Triphenyl Tetrazolium Chloride Ringtest

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Triphenyl tetrazolium chloride, TTC, is a redox indicator used to indicate cellular respiration. Its solution in water is colourless but in living tissues the TTC is reduced to a red substance thus dyeing living tissues in red. The test is commonly used for testing seed quality with various instructions produced by, e.g., the International Seed Testing Association. Certain adaptations for specific seeds are commonly made. In case of common ragweed we hypothesised that the variance between seed populations collected in Hungary, Austria, and Germany would be larger than the variance between the participating labs. For our ring trial we followed in the first round our protocol 1st edition.

The experiment consisted of two rounds. In the first round, four populations of common ragweed seeds were tested. These populations were provided by JKI (Hordorf, sampled 2011, and Herbiseed, bought in 2011) and BOKU (Unterpurkla, sampled 2010, and Hagenbrunn, sampled 2010) and sent to each partner. One hundred achenes per population were required (4 replicates, each with 25 achene halves).

Materials:

- 100 achenes per population
- Tap water
- An instrument to cut achenes in halves. A nail clipper was very reliable or a surgical scalpel or similar instrument
- Distilled water
- 16 glasses of 5-10ml volume which can be covered
- Incubator or drying chamber
- Refrigerator
- 1% TTC-solution (i.e. 100 ml)
- dissecting microscope/binocular

Implementation:

- Common ragweed achenes were imbibed in tap water at room temperature for 24 hours
- The achenes were cut open with a surgical scalpel or similar instrument in such a way that the endosperm was exposed
- The biggest part of the achene is used for testing, the other part is discarded
- 25 achene halves are put into one glass and filled up with TTC solution (per replicate)

- Closing the glass tight
- Glasses are put to react at 30°C for 6 hours in absolute darkness, because TTC is light sensible, avoid unnecessary light input
- If it is not possible to keep on with the protocol after these 6h, the closed glasses can be stored in a refrigerator (6-8°C) over night
- TTC solution is poured off and halves are rinsed under distilled water.

Under a dissecting microscope, seeds were counted in three classes: a) stained (=alive), b) intermediate cases that are only lightly or partly stained, c) not stained resp. no fully developed embryo present (=dead) (Fig. 1).

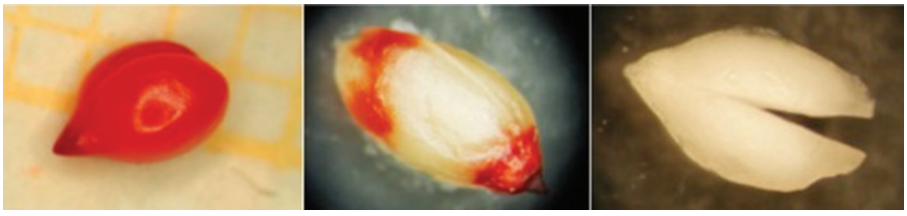

Fig. 1: Common ragweed embryo staining intensity and coding: left: stained = 1, middle: intermediate = 0.5, right: dead = 0

The differences in classification of the different seed lots by different labs were higher than the variation between the seed origins (Starfinger *et al.* 2012).

Intending to reduce variation of individual differences in classifying various stages of staining of common ragweed seeds we started a questionnaire of how differently stained seeds after TTC treatment were classified as "stained" (=class 1), "unstained" = dead (class 0), and "intermediate" (class 0.5; only parts of the embryo stained) by the various labs. The interpretations varied at high levels (Tab. 1). Before starting a second round of the TTC-test we defined the 3 classes (1; 0; 0.5) on base of this comparison of individual assessments. The results were accounted for the 2nd edition of the manual for TTC-testing, including pictures of various stages of staining and their recommended classification (last column in Fig. 2).

	figure	NL	NL 2	DK	UKI (Martina)	KU	BOKU (Felicia)	BOKU (Hannes)	BOKU(Martin)	BOKU (Nina)	BOKU (Gerhard)	KIS	Mittel	class
	1	1	1	1	1	1	0,5	1	1	1	0,5	0,5	0,86364	**1**
	2	0,5	0	0	0,5	0	0,5	0	0	0	0	0,5	0,182	**0,5**
	3	1	0,5	1	0,5	0,5	0,5	1	1	1	0,5	0,5	0,72727	**0,5**
	4	1	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,54545	**0,5**
	5	0,5	0	0	0,5	0	0,5	0,5	0,5	0,5	0,5	0,5	0,36364	**0,5**
	6	1	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,54545	**0,5**
	7	1	1	1	1	0,5	1	0,5	1	1	1	1	0,90909	**1**
	8	1	1	1	1	0,5	1	1	1	1	1	1	0,95455	**1**
	9	0	0	0	-	0	0	0	0	0	0	0	0	**0**
	Mean	0,78	0,5	0,55556	0,6875	0,38889	0,555556	0,5555556		0,61111	0,5	0,55556	0,56566	0,611111

Fig. 2: Standardisation of classification of stained embryos of common ragweed after TTC treatment

In the second round (2013) we tested seed lots provided by KU (Kaposvar, sampled in 2011) and BOKU (Zillingtal, sampled in 2011 and Unterpurkla, sampled in 2010).

The results for the BOKU trials are summarized in Fig. 3. The number of viable seeds (class 1) was

high in the seed lots from Kaposvar and Zillingtal. In the seed lot from Unterpurkla (Austria, Styria) the amount of intermediates (class 0.5) was higher than the number of stained (viable) seeds.

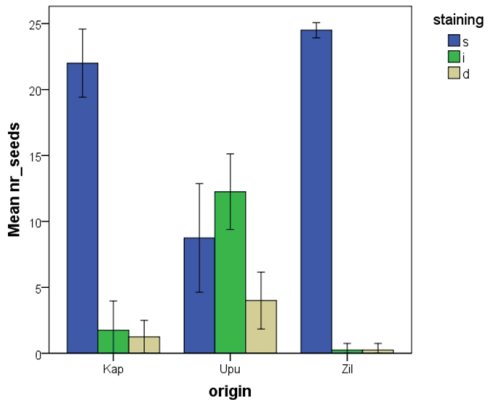

Fig. 3 : Mean number of common ragweed seeds per staining category and place of origin (Error Bars: +/- 2. SE) classified by BOKU; Seed origin: Kap = Kaposvar, Upu = Unterpurkla, Zil = Zillingtal. s – stained, I – intermediate, d – dead

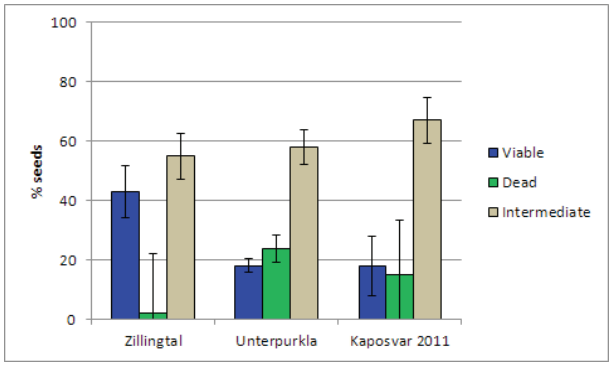

Fig. 4: Mean percentage of common ragweed seeds per staining category and place of origin (Error Bars: +/- 2. SE) classified by AU;.

The Danish lab (AU) classified the same seed lots after the same treatment rather different (Fig. 4). I. e., the number of intermediates is relatively high compared to the Austrian estimation indicating differences in the interpretation of the classification of stained embryos. .

Kaposvar and Zillingtal samples were collected in 2011 whereas the Unterpurkla seeds were collected in 2010 and stored at 4°C in darkness. The Unterpurkla seeds might have suffered from spontaneous death by ageing (Béres 2004).

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