2. BLASCO, M. Composición de la quinua cultivada en el Altiplano de Puno, Perú. Turrialba 29: 219-221. 1979

3 CANO, J. y ROSAS, M. Evaluación cualitativa y cuantitativa del comportamiento de siete variedades de quinua. *In* II Convención Internacional de Quenopodiáceas: Quinua-Cañahua Potosí, IICA, Informes de Conferencias, Cursos y Reuniones n. 96 pp 106-112. 1976

4 TAPIA, M. et al. La quinua y la kañiwa. San José. IICA-CIID. Materiales y Libros Educativos n 40. 1979. 227 p.

5 ZVIETCOVICH, G. Identificación del comportamiento fisiológico de la quinua. *(Chenopodium quinoa* W.) y cañihua *(Chenopodium pallidicaule)* como plantas eficientes C_4 o ineficientes C_3. In *II* Convención Internacional de Quenopodiáceas: Quinua-Cañahua Potosí, IICA, Informes de Conferencias, Cursos y Reuniones n 96, 1976. pp 70-77

Some observations on tetrazolium-testing of coffee seed (Coffea arabica and C. canephora)

Sumario En semillas de café hay correlación entre la prueba de germinación y los resultados de la prueba de tetrazolio (TTZ), si los lotes tienen alta viabilidad. En el rango de 30 a 70% aproximadamente de germinación, los resultados de la prueba de TTZ dan generalmente valores más altos.

La prueba del TTZ de embriones extraidos de las semillas es más clara que si se hace en semillas cortadas, pues en el primer caso la coloración es más uniforme y los resultados más confiables. En las semillas cortadas hay la tendencia a que no se desarrolle color en la superficie cortada del embrión. La coloración se completa después de 3-4 horas en una solución del 1% de TTZ.

Los embriones de café Robusta son más susceptibles a daño mecánico que los de café arábica. Se dan las técnicas para su preparación correcta

Introduction

Coffee seeds are relatively short-lived and may lose their viability within a short period, especially when stored under adverse conditions (2). It is therefore important to check the viability of seed lots sown several months after harvest. However, conventional germination tests are unsuitable because they require at least 40 to 50 days to complete. During this time the seed deteriorates further, and a more rapid testing method is required.

The most commonly used method is the tetrazolium staining technique, first developed by Lakon and coworkers, (4) This test involves the reduction of the water-soluble, colourless tetrazolium salt to insoluble red formazan by the dehydrogenases present in living tissues. Dead tissue remains unstained. By comparison of the amount and distribution of dead and living tissue within the embryo, the viability of the seed can be judged.

The following communication summarises experience gained at CATIE, Turrialba with TTZ testing of coffee seed

Materials and methods

During 1978 and 1979 tetrazolium (TTZ) testing was carried out on a large number of different seed lots of *Coffea arabica* L., cvs. 'Caturra' and 'Geisha' of the 77/78 and 78/79 harvest and ranging in viability from 0% to 100%. In one occasion a seed lot of *C. canephora* was also tested.

Carefully processed, hand selected seeds were used throughout. Differences in viability were due only to different seed age and varied storage conditions. Four repetitions of 50 seeds each were used for testing.

The optimum TTZ concentration proved to be 1%, using a pH-7 phosphate buffer solution prepared according to the ISTA-Rules (3). In preliminary tests it was found that embryos cut longitudinally as described by Mondoñedo (5) remained almost white at the cutting surface, and the evaluation of excised whole embryos gave more reliable results. Thereafter, the excised embryo method was used throughout. Excised embryos stained completely after about 4 hours at 35°C, and fresh embryos took even less time to become adequately stained. Much more time was required for longitudinally cut seeds, where twelve to eighteen hours were needed for the solution to penetrate to the still embedded parts of the embryos.

To minimize embryo damage during preparation seeds were presoaked in lukewarm water until the perisperm was soft enough to cut easily. This took up to 4 days in the case of dry and old seeds and the water was replaced three times a day throughout the soaking period in order to avoid fermentation and oxygen stress, which may impair the seed viability.

Water uptake of dry seeds may be accelerated by scarifying the perisperm near the embryo with a sharp scalpel blade, or by rubbing it against an abrasive.

In thirteen cases the results of the TTZ-testing were compared with germination test results of the same seed lots. The germination tests were started about four weeks before the TTZ test was carried out. This compensates partially for the delay in germination of coffee seeds (not less than 40 to 50 days and, in older seeds, up to there months). Germination tests were made at a constant temperature of 25°C, with four replicates of 50 seeds each. Seeds were sown in trays with clean graded sand, moistened to about 70% of its water holding capacity.

Results and discussion

Table 1 shows the results of several storage experiments; see also Aguilera (1). Generally the viability percentage as measured by the TTZ test tends to be higher when compared with the corresponding germination test result. This can be explained by the fact that it takes about 2 to 3 months to complete germination, (in dry old seeds even more), and that during this period the weaker seeds die. The TTZ test however, indicates the current viability of a seed lot, and the final germination percentage will consequently be lower. This is especially true for seed lots

with medium viability percentages In our experiments, additional storage of one month did not quite compensate for the losses between sowing and germination Only in lots 2, 3, 4, 5 and 10, were losses during one month storage equalled or surpassed by the losses occurring during germination testing

Table 1.—Comparison of coffee seed (*Coffea arabica* cv. 'Caturra') viability as measured by the Tetrazolium-test after six months of seed storage with germination percentage after five months of seed storage

Seed Lot Nº	Tetrazolium-Testing (%) at six months storage	Germination (%) at five months storage
1	0	0
2	6	16
3	10	17
4	11	52
5	20	18
6	25	19
7	30	22
8	38	20
9	51	40
10	56	74
11	84	69
12	90	80
13	92	94

r = 0 81

Note: Seed lots 1 through 13 were stored under different conditions

In preliminary tests, it was found that the results of germination test and TTZ-test coincided quite well for lots of fresh, high quality seeds. This was also observed by Mondoñedo (5), and, when the viability had fallen to almost nil, this was equally indicated by both methods. Only medium quality seed lots frequently gave substantial deviations for the reasons explained above.

One difficulty in TTZ-testing of coffee seeds is to bring the embryo in contact with the solution without damaging it. Mondoñedo (5) pointed out, that excised whole embryos gave the best results when testing coffee seeds with tetrazolium Because the procedure for exposing the embryo is somewhat time consuming, he proposed slicing through the seeds in such a way that part of the embryo is removed and a larger part, still embedded in the perisperm, is used for TTZ- testing It was found this latter method less useful, as the cutting surface of the embryo always remained white and penetration of the TTZ solution was irregular.

Fractioning of the embryos may alter their reaction to TTZ, as was clearly shown by Rivas and Morillo (6) The susceptibility of coffee embryos to mechanical damage is also the reason why the method of exposing the embryos of presoaked seeds by thumb-pressure sometimes results in reduced staining. In one case, less than 50% of the viable embryos stained when using this method, while 100% of carefully exposed embryo, stained (6) It was found that *C canephora* embryos are even more susceptible to mechanical injury.

The method of exposing or excising the whole undamaged embryo is naturally more time consuming than slicing whole seeds and requires certain manual skill, and this is especially so for *C canephora*, where the embryo adheres closely to the perisperm-layers

Two preparation techniques were used for excising the embryo from soaked, softened seeds:

— Three superficial cuts are made through the upper perisperm layer near to the embryo, so that this layer can be lifted up with a scalpel or a dissecting needle, (Fig. 1a), or

— the seed is sliced apart near to the embryo and one or two superficial cuts are made through the upper perisperm layer, which can then be lifted with a dissecting needle (Fig. 1b)

Once the embryo has been exposed it may be taken out carefully and be immersed in the TTZ solution. Alternatively a small part of the seed, with the embryo still attached, can be immersed in the TTZ solution We found it more convenient in routine operations to immerse seed fragments into the solution rather than to apply the solution dropwise to part seeds as proposed by Rivas and Morillo (6, 7). During staining, some embryos become detached from the periderm fragment and drop to the bottom of the vial.

After some practice, a skilled analyst can prepare about 100 seeds per hour (or even more), provided the seeds are soft enough.

When assessing the stained embryos (Fig 2), white or pink mottled embryos were considered as non-viable. If only small, unimportant parts of the embryo remained white, e.g. the extreme root tip or cotyledon margin, they were counted as viable. Non viable embryos frequently showed a greyish palegreen colour and had a flaccid appearance. With some experience, damage caused during the excision of the embryos can be reliably identified, mainly by the unusual location and shape of unstained spots If the rest of the embryo was well stained, such seeds were counted as viable.

Despite the time required for soaking and excision of the embryos, the estimation of coffee seed viability by TTZ staining seems to be the most reliable rapid method.

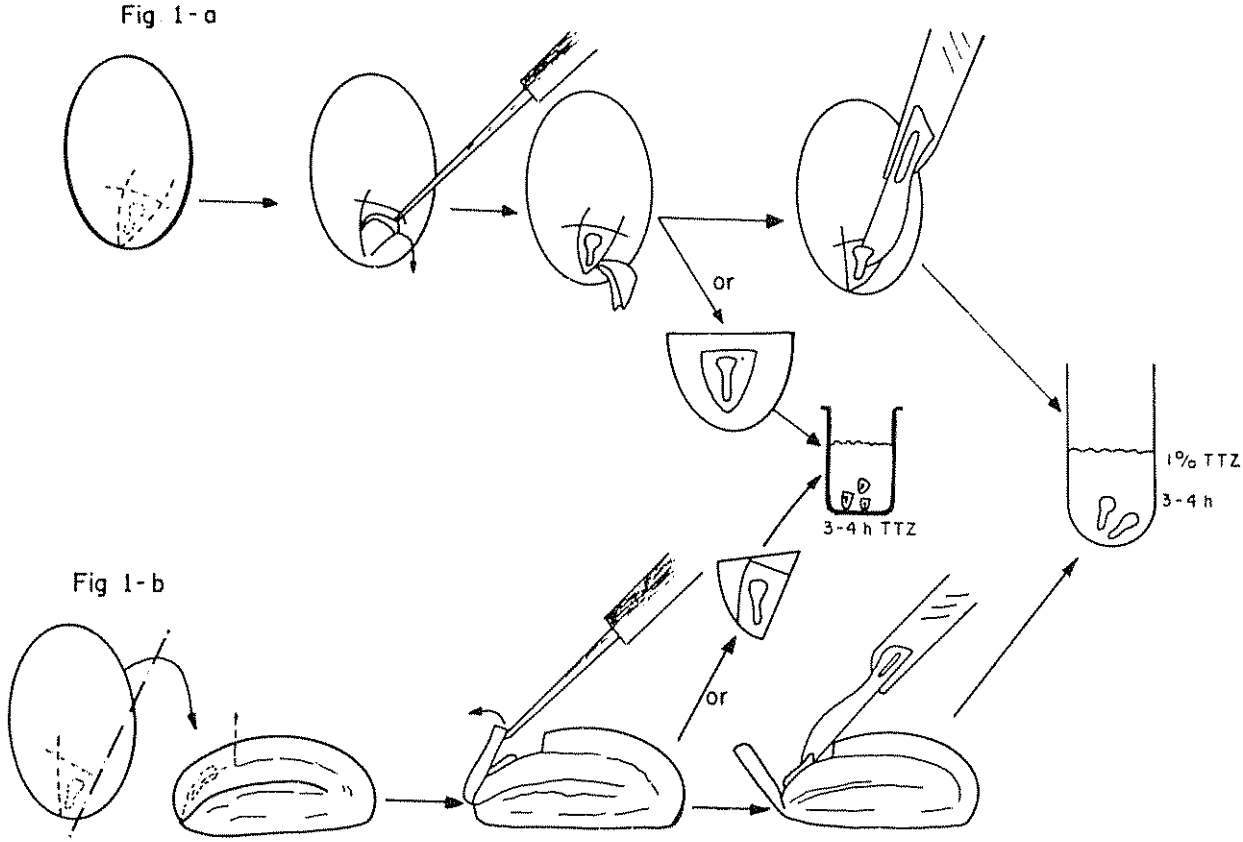
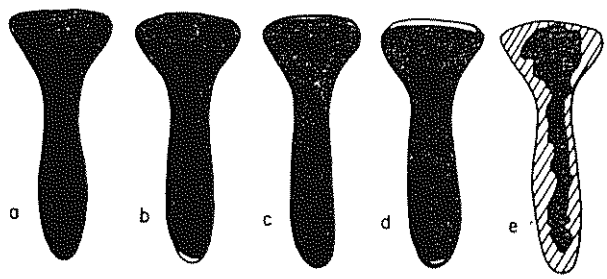


Fig 1 —*Exposure and excision of coffee embryos by lifting the upper perisperm layer after:*
1-a. Three superficial cuts through the upper perisperm layer.
1-b. Sectioning through the seed parallel to the embryonic axis and exerting superficial cuts through the perisperm

Seed parts with the embryo exposed (Alternative I) or excised embryos (Alternative II) may be subjected to the TTZ staining.

More comprehensive tests for improvement of reliability and correlation with actual germination test results of medium quality seed lots are under way and will be reported later.



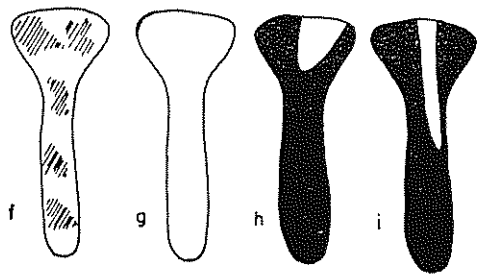


Fig. 2 —*a) Embryo viable, completely stained*
b) Embryo viable, only extreme tip of radicle unstained
c) Embryo viable, only extreme margin of cotyledons unstained
d) Embryo viable, only extreme margin of cotyledons and tip of radicle unstained
b) Through d) still uncertain, whether due to damage during preparation
e) Viability questionable, embryo pink stained with red mottling
f) Embryo non-viable, pink mottling, frequently on a greyish-green background
g) Embryo non-viable, unstained, frequently greyish-green colour
h) and i) Embryo viable, unstained areas caused by damage during embryo excision

Summary

The correlation between viability of coffee seeds as estimated by the Tetrazolium (TTZ) testing and the results of standard germination testing is close for high viability seed lots. In the range of about 30 to 70% germination, the TTZ results tend to be higher.

TTZ-testing of excised embryos of coffee seeds seems to be superior to testing of halved seeds; staining is more uniform and gives more reliable results whereas, in cut seeds, the cut surface of the embryo tends to remain unstained. Staining of embryos is complete after 3-4 hours at 35°C in a 1% solution. Care has to be taken not to injure the embryos during preparation. Robusta embryos are more susceptible to mechanical damage than those of Arabica coffee. Preparation techniques for embryo extraction are briefly described.

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HEINER GOLDBACH*
HUMBERTO AGUILERA VIZCARRA**

* Fisiólogo de semillas, Unidad de Recursos Genéticos CATIE/GTZ CATIE, Turrialba, Costa Rica.
** Instituto Salvadoreño de Investigaciones del Café. Santa Tecla, El Salvador

REFERENCES

1 AGUILERA V., H. E. Almacenamiento de semillas de café. M. Sc. Thesis. Turrialba, Costa Rica. Universidad de Costa Rica/ Centro Agronómico Tropical de Investigación y Enseñanza. 1979.

2 BENDAÑA, F. E. Fisiología de las semillas de café. I. Problemas relativos al almacenamiento. Café (Costa Rica) 4(15): 93-96. 1962.

3 INTERNATIONAL SEED TESTING ASSOCIATION. International rules for seed testing. Seed Science and Technology 4: 29-30 and 133-134. 1976.

4 LAKON, G. The topographical tetrazolium method for determining the germinating capacity of seeds. Plant Physiology 24: 389-394. 1949.

5 MONDOÑEDO, F. R. Quick test with tetrazolium chloride on coffee seed viability. Journal of Agriculture of the University of Puerto Rico 54 (2): 370-376. 1970.

6 RIVAS V., A. and MORILLO, A. R. Uso del tetrazolium en la determinación del poder germinativo de la semilla de café. Agronomía Tropical (Venezuela) 14(1): 25-32. 1964.

7 RIVAS V. A. and MORILLO A. R. Duración de la prueba de germinación en semillas de café de tres edades diferentes tratadas con Tetrazolium. Agronomía Tropical (Venezuela) 16 (3): 205-208. 1966.

Fertilización del cultivo de quinua en condiciones del Altiplano de Puno, Perú*

Summary. The purpose of this study was to determine quinua (**Chenopodium quinoa**) yield responses to applications of N, P, K, in conditions of Puno Highlands, Peru. Quinua production was significantly increased by N applications, while no statistical differences were found with P and K fertilization. Data confirm nitrogen deficiency in Puno Highlands as a major limitation to obtain substancially increased quinua production.

Introducción

Como se indicó en una publicación previa (1), los suelos del Altiplano de Puno, Perú, son pobres en nitrógeno, deficientes en fósforo y muy variables en cuanto al contenido de potasio, lo cual significa que la producción de quinua *(Chenopodium quinoa)* tendrá ciertas dificultades derivadas de la disponibilidad de esos tres elementos, en especial de los dos primeros. La revisión de literatura hecha por Tapia, *et al.* (3) tiende a señalar que la quinua, en los Altiplanos de Bolivia y Perú, responde bien a la fertilización nitrogenada, de manera intermedia al fósforo, y poco o nada a la fertilización potásica. Para el Altiplano de Puno, a nivel de agricultor se ha venido recomendando (2) la fórmula 80-40-00, aplicada al momento de la siembra, de ser posible, dejando la mitad del nitrógeno para incorporarlo en la época del deshierbo.

La presente investigación tuvo por objetivos definir una fórmula de fertilización basada en la experimentación y tratar de concretar el comportamiento del fósforo, para lograr las mejores producciones con la menor cantidad del insumo posible.

Dosis de N-P-K-

Se estudiaron 3 niveles (0-50-100) kg/ha de N-P-K utilizándose el diseño de bloque completo al azar con 27 tratamientos y 4 repeticiones. De acuerdo con el análisis de variancia que aparece en el Cuadro 1, hubo diferencias altamente significativas entre tratamientos, lo mismo que para las aplicaciones de N, mientras que no hubo diferencias significativas para el P y K y sus interacciones. La prueba de Duncan (p 0,05) mostró que la dosis N_{100} y N_{50} se comportan de manera similar superando significativamente a la dosis de N_0 que tuvo el rendimiento más bajo.

De acuerdo con el análisis estadístico se recomendaría emplear 50 kg/ha/cosecha. Sin embargo, de acuerdo a los datos de extracción de N por la quinua (1), una biomasa total media de 5000 kg/ha requiere 80 kg/N. Si el agricultor cambia el sistema tradicional de cosecha consistente en arrancar las plantas por segar con hoz, el suelo recibe de esa biomasa total

* Estudio realizado dentro del Proyecto Instituto Interamericano de Ciencias Agrícolas-Fondo Simón Bolívar, Oficina del IICA en Perú