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# Influence of storage conditions on change of hemp seed quality

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# ABSTRACT

Hemp (Cannabis sativa L.); is an alternative fiber crop has been developed mostly in highland. Because seed production are varies in area and time consuming, storage conditions of surplus seed is needed to determine for mass fiber production. The splitsplit plot was designed which main plot were hemp 4 cultivars, sub-plot were 5 conditions, and storage time were sub-sub-plot. The seeds were packed in aluminum foil and stored at room temperature and 15, 4 and -4 °C compared with seal plastic bag condition kept at room temperature. The seed was sampling and tested the qualities before and each month after storage for 12 months. The result found that hemp varieties, storage conditions, time and the interaction between them had significant affect on hemp seed qualities. Before storage, the seed moisture content (mc) of cv. RPF 1, RPF 2, RPF 3 and RPF 4 were 7.7, 8.62, 6.52 and 7.68%, respectively and the germinations were 92, 85, 90 and 91%. During storage, the mc of seed stored in plastic bag was varies due to seed moisture equilibrium. The germination and vigor of seed cv. RPF 1, RPF 3 and RPF 4 packed in both materials type at room temperature was unchanged during 6 months storage, while the seed contained in plastic bag showed the most adverse effect on decrease of viability. During first 3 months, the conditions also significantly affected on decline of germination and vigor of hemp seed cv. RPF 2 which decline to be lesser than 30% during 8 - 12 months. The change of germination and vigor of hemp seed cv. RPF 1, RPF 3 and RPF 4 stored at 15, 4 and -4 °C were not significantly difference during a year of storage. Therefore, type of cold room (15 °C) was suggested to be the best condition for hemp seed storage. © 2015 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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# 1. Introduction

Hemp (*Cannabis sativa* L.); is an annual herb plant which is used for production fibre for such as rope, sail and clothing as well as the seed crushed for oil, food and feed. Due to the outer portion of the hemp stem contains strong and long bast, fibre provides a strength and high quality textile, while inner of the stem so called hurd also used for paper and building material (Mooleki, 2006). After Thai royal project interested in developing industrial hemp as an economic crop to support the hill tribes, the quality seed are needed to propagate and disperse for the farmer. In addition, the cultivation of hemp is being permitted in Thailand especially in the north, but under strict control and monitoring by office of the narcotics control board of Thai. Hemp is determined a dioecious crop and seed production is required a wider space from plant to plant than producing as fibre. Consequently, large area and high cost are necessary. In addition as an annual crop, cultivated seed are usually sown in early March and late of May, and harvested in the end of November under preferable natural temperature 14 - 27 °C. Therefore, they are attractive to produce exceed seed supply and then stored for one or several years for cultivation.

Storage of seed is an important process of plant production to avoid unfavourable environmental conditions and the acceleration of the deteriorations, which is started after harvest. Storage conditions play an important role to maintain high seed quality, which directly related to environmental conditions (mainly temperature and relative humidity). However, the sensitivity of seed to high temperature is depended on the water content that the higher moisture content, the looser viability (McDonald, 1999). Seed deterioration is associated with the genotype, seed history and their physiological and chemical compositions (Copeland and McDonald, 2001). As in hemp, seed is determined as an orthodox seed that can be dried to low moisture content (mc) and stored at low temperature for several years (Robert and Ellis, 1989). Nonetheless, the seed contain 20 - 25% protein, 20 - 30% carbohydrates, 10 -15% insoluble fibre and especially 25 -35% oil (Deferne and Pate, 1996) which was considered to be the major contributors to seed deterioration as previous demonstrations of soybean (Sung, 1996), peanut (Sung and Jeng, 1994) and sunflower (Bailey et al., 1996). For hemp seed, it was suggested that seed should be dried until the moisture content lower than 12%. Consequently, for further storage, either high or low relative humidity affected the moisture equilibrium of seed due to hygroscopic property. Then, the type of container was important for protecting moisture immigration (McPartland et al, 2000). As in closely fibre species such as kenaf (Hibiscus cannabinus), seed stored at 8% mc remains viable for up to 5.5 years when stored at 8 °C and stored above 10 °C and 12% mc showed significant decrease in seed viability (Toole, 1960). In parallel, Meints and Smith, (2003) also found that germination of kenaf under ideal conditions remained high in seed stored up to 4 years at 10 °C and did not show appreciable differences in field emergence or performance through the growing season. Determination of hemp seed qualities which were derived from segregation of the local landrace would assist the Royal project and further seed supplier in reliable on fibre hemp production. The objectives of this study were to determine the impact of storage conditions and hemp seed quality losses during year storage.

# 2. Materials and methods

# 2.1. Experiments

Hemp seed 4 cultivars which was RPF2 produced by the Royal agricultural station AngKhang ( $19^{\circ}54'27.37''N$ ) and RPF1, RPF3, RPF4 produced by the Royal project development centre MaeHae ( $18^{\circ}47'25.97''N$ ), PangUng ( $18^{\circ}46'57.10''N$ ), and MaeSaMai ( $18^{\circ}53'3.73''N$ ). The seeds were produced in each location during May to December, 2010 and harvested in February, 2010. The split-split plot was designed which main plot were hemp 4 cultivars and sub-plot were 5 conditions as well as storage time were sub-sub-plot. For storage conditions, the seed were stored in different types and temperatures as: sealed polypropylene (PP) bag at room temperature (T1); aluminium foil at room temperature (T2); aluminium foil stored at  $15 \,^{\circ}$ C (T3); aluminium foil stored at  $4 \,^{\circ}$ C (T4) and aluminium foil stored at  $-4 \,^{\circ}$ C (T5). The seeds were sampling before and 12 times at one month interval after storage for 12 months (March, 2010 through March, 2011) after that the sample were divided by gravimetric method to test the seed qualities.

#### 2.2. Determination of seed quality

The moisture content of hemp seed sample was determined by standard method according to ISTA (2006) by using a high constant temperature oven method at 105 °C for 20 hr. Standard germinations were conducted with four replications. Each replication consists of 100 seeds were germinated between moistened paper and incubated for 7 day at 20 °C, then seedlings were counted according to the methods described by ISTA rule (2006). The Accelerated aging test (AA-test) was applied from Deluche and Baskin (1973). Two hundred seeds sample were placed in the inner chamber method and incubated at 41 °C for 72hr, then the seed germination was tested and calculated as percentage.

#### 2.3. Weather data collection

Weather temperature and relative humidity were measured 2 times a day (8.00 am and 16.00 pm) by digital thermometer and these records regulated at temporary seed storage room at Seed Science and Technology laboratory of Faculty of Agriculture, Chiang Mai University. Then, the data were calculated to show in average value.

#### 2.4. Data analysis

Data of hemp seed quality were subjected to analysis of variance which means from each treatment were separated using least significant difference (LSD) test at the probability level of 0.01.

#### 3. Results

#### 3.1. Weather condition during storage

Change in the average temperatures and humidity during the storage period in temporary room condition were presented in Fig. 1. In general, room temperature varied parallel to weather temperature. Mass temperature increased in summer (April to June) and decreased in rainy through the end of the year. While mass humidity in the store room also varied parallel to changing weather relative humidity. Mass humidity in the room storage increased especially during May to October due to the rains in this period. Mass humidity decreased again in November, 2010 through March, 2011

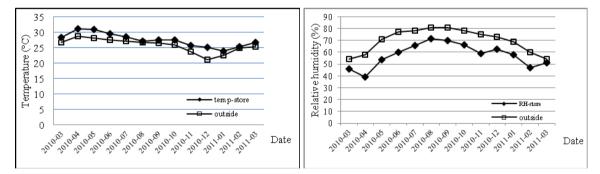


Fig. 1. Weather data in Chiang Mai (outside) and storage room (temp store) for temperature (a) humidity (b)

#### 3.2. Moisture content

Before storage, the seed moisture content (mc) of cv. RPF2, RPF1, RPF4 and RPF3 were 8.62, 7.7, 7.68 and 6.52%, respectively. Analysis of variance showed that storage time, hemp cultivar and interaction between them significantly affected on change of mc (Table 1). The moisture content of seed stored in polypropylene bag plastic bag at room temperature was significantly higher than those conditions. The moisture content of the seeds decreased

during storage for first 7 months, after that it becomes increased in 7 - 12 months. Storage for 12 months affected the decrease of moisture content of hemp seed cv. RPF 2, RPF1 and RPF4 to the average of 8.3, 7.6 and 7.1%, respectively while the seed of cv. RPF3 maintained at the lowest (nearly 6.5%). (Fig. 2)

Source of variation		Р		
	Degree of freedom	Moisture content	Germination	AA-test
Storage time (M)	11	**/1	**	**
Variety (V)	3	**	**	**
MxV	33	**	**	**
Storage treatment (T)	4	**	**	**
ΤxΜ	44	**	**	**
T x V	12	**	**	**
TxMxV	132	**	**	**

Table 1 Analysis of variance

<sup>/1</sup> significant level at  $P \le 0.01$ 

During initial 2 months storage, the high moisture containing seed such RPF 2, RPF1 and RPF4 in all condition tended to reduce especially PP package after that there were not in the pattern due to air exchange between the atmospheres and the seed which result from hygroscopic property. However, the change of moisture of seed packed in aluminum foil at 15, 4 and -4 °C showed no difference during 3 - 12 month storage. However, the low seed moisture (cv. RPF3) packed in PP bag was unchanged during first 6 month (Fig. 2c). For the interaction between seed cultivars and storage conditions, it was found that PP plastic bag affected on increase of moisture content of cv. RPF1 (Fig. 2a), RPF3 (Fig. 2c) and RPF4 (Fig. 2d) during storage in room temperature between 7 to 12 months while mc of the seeds stored at 4 and -4 °C was not different. The seed cv. RPF3 contains low mc (6.5%) stored in seal PP plastic bag at ambient temperature showed sharply increased to 7.2 - 7.5% during 7 to 9 months, afterward it declined to 7.15% again. This result suggested that the rains period and high humidity during month 7<sup>th</sup> - 9<sup>th</sup> influenced on water absorption of the seed, thus the seed attained moisture equilibrium with surrounding air.

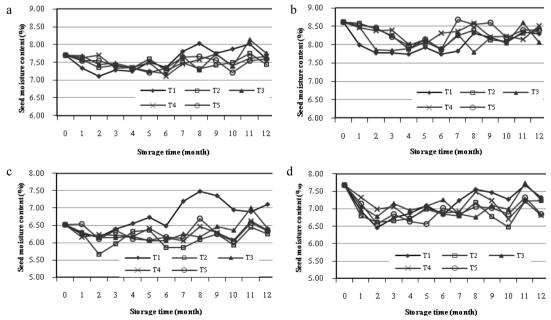


Fig. 2. Moisture content of hemp seed 4 varieties; (a) RPF 1, (b) RPF 2 (c) RPF 3 and (d) RPF 4 which stored at different conditions for 12 months (T=Treatment)

#### 3.3. Seed germination

Effect of storage conditions on hemp seed germination after storage up to 12 months was illustrated in Fig. 4. The storage time and conditions as well as cultivar and the interaction between them significantly resulted in seed germination as shown in Table 1. The germinations of cv. RPF1, RPF4 and RPF3 and RPF2 at initial time of storage were 92, 91, 90 and 85%, respectively. The seed remained high germination when stored in aluminum foil at 15, 4 and -4 °C followed by storage at room temperature. On the other hands, storing seed in seal PP plastic bag had an adverse effect on decrease of seed germination. For the interaction between storage condition and variety, the germination of seed cv. RPF1 (Fig. 3a) and RPF4 (Fig. 3d) packed in both aluminum foil and plastic bag stored at room temperature were unchanged during first 6 months, after that PP plastic bag showed more negative effect on germination than aluminum foil. The effect slightly decreased germination of the seed cv. RPF2 during first 6 months and sharply reduced to be less than 30% during 8 - 12 months (Fig. 3b). However, all conditions of package were not effect on the viability of seed cv. RPF3 through 11 months (Fig. 2c). The germination of seed all cultivars stored at 15, 4 and -4 °C was not significant different over a year of storage and the germination remained unchanged.

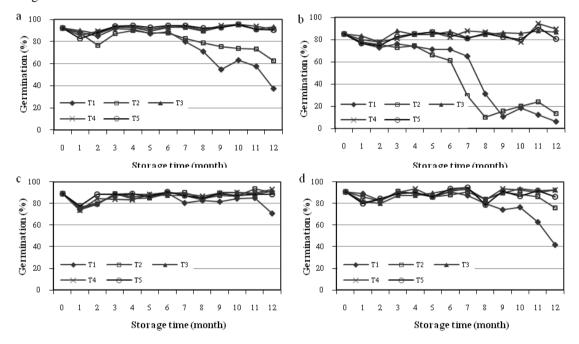


Fig. 3. Germination of hemp seed 4 cultivars; (a) RPF1, (b) RPF2, (c) RPF3 and (d) RPF4 which stored at different conditions for 12 months (T=Treatment)

### 3.4. Accelerated aging test

According to ISTA (1995) mentioned that the high germinating seed lots, the germination test result alone might not provide enough information as to potential seed lot performance. It is in this circumstance that the vigor status of the seed lot becomes important and vigor testing necessary. Accelerated aging test before germination resulted in further reduction in hemp seed germination while the seed was low vigor. From this experiment the result confirmed that the analysis of variance as shown in Table 1 illustrated that hemp varieties, storage conditions, time and the interaction between them had significant affect on hemp seed germination after AA test. AA of all hemp seed stored in all condition was unchanged during 5 months storage, after that it significantly decreased till 12 month (Fig. 3). Hemp seed cv. RPF2 showed lower an average of AA than the others after one year storage while cv. RPF3 showed the highest vigor. Packaging the seed in both aluminum foil and PP plastic bag has an effect on decrease of AA. The

germination from AA test of cv. RPF1, RPF3 and RPF4 during 7 months storage were not different from standard test, however these decreased after 8 - 12 months. Storage seed in aluminum foil and incubated at 15, 4 and -4 °C maintained high seed vigor of all hemp cultivars. Storage at room temperature showed highly impact on germination of cv. RPF1, RPF2 and RPF4 packed in both aluminum foil and PP which gradually declined after storage for 7 - 12 months. However, the condition affected vigor of cv. RPF3 only the seed contained in plastic bag at ambient temperature (Fig. 3c).

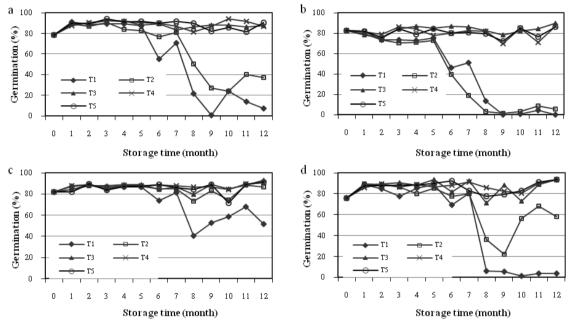


Fig. 4. Accelerated aging (AA) test of hemp seed 4 cultivars; (a) RPF 1, (b) RPF 2 (c) RPF 3 and (d) RPF 4 which stored at different conditions for 12 months (T=Treatment)

#### 4. Discussion

These experiments were conducted to compare storage condition affecting different hemp seed cultivars by observing the physiological of seed qualities. The moisture contents at which these changes are observed among cultivars, and continuously involve in the change of stored seed quality. The seed cv. RPF2 contained higher moisture content (8.62%mc) than the others and also showing lower initial germination (85%) illustrated significantly decreased with storage time when packed in both seal aluminium foil and plastic bag at ambient temperature (25 - 35 °C). McDonald (1999) stated that the effect of high temperatures on seeds quality is highly dependent on their water content and the loss of viability was more rapid when the seed contain high moisture. However, the quality of seed packed in both materials was not different during 5 month, but after that aluminium foil had an adverse effect on reduction of seed viability and vigour than plastic bag during 5 - 8 month. This suggested that high moisture content seeds might be closely oppressed to dry air and then water remove to the surface because of hygroscopic of which higher moisture content was observed in seed packed in aluminium foil (Fig. 2b). The change of moisture content was not different when seed stored in aluminium foil at 15, 4 and -4 °C, while the seed germination and vigour was also remained. The result revealed that aluminium foil has better water and oxygen proved properties than plastic bag made from polypropylene which the exchange were 0.0914/m<sup>2</sup>/hr and 0.2472/m<sup>2</sup>/hr for oxygen; 65 g-water/m2/hr and 1378 g-water/m2/hr for water, respectively. This suggested that initial moisture content and germination are the main factor for providing seed longevity. Hemp seed containing high moisture content stored at room temperature maintained the germination and vigour only up to 5 months of storage, after that the conditions and aging was deleterious to seed viability. Seed deterioration is associated with various biochemicals, metabolic and physiological alteration including loss of membrane integrity and cell

compartmentalization, decreasing in ATP production and impairment of RNA and protein biosynthesis (McDonald, 1999). Moreover, lipid peroxidation and free radicals were considered to be the major contributors to seed deterioration (Hendry, 1993), especially, at high temperature, it was mainly related to membrane damage and alteration of energy metabolisms as demonstrated in sunflower (Corbineau et al., 2002). In the other hand, seed germination and vigour of low seed moisture content as cv. RPF3 contained in aluminium foil at room temperature remained viable. This previous study mentioned that the dehydration of seed could prevent thermal denaturation (Ellis et al., 1988).

# 5. Conclusion

The result exhibited that storage all hemp seeds even in cold at 15 °C or cooler down to 4 and -4 °C were the promising conditions to maintained high seed quality in one year. The evident coincide with the previous research by Meints and Smith (2003) also found that germination of kenaf under ideal conditions remained high in seed stored up to 4 years at 10 °C and did not show appreciable differences in field emergence or performance through the growing season. Furthermore, the result suggested speculations regarding the confidence on the information obtained from the germination and vigour of 4 different varieties. Storage seed at 15 °C was considered as one of the most efficient to maintain seed viability. Unfortunately, seed storage at 0 °C or lower are not currently practical for seed producers due to lack of facilities and high maintenance cost.

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