EFFECT OF TETRAZOLIUM AND CONVENTIONAL GERMINATION TESTS ON FIVE DIFFERENT SHORT TERM HIGH YIELDING RICE VARIETIES

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ABSTRACT

This study was conducted in the net house of Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan Campus from April 2014 until October 2014 to determine the effect of tetrazolium and conventional germination tests on five short terms high yielding rice varieties. The objectives of this study were to determine the efficiency of both tetrazolium quick test and conventional germination test on the percentage/ degree of seed viability on different varieties of rice seeds and to compare the efficiency between the seed tetrazolium quick test and conventional germination test on different varieties of rice seeds. The freshly harvested TQR-1, TQR-2, IR 72, TR 8 and TR 9 rice seeds were bisected and half of the seed was soaked in tetrazolium chloride for 2 to 4 hours and decanted before placed in an oven. The seeds also were germinated in germination boxes and were observed for 14 days. Completely Randomized Design was used in this experiment with three replicates for each test. Data for important parameters such percentage of germination and the number of viable seeds were analysed by two-way between group ANOVA while the parameters such as percentages of normal and abnormal seedlings, percentage of seedlings with plumule, percentage of seedlings with radicle, length of roots, height of seedlings and percentage of dead seeds for conventional germination test were compared with number of viable seeds. IR 72 (87.5%) resulted better in seed viability followed by TQR-2 (87%) when tested with tetrazolium quick test, while when tested with conventional germination test, TQR-2 (95.25%) resulted better in seed viability followed by TQR-1 (90.92%). The lowest mean seed viability for both tests was TR 8 (85.42% for tetrazolium and 72.84% for conventional germination tests). However, there were significant differences between both tests and rice varieties on Week 1 and 3 while on Week 5 and 7, there were no significant differences between both tests and rice varieties. Taking into consideration the percentages of seed viability tested with tetrazolium were compared to other parameters, it can be concluded that as seed viability increased, the percentage of normal and abnormal seedlings, height of seedlings, percentage of seedlings with plumule, percentage of seedlings with radicals also increased in all rice varieties tested but decreased in length of roots and the percentage of dead seeds. Therefore, combination with conventional test can be useful for testing dormant seed while the tetrazolium quick test gave the percentage of viable seed and the difference between both tests represent the percentage of dormant seeds. Besides, both tests can be used to determine the seed viability. It is also be recommended use both tests in determining the seed viability which may give more accurate results and faster and also it is recommended to use tetrazolium test and followed or supported by conventional germination test in determining the seed viability due to some rice variety having seed dormancy.

KESAN UJIAN TETRAZOLIUM DAN PERCAMBAHAN KONVENSIONAL KE ATAS LIMA VARIETI TANAMAN PADI JANGKA PENDEK

ABSTRAK

Kajian ini telah dijalankan di dalam rumah jaring di Fakulti Pertanian Lestari, Universiti Malaysia Sabah, Kampus Sandakan dari April 2014 hingga Oktober 2014 bagi menentukan kesan ujian tetrazolium dan percambahan konvensional pada lima varieti padi berhasil tinggi. Objektif kajian ini adalah untuk menentukan kecekapan kedua-dua ujian tetrazolium dan percambahan konventional ke atas peratus atau tahap viabiliti varieti benih padi dan untuk membandingkan kecekapan antara ujian tetrazolium dan ujian percambahan benih konvensional pada varieti padi yang berbeza. TQR-1, TQR-2, IR 72, TR 8 dan TR 9 varieti padi yang baru dituai telah dibelah dua dan sebahagiannya telah direndam dalam tetrazolium klorida untuk 2 hingga 4 jam dan dibuang larutannya sebelum diletakkan di dalam oven. Benih ini juga telah dicambahkan di dalam kotak percambahan dan diperhatikan selama 14 hari. Rekabentuk secara rawak (Complete Randomized Design) diaplikasikan dalam eksperimen ini dengan tiga replikasi bagi setiap ujian. Pada akhir eksperimen, parameter penting seperti peratusan percambahan dan jumlah viabiliti benih yang dianalisis oleh Analisis Varian (ANAVA) dua hala antara kumpulan manakala parameter seperti peratusan anak benih normal dan tidak normal, peratus anak benih dengan plumula, peratusan anak benih dengan radikel, panjang akar, tinggi anak benih dan peratusan benih mati untuk percambahan konvensional telah dibandingkan dengan jumlah viabiliti benih. IR 72 (87.5%) menghasilkan viabiliti benih yang tertinggi diikuti oleh TQR-2 (87%) apabila diuji dengan menggunakan tetrazolium klorida, sementara TQR-2 (95.25%) menghasilkan viabiliti yang paling tinggi diikuti oleh TQR-1 (90.92%) apabila diuji dengan ujian percambahan konvensional. TR 8 (85.42% untuk ujian tetrazolium dan 72.84% ujian percambahan konvensional) merupakan varieti padi yang menunjukkan viabiliti yang paling rendah apabila diuji dengan menggunakan kedua-dua ujian viabiliti. Walau bagaimanapun, terdapat perbezaan yang signifikan antara kedua-dua ujian dengan varieti padi pada minggu pertama dan ketiga manakala pada minggu kelima dan ketujuh, tiada perbezaan yang signifikan antara kedua-dua ujian dengan varieti padi. Dengan mengambil kira peratusan viabiliti benih yang diuji dengan tetrazolium telah dibandingkan dengan parameter lain dan ia dapat disimpulkan bahawa semakin meningkat viabiliti benih, peratusan anak benih normal dan tidak normal, tinggi anak pokok, peratusan anak benih dengan plumula, peratusan anak benih dengan radikel juga meningkat dalam semua varieti padi diuji tetapi berkurang dalam panjang akar dan peratusan benih mati. Oleh itu, kombinasi dengan ujian konvensional boleh digunakan untuk menguji benih dorman manakala tetrazolium memberikan peratusan viabiliti benih dan perbezaan antara kedua-dua ujian mewakili peratusan benih dorman. Selain itu, kedua-dua ujian boleh digunakan untuk menentukan viabiliti benih. Ia juga disyorkan menggunakan kedua-dua ujian dalam menentukan viabiliti benih yang boleh memberikan hasil yang lebih tepat dan lebih cepat dan disyorkan juga untuk menggunakan ujian tetrazolium dan diikuti atau disokong oleh ujian percambahan konvensional dalam menentukan viabiliti benih kerana beberapa varieti padi mempunyai benih yang dorman.

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%	Percentage
ANOVA	Analysis of Variance
cm	Centimetre
CSAAS	Commercial Seed Analysis Association
CRD	Completely Randomize Design
DAS	Date after sowing
EMC	Equilibrium moisture content
Ha	Alternate hypothesis
Ho	Null hypothesis
IRRI	International Rice Research Institute
ISTA	International Seed Testing Association
kg	Kilogram
MAGB	Maltsters's Association of Great Britain
MC	Moisture content
PSP	Photoperiod Sensitive Phase
SPSS	Statistical package for the social science
FSA	Faculty of Sustainable Agriculture
TZ	Tetrazolium
UMS	Universiti Malaysia Sabah
USA	United State of America
USDA	United State Department of Agriculture

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Background of Rice

Oryza sativa L., commonly known as rice from the family Poaceae is the primary staple food for more than half the world's population with Asia and Africa the largest consuming regions. Rice is a direct supply more than 50% calories consumed by the entire human population, second only to wheat. India has dominated the rice production next to China which accounts for 22% of the total cropped area, 39% of the total area under cereal and 31% of the total area under food grains. However, the average yield of rice in India is 1910 kg/ha whereas in Korea is 6000 kg, 5800 kg in Japan, 5700 kg in China and 5700 kg in Egypt (Gururajan *et al.*, 2008). According to International Rice Research Institute (IRRI) the world consumption in 2010 was 443.8 million metric tons and has increased steadily to an estimated 463.0 million metric tons in 2011. In 2012, the world consumption has decreased to 463.2 million metric tons. The production trends mirror the world consumption.

Each seed will mature depending on crops and varieties. When the seed matures, germination process will follow but the interval between these two processes may vary because not all matured seed will germinate at the same time due to seed dormancy. There are two types of seed dormancy which are seed coat dormancy (external) and internal dormancy. External dormancy can be resulted from the seed coat that impermeable to water and gases. Meanwhile, internal dormancy is a general term encompassing a number of physiological conditions that delay germination and the most common one is after ripening (Emery, 1987). Since all seeds are not genetically identical to each other, so it would be common if all viable seed that being planted not germinated. Seed may express their heredity in the form of germination by depending on the environment during seed formation, maturity, and the variable germination blocks (Babasaheb, 2004).

The seed structure must be understandable by seed technologist in order to know how seed may respond during harvesting, conditioning, germination, and seedling emergence. Seed can be divided into two major classifications which are monocotyledons (monocots) and dicotyledons (dicots), based on the number of cotyledons (seed leaves) in a seed. Rice is a monocots plant since monocots means has one cotyledon, while dicots have two. Seeds are composed of 3 basic structures which are the seed coat, embryonic axis and supporting tissue. The location of seed structures plays an important role in determining the seeds' susceptibility to mechanical injuries and weather damage. The position of embryonic axis is just often below the seed covering. Impacts to the embryonic axis can cause severe damage, resulting on abnormal seedling or death of the seed.

Rice cultivation in the country is undertaken in the humid tropical and subtropical climate characterized by high temperature and high relative humidity, resulting in changes in genetic integrity leading to more rapid deterioration of seeds (Kapoor *et al.*, 2011). This can be a greater problem among farmers in Malaysia because they cannot produce rice at minimum level. Hence on behalf of the farmers, the seeds do not grow up to be a waste. Seed deterioration is a natural process that expresses as the loss of quality, viability and vigour during ageing or adverse environmental conditions. It is irreversible degenerative process that occurs during storage. The rate of deterioration is influenced by the seed moisture content, and the temperature of the storage, increasing of these factors leading to more rapid deterioration (Ellis *et al.*, 1992).

To increase the world production of rice, the seed must be in good condition and has high quality. Seeds are hygroscopic which mean the seeds have the ability to absorb and release the moisture in any environment with which it is not equilibrium. Besides, the seeds those being planted have to germinate in order to accommodate human consumption. Therefore the germination test is the best indication of the potential of a seed lot of emerges under field conditions. However, it takes from days to weeks and in some cases even months to complete. To overcome this problem, tetrazolium test method is introduced in the 1940 by German scientist. Tetrazolium is use to test seed viability instead of germination test and have been developed to furnish quick estimates of seed germinability. The test is very useful in processing, handling, storing and marketing large quantities of seed in a short time, testing dormant seed lots, vigour rating of seed lots, supplementing germination test results and diagnosing the cause of seed deterioration (Patil and Dadlani, 2009).

1.2 Justification

Most farmers when sowing rice do not know the level of purity and quality of the seeds. Rice farming is one of the challenging works because farmers have to face many challenges in terms of seed germination when using less effective methods. Therefore, the germinated seeds will germinate not necessarily due to the fertility factors and its low growing seedlings, pre-germination of seeds, abnormal seed germinated and dead seeds are infected and so on.

Rapid seed deterioration during storage is one of the important constraints encountered by farmers since they usually stored their seeds under ambient temperature which may increase the seed deterioration. Since Malaysia has tropical climate, storing seeds under ambient temperature would be more difficult due to its nature being hygroscopic. Most of the seeds companies and rice seed centres produce rice seeds and keeps in stock for sell to famers for sell to the farmers where the storage seeds are at least kept for 6 months to 1 year in the warehouses. Although the germination test are being carried out, but the test results are obtain from the seeds which have been placed under favourable conditions. Seldom are these favourable conditions encountered in the fields and germination result often overestimate field emergence (AOSA, 1983).

Seed quality has become more important to farmers because criteria needed for a quality seeds are including free from disease, high germination percentage, pure seeds, free from other crop and weed seeds and more. These criteria can be checked by using both conventional and tetrazolium tests. However there are advantages and disadvantages for both tests which are interdependent. The advantage of conventional test is dormant seed can be seen, while the disadvantages are very time consuming, need several requirements to be fulfilled, and need more care than tetrazolium test.

For tetrazolium test, the advantages are fast where the results are available within few hours, or at the most two days, very little equipment is needed, the close examination of individual seeds will often reveal the reason for poor germination result and can determine the potential viability of seeds with deep and prolonged dormancy. The advantage of tetrazolium on seed dormancy is very important to many seed producers and rice seed centres because even the newly harvested seeds have dormancy and this may help them to determine the percentage of dormant seed when a separated standard germination test is required and estimate viability of a seed lot before completion of a germination test or to estimate viability independently of a germination test. The disadvantages of tetrazolium test are more workers are needed, dormant seed is not disclosed, difficulty and the experience require to interpret it correctly, damage seeds by fungicides, insecticides and fumigants as well as by frost or heat does not always show and microorganisms which might harm the seeds are not detected. McLeod (1950) found that under a narrow range of conditions of grain moisture and temperature, tetrazolium chloride results grossly overestimated germination. This overestimation was due to the fact that seed germination was more sensitive to heat damage than was enzymatic activity. The disadvantages may seem many but the tetrazolium test is a tool well worth acquiring (Chalam *et al*, 1967; Copeland and McDonald, 1995).

A standard method of germination may not be the one that measures the true germinability of a particular kind of seed. For example, in the tests with four varieties of rice the standard method failed to measure the maximum germinability of the seed of three varieties. When the seed are planted, the percentage germination compared closely with the percentage of stained embryos that were soaked in the tetrazolium chloride solution. These may help the seed companies and rice seed centre to solve problem that could be encountered during germination testing such as when the reasons of abnormalities appearance are unclear, for slowly germinated seed, determine the different types of damage, when the information on potential seed germinability is needed urgently, and when sowing must be performed immediately after harvest in very dormant seed.

Moreover, this research is to be carried out in order to know whether the tetrazolium quick test is comparable to conventional germination test and to see the effect in efficiency of tetrazolium test against conventional germination test on five high yielding rice varieties.

1.3 Problem Statement

The problem faced by seed centres and seed producers is to identify the seed viability during short period of time. Tetrazolium may help them to identify the seed viability within 48 hours while conventional germination test would give results after 14 days.

1.4 Objectives

The objectives of this research were:

1. To evaluate the effect of tetrazolium and conventional germination tests on the percentage/ degree of seed viability on different varieties of rice seeds.
2. To compare the efficiency between the tetrazolium and conventional germination tests on different varieties of rice seeds.

1.5 Hypotheses

Ho1: There is no significant different on the percentage of seed viability for both tetrazolium and conventional germination tests on different varieties of rice seeds.

Ha1: There is significant different on the percentage of seed viability for both tetrazolium and conventional germination tests on different varieties of rice seeds.

Ho2: There is no significant different in term of efficiency for both tetrazolium and conventional germination tests on different varieties of rice seeds.

Ha2: There is significant different in term of efficiency for both tetrazolium and conventional germination tests on different varieties of rice seeds.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice

Rice is a self-pollinated crop that have been grown by more than a hundred countries with 24 species where 22 are wild species and 2 are most important cultivated rice namely *Oryza sativa* and *Oryza glaberrima* . These two cultivated species is native to tropical and subtropical southern and south-eastern Asia and to Africa. There are three sub species of rice in which are *Indica* (long grain), *Japonica* (round grain), and *Javanica* (medium grain). *Indica* rice is the major type grown in warm climate zone such as Indo-China, India, Pakistan, Thailand, Brazil, and Southern U.S.A., *Japonica* is mostly grown in cold climate zone of the subtropics and in the temperate zone. The *Javanica* is grown in Indonesia only. The characteristics of each rice sub species is shown in Appendix A.

2.1.1 Taxonomy

All plants which are multicellular, eukaryotic, producer and photosynthetic are put under kingdom *Plantae* where the cell walls possess mainly cellulose. Rice is put under kingdom *Plantae* of the domain *Eukarya.* The ability of rice to flower, classify it as angiosperm in the division *Magnoliophyta.* Then, rice is classified as monocotyledons which only possess one cotyledon in their seed. The *Cyperales* are under subclass *Commdlinidae* means mostly self-pollinated flowers that have a unilocular, two or three carpellate ovary bearing a single ovule. This order then bought rice into genus *Oryza* L. (USDA, 2013). The summary for this taxonomy classification order is summarized as in Table 2.1.

Table 2.1 Classification of rice according to its taxa

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed Plants
Division	*Magnoliophyta* – Flowering plants
Class	*Liliosida* – Monocotyledons
Subclass	*Commelinidae*
Order	*Cyperales*
Family	*Poaceae* – Grass family
Genus	*Oryza* L. – rice

Source: USDA, 2013

2.1.2 Morphology and Structure of Rice Seed

Rice is a monocot and annual plants that may grow until 1 to 1.8 m height with long slender leaves 50 to 100 cm long and 2 to 2.5 cm broad. The vegetative organ of rice consists of roots, culms, and leaves. Rice has fibrous root that possesses rootlets and root hairs. The culm is the jointed stem of rice plant which made up of a series of nodes and internodes. This node supports a leaf and a bud. The terminal shoot of rice plant is a determinate inflorescence, the panicle which later becomes spikelet. The rice seeds consist of the true fruit or brown rice (caryopsis) and the hull, which encloses the brown rice. The grain is ripened ovary, with the lemma, palea, rachilla, sterile lemmas, and the awn, if present, firmly attached to it (Figure 2.1).

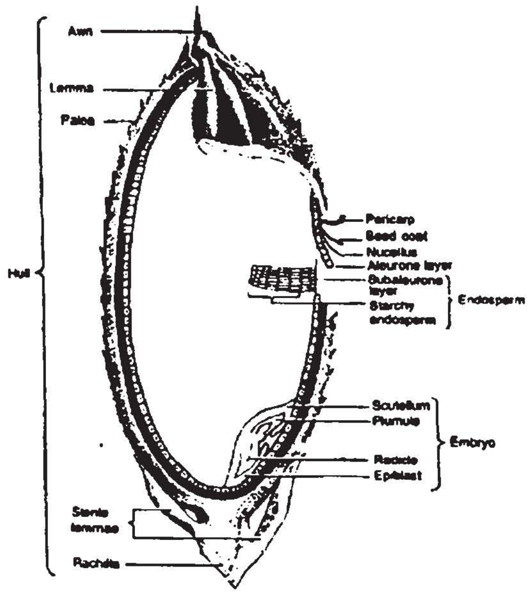

Figure 2.1 Structure of rice grain
Source: Food and Agriculture, 1993

2.1.3 Rice Growth Phases

Physiologically the growth phase of rice can be divided mainly in vegetative, reproductive (Dunand, 2014) and ripening phases (Narendra and Ramadhar, 2002; Reddy, 2004) as shown in appendix B.

a) Vegetative Phase

This phase refers to a period from germination to the initiation panicle primordial. It is characterised by active tillering, gradual increase in plant height and leaf emergence at regular intervals. It is further divided into: (1) Seedling stage where the seed sprout into young, (2) Transplanting stage which covers the period from uprooting, transplanting up to the full recovery of the seedlings, (3) Tillering stage comes between 35 to 40 days after transplanting depending upon the variety and usually stop producing tillers at about maximum tillering stage after tertiary tillers have been produced and (4) Vegetative lag phase which is also known as Photoperiod Sensitive Phase (PSP) that occur between maximum tillering and panicle primordial

b) Reproductive Phase

It is characterised by culm elongation (which increase plant height), decline in tiller number, emergence of flag leaf (the last leaf), booting, heading and flowering of spikelets. This stage can be further divided into: (1) Panicle initiation stage is the beginning of the reproductive stage succeeding the maximum tillering stage and comes 25 days before heading when the panicle can be recognised visually (grown about 1mm long), (2) Booting stage is the elongation of internodes due to increased auxin activity and when the developing panicle causes bulging in the leaf sheath, (3) Heading stage is the emergence of panicle or heading takes place after the booting stage and (4) Flowering stage which occurs 20 to 25 days after booting and continues until spikelets in the panicle bloom.

c) Ripening Phase

The rice ripens in 25 to 40 days after passing through: (1) Milky stage occurs after anthesis where the watery content of the grain begins to turn thick milky between 7 to

12 days, (2) When the milky grains turn into the soft dough and later into hard dough stage in 14 to 21 days and lastly (3) Maturity stage is when the grains turn into hard, clear and translucent color.

2.2 The Physiological Maturity of Seeds

Rice reaches it physiological maturity when three-quarters of ear changes from green to yellow color (Vearasilp *et al.* 2001). Seed quality may be reduced if the rice seeds have more than 20% of moisture content such as cracking and susceptibility for insects and diseases (Insompan, 1998). Seed harvesting with high moisture content about 22-30% is not suitable for harvest and storage. Rice seed drying up to 14% moisture content is good for storage, handling and processing (Shinasuwan *et al.* 1995). Therefore, the seeds must be harvested at their maturity as the moisture content is still low.

2.3 Seed Quality

Seed quality describes the potential and performance of a seed lot. The important aspects of seed quality are germination percentage, free from seed borne disease, vigour, pure variety, the presence of inert matter, seed of other crops, or weed seeds and appearance. According to Santos (1995), quality seed can be defined as varietally pure with a high germination percentage, free from disease and disease organisms, and with a proper moisture content and weight. Furthermore, good quality seeds will ensure good germination, rapid emergence, and vigorous growth. These aspects translate to a good stand (whether greenhouse or field). Poor quality seed results in 'skips,' excessive thinning, or yield reductions due to overcrowding, all which reduce profitability. The problem of poor seed arises as most farmers in Asia keep their own seed and do not tend to do any seed processing to ensure varietal purity or seed quality.

The used of good quality seed is important in order to achieve the yield potential of any rice variety. A good quality seed can increase yields by 5 to 20 % and have more than 80% germination percentage (IRRI, 2003). Besides, high quality seed enables farmers to attain crops which have the most economical planting rate, rapid emergence, high resistance to stress and disease, uniformity in ripening and minimum of replanting.

2.3.1 Seed Germination

To the seed analyst, germination has a definite and standardized meaning, which must lead to uniform interpretations made by analyst in different laboratories. In the Rules for Testing Seed of the Association of Official Seed Analyst (AOSA), seed germination is described as "the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce normal plant under favourable conditions" (AOSA, 1978). The criteria of germinated seeds are the emergence of an embryonic plant that must consist of a complete root and shoot axis that has the capacity of normal growth under favourable conditions. Germination is more than just protrusion of the root or shoots from the seed covering. It is important that all seedling structures necessary for continuation of the next generation be present and healthy.

Based on the fate of the cotyledons, there are two kinds of seed germination: a) epigeal germination and b) hypogeal germination. Rice is a hypogeal germination where the cotyledon stays under the soil while the plumule pushes upward and emerges above the ground. Once germination has begun, water, temperature, and light stress can be fatal meanwhile the seed maturity and oxygen become important requirement to be fulfilled for seed germination to take place. Therefore the best possible conditions during germination and establishment period are crucial (Schmidt, 2000).

a) Seed Maturity

   Seed maturation is a process that comprises a set of morphological, physical, physiological and biochemical events that occur from ovule fertilization until the seeds become physiologically independent of the parent plant (Delouche, 1971).

b) Oxygen

   Excessive moisture can block the gas exchange of the germination seed. Therefore seed require adequate supply of oxygen due to increase in respiration during seed germination.

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