

PERSISTENCE OF ANATOMIC SECTIONS FROM REVERTED HOMEOTIC SEGREGANTS OF THE WINGED BEAN [*PSOPHOCARPUS TETRAGONOLOBUS* (L.) DC] UNDER LABORATORY CONDITIONS

Edward G.F. Benya

Key words: Homeotic gene, morphological reversion, metabolic persistence, winged bean.

Gene homeótico, reversão morfológica, persistência metabólica, feijão-de-asa.

Abstract

*Laboratory tests concerning anatomic sections, both morphologically reverted and normal, from reverted segregants, homozygous for a recessive homeotic gene in the winged bean [*Psophocarpus tetragonolobus* (L.) DC] revealed significant differences in metabolic persistence as measured by treatment time span. The six major classes of anatomic sections studied included normal flowers and stems, plus the four recognized abnormal classes: reverted stems, fusions, reverted flowers and reverted flower buds. Persistence increased in significant steps according to: determinate (e.g. floral) or indeterminate (e.g. stem) growth, reverted or non-reverted condition, extra-morphologically active (i.e. extra-rhizogenous and/or extra-vegetative) or inactive condition, and purity of water (i.e. plain or deionized) as the medium of culture.*

Author's address: Escola Agrícola Santo Afonso Rodriguez; Socopo, C.P. 421, 64.000-972, Teresina, Piauí, Brazil.

Acta Biologica Leopoldensia	Vol. 21	Nº 2	julho/dezembro	1999	p. 183-193
-----------------------------	---------	------	----------------	------	------------

Resumo

PERSISTÊNCIA DAS SECÇÕES ANATÔMICAS ORIGINADAS DOS SEGREGANTES HOMEÓTICOS REVERTIDOS DE FEIJÃO-DE-ASA [*PSOPHOCARPUS TETRAGONOLOBUS* (L.) DC] EM LABORATÓRIO. Experiências no laboratório tratando pedaços anatômicos, tanto morfológicamente revertidos quanto morfológicamente normais, originados dos segregantes revertidos homozigóticos para um gene homeótico recessivo em feijão-de-asa [*Psophocarpus tetragonolobus* (L) DC)] revelaram diferenças estatisticamente significativas na persistência metabólica medida por espaço do tempo do tratamento. As seis classes principais de pedaços anatômicos investigados incluíram flores e caules normais, além de quatro classes anormais reconhecidas: caules revertidos, fusões, flores revertidas e botões revertidos. A persistência aumentou em passos significativos segundo: o crescimento determinado (e.g. flores) ou indeterminado (e.g. caules), a condição revertida ou não revertida, a condição extra-morfológicamente (e.g. extra-rizógena e/ou extra-vegetativa) ativa ou inativa, e a pureza da água (i.e. comum ou deionizada) usada para a cultura.

Introduction

Investigations pertaining to a homeotic recessive mutant gene associated with morphologic reversion in the winged bean [*Psophocarpus tetragonolobus* (L.) DC] (BENYA, 1995) have revealed two basic morphologic changes primarily associated with that reversion: digonolobanous (rather than tetragonolobanous) pod formation (100% of cases) and virescence (i.e. chlorophyllization) of normally pigmented newly formed flowers (99.4% of cases). Flowers revert from the sexual reproductive function to a vegetative photosynthetic function (BENYA, 1995). These changes are most apparent in flowers, floral buds and parts thereof. They reflect a genetic influence, manifest in different degrees of phenotypic plasticity, in individual segregants homozygous for the gene in its activated state (BENYA, 1999; BRADSHAW, 1965).

Purpose of these investigations was to observe specific floral and vegetative parts, both normal and reverted, all originating from reverted segregants and compare persistence in terms of metabolic activity as measured by length of treatment time spans between groups. This was an effort to understand any prerequisites to extra-morphological (i.e. extra-rhizogenous and/or extra-vegetative) activity, recognizing such activity qualitatively but not yet addressing quantitative specifics of it.

Material and Methods

A total of 172 specimens representing six basic anatomic and/or organ categories (types) were tested under controlled laboratory conditions of 43 treatments usually having four repetitions (but varying between two and five repetitions) for each treatment. Treatments and repetitions represented five segregants and two varieties. They were composed of the 172 specimens tested at different "reversion ages" (i.e. time after the onset of reversion). An individual segregant could, at any one time, contain one or more examples of all six categories of anatomic types. Thus comparison of persistence of specimens between segregants and within the same segregant was possible and desirable. However for reasons of clarity, this research treated specimens according to anatomic type only (reverted and non-reverted); regardless of variety, reversion age, age of segregant and specificity of segregant which can all be treated in future analysis. The research simply measured the degree of persistence under laboratory conditions. Viability of specimens, as reflected in the treatment time span, was taken as a measure of metabolic persistence among the six basic types or categories of anatomic structures studied. These six categories were: normal, non-reverted flowers; cut, normal and abnormal stems (i.e. having viviparous nodes, nodes showing profuse vegetative growth); fused floral and non-floral parts (i.e. fusions); reverted flowers and reverted flower buds (BENYA, 1999). Researchers then compared persistence of anatomic parts (i.e. stem sections), organ systems (i.e. flowers, buds) and fused sections from reverted segregants and/or sections of reverted segregants.

Each treatment was conducted within individual plastic cups and/or glass test-tubes using plain or deionized water within a double chambered laboratory. Temperature and humidity of the chambers closely followed those of external ambiental conditions during the winter and spring seasons in Teresina, Piauí, Brazil from early August to late November (GADELHA DE LIMA, 1987), there being no internal climate control. Temperatures in the chambers ranged between 19°C and 41°C while relative humidity ranged between 40% and 100%.

Pure, deionized water, was used in three treatments. This served as a further control (of an external ambiental variable) in order to test for any variability in persistence of similar specimens under different conditions of aquatic purity.

Data were collected and recorded as results appeared. Statistical analysis i.e. ANOVA, X^2 , t-tests (GOMEZ & GOMEZ, 1984; MENDENHALL, 1968) were used to identify and verify any significant differences.

Results

Morphologically normal specimens (i.e. normal flowers and non-reverted stems) served as a control. Analysis of variance (ANOVA) indicated significant statistical differences ($F = 8.91$, $P < 0.01$) between persistence levels of the two principal morphological groups being tested; control or non-reverted specimens (i.e. normal flowers and stems) and reverted specimens (i.e. reverted stems, fusions, buds and flowers). Chi-square test for independence of morphological categories then confirmed similarity (i.e. lack of independence) among specimens within those two morphological groups ($X^2 = 9.45$, $P < 0.01$). The statistical t-test analyses were then used to detect and confirm any significant differences between categories of specimens occurring within the two morphological groups. Results of these tests are summarized in the tables presented here. The t-tests showed normal flowers had significantly lower ($P < 0.01$) mean persistence (8.000 days) than did normal stems (13.125 days).

Average or mean metabolic persistence for the entire group of 172 specimens was 19.890 days. However mean persistence of non-reverted specimens (i.e. normal flowers [8.000 days] and stems [13.125 days]), even though originating from reverted recombinants, was significantly lower ($P < 0.01$) (TABLE I) than that for all reverted specimens (20.846 days).

TABLE I – Persistence of major reverted and non-reverted structures.

Structure	number	persistence (total days)	mean treatment span (days)	P
Normal flowers	8	64	8.000	< 0.01
Stems (normal)	8	105	13.125	< 0.01
Stems (reverted)	6	112	18.667	NS
Fusions	4	67	16.750	NS
Buds (total)	23	448	19.478	NS
Reverted flowers (total)	123	2,625	21.342	NS
t-test				

Specimens from the four categories of reverted structures (i.e. reverted stems, fusions, reverted flowers and buds) accounted for 156 of the 172 specimens in this study. Among all reverted structures, morphologically active and inactive, mean persistence for reverted stems (18.667 days), fusions (16.750 days), buds (19.478 days) and for all reverted flowers (21.342 days) showed no significant difference (TABLE I).

Structures having vegetative, photosynthetic and/or supportive functions included fusions, stems (reverted and normal), reverted flowers and reverted buds. All reverted flowers (21.342 days), buds (19.478 days) and fusions (16.750 days) showed significantly higher mean persistence ($P < 0.01$) from that of normal stems (13.125 days). Even reverted stems showed a significantly higher mean persistence (18.667 days, $P < 0.05$) from that of normal stems (TABLE II).

TABLE II – Persistence of vegetative, photosynthesizing and/or supportive structures.

Structure	number	persistence (total days)	mean treatment span (days)	P
Stems (normal)	8	105	13.125	—
Stems (reverted)	6	112	18.667	< 0.05
Fusions	4	67	16.750	< 0.01
Reverted buds (total)	23	448	19.478	< 0.01
Reverted flowers (total)	123	2,625	21.342	< 0.01
t-test				

Among stems, there was a significant difference in mean persistence between all stems (15.500 days) and normal stems (13.125 days $P < 0.05$) and between reverted stems (18.667 days) and normal stems ($P < 0.05$). However mean persistence between reverted stems (18.667 days) and all stems (15.500 days) showed no significant difference.

Persistence within structural types showed significant differences. The normal flowers (8.000 days) had significantly lower mean persistence ($P < 0.01$) than did all reverted flowers (21.342 days) (TABLE I).

A selected group of 112 reverted flowers, individually identified for extra-morphological activity or inactivity, showed significant differences in metabolic persistence. Morphologically inactive reverted flowers (12.605 days) had significantly lower mean persistence ($P < 0.01$) from that of morphologically active reverted flowers (28.230 days) (TABLE III).

Among the three categories of morphologically active reverted structures, active reverted flowers (28.230 days) had significantly higher ($P < 0.05$) mean persistence from that of either reverted stems (18.667 days) or fusions (16.750 days).

TABLE III – Persistence of the four major types of reverted structures.

Reverted Structure	number	persistence (total days)	mean treatment span (days)	P
Stems	6	112	18.667	NS
Fusions	4	67	16.750	NS
Flowers (selected)	112	2,568	22.929	NS
inactive	38	479	12.605	< 0.01
active	74	2,089	28.230	< 0.01
Buds (total)	23	448	19.478	NS
inactive	15	130	8.667	< 0.01
active	8	318	39.750	< 0.05
t-test				

Within the group of all reverted floral structures (i.e. reverted flowers and buds) taken as a whole, persistence varied. For purposes of distinction a flower, reverted or normal, is defined as any floral structure showing 100% emergence of petals and reproductive organs from the calyx. A bud is a floral structure showing less than 100% emergence of those parts. Mean persistence for all reverted flowers (21.342 days) was not significantly different from that for all reverted buds (19.478 days) (TABLE II). However morphologically active reverted buds showed significantly higher mean persistence (39.750 days, $P < 0.05$) from that of all reverted flowers (21.342 days) while inactive reverted buds showed significantly lower (8.667 days, $P < 0.01$) mean persistence from both groups (TABLE IV). Both active reverted buds (39.750 days, $P < 0.05$) and selected active reverted flowers (28.230 days, $P < 0.01$) showed significantly high persistence, although the difference between the two groups was not significant (TABLE III).

TABLE IV – Persistence among reverted flowers and buds.

Reverted Structure	number	persistence (total days)	mean treatment span (days)	P
Reverted flowers (all)	123	2,625	21.342	NS
Reverted buds (all)	23	448	19.478	NS
Reverted buds (active)	8	318	39.750	< 0.05
Reverted buds (inactive)	15	130	8.667	< 0.01
t-test				

Water quality significantly affected metabolic persistence (TABLE V). All reverted flowers, morphologically active and inactive, in plain water, showed significantly lower persistence (19.51 days) from that of reverted flowers, both active and inactive in deionized water (51.417 days) ($P < 0.01$). Active reverted flowers in deionized water had the highest mean metabolic persistence (53.091 days), significantly higher ($P < 0.01$) than any other group. Even the single inactive reverted flower in deionized water showed high metabolic persistence of 33 days although significance could not be calculated.

TABLE V – Persistence among reverted flowers by water quality.

Reverted Flower class	Water quality	persistence number	mean treatment (total days)	span (days)	P
active & inactive	plain	100	1,951	19.510	< 0.01
inactive	plain	37	446	12.054	< 0.05
active	plain	63	1,505	23.889	—
active & inactive	deionized	12	617	51.417	< 0.01
inactive	deionized	1	33	33.000	—
active	deionized	11	584	53.091	< 0.01

t-test

Discussion

Normal flowers and normal stems showed quick demise (i.e. relatively short mean metabolic persistence). They maintained their normal, delicate, vascularized composition necessary for sexual reproduction and/or rapid elongative growth. Secondary structuring in terms of extensive tissue differentiation, mature fiber formation and overall maturation (WILSON & LOOMIS, 1967) was probably minimal although specific fiber analysis (i.e. acid and neutral detergent fiber) was beyond the scope of this research.

The significant difference in mean persistence between normal flowers and normal stems reflected their basically different types of growth. Flowers have a determinate growth while stems have an indeterminate growth as reflected in their essentially, distinct tissue differences. Flowers have a complete, yet immature, sensitive (vascularized) reproductive tissue of limited (i.e. determinate) growth (WILSON & LOOMIS, 1967) necessary to the amphigonic fertilization function of sexual reproduction. This tissue is essentially terminal, giving way to a more permanent, durable, differentiating tissue only when fertilization has been achieved and basic organ development has begun in the fertilized ovary. Lacking such fertilization, the flower naturally dies.

New, non-reverted stems also contain immature, sensitive tissue, but it is of a continuing (i.e. indeterminate) elongative, vegetative, differentiating nature destined at maturity to fulfill supportive, conductive and (to a minor degree) photosynthetic functions. These differences in essential structure were reflected in the significant differences of mean persistence between specimens of these two control groups.

Secondary, more fibrous structure (i.e. tissue reinforcement) was probably the principle initial cause (i.e. first step) of significantly longer mean metabolic persistence in all reverted specimens. The same delicate, high-moisture, low-density tissue found in normal, non-reverted flowers and stems was replaced by more durable, fibrous (and thus less sensitive) tissue (WILSON & LOOMIS, 1967) as reverted specimens were transformed from the sexual reproductive or from the morphological elongative functions, to the vegetative, photosynthetic or supportive functions.

Reverted structures as a group (i.e. fusions, reverted flowers and buds, reverted stems), morphologically active or inactive, presented no significant differences in mean persistence. They simply represented a more sturdy, fibrous, virescent tissue common to mature, durable, vegetative structures. However within this reverted group, a second structural step significantly distinguished specimens. This second step was that of anabolic or extra-morphological activity (i.e. extra-rhizogenous and/or extra-vegetative).

Fusions and stems by definition always showed morphologic activity, extra or merely normal. Reverted flowers and reverted flower buds however, showed distinctions between morphologically active and morphologically inactive specimens within each group. Anabolic activity significantly contributed to increased persistence in any specimen. Reverted specimens not only showed photosynthetic capacity (as qualitatively identified in virescence of usually non-chlorophyllized parts), but many of these specimens demonstrated a mass volume increase as qualitatively identified in extra-rhizogenous and extra-vegetative growth. Especially concerning reverted flowers and buds, highly significant differences in persistence of specimens between these two groups were clearly linked to this second or anabolic step in activity.

Water purity further contributed significantly to metabolic persistence. This is a factor external to the nature of reverted segregants but it demonstrates a point concerning pure ambiental effects on such segregants. The natural extra-rhizogenous activity documented in field plantings containing reverted segregants (BENYA, 1999) was significantly correlated with ambiental moisture levels (i.e. precipitation and atmospheric humidity percentages) in the months immediately preceeding and coinciding with actual manifestation of rhizogeny. Precipitation would not be as pure as the deionized water used as a control in this experiment. However it would probably be more pure than the plain water used for the bulk of this experiment.

Conclusion

The significantly different persistence levels between normal (i.e. non-reverted) control specimens (i.e. flowers and stems) probably reflected the essential structural and functional differences that distinguish these two groups; the determinate, sexual reproductive function of normal flowers; the indeterminate vegetative, elongative and photosynthetic function of normal stems. The further significant difference between the normal specimens of these two structural categories as a group, and reverted specimens of the four major structural categories as a group (i.e. reverted stems, reverted flower buds, reverted flowers and fusions) then reflected the overall effect or effects of reversion when compared with non-reversion.

Within the group of all reverted specimens, further significant differences were clearly seen between the morphologically active and the morphologically inactive. Fusions and reverted stems by definition were always active. Reverted flower buds and reverted flowers might be or might not be active. Morphologically active reverted flower buds and reverted flowers then showed further significant increases in mean persistence from their inactive counterparts thus reflecting anabolic functions of that activity.

Water purity represented an ambiental variable external to any segregant, reverted or not. Deionized water further affected or significantly contributed to increases in metabolic persistence among reverted specimens treated with same. It suggested possible influences of other ambiental variables to management of this particular form of reversion.

Metabolic persistence of reverted segregants is an essential quality to any possible practical application of this phenomenon. Factors both internal and external to the segregant, influencing persistence, merit identification and quantification. This experiment has begun to identify a few of those factors.

Acknowledgements

I thank E.M. Moreira, H. Pietrogrande, J.P. Cornado and Escola Agrícola Santo Afonso Rodriguez for providing time and facilities for this research. The "Universidade do Vale do Rio dos Sinos" (UNISINOS) and "Instituto de Pesquisa de Planárias" (IPP) furnished time and facilities for statistical analysis of data. A. De Paula assisted with technical work. M.C. Moura Carvalho and V.A. Rodrigues helped with manuscript preparation.

References

- BENYA, E.G.F. 1995. Genetic aspects of flower reversion in the winged bean [*Psophocarpus tetragonolobus* (L.) DC]. **Acta Biologica Leopoldensia** **17**: 65-72.
- BENYA, E.G.F. 1999. Inter-organismal morphological aspects of flower and bud reversion in the winged bean [*Psophocarpus tetragonolobus* (L.) DC]. **Acta Biologica Leopoldensia** **21**: 71-82.
- BRADSHAW, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. **Adv. Genet.** **13**: 115-155.
- GADELHA DE LIMA, M. 1987. **O clima de Teresina**. Fundação Universidade Federal do Piauí, Pro-Reitoria de Extensão, Dep. de Engenharia Agrícola e Solos (DEAS), 6p.
- GOMEZ, K.A. & GOMEZ, A.A. 1984. **Statistical procedures for agricultural research**. John Wiley & Sons, New York, 680p.
- MENDENHALL, W. 1968. **Introduction to probability and statistics**. Wadsworth Publishing Company, Inc., Belmont, California, 393p.
- WILSON, C.A. & LOOMIS, W.E. 1967. **Botany**. Holt, Rinehart and Winston, New York, 626p.

Recebido em 10/02/99

Aceito em 22/07/99

APPENDIX – Description of treatments.

Treatment	variety	segregant	Reversion age (days)	Specimens						
				Normal reps	Normal flowers	Reverted stems	Reverted stems	Fusions	Reverted buds	Reverted flowers
1	SLS-7	1	6	4						4
2	SLS-7	1	6	4					4	
3	SLS-7	1	6	4		4				
4	SLS-7	1	6	4	4					
5	Tpt-2	2	15	4					4	
6	Tpt-2	2	15	4		4				
7	Tpt-2	2	15	4	4					4
8	SLS-7	1	12	4						4
9	SLS-7	3	18	4						4
10	SLS-7	4	1	4						4
11	SLS-7	1	19	4						4
12	SLS-7	3	25	4						4
13	SLS-7	4	8	4						4
14	SLS-7	1	26	4						4
15	SLS-7	3	32	5				1		4
16	SLS-7	4	15	4						4
17	SLS-7	1	33	4						4
18	SLS-7	3	39	5						5
19	SLS-7	4	22	4						4
20	SLS-7	5	27	3						3
21	SLS-7	1	40	4						4
22	SLS-7	3	46	4						4
23	SLS-7	4	29	4						4
24	SLS-7	1	47	4						4
25	SLS-7	3	53	4				1		3
26	SLS-7	4	36	4						4
24 a *	SLS-7	1	48	4						4
25 a *	SLS-7	3	54	4					1	3
26 a *	SLS-7	4	37	4					4	
27	SLS-7	1	54	4						4
28	SLS-7	3	60	4						4
29	SLS-7	4	43	4						4
30	SLS-7	1	61	4						4
31	SLS-7	3	67	4						4
32	SLS-7	4	50	4					1	3
33	SLS-7	1	68	4					3	1
34	SLS-7	3	74	4					2	2
35	SLS-7	1	75	4						4
36	SLS-7	3	81	5					4	1
37	SLS-7	1	82	4						4
37 a	SLS-7	1	82	2				1		1
38 a	SLS-7	3	88	4			4			
39 a	SLS-7	1	89	4			2	1		1

$$\Sigma = 172 = (8 + 8 + 6 + 4 + 23 + 123)$$

* = Treatments in deionized water; all other treatments done in plain water.