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Research Article

Novel Colorimetric Method to Measure Aliveness of Seed Samples via Tetrazolium Salt as Analytical Reagent

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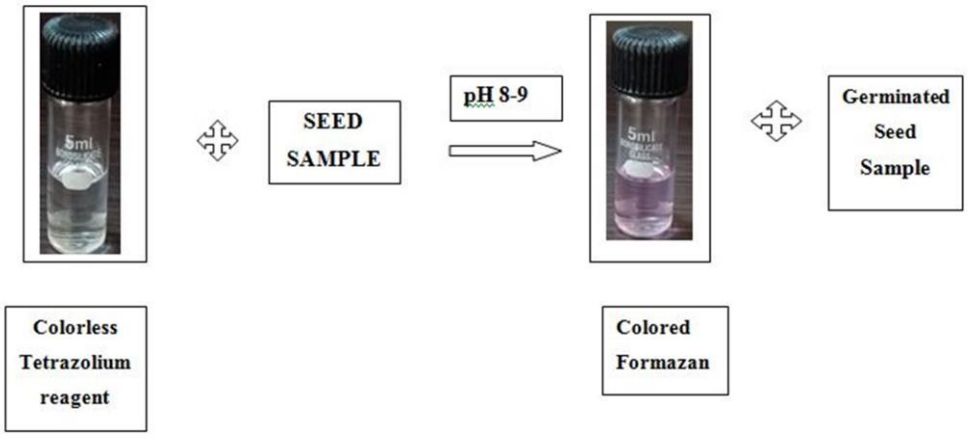
ABSTRACT

The seed is a significant part and the quality seed plays a urgent job in farming creation just as in national economy. In this manner, the great quality seed is essential to improve the creation and profitability. Seed have most extreme life at the hour of physiological development, from that point the seed steadily matured and decreases in feasibility and energy. Seed maturing prompts decrease in seed quality execution and stand foundation. So different preparing procedures and strategy is utilized to test the aliveness of seeds. Present technique depict the germination of seeds by means of decrease of drab tetrazolium salt reagent into hued formazan. The quantification of germination i.e. how much the seed is germinated can be test by measuring the absorbance of colored formazan using uv-visible spectrophotometer. Improved seed empowerment strategies being utilized in numerous pieces of world to decrease the germination time, synchronize germination, improve germination rate and increment seedling stand.

Keywords: *Seed; Ageing; Tetrazolium Salt; Spectrophotometer*



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INTRODUCTION

Seed has been significant for horticulture since the time the plants were trained. Plant raisers have built up various high yielding assortments and to collect their latent capacity, quality seed has been perceived as a significant and least expensive info. The nature of seed is for the most part estimated by its hereditary virtue and ability to form into a sound plant. Worldwide Seed Testing Association (ISTA, 1999) has proposed various institutionalized power and feasibility tests to check the potential degree of movement what's more, execution of the seed during germination and seedling rise. The nature of the seed and capacity limit are likewise related to one another. The seed is a significant segment and the quality seed plays an essential job in agrarian creation just as in national economy. In this way, the great quality seed is essential to upgrade the creation and efficiency. It has been exhibited to understand that utilization of value seeds expanded profitability of yield by 15-20 percent (Sidhawani, 1991). Seed have most extreme life at the hour of physiological development (Meena et al., 1994); from that point the seed step by step matured and decreases in reasonability and power. Seed maturing prompts decrease in seed quality execution and stand foundation (Christiansen and Rowland, 1981). Weakening of seed is not kidding issue in tropical and subtropical nations like India where high temperature and dampness quicken the seed maturing wonder. Subsequently, in the wake of knowing the nature of seed part, the significant viewpoints came in picture is to characterize the capacity or time span of usability of the seed. Monetarily seed disintegration is a significant issue in farming creation (McDonald, 1999). As seed age, they came to sprout more gradually than crisp seeds, breathe increasingly slow progressively vulnerable to illness, chromosomal variations from the norm and expanded extent of morphologically strange seedlings. Feasibility and force are the most significant elements of seed quality which are constrained by both genetical and condition factors. Seed have most noteworthy power at the hour of physiological development, from that point the seed bit by bit decays (Meena et al., 1994). Seed weakening prompts decrease in seed quality, execution and stand foundation which is serious issue in horticulture generation (Christiansen and Rowland, 1981). Along these lines, to survey the capacity limit of the seed, the quickened maturing test was accounted for by ISTA which considers with outrageous condition of relative moistness and temperature (Indira et al., 2000). Handbook of Force Testing strategies has a proposal for the quickened maturing for wheat whereas (Modarresi et al., 2002) recommended new mix of temperature and time (41 ºC/72 hours) for leading quickened maturing of wheat. Quickened maturing of seeds more than a few days of introduction to high temperature and high stickiness has been perceived as acceptable indicator of seed storability (Chiu et al. 1995). (Hampton et al. 2004) contemplated the impact of different mixes of time and temperature for quickened maturing test of pea seed. Seed preparing (Pre-planting hydration medications of seeds) is a generally utilized procedure to upgrade seed execution, prominently regarding rate and consistency of germination in this manner empowering better yield foundation (Taylor et al. 1998). Various investigations have exhibited that preparing is related with an expansion in protein blend (Bray et al. 1989). Improved seed strengthening procedures are being utilized in numerous pieces of world to lessen the germination time, synchronize germination, improve germination rate and increment seedling stand (Khan 1992, Lee and Kim, 2000). Preparing of wheat seed in osmoticum or water may improve germination what's more, rise (Ashraf and Abu-Shakra, 1978) and advance vigourous root development (Cerceller and Soriano 1972). Osmotica that have demonstrated great potential to upgrade germination development; development and grain yield of wheat incorporate arrangement Potassium chloride (Misra and Dwibedi 1980). On ranch preparing of wheat seeds has been

found as a mean of advancing fast germination rise and to expand seedling power also, yield (Harris et al. 2001). In this manner, it become important to describe the adjustment in seed put away nourishment which may get influenced by characteristic and quickened maturing and would result in either reclamation or loss of feasibility under confined hydration condition or preparing. In this way, keeping in see the significance of harvest, the present work is intended to decide the impact of maturing and preparing on seed quality with the accompanying destinations. To consider the physiological and biochemical reason for misfortune in practicality during characteristic and quickened maturing To contemplate the adjustment in compounds exercises and seed stockpiling protein profile during capacity To consider the impact of preparing on seed nature of minimal seed parts Seed has most elevated practicality and force at physiological development (Meena et al., 1994). From that point, the seed progressively ages and decrease in practicality and power. Seed disintegration prompts decrease in seed quality, execution and stand foundation (Christiansen and Rowland, 1981). The climatic states of India significantly quicken the seed maturing wonder under surrounding stockpiling condition, causing resulting crumbling and loss of seed practicality (Basu, 1976). The specific reasons for misfortune of seed practicality are as yet obscure. However, the free radicals actuated nonenzymatic peroxidation which can possibly harm layer is prone to be the essential driver of decay (Larson, 1997). During capacity, the practicality is influenced by different factors, for example, hereditary, preharvest climatic conditions, seed type, seed structure, seed wellbeing, temperature, relative mugginess of climate, seed dampness content and seed medicines and so on. Ecologically, high temperature and high family member dampness during capacity improves seed weakening. There are different techniques and procedures used to improve the germinability of the seed by different medications, for example, osmo-preparing and hydro-preparing of seeds (Maity et al., 2000). Preparing of seeds has been utilized as pre-planting or then again mid-capacity treatment for seed parcels that have lost energy because of inappropriate capacity conditions.

Crumbling of seed is not kidding issue in tropical and subtropical nations like India where high temperature and stickiness quicken the seed maturing wonder. Accordingly, in the wake of knowing the nature of seed parcel, the significant viewpoints came in picture is to characterize the capacity or time span of usability of the seed. Monetarily seed disintegration is a significant issue in rural creation (McDonald, 1999). As seed age, they came to sprout more gradually than crisp seeds, breathe increasingly slow progressively vulnerable to ailment, chromosomal variations from the norm and expanded extent of morphologically strange seedlings. Thusly, it become important to describe the adjustment in seed put away nourishment which may get influenced by normal and quickened maturing and would result in either reclamation or loss of feasibility under limited hydration condition or preparing. Therefore, keeping in see the significance of yield, the present work is intended to decide the impact of maturing and preparing on seed quality.

Experimental

Reagents and Solutions

Tetrazolium salt solution was prepared using double distilled water. All other samples were purchase from a well-branded store.

Instrumentation

The measurements of absorbance were carried out using a double beam systronics uv-visible spectrophotometer 117 equipped with quartz cell of 1cm light path. The absorption spectrum was obtained by measurement of absorbance at different wavelengths from 400-700 nm with the difference of five at each measurement. The pH of the resulting complex was measured by using a Hanna-H19800 pH meter, which is calibrated by standard buffer solution of pH 4.

Solutions

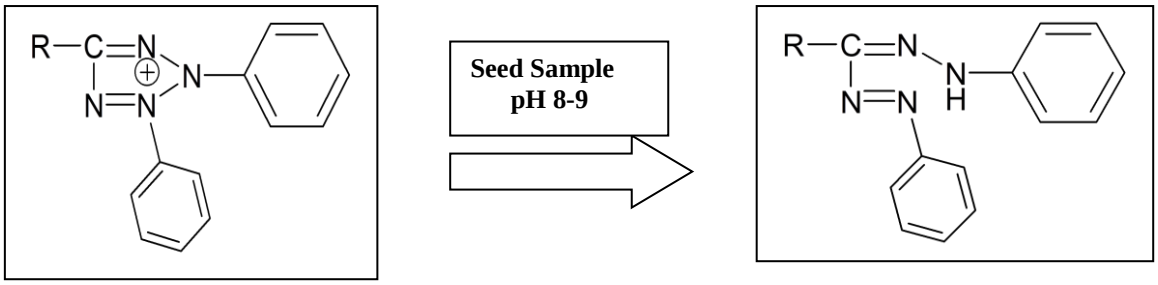
Experimental Procedure

Germination or aliveness of the seed was tested using the tetrazolium salt reagent. The present procedure based upon the reducing property of the germinated seeds. The germination process was studied using this reducing character of the germinated seed. To testify it simply soak the seed sample in 2% tetrazolium salt solution with a few drops of dillute sodium hydroxide solution. As the germination starts the colorless tetrazolium salt solution start converting into the colored formazan. As the germination proceeds the color becomes dark and dark. After complete germination the color of solution gives maximum intensity. The process can be studied by measuring the absorbance of colored formazan using uv-visible spectrophotometer. As the germination proceed the absorbance of the colored solution increases after that reach to a maximum value and the starts decreasing due to decaying of seed quality. The absorbance value gives the germination index of the seed sample.

RESULT AND DISCUSSION

In the mid 1940s, Lakon built up the tetrazolium test as a fast technique for assessing the germination limit of the seeds. Because of quick forecast of 'aliveness' or 'germinability' of seeds, the seed examiner have been keen on techniques for estimating the germinability of seeds without the need of routine germination test, especially when managing torpid seeds or with seeds requiring an extensive stretch for the finishing of a test. The tetrazolium test has increased wide acknowledgment not just as a quick system for evaluating feasibility yet in addition a force full instrument for surveying force. Based on geographical example of recoloring tetrazolium test as a proportion of seed feasibility has been utilized (Moore, 1973). Deswal and Chand (1997) saw that the immediate dousing of preconditioned rice bean (Vigna umbellate) seeds in 1.0 percent tetrazolium arrangement at 40^{o}C for 3h delivered great shading force. Perez and Arguello (1997) announced that use of tetrazolium test to groundnut seeds demonstrated that the test was a precise technique for assessing the nature of seed parcels. Dahiya et al. (1999) uncovered that critical emphatically connection in tetrazolium with standard germination in sunflower. Verma et al. (2003) saw that germination and feasibility of four-year-old seeds diminished forcefully in Brassica juncea.

The present procedure describe the instantaneous reduction of colorless tetrazolium salt into colored formazan in the range of pH 8-9.



Colorless tetrazolium reagent **Colored Formazan**

Comparison of the proposed method with other existing methods

Almost all the existing methods for testing the germination of seeds are time consuming and difficult to handle for example Lakon developed the tetrazolium test as a quick method for evaluating the germination furthest reaches of the seeds. In view of speedy figure of 'aliveness' of seeds, the seed inspector have been enthusiastic about procedures for assessing the germinability of seeds without the need of routine germination test, particularly while overseeing lethargic seeds or with seeds requiring a broad stretch for the

completing of a test. The tetrazolium test has expanded wide affirmation not similarly as a snappy framework for assessing plausibility yet what's more a power full instrument for reviewing power. In light of land case of recoloring tetrazolium test as an extent of seed practicality has been used (Moore, 1973). Deswal and Chand (1997) saw that the prompt drenching of preconditioned rice bean (Vigna umbellate) seeds in 1.0 percent tetrazolium game plan at 40 ^{o}C for 3h conveyed extraordinary concealing power. Perez and Arguello (1997) declared that utilization of tetrazolium test to groundnut seeds showed that the test was an exact procedure for surveying the idea of seed bundles. Dahiya et al. (1999) revealed that basic insistently association in tetrazolium with standard germination in sunflower. Verma et al. (2003) saw that germination and attainability of four-year-old seeds lessened commandingly in Brassica juncea.

But, the present methodology depict the immediate decrease of vapid tetrazolium salt into shaded formazan in the scope of pH 8-9.

CONCLUSION

The proposed method for the assay of germination of seed is more sensitive than other existing methods. The quantification is done by using simple, cost-effective spectrophotometric method, which is less time consuming and simple to use in comparison with the literature techniques such as HPLC, UPLC, Gas Chromatography, etc. which are quite expensive and difficult to handle. The proposed tetrazolium method is excellent for the measurement of aliveness of seed samples.

Competing interests

The ‘authors declare no competing interests.

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Compliance with Ethical Standards

Conflict of interest The Authors declares that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

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