reported that light was a decisive factor in improving germination under osmotic stress

From these experiments, it may be concluded that in the natural environment germination of these seeds will occur only when adequate water is available; in this case it would almost certainly be in the free state The results further indicate that water availability is very crucial for the species considered Although germination under osmotic stress cannot be used to predict the behaviour of seeds in dry soil (6) it is a fair indicator of which seeds would germinate under a given moisture level.

Tolerance of low water potentials during germination is an adaptive factor contributing to the growth of weed and crop plants The suggestion that there is a characteristic water potential below which seeds of a given species will not germinate underscores the need for a more comprehensive study of the moisture stress-germination relationships of seeds in the Sahelian tropics

Summary

Seeds of eight tropical crop and weed species were germinated at 0 to -3.5 bar water solutions of polyethylene glycol-6000 to simulate drought Germination of all species decreased with decreasing potentials A moisture stress of 1 bar led to a significant drop in the percentage germination of 70-100 at 0 bar to about 0-10%.

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Purification and serology of bean common mosaic virus

Sumario. La cepa tipo del virus del mosaico común del frijol (BCMV) fue purificada de hojas infectadas de frijol mediante clarificación con cloroformo-tetracloruro de carbono, precipitación con polietilenglicol, y centrifugación en gradientes de cloruro de cesio Un examen de la suspensión final con el microscopio electrónico, demostró la presencia de partículas de 730-740 nm de longitud características de este virus Al ser analizada en el espectrofotómetro, la suspensión del virus purificado mostró una relación 260/280 nm de 1,27 Tres componentes de peso molecular 29,0, 32,5 y 34,4 x 10^3d fueron observados mediante electroforesis del virus en medio de poliacrilamida al 10% con sulfato dodecilo de sodio (SDS) Se produjo un antisuero según la técnica de inmunización en la yema de la pata trasera de un conejo, el cual demostró poseer gran especificidad en pruebas de inmunodifusión doble en medio de agar con SDS

The serological characterization of bean common mosaic virus (BCMV) has often been hindered by the lack of a suitable purification method. The two major problems encountered in the isolation of this virus are, first, its tendency to aggregate and precipitate at low centrifugal forces and, secondly, the incomplete separation of the virus from major plant contaminants (1, 4, 8, 10).

This report describes a purification method that has permitted the isolation of BCMV with a high degree of purity and capsid protein integrity, and the production of a specific antiserum from minimum amounts of purified virus

Material and Methods

Virus source

The type strain of BCMV (BCMV 1) had been preserved in and was subsequently reisolated from infected 'Diacol Calima' bean *(Phaseolus vulgaris* L.) seed at the Centro Internacional de Agricultura Tropical (CIAT), Palmira, Colombia.

Virus multiplication

The primary and first trifoliate leaves of 18-day old plants, grown from infected seed, were harvested and homogenized in 0.01 M potassium phosphate buffer, pH 7.5 (1:10 w/v). This inoculum was then manually applied to the primary leaves of 10-day old 'Bountiful' bean seedlings used as propagation plants for subsequent virus purification.

Virus purification

The purification procedure involved a modification of the method developed by Hiebert and McDonald (6) to purify other potyviruses. Fifteen days after BCMV inoculation, 100 g of primary and first trifoliate leaves were harvested from 30 systemically infected 'Bountiful' plants. The tissue was then homogenized for 3 min with a blender in 200 ml of cold 0.5 M potassium phosphate buffer, pH 7.5, containing 0.5 g sodium sulfite (Na_2SO_3), 50 ml chloroform and 50 ml carbon tetrachloride. The homogenized mixture was centrifuged at 4,080 g for 5 min, after which the pellet was discarded and the supernatant filtered through glasswool. The virus was then precipitated from the supernatant by addition of 6% (w/v) polyethylene glycol (PEG, MW 6,000). After stirring for one hour at 4 C, the virus was concentrated by centrifugation at 11,700 g for 10 min. The virus pellet was then allowed to resuspend for at least 6 h prior to a 10 min clarification centrifugation at 12,350 g.

The resulting supernatant was treated with 20% PEG in 0.02 M Tris buffer, pH 8.2, using 2 ml of PEG per 5 ml of the virus preparation. The mixture was maintained at 4 C for 1 h, and the virus then concentrated by centrifugation at 17,300 g for 10 min. The precipitate was resuspended in 0.25 M potassium phosphate buffer, pH 7.5, and maintained overnight at 4 C before a 10 min clarification centrifugation at 12,350 g.

The virus was further purified by equilibrium density gradient centrifugation (120,000 g for 17 h) in a 30% (w/w) suspension of cesium chloride (CsCl) prepared in 0.05 M potassium phosphate, pH 7.5. The visible virus zone, located approximately 12 mm from the bottom of the CsCl gradient (Fig. 1A) after centrifugation, was collected in a dropwise manner through a needle hole punched in the bottom of the tube. The volume thus collected was diluted in 0.25 M potassium phosphate buffer, pH 7.5, and the virus was concentrated by ultracentrifugation at 84,500 g for 90 min. The virus pellet was allowed to resuspend overnight in 0.5 ml of 0.02 M Tris-HCl buffer, pH 8.2, and then given a 5 min clarification centrifugation at 12,350 g.

Electron microscopy

The presence, purity, and structural integrity of the purified virus were assayed with a Philips Model 200 electron microscope. Purified preparations were prepared in 1% potassium phosphotungstate for observation.

Spectrophotometry

The 260/280 nm absorbance ratio of the purified BCMV preparation was determined with a Beckman Model 25 spectrophotometer to check the virus purity. The virus concentration was determined from the optical density at 260 nm using an extinction coefficient of 2.4 mg/ml/cm (11). Corrections for light scattering were made according to the method of Englander and Epstein (2).

Polycrylamide gel electrophoresis

The electrophoretic analysis of the BCMV coat protein in polyacrylamide gels containing sodium dodecyl sulfate (SDS), was performed acording to the method of Weber and Osborn (13) but as modified by Hiebert and McDonald (6). Electrophoresis was done in the Ortec 4010/4011 vertical apparatus (Ortec Inc., Oak Ridge, Tenn.), with gel slabs 75 mm in height cast to a 10% acrylamide concentration. The coat protein subunits were dissociated for electrophoresis by incubation of one volume of a 1 mg/ml purified virus preparation in two volumes of a dissociation solution containing 0.1 ml sodium phosphate buffer (13), 0.25 ml 10% SDS, 0.025 ml 2-mercaptoethanol, and 0.25 ml 60% sucrose. Serum albumin (67,000 d) glutamate dehydrogenase (53,000 d) and carbonic anhydrase (29,000 d) were used as markers (5 mg/ml concentration) for molecular weight determinations.

Preparation of antiserum

The antiserum of BCMV was prepared by injecting a New Zealand white rabbit with intact purified virus possessing a high degree of capsid protein integrity as determined by SDS - PAGE (6).

The virus preparation was standardized to a concentration of approximately 1 mg/ml, and divided into four 0.15 ml aliquots which were kept frozen until use. A series of three injections were given to the rabbit at weekly intervals using the toe-pad immunization technique (14). Each injection consisted of 0.15 ml purified virus preparation emulsified with an equal

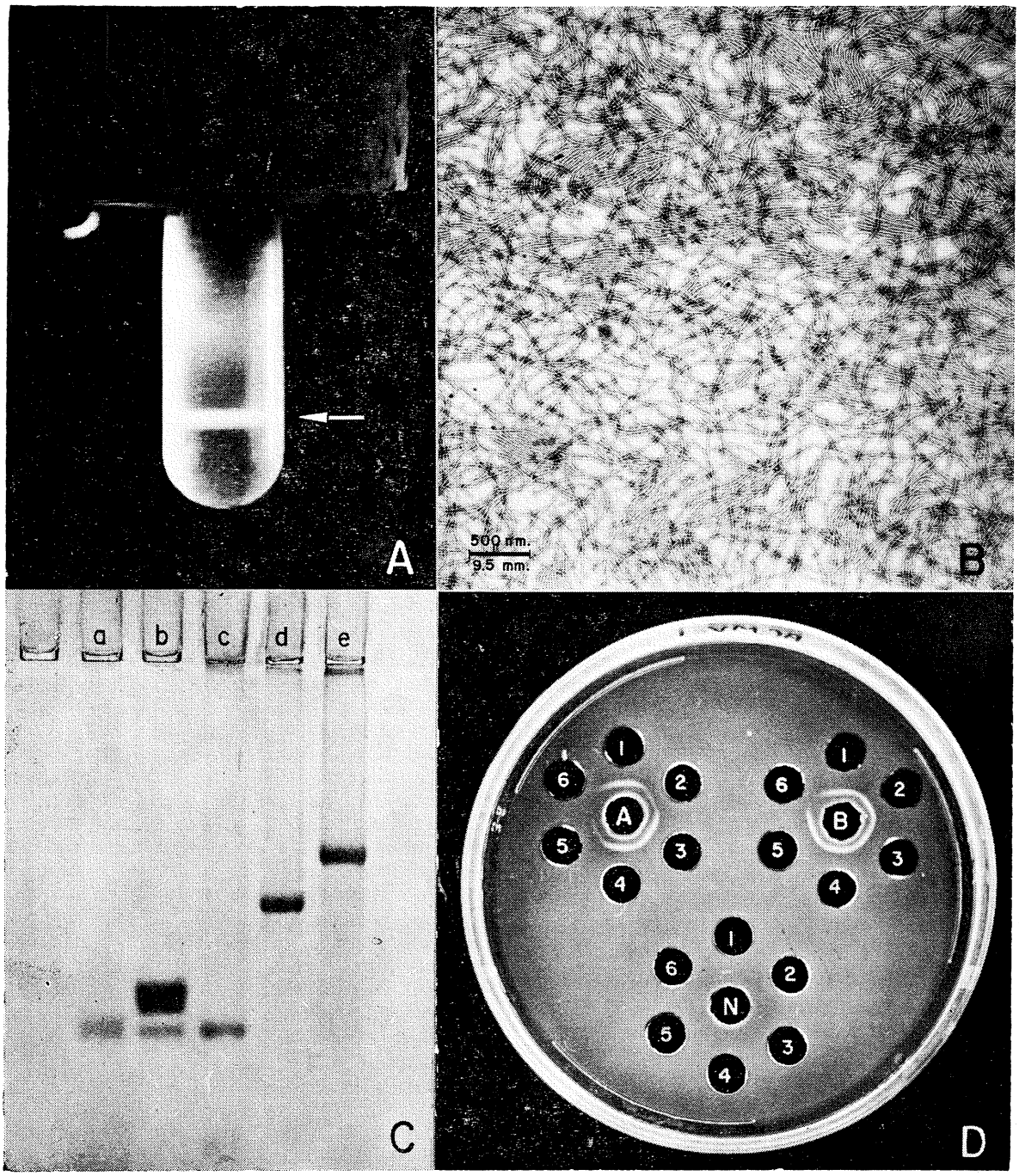

Fig. 1.—*A. Light-scattering band (arrow) in a CsCl gradient.*

B. Filamentous virus particles in a purified preparation.

C. SDS - Polyacrylamide gel electrophoresis: a) Degraded potato virus Y coat protein subunit; b) Electrophoretic forms of the SDS-dissociated capsid protein of purified BCMV; c) Carbonic anhydrase; d) Glutamate dehydroginase; and e) Bovine serum albumin.

D. Double immunodiffusion test. Center wells contain: A and B) BCMV antiserum; N) normal serum. Peripheral wells contain: A) sap from a BCMV-infected 'Diacol Calima' plant (1, 3 and 4), purified BCMV at 0.02 mg/ml (2, 5), and sap from a BCMV-free 'Diacol Calima' plant (6); B) sap from a BCMV-infected 'Diacol Calima' (1, 2) and 'Bountiful (3, 4) plant, purified BCMV at 0.1 mg/ml (5), and sap from a BCMV-free 'Bountiful' plant (6); and N) sap from a BCMV-infected 'Diacol Calima' (1, 2) and 'Bountiful' (3, 4) plant, and from a BCMV-free 'Diacol Calima' (1, 2) and from a BCMV-free 'Diacol Calima' (5) and 'Bountiful' (6) plant.

volume of Freund's complete (first injection), or incomplete (subsequent injections) adjuvant. A booster injection was given one month after the third injection, and the first bleeding took place one week after the third injection.

Serological tests

Double immunodiffusion tests (Ouchterlony) were performed in a medium containing 0.08% Noble agar, 0.25% SDS, and 1% sodium azide (NaN_3) all in water (w/v) (5). The agar medium was poured into 9 cm plastic petri diches, and the wells were punched in a hexagonal arrangement with a center well spaced 4-5 mm from its edge to the inner edges of the peripheral wells. The antigen was prepared by homogenizing approximately 1 g of BCMV-infected plant tissue in a pestle and mortar containing a suspension of 1 ml deionized water and 1 ml 3% SDS. The reactants were pipetted into their respective wells, and the plates were incubated in a moist chamber at 24 C. Reactions were observed 24 h after preparation of the plates.

Results

As can be observed in Figure 1A, a sharp and distinct band could be observed in CsCl gradients following equilibrium centrifugation. Upon recovery and further purification, this band was thrown by electron microscopy to contain filamentous particles 730-740 nm in length (Fig 1 B) characteristic of BCMV and other members of the potyvirus group (3). The absence of major contaminants was also evidenced by spectrophotometric analysis of the virus preparation which had an absorbance 260/280 ratio of 1.27. This value is in agreement with similar figures reported for other purified potyviruses (9, 12). The correcetd yield of the virus was 0.8 mg per 100 g of infected tissue.

The SDS-PAGE analysis of purified BCMV revealed the existence of three molecular weight components (Fig. 1 C). The phenomenon of coat protein heterogeneity has already been reported for other potyviruses although, the number and position of the components resolved have varied for each virus. The 29.0×10^3 d component, however, occurs in most potyvirus preparations analyzed by SDS-PAGE, and has been shown to originate from the heavier molecular weight component(s) by enzymatic degradation (6, 9).

The results obtained in this study suggest that the molecular weight of the capsid protein subunit of BCMV is close to the $32.5 - 34.4 \times 10^3$ d values obtained for the predominant electrophoretic components resolved.

Clear precipitin reaction were observed in double immunodiffusion tests between BCMV antiserum and its homologous antigens (Fig 1 D). Extracts of healthy bean plants did not react with antiserum in any of the tests.

Discussion

Bean common mosaic virus can be isolated from infected bean plants in adequate quantities and sufficiently pure form to allow production of a highly specific antiserum. The purity of the virus was confirmed by spectrophotometrical, electron microscopical, electrophoretical, and serological assays. The results obtained in the SDS-PAGE analysis of BCMV are important, considering that some potyviruses having their coat protein predominantly in the 29,000 d form (degraded form) have been shown to possess distinct serological properties (7). The purification method yielded virus with the coat protein in a predominantly undegraded form and is therefore, suitable for serological studies.

The 'toe-pad' method of immunization proved highly efficient in the utilization of the antigen. In this study, one hundred grams of BCMV-infected bean leaves provided enough virus to produce an antiserum. This constitutes a substantial saving in materials and time when compared with the traditional intramuscular and intravenous immunization methods.

The distinct precipitin reactions observed in doble immunodiffusion tests clearly demonstrated the specificity and high titer of the antiserum. The double diffusion test, unlike the microprecipitin tests conducted in the past, will permit investigators to study the serological relationships between BCMV strains, and to develop an alternative and more efficient system to group these strains.

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Notas y Comentarios

Ayuda técnica a la agricultura tropical

El Tropical Products Institute, de Londres, es la primera institución británica en ser nominada como centro de adiestramiento de la Universidad de las Naciones Unidas (UNU) al firmarse en 1978 el acuerdo que establece la asociación entre ambos organismos, por el Dr. Philip Spensley, Director del TPI y el Dr. Nevin Scrimshaw, Consejero Principal del Rector de la UNU. Así, el TPI será una de las instituciones asociadas, como la es ya el CATIE en Costa Rica (Cf., *Turrialba* 27:220 y 28:8) que suministran adiestramiento para especialistas en varios aspectos de ciencia, tecnología y economía poscosecha para becados de la UNU procedentes de países en desarrollo.

El TPI ha organizado cursos diseñados específicamente para individuos o grupos, tanto en el Reino Unido como en los países en desarrollo. Su amplia gama de actividades lo califica para participar en el Programa Mundial contra el Hambre de la UNU, que tiene las siguientes cuatro áreas de prioridad de acción: a) necesidades alimenticias humanas y su satisfacción en las condiciones prevalentes en los países en desarrollo; b) conservación poscosecha de los alimentos; c) objetivos alimenticios en los planes de desarrollo nacional; d) intercambio de información sobre producción agrícola y ciencia de los alimentos y nutrición.

Para apreciar mejor la labor de ayuda del TPI a la agricultura tropical americana, se pueden mencionar algunas recientes actividades mencionadas en el *TPI Newsletter* (Nº 12, September 1978).

David Dendy y Brian Jones, tecnólogos en cereales, visitaron el Perú en abril como consultores de la Junta del Acuerdo de Cartagena. Con la cooperación del Rupert Best, ingeniero de Procesos del TPI que está en el Perú por un período de un año, se proveerán consejos sobre el procesamiento de cereales producidos en la Sierra Andina, a saber, maíz, avena, cebada, trigo y quina. El objetivo es organizar proyectos pilotos para comunicar y aplicar tecnología apropiada, para difundir las técnicas mejoradas a otros países del Pacto Andino, particularmente Bolivia y Ecuador. El lugar seleccionado para el primer proyecto es Conchan, sobre el río Mantaro, en el Perú.

Geoffrey Pickering visitó el Instituto de Pesquisas Veterinarias "Desiderio Finamor" (IPVDF) de Río Grande do Sul, Brasil, para evaluar las prácticas locales en el uso de plaguicidas, particularmente acaricidas en baños de animales y para asesorar al IPVDF sobre los pasos necesarios para tratar los problemas asociados.

Geoffrey Ames visitó Jamaica para estudiar la forma en que el TPI puede cooperar con el Departamento Químico de la University of the West Indies para desarrollar un nuevo curso sobre alimentos. Recomendó que dos miembros del personal del Departamento deberían visitar el TPI para programas de adiestramiento especializado en enero de 1979. El TPI va a organizar en Jamaica un curso corto sobre la evaluación sensorial así como también cooperar en el funcionamiento de talleres experimentales sobre enlatado de frutas que serán incluídas en el nuevo curso de ciencia de alimentos.

Evolución de áfidos bajo la presión de insecticidas

Cambios genéticos, previamente observados sólo en el laboratorio han tenido lugar, literalmente, en el campo. Los agricultores que usan cantidades generosas de insecticidas han puesto tanta presión sobre un áfido, el pulgón del melocotonero *(Myzus persicae)*, que este ha comenzado a prepararse para un cambio evolucionario.

A. L. Devonshire y R. M. Sawiscki, de la Estación Experimental de Rothamsted, han encontrado que la mayor parte de estos áfidos han duplicado algunos de sus genes. Los experimentos de laboratorio han mostrado que cuando las bacterias son forzadas a vivir en condiciones desfavorables (si se les dá, por ejemplo, un alimento inapropiado), forman un juego extra de genes.

El gen original continua entonces produciendo una proteína funcional mientras que el duplicado queda libre para evolucionar hasta hacer un nuevo y mejor producto. Esto va en apoyo de una importante teoría evolucionaria que se propone explicar la forma como los genes pueden evolucionar al azar sin someter al organismo a largos períodos de seria desventaja selectiva mientras que el gen en evolución estaba pasando por una serie de cambios que no benefician al ser viviente *(Nature* Vol 280, p. 140).

Sawicki y Devonshire comenzaron a sopechar que el proceso estaba funcionando en el áfido cuando encontraron que las líneas resistentes del insecto contenían una cantidad mayor de la enzima que descompone el insecticida (carboxil esterasa) que las líneas no resistentes. Han medido cuánto más activa es la enzima carboxilesterasa en las líneas resistentes del áfido. Los resultados indican que la actividad enzimática aumentó en pasos bien definidos en relación con la resistencia al insecticida.

En algunas líneas, la actividad enzimática fue doblada, en algunas cuadruplicada, y en las líneas más resistentes la actividad aumentó 64 veces. Devonshire y Sawiscki piensan que este marco exponencial significa que esta resistencia incrementada se produjo debido a duplicaciones de genes. Los áfidos con la mayor duplicación del gen de la carboxilesterasa son aquellos que viven en los invernaderos, donde los insecticidas están en su máxima concentración. En ausencia de insecticidas, el número de genes tiende a bajar de nuevo.

Casi todos los pulgones del melocotonero británicos tienen ahora dos, cuatro o más copias de sus genes de carboxilesterasa. Quizás uno de ellos encontrará pronto algo original y provechoso que hacer con una de las copias extras.