ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 08, Issue, 12, pp.24683-24687, December, 2018

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

GERMINATION OF CORN SEED PERSEVERED IN OXYGEN DEPLETED MINIENVIRONMENT

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ARTICLE INFO

Article History:
Received 21st September, 2018
Received in revised form
16th October, 2018
Accepted 28th November, 2018
Published online 31st December, 2018

Key Words:
Poor Oxygen Storage,
Corn Seed, Germination.

ABSTRACT

Two corn seed samples of moisture content 11.7% and 13.2% were preserved in an air oxygen depletion minienvironment, with volume concentration <2%, control samples of moisture content 11.7 % was preserved in natural atmosphere at oxygen concentration of 20.9%. Germination of the seeds was periodically examined during the preservation, at 0, 3, 6, and 9 months. The obtained test results showed that the germination, the normal and the growth of young trees, were dependent mainly on the oxygen concentration and moisture content during 9 month storage. After storage at oxygen concentration <2% continuously during 9 months, the germination was 100% only for the seed of 11.7 % moisture content preserved in < 2 % oxygen, however for the seed of moisture content 13.2 % moisture content the germination í acceptable but the growth of young trees was slower. The germination of control sample (preservated in 20.9 % oxygen) decreases unacceptably down.

Citation: Khanh Le Quoc, Duong Nguyen Van, Chi Le Linh, Huong Do Tra and Que Le Xuan, 2018. "Germination of corn seed persevered in oxygen depleted minienvironment", *International Journal of Development Research*, 8, (12), 24683-24687.

INTRODUCTION

Maize is one of the most important food crops in the world as well as in Vietnam. Maize is ranked third in terms of area, second in terms of annual yield and top in productivity. In order to improve the value of maize, post-harvest technology such as drying, preserving and processing to reduce losses and maintain maize quality, is extremely important and necessary.Especially after harvest, the respiration - oxidationstill affects the ability to preserve, contributs strongly to reduce both quantity and quality. Farmers were very interested in the quality of seeds because seeds were the first basis for their cultivation (Roberts, 1973; Vu Van Liet and Nguyen Van Hoan, 2007). The seed embryo contains many valuable nutrients such as protein, lipid, sugar, vitamins, enzymes ... which are physiologically active, very susceptible to moisture adsorption and easy and fast to lose quality (Roberts, 1973 Bonner, 1990), and then reducing significantly the seed quality, in turn affects the germination and crop yield.Therefore the preservation of seeds plays a particularly important role. The providing a wide range of quality seeds, including maize seed, with acceptable cost, is alway essential for any agricultural countries.

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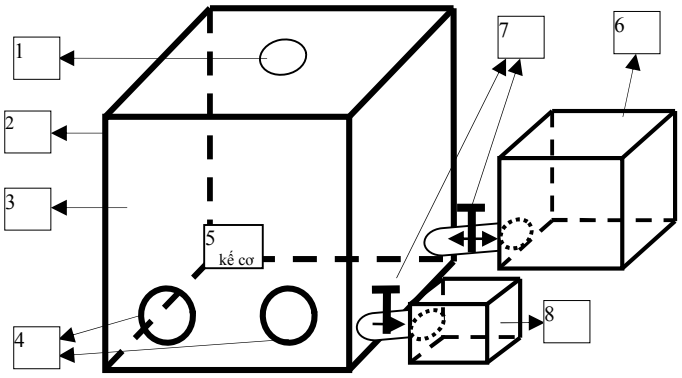
However the seeds will only have its high vigor if being well-preserved under suitable condition with appropriate three principal environment factors: temperature, humidity and oxygen levels. The effects of these three factors have been investigated and applied in tropical preservation (Justice and Louis, 1979; Larry *et al.,* 2001; Que Le Xuan*et al.,* 2018).Germination process of corn, with different stages and visual indicators, has been well introduced (Nielsen, 2010); Uniform germination and emergence of corn has been discussed (Nielsen, 2010; Turnipseed, 2016). The present study dealt with an effect of oxygen poor preservation on corn seed viability and germination.

EXPERIEMT

Minienvironmental preservation

The storage environment is designed as shown in Fig. 1 (Que Le Xuan*et al.,* 2018). A built storage minienvironment is presented in Fig. 2.

Figure 1. Schema of Oxygen-poor hermeric minienvironmental:



1. Top surface for placing equipments (temperature-humidity and oxygen sensors)
2. Minienvironment frame;
3. 2 layers safety glass, thickness of 3 mm to 5 mm + PE film (or PVC); The side plate can be fitted with lightweight aluminum panels.
4. Operating door with rubber glove (air tight);
5. Moisture metter and fan;
6. Pressure regulator
7. Valve - lock with (for 6 and 8);
8. Deoxygenation chamber.

Fig. 2. Preservation minienvironment, airtightness checked (National Technical Standard).

Air moisture in the minienvironment was measured by a Hair Hygrometer, tolerance of ± 0.2%. The experiment was carried out at 25 ^{o}C ± 2 ^{o}C, moisture content of 70 % - 72 %, oxygen concentration always <2% (but 20.9 % for the control sample in the natural armosphere).

MATERIALS

- The maize seed was selected in the Maize Research Institute of Vietnam (MRI), maize variety is LVN17, harvested in 2017, handled against Sitophilus zeamais Motsch
- Seed trays
- Water: Water used to moisten the environment must be treated free of organic and inorganic impurities, pH 6.7 to 7.2. Distilled water, deionized water, demineralized water can be directly used.
- Seedbed for the germination
- Moisture content Meter: dedicated moisture meter for grains, Figure 3.

Fig. 3. Moisture Meter Wile 55

Experiment steps

Place: Institute for Tropical Technology (ITT), VAST.

Step 1: Collecting samples, selectively removing bad quality seeds.
Step 2: Dry to a moisture content of 11.7% (sample W1) or 13.2% (Sample W2).
Step 3: Preparing minienvironment for oxygen poor preservation (using oxygen reducer).
Step 4: Put corn seeds into the minienvironment.
Step 5: After each 3 months maize samples were collected for a germination evaluation according to National Standard.

The seeds are placed on a damp sand layer and covered with another layer thick about 10 mm - 20 mm; to ensure good ventilation it is nessecery to soften the sand (National Standard TCVN 8548); after incubation, the seedlings and germinated seeds should be frequently inspected.

Experimental samples

- Mode 1: Preservation in a minienvironment of oxygen concentration of 20.9%
- Mode 2: Preservation in the minienvironment with oxygen concentration <2%. Take two samples of maize with moisture content of W1, W2, each sample was divided into 4 equal parts of seeds corresponding to 4 sample collections every 3 months, that means after being preservated 3 , 6, 9 and 12 months. Prepare 4 minienvironment with oxygen concentration < 2% corresponding to 2 seed moisture contents W1 of 11.7% and W2 of 13.2%.

Evaluation of results

The ratio of germination was calculated according to formula (National Standard TCVN 8548):

$$G_P\,(\%) = 100\frac{N}{N_0}$$

Where G_P (%) is germination ratio, N is Number of germinated seeds, and N_0 is total number of tested seeds

Normal growth has been evaluated using organoleptic estimation and measurement of height of young trees (Nielsen, 2010; National Standard TCVN 8548).

RESULTS AND DISCUSSION

Germination before storage – sample S0

The germination test was firstly performed on the control sample, both W1 and W2 (moisture content of 11.7 % and 13.2%, respectively). Obtained data on the germination ratio G_P and height of young trees are presented in Table 1.

Table 1. Germination ratio G_n (%) and mean height of young seedling d (cm) ±SD (Standard Deviation) of corn seeds before test (0 months), 2 – 5 days after planting seeds

Time after planting, day		2 days	3 days	4 days	5 days
Para-meters	G_p (%)	98.04	100	100	100
	d, cm	17.31	61.65	113.84	164.9
		±2.04	±1.70	±0.47	±8.36

Young corn plants after germinating were uniform in shape and color, without defect, the average height has developed well each day after emergence; small SD value shows fairly uniform height of 100 corn young plants. There is no germination difference between seed samples W1 and W2 off moisture contents 11.7 % and 13.2 %. Respectively With G_P being 100% and good quality of young seedlings, maize seeds were selected for next test.

Controling oxygen concentration

Oxygen concentration inside of preservation minienvironment was controlled using oxygen reducer FOCOAR (Le Quoc Khanh *et al.,* 2017). The volume ratio of oxygen reducer (g/L) in a preservation minienvironment was 3.0g/L. Data of the variation of oxygen concentration in preservation minienvironment by time are shown in Tables 2, and graphically represented in Figure 4. It is evident that oxygen concentration in the minienvironment decrease from 20.9 % down to 1.5% during over 1550 min, meanly 0.0125 % /min, then the oxygen level reduces slowly down to 0.2%. The high ratio 3g FOCOAR / L has ensured the residue of oxygen reducer over 200% for remaining low oxygen concentration even any air diffusion or penetration through hermetic walls separating the minienvironment up to 24 months (Que Le Xuan *et al.,* 2018; LeQuocKhanh *et al.,* 2017). On the curve C – t (Figure 4) there 4 sections representing 4 different kinetics of the oxygen reduction using of FOCOAR which has been studied in detail previously (LeQuocKhanh *et al.,* 2017).

Table 2. Oxygen concentration over time in preservation microenvironments

With 10 kg of corn grain			
t (min)	C, %	t (min)	C, %
0	20.9	407	6.0
28	19.0	454	5.5
59	17.0	511	5.0
100	15.0	572	4.5
155	13.0	638	4.0
217	11.0	714	3.5
286	9.0	805	3.0
307	8.5	969	2.5
324	8.0	1550	*1.5*
343	7.5	1869	*1.1*
365	7.0	2229	*0.5*
385	6.5	2403	*0.2*

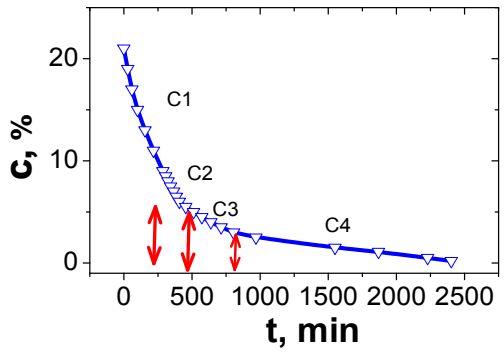


Fig. 4. Oxygen concentration (C, %) variation as a function of oxygen reduction time (t, min), the ratio: 3g FOCOAR / L

Germination and height of seedling - Samples S3 – S12

- **Visual evaluation (National Standard TCVN 8548; Ransom *et al.,* 2014):** The corn seeds have been preserved for 12 months, evaluated each 3 months according to three criteria (visual indicators): 1- Round shape, no cracks, no pests (This criterion is denoted by d), 2) the color of kernel is not tarnished (this criterion is c), 3) good polish (symbol b). All three criteria (dcb) were evaluated in three grades: good (dcb), acceptable (d-c-b-), and bad (- -).

Table 3. Visual evaluation of the corn seed during 12 months storage

STT	Sample	t, month	Moisture content H, %			
			11.7		13.2	
			20.9	< 2	20.9	< 2
1	S0	0	dcb	dcb	dcb	dcb
2	S3	3	dcb-	dcb	dc-b-	dcb
3	S6	6	d-c-b-	dcb	d- - -	dcb
4	S9	9	d-c- -	dcb	- - -	dcb-
5	S12	12	d- - -	dcb	- - -	dc-b-

- **Germination:** Germination G_p depends principally on preservation time, then on moisture content of maize kernel and oxygen concentration inside preservation minienvironment, Figure 5. It is evident that only G_P of 2 samples preserved at oxygen concentration <2 % (samples 11.7-<2 and 13.2-<2, figure 5) and sample 11.7-20.9, still remain acceptable values closed to 100% after 6 months preservation, indicating a good quality of seed. However after 9 and 12 months preservation only G_P of sample 11.7-<2 corresponds to requirements of seed quality. In general, seeds of moisture content 13.2 % were not suitable for the long storage even in low oxygen concentration.

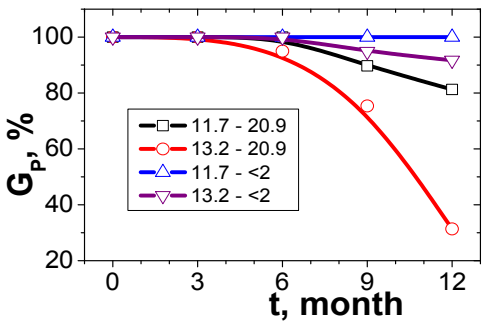


Fig. 5. Germination G_P variation as a function of preservation time; oxygen concentration (20.9% and <2%) and seed moisture content H (11.7% and 13.2%) are noted inside figure)

- **Height d_n of young corn plants:** The height of young corn plants d were measured each day after emergence, and number of the measurement day is indexed as n (d_n). Variation of d_n after emergence 3 days, 4 days and 5 days are represented in figure 6, 7, and 8, respectively.

In general the d_n values decrease evidently with preservation time in comparison with control sample S0, exception of sample of H=11.7 % and C<2%. This sample possesses the heights of seedling closely to the original corn seeds (S0), continuously up to 12 months storage.

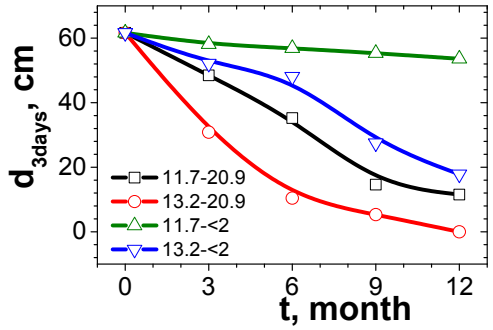


Fig. 6. Variation of d_{3days} as a function of preservation time, the same notation for Figure 5.

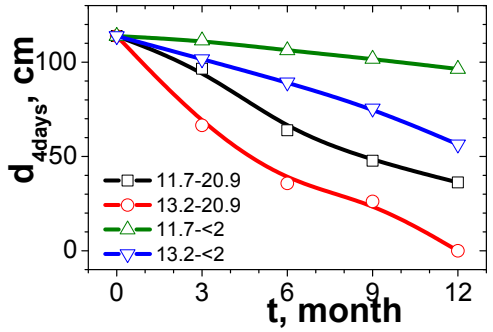


Fig. 7. Variation of d_{4days} as a function of preservation time, the same notation for Fig. 5.

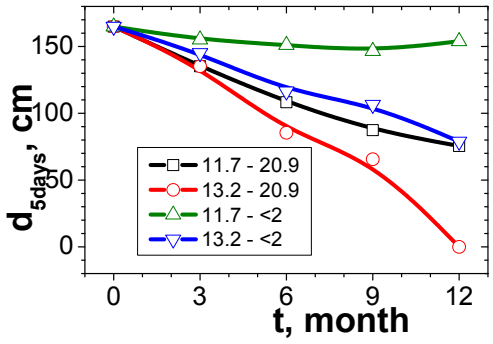


Fig. 8. Variation of d_{5days} as a function of preservation time, the same notation for Fig. 5.

Beside preservation time the oxygen concentration impacts considerably on the germination GP and height dn of young corn plants. Indeed the principal role of the most of seed preservation is antioxidant; however the most oxidation consumes oxygen, including corn seed respiration. In the case of critically low oxygen concentration any oxidative reaction shought be prolonged its incubation and small reaction rate. In order to emphasize the oxygen impact, the quality of corn seed preserved at oxygen concentration of 20.9 % and < 2% is considered to relatively compare the germination and young seedling height of the samples.

In fact, the germination and height of the saplings have been decreased with the storage time, so it is possible to calculate the rate of germination degradation and the rate of decline of saplings over storage time then to compare them in correlation concerning oxygen level in storage minienvironments, between maximum 20.9% and minimum <2 %.

The decrease rate of germination G_P, denoted r_g (unit of % / m), is calculated by the formula:

$$r_g = -\frac{G_P(i) - G_P(i-1)}{t(i) - t(i-1)} \text{ (\%/m)}$$

where m is time (month).

The decrease rate of young seedling height d_{dn}, denoted r_{dn} (unit of cm/m), is calculated by the formula:

$$r_{dn} = -\frac{d_n(i) - d_n(i-1)}{t(i) - t(i-1)} \text{ (cm/m)}$$

Variation of r_g and r_{dn} as a function of preserving time are represented in Figure 9 and 10, respectively.

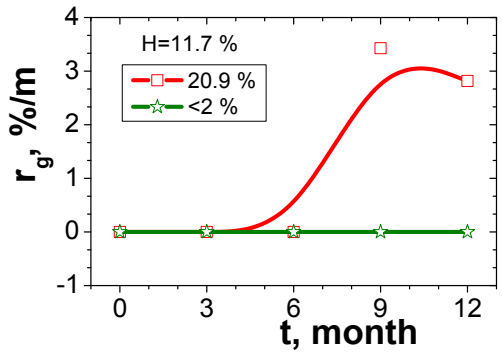


Fig.9a.

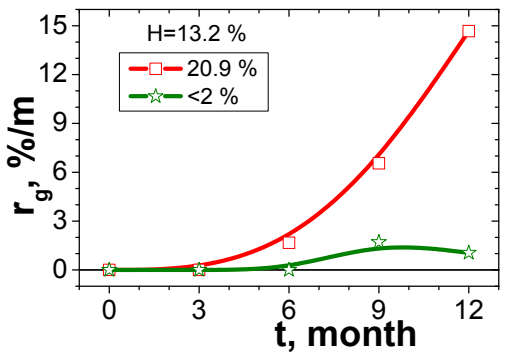


Fig.9b.

Fig. 9. Variation of r_g as a function of preservation time, oxygen concentration 20.9 % and <2 %. a/ H = 11.7 %, b/ H = 13.2 %

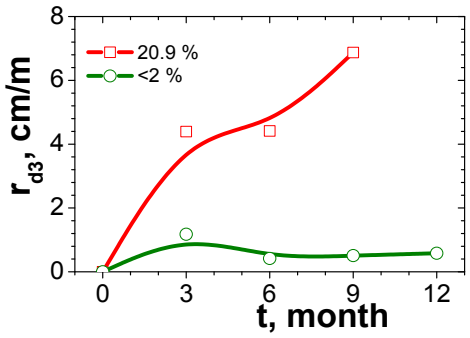


Fig.10a.

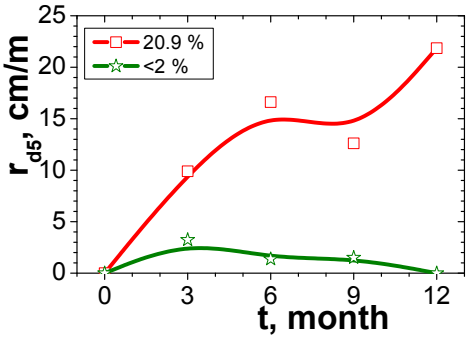


Fig.10b.

Fig. 10. Variation of r_{d3} (a) and r_{d5} (b) as a function of preservation time, oxygen level of 20.9 % and <2 %, H = 11.7 %

Considering Figures 9 and 10 it is clear that the degradation rate of germination and of seedling height strongly depend on the oxygen concentration in the storage minienvironment. These rates, in the case of preservation at 20.9% oxygen concentrations are much higher than that stored at low oxygen concentrations <2%. Poor oxygen preservation provides an strong antioxidant effect that maintains long-term grain quality and, at the same time, it slows the fermentation process and asphyxiation killing insects without the use of toxic chemicals (Le Xuan Que *et al.,* 2012). Although there are many factors influencing the quality of germination and young plants of maize (Parminder *et al.,* 2016; Daniela Vieira dos Anjos Sena *et al.,* 2017), preservation of seeds for the next season is still extremely important. The application of oxygen poor preservation for seed allows for more proactive farming and reducing initial costs.

Conclusion

Maize seed sample were selected according to National standard. After 6 months of preservation in the oxygen poor environment, at oxygen concentraion <2%, corn seed samples with a moisture content of 11.7% and 13.2% possesses the maximum germination G_P, normal seedling and growth, well coresponding to standards. However, in the high oxygen level of 20.9% even the germination ratio was high, but the young plants showed the slow growth pattern, especially for the sample of moisture content of H = 13.2%. Only the sample of corn seed with moisture content of H = 11.7%, preserved 12 months in low oxygen level (<2%), has kept its germination in the best quality, and the good normality of seedling growth.

Acknowledgments: This article is supported by the Vietnam Academy of Science and Technology (Grant code CCVCC13.03 / 18-18).

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