Chemical Regulation of Endogenous Dormancy in Seeds of *Stylosanthes humilis* H.B.K.[1]

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ABSTRACT

Freshly-harvested and scarified seeds of *Stylosanthes humilis* exhibit endogenous dormancy that is broken by two complementary principles acting synergistically: a cytokinin (supplied as benzyladenine) and ethylene (as ethrel). When applied alone, ethrel was much more efficient than benzyladenine. Cobalt ions inhibited the germination of the old seeds which do not show endogenous dormancy. The inhibition caused by abscissic acid on the germination of old seeds was partially reversed by ethrel or benyladenine, and completely overcome by the combination of both promoters. While potassium nitrate and gibberellic acid were inefficient in breaking endogenous dormancy, thiourea completely substituted for the combination of ethrel plus benzyladenine.

COMPENDIO

Semillas recién cosechadas y escarificadas de *Stylosanthes humilis* presentan un letargo endógeno que puede ser quebrado por dos principios complementarios actuando sinérgicamente: una citocinina (suministrada en la forma de benziladenina) y etileno (en la forma de etrel). Aisladamente, el etrel se muestra más eficiente en la quiebra del letargo. La inhibición de la germinación causada por el ácido abscísico en semillas viejas puede ser interrumpida parcialmente por el suministro de etrel o benziladenina y completamente por la combinación de los dos promotores. Nitrato de potasio y ácido giberélico son ineficientes para romper el letargo seminal endógeno. La tiourea sustituye en eficiencia la combinación etrel-benziladenina.

INTRODUCTION

Freshly-harvested seeds of the tropical leguminous forage plant, *Stylosanthes humilis* H.B.K., exhibit tegumentary and endogenous dormancies, while in seeds one year old or older only the first type is observed. The tegumentary dormancy is broken by simple scarification, and is well-documented (3, 7, 8, 12). The endogenous dormancy has been little studied (3, 5), probably because, under natural conditions, only the old seeds germinate and/or the freshly-produced seeds have their endogenous dormancy broken by one or more natural factors.

The purpose of the present study was to investigate the action of some GRs on the endogenous dormancy of *S. humilis* seeds.

MATERIALS AND METHODS

Seeds of *S. humilis* were harvested from plants grown in a greenhouse, and were kept in the laboratory until the beginning of the experiments. By that time, the old seeds (without endogenous dormancy) were 12-14 months old and the endogenously dormant ones, one to four months old. Just before the germination tests, the seeds were freed from their husks, scarified with fine sandpaper, sterilized with 0.5% Na OCl for 10 min, and thoroughly washed with distilled water. The germination tests were carried out in 9.0 cm sterilized Petri dishes containing two sheets of Whatman No. 1 filter paper and 10.0 ml of test solution, and kept in the dark at 30°C. Germination was regarded as complete when the extruded radicles attained 3.0 mm (12); a period of 96 h was established for the final estimation of total germination. To characterize the state of activity of the seeds, the progress of water absorption by old and freshly-harvested scarified seeds was also monitored.

All the GRs employed to stimulate or inhibit the germination —ABA, BA, B-995, CCC, $Co(NO_3)_2$, ethrel, GA_3, KNO_3 and thiourea— were dissolved in 10.0 mM phosphate buffer at pH 6.0 and applied alone or in combination, in the ranges of concentrations shown under Results and Discussion. A completely randomized design was employed consisting of four replicates per treatment, and 50 seeds per Petri dish. Comparable means followed by different letters in Tables 1 to 3 were significantly different (P = 0.05) by the Tukey test.

RESULTS AND DISCUSSION

The biphasic pattern of water absorption by freshly-harvested seeds strongly suggests the existence

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of endogenous dormancy (Fig 1) The old seeds, however, showed and additional phase of water absorption which, according to Bewley and Black (2), means that the process of germination was apparently under way.

Ethrel, which once inside the cells liberates ethylene, considerably overcomes the endogenous dormancy as shown in Fig. 2, in a similar manner to that observed in seeds of lettuce (1, 16, 17) and peanut (9). Figure 2 also shows that BA was less efficient than ethrel in breaking that kind of dormancy. When applied in combination, the regulators (ethrel plus BA), had a synergistic effect upon germination. The treatment of endogenously dormant seeds with 10 $\mu g/ml$ of ethrel or BA, for instance, resulted respectively in 60 and 20% germination, while the simultaneous application of both regulators, at the same concentrations, produced about 100% germination. As Fig. 2 shows, the synergistic effect was still more evident when lower concentrations of the GRs were employed. Identical results were also reported in some varieties of lettuce (14, 17). Hence it is likely that two complementary principles, a cytokinin and ethylene, are essential for the release of endogenous dormancy in seeds of S. humilis. Since a proportion of freshly-produced seeds germinated in response to just one applied principle (ethrel or BA, Fig. 2), it may be speculated that the germinated seeds should contain (or synthesize or activate) the complementary one. If those two principles are required for germination, the elimination of one of them would render the old seeds unable to germinate. Cobalt ions, which inhibit ethylene synthesis (10, 15), also impaired the germi-

nation of old seeds (Fig. 3). This supports the suggestion that old seeds are able to produce ethylene, as has been observed in peanut (9) and lettuce (13) seeds Fig. 3 also shows that the inhibitory effect of cobalt ions could be reversed by ethrel or BA alone, a quite unexpected result. Table 1 shows, however, that only ethrel was able to induce germination of endogenously dormant seeds treated with cobalt ions, and that ethrel plus BA produced the typical synergistic interaction. Thus, the reversal by BA of the cobalt-inhibited germination of old seeds (which do not exhibit endougenous dormancy) can be explained as follows: to avoid problems of toxicity, they were treated with 100 $\mu g/ml$ (Co (NO$_3$)$_2$, which still yielded 35% germination. Thus the seeds were still able to produce or activate the two principles. Consequently, the addition of one of those principles would lead to the establishment of the synergistic effect.
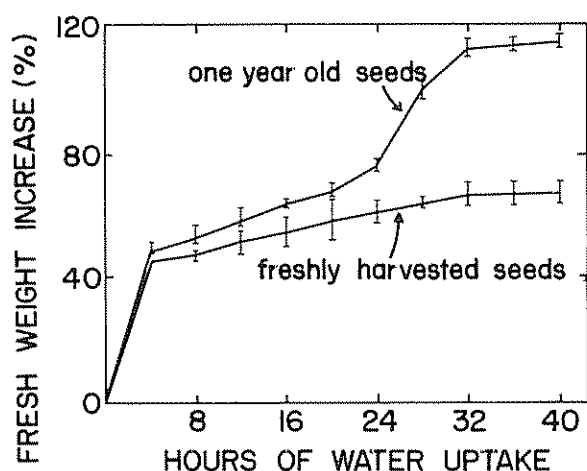
The inhibition of the germination of old seeds by ABA was completely reversed only by the simultaneous addition of ethrel and BA (Fig. 4). Considering this response and the observation that only the joint application of ethrel and BA could break the endogenous dormancy of seeds treated with ABA (Table 2), it may be speculated that only the two principles
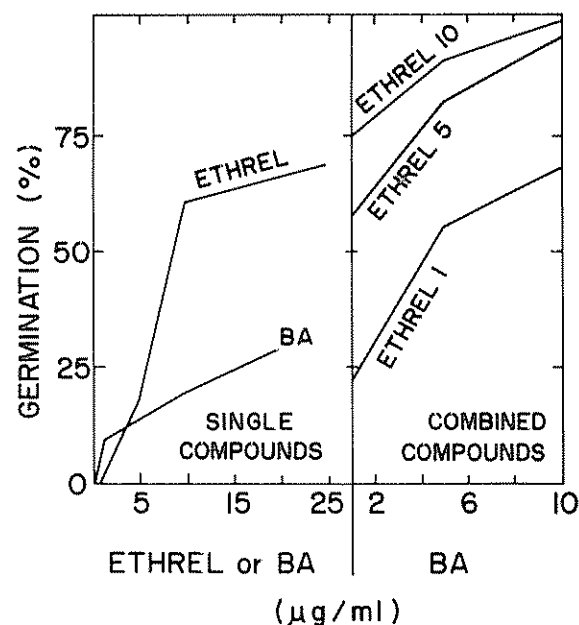

Fig. 1. Time course of water absorption by freshly-harvested (exhibiting endogenous dormancy) and old (without endogenous dormancy) seeds of S humilis, expressed as percentage increase over the original fresh weights

Fig. 2 The effects of ethrel and BA, applied singly or in combination, in the germination of endogenously dormant seeds of S. humilis Arrows on the left of the figure indicate the concentrations of the regulators employed in the combinations shown on the right (In this plot, and the subsequent plots, the figures accompanying each regulator refer to their concentrations, in $\mu g/ml$ employed in the experiments).

together would break the natural endogenous dormancy of *S humilis* seeds. Similar results were also found by Rao *et al* (14) in that the application of ethrel and kinetin showed a synergistic effect upon the reversal of the inhibited germination caused by ABA in lettuce seeds. The partial reversal of the ABA inhibited germination of old seeds by ethrel or BA alone (Fig 4) is due to the fact that, at the concentration employed of 20 μg/ml ABA, some germination was still observed; hence, the explanation for the reversal of the cobalt-inhibited germination by just one principle (Fig 3), can be extrapolated to this new situation. With ABA, however, the reversal caused by a single regulator was much smaller than in the case of the reversal of the cobalt ion effects, and this may be attributed to a probable natural role of ABA as a growth inhibitor and that the two principles are required to overcome the ABA effect in endonenously dormant seeds (Table 2) The reversal of ABA plus Co $(NO_3)_2$-inhibited germination of dormant seeds caused by the promoters followed a similar pattern to the reversal described for seeds treated with ABA alone (not shown).

Thiourea, which substitutes for the light requirement in breaking dormancy and reverses the secondary dormancy imposed by high temperatures in lettuce seeds (11), was highly efficient in overcoming the endogenous dormancy in seeds of *S humilis*, as shown in Table 3. Old seeds treated simultaneously with 20 μg/ml ABA and 100 μg/ml Co $(NO_3)_2$ exhibited 82% germination when 7 500 μg/ml thiourea was added to the medium (data not shown). Thus, thiourea alone substitutes for the two principles apparently involved in breaking the endogenous dormancy of *S humilis* seeds.

GA$_3$, which stimulates the production of ethylene in lettuce seeds (1), did not alter the state of endogenous dormancy of the seeds of *S humilis* at a concentration range of 10-1 000 μg/ml When applied together with ethrel or BA, it can be seen that the effect on the germination of endogenously dormant seeds is entirely due to the action of the two last promoters (Fig 5). On the other hand CCC (10-2 000 μg/ml) and B-995 (10-1 000 μg/ml) did not affect the germination of old seeds As long as the retardants are supposed to interfere with the synthesis of gibberellins, it is yet to be demonstrated if ethrel and/or BA promote the release and the physiological activation of some gibberellins other than GA$_3$, which would be conjugated prior to the break of endogenous dormancy

KNO$_3$, which substitutes for the light requirement in the germination of sorghum seeds (4), did not affect the endogenous dormancy of *S humilis* seeds when employed in a concentration range of 10-10 000 μg/ml The addition of some promoters to
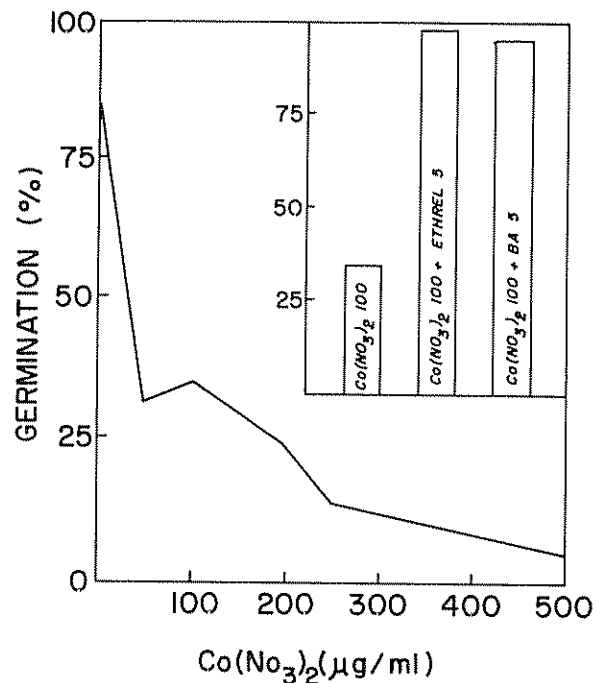

Fig 3 The germination response of old (without endogenous dormancy) seeds of *S humilis* to different concentrations of Co(NO$_3$)$_3$ The inset shows the germination response when ethrel and/or AB were added to the medium containing 100 μg/ml Co(NO$_3$)$_2$

Table 1. The effects of ethrel and/or BA on the germination of endogenously dormant seeds of *S humilis* treated with cobalt nitrate (100 μg/ml). (In this table, and subsequent tables, the figures in brackets refer to the concentrations of the GRs employed).

Treatments (µg/ml)	Germination %
Control, Co(NO$_3$)$_2$ only	0 0 c
BA (10)	0 0 c
Ethrel (10)	39 0 b
Ethrel (10) + BA (10)	59 5 a

Table 2. The effects of ethrel and/or BA on the germination of endogenously dormant seeds of *S humilis* treated with ABA (20 μg/ml).

Treatments (µg/ml)	Germination %
Control, ABA only	0 0 b
Ethrel (10)	7 5 b
BA (10)	0 0 b
Ethrel (10) X BA (10)	63 5 a

dormant seeds treated with KNO_3 resulted in lower levels of germination (Fig 5) than when in the presence of the promoters alone, which suggests that KNO_3 had a toxic effect.

Table 3. The effect of thiourea on the germination of endogenously dormant seeds of *S humilis*

Thiourea (µg/ml)	Germination %
0 (control)	2 5 dc
750	0 0 d
1 850	8 0 c
3 750	23 5 b
7 500	91 5 a
8 750	0 0 d

Fig. 5 Effects of ethrel and/or BA on the germination of endogenously dormant seeds treated with GA_3 and KNO_3 (Both GA_3 or KNO_3 alone were ineffective in releasing endogenous dormancy in seeds of *S humilis*)
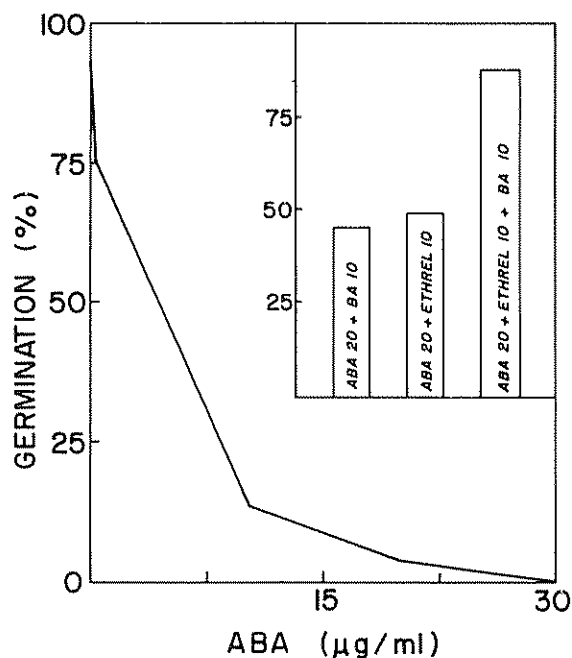

Fig. 4. The germination response of old (without endogenous dormancy) seeds of *S humilis* to different concentrations of ABA. The inset shows the germination response when ethrel and/or BA were added to the medium containing 20 µg/ml ABA.

CONCLUSIONS
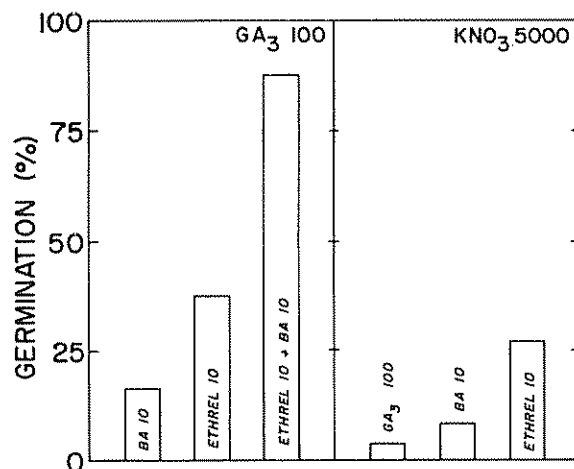
The tegumentary and endogenous dormancies constitute adaptative mechanisms developed by *S humilis* and prevent germination of the bulk of the seeds shed to the soil, with the result that the seedlings do not become established during the dry season (6). From the results with application of GRs one is led to suggest that the production or activation of ethylene and cytokinin is required to break the endogenous dormancy of the seeds. Since a proportion of freshly-produced seeds germinated in response to just one principle (ethrel or BA), it may be speculated that three populations of freshly-produced seeds seem to exist: a major proportion of the endogenously dormant seeds can synthesize (or already has or activates) enough cytokinin; a smaller proportion synthezises (or already has or activates) sufficient ethylene; and finally a certain percentage are deficient in both principles. As the seeds age, some intermediate phase(s) seem(s) to exist. With eight-month old seeds, for instance, just one factor (high temperature), or just one promoter (ethrel) was able to break the endogenous dormancy (unpublished results). Further work is necessary to ascertain the factors that affect the availability of the two principles in endogenous dormant seeds.

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Notas y Comentarios

Cultivos alimenticios andinos para Etiopía

La sequía de la región sub-Sahara de Africa, ocurrida no hace mucho tiempo, estimuló a muchos grupos a prestar su ayuda inmediata para enviar víveres y motivó a establecer planes a plazos más largos. Uno de los proyectos que ha llegado ya a una etapa crucial podría convertir algunos tubérculos andinos, poco conocidos fuera de la zona andina de América del Sur, en cultivos apropiados para las altiplanicies de Etiopía.

Según informa **New Scientist** 110(1505):34, Allan Brunt y sus colegas del Glasshouse Crops Research Institute, cerca de Littlehampton, Inglaterra, han estado trabajando sobre las virosis de los cultivos de los tubérculos oca (*Oxalis tuberosa,* Oxal.), arracacha (*Arracacia xanthorhiza,* Umbel.) y olluco (*Ullucus tuberosus,* Basell.). Algunas selecciones libres de virus están ya listas para ser ensayadas en sus nativos Andes. Eventualmente, podrían ser plantados en Etiopía y otros altiplanos tropicales.

Cuando los colonos españoles fueron a América del Sur, la oca, la arracacha y el olluco, junto con la papa, eran alimento cotidiano en los dominios de los Incas. Según **New Scientist,** por razones desconocidas, los españoles eligieron a la papa para llevarla a Europa y ahora unos 130 países la cultivan. Los otros tubérculos permanecieron como cultivos menores en manos de pobres campesinos en Perú y otras partes del Altiplano Andino.

La revista, por supuesto, está equivocada; esto nos muestra que aún los periodistas científicos cometen errores cuando se extienden en sus escritos sobre temas que no dominan. Todos estos tubérculos andinos, a los que se puede agregar otro tubérculo, la mashua (*Tropaeolum tuberosum*), se cultivan todavía en los Andes y se encuentran en los mercados de ciudades como Lima, Bogotá, La Paz y Quito; se consumen en apreciable cantidad en las mesas hogareñas. Los he

consumido en Lima y estando en Bogotá, fui servido en una mesa amiga un plato de arracacha.

En lo que la revista se refiere a que no se sabe las razones por las cuales los españoles escogieron la papa para llevarla a Europa, hay que recordar la historia de los principales alimentos de las grandes zonas en las que se ha establecido el hombre. En general, el alimento básico, el que ha predominado en la historia de las civilizaciones, ha tenido la particularidad de no tener un sabor pronunciado; ello ha permitido servir de base de platos en los que los sabores los han dado otros alimentos: aves, pescados, verduras, etc. y diversos condimentos.

Desde el arroz en el Lejano Oriente, al trigo en el Medio Oriente, los millos y sorgos en el Africa, la col en la Europa Central, el maíz en la América y otros más, todos se caracterizan por tener un sabor poco dominante. La papa comparte esa cualidad: puede servir de base para muchos platos, sopas, ensaladas, y bocaditos salados. Estas cualidades no la compartían los otros tubérculos, los que se siguieron cultivando en sus lugares de origen. Recordemos, de paso, que esta misma observación la hizo Salaman en su libro clásico sobre la papa (2), al discutir el porqué en la Europa del siglo XVI, la popularidad de la papa fue compartida al comienzo por otra planta con órganos subterraneos comestibles, el topinambur (*Helianthus tuberosus*), a la que se llamaba también "alcachofa de Jerusalen", un nombre doblemente inapropiado. No es alcachofa ya que ésta es un cardo (*Cynara*) mientras que el topinambur es un girasol; no es de Jerusalen sino americana, llevada a Europa por los franceses de la Nouvelle-France, conocida hoy como Canadá. Según Hyams (1), "Jerusalen" es en este caso una corrupción en la pronunciación del nombre italiano "girasol". Esta popularidad, que fue en un momento mayor que la de la papa, cesó poco después. Al parecer, el sabor no pronunciado de la papa no aburrió al consumidor, como parece que lo hizo el sabor del topinambur.

Es probable que al sembrarse fuera del altiplano, todos estos tubérculos andinos han sido contaminados con virus. Aún la papa ha sufrido este destino; por años se han sembrado selecciones libres de virus en las partes altas, como en Escocia, las que surten de semillas a los cultivadores de las zonas menos altas del Reino Unido.

Brunt ha tratado de preparar plantas libres de virus mediante una técnica llamada cultivo de puntas de meristema. Para esto, se corta un nuevo brote apenas comienza a desarrollar, y se le hace crecer en un tubo de prueba hasta que se transforme en una nueva planta. La idea es lograr la planta antes de que sea infectada. Entre 10 y 70 por ciento de las plantas logradas de esta manera resultan estar libres de virus. A veces, ayuda el poner en el medio de cultivo una sustancia con propiedades antivirales.

Brunt espera que los rendimientos de estos tubérculos libres de virus suban en un 30 a 40 por ciento. Se observarán para ver cuánto tardarán en llegar a ser nuevamente infectadas con virus. Esto determinará si hay necesidad de formar núcleos de variedades libres de enfermedad en lugares aislados que servirán para proveer semilla sana, que pueda servir a otras zonas del Tercer Mundo.

En vista del progreso obtenido con los tubérculos andinos Brunt piensa en otras plantas de reproducción vegetativa como los camotes (*Ipomoea batatas*) y los ñames (*Dioscorea* spp.), alimentos básicos para cientos de millones de personas. Tales plantas podrían ser sometidas a este proceso para obtener núcleos de semillas libres de enfermedades que aumenten sus rendimientos en el campo. A.G.

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