Hemp and Tetrazolium Test: a new tool to evaluate the viability of seed

Pasquini S.[1], Perri E.[2], Barbante A.[2], Arioli A.[2], Cassinetti L.[2], Enea N.[2], Seminari A.[2], Andriani V.[1], Baldin C.[3], Alberti I.[3]

1) **Centro Nazionale Carabinieri Biodiversità - Via del Ponte, 256 - 37020 Peri (VR) - Italy**
2) **Council for Agricultural Research and Economics - Research Centre of Plant Protection and Certification - Via Emilia, Km 307 - 26838 Tavazzano con Villavesco (LO) - Italy**
3) **Council for Agricultural Research and Economics - Research Centre of Cereals and Industrial Crops - Viale Giovanni Amendola, 82 - 45100 Rovigo (RO) - Italy**

Introduction

Hemp (*Cannabis sativa* L.) cultivation in Europe has significantly increased from 20,540 hectares in 2015 to 28,000 hectares in 2023, with France leading in cultivated areas, followed by Germany and the Netherlands. The European Green Deal's goals, such as carbon dioxide storage, disease cycle interruption, soil erosion prevention, biodiversity, and reduced pesticide use, drive this renewed interest.

Hemp is versatile, used for textiles, food and feed production, green building materials, paper, cosmetics, biofuels, and extracts for cosmetic, food, and health purposes. The THC content in hemp must be below 0.3%, and farmers must use certified seeds from the EU's common catalogue, which lists 116 varieties as of 2024.

This study aims to evaluate the feasibility of using the tetrazolium test and an organic substrate to assess the viability and the germination rate of hemp seeds, primarily intended for research studies and breeding purposes especially when it is important to obtain the highest number of viable seedlings from seed lots stored for several years.

Materials & Methods

The germination tests (four replicates of 50 seeds) were carried out as described in Chapter 5 of ISTA Rules (2025) on top of paper (TP) in a transparent boxes (Fig. 1), and with an alternative method in plastic pots filled with an organic substrate (O) (Fig. 2) composed with: Coco Mix® 45% (Coco fibre), BioBizz® 30% (Worm Humus) and Perlite 25%.

The temperature was set at 20°C with a photoperiod of 16 hours of light and 8 hours of darkness. The trial was replicated twice in three laboratories

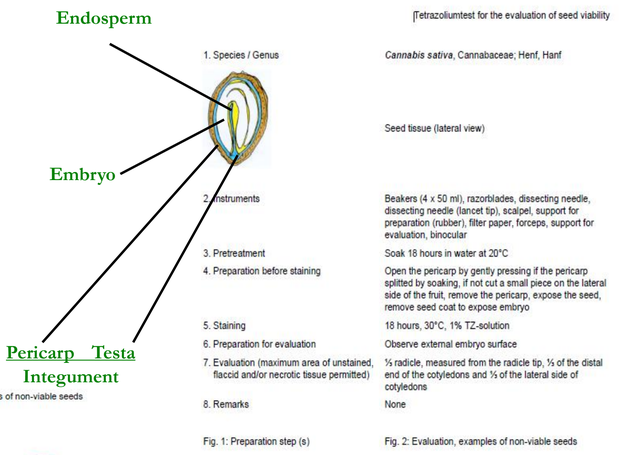
Fig. 1 – Transparent box used for determination of germination rate

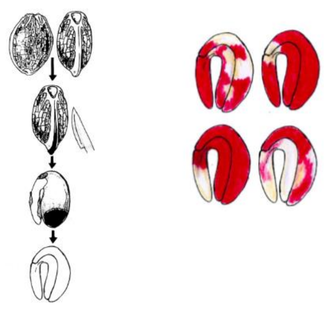
Fig. 2 – Plastic pots containing the organic substrate

The study was conducted on 5 hemp varieties, listed in the European Common Catalogue of varieties, coming from different reproductive areas and with different ages of conservation:

Variety	Cultivation area	Year
Carmagnola	Italy	2014
Eletta Campana	Italy	2017
Futura 75	France	2021
USO 31	France	2023
Santhica 27	France	2016

The biochemical test for viability was carried out with the Topographical Tetrazolium Test as described in Chapter 6 of ISTA Rules (2025). The seeds (four replicates of 25 seeds) were immersed in water for 18 hours at 20°C. The trial was replicated twice in three laboratories.





The integument (pericarp and testa) was removed for exposing the embryos, subsequently immersed in a solution of 2,3,5-thriphenil tetrazolium chloride (1%) for 18 hours at 30°C. The evaluation was done observing external embryos surface.

Results

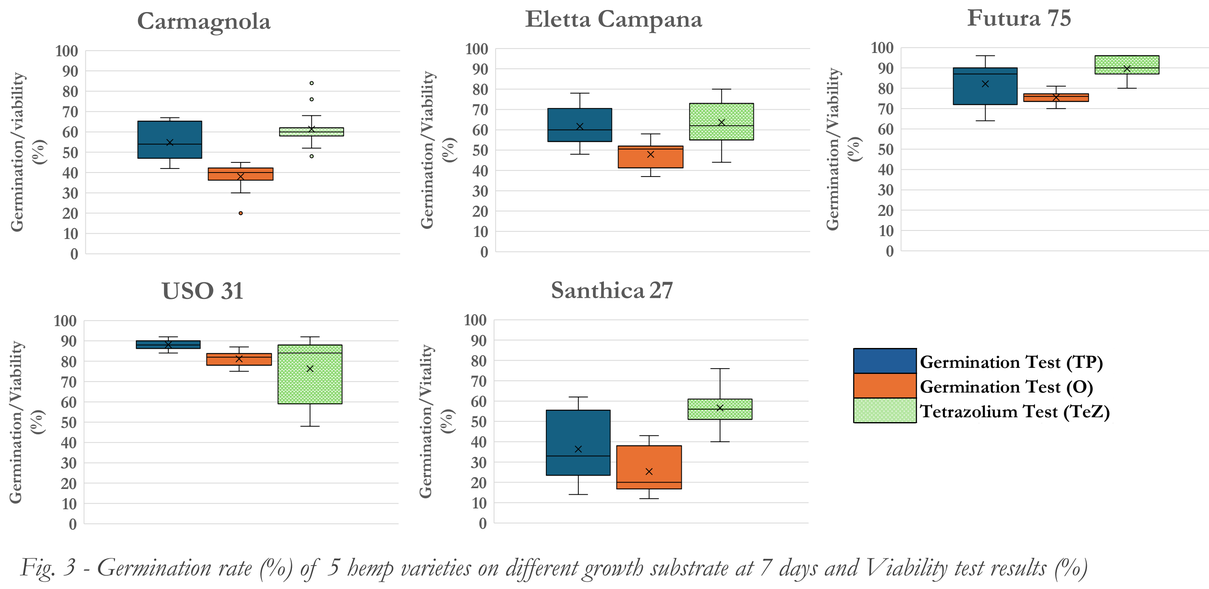


Fig. 3 - Germination rate (%) of 5 hemp varieties on different growth substrate at 7 days and Viability test results (%)

Germination Test (Fig.3)

Data distribution: the median of the tests conducted on TP shows higher values compared to the organic substrate in all the analyzed varieties.

Interquartile Range (IQR): The IQR of the tests on TP is wider compared to the organic substrate (O) in all varieties, except for USO 31 (younger seeds).

Outliners: one outlier was detected on organic substrate (O) in vr. Carmagnola.

Symmetry and Skewness: The data distribution appears fairly symmetrical in Carmagnola and USO 31 on TP and O, Eletta Campana on TP and in Futura 75 on O.

Tetrazolium Test (Fig. 3)

Interquartile Range (IQR): The IQR of the tests in USO 31 is wider compared to the other varieties

Outliners: three outliners were present in vr. Carmagnola.

Symmetry and Skewness: The data distribution appears fairly symmetrical in all varieties except in USO 31, where the skewness is shifted upwards.

Discussion & Conclusion

The study point out that the ISTA method TP is more effective than the organic substrate (O) for conducting germinability assays on *C. sativa*. However, the organic substrate is more leading to evaluate the seedlings, despite the highest germinability percentage being attained after a period longer than specified by the TP method (data not shown). Seeds of the Futura 75 and USO 31 varieties exhibit higher germinability percentages compared to older varieties, corroborating the high decline in germination rates of the hemp seeds over time (Suriyong *et al.*, 2015). From a technical standpoint (Fig. 4), the preparation of samples for the Tetrazolium test becomes increasingly labour-intensive as seed age advances. In younger seeds, the integument is more readily removed, mitigating preparation damages that could potentially distort test results. The Tetrazolium test remains essential for evaluating the viability of long-stored seed pools which are critical for breeding programs and thus must be preserved. Some pools contain only a few seeds, making it imperative to determine their viability percentage to guide production and crossing activities, thereby conserving valuable seeds. This study reaffirms the significance of the Tetrazolium test in supporting breeding programs.

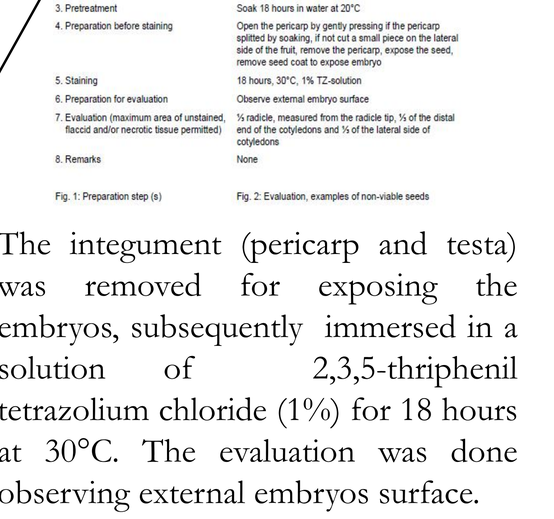


Fig. 4 – Tetrazolium effects on hemp seeds

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References

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