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MORPHOLOGY AND VIABILITY OF SEEDS OF *Bombacopsis quinata* AND *Anacardium excelsum*

Morfología y viabilidad de semillas de *Bombacopsis quinata* y *Anacardium excelsum*

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ABSTRACT. Most of tropical forest species are propagated by sexual seed, but in many cases, their morphology is unknown and there is no standardized rapid measurement of viability with biochemical tests. The aim of the study was to describe the morphological characteristics and viability of the species seed *Bombacopsis quinata* and *Anacardium excelsum*. The study was conducted at the Córdoba University, Montería - Colombia, between April 2012 and July 2014. In forest plantations in five locations in Córdoba department, five trees by species were randomly selected, and each tree, five samples of 100 seeds were taken randomly. For morphometric description of the parts of seeds, 10 seeds by species were used. Biochemistry tetrazolium test was done under a completely randomized design, with six treatments: three tetrazolium concentration, 0,5; 1,0 and 1,5 % with two times of immersion in the solution: two and three hours, and four replications of 25 seeds each. The viability obtained with tetrazolium test was compared with a conventional test of germination in laboratory. External characteristics of the seeds of the species *B. quinata*, showed less variation than *A. excelsum*, especially those related with the weight of them. Three topological patterns were identified in the seeds of each species. The concentration of 1,0 % tetrazolium for three hours, proved to be effective to determine the seed viability of these two species.

Key words: forest trees, seed germination, tetrazolium test, sexual reproduction

RESUMEN. Gran parte de las especies forestales del trópico se propagan por semilla sexual, pero, en muchos casos, se desconoce su morfología y no se tiene estandarizada la medición rápida de su viabilidad con pruebas bioquímicas. El objetivo del estudio fue describir las características morfológicas y la viabilidad de las semillas de las especies *Bombacopsis quinata* y *Anacardium excelsum*. El estudio se realizó en la Universidad de Córdoba de Montería – Colombia, entre abril de 2012 y julio de 2014. En plantaciones comerciales de cinco localidades del Departamento de Córdoba, se seleccionaron al azar cinco árboles/especie y, de cada árbol, se tomaron al azar cinco muestras de 100 semillas. Para la descripción morfométrica de las partes de las semillas, se utilizaron 10 semillas/especie. La prueba bioquímica de tetrazolio se hizo bajo un diseño completamente al azar, con seis tratamientos: tres concentraciones de tetrazolio, 0,5; 1,0 y 1,5 % con dos tiempos de inmersión en la solución: dos y tres horas, y cuatro repeticiones de 25 semillas cada una. La viabilidad obtenida con la prueba de tetrazolio se comparó con la de una prueba de germinación convencional, en laboratorio. Las características externas de las semillas de especie *B. quinata*, presentaron menor variación que las de *A. excelsum*, especialmente aquellas relacionadas con el peso. Tres patrones topológicos fueron identificados en las semillas de cada especie. La concentración de 1,0 % de tetrazolio durante tres horas, resultó ser efectiva para determinar la viabilidad de las semillas de estas dos especies.

Palabras clave: árboles forestales, germinación de las semillas, prueba de tetrazolio, reproducción sexual

INTRODUCTION

Studies on morphological characteristics, dimensions, weight, determination of viability and conservation of the seeds of native and exotic forest species, by several authors, show the importance of using this knowledge in nursery and forestry studies. The studies are based on seeds of *Pinus brutia* (1); *Pinus tropicalis* (2); *Anadenanthera Colubrine* (3); *Tabebuia rosea* (4); *Stizolobium aterrimum* (5), *Bumelia obtusifolia* (6); *Plinia trunciflora* (7); *Gliricidia sepium* (8); *Jatropha curcas* (9); *Piptadenia moniliformis* (10); *Ceiba speciosa* (11), *Coffea arabica* (12) and *Alibertia patinoi* (13).

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In Colombia, work on seeds of the species *Alnus jorullensis, Cariniana piryformis, Cedrela odorata, Cordia alliodora, Tabebuia rosea, Anacardium excelsum, Cedrela montana, Enterolobium cyclocarpum, Erythrina edulis, Erythrina fusca, Jacaranda copaia, Juglans neotropica, Lafoensia speciosa*, *Samanea saman, Tabebuia chrysantha., Cariniana micrantha, Manilkara bidentata and Peltogyne paniculata* are important (14-16). This shows that the number of native forest species under study continues to increase nationally, as in the different areas with natural forests it is investigated and knowing its characteristics, benefits and forestry and industrial potential

The forest species: *Bombacopsis quinata* (Jacq.) Dugand and *Anacardium excelsum* (Bertero & Balb. ex Kunth) Skeels) are prioritized in the Colombian Caribbean by all the actors of the Forest Chain of Córdoba, for their adaptation and dispersion in the tropical, contributions to the conservation and regeneration of natural forests, benefits in the processes of wood production, reforestation, ecosystem services, forestry, nurseries, excellent quality and nobility of wood, and significant demand for this type of wood in the markets local, national and international (14-16). In spite of the previous utilities, the characteristics of the seeds of these forest species in the Department of Córdoba are little known and studied, in comparison those of the exotic commercial forest species and with that of agricultural crops.

The knowledge and interpretation of the integral quality of the seed and its optimal management in nursery, is important in agricultural and forestry production, since it is the first step to achieve success in forestry management, growth, yield, quality, productivity, competitiveness and sustainability of plantations and wood production (17,18).

The objective of this work was to describe the morphological characteristics and the viability of *Bombacopsis quinata* (Jacq.) Dugand and *Anacardium excelsum* (Bertero & Balb. ex Kunth) Skeels) seeds by means of biometric characterization and viability test with tetrazolium.

MATERIALS AND METHODS

LocaLization and evaluation period

The research was carried out at the University of Córdoba, in Montería-Colombia, located in the middle zone of the Sinú Valley, between April 2012 and July 2014.

Genetic material

Free-pollinated sexual seed was used from five trees sampled at random in plantations of *B. quinata* in Planeta Rica (m. a.s.l: 87 m, LN: 08º24’47,6 “and LO: 075º36’16.9”) and San Antero (m. a.s.l: 48 m, LN: 09º19’39.95 “and LO: 075º49’54.06”), and *A. excelsum* in Montería (m. a.s.l: 13 m; LN: 08º42’27,73 “and LO: 075º40’06,47”), Ciénaga de Oro (m.a.s.l: 13 m, LN: 08º51’16,75 “and LO: 075º35’21,06”) and San Carlos (m, a.s.l: 50 m, LN: 08º42’23.67 “and LO: 075º40’13,19”), municipalities located in the tropical dry forest ecological zone with an average temperature of 27-28 ºC, relative humidity of 84-86 % and annual precipitation 1200-1400 mm, in the Department of Córdoba-Colombia (19). Therefore, the seed of each tree constituted a family of half-brothers (20). At the time of the seed collection, the trees presented ages of 10-22 years, plant height of 8-18 m and diameter to 1,3 m height, from 20 to 53 cm.

Procedure

The process of tree sampling and seed collection in each of the species was carried out according to the specific protocols proposed (21), adjusted and validated (18).

For the morphometric characterization of the seeds of the two species, five trees/species were taken at random and, from each tree, five samples of 100 seeds, for a total of 500 seeds/species. Next, the essential parts of seeds such as embryo, endosperm and radicle were identified, on ten seeds/species, based on the proposed methodology for tree and shrub seeds (22,23).

The biometric characteristics maximum width (AS), maximum length (LS), width / length ratio (RALA), were measured in centimeters (cm); while those associated with seed weight (PES) and weight of 100 seeds (P100S) were measured in grams (g). The number of seeds/kg (NSKG) was estimated by counting the number of seeds in five samples of 100 seeds/species, and then the average was taken to kilogram (kg), by proportionality.

For the identification and description of the seminal tissues, and the determination of topological patterns in the laboratory, ten complete and healthy seeds, taken at random were used for each species. In the description of the embryo structure, a pre-conditioning was performed, by immersion in distilled water at a temperature between 25 and 30 ºC. The separation of the seminal cover allowed total exposure of the embryo and the parts considered “essential” and of importance for the tetrazolium test (24). The observations were made with a stereoscope (Vista Visión®).

For the study of the viability of the seeds, a stain with 2, 3, 5 triphenyl tetrazolium chloride(tetrazolium) was performed by immersing the seeds in 1% solution, with a staining time of two hours, in the absence of light and at a temperature of 40 °C (24). The seeds were then washed three times with distilled water to remove the excess dye and the viability was evaluated with the help of a stereoscope (Vista Visión®) to improve the visualization of the embryo structure.

Viability was assessed by identifying three categories of seeds, according to the recommendations for the interpretation of topological staining patterns (25):

Category 1. Viable seeds: a) those with the embryo and endosperm completely stained; b) those that present superficial necrosis in the middle of the endosperm, mainly in the remote parts of the embryo; c) those with unstained areas (dead) in the endosperm, in areas opposite the radicle.

Category 2. Non-viable seeds. a) those with the unstained embryo and endosperm (dead); b) those that present the unstained embryo, even if the endosperm is stained; c) those that present acute necrosis in the embryo; d) those that present the dyed and unstained endosperm embryo; e) those that present necrosis at the tip of the radicle; f) those with serious damage in more than half of the essential parts of the seed.

Category 3. Doubtful seeds: partially stained seeds that will produce normal or abnormal seedlings, depending on the intensity and pattern of the staining. In this category are seeds that have less than half stained and healthy essential parts.

For the efficiency evaluation of the embryo staining, based on the intensity and color uniformity (26), an experiment was established under a completely randomized design, with six treatments and four repetitions of 25 seeds each. The pre-conditioning, removal of the covers and exposure of the embryos was done in the same way as was described in the determination of the topological patterns. The treatments were structured on the basis of the combination of three tetrazolium concentrations (0,5; 1,0 and 1,5) and two staining times (two and three hours) and they were randomized as six levels of a factor. Viability was assessed by using the categories described for the staining patterns, defined for each species (25).

To measure the reliability of the tetrazolium test, a conventional germination test was performed under laboratory conditions for each species.

A germination chamber (Dies®) was used at a temperature of 28 ºC, relative humidity of 80 %, with a light period of 10 hours per day. Four repetitions of 25 seeds each per species were used, arranged on paper towel in aluminum trays, with uniform irrigation supply for 45 days. The viability of the seeds was evaluated with the record of the number of normal and healthy seedlings emerged during the test. The comparison with the tetrazolium test was done by assuming as viable seeds those of the 'Viables' category, plus half of the seeds of the 'Doubtful' category, according to what were used by several authors (11, 12, 14).

Statistical analysis

For the morphological characteristics, descriptive statistics were made and confidence intervals of 95 % probability were estimated, and for effect estimation of the treatments and the reliability of the tetrazolium test in these two species, an analysis of variance factor with six levels (treatments) and Duncan's multiple range tests at 5 % probability was carried out (27). The GENES computer program version Windows GENES V, 2013, 5.1 (28) was used. The validation of the tetrazolium test was made by multiple comparison of the viability means of the six treatments, plus the average viability obtained with the conventional germination chamber germination test, as an additional treatment.

RESULTS AND DISCUSSION

Biometric characterization of the seeds

The seeds of *B. quinata*, a species known in the Colombian Caribbean as red kapok, had averages of 0,38 ± 0,015 cm wide (AS), 0,48 ± 0,015 cm long (LS), 0,80 ± 0,014 AS/LS ratio (RALA), 0,0315 ± 0,0017 g seed weight (PES) and 3,15 ± 0,173 g weight of 100 seeds (P100S). According to the weight of 100 seeds, the number of seeds/g (NSKG) was approximately 33,876 ± 2,480 (Table I). The seed had a rounded and smooth shape, with a dark brown seed coat (Figure 1a and 1b). According to the observations, and taking into account the classification carried out by Martín (22), the embryo was defined as axial, foliated, folded, convoluted, curved and with thick plano-convex cotyledons; white to cream, folded over the hypocotyl (Figure 1b), agreeing with the descriptions made (29,30).

Table I. Descriptive statistics for the dimensions and weight of the seeds of two native forest species of the Department of Córdoba (Colombia)

Variable	Media	Mín.	Máx.	CV	DE	LI	LS
B. quinata (Jacq.) Dugand (Ceiba roja)							
AS (cm)	0,38	0,35	0,41	4,00	0,0152	0,377	0,385
LS (cm)	0,48	0,45	0,51	3,12	0,0149	0,474	0,482
RALA*	0,80	0,76	0,84	1,77	0,0142	0,794	0,802
PES (g)	0,0315	0,0253	0,0343	5,49	0,0017	0,031	0,032
P100S (g)	3,146	2,533	3,432	5,49	0,1728	3,098	3,190
NSKG (#)	33,876	29,998	44,407	7,32	2,4799	33,1910	34,5164
A. excelsum (Bertero & Balb. ex Kunth) Skeels) (Caracolí)							
AS (cm)	1,76	1,66	1,88	3,20	0,0565	1,748	1,778
LS (cm)	3,25	3,06	3,46	3,45	0,1119	3,215	3,275
RALA*	0,55	0,50	0,60	4,65	0,0254	0,539	0,553
PES (g)	2,37	1,67	2,91	17,17	0,4075	2,261	2,479
P100S (g)	37,35	166,57	291,44	17,17	40,75	226,090	247,867
NSKG (#)	454	352	609	18,33	83,2	430,8	475,3

AS = seed width; LS = seed length; RALA = ratio width / length of seed; PES = weight of seeds; P100S = weight of one hundred seeds; NSKG = number of seeds per kilogram; Min = minimum value; Max = maximum value; C.V. = coefficient of variation; IC = confidence interval; LI = lower limit; LS = upper limit

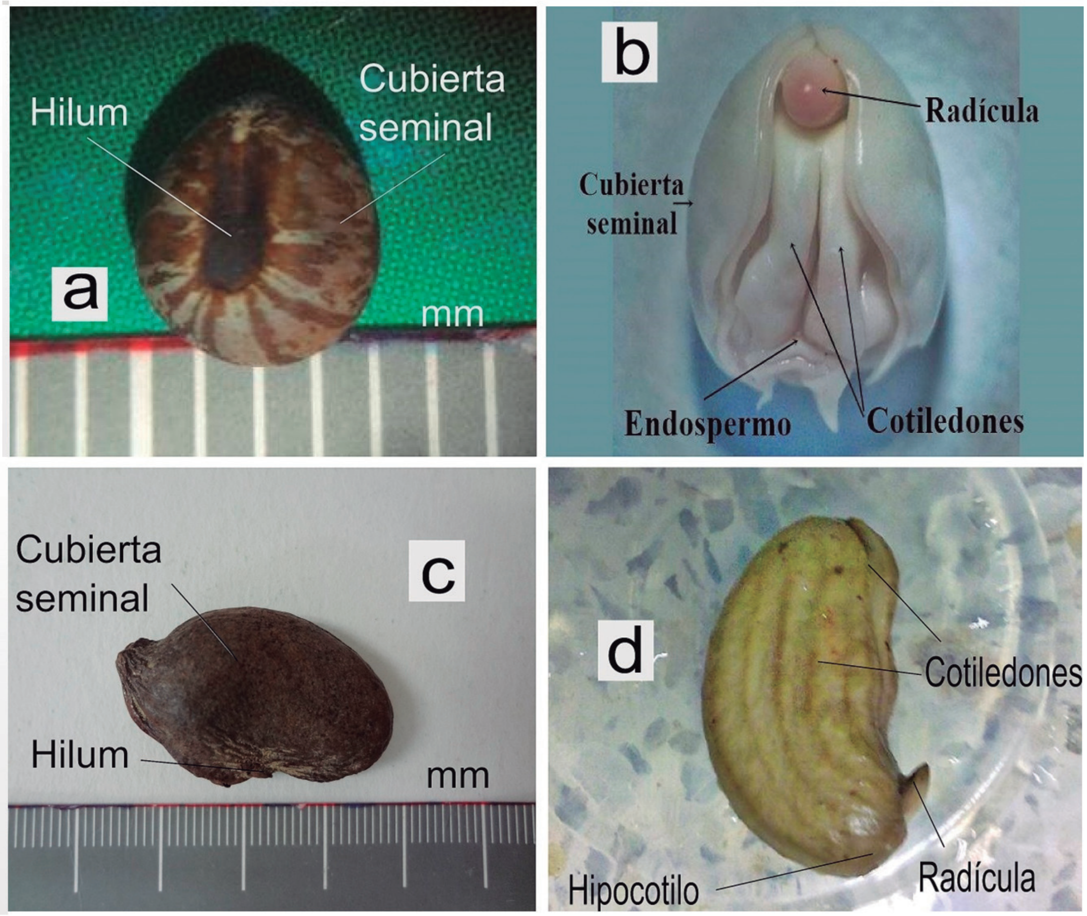

Figure 1. External and internal morphology of the seeds of *B. quinata* (a and b) and *A. excelsum* (c and d)

The seeds of *A. excelsum*, known in Colombia as a “caracolí”, had averages of 1,76±0,057 cm wide (AS), 3,25±0,112 cm long (LS), 0,55±0,025 AS/LS ratio (RALA), 2,37±0,408 g seed weight (PES) and, 237,35±40,75 g for weight of 100 seeds (P100S). According to the P100S, the average number of seeds/kg (NSKG) was approximately 454 ± 83 (Table I). These characteristics are in agreement with those found in other investigations (22,23). According to the observations and taking into account the classification (22,23), the endosperm was very thin; the massive embryo provided with thick cotyledons of creamy and fleshy color, rounded apex and partially joined at the base on the insertion of the radicle (accumbents) (Figure 1c and 1d), similar to those reported in other studies (28).

Topological patterns

The staining patterns identified in the seeds of *B. quinata* and *A. excelsum* are shown in Figures 2 and 3, with three categories defined on the percentage basis of stained tissue: viable, doubtful and non-viable, similar to what reported in other tree species, such as: *T. roseoalba* (4); *Enterolobium contortisiliquum* (31); *Bumelia obtusifolia* (6); *Plinia trunciflora* (7).

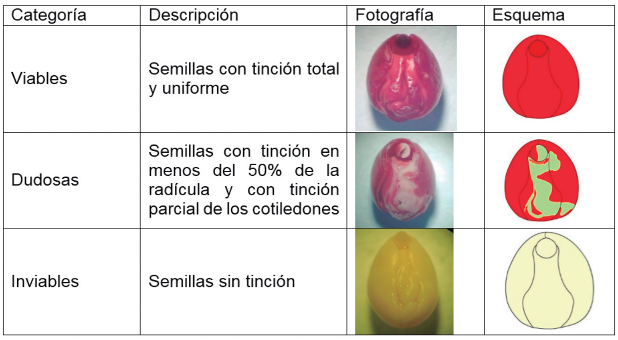

Figure 2. Topological patterns identified in the tetrazolium test in seeds of *B. quinata*

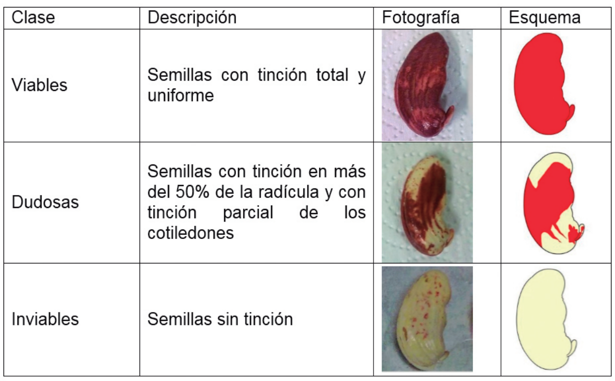

Figure 3. Topological patterns identified in the tetrazolium test in seeds of *A. excelsum*

The seeds showed variations in the staining intensity, because the tetrazolium salt allows determining the presence, location and nature of the alterations in the live tissue of the seeds (24), when they produce triphenyl formazan (red) that evidences the respiratory activity of the mitochondria, and with it, the cellular viability. Therefore, the red color in the embryos is a positive indicator of the seed viability (32), while those regions faintly colored in some parts of the embryo indicate that the cells have a decreased respiratory activity and, therefore, lower activity of dehydrogenase enzymes (26,32).

Evaluation of seed viability with the tetrazolium test

The mean comparisons of the viable, doubtful and nonviable categories, as responses to tetrazolium concentrations and staining time, are recorded in Table II. Likewise, the intensity and uniformity of the staining is recorded in Figures 4 and 5. In the two species, the viability means in each of three categories differ from each other, indicating that the seed tissues are influenced by differential form by tetrazolium concentrations along with staining times. These results are similar to those obtained in *Tabebuia roseoalba* (4), *Enterolobium contortisiliquum* (29), *Pinus tropicalis* (2), *Plinia trunciflora* (7), *Gliricidia sepium* (8) and *Piptadenia moniliformis* (10).

Table II. Mean values of three categories associated with seed quality of *B. quinata* and *A. excelsum*, under three concentrations of tetrazolium and two staining times

Especie	Tratamientos	Viables	Dudosas	Inviables
B. quinata	[0,5]* y 2 h	81,0 b	15,0 abc	4,0 b
	[0,5]* y 3 h	91,0 a	7,0 c	2,0 b
	[1,0]* y 2 h	75,0 b	25,0 a	0,0 b
	[1,0]* y 3 h	77,0 b	5,0 c	18,0 a
	[1,5]* y 2 h	79,0 b	18,0 ab	3,0 b
	[1,5]* y 3 h	84,0 ab	11,0 bc	5,0 b
A. excelsum	[0,5]* y 2 h	1,0 c	27,0 ab	72,0 a
	[0,5]* y 3 h	56,0 a	35,0 a	9,0 c
	[1,0]* y 2 h	57,0 a	31,0 ab	12,0 c
	[1,0]* y 3 h	64,0 a	19,0 b	17,0 c
	[1,5]* y 2 h	34,0 b	32,0 ab	34,0 b
	[1,5]* y 3 h	21,0 b	36,0 a	43,0 b

*Concentration of tetrazolium (%); Different letters in the same column, in each seed category, indicate significant differences, according to the Duncan test at 5% probability

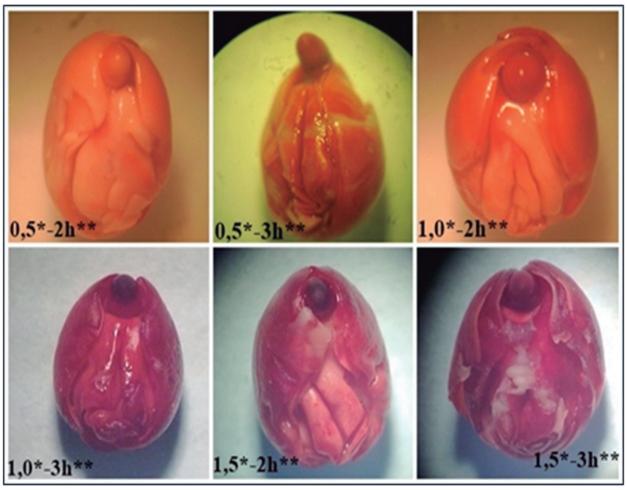

Figure 4. Seeds of *B. quinata* subjected to the tetrazolium test; * tetrazolium concentration in percentage (%); ** staining time in hours

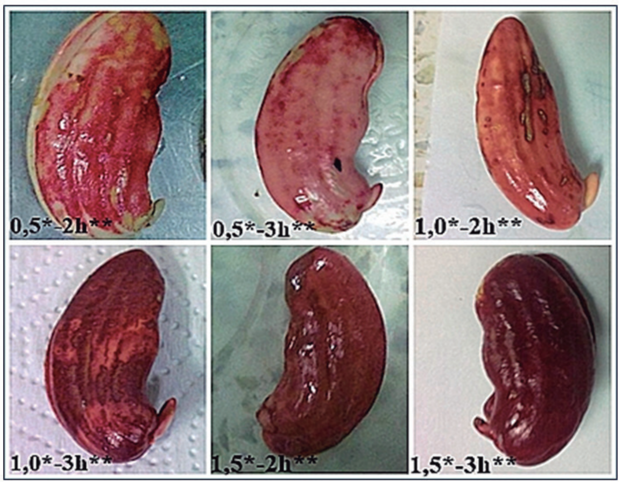

Figure 5. Seeds of *A. excelsum* subjected to the tetrazolium test; * tetrazolium concentration in percentage (%); ** staining time in hours

In seeds of *B. quinata*, the highest percentage of seeds of the viable category was observed at the concentration of 0,5 % of tetrazolium and the staining time of three hours, without significant differences with respect to the concentration of 1,5 % and equal staining time. These same treatments were statistically similar to the percentages of the doubtful and unviable categories. However, in the unviable category, the concentration of 1 % tetrazolium and three hours of staining registered the highest and statistically different value of the rest of the treatments, which in turn, did not differ from each other. On the other hand, the percentage of seeds of the doubtful category was less than 1 % of tetrazolium and three hours, without deferring from the treatments to 0,5 and 1,5 % of tetrazolium and three hours of staining.

Therefore to determine the viability of *B. quinata* seeds it is necessary three hours of contact with the tetrazolium solution at concentrations between 0,5 and 1,5 %.

In seeds of *A. excelsum*, the highest percentage of seeds of the viable category was observed in three of the six treatments: 0,5 % tetrazolium and three hours, 1 % tetrazolium and two hours, 1 % tetrazolium and three hours. However, in the unviable category, these same treatments registered the lowest values and, in the category doubtful values in the range of 19 to 32 %. Therefore, for *A. excelsum*, an immersion in the tetrazolium solution at a concentration of 0,5 % for three hours or 1,0 % tetrazolium for two hours is necessary to determine the viability of its seeds.

In general, the stains corresponding to the treatments mentioned for each species, have an optimal coloration, whose uniformity and intensity are in accordance with the recommendations made in other investigations (11,12,14). In addition, they allow to efficiently evaluating the viability of the seeds through this test; which is consistent with that found in seeds of mortiño (*Acca sellowiana*), in which with the concentration of tetrazolium at 1 %, adequate staining of the embryo was achieved (33). Likewise, other authors affirm that this concentration is the most recommended to perform the tetrazolium test (12,14, 25).

According to the above, it can be noted that, as the concentration of tetrazolium increases, the embryos show a greater intensity of color, from a low tonality in the treatments with 0,5 % tetrazolium to a very intense coloration in the treatments with a concentration of 1,5 %, due to increased activity of tetrazolium in living tissues. Likewise, as the staining time increases, under a same concentration, the colors become darker, which indicates that the exposure time of the tissues to the tetrazolium salt influences the imbibition and the reactions of oxide-reduction. Similar trends were reported in the species: *Pinus tropicalis* (2), *Enterolobium contortisiliquum* (32), *Plinia trunciflora* (7) and *Piptadenia moniliformis* (10), among others.

When comparing the means of the tetrazolium treatments, with each other and with the conventional germination test, it was observed that the use of a 1 % tetrazolium concentration during three hours, for the seeds of *B. quinata*, it is indicated, since the average percentages do not differ significantly from each other (Table III).

For *A. excelsum*, the percentage of germination obtained with the conventional test does not differ statistically from the percentage of viable seeds estimated with 0.5 and 1% of tetrazolium with staining times between two and three hours, respectively.

Therefore, any of the two concentrations is valid in its application and obtain reliable results in verifying the viability of said seeds, in an analysis of their quality.

Investigations with seeds of *Ricinus comunis*, *Poincianella pyramidalis* and *Crambe abyssinica* (34-36), indicate that with lower concentrations of the tetrazolium salt, the results of the viability evaluation seem to have greater correlations with the conventional germination test.

The consistency in the results of the viability test with tetrazolium and that of conventional germination in this study, in the two species studied, indicates that the first one estimates the viability of the seeds in a safe, fast and economic way, since the differences in the results of both tests are statistically due to chance and they are within the range admitted and reported for other forest, tree and agricultural species (2,4-12,31,35,37-39). In spite of the above, in the species with problems of dormancy in their seeds, the tetrazolium test and the germination test can produce very different results, when the appropriate pre-germinative treatments are not applied (4,5,31,35) .

Table III. Average values of germination and viability with different concentrations of tetrazolium in two native forest species

Tratamientos	*B. quinata*	*A. excelsum*
PG	74 b	81 a
[0,5 %] y 2h	88 a	15 c
[0,5 %] y 3h	94 a	74 a
[1,0 %] y 2h	87 a	73 a
[1,0 %] y 3h	79 b	74 a
[1,5 %] y 2h	88 a	50 b
[1,5 %] y 3h	89 a	39 bc
Significancia estadística	*	*
CV (%)	11,8	14,6

PG = Germination test; CV: coefficient of variation; means identified with the same letter do not differ statistically, according to the Tukey test at 5 % (40). *Significant at 5 % probability, according to the F test

These results constitute an important contribution to the certification process and physiological quality control of the seeds of the two species studied. Likewise, its use contributes to speed up decisions of purchase, sale, benefit, conservation, storage or disposal of seeds in such species. Additionally, it should be noted, as they have proposed (2,5,35,37-39) that this test can also be used to estimate the vigor of the seeds, given the direct relationship between vigor and intensity and distribution of the stain in vital structures of the same.

However, the selection of the tetrazolium concentration and the staining time of the seeds in this test should always be done in accordance with the ease and safety to differentiate viable from unviable seeds.

CONCLUSIONS

- The external characteristics of the seeds of *Bombacopsis quinata* present less variation than those of *Anacardium excelsum*, especially those related to weight.
- The staining patterns identified with the tetrazolium test allow efficient evaluation of the seed viability in the two native forest species studied.
- The viability of the seeds for *B. quinata* and *A. excelsum* can be determined at a concentration of 1,0 % tetrazolium and a time of three hours.
- Tetrazolium and conventional germination tests did not present significant differences in the two species and guarantee the reliability of the biochemical test to measure the physiological quality of the species studied.

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