange sectors in the green alba plants even further. Formation of orange sectors in *vao*^{A}/*vao*^{A} plants would require the inactivation (or back mutation to *vao*^{a}) of both *vao*^{A} alleles of the cell (an unlikely event), or a reversion of both *vao*^{A} into *vao*^{a} alleles. These "inactivated, back mutated, or revertant" green and orange sectored alba plants should have produced, upon self-fertilization, some orange alba plants (*vao*^{a}/*vao*^{a}), which, to date, has not been observed.

There is another possible explanation for the unusual behavior of the green alba mutants. Some of the orange variegation (*vao*) alleles are highly reversible - the same is true for the *vao*^{g} alleles from which the *vao*^{a} alleles originated (Goršič 1998). Orange and green sectored plants (*vao*^{R}/*vao*^{R}, *vao*^{gR}/*vao*^{gR}) with highly reversible *vao* or *vao*^{g} alleles, when self-fertilized, produce mainly green plants with no or barely detectable orange patches and rarely a pure orange plant (Goršič 1998, Goršič and Kerby, 1996).

The orange alba allele (*vao*^{a}) behaves as a stable, non-reversible gene even in the presence of a dominant activator *Ac*, which brings about the *vao* and *vao*^{g} gene reversion (Goršič 1998). However, the *vao*^{a} allele may be associated with another, non-functional reversion factor, which occasionally shifts to the functional form, thus making the *vao*^{a} reversion to *vao*^{aR} possible. The observation that the pattern of green and orange sectors in alba plants is retained from lower to higher leaves (plants have decussate leaf arrangement) on the same side of the stem during plant growth suggests that the green and orange tissues are produced by mitotic propagation of revertant (*vao*^{aR} - green) and non-revertant (*vao*^{a} - orange) cells of seedlings' apical meristem (Fig. 1D).

If the *vao*^{A} alleles are actually revertant orange alba alleles (*vao*^{aR}) in which the chlorophyll synthesizing capacity has been restored (as in reverted *vao*^{R} and *vao*^{gR} alleles) but the control of alba dilution pattern retained, the behavior of the green alba plants can be explained without resorting to inactivation, back mutation, or reversion of the dominant *vao*^{A} allele to the recessive *vao*^{a} allele.

The green alba plants (*vao*^{A}/*vao*^{A} = *vao*^{aR}/*vao*^{aR}) with both *vao*^{a} alleles highly reversible, that reverted to the *vao*^{aR} during gamete formation or in the early stage of embryonic development, would produce, upon self-fertilization, only green alba plants. Plants carrying both a highly reversible *vao*^{a} allele and a less reversible *vao*^{a} allele that reverts to *vao*^{aR} at a later stage of embryonic development would produce, upon self-fertilization, green alba plants without orange sectors and green and orange sectored alba plants in a 3:1 ratio. The green and orange sectored alba plants (*vao*^{A}/*vao*^{a} = *vao*^{aR}/*vao*^{a}) carrying a highly reversible *vao*^{aR} allele and an orange alba allele (*vao*^{a}) would produce, upon selfing, green alba (*vao*^{aR}/*vao*^{aR}, *vao*^{aR}/*vao*^{a}) and orange alba plants (*vao*^{a}/*vao*^{a}) in a 3:1 ratio. The green and orange sectored alba plants (*vao*^{aR}/*vao*^{a}) carrying a less reversible *vao*^{aR}

allele would produce, upon self-fertilization, green alba (possibly with barely detectable orange streaks, vao^{aR}/vao^{aR}), green and orange sectored alba (vao^{aR}/vao^{a}), and orange alba plants (vao^{a}/vao^{a}) in a 1:2:1 ratio (Table 1).

In one aspect the revertant vao^{R} (or vao^{gR}) and vao^{aR} alleles behave differently: the vao^{R} and the vao^{gR} are dominant over *vao* allele (producing green sectors in orange leaves of vao^{R}/vao and vao^{gR}/vao heterozygotes), whereas, the vao^{aR} allele behaves as a recessive to *vao* regarding alba dilution pattern but dominant regarding chlorophyll production, resulting in green sectored non-alba orange hybrids (vao^{aR}/vao). When these hybrids are crossed with orange alba plants (vao^{a}/vao^{a}) they produce the green alba or green and orange sectored alba (vao^{aR}/vao^{a}) and orange non-alba plants (vao/vao^{a}). It appears that the identifying feature of the vao^{aR} allele is not its dominant capacity to produce chlorophyll (green sectors), but the production of alba dilution pattern, which, as the crosses attested, is recessive to the non-alba orange allele (*vao*). The recessive *vao* gene overrides the action of the recessive alba dilution pattern but not the dominant chlorophyll producing aspect of the vao^{aR} allele in the vao^{aR}/vao hybrids. This selective action of *vao* upon vao^{aR} gene in the vao^{aR}/vao hybrids points to the intricate relationship between the alleles of the orange variegation locus (*vao*).

SUMMARY

The newly identified green alba allele (vao^{A}) behaves as dominant to orange alba allele (vao^{a}) but as recessive, regarding alba dilution pattern, to orange variegation (*vao*), revertant orange (vao^{R}), and the wild-type (*Vao*) alleles.

The progenies of some selfed vao^{A}/vao^{A} plants segregated in 3:1 ratios for green alba and green and orange sectored alba plants, suggesting that some vao^{A} alleles may back mutate, or be inactivated, or reverted to vao^{a}, thus permitting the expression of orange sectors. The progenies of selfed vao^{A}/vao^{a} green alba plants segregated either into green alba (vao^{A}/--) and orange alba (vao^{a}) plants in 3:1 ratios, or into green alba (vao^{A}/vao^{A}), green and orange sectored alba (vao^{A}/vao^{a}) and orange alba (vao^{a}/vao^{a}) in a 1:2:1 ratio, giving an impression that some vao^{A} alleles are incompletely dominant over orange alba allele (vao^{a}). Some Vao/vao^{A} heterozygotes, when selfed, produced wild-type (*Vao*/--) and green alba plants (vao^{A}), others wild-type and green and orange sectored alba plants in a 3:1 ratio. The stomatal guard cells of white areas of leaves of green alba plants carry no chloroplasts, diluted areas carry a variable number of different sized chloroplasts, and the green areas carry 10-14 wild-type chloroplasts.

Production of orange sectors in green alba plants can be made possible by inactivation, back mutation, or reversion of the vao^{A} allele to vao^{a} allele. More in line with the behavior of *vao* alleles would be to assume that the green alba alleles (vao^{A}) are actually revertant vao^{aR} alleles: highly reversible vao^{aR} alleles, reverting during gamete formation or in an early stage of embryogenesis, would produce green alba plants, and less reversible vao^{aR} alleles, reverting in later stages of embryogenesis, would produce alba plants with variable mixtures of green and orange sectors in their cotyledons and leaves.

LITERATURE CITED

Delool, R.A.H., and R.A.E. Tilney-Basset. 1986. Germinal reversion in three variegated-leaf mutants of *Antirrhinum majus* L. Jour. Her. 77:236-240.

Goršič, J. 1994. Inheritance of eleven new variants of *Collinsia heterophylla*. Jour. Her. 85:314-318.

Goršič, J. 1998. Inheritance of green variegation and other new variants of *Collinsia heterophylla*. Ill. State Acad. Sci., Transactions 91:19-31.

Goršič, J. 2000. Further studies of variegation and other leaf pigmentation mutants of *Collinsia heterophylla*. Ill. State Acad. Sci., Transactions 93:89-93.

Goršič, J. and K. Kerby. 1996. Inheritance of variegation in *Collinsia heterophylla*. Ill. State Acad. Sci., Transactions 89:7-19.

Martinez-Zapater, J.M. 1993. Genetic analysis of variegated mutants in *Arabidopsis*. Jour. Her. 84:138-140.

Figure 1. Alba mutants of *Collinsia heterophylla*. A. Orange alba (vao^{a}). B. Green alba ($vao^{A}=vao^{aR}$), left; Green and orange sectored alba (vao^{A}/vao^{A} or vao^{A}/vao^{a}), right. C. Leaves of the second and third pair: Green and orange sectored alba (vao^{A}/vao^{A} or vao^{A}/vao^{a}), top; Revertant orange variegation mutant (vao^{R}/vao, vao^{A}/vao or vao^{A}/vao^{g}), bottom. D. The second, third, and fourth leaf pairs of the green and orange sectored alba (vao^{A}/vao^{A}): Leaves of one side of stem exhibiting greater amount of orange (top row) than leaves of the opposite side of stem exhibiting lesser amount of orange (bottom row).