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Seed Quality and Dormancy of Hemp (*Cannabis sativa* L.)

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Seed Quality and Dormancy of Hemp (*Cannabis sativa* L.)

Cover Page Footnote

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ABSTRACT

Hemp has great potential as a cash crop for growers in the US because of its multiple uses in food, pharmaceutical, and industrial products. Seed quality and dormancy research had been put off for decades. The indeterminate flowering pattern of hemp results in harvesting seeds with varying maturity levels, affecting their quality. The objectives of this study were to: 1) investigate the differences in quality of seeds harvested from different locations on the same plant, 2) determine suitable viability and vigor tests for evaluating hemp seeds, and 3) examine the extent of dormancy in newly harvested seeds and develop a dormancy-breaking method. Two hemp varieties, Merlot (ML) and Berry Blossom (BB) were evaluated. BB had smaller seeds than ML. Seeds from the upper 2/3 part of the plants (more mature) were harvested separately from the lower 1/3 (less mature). The lower part was harvested 7 d after the upper part. Seeds were tested 21 d after harvest. Seed quality was measured by standard germination (SGT), tetrazolium (TZ), accelerated aging (AAT), and speed of germination (SGI) tests. Dormancy was measured in weekly intervals. Difference in seed size did not affect seed quality. Seeds harvested from the upper part of plants had better quality than those from the lower part, however, it became similar to the upper part after 8 d. TZ, SGT, AAT, and SGI were suitable for measuring seed quality. Both varieties had a short-lived dormancy of 35-42 d. Prechilling treatment at 10°C for 5 d was effective in breaking dormancy.

Abbreviations: AAT, Accelerated aging test; AOSA, Association of Official Seed Analysts; BB, Berry Blossom; ISTA, International Seed Testing Association; ML, Merlot; SGI, speed of germination index; SGT, standard germination test; and TZ, tetrazolium test.

INTRODUCTION

Hemp, *Cannabis sativa* L., is an annual dicotyledonous plant belongs to Cannabaceae family (Chase, 1998). The seed of hemp is botanically called achene fruit (Bócsa and Kraus, 1998). Hemp has a wide range of applications, including pharmaceutical products, food, fiber, and other industrial usage such as textile, rope, bioplastics, insulation, biofuel, and other uses. In addition to being rich in unsaturated fatty acids, hemp seeds are also rich in proteins, (USDA, 2016). Currently, more than 30 nations grow industrial hemp as an agricultural commodity. Although global hemp production declined significantly from its peak in the 1950s, over the past decade it has rebounded in response to consumer demands and policy changes. According to 2018 U.S. market and import data for hemp products and ingredients, the demand for hemp has grown exponentially in the last few years (Johnson, 2018; Mark, and Snell, 2019). Hemp and marijuana belong to the same genus and species, *C. sativa*. However, they are genetically distinct in their chemical composition, uses, and biological effects. Hemp contains less than 0.3% Δ^9 -Tetrahydrocannabinol (THC), which differentiates it from marijuana that has higher levels of THC. Since the hemp prohibition in the US by the Congress legislation in 1937, research on hemp had been suspended for decades until the 2018 Farm Bill legislation (Hemp History, 2019; USDA, Hemp Information for Producers, 2018). Hemp is now once again a popular crop, but it is not easy to grow. Autoflowering, feminized seeds, photoperiodism, dormancy, and seed quality are just some of the production issues in urgent need of comprehensive studies (Salentiji et. al., 2019; CHTA, 2019).

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Hemp plants have an indeterminate flowering pattern, where flowers continue to form over a period of time, developing seeds with different ages on the same plant at the time of harvest (CHTA, 2019). Peanut (*Arachis hypogaea*), canola (*Brassica napus*), and Lentil (*Lens culinaris*), are examples of the crops that have an indeterminate growth habit similar to hemp (Barkley et al., 2016; Elias and Copeland, 2001; Nleya et al., 2016). Female plants develop their inflorescences at the apex of the central stem and appear in racemes (Bouloc, 2013). Seeds at the top of the plant mature earlier than those in the lower part. This flowering system produces a mixture of well-developed and under-developed seeds at the time of harvest, which may affect the physiological quality of seeds. Examining the difference in the quality of hemp seed harvested from the top and lower parts of the plants was one of the focal objectives in this study. Mishchenko et al. (2017) reported that hemp achene fruits begin to ripen at BBCH 81 and reach full maturity at BBCH 89. The BBCH (Biologische Bundesantalt, Bundes-sortenamt und Chemische Industrie, Germany) is a scaling system for the uniform coding of phenological stages of growth and development in different species (Meier, 2001). In addition to the BBCH scale, we used the visual coloring scheme in describing the stage of maturity at which we harvested the hemp seeds for the study. Hemp is a short-day plant, which is critical for inducing flowers (Amaducci et al., 2015; Bócsa and Kraus, 1998). Since different geographical regions have different lengths of dark and light, breeding genotypes for photoperiodism that matches the area of hemp production is needed. Autoflowering which happens when plants automatically switching from vegetative to flowering mode after certain period of vegetative growth without photoperiodism requirement and takes place in cloned plants offers an option for hemp growers to bypass the photoperiodism requirement.

Seed dormancy is a natural mechanism in many plant species to increase the chance of survival. Breeding programs of new varieties constantly aim to reduce dormancy to achieve fast, uniform field emergence. Dormancy can be morphological, related to the seed coat impermeability to water; physical, related to hard seed coat (i.e., shell) as physical barrier to the emergence of radicle and leaves; or physiological, related to hormonal imbalance (Baskin and Baskin, 2014; Copeland and McDonald, 2001). Degree of dormancy varies among species and varieties (Soares et al., 2016). Jovičić et al. (2019) reported that hemp seed may have certain residual dormancy that needs to be investigated in detail. Wild cannabis, also known as feral or ditch weed, is very low in THC, and can stay dormant, viable in soils for up to 10 years. Initial seed moisture content and temperature is imperative for safe storage of hemp seeds (Small and Brooke, 2012; Way of life, 2019). This study was conducted to characterize dormancy and quality of seeds with different maturity stages (ages) grown on the same plant due to the indeterminate flowering pattern. No published data are available in comparing the magnitude of dormancy and the quality of seeds harvested from the upper and the lower hemp plants, not before the hemp prohibition legislation in 1937, or after the 2018 Farm Bill legislation.

The use of high quality seeds is critical in any successful farming system. Selecting improved varieties with desirable traits, adapted to the area of production is key to optimize crop performance, hemp is not an exception. Quality of hemp seed, including viability, vigor and dormancy, has not been well studied. Viability and vigor are fundamentals of seed quality. Environmental and growing conditions during seed development and maturation, along with harvest methods, post-harvest managements, including cleaning, drying, and storage affect seed quality (Elias, 2018). Research related to hemp seed quality is scarce due

to decades of restriction in cultivating this crop. Although seed testing rules include standard germination test procedures (AOSA, 2019; ISTA, 2019), no vigor test is available to measure the quality of hemp seeds in either organizations. This study introduced a new protocol for an accelerated aging test to measure seed vigor of hemp. The test imposes stress conditions of high temperature and relative humidity (RH) on seeds. After the stress period, high quality seeds produce higher percentage of normal seedling at the end of the germination test period than lower quality seeds. This will be the only vigor test currently available for hemp seeds, and possibly can be adapted by AOSA for laboratories and growers use. This study evaluated the quality and dormancy of hemp seed over a six-week period after harvest.

The objectives of this study were to: 1) examine the extent of dormancy in newly harvested seeds of two hemp varieties and develop a dormancy-breaking method, 2) measure the quality of seeds harvested from the upper 2/3 part of the plants (more mature) and the lower 1/3 part (less mature), and 3) identify suitable tests for measuring seed viability and vigor.

MATERIALS AND METHODS

Seed Materials

Two hemp varieties, Merlot (ML) and Berry Blossom (BB), grown in Jefferson, Oregon (44°43'5"N 123°0'34"W), elevation 70 m, were used in the study. Cloned plants of the two varieties were implanted in a field during the first week of April using standard hemp tillage and fertility practices. Seeds from the upper 2/3 part of the plants (mature early) were harvested separately from the lower 1/3 part of the plant. Seeds from the upper 2/3 part were harvested on 1 July 2019, and on 9 July from lower 1/3 part. Seeds from the upper part of the plant were harvested when seeds reached full maturity stage (BBCH 89), and the color of seeds turned brown. Seeds from the lower part of the plant were harvested at medium maturity stage (BBCH 85), and the color of seeds were light brown. Seeds were stored in polyethylene bags from the time of harvest until evaluating the quality of seeds at room 21°C and relative humidity 42%.

The initial quality of ML and BB seeds was measured for moisture content, viability, and vigor tests 21 days after harvest. Seeds harvested from the upper and lower parts of the plants were evaluated independently at weekly intervals for four consecutive weeks. Seed size of ML was slightly bigger than BB at 73 and 78 seeds g⁻¹, respectively. All the experiments were conducted at the Oregon State University Seed laboratory.

Seed Quality Evaluation Tests:

The following tests were used to evaluate the initial quality of seeds:

Standard Germination Test (SGT): It is a physiological test used to evaluate the viability of seeds, where seeds are germinated under optimum conditions of temperature, moisture, and light. At the end of the SGT test period, the ungerminated seeds were evaluated by the TZ test to determine whether they are viable. Four replications of 100 seeds of each treatment were planted on germination paper towels (Anchor Paper Co. St. Paul, MN) moistened with 0.2% potassium nitrate (KNO₃) solution. The paper towels were rolled and placed in plastic bags to keep the moisture intact. The bags were then placed at alternating temperatures of 20-30°C (16 h at 20°C in dark; 8 h at 30°C in light) as prescribed in the AOSA rules for testing seeds (2019). Pre-chilled seeds at 10°C for 5 d and non-chilled seeds of each treatment were germinated and

the results were compared. Final germination was counted 7 d after planting. Seedlings were considered normal when they developed healthy roots (i.e., primary root not stubby or malformed and/or numerous secondary roots are developed) and healthy shoot (well-developed epicotyl, hypocotyl, and leaves, not deformed) structures. In evaluating the SGT, seedlings are classified into normal and abnormal based on the development of root and shoot structures, and dead seeds, with no growth at all (AOSA rules for testing seeds, 2019). The SGT provides a more comprehensive expression of seed viability than the biochemical TZ test, because the root and shoot developments are visible and evaluated, therefore it relates more to field emergence. Healthy, vigorous seeds are expected to produce normal seedlings under optimum environmental conditions.

Tetrazolium Test (TZ): It is a quick biochemical test that determines seed viability based on the activity of dehydrogenase enzymes regardless of the dormancy level (AOSA, 2010, Copeland and McDonald, 2001; Elias et al., 2012). The initial quality of seeds was determined by the TZ test. Unlike the SGT, TZ test reveals viability even if seeds are dormant. Two replicates of 100 seeds from each treatment were used. Seeds were soaked in water for 24 h to soften the tissues and activate the enzymes, and then cut longitudinally, and soaked in 1% 2,3, 5-triphenyl tetrazolium chloride (Sigma-Aldrich, Inc., St. Louis, MO) solution for 16 h at 30°C. Seeds were evaluated under a 7x microscope and classified as viable when the entire embryo (embryonic axis and cotyledons) was evenly stained red (AOSA tetrazolium testing handbook, 2010). Classifying seeds into viable and nonviable in the TZ test is based on the pattern and intensity of staining of the embryonic axes and the cotyledons (Elias and Garay, 2004). When seeds were completely or partially unstained, blotchy, and/or more than one-half of the cotyledons unstained, they were classified as nonviable. Two main advantages of measuring viability by TZ over the SGT: 1) speed because the test can be completed in 48 hours, and 2) the ability to identify viability of dormant seeds without the need to prechilling treatment. However, the actual physiological development of seedlings is not expressed.

Dormancy Evaluation

Dormant seeds are viable, but do not germinate until the dormancy is broken. Exposing wet seeds to cold temperature for a specific period of time is known to break dormancy of many species (Baskin and Baskin, 2014; Elias et al., 2012). In this study, prechilling treatment was used to evaluate its effectiveness on breaking dormancy in hemp seeds. The null hypothesis was that no dormancy in hemp seeds takes place, and thus no difference in germination between chilled and unchilled seeds; the alternative hypothesis was that hemp seeds possess some levels of dormancy, and the pre-chilling treatment would break dormancy and lead to a better germination rate. Seeds of ML and BB from the upper and the lower parts of the plants were germinated with and without prechilling treatments. In the prechilling treatment, wet seeds were incubated at 10°C for 5 d before moving to 20-30°C for 7 d (SGT). Comparing the germination results with and without prechilling treatments revealed the effectiveness of prechilling in breaking dormancy. At the end of each germination test, the ungerminated seeds from both chilled and unchilled treatments were tested by TZ to determine whether these seeds were dead or still dormant.

In order to determine the period of “after ripening” at which all dormancy was broken (Copeland and McDonald, 2001), two subsamples of each treatment were planted with and without prechilling treatments at weekly intervals for four consequent weeks. Similar

percentage of germination of prechill and non-chilled seeds were indicative that dormancy has been broken.

Vigor Tests: Speed of germination index (SGI), and accelerated aging test (AAT) were used to evaluate the vigor of seeds harvested from the upper and the lower parts of the plants.

Speed of Germination Index (SGI): it is a vigor test that measures the speed at which seed germinate and produce normal seedlings, which is taken as an index of seed vigor. Higher SGI indicates faster germination, thus good vigor and quality index. To determine the SGI, four replications of 50 seeds from each treatment were planted. Number of seeds germinated were counted and recorded daily from day two to day seven (the end of test period). Seedlings are considered normal when they have healthy (not deformed) roots and shoot length 5 mm or more. The following formula was used to calculate the SGI (AOSA Seed Vigor Testing Handbook, 2009; Maguire, 1962):

$$SGI = \frac{\text{number of normal seedlings}}{\text{days of first count}} + \dots + \frac{\text{number of normal seedlings}}{\text{days of final count}}$$

Accelerated Aging Test: AAT is a stress test that measures seed vigor by exposing seeds to high temperatures and relative humidity (RH), and then germinating them at optimum temperature (20-30°C) as prescribed in the AOSA, seed vigor testing handbook (2009) and the AOSA rules for testing seeds (2019). At the end of the test period (7 d), high quality seeds are expected to achieve better germination than lower quality seeds. Preliminary trials were conducted using varied combinations of temperatures (ranging from 41 to 45°C) and time span (ranging from 24 to 72 h) to determine the AAT parameters. Two replications of 100 seeds from each treatment were placed as a single layer on a wire mesh tray inside a 12 x 12 cm plastic box (Hoffman manufacturing, Jefferson, OR). 50 ml of distilled water was added in the plastic boxes to raise RH. The boxes were placed in an oven (Sheldon Manufacturing, Inc., Cornelius, OR) at 43°C and near 100% RH for 48 h. After the incubation period, seeds were planted following the SGT procedure at alternating 20-30°C for 7 d. Normal seedlings were counted and recorded at the end of the test period.

Statistical analysis

Four-factor completely randomized design, with four replications was used in the SGT and the SGI experiments. The factors were, varieties, weeks of evaluation (4 wk in the SGT and 2 wk in the SGI), location of seeds on the plant (upper 2/3 and lower 1/3), and prechilling treatment (chill vs. no chill). Two-way ANOVA with two replications was used in the TZ and the AAT experiments. Factors were the variety and location of seeds on the plant, i.e., upper 2/3 or lower 1/3.

The data from SGT, SGI, TZ, and AAT were subjected to analysis of variance (ANOVA). When ANOVA indicated significant effect of treatments on viability or vigor (as tested by respective tests), means were separated using the LSD test at $P \leq 0.05$. The statistical package MSTAT (Michigan State University) was used in analyzing the data.

RESULTS AND DISCUSSION

Initial quality of seeds

The results of the SGT showed that there was no significant difference ($P \leq 0.05$) in the initial germination percentage between seeds from ML and BB (Table 1). However, the germination of seeds from the upper and the lower parts of the plants was significantly different at $P \leq 0.001$ (Table 1). The results of the viability by the TZ test showed that seeds harvested from the upper 2/3 had significantly higher viability by TZ at $P \leq 0.01$ (Table 1) at 97% and 96% than seeds from the lower 1/3 of the plants, which had 86% and 88% for ML and BB, respectively (Fig. 1).

Table 1. Analysis of variance (ANOVA) for the effects of varieties, location of seeds on the plant, prechilling treatment, and weekly germination on dormancy, viability, and vigor as measured by standard germination test (SGT), speed of germination index (SGI), tetrazolium test (TZ), and accelerated aging test (AAT) of two hemp varieties germinated over 4-week period, with and without prechilling treatments.

Source of variation	df	SGT	df	SGI	TZ	AAT
Main effects						
Week (W)	3†	***	1	ns‡		
Variety (V) §	1	ns	1	ns	ns	ns
Seed Location on plant (L) ¶	1	***	1	ns	**	ns
Chill/No Chill (CH) #	1	ns	1	***		
Interactions						
WV	3	**	1	ns		
W x L	3	*	1	***		
W x CH	3	ns	1	***		
V x L	1	ns	1	***	ns	***
V x CH	1	ns	1	ns		
L x CH	1	***	1	***		
W x V x L	3	ns	1	*		
W x L x CH	3	*	1	*		
W x V x L x CH	3	**	1	ns		

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively.

† In the SGT, seeds were tested at weekly intervals for four weeks (df=3), and for two weeks in SGI (df=1).

‡ ns, non-significant.

§ Merlot and Berry Blossom.

¶ Seeds harvested from the upper 2/3 and the lower 1/3 part of the plant.

Seeds chilled at 10°C for 5 d before they were moved to 20-30°C for 7 d.

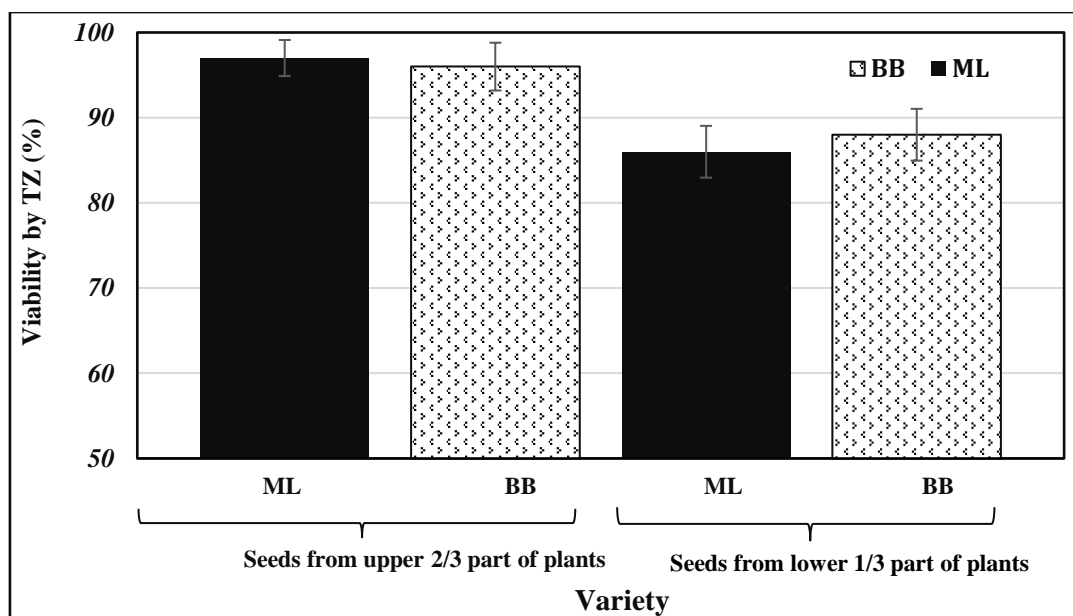


Figure 1. Viability by TZ test of seeds harvested separately from the upper 2/3 and the lower 1/3 parts of the plants of Merlot (ML) and Berry Blossom (BB) hemp varieties. Means (columns) with overlap error bars are not significantly different from each other ($P \leq 0.05$).

The difference in initial quality between the upper and the lower parts of the plant indicated that the seeds from the lower part were not fully matured and may need some extra “after ripening time” to reach maximum potential germination. Approximately 10% of the seeds from the lower part of the plants were slightly smaller than the seeds from the upper part. The indeterminate flowering pattern of hemp inflorescence results in fully matured seeds in the upper part of the plants, displaying better quality than seeds from the lower part of the plant, which needed more time to reach full maturity. Fully matured seeds are expected to have better quality than underdeveloped seeds probably because of the incomplete physiological and biochemical development of immature seed, which affect seed viability, vigor, and consequently seed performance. Although the actual physiological development of seedlings is not expressed in the TZ test, there is a wide interest in this test because of the speed and the determination of seed viability even when seeds are dormant. In the modern, fast-paced global economy, both accuracy and speed of seed testing are crucial. Seed producers, dealers, and companies rely on the availability of quick testing results to make swift and well-informed marketing decisions.

Effects of prechilling treatment and location of seeds on plants on germination

Seeds of ML and BB were germinated at weekly intervals for four weeks. The ANOVA showed that germination rate differed significantly ($P \leq 0.001$) from week to week and from the upper to the lower seeds on plants at $P \leq 0.001$. However, the differences attributable to varieties and prechilling treatment were not significant at $P \leq 0.05$ (Table 1). Nevertheless, the

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interaction between location of seeds on plants and prechilling treatment was highly significant ($P \leq 0.001$) indicating that the effect of prechilling treatment differed between seeds harvested from the upper and the lower parts of the plants (Tables 1 and 2). Moreover, the 3-way interactions between location of seeds on the plants, prechilling treatment, and weekly germination were significant at $P \leq 0.05$ (Table 1), indicating that germination did not follow a consistent pattern in response to seed location on the plants and the prechilling treatment over the 4-wk study period. This suggested that the response of seeds with different level of maturity to the prechilling treatment over time is not consistent. Therefore, prechilling the seeds if there is any doubt of dormancy presence is recommended. The significant difference ($P \leq 0.01$) in interactions among week, variety, location, and chill treatment suggested that varieties contributed to the difference in germination over the 4-wk period of the study in response to prechilling and seed location (Table 1 and 2).

Table 2. Germination of chilled and non-chilled seeds of two hemp varieties tested 21 days after harvested. Seeds were harvested separately from the upper 2/3 and the lower 1/3 parts of the plants.

Position of seeds on the plant at harvest	Variety	No chill		Dormant seeds	Pre-chill‡		Dormant seeds
		Germ	Germ + Viable seeds by TZ†		Germ	Germ + Viable seeds by TZ	
		%					
Upper 2/3	ML	79	94	15	95	99	4
	BB	91	98	7	93	98	5
Lower 1/3	ML	95	98	3	91	96	5
	BB	96	98	2	96	98	2
<i>LSD (0.05)</i>		4.1					

† Ungerminated seeds at the end of the germination test were tested by TZ to determine if they were dead or dormant.

‡ Seeds were prechilled at 10°C for 5 d, and germinated afterwards for 7 d at 20-30°C.

In the first week, the SGT results of ML and BB seeds from the upper 2/3 part of the plants were 79% and 91%, respectively (Table 2), which did not show maximum potential viability as indicated by the TZ results which were 97% and 96% for ML and BB, respectively (Fig. 1). Surprisingly, seeds from the lower part of the plants showed little dormancy and higher germination after 21 days than seeds from the upper of the plants (Table 2). This might suggest

the presence of short lived dormancy in ML and BB. It may also be attributed to the random distribution of dormancy seeds within the seed lot from where the 400 seeds were used for the SGT. The seed used for the SGT happened to have a low number of dormant seeds. It is worthy to note that viability and vigor were tested three weeks after seeds were harvested, therefore, some after ripening had happened to the seeds before the beginning of testing.

The germination test results were lower than the TZ results in newly harvested seeds while dormancy was at its peak. The difference between TZ and SGT results was probably because of dormancy. The dormancy observed in the hemp seeds was not unusual as different species and varieties may have different levels of dormancy (Soares et al., 2016). In our study, TZ tests were conducted on the ungerminated seeds at the end of the SGT to determine whether they did not germinate because they were dead or dormant. Results showed that ML had more dormant seeds than BB, especially for non-chilled seed (Table 2). Apparently, prechilling treatment was effective in breaking dormancy (Table 2). The percentage of germination of seeds that received prechilling treatment was similar to the TZ test results for both ML and BB (Fig. 1 and Table 2). Some crops are known to exhibit various levels of dormancy, especially for newly harvested seeds (Soares et al., 2016). This type of dormancy disappears gradually over time by a physiological phenomenon called “after ripening” (Copeland and McDonald, 2001). The maximum germination potential of such seeds is attained when the dormancy is completely broken. Since all viable seeds are stained red in the TZ test regardless of the dormancy level, it is not uncommon to observe higher TZ results than that of SGT in newly harvested seeds. In SGT, some seeds may not reach maximum germination potential because of dormancy, therefore the TZ test is a useful tool to differentiate between viable and nonviable seeds when seeds are dormant.

The germinability of seeds from the upper and the lower parts of the plants were similar in ML and BB from 28 d to 42 d after harvest (Table 3). The germination of non-chilled seeds of ML harvested from the upper 2/3 part of the plants ranged between 93-97%, compared to 92-98% for the lower 1/3 part of the plants.

Similar results were obtained for the prechilled seeds, where the germination ranged between 94-96% in the upper 2/3 of the plants, and 92-98% in the lower 1/3 of the plants. Similar germination results were observed in BB (Table 3). The high germination 28 d to 42 d after harvest suggested that the dormancy of most seeds had been broken by after ripening, and that ML and BB have short-lived dormancy periods. The similarity in germination between non-chilled and prechilled seeds after 28 d of harvest was a clear indication of the short-lived dormancy in these two varieties (Table 3). As such, the prechilling treatment becomes optional five weeks after harvest, since no increase in germinability was observed. Nonetheless, if there is any doubt that seeds might have dormancy, or the age of seeds is not known, prechilling is recommended to obtain maximum potential germination. Even if prechilling treatment did not increase germinability, it was observed that prechilled seeds of perennial ryegrass grew faster and more uniform than non-chilled seeds (Elias and Garay, 2008). In general, the SGT gives more accurate determination of seed viability than the TZ test because the physiological development of root and shoot structures is evaluated rather than just evaluating the color reaction of enzymatic activities in seeds, which can be more subjective in comparison with evaluating actual seedlings.

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Table 3. Germination test results (%) of chilled and non-chilled seeds of two hemp varieties tested 28-42 days after harvested. Seeds were harvested separately from the upper 2/3 and the lower 1/3 part of the plants.

Position of seeds on the plant at harvest	Variety	Days after harvest					
		28		35		42	
		No chill†	Pre-chill†	No chill	Pre-chill	No chill	Pre-chill
Upper 2/3	ML	97	94	94	96	93	94
	BB	93	94	93	96	90	92
Lower 1/3	ML	98	98	95	92	92	92
	BB	98	95	96	93	95	95
<i>LSD (0.05)</i>		2.4					

† Seeds were prechilled at 10°C for 5 d, and germinated for 7 d at 30-30°C. No TZ test was performed at the end of the germination tests on the seeds that did not germinate.

Vigor Tests

Accelerated aging test (AAT): Preliminary trials were conducted using varied combinations of temperatures (ranging from 41 to 45°C) and time span (ranging from 24 to 72 h) to determine the AAT parameters. It was found that 43°C for 48 h is suitable to differentiate among seeds with different quality levels. The ANOVA showed that neither varieties nor location of seeds on the plant significantly ($P \leq 0.05$) affected seed vigor as measured by the AAT (Table 1). However, the interaction between these two factors was highly significant ($P \leq 0.001$), indicating that germination of seeds harvested from the upper and the lower parts of the plants did not show a similar pattern in ML and BB (Fig. 2). For example, the AAT result of ML was better in seeds from the lower part of the plants (70%) than from the upper part (64%), and vice versa in BB (Fig. 2). Seeds from the lower 1/3 part of the plants were harvested 7 d after the upper 2/3 of the plant, which gave seeds time to catch up with maturation, and thus improving their quality. In addition, random sampling variation and variability in vigor within individual seeds within the same seed sample may have contributed to the inconsistency in germination and vigor between the upper and the lower parts of the plants, and between varieties as well.

The AAT results suggested seed vigor from the lower part of hemp plants can be improved if harvest is delayed by 7 d. This will give seeds in the lower part of the plants an opportunity to mature. However, delaying harvest may result in increasing seed shattering from the upper part of the plants, especially under warm, dry, windy conditions. Therefore, finding the balance between maximum seed yield, optimum maturity, and minimum shattering is critical when determining the optimum harvest date.

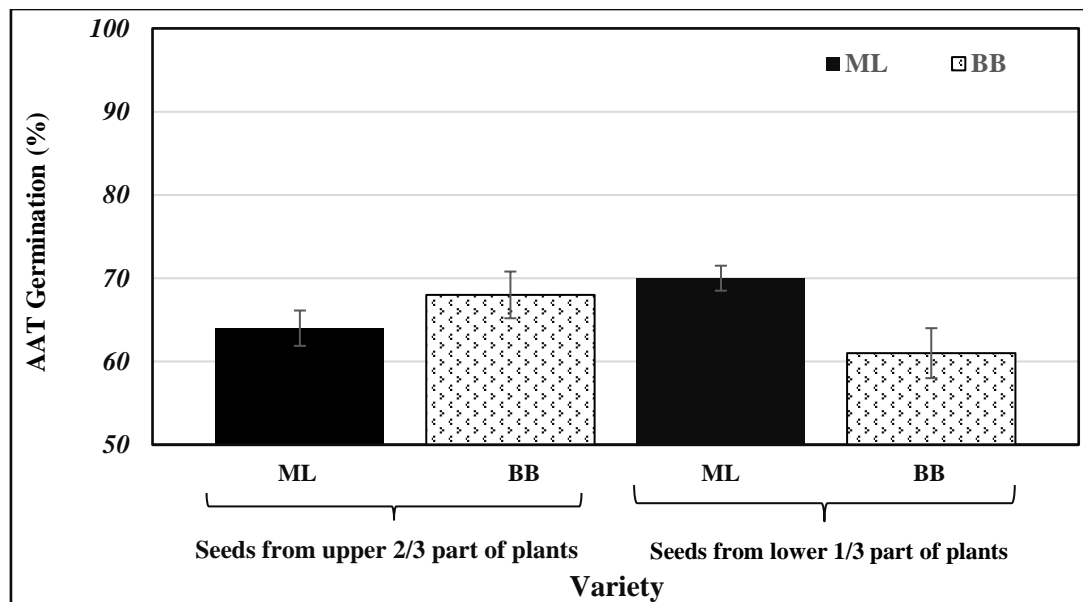


Figure 2. Vigor by accelerated aging test (AAT) of seeds harvested separately from the upper 2/3 and the lower 1/3 of the plants of Merlot (ML) and Berry Blossom (BB) hemp varieties. Means (columns) overlapped bars are not significantly different from each other ($P \leq 0.05$).

When the TZ test was conducted on the ungerminated seeds at the end of the AAT, it was found that 19-25% of the remaining seeds were viable (data not shown). This suggests that the stress caused by high temperature and RH in the AAT may have induced secondary dormancy, where seeds did not germinate after being exposed to such adverse conditions. This might also explain the low AAT results compared to the initial quality of seeds which ranged between 97- 98% by the TZ test.

Speed of germination index (SGI): Number of germinated seeds was counted daily starting from day two until the end of germination test (day 7). Data for the SGI were collected in the first two weeks of study, i.e., seeds that was planted after 21 and 28 d after harvest. The ANOVA showed that neither varieties nor location of seeds on the plant significantly affected ($P \leq 0.05$) SGI, but prechilling treatment significantly ($P \leq 0.001$) improved speed of germination (Table 1 and Fig. 3). The interactions among seed location in the plants (i.e., upper and lower parts), prechill treatment, and variety were significant ($P \leq 0.001$), indicating that the speed of germination of seeds of different varieties, from different locations on the same plant, whether or not they were chilled, they did not necessarily follow similar pattern (Table 1).

In the first week, the average SGI of non-chilled seeds of ML and BB ranged between 15-19, whereas the average SGI of chilled seeds ranged between 38-43 (Fig. 3).

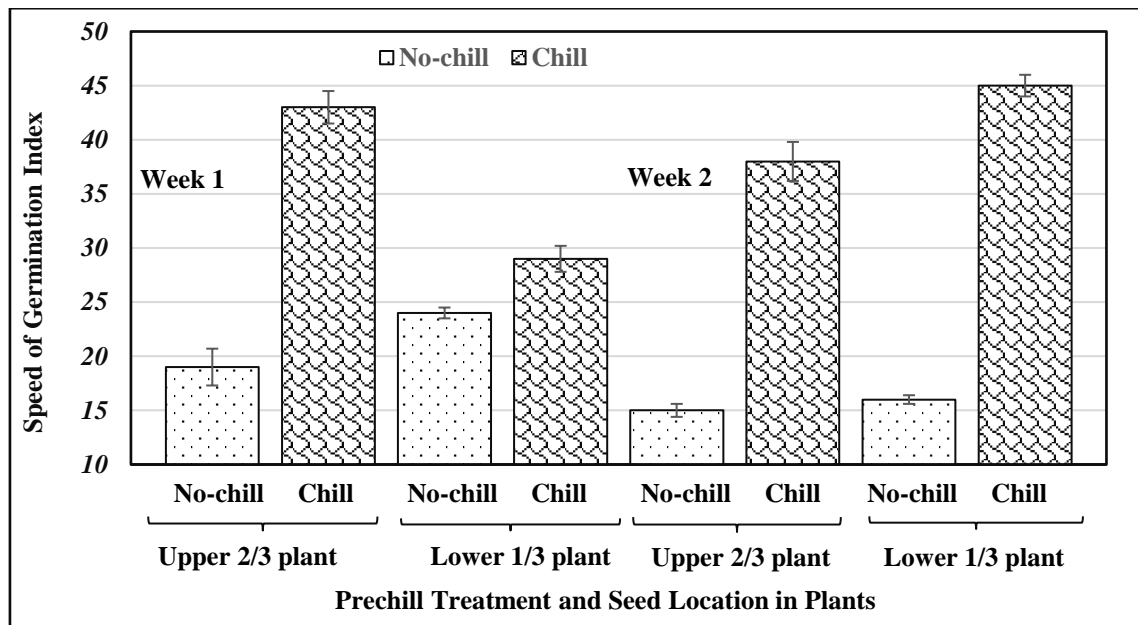


Figure 3. Speed of germination index (SGI) of seeds harvested separately from the upper 2/3 and the lower 1/3 parts of the plants of chilled and no-chilled seeds averaged over two hemp varieties. Means (columns) with overlapped bars are not significantly different from each other ($P \leq 0.05$).

These results indicate that the emergence of chilled seed was significantly faster than non-chilled seeds. Similar trend was observed in seeds planted at week two, where the average SGI of non-chilled seeds ranged between 16-24, and ranged between 29-45 for chilled seeds (Fig. 3).

Prechilling treatment at 10°C for 5d was adequate in breaking seed dormancy. In addition to breaking dormancy, prechilling serves as hydropriming because during prechilling, the germination enzymes start to be activated and the stored food begins to break down and move into the embryonic axes. Once seed is moved to warmer temperature, i.e., 20-30°C, it grows fast and uniformly. This agrees with a report by Elias and Garay (2008) who observed that prechilled seeds of perennial ryegrass grow faster and more uniform than non-chilled seeds.

CONCLUSIONS

ML and BB hemp varieties had high initial seed quality as indicated by the TZ viability test. The difference in seed size between the two varieties did not affect seed quality. Both varieties had short-lived dormancy of approximately 35-42 d. The germination test results were lower than the TZ results in newly harvested seeds while dormancy was at its peak. The prechilling treatment at 10°C for 5 d was effective in breaking the dormancy. We recommend prechilling newly harvested seeds and whenever the age of seeds is not known at 10°C for 5 d before the standard germination test to break dormancy and achieve maximum germination.

The AAT at 43°C for 48h, followed by germination at 20-30°C for 7 d was a good indicator of seed vigor. The results suggested that delaying harvest seeds by 7 d would give the lower part of hemp plant time to mature; and improved the overall quality of seeds. This makes the whole plant, upper and lower parts, available for harvest, increasing the yield by 1/3 because the current practice of some hemp farming systems is to harvest only the upper 2/3 part of the plants to avoid the underdeveloped seeds from the lower part.

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