INTERORGANISMAL MORPHOLOGICAL ASPECTS OF FLOWER AND BUD REVERSION IN THE WINGED BEAN [*PSOPHOCARPUS TETRAGONOLOBUS* (L.) DC]

Edward G.F. Benya

Key words: **Homeotic mutant, morphological reversion, reversion sequence, winged bean.**
Mutante homeótico, reversão morfológica, seqüência da reversão, feijão-de-asa.

Abstract

Morphologically reverted segregants, homozygous for a recessive homeotic mutant gene that permits reversion, field-grown in the tropical, semiarid, equatorial region of Teresina, Piauí, Brazil showed a variable sequence of inter-organismal morphological characteristics associated with that reversion. Those characteristics and their sequence provide further information about this particular reversion phenomenon and its effects which overall must be considered deleterious to populations and individuals that are homozygous for this gene.

Resumo

*ASPECTOS MORFOLÓGICOS INTER-ORGANISMAIS PERTENCENDO À REVERSÃO DAS FLORES E BOTÕES NO FEIJÃO-DE-ASA [**PSOPHOCARPUS TETRA-***

Endereço do autor: Escola Agrícola Santo Afonso Rodriguez; Socopo; C.P. 421; 64001-970, Teresina, PI.

GONOLOBUS *(L.) DC] Segregantes morfologicamente revertidos, homozigóticos para um gene homeótico mutante, recessivo que permite aquela reversão, cultivados no campo na região tropical, semiárida, equatorial de Teresina, Piauí, Brasil mostraram uma seqüência das características morfológicas inter-organismais, associadas com aquela reversão. Estas características e sua seqüência forneceram mais informação sobre este fenômeno de reversão e seus efeitos que, sobre tudo, devem ser considerados deletérios às populações e aos indivíduos que são homozigotos para este gene.*

Introduction

Homeotic mutant genes have been identified in a number of plant species (COEN et al., 1990; MANDEL et al., 1992; MENA et al., 1996; SCHWARZ-SOMMER et al., 1990; WEIGEL & MEYEROWITZ, 1993, 1994). Research concerning the winged bean [*Psophocarpus tetragonolobus* (L.) DC] in the semiarid, tropical equatorial climate (GADELHA DE LIMA, 1987) of Teresina, Piaui, Brazil (05°05'S; 42°49'W) has revealed the presence of a recessive, homeotic, mutant gene that underlies and permits a plasticity of form in segregants homozygous for the gene (BENYA, 1995). The change in form is manifested principally but not exclusively in reversion of flowers and flower buds from the sexual reproductive function to a vegetative, photosynthetic function. That reversion is initially characterized by a transition in ovary morphology (from a tetragonolobanous to a digonolobanous form) and by virescence of normally pigmented flower parts (i.e. petals), and at times by viviparous growth (BENYA, 1995).

However these initial changes (i.e. reversion) were followed by further transitions in form and capacity, a sequence of which may further reveal factors and influences involved in the activation of this gene and manifestation of this plasticity of form in individual specimens (i.e. segregants) homozygous for the gene. This research aimed to recognize and chronicle any sequence or sequences of changes following initial reversion, their association with plasticity and their variability.

Material and Methods

Field plantings of about 100 to 300 m^2 each for this species have been used as sources of data for growth, yields and morphological abnormalities since 1987, when this form of reversion was first noted. Plantings initially contained a mixture of heterozygous and homozygous specimens for the recessive gene under study. More recent studies have used only homozygous specimens recessive for the gene, in an effort to more clearly understand the reversion

process and any accompanying sequence or sequences of morphological transitions associated with that process.

Data collection was made at varying intervals, generally every two to ten days during the cropping season and then at greater intervals of about five to fifteen days during the post-harvest season. Aberrations were chronicled according to form, segregant and date of verification.

For this study, 35 segregants were examined in order to determine any further abnormalities besides the three (i.e. digonolobanization of ovary, virescence and vivipary) that initially mark reversion.

Results

Morphological flower reversion from the sexual reproductive function to the vegetative photosynthetic function is the most obvious sign of flower and flower bud reversion in this species (FIGURE 1). Of 284 flowers sampled, this reversion was always (100% of samples) accompanied by a structural shift in ovary form from the usual tetragonolobanous form to a digonolobanous form, and almost always (99.4% of samples) accompanied by virescence of petals. Vivipary (FIGURES 2a, 2b) in the form of profuse vegetative growth is an extra-vegetative, proliferous (usually photosynthetic) activity (WILSON & LOOMIS, 1967). It could arise simultaneously with or just after the initial signs.

FIGURE 1 – Normal flowers (bottom) having sexual reproductive function. Reverted flower and flower bud (center) having vegetative, photosynthetic function, virescent organs.

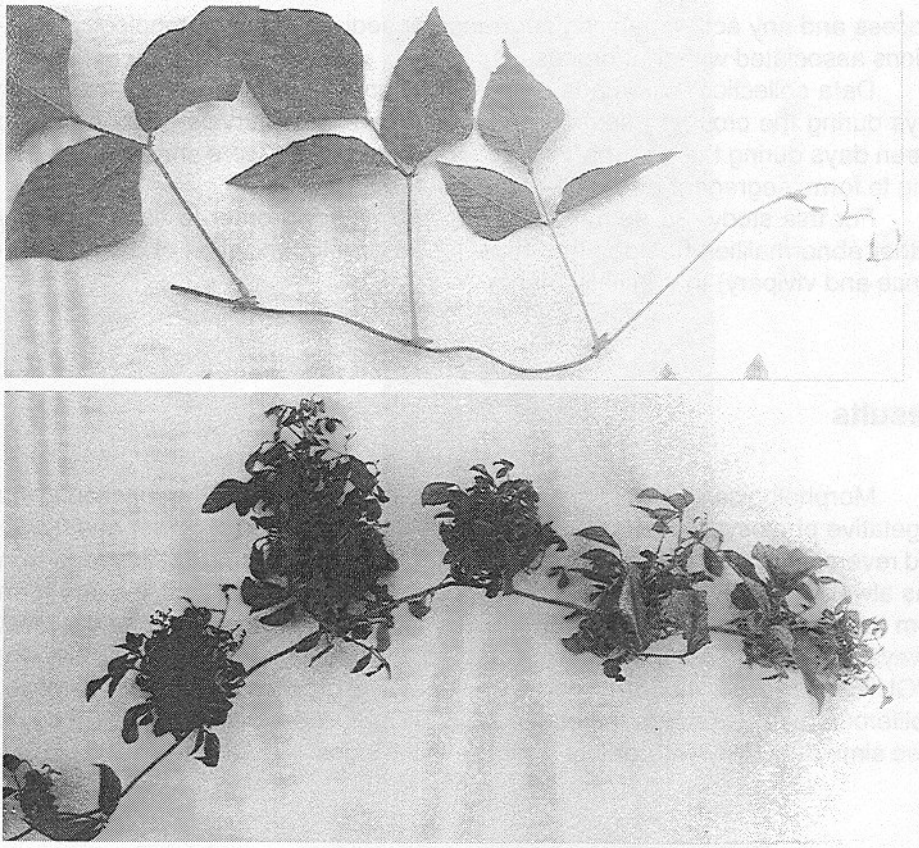

FIGURE 2 – a) Normal vegetative and foliar growth; b) Vivipary; profuse apical, foliar and vegetative growth.

However, a variable sequence of other morphological changes and metabolic capacities (i.e. abnormalities) then followed these initial changes. These are summarized in TABLE I where they have been classified according to morphological or metabolic function, and according to structural occurrence. These are inter-organismal morphological, metabolic and intra-structural abnormalities.

Reversion in the 35 segregants under study showed significant correlation with age of the plant (TABLE II). Reversion in eight month old plants was significantly greater ($P < 0.01$, $r = 0.986$) than in any other age group.

TABLE I – Frequency of morphological, metabolic and intra-structural abnormalities associated with the reversion sequence on the 35 segregants studied.

Abnormality	Number of segregants	Frequency	Mean time (months) of appearance from initial date of reversion. mean	range
A. Inter-organismal Morphological				
Floral & bud reversion	29	(29/35) = 82.86	0.00	(0.0 – 0.0)
Atrophied fruit	7	(7/35) = 20.00	0.21	(0.0 – 1.0)
Vivipary	32	(32/35) = 91.43	0.27	(0.0 – 1.0)
Meristematic apices on reverted flowers	3	(3/35) = 8.57	0.75	(0.5 – 1.0)
Rhizogeny of reverted structures	2	(2/35) = 5.71	0.75	(0.5 – 1.0)
Fusion of anatomic parts	6	(6/35) = 17.14	1.08	(0.0 – 2.0)
B. Inter-organismal Metabolic				
Renewal of segregants	2	(2/35) = 5.71	1.25	(1.0 – 1.5)
Death of segregants from reversion	31	(31/35) = 88.57	2.16	(0.5 – 4.0)
C. Intra-structural				
Ovary (pod) digonolobanization	284	(284/284) = 100.00	---	---
Virescence (chlorophyllization) of flower parts	336	(336/338) = 99.4	---	---

All new plantings (less than one year old) during this study demonstrated reversion of flowers and/or flower buds. A planting or part thereof could extend from one planting season to another as individual specimens of the species manifested a perenniality (BENYA et al., 1985). Thus for this study a distinction is made between new plantings (less than one year old) and older ones. However two segregants from older plantings (i.e. more than 12 months old) are represented here (TABLE II). Vivipary of buds arose an average of 0.27 months (range 0.0 – 1.0 months) after reversion but sometimes accompanied the initial signs of reversion.

Atrophied fruits (green pods) are elongating pods that cease development and remain as such for two, three or more weeks until desiccation and death. They appear exactly like normally elongating pods. These were few (i.e. some 30 fruits on seven of the 35 segregants), but were noted an average of 0.21 months (range 0.0 to 1.0 months) after reversion, being at times simultaneous with the initial signs of reversion.

TABLE II – Reversion frequency by age of the 35 segregants.

Age[a] (months)	Number of plants (segregants)
1.0	0
2.0	0
3.0	0
4.5	1
5.5	1
6.5	2
7.0	1
7.5	2
8.0	11**
8.5	2
9.0	3
9.5	1
10.0	3
10.5	4
11.0	0
11.5	1
12.0	1
12.5	1
13.0	0
14.0	0
14.5	1
Total	35
mean = 8.83 months sd = 1.99 months	

[a] age is rounded to the nearest half month;
** significant at the 1% level ($P < 0.01$)

Rhizogeny (i.e. rooting) (FIGURE 3) of reverted structures, especially reverted flowers, is an extra-rhizogenous activity when it occurs in plant parts (e.g. bracts, ovaries, calyces) normally associated exclusively with aerial, photosynthetic and/or reproductive activity. It appeared an average of 0.75 months (range 0.5 – 1.0 months) after reversion. Natural rhizogeny was minimal (two segregants) as reverted flowers do not initially have rhizogenous capacity. It develops only after the beginning of reversion, after a period of "capacitation" of already reverted flowers and buds. Further, such rhizogeny of field specimens depends on high ambient moisture during the month of capacitation (i.e. the month immediately preceeding rhizogeny) (TABLE III).

TABLE III – Linear correlation (r) of monthly values (for the 15 months involving one or more segregants showing reversion) for major ambiental variables, with the number of segregants (2) showing natural rhizogeny of reverted parts (d.f. = 13).

Variable	Correlation (r) with rhizogenous capacity in month preceeding rhizogeny (i.e. month of reversion)	Correlation (r) with rhizogeny in month when roots actually appeared (i.e. month succeeding reversion)
Evaporation (mm)	- 0.620*	- 0.528*
Mean daily range in temperature (°C)	- 0.641**	- 0.537*
Mean Temperature (°C)	- 0.370 NS	- 0.454 NS
Mean humidity (%)	0.643**	0.554*
Precipitation (mm)	0.789**	0.595*
Evapo-Transpiration Potential (ETP)	- 0.631*	- 0.607*
Photoperiod (h)	- 0.059 NS	- 0.350 NS
Change in photoperiod (h)	- 0.693**	- 0.529*

NS = not statistically significant; * = statistically significant ($P < 0.05$); ** = statistically highly significant ($P < 0.01$)

Vegetative meristematic apices (FIGURE 4) arose on reverted flowers. As with rhizogeny, their appearance occurred in an average of 0.75 months (range 0.5 – 1.0 months) after reversion. This seemed to be due principally to ovules within reverted pods or ovaries that became meristematically active. However meristematic apices also arose on reverted flowers at the region of the calyx-ovary convergence (i.e. receptacle) that had elongated from a point of contact in normal flowers to a length of as much as 30 mm in reverted flowers (FIGURE 4). Both of these apical meristematic origins seemed to contribute to, but not totally account for the vivipary of reverted segregants.

Fusion of floral and non-floral parts presented a complexity of structures that challenged exact classification (FIGURE 5). These fusions arose an average of 1.08 months (range 0.0 – 2.0 months) after reversion. They could include flowers, stems, leaves, apices and any parts or combinations therein.

Two reverted segregants recovered from all symptoms of reversion although their reverted portions died. This phenomenon was termed "renewal". Recovery seemed to occur in segregants whose normal vegetative growth remained at 50% or more (i.e. total reverted portion of less than 50%). One of these renewed specimens later died after a nematode infection of the roots. The other specimen first reverted at 5.5 months of age, recovered, resumed normal pod production, then reverted again at eight months of age. It is here treated as two cases. It finally died at ten months of age showing only symptoms of reversion. Renewal arose an average of 1.25 months (range 1.0 – 1.5 months) after reversion.

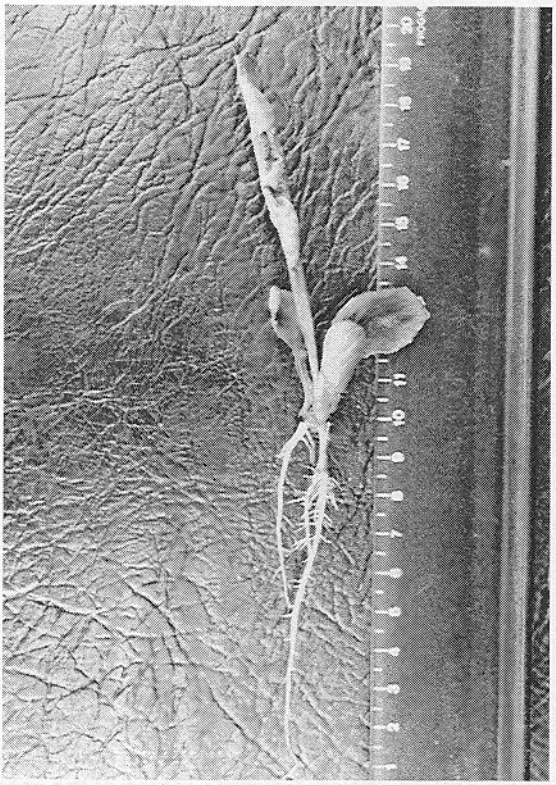

FIGURE 3 – Rhizogeny: Rooting of reverted flower at calyz, stem and bract.

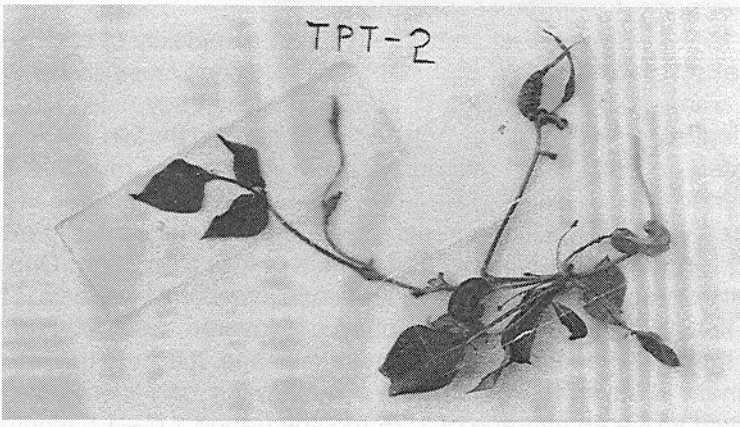

FIGURE 4 – Vegetative, meristematic apex (winged bean variety TPT-2): single apex on reverted flower (left) originating from elongation of the receptacle (center).

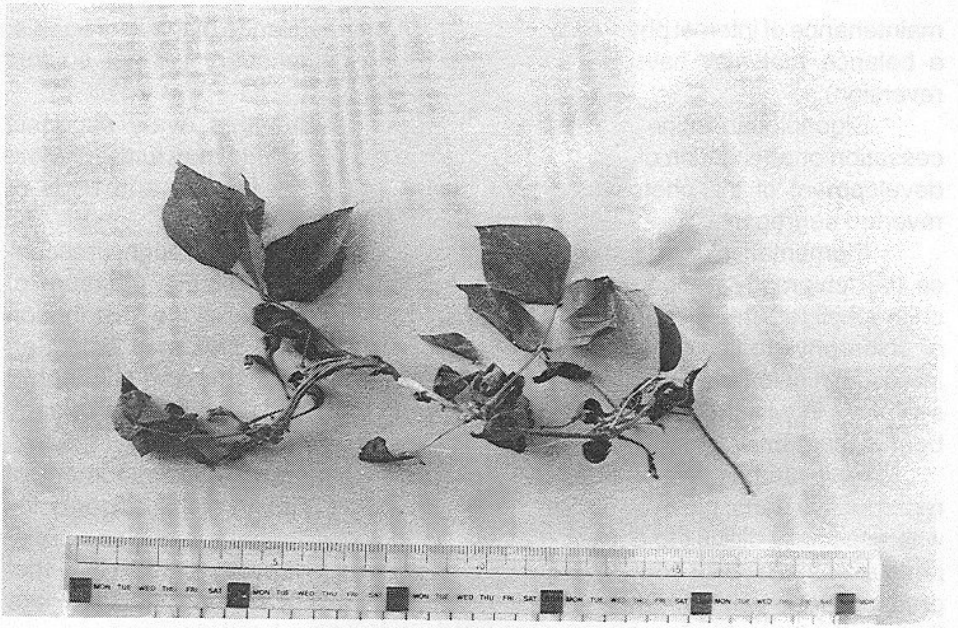

FIGURE 5 – Fusion: flowers, flower parts, stems.

Of 35 reverted segregants studied here, 31 died showing symptoms of reversion while no other symptoms were noted. The other four died while showing symptoms of parasite infection (e.g. nematodes) or mechanical damage (e.g. root cut). Death apparently due solely to reversion occurred an average of 2.16 months (range 0.5 – 4.0 months) after reversion.

Although this study dealt principally with the effects of reversion on segregants producing immature (i.e. green) pods, examination of 15 mature or maturing pods from reverted segregants revealed four pods with desiccating but live seeds. Of 44 seeds examined from these pods, about 90% were germinating within the pods while about 80% showed virescence of seeds to some degree.

Discussion

The significantly high level of reversion in eight month old segregants may reflect metabolic and/or structural changes internal to the segregants at or just prior to this age; changes that permit or even promote activation or manifestation of effects for the recessive mutant gene under study. The recovery (renewal) of two segregants at 1.0 and 1.5 months respectively after reversion suggests

maintenance of internal physiological and/or structural balance of the segregants, a balance that may have been such as to permit recovery (or not sustain reversion).

Digonolobanization of the normally tetragonolobanous ovary suggests cessation or attenuation of a complex formative process. This may further reflect development of the photosynthetic function in reverted flowers and buds of reverted segregants.

Pigmentation transition of floral parts (especially petals) through virescence (i.e. chlorophyllization of said parts) was a simple, qualitative measure of chlorophyll redistribution (i.e. extension) in reverting segregants (i.e. distribution of chlorophyll to flowers as well as to leaves and stems). This may reflect an increase in chlorophyll synthesis by reverting segregants. Increased chlorophyll synthesis in winged bean leaves in response to thermal variability has already been documented by HERAT & OMROD (1979).

Fruit atrophy reflects a simple cessation in the normal development of reproductive parts; the ovary or seed pod in this case. Timing of this abnormality was especially difficult to calculate as atrophied pods appear exactly as normal pods except that their continuing development ceases leaving a green pod that gradually toughens and then desiccates over a period of weeks. Exactly where that continuity of development ceases is very difficult to determine.

Natural rhizogeny of field specimens was rare (two of 35 sites). The exact internal mechanism involved is perplexing. However the mere possibility of this capacity indicates that this type of reversion extends not only to the aerial (e.g. photosynthetic) mechanism of plant function but also to the suberranean (e.g. rhizogenous) mechanism of that function.

The vegetative, apical meristematic activity (i.e. vivipary) seemed to have its origin in a number of anatomic regions, including ovules and the point of calyx-ovary convergence (i.e. receptacle) which had elongated in most reverted flowers (FIGURE 4). This seemed to be due to the degree to which normal vegetative buds had been induced or evoked (BATTEY & LINDON, 1990; BENYA, 1995) to the floral function. This activity further attests to the vegetative function of reverted flowers and buds.

Fusion of floral and non-floral parts is a major indicator of the conjunctive, destructive aspects of reversion. Organizational unity of process or processes is dramatically altered, even destroyed in reverted segregants.

Use of pods for mature seed rather than green pods diminished rate of reversion significantly. The immaturity factor and maintenance of same through harvest of green (i.e. immature) pods seems to promote reversion.

Renewal (or recovery) of two segregants, except for their reverted segments, presented a variability factor in terms of reversion exactitude. This reversion phenomenon is apparently reversible in some, perhaps all segregants, depending on age and/or external factors. Renewal does not extend to portions or regions of a segregant already demonstrating any or all morphological signs

of reversion. These always ended in death. Renewal thus helped researchers recognize the essential deleterious nature of this type of reversion.

Virescence of maturing seed and germination of that seed while still in the pods of reverted segregants indicated an absence of seed and/or seed-coat dormancy. This is a further disruptive effect (i.e. of maturing seed in this case) of this type of reversion.

Conclusion

Research on 35 homozygous segregants, recessive for a homeotic mutant gene causing morphological reversion in the winged bean revealed a variable sequence of morphological events. These events occurred to various degrees and furnished further information concerning orientation and depth of reversion.

The significantly high level of reversion in eight month old segregants may reflect a senility factor in specimens at that age or older. Digonolobanization of normally tetragonolobanous ovaries, virescence of flower parts, vivipary of buds, ovules and several reverted regions of reverted flowers (e.g. receptacle) tended to confirm the disruption of normal function first manifest in the shift of flower and flower bud activity from sexual reproduction in normal flowers to a vegetative (i.e. asexual), photosynthetic capacity in reverted flowers. This asexual capacity seems even to include certain subterranean functions as demonstrated by a rhizogenous capacity developed by various reverted flowers after a period of capacitation. A further disruptive function is indicated in the fusion of floral and non-floral parts into almost indistinguishable tissue and organ masses.

Fruit atrophy of green pods in various stages of development demonstrated an attenuating function by this reversion process on the segregants. Recovery of various segregants suggested the maintenance of at least some normal internal systems and/or processes that thus prevented fixation and maintenance of the reversion phenomenon. However death of all reverting segregants and/or portions of segregants showing reversion indicates that this type of reversion must be considered deleterious to all individuals manifesting same. The presence of the homeotic, mutant recessive gene, especially in homozygous form, in any individual or population must therefore be considered a negative quality.

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