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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 3543.9-2025

Replacing GB/T 3543.7-1995

Rules for agricultural seed testing - Part 9: Sowing quality - Viability test

农作物种子检验规程 第9部分：播种质量 生活力测定

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1 Scope

This document describes the tetrazolium method for determining the viability of agricultural seed.

This document applies to the testing of agricultural seed, and mainly to the following cases: seed that has to be sown as soon as possible after harvest; seed that has already begun to germinate and seed damaged during harvest; deeply dormant seed and slow-germinating seed; and seed whose germination potential has to be known quickly, together with the part of the seed at the end of a germination test that is suspected of being dormant.

3 Terms and definitions

3.1 seed viability: the potential germination capacity of the seed, or the vital force possessed by the embryo.

4 General

The tetrazolium test is a rapid method of determining seed viability. Whether a seed is judged viable depends chiefly on the position and the extent of the necrotic areas in the embryo, the endosperm, the perisperm, the gametophyte and similar tissues, and not necessarily on how deep the stain is; the depth of colour serves to judge whether the tissue is sound, weakened or dead.

The viability shown by the tetrazolium test is a property peculiar to seed in the dormant state. In dormant seed there is a marked difference between viability and germination percentage. Where the following six conditions are met, however, there is no significant difference between viability and germination percentage: the seed is neither dormant nor hard, or dormancy and hardness have been broken by suitable pre-treatment; the seed is not infected, or has been suitably cleaned; the seed was not exposed to unfavourable conditions during processing and was not treated with harmful chemicals during storage; germination has not yet begun; no deterioration occurred during the normal or the extended germination test; and the germination test was carried out under suitable conditions.

5 Principle

A colourless solution of 2,3,5-triphenyl tetrazolium chloride, or of the bromide, is used as the indicator. It takes part in the reduction reaction of the living cells within the seed tissue, accepting hydrogen ions from dehydrogenase; by this hydrogenation the colourless tetrazolium produces, in the living cells, a red, stable formazan that does not diffuse readily and is insoluble in water. According to the position and the colour of the tetrazolium staining, seeds are separated into completely stained viable seed, completely unstained non-viable seed, and seed with necrotic tissue or only partly stained.

6 Apparatus and reagents

6.1.1 Electrically heated thermostatic cabinet or germination cabinet, with a control range of 5 degrees Celsius to 40 degrees Celsius and able to hold 35 degrees Celsius plus or minus 2 degrees Celsius.

6.1.2 Refrigerator, with a control range of 5 degrees Celsius to 10 degrees Celsius.

6.1.3 Balance, readable to 0.001 g.

6.2.1 Tetrazolium solution: the concentration should be 0.1 percent to 1.0 percent by mass and the pH shall be 6.5 to 7.5. It may be made up directly with distilled or deionised water, provided the pH stays within 6.5 to 7.5; where it does not, phosphate buffer should be used in place of the distilled or deionised water. The prepared solution shall be stored cold in darkness or in a brown bottle, and under dark conditions at 5 degrees Celsius to 10 degrees Celsius it will normally keep for a year.

6.2.2 Phosphate buffer, pH 6.5 to 7.5, prepared as follows. Solution one: weigh 9.078 g of potassium dihydrogen phosphate and dissolve it in 1 000 mL of distilled or deionised water. Solution two: weigh 9.472 g of disodium hydrogen phosphate, or 11.876 g of the dihydrate, and dissolve it in 1 000 mL of distilled or deionised water. Mix two parts of solution one with three parts of solution two to obtain the phosphate buffer.

7 Test procedure

7.1 The working sample shall be taken at random from thoroughly mixed pure seed complying with GB/T 3543.3, 400 seeds in all, divided into four replicates of 100 seeds each. Where only the part of the seed suspected of being dormant at the end of a germination test is to be tested, all the fresh ungerminated seed and hard seed from the end of the germination test may be used.

7.2.1 Before staining, the seed should be pre-moistened. Seed whose coat hinders the uptake of moisture, and seed that needs to be stained more evenly, shall be treated accordingly beforehand. External appendages may first be removed, as the hulls are removed from rice; where the coat hinders imbibition, it is pierced at a point that is not vital, as in cotton and hard legume seed.

7.2.1 Pre-moistening may be done in either of two ways. Seed that splits or is damaged when soaked directly in water, old seed and dry seed should be moistened slowly, following the germination test procedure of GB/T 3543.4 and placing the seed on top of paper or between paper. Seed that cannot be fully imbibed by slow moistening and that will not have its tissue split or damaged by direct immersion should be soaked in water until fully imbibed, the water being changed in good time where soaking runs beyond 24 h. The method and duration of pre-moistening for each kind of seed shall follow Table A.1. Some seeds may give off a viscous substance during pre-moistening, which shall be removed by surface drying, by wiping between cloth or paper, or by soaking for 5 min in a 1.0 percent to 2.0 percent solution of aluminium potassium sulfate dodecahydrate.

7.2.2.1 The seed of most crop species needs pre-treatment; where the coat hinders staining, piercing, longitudinal cutting, transverse cutting, transverse sectioning, excision of the embryo or removal of the coat may be used to expose the tissue. The pre-treatment for each kind of seed follows Table A.1. Pre-treated seed shall be kept moist until every replicate has been dealt with.

7.2.2.2 Piercing may be done with a dissecting needle or scalpel; in pre-moistened seed and in hard seed the piercing shall be done at a part of the tissue that is not vital.

7.2.2.3 Longitudinal cutting may follow one of three patterns. In cereal seed, cut lengthwise from the base of the seed along the midline of the embryonic axis, the cut running to about three quarters of the length of the endosperm. In seed of dicotyledons without endosperm and with an erect embryo, cut lengthwise through about half of the cotyledons, slightly to

one side of the central axis, without injuring the embryonic axis. In seed whose embryo is surrounded by living tissue, cut lengthwise close to the embryo.

7.2.2.4 Transverse cutting may follow one of three patterns. In grass seed, cut across at a part of the tissue that is not vital, immediately above the embryo, and immerse the part containing the embryo in the tetrazolium solution. In dicotyledon seed with a straight embryo and no endosperm, cut off one third to two fifths of the distal end of the cotyledons. In small seed, a transverse section that is cut into but not cut through may be used instead of a full transverse cut.

7.2.2.5 In seed of Hordeum, Secale and Triticum the embryo may be separated with a dissecting needle: pierce the endosperm slightly off centre above the scutellum and twist gently so that the endosperm splits lengthwise, then lift out the embryo with the scutellum attached.

7.2.2.6 In seed unsuited to piercing or cutting, the whole coat, and certain other covering tissues, may be removed. Seed with a hard outer covering, such as nuts and drupes, may be split or cracked open dry or after pre-moistening, care being taken not to injure the embryo. After pre-moistening the coat is carefully torn away with a dissecting needle or scalpel.

7.3 The prepared seeds or embryos shall be fully immersed in the tetrazolium solution and stained in darkness or dim light in the thermostatic cabinet, the germination cabinet or the refrigerator. Small seed that is hard to handle may first be pre-moistened and exposed on a paper strip, which is then folded or rolled and immersed in the solution. The concentration, temperature and time given in Table A.1 should be used for each type of seed. Staining time varies with the kind of seed, the way it was prepared, the strength of its viability, the concentration of the solution and the temperature; in general, the higher the concentration and the temperature, the shorter the staining time. The temperature may be varied around the value given in Table A.1 but shall stay between 20 degrees Celsius and 40 degrees Celsius. Where the optimum of 30 degrees Celsius cannot be used, the duration should be adjusted accordingly: an increase or decrease of 5 degrees Celsius corresponds to halving or doubling the staining time. Seed that is still incompletely stained within the prescribed time may be left longer, but it shall not be overstained, since overstaining can mask the different staining patterns produced by particular injuries such as frost damage, or by inherent weakness, and so affect the evaluation. Foam with a black deposit may appear during staining, and a trace of bactericide or antibiotic may be added to the solution to remove the interference. When staining is over the solution is poured off and the seed is rinsed with clean water and examined before evaluation.

7.4.1 Evaluation shall follow immediately after staining. Before evaluation the stained seed may be prepared so that the main structures of the embryo and the other living nutritive tissues are exposed and can be examined and counted; the preparation for each kind of seed follows Table A.1.