



Effects of Seed Storage Duration and Matriconditioning Materials on Germination and Seedling Characteristics of Maize

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ABSTRACT

Matriconditioning treatments have been reported to improve seed and seedling qualities of many crops after certain storage periods. The research was conducted to evaluate different matriconditioning substances on seedlings characteristics of seeds that previously stored in different periods. The research was carried out from October 2015 to April 2016 at the Indonesian Cereal Research Institute (ICERI). The experiment was arranged in factorial completely randomized design to facilitate the combination of two factors. The first factor was seed storage period, i.e. 4, 36 and 72 months, while the second factor dealt with matriconditioning substances, i.e without matriconditioning, sawdust, carbonized rice hull and rice straw. The results showed that matriconditioning treatments improved seed and seedling qualities of the maize seeds derived from different storage periods. Shorter seed storage period produced seedlings with higher percentage of germination, germination rate, seedling dry weight, shoots and roots lengths and lower EC. Among the tested matriconditioning substances, carbonized rice hull provided more suitable condition to improve seedling qualities in any seed storage period than saw dust and rice straw.

INTRODUCTION

The use of qualified planting material is one determining factor in the success of all crops production, including maize. Related with the seed, there are many factors responsible for the low level of yielding: some of these are low quality seed, poor seed germination and poor seedling vigour depending on the agro-ecological zones (Sulewska et al., 2014). In remote areas, farmers often find difficulties to obtain qualified seeds in the planting period. Thus, farmers usually stored the existing available seeds for the next planting seasons (Wambugu, Mathenge, Auma, & VanRheenen, 2012).

Generally seeds are very susceptible and sensitive to the adverse environmental conditions, thus the storage environment where the seeds are stored, greatly influences the time period of the survival of the seeds. Unfavorable storage environment might deteriorate the seeds resulting the decrease of seed vigor. The deterioration of seeds might be due to interactions of several factors,

like initial moisture content, oxygen, temperature and relative humidity (RH) of storage room, pest and disease attacks and others (El-Abady, 2014; Govender, Aveling, & Kritzing, 2008; Mariucci et al., 2018).

During storage, the increase of temperature and relative humidity would induce higher respiration rate. These oxidative reactions include free radical oxidation, enzymatic dehydrogenation, aldehyde oxidation of proteins, and Maillard reactions (Wang et al., 2018). Oxygen has been suggested to be an important factor to regulate these series of physiological processes, which was associated with seed deterioration. Oxygen accelerates the accumulation of super oxide and leads to O₂ injury of seeds during storage (Kurek, Plitta-Michalak, & Ratajczak, 2019). Wiebach, Nagel, Börner, Altmann, & Riewe (2020) reported that free radicals damage the membrane, and cause decreased viscosity and permeability. The dissolved membrane in the seed leakage is an indication that the membrane has been

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damaged, thus accelerates the seed deterioration. They also observed that RH plays an important role in controlling the longevity of the seed during storage. High RH along with high temperature quickly accelerate the deterioration of the seed and thereby cause aging. RH has been reported to be interacts with oxygen in determining the seeds longevity during storage (Suma, Sreenivasan, Singh, & Radhamani, 2013). Suleiman, Rosentrater, & Bern (2013) reported that the presence of O₂ severely decreases the longevity of primed seeds under low RH, while such phenomena was not observed under high RH.

Based on the above reports, the seed deterioration due to high oxidation reaction might result in the decrease of vigor and viability of seeds, especially when stored in longer period. Efforts have been made to prevent and minimize the seed deterioration during storage and one promising method is called invigoration. Invigoration was pre-planting treatment for seed in order to increase the rate of germination, vigor and viability (Mir-Mahmoodi, Ghassemi-Golezani, Habibi, Paknezhad, & Ardekani, 2011; Khan *et al.*, 2017). Invigoration can also be referred as conditioning or priming. Depending on the seed types, the conditioning methods can be applied through hydrospriming or hydration-dehydration, osmoconditioning or osmopriming that using osmotic solution, and matriconditioning which is also called a solid matrix priming that using a damped solid material (Bakhtavar, Afzal, Basra, Ahmad, & Noor, 2015; Čanak *et al.*, 2016).

Many reports has revealed the succesfull the application of conditiniong on the increase of gemination percentage, uniformity of germination and vigor improvement of seedlings and yield on several crops, like maize (Soleimanzadeh, 2013), rice (Mamun, Naher, & Ali, 2018), soybean (Arif, Jan, Marwat, & Khan, 2008; Miladinov *et al.*, 2018), wheat (Jafar *et al.*, 2012; Sarlach, Sharma, & Bains, 2013), black gram (Benaseer, Ahamed, & Sujatha, 2017), lentils (Toklu, 2015) and others. The preferred medium for matriconditioning are materials that have low potential matrix, negligible osmotic potential, low solubility, able to remain intact during treatment, non-toxic, high water absorption and water holding, high water flow capability, has a large surface area, low specific weight and able to adhere to the seed coat (Hasan, Abdullah, & Duka, 2018; Ruliyansyah, 2011). The waste materials like

sawdust, rice hull and rice straw was potential to be use for seed matriconditioning media. These materials were considered abundant, less utilized, cheap and easy to obtain. Rice husk had been used to invigor tomato seedlings (Sutariati, Madiki, & Khaeruni, 2014) while, sawdust has been reported to improve in seed vigor on vegetable (Ilyas, 2006) and long-yard bean (Erinnovita, Sari, & Guntoro, 2008). Matriconditioning with rice hull ash on yard longbean improve viability and vigor of aged seed in term of germination and seedling emergence (Ilyas & Suartini, 1998). The research was then carried out to find the effects of these matriconditioning materials on maize seeds that had previously been stored in different periods.

MATERIALS AND METHODS

The research was conducted from October 2015 to April 2016 at Seed Laboratory of Indonesian Cereal Research Institute (ICERI), Maros, South Sulawesi, Indonesia. Maize seed 'White Srikandi' obtained from different storage periods with temperature of 18-21°C and relative humidity of 40-50% were used for testing material. The research was designed in a factorial completely randomized experiment to facilitate the combination of two factors with three replicates. The first factor was the storage period of the tested seeds, i.e. 4, 36 and 72 months, while the second factor dealt with matriconditioning substances, i.e without matriconditioning, sawdust, carbonized rice hull and rice straw. The procedure of experiment was described in the following details.

Preparation of Seeds and Matriconditioning Substances

The seeds were obtained the cold storage room from different storage periods and weighed at 100 g each. The weighted seeds were then put into plastic clips and placed at room temperature for a while. The matriconditioning substance, sawdust, was obtained from the the sawmill and a mixture of various types of sawn wood powder. The carbonized rice hull was taken from the ICERI nursery and the rice straw was bought from the rice farmers surrounding the experimental site. Before applied, the rice straw was chopped with the size of ± 0.5 cm to facilitate the matriconditioning treatment.

Application of Matriconditioning Treatments

All matriconditioning materials are weighted at 150 g each. The matriconditioning was then mixed with 100 ml aquadest and 100 g maize seeds

based of the design treatments. The mixtures were then stirred for 15 minutes. After stirred, the mixture was put into a plastic clip and tightly closed. After stored for 24 h inside the cold room (18°C, RH = 40%), the mixture was then washed with running water and the seeds were separated from the matriconditioning substances. The seeds were then aerated for 1 h.

Sand as planting media was put into porous plastic box (50 × 16 × 13 cm) at the depth of 1.5 cm. Three replications of 50 seeds of each treatment combination was planted in planting media. Each box were then gently poured with 500 ml water to facilitate humidity. Normal seedlings were evaluated after 7 days.

The observed parameters included percentage of germination, germination rate, shoot length, root length, seedling dry weight and electrical conductivity. Percentage of germination was calculated by the comparison of number of germinated seeds and number of planted seeds after 7 days. Germinated rate was expressed by the margin germinated seed in every observation time. In every observation, total percentage of normal shoot is divided by etmal (24 hours). The germination rate was calculated using the following formula (Maguire, 1962).

$$KT = \frac{(X_i - X_{i-1})}{T_i} \dots\dots\dots 1)$$

Where: KT = germination rate (%/etmal); X_i = the percentage of normal seed etmal I; T_i = time of observation (etmal)

Shoot and root lengths were observed from selected 10 normal seedlings from each treatment combination after 7 days germination periods. Seedling dry weight was measured from the weight of 10 normal seedlings after being forced dried using oven at 1100C for 17 h. Electrical conductivity was measured weighing 50 seeds of each replication. The seeds of each replication were placed in 200 ml beaker and 50 ml of deionized water was added. Seeds were stirred gently to ensure that all seeds were completely immersed and evenly distributed. The beakers were placed at temperature of 20oC for 24 h. The electrical conductivity of the leachates of each replication was measured by using a conductivity meter (Sension5) and conductivity per gram of seed weight was calculated using the following formula (AOSA, 2002).

$$\text{Conductivity } (\mu\text{S/cm/g}) = \frac{\text{conductivity reading- blank reading}}{\text{weight of replicate (g)}} \dots\dots\dots 2)$$

RESULTS AND DISCUSSION

Analysis of variance revealed that the single effects of matriconditioning materials and seed storage periods treatments were observed significant in all parameters observed. Similar results were also observed in the interaction effects of the treatment being applied.

Seed Moisture Content

The single effects of matriconditioning and seed storage period on the seed moisture content was significant based on analysis of variance. Similar results was obtained on the interaction effects of both treatments as presented on Table 1. Treated in different matriconditioning types, the seed moisture contents derived from various storage periods showed dissimilar trends. Under saw dust and rice straw matriconditioning treatments, higher moisture content was detected on the seeds stored at 4 months followed by 36 and 72 months storage periods. While under carbonized rice hull treatment, higher moisture content was detected at the seeds derived from 36 and 72 months storage. In the absent of matriconditioning treatments, the higher moisture content was detected on the seed stored at 72 months followed by the shorter storage periods.

Similar phenomena were also observed on seed moisture contents under different storage period in each matriconditioning type. After 4 months storage, highest moisture content was detected on the seeds under rice straw matriconditioning treatment, followed by carboinized rice hull, saw dust and no matriconditioning treatments (Table 1). While the moisture contents of the seeds derived from 36 and 72 months storage were highest after treated with carbonized rice hull matriconditioning, followed by saw dust, rice straw and no matriconditioning treatments.

Before the seeds were stored, the seeds underwent certain treatments to lower their moisture contents. These low seed mositure contents was dedicated to control respiration rates and prevent the physiological integrity of seeds during storages (Horbach, Dranski, Malavasi, & Malavasi, 2018). These indicated that under prolonged storages, the moisture content of seeds were decreased untill certain periods, the seed moisture content was unable to facilitate and maintain the supply of energy, resulting the death of the seed embryos (Yuniarti & Nurhasybi, 2015). The process of water imbibition is the initial process on seed germination

and the process was dedicated to stimulate other biochemical process regarding seed germination (Costa, Dias, & Dias, 2019). The higher seed moisture content after carbonized rice hull matriconditioning treatment on the seed derived from 36 and 72 months storage indicated that carbonized rice hull was able to provide more conducive environment for the maize seed to absorb the water during matriconditioning process. Carbonized rice hull has a bulk density of about 0.150 g/cm³ and consist of a very light material with a micro-porous structure (Haefele, Knoblauch, Gummert, Konboon, & Koyama, 2009). The carbonization process also improves the water-holding capacity of the rice hull (Oshio, Nii, & Namioka, 1981). These conducive environment was predictably derived from higher water holding capacity, thus better facilitate water absorption by the seeds (Sonhaji, Surahman, Ilyas, & Giyanto, 2014). Carbonized rice hull as matriconditioning has higher

water holding capacity significantly enhanced vigor of some vegetable seeds i.e. tomato (Sutariati, Madiki, & Khaeruni, 2014), yard longbean (Ilyas & Suartini, 1998).

Percentage of Seed Germination and Germination Rate

Percentage of seed germination of maize seeds from different storage periods after 7 days under various matriconditioning treatments was presented in Table 2. In all matriconditioning treatments, percentage of germination was higher in 4 months seed storage, followed by 36 and 72 months. On the seeds from 4 months storages, the percentage of germinated seed were significantly boosted by matriconditioning treatments. On the seeds from 36 and 72 months storages, however, only the carbonized rice hull matriconditioning treatment significantly induced higher percentage of seed germination.

Table 1. Effects of matriconditioning treatments on moisture content (%) of maize seeds that previously stored under different periods.

Matriconditioning	Moisture content of maize seed (%) previously stored in different storage period (months) ^{*)}		
	4	36	72
No matriconditioning	11.28 d C	13.40 d B	14.27 d A
Saw dust	29.53 c A	28.65 b B	28.90 b B
Carbonized rice hull	30.30 b B	31.68 a A	30.91 a B
Rice straw	31.02 a A	26.49 c B	25.23 c C

Remarks: ^{*)} Values in the same row followed by different capitalized letters differ significantly by DMRT ($\alpha \leq 5\%$); ^{**)} Values in the same column followed by different undercase letters differ significantly by DMRT ($\alpha \leq 5\%$).

Table 2. Effects of matriconditioning treatments on percentage of germination (%) of maize seeds that previously stored under different periods.

Matriconditioning	Percentage of seed germination (%) that previously stored in different storage period (months) ^{*)}		
	4	36	72
No matriconditioning	89.32 b A	58.67 b B	30.21 b C
Saw dust	92.67 a A	43.67 d B	27.67 c C
Carbonized rice hull	93.33 a A	87.67 a B	34.67 a C
Rice straw	94.33 a A	45.67 c B	33.33 a C

Remarks: ^{*)} Values in the same row followed by different capitalized letters differ significantly by DMRT ($\alpha \leq 5\%$); ^{**)} Values in the same column followed by different undercase letters differ significantly by DMRT ($\alpha \leq 5\%$).

Germination rate of maize seeds from various storage period after matriconditioning treatments was presented in Table 3. In all matriconditioning treatments, the germination rates of maize seeds decreased along with the lengthened storage periods, though in certain matriconditioning treatments, i.e. sawdust and rice straw, the diminishing germination rates of the seeds taken from 36 and 72 months storage were not significant. In every storage period, the seeds treated by carbonized rice hull had higher germination rates, compared to other matriconditioning treatments.

Higher percentage of seed germination and germination rates of maize seeds taken from longer storage period when treated with carbonized rice hull indicated that these substances had more suitable potential matrix properties to induce embryo response and promote seed viability. The characteristics include low osmotic retention, non toxic to the seeds and stable in term of form during matriconditioning process, low solubility in water, high water holding capacity and good aeration in field capacity (Sucahyono, Sari, Surahman, & Ilyas, 2014). These conditions ensure the water imbibition process would be slower and take place longer in aerobic conditions than direct water soaking or submersion (Pallaoro, Camili, Guimarães, & de Figueiredo e Albuquerque, 2016). Slower and longer imbibition process prevented the disruption of cell membran, thus the membrane stability is maintained to facilitate further biochemical and physiological processes for seed germination (Goswami, 2019). Carbonized rice hull had been used to invigor tomato seedlings (Sutariati, Madiki, & Khaeruni, 2014). Research by Ilyas & Suartini (1998) found that matriconditioning with carbonized

rice hull on yard longbean improve viability and vigor of aged seed in term of germination and seedling emergence.

Shoot and Root Lengths

Shoot length of germinated maize seeds after 7 days were varied among the matriconditioning and seed storage period treatments. The longer shoot length was detected on the germinated seeds derived from 4 months storage, followed by 36 and 72 months (Table 4). These phenomena was observed in all matriconditioning treatments, including on the seeds with no matriconditioning treatment.

In every storage period treatment, the shoot length of germinated seeds showed almost similar trends. The germinated seeds that were previously matriconditioned with carbonized rice hull had longer shoot than those treated by other matriconditioning substances. Similar condition was also detected on the root length after 7 days germination period (Table 5). These longer shoot and root growths indicated that carbonized rice hull provided more optimum conditions to the treated seeds to make use of all their potential components to support growth during germination period. These includes the biochemical and physiological processes, like digestion of stored carbohydrate, protein and lipid into their simpler forms and then transferred into growing point to facilitate meristematic activities/cell division, cell differentiation, tissue and organ formations (Khalid, Elballa, Jinghua, & Zenda, 2018). Seedling emergence and germination rate of yard longbean was increased by giving carbonized rice hull as seed matriconditioning on aged seeds (Ilyas & Suartini, 1998).

Table 3. Effects of matriconditioning treatments on germination rate of maize seeds that previously stored under different periods.

Matriconditoning	Germination rate of maize seeds that previously stored in different storage period (months) ^{*)}		
	4	36	72
No matriconditioning	24.37 b A	14.97 b B	7.77 a C
Saw dust	29.08 a A	11.48 b B	7.65 a B
Carbonized rice hull	30.63 a A	18.23 a B	10.15 a C
Rice straw	29.40 a A	11.18 b B	8.47 a B

Remarks: *) Values in the same row followed by different capitalized letters differ significantly by DMRT ($\alpha \leq 5\%$); **) Values in the same column followed by different undercase letters differ significantly by DMRT ($\alpha \leq 5\%$).

Table 4. Effects of matriconditioning treatments on shoot length of germinated maize seeds that previously stored under different periods.

Matriconditioning	Shoot length of germinated maize seeds (cm) originated from different different storage period (months) ^{*)}		
	4	36	72
No matriconditioning	15.46 c A	11.32 b B	9.37 c C
Saw dust	17.02 b A	11.93 b B	10.38 b C
Carbonized rice hull	18.39 a A	14.87 a B	12.27 a C
Rice straw	17.32 b A	12.54 b B	10.67 b C

Remarks: *) Values in the same row followed by different capitalized letters differ significantly by DMRT ($\alpha \leq 5\%$); **) Values in the same column followed by different undercase letters differ significantly by DMRT ($\alpha \leq 5\%$).

Table 5. Effects of matriconditioning treatments on root length of germinated maize seeds that previously stored under different periods.

Matriconditioning	Shoot length of germinated maize seeds (cm) that previously stored in different storage period (months) ^{*)}		
	4	36	72
No matriconditioning	7.8 c A	6.0 d B	2.8 d C
Saw dust	8.1 b A	6.2 c B	3.1 c C
Carbonized rice hull	8.5 a A	8.0 a B	4.8 a C
Rice straw	8.3 b A	6.8 b B	3.3 b C

Remarks: *) Values in the same row followed by different capitalized letters differ significantly by DMRT ($\alpha \leq 5\%$); **) Values in the same column followed by different undercase letters differ significantly by DMRT ($\alpha \leq 5\%$).

Table 6. Effects of matriconditioning treatments on seedling dry weight of germinated maize seeds that previously stored under different periods.

Matriconditioning	Seedling dry weight of germinated maize seeds (g) that previously stored in different storage period (months) ^{*)}		
	4	36	72
No matriconditioning	7.67 b A	7.13 c B	3.27 a C
Saw dust	8.17 a A	5.07 b B	2.83 b C
Carbonized rice hull	8.63 a A	7.53 a B	3.53 a C
Rice straw	8.43 a A	5.23 b B	3.37 a C

Remarks: *) Values in the same row followed by different capitalized letters differ significantly by DMRT ($\alpha \leq 5\%$); **) Values in the same column followed by different undercase letters differ significantly by DMRT ($\alpha \leq 5\%$).

Table 7. Effects of matriconditioning treatments on electrical conductivity of maize seeds that previously stored under different periods.

Matriconditioning	Electrical conductivity of maize seeds ($\mu\text{S}/\text{cm}/\text{g}$) that previously stored in different storage period (months) ^{*)}		
	4	36	72
No matriconditioning	17.53 b C	22.06 b B	30.80 a A
Saw dust	15.83 c B	20.08 b A	21.09 c A
Carbonized rice hull	13.85 d C	16.83 c B	20.67 c A
Rice straw	18.45 a C	25.56 a B	28.38 b A

Remarks: *) Values in the same row followed by different capitalized letters differ significantly by DMRT ($\alpha \leq 5\%$). **) Values in the same column followed by different undercase letters differ significantly by DMRT ($\alpha \leq 5\%$).

Seedling Dry Weight

In line with percentage of germination, germination rate, shoot and root lengths, the dry weight were higher at the seedlings derived from the seeds that previously stored at 4 months, followed by 36 and 72 months storages (Table 6). These phenomena were detected in all matriconditioning treatments. Among the matriconditioning treatments, carbonized rice hull was apparently still to be the most suitable substances to improve seedling growth quality derived from the stored seed, especially dry weight. Higher seedlings dry weight indicated that tissues and organs formations were optimally promoted through active respiration and biosynthesis from the carbohydrate mobilization (Sánchez-Linares et al., 2012) and perhaps a part from photosynthesis from the newly developed leaves.

Electrical Conductivity

Electrical conductivity (EC) measurement is dedicated to determine the seed vigor through electrolyte leakage from the seed. When the seeds are soaked in the water, the seeds exude ions, sugar and other metabolites. Electrolyte leakage is usually happened due to the loss of membrane integrity and according to some reports, the values were in line with the seed ageing and or storage (Hussein, Shaheed, & Yasser, 2012). High-vigour seeds are able to reorganize their membranes during imbibition more rapidly and repair any damage to a greater extent than low vigour seeds, so electrolyte leakage from the seed is usually low. The greatest leakage is to be expected from dead seeds. In dead seed, the repair mechanism is absent or inefficient, or the membranes are

completely damaged, thus permitting leaching of larger electrolyte amounts (Sena, Alves, & de Medeiros, 2017).

The EC values of maize seeds derived from various storage periods and treated by different matriconditioning substances were varied. In general, the seeds stored in shorter periods had lower EC values (Table 7), indicating the shorter storage period might minimize the seed deterioration, thus produce more vigor and normal seedlings (Borza, Barros, Moldovan, & Micu, 2017; Domin et al., 2020). In each storage period, maize seeds treated by carbonized rice hull matriconditioning showed less EC values than other matriconditioning treatments. These indicated that carbonized rice hull might provide more conducive environment for maize seeds to minimize leakage and induce the repairment of deteriorated parts, thus enable to produce more normal seedlings (Table 2 and Table 3).

CONCLUSION

Matriconditioning treatments improved the seed and seedling qualities of the maize seeds derived from different storage periods. Shorter seed storage period (4 months) produced better seed and seedling qualities for 33.49 – 60.9% higher of seed germination, 14.41 – 19.86 % higher germination rate, 34.34 - 59.6% longer shoots, 21.11 – 93.12% longer roots, 31.8 – 95.25 % higher seedling dry weight, 28.72 - 53.73 % lower EC than seeds stored at 36 and 72 months. Matriconditioning treatments also improved seedlings qualities in any seed storage period. Carbonized rice hull matriconditioning seemed to provide more suitable conditions than saw dust and rice straw for seed

germination that induced 4.37-12.26% higher seed moisture content, 26.17 – 33.33% higher germination, 20.31 – 22.65% higher germination rates, 12.36 – 15.79% longer shoots, 15.82 – 22.41% longer roots, 15.49 – 22.39% higher dry weight and 10.98 – 40.95% lower EC compared to saw dust and rice straw mantriconditioning.

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