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Viability and vigor in caryopses of grasses from semi-arid regions

Viabilidad y vigor en cariópsid es de gramíneas de zonas semiáridas

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ABSTRACT

Few studies have evaluated seed quality in native to semi-arid zones' grasses. The objective was to validate published methodologies to carry out viability tests using Tetrazolium (TZ) test for caryopses (Cs) of *Bouteloua curtipendula*, *Leptochloa dubia*, *Digitaria californica*, *Setaria macrostachya* and *Pennisetum ciliare*, considering the imbibition time, TZ concentration and cut in Cs. In addition, showing staining patterns according to evaluated treatments. Viability and vigor data was expressed as percent and hence was transformed to arcsine. A completely randomized experimental design was carried out using a factorial arrangement: A) imbibition time before cutting; B), cut type; C), TZ concentration, and Tukey's test ($p<0.05$) for mean comparisons. Cs longitudinal cut was the most adequate ($P<0.05$), it allows observing Cs' embryonic structures and determining damaged sites; however, Cs of *D. californica* and *S. machrostachya* are permeable to TZ and it was not necessary to section Cs, which facilitates its evaluation. ISTA protocols for viability in Poaceae are not directly applicable for the species evaluated, because the imbibition time for these species is not considered, which 3 to 4 hours was. It is recommended to use a 0.5 % TZ concentration and evaluate 12 hours after the imbibition time between Cs and TZ solution.

Keywords: embryonic damage, ISTA, propagules, benefited seed, seed vigor.

RESUMEN

Pocos trabajos han estudiado la calidad de semilla en pastos nativos de zonas semiáridas. El objetivo fue validar metodologías publicadas para otras Poaceae, para pruebas de viabilidad por Tetrazolio (TZ) en cariópsides (Cs) de *Bouteloua curtipendula*, *Leptochloa dubia*, *Digitaria californica*, *Setaria macrostachya* y *Pennisetum ciliare*; lo anterior, considera tiempo de imbibición, concentración de TZ y tipo de corte del

Cs y muestra los patrones de tinción de tratamientos. Viabilidad y vigor se expresaron en porcentaje y se transformaron al arcoseno. Se utilizó un diseño experimental completamente al azar con arreglo factorial: A) tiempo de imbibición antes del corte; B), tipo de corte; C), concentración de TZ (V:V) y Tukey ($P<0.05$) para comparar medias. El corte longitudinal fue más adecuado ($P<0.05$), permite observar estructuras embrionarias y determinar sitios dañados. Cariópsides de *D. californica* y *S. machrostachya* son permeables a TZ y no requieren seccionado, lo que facilita su evaluación. Los protocolos ISTA para viabilidad en Poaceae no son directamente aplicables para las especies evaluadas, no consideran tiempo de imbibición de Cs; el cual, fue de 3 a 4 horas. Se recomienda usar concentración de TZ al 0.5 % y evaluar 12 horas después del contacto entre Cs y la solución de TZ para las especies aquí evaluadas.
Palabras clave: daño embrionario, ISTA, propágulos, semilla beneficiada, vigor de semilla.

INTRODUCTION

Due to pasture degradation, the establishment of semi-arid rainfed pastures with native species is required (Quero & Flores, 2023). There are few studies on seed quality in grasses for dryland conditions in arid zones (ISTA, 2016) this, despite the institutional registry of varieties (SNICS- CNVV, 2023). There is a wide diversity of propagules that can be used for sowing grasslands in pastures and it is essential to know the physical, genetic, sanitary and physiological quality of the propagule to be used (Quero *et al*., 2017). The seed industry requires effective analysis techniques to determine the quality of seed lots and provide certainty in making decisions on sowing and storage. Tetrazolium test (2,3,5-triphenyl tetrazolium chloride; TZ) is a rapid test used to determine viability and vigor associated with germination capacity, especially for seed lots with high dormancy; however, it has some disadvantages: the interpretation of staining is visual (subjective) and requires training, especially in small seeds (Lopez *et al*, 2017). Evaluating with TZ involves cutting and grading Cs under stereo microscopy. Viability is determined according to the staining pattern and intensity of embryo coloration. A viable seed indicates that it is capable of producing a normal seedling (ISTA, 2016). When accessory bracts eliminate evaluating Cs, dormancy; however, in the field, the propagule used during sowing is the spikelet or twig; therefore, germination is expected to be slow and lower. This test discriminates, on the embryonic axis, live and dead tissue, based on enzymatic activity (dehydrogenases), which increases when hydrolyzed during imbibition, producing H^+ release and, with it, the chemical reduction of the TZ solution (colorless) to formazan (red color).

Viability is determined according to the staining pattern of the embryonic axis and intensity of coloration: live and vigorous Cs will present intense red coloration and the dead ones, no coloration. For the TZ test, by hydration, dehydrogenase activity and tissue softening are induced in Cs to facilitate cutting (longitudinal, transverse or puncture) and thus allow TZ-embryo tissue contact. Cs are immersed in TZ and kept in darkness, reading can be done from 2 to18 hours later (ISTA, 2016), depending on the species evaluated. A problem in Cs of Banderita *Bouteloua curtipendula* (Michx.) Torr., Gigante *Leptochloa*

dubia Kunth, Punta Blanca *Digitaria californica* (Benth.) Henrard, Tempranero *Setaria macrostachya* Kunth and Buffel *Pennisetum ciliare* (L.) Link) and other Poaceae, lies in obtaining adequate material (Cs) for the test; which, must be representative of the sample to be analyzed; in addition, these species are not included in proposed procedures, (ISTA, 2012); with the exception of Buffel, included in Tetrazolium Worksheets (ISTA, 2003). The objective was to validate published protocols for Poaceae, such as *Avena sativa*, *Hordeum vulgare*, *Secale cereale*, *Triticum aestivum*, *Megathyrsus maximus* and *C. ciliaris*, the latter was included as a control to propose specifications, when using the TZ test in Cs of Banderita, Gigante, Punta Blanca and Tempranero for: imbibition time, TZ concentration and cut type to apply in Cs and to show staining patterns according to the treatments performed.

MATERIAL AND METHODS

Seed was harvested at Postgraduate College in 2017, stored in paper bag at 25 °C temperature and 15 % constant humidity until evaluation. Caryopses (Cs) obtained by physical scarification of florets, twigs and/or spikelets of Banderita, Gigante, Punta Blanca, Tempranero and Buffel were evaluated, removing torn or damaged Cs, supported in stereoscopic microscope (Zeiss, Model 464002-9901). A completely randomized experimental design with factorial arrangement was used: A) imbibition time before cutting (1, 2, 3, 4, 6, 6, 8, 10 and 12 h); B) type of cut (longitudinal, transverse and without cut; i.e., complete Cs); C) percentage of TZ concentration (1, 0.5 and 0.1 %; V:V), 72 combinations or treatments. The experimental unit consisted of a beaker with 25 Cs, with ten replicates. The results were analyzed by Analysis of Variance (ANAVA) with factorial arrangement (SAS, 2013) and Tukey ($P < 0.05$). For imbibition time, Cs were placed in beakers with distilled water until completely covered. At the end of each imbibition time, Cs were cut, according to treatment, TZ solution was added at the concentration to be evaluated until completely covered, in darkness at room temperature (25° ± 2 °C) for 12 h; subsequently, Cs were removed from the TZ solution, to evaluate staining, as recommended for small Cs (ISTA, 2016). The embryonic axis pieces were rinsed with water. The embryonic axis pieces were rinsed with running water and were placed on absorbent paper, previously moistened, which prevents dehydration. Cs staining patterns were documented, taking into account the type of cut (TC), TZ concentration (CTz) and imbibition time (h). Pigmentation intensity and site on the embryonic axis were analyzed: a) absence of staining (no color), b) initial staining (pink tone), c) weak staining (strong pink), d) adequate staining (red) and e) intense red. It was documented by stereo microscope and cell phone camera photographs of Cs. Cs were classified as viable (viable vigorous and viable medium vigor) and non-viable, according to staining in embryonic

tissues and as reference, published viability protocols (ISTA, 2016; ISTA, 2003) and grouped by interpretation (Delouche y Baskin, 1973; ISTA, 2016).

RESULTS AND DISCUSSION

Staining patterns and structures of importance to be considered in viable, defect-free Cs were evaluated (Figure 1). The staining observed is similar to ISTA (2003), although they do not express the totality of damage observable in Cs.

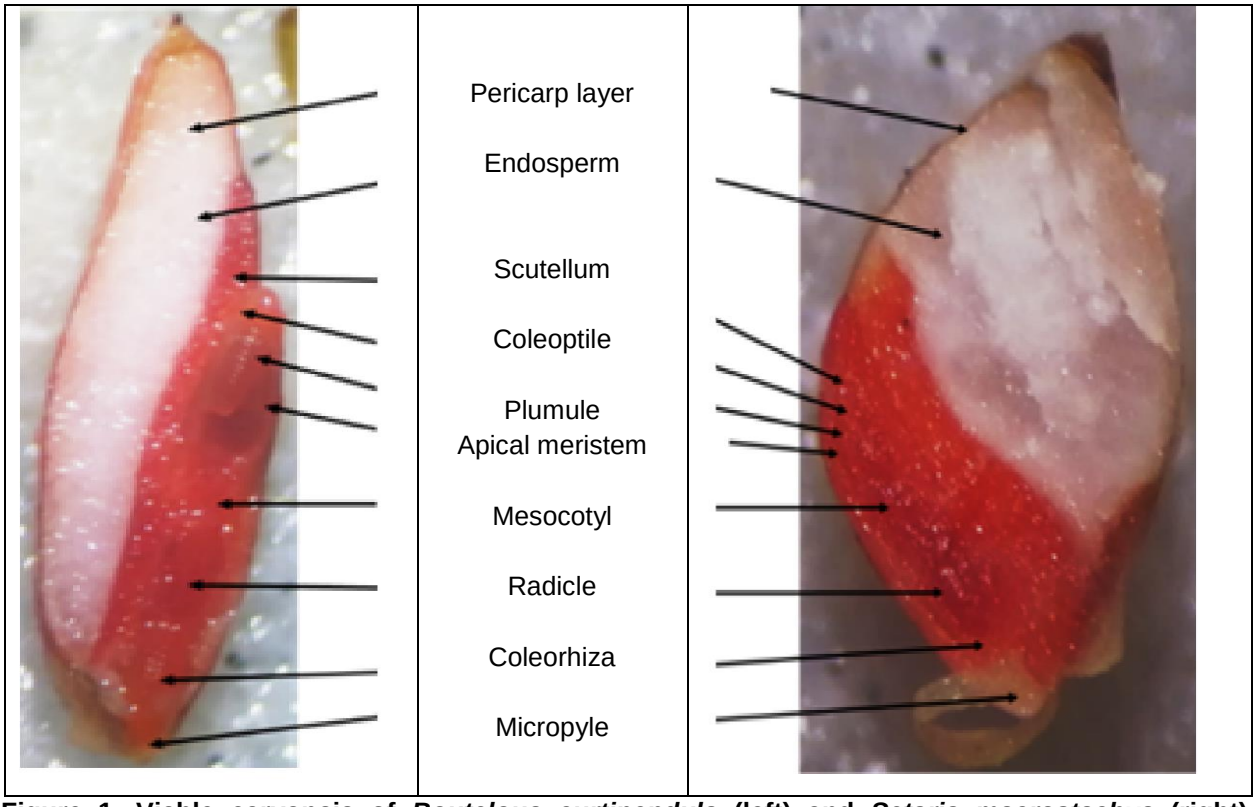

Figure 1. Viable caryopsis of *Bouteloua curtipendula* (left) and *Setaria macrostachya* (right), completely stained with Tetrazolium. Parts on the embryonic axis are shown, for viability determination

To facilitate the evaluation of Cs, three classes were defined according to the area and intensity of staining as an indicator of vigor: Class 1. Viable and vigorous embryos. Firm tissues, without visible lesions and uniform pink to bright red coloration. Light superficial damage located in the external part of the endosperm (Figure 2). Class 2. Viable embryos with medium vigor. Pink color, with firm tissues. Embryonic axis with light and superficial internal damage, discolored radicle and plumule, with less than 50 % dead tissue (white color; Figure 2). Class 3. Non-viable embryos. Radicle and plumule not stained. Embryo with lesions or dead areas in the embryonic axis. Includes embryos with areas greater than 50 % dead tissue (Figure 2).

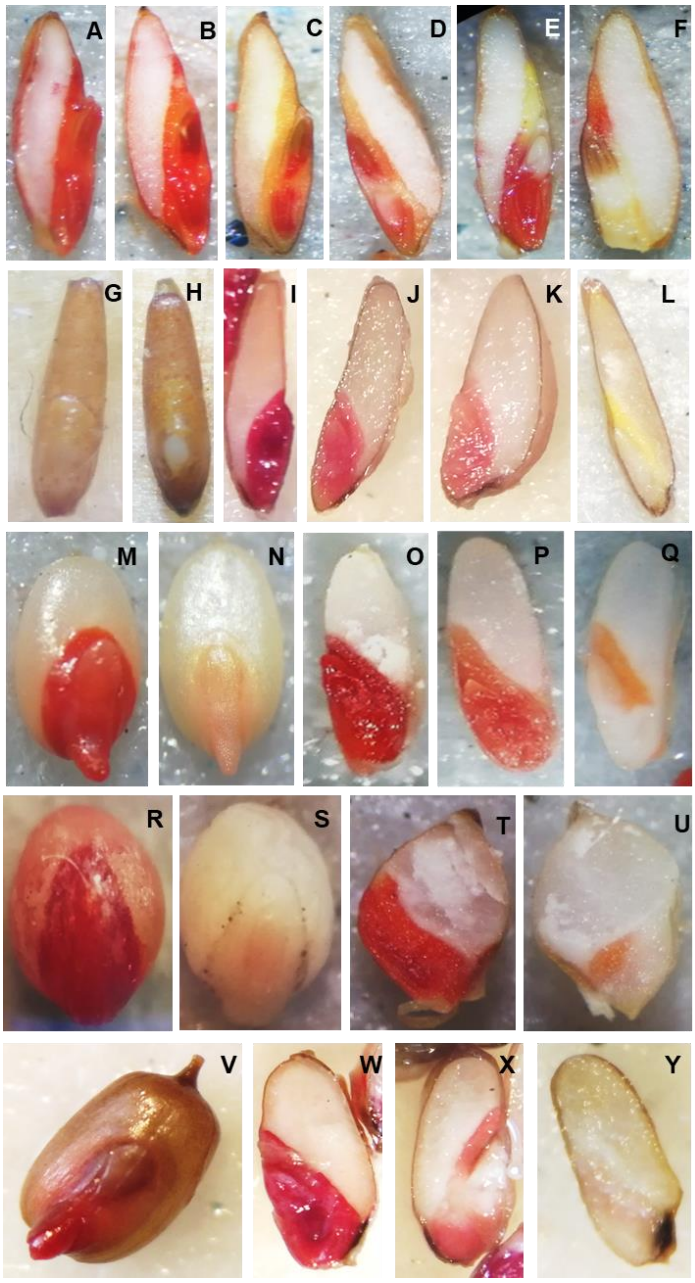

Figure 2. Complete and longitudinally cut caryopsis. Different tissues of the dead embryonic axis are observed (without staining; F, L, Q, U, Y). A-F) Cs of Banderita; G-L) Cs of Gigante grass; M-Q) Cs of Punta Blanca; R-U) Cs of Tempranero; V-Y) Buffel grass

Banderita *Botueloua curtipendula* (Michx.) Torr. ANAVA based on staining patterns for TZ concentrations, cutting (or not) of the embryonic axis, and imbibition time showed differences ($P \leq 0.0001$) between factors for viable and vigorous, medium vigor, and nonviable Cs. Double interactions showed differences ($P \leq 0.0001$) for TC × CTz, TC × h, CTz× h, for all variables. The triple interaction TC × CTz × h, was significant ($P \leq 0.0001$). TZ concentrations of 0.5 %, resulted in 56 % of viable and vigorous Cs and with 1 and 0.1

%: 51.8 and 44.3 % (Table 1). When analyzing cut types in Cs, it was observed that Cs are semi-permeable to TZ, since in intact Cs showed 39.8 % of viable and vigorous Cs; therefore, it is necessary to cut for evaluation (P<0.05). In relation to imbibition time of Cs, Banderita increased the number of viable and vigorous seed at longer imbibition time (P<0.05). At one hour of imbibition, 48 % was observed, which contrasts at 4 and 12 h (52.9 and 53.1 %) of viable and vigorous embryos. It is recommended to imbibe for 4 h and subsequently, to perform the cutting.

Table 1. Response (%) to Tetrazolium (TZ) concentrations, cut-off and imbibition time (h) in caryopses of *Bouteloua curtipendula* (Michx.) Torr

Variable	Concentration of TZ (%)				
	1	0.5	0.1	Mean	DMSH
VV (%)	51.8^{b}	56.0^{a}	44.3^{c}	50.7	1.76
VVM (%)	18.3^{b}	20.8^{a}	9.8^{c}	16.3	1.58
NV (%)	29.9^{b}	23.2^{b}	45.8^{a}	33.0	2.21
	Caryopsis cut				
	Whole	Longitudinal	Transversal	Mean	DMSH
VV (%)	39.8^{c}	57.9^{a}	54.5^{b}	50.7	1.76
VVM (%)	13.3^{c}	15.5^{b}	20.2^{a}	16.3	1.58
NV (%)	47.0^{a}	26.6^{b}	25.3^{b}	33.0	2.21

	Horas de imbibición									
	1	2	3	4	6	8	10	12	Mean	DMSH
VV (%)	48.0^{bc}	47.9^{c}	51.6^{abc}	52.9 ^{a}	50.7^{abc}	50.0^{abc}	51.7^{ab}	53.1^{a}	50.7	3.73
VVM (%)	11.7^{c}	14.7^{bc}	15.0^{bc}	16.6^{ab}	19.0^{a}	18.7^{a}	17.8^{ab}	17.2^{ab}	16.3	3.36
NV (%)	40.3^{a}	37.4^{ab}	33.4^{bc}	30.6^{c}	30.3^{c}	31.3^{c}	30.6^{c}	29.7^{c}	33.0	4.68

± Means with the same letter in each row are statistically equal (Tukey p≤ 0.05). VV= Viable vigorous; VVM= Viable medium vigor; NV= Non-viable

Gigante *Leptochloa dubia* (Kunth) Nees. ANAVA showed differences (P ≤ 0.0001) between factors for viable and vigorous, medium vigor and nonviable Cs. The two-way interactions showed significance (P ≤ 0.0001) for TC × CTz, CTz× h for all variables and for the TC × h interaction (P ≤ 0.005). The three-way interaction TC × CTz × h was significant (P ≤ 0.0001). Cs viability and vigor showed that TZ concentrations of 1 %, 65.9 % viable and vigorous Cs were obtained; whereas, with 0.5 and 0.1 %, 63.2 and 54.3 % were obtained, respectively (Table 2). Moderate pericarp permeability to TZ was observed; in intact Cs, 51.7 % of viable and vigorous seeds were obtained and 73.6 and 58 % in longitudinally and transversally cut Cs, respectively. Longitudinal cutting results in the correct evaluation in this species (P<0.05). At one hour of imbibition, 39.5 % were observed; while at 4, 6 and 12 h: 68.6, 12.4 and 68.9 % (P<0.05) of viable and vigorous embryos, respectively.

Table 2. Response (%) to different concentrations of Tetrazolium (TZ), cut-off and imbibition time (h) in caryopses of *Leptochloa dubia* (Kunth)

Variable	Concentration of TZ (%)				
	1	0.5	0.1	Mean	DMSH
VV (%)	65.9[a]	63.2[b]	54.3[c]	61.1	1.8
VVM (%)	12.2[c]	16.7[a]	17.0[a]	15.3	1.8
NV (%)	21.9[b]	20.1[b]	28.7[a]	23.6	2.1
	Caryopsis cut				
	Whole	Longitudinal	Transversal	Mean	DMSH
VV (%)	51.7[c]	73.6[a]	58.0[b]	61.1	1.8
VVM (%)	19.9[a]	10.1[c]	16.0[b]	15.3	1.8
Non- viable (%)	28.4[a]	16.3[c]	26.0[b]	23.6	2.1

	Imbibition time (hours)									
	1	2	3	4	6	8	10	12	Mean	DMSH
VV (%)	39.5[c]	42.8[c]	65.0[b]	68.6[ab]	68.9[a]	68.1[ab]	67.7[ab]	68.4[ab]	61	3.9
VVM (%)	24.1[a]	19.2[b]	11.6[d]	12.4[cd]	12.0[cd]	15.8[bc]	16.6[b]	10.9[d]	15	3.9
NV (%)	36.4[a]	38.0[a]	23.4[b]	19.0[cd]	19.1[bcd]	16.1[d]	15.8[d]	20.7[bc]	23	4.4

± Means with the same letter in each row are statistically equal (Tukey p≤ 0.05). VV= Viable vigorous; VVM= Viable medium vigor; NV= Non-viable

Punta Blanca *Digitaria californica* (Benth.) Henrard. Staining ANAVA showed differences (P ≤ 0.0001) between factors evaluated for viable and vigorous Cs, with medium vigor and non-viable, the above, except for TC (P>0.05). In double interactions TC × CTz (P ≤ 0.006), TC × h (P ≤ 0.03), for live and vigorous Cs CTz× h and (P ≤ 0.0003) for viable Cs medium vigor. The three-way interaction TC × CTz × h was significant (P ≤ 0.0001). Viability and vigor of Cs at 1 % CTz was 83.2 % of viable and vigorous Cs; whereas, at 0.5 and 0.1 %, 80.4 and 72.4 % were obtained, respectively (Table 3). Cs cuttings showed 76.6 % of viable and vigorous seed, 4.2 and 2.5% lower with respect to whole and cross-cut Cs; the above, allows the use of whole Cs (P<0.05). Testa permeability to TZ needs to be monitored, since it could be related to vigor. Tempranero and Punta Blanca showed lower viability at longer imbibition time: Cs with one hour of imbibition showed 93.8 % and at 4, 8 and 12 h: 83, 70 and 61 % of vigorous viable embryos, respectively.

Tempranero *Setaria macrostachya* (Kunth). ANAVA of staining patterns obtained when evaluating CTz and h of imbibition showed differences (P ≤ 0.0001) except for CT (P ≤ 0.0243) for viable and vigorous, medium vigor and non-viable Cs. Double interactions showed significance (P ≤ 0.0001) for TC × CTz, CTz× h, not so for TC × h (P ≤ 0.0007, 0.0327 and 0.0009 for viable and vigorous Cs, with medium vigor and non-viable, respectively). The three-way interaction TC × CTz × h, was significant (P ≤ 0.0007; P ≤ 0.0175, for viable and vigorous Cs, with medium vigor), but was highly significant for nonviable Cs. Viability and vigor of Cs showed differences (P≤ 0.02) at 0.5 % TZ concentrations, with 6.7 and 8.3 % higher viable and vigorous Cs, compared with 1 and 0.01 % TZ concentration (Table 4). The same behavior was observed for Cs of medium

viability and vigor, with faint pink coloration and tissue stained entirely over the embryonic axis. When evaluating Cs cut types, differences were obtained (P≤ 0.0001) and it was observed that Cs of this species is permeable to TZ. In complete Cs, 31.1 % of viable and vigorous seed was obtained, only 1.9 and 1.3 % higher than Cs subjected to longitudinal and transverse cutting. It is possible to perform the test in intact Cs. Regarding Cs imbibition, differences were observed (P≤ 0.0001), when immersing Cs in distilled water for 4, 8 and 12 h, 28.9, 15.4 and 9.6 % of viable and vigorous embryos were recorded, compared to those subjected to one hour of imbibition, with 58.3 % of viable and vigorous embryos. The longer the imbibition time Cs showed embryo abortion. It is important to mention that this seed is especially delicate and susceptible to manual scarification, which makes it difficult to obtain intact Cs for testing; therefore, intact Cs are a good alternative.

Table 3. Response (%) to different concentrations of Tetrazolium (TZ), cut-off and imbibition time (h) in caryopses of *Digitaria californica* (Benth.) Henrard

Variable	Concentration of TZ (%)				
	1	0.5	0.1	Mean	DMSH
VV (%)	83.2^{a}	80.4^{b}	72.4^{c}	78.7	1.8
VVM (%)	11.0^{b}	8.8^{c}	14.4^{a}	11.4	1.7
NV (%)	5.88^{c}	10.9^{b}	13.2^{a}	10.0	1.9
	Caryopsis cut				
	Whole	Longitudinal	Transversal	Mean	DMSH
VV (%)	76.6^{c}	81.0^{a}	78.4^{b}	78.7	1.8
VVM (%)	11.5^{a}	11.4^{a}	11.2^{a}	11.4	1.7
NV (%)	12.0^{a}	7.8^{b}	10.2^{a}	10.0	1.9

	Imbibition time (hours)									
	1	2	3	4	6	8	10	12	Mean	DMSH
VV (%)	93.8^{a}	90.7^{ab}	87.6^{b}	82.9^{c}	76.7^{d}	69.7^{e}	67.4^{e}	60.6^{f}	78.65	3.76
VVM (%)	2.9^{e}	4.2^{de}	7.0^{cd}	10.0^{c}	9.8^{c}	15.4^{b}	18.9^{b}	22.7^{a}	11.36	3.64
NV (%)	3.3^{b}	5.1^{b}	5.4^{b}	7.1^{b}	13.6^{a}	14.9^{a}	13.7^{a}	16.8^{a}	9.99	3.94

± Means with the same letter in each row are statistically equal (Tukey P≤ 0.05). VV= Viable vigorous; VVM= Viable medium vigor; NV= Non-viable

Buffel (*Pennisetum ciliare* (L.) Link. ANAVA of observed staining patterns showed differences (P ≤ 0.0001) between factors evaluated for viable and vigorous, medium vigor and nonviable Cs. Double interactions showed high significance (P ≤ 0.0001) for TC × CTz, TC × h, CTz× h for all variables. However, not so for the triple interaction TC × CTz × h (P < 0.0117; P < 0.0004; P < 0.0003; for viable and vigorous, medium vigor and non-viable Cs, respectively). Viability and vigor in Cs of Buffel showed that with TZ at 0.5 %, 30.5 % of viable and vigorous Cs were obtained; whereas, with 1 and 0.1 %, 27.7 and 30.1 %, respectively (Table 5). When analyzing different cuts of Cs, it was observed that the testa is not permeable to TZ; therefore, any type of cut can be performed for the correct evaluation (P< 0.05). The imbibition time of Cs is important for this species; at one hour of imbibition, 21.4 % was observed; while, at 12 h, 37.2 % of embryos were viable and

vigorous. Therefore, twelve hours established to perform the reading, allows the correct evaluation.

Table 4. Response (%) to different concentrations of Tetrazolium (TZ), cut-off and imbibition time (h) in caryopses of *Setaria macrostachya* (Kunth)

Variable	Concentration of TZ (%)				
	1 %	0.5 %	0.1 %	Mean	DMSH
VV (%)	28.3^{b}	35.0^{a}	26.8^{b}	30.0	1.6
VVM (%)	15.8^{b}	21.3^{a}	14.9^{b}	17.4	1.8
NV (%)	55.9^{b}	43.7^{c}	58.3^{a}	52.6	2.3
	Caryopsis cut				
	Sin corte	Longitudinal	Transversal	Mean	DMSH
Viable vigorous (%)	31.1^{a}	29.2^{b}	29.8^{ab}	30.0	1.7
Viable medium vigor (%)	21.6^{a}	16.1^{b}	14.3^{c}	17.4	1.8
Non-viable (%)	47.3^{b}	54.7^{a}	55.9^{a}	52.6	2.3

	Imbibition time (h)									
	1	2	3	4	6	8	10	12	Mean	DMSH
VV (%)	58.3^{a}	52.3^{b}	40.0^{c}	28.9^{d}	24.2^{e}	15.4^{f}	11.4^{g}	9.6^{g}	30.0	3.5
VVM (%)	13.8^{de}	16.9^{cd}	23.1^{b}	27.1^{a}	18.1^{c}	15.3^{cde}	12.8^{e}	11.7^{e}	17.4	3.8
NV (%)	27.9^{f}	30.8^{f}	36.9^{e}	44.0^{d}	57.7^{c}	69.2^{b}	75.8^{a}	78.8^{a}	52.6	4.9

± Means with the same letter in each row are statistically equal (Tukey p≤ 0.05). VV= Viable vigorous; VVM= Viable medium vigor; NV= Non- viable

Table 5. Response (%) to different concentrations of Tetrazolium (TZ), cutting and imbibition time (h) in caryopses of *Pennisetum ciliare* (L.) Link

Variable	Concentration of TZ (%)				
	1	0.5	0.1	Mean	DMSH
VV (%)	27.7^{b}	30.5^{a}	30.1^{a}	29.5	1.7
VVM (%)	8.2^{c}	15.0^{a}	10.3^{b}	11.2	1.5
NV (%)	64.1^{a}	54.5^{c}	59.5^{b}	59.4	2.0
	Caryopsis cut				
	Whole	Longitudinal	Transversal	Mean	DMSH
VV (%)	0.0^{b}	44.8^{a}	43.6^{a}	29.5	1.7
VVM (%)	1.2^{c}	17.6^{a}	14.7^{b}	11.2	1.5
NV (%)	98.8^{a}	37.6^{c}	41.7^{b}	59.4	2.0

	Imbibition time (hours)									
	1	2	3	4	6	8	10	12	μ	DMSH
VV (%)	21.4^{d}	22.4^{d}	24.8^{cd}	26.4^{c}	30.0^{b}	36.1^{a}	37.2^{a}	37.2^{a}	29.5	3.5
VVM (%)	17.2^{a}	13.8^{b}	10.2^{cd}	11.8^{bc}	11.6^{bc}	8.7^{cd}	7.2^{d}	9.0^{cd}	11.2	3.3
NV (%)	61.3^{ab}	63.8^{a}	65.0^{a}	61.8^{ab}	58.4^{bc}	55.2^{cd}	55.6^{cd}	53.8^{d}	59.4	4.3

± Means with the same letter in each row are statistically equal (Tukey p≤ 0.05). VV= Viable vigorous; VVM= Viable medium vigor; NV= Non-viable

For the concentrations of TZ evaluated, the embryos acquired adequate tonalities for final counting. At no concentration tested was intense red observed in embryonic tissues that would have prevented the identification of damage to embryonic structures. Cs exposed to TZ concentrations of 0.1, 0.5 and 1 % for 12 h, showed to be adequate, given the similar results, since living and vigorous tissues acquire uniform coloration. The above, indicates that tissues in contact with TZ reacted with the H^{+} product of cellular respiration, which

facilitates rapid and uniform pink coloration. For this reason, the TZ test is considered a reliable colorimetric method, since it indicates the presence of active enzymes: peroxidase, esterase and dehydrogenase (Carvalho *et al*., 2013); however, this contradicts what was found in barley where weak coloration was observed independent of imbibition time (Lopez *et al*., 2017).

In Poaceae, forage plants such as: *Urochloa* spp., *Chloris gayana*, *Dactylis* spp., *Panicum* spp. and *B. gracilis*, among others, the ISTA rules (ISTA 2003; ISTA, 2012) state that spikelets without Cs (true seed) should be considered non-viable, but do not specify how to express empty florets when performing the viability test by TZ. Some authors have expressed that ISTA demands for purity assessment, to determine percentage of filled florets, with caryopsis inside (Agüero *et al.*, 2017). It is suggested, prior to staining, to make cuts in Cs, in such a way that the tissues are exposed to the TZ (ISTA, 2012); however, it does not refer to the optimal imbibition time of Cs for tissue softening (testa and endosperm) to perform an accurate cut and therefore a standardized, correct and repeatable methodology. For small Cs, the longitudinal or transverse cut should be performed; however, the possibility that some species possess TZ-permeable testa is not considered; the above, given that the cut allows evaluating and differentiating dead seeds from dormant ones (Navarro *et al*., 2015). Several factors can influence the results in the TZ test, many related to the methodology used; in addition, the concentration of TZ in the solution, time and temperature of exposure to TZ and interpretation criteria (Lopez *et al*., 2017; Pereira *et. al*., 2017; Steinbrecher & Leubner, 2017). Therefore, correctly determining staining patterns (Figure 2 to Figure 8) in Cs, at different staining concentrations and imbibition times, applicable to evaluate embryonic axis structures based on the obtained staining, will allow rapid establishment of seed lot quality. Under field conditions, a higher imbibition rate can be advantageous for the seed because the endosperm takes longer to hydrate than the embryo (Carvalho *et al*., 2013; Lopez *et al*., 2017). *Panicum* protocols (ISTA, 2012) are not applicable to Banderita, Gigante, Punta Blanca, Tempranero and Buffel, the above, because imbibition time is species specific. Performing correct cuts in Cs implies training, which allows observing dead or necrotic structures on the embryonic axis, which agrees with Agüero *et al*. (2017) for Buffel. Depending on species and size of Cs will be the concentrations of TZ. For *Solanum licopersicum* and *Mimosa bimucronata,* this is 0.075 % (Salazar *et al*., 2020; Ferreira *et al*., 2020), in 17 high Andean species, it was found that the best evaluation condition was 1 to 1.5 % TZ, 40 °C and 24 hours of exposure to the solution (Mancipe *et al*, 2018). For Barley, it is recommended to precondition seed in direct immersion in H_2O and staining by immersion in 0.1 % or 0.5 % concentration of TZ or staining on filter paper moistened with 1.0 % TZ (Lopez *et al*., 2017). In wheat, it is recommended to perform seed

preconditioning on paper towels for 18 hours, at 20 °C and subsequently, staining with TZ can be efficient from 0.075 to 1.0 % (Carvalho *et al*., 2013). In *Cucumis anguria*, it is recommended to perform the viability test at 0.05 % concentration for six hours at 35 °C or four hours at 40 °C (Pereira *et al*., 2017). The above shows the importance of proposing specifications according to the species of interest, since they all respond to different TZ concentrations.

CONCLUSIONS

ISTA protocols for viability in Poaceae are not directly applicable for *B. curtipendula*, *L. dubia*, *D. californica*, *S. macrostachya* and *C. ciliaris*. The imbibition time for these species is 3 to 4 h, it is recommended to use 0.5 % TZ and evaluate 12 h post initiation of Cs-TZ contact. The complete longitudinal cut is adequate for a correct evaluation of embryonic structures in these species. Punta Blanca and Tempranero have a permeable testa to TZ, so it is possible to perform the evaluation with intact Cs.

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Errata Erratum

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