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Determination of Germination and Emergence Performance of Hemp (*Cannabis sativa* L.) Seeds at Different Maturity Stages

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ABSTRACT

Seed maturation in hemp is not homogeneous, and the harvest is done when the seeds are 70-80% mature. This study was carried out to determine the germination and emergence performance of hemp (*Cannabis sativa* L.) seeds at different maturation stages. Mature, semi-mature and immature seeds were used as material in the study. The study was carried out as two separate experiments, germination and emergence. The germination test was carried out in petri dishes and the emergence test in viols. Both experiments were carried out according to the randomized complete block design with 3 replications. Germination rate (%), shoot and root length (mm), shoot and root fresh weight (mg plant^{-1}) and shoot and root dry weight (mg plant^{-1}) parameters were investigated in the germination study. In the emergence study, the emergence rate (%), seedling and root length (mm), seedling and root fresh weight (mg plant^{-1}), and seedling and root dry weight (mg plant^{-1}) values were examined. In the germination study, semi-mature seeds germinated but did not develop. Therefore, no data could be obtained from other parameters except germination rate. In germination study, germination rate varied between 6.67 and 84.67%, shoot length 27.2-38 mm, root length 39.4-50.8 mm, shoot fresh weight 30.66-49.89 mg plant^{-1}, root fresh weight 4.32-7.69 mg plant^{-1}, shoot dry weight 3.25-7.99 mg plant^{-1}, root dry weight 0.68-2.03 mg plant^{-1}. In the emergence study, emergence rate ranged between 5.18-82.69%, shoot length 68-136.4 mm, root length 37.4-69.6 mm, shoot fresh weight 60.33-154.80 mg plant^{-1}, root fresh weight 27.30-46.73 mg plant^{-1}, shoot dry weight 3.97-10.24 mg plant^{-1}, root dry weight 2.61-5.43 mg plant^{-1}. In both experiments, the highest values obtained from all the examined traits were obtained from mature seeds. In both studies, the highest values were obtained from mature seeds for all traits examined. Semi-mature and immature seeds gave similar results in terms of the traits examined.

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Introduction

Hemp (*Cannabis sativa* L.) is an annual, dioecious, and cross-pollinated plant that humans have cultivated since ancient times (Yilmaz et al., 2023). Hemp, as a fiber plant, has been used by humans mainly as a source of fiber, but its seeds and oil obtained from the plant extracts have also found widespread use. In this sense, it has been reported that different parts of hemp can be used in the medical, automotive and food sectors, especially in the textile industry (Pounds, 2010; Antonisomy et al., 2015).

In a successful production, obtaining the desired plant density and high yield depends on the fast, uniform and complete germination and emergence of the sown seeds (Elkoca, 2007). Hemp can be cultivated at varying plant densities depending on whether it is grown for seed or fiber production (Benevenute et al., 2022). Plant density in hemp affects yield and yield criteria by enabling the plant to benefit from light, water and nutrients effectively (Westerhuis, 2016; Tang et al., 2017; Yazici et al., 2020). Studies on the effect of plant density on fiber and seed yield in hemp have been conducted on a large scale such as 90 (Ranalli, 1999), 150-225 (Townshend & Boleyn, 2008) and 300 plants/m^2 (Hall et al., 2014). However, even if the sowing is done at a level to obtain the appropriate plant density, the chance of obtaining the desired quality fiber and seed decreases if uniform germination does not occur due to environmental or seed-borne factors.

In plant production, the first step of cultivation is sowing the seed under suitable conditions and ensuring its emergence (Karakurt et al., 2010). However, germination is affected by environmental factors such as temperature, humidity, and soil salinity on one hand (Karakurt et al., 2010), and lack of uniformity in genetic structure, seed maturity, and seed size, on the other hand (McDonald, 2000; Karakurt et al., 2010). Therefore, the rate of germination and emergence decreases and the desired plant density is not achieved (Elkoca, 2007).

Seed maturation in hemp is not homogeneous, it occurs from the bottom up in the plant. It is risky to wait for all the seeds to mature for harvest, as early maturing seeds tend to shed (Madia et al., 1998; Marzocchi & Caboni, 2020). It has been observed that even in the harvest made when the seeds are 70-80% mature in hemp, some seeds cannot fully mature (Yılmaz et al., 2021). For this reason, this study aimed to determine the germination and emergence parameters of hemp seeds at different maturity levels.

Materials and Methods

Materials

This study was carried out in Yozgat Bozok University, Faculty of Agriculture laboratories in two different ways, as germination and emergence trials in 2022. The seeds used in this study were obtained from the studies carried out for seed production in 2021 by Yılmaz et al., (2021) in Yozgat Bozok University, Institute of Hemp Research, Department of Agriculture and Food. In the study, seeds belonging to the Narlısaray hemp population were used as material. Seeds were divided into 3 different groups according to their maturity, and the characteristics of each group are given in Table 1 and Figure 1.

Methods

The germination study was carried out in the germination chamber, using petri dishes (100 × 20 mm). The study was carried out according to a randomized complete block design with 3 replications and a total of 50 seeds were used for one replication of each treatment. The selected seeds were first sterilized with 5% Sodium Hypochlorite for 5 minutes, then placed between blotting paper (as a germination medium) in petri dishes. The Petri dishes were incubated at 20°C (Suriyong et al., 2015) and 8 h light, 16 h dark cycle (Geneve et al., 2022) in germination chamber. The seeds in the germination medium were monitored and the embryonic roots emerging from the testa were considered germinated when they reached 1 mm and the germinated seeds were counted daily for seven days (Suriyong et al., 2015; Hu et al., 2018). At the end of the germination study, germination rate (%), shoot and root length (mm), shoot and root fresh weight (mg plant^{-1}) and shoot and root dry weight (mg plant^{-1}) were measured.

The emergence study was carried out in the climatic chamber, in peat-filled viols (50x50 mm), with 45 seeds in each viols according to a randomized complete block design with 3 replications. The seeds were sowed at a depth of 4 cm and the ambient temperature was adjusted to 18-23 °C (day and night), and the humidity was constantly controlled. The emergence study was terminated on the 21st day and, emergence rate (%), seedling and root length (mm), seedling and root fresh weight (mg plant-1), and seedling and root dry weight (mg plant-1) were measured.

Statistical Analysis

The data obtained from both studies were analyzed with the JMP statistical package program according to the randomized complete block design (RCBD), and mean values were compared according to LSD.

Figure 1. Hemp seeds at different maturity levels (A: Mature, B: Semi-mature C: Immature).

Table 1. Information on hemp seeds at different maturity stage

Seed group	Seed characteristics
Mature seeds	Seeds that have completed their physiological maturity, have reached the ideal seed size and seed coat hardness, have completed formation of ideal testa (seed coat) color (brown color) and have a moisture content of around 10% (±2). The average 1000 seed weight is 17.04 g and the width and length dimensions of the seeds are 3.24-4.81 mm.
Semi-mature seeds	Seeds that although have completed the green-yellow maturation period, have not completed their physiological maturity. The testa (seed coat) color is between green and light brown, the seed coat has not reached the ideal hardness, and the moisture content is around 15% (± 2%). The average 1000 seed weight is 7.01 g and the width and length dimensions of the seeds are 2.76-4.47 mm.
Immature seeds	Seeds that are still in the green-yellow maturation period and have a green color testa (seed coat). These seeds have not reached the ideal seed coat hardness and seed size, and have a moisture content of around 20% (± 2). The average 1000 seed weight is 6.89 g, and the width and length dimensions of the seeds are 1.97-3.42 mm.

Table 2. Variance analysis results (F values) and significance levels of the germination study of hemp seeds at different maturity stage

SV	df	GR	SL	RL	FSW	FRW	DSW	DRW
Maturity stage	2	394.57*	610.45*	647.56*	832.12*	124.47*	1311.19*	1391.27*
Replication	2	0.00	0.70	0.39	0.01	1.56	0.01	3.15
CV		10.59	6.31	6.09	5.63	14.95	5.13	5.30

SV: source of variation, df: degree of freedom, GR: germination rate (%), SL: shoot length (mm), RL: root length (mm), FSW: fresh shoot weight (mg plant^{-1}), FRW: fresh root weight (mg plant^{-1}), DSW: dry shoot weight (mg plant^{-1}), DRW: dry root weight (mg plant^{-1}). *: p<0.05

Table 3. Average germination values of hemp seeds at different maturity stages

MS	GR	SL	RL	FSW	FRW	DSW	DRW
Mature seeds	84.67a	38.0a	50.8a	49.89a	7.69a	7.99a	2.03a
Immature seeds	14.67b	27.2b	39.4b	30.66b	4.32b	3.25b	0.68b
Semi-mature seeds	6.67b	-	-	-	-	-	-
LSD Value	8.48	3.11	4.07	3.42	1.36	0.44	0.11

MS: maturidy stage, GR: germination rate (%), SL: shoot length (mm), RL: root length (mm), FSW: fresh shoot weight (mg plant^{-1}), FRW: fresh root weight (mg plant^{-1}), DSW: dry shoot weight (mg plant^{-1}), DRW: dry root weight (mg plant^{-1}). -: Data could not be obtained because root and shoot formation after germination was not possible.

Table 4. Variance analysis results (F values) and significance levels of the emergence study of hemp seeds at different maturity stage

SV	df	ER	SL	RL	SFW	RFW	SDW	RDW
Maturity stage	2	1005.13*	589.06*	93.15*	304.85*	69.02*	325.98*	69.37*
Replication	2	0.70	0.19	0.08	0.68	0.19	0.86	0.26
CV		6.90	2.93	5.66	5.83	6.81	5.60	8.99

SV: source of variation, df: degree of freedom, ER: emerence rate (%), SL: seedling length (mm), RL: root length (mm), SFW: seedling fresh weight (mg plant^{-1}), RFW: root fresh weight (mg plant^{-1}), SDW: seedling dry weight (mg plant^{-1}), RDW: root dry weight (mg plant^{-1}). *: p<0.05

Results and Discussion

Germination

The variance analysis results of the data obtained from the germination study are given in Table 2. As shown in Table 2, statistically significant differences (p< 0.05) were determined in all parameters examined among hemp seeds of different maturity.

The highest germination rate (GR) (84.67%) in hemp seeds of different maturity was obtained from mature seeds, while the lowest value (6.67%) was obtained from semi-mature seeds. Mature seeds exhibited high values in shoot length (SL) (38.00 mm) and root length (RL) (50.80 mm), while immature seeds had the low values (27.2 and 39.4 mm, respectively). Fresh shoot weight (FSW) obtained from the germination study ranged from 30.66 to 49.89 mg plant^{-1}, and the fresh root weight (FRW) ranged from 4.32 to 7.69 mg plant^{-1}. In both parameters, the high values were obtained from mature seeds, and the lower values were obtained from immature seeds. High values in dry shoot weight (DSW) and dry root weight (DRW) (7.99 and 2.03 mg plant^{-1}, respectively) were obtained from mature seeds, while immature seeds had low values (3.25 and 0.68 mg plant^{-1}, respectively) (Table 3).

Emergence

The variance analysis results of the data obtained from the emergence study are given in Table 4. Based on the results it was determined that there were statistical differences (p<0.05) among the hemp seeds of different maturity in terms of all parameters examined.

In the emergence tests with hemp seeds of different maturity, the highest emergence rate (ER) (82.96%) was obtained from mature seeds. The lowest ER (5.18%) was obtained from semi-mature seeds, while this rate was determined as 13.33% for immature seeds. In the emergence study, the longest SL and RL were obtained from mature seeds with 136.4 and 69.6 mm, respectively, while semi-mature seeds had the lowest values (68.0 and 37.4 mm, respectively). While the highest value in terms of SFW (154.8 mg plant^{-1}) was obtained from mature seeds, this value was determined as 61.57 and 60.33 mg plant^{-1} for immature and semi-mature seeds, respectively. In terms of RFW, mature seeds reached the highest value with 46.73 mg plant^{-1}, the lowest values were 27.76 mg plant^{-1} for immature seeds and 27.30 mg plant^{-1} for semi-mature seeds. The SDW obtained from the emergence study ranged from 3.97-10.24 mg plant^{-1}, and the RDW was between 2.61-5.43 mg plant^{-1}. The highest values in both SDW and RDW (10.24 and 5.43 mg plant^{-1}, respectively) were obtained from mature seeds (Table 5).

In this study, mature seeds reached the highest values in terms of all parameters examined in both germination and emergence experiments. The effect of seed maturity on germination and vigor has been studied on many plants. It has been reported that maximum germination in most plants occurs when the seeds reach maximum dry weight, which is defined as physiological maturity (Samarah & Mullen, 2004). In most seeds, the transition from the developmental stage (in which dry matter and nutrient accumulation are complete) to a germination/growth-only stage is necessary for seed maturation and ideal germination. Although immature embryos have a certain germination capacity, they often cause abnormal seedling formation because the necessary developmental metabolism cannot be completed (Kermode, 1990; Rao et al., 2005).

Table 5. Average values of emergence study of hemp seeds at different maturity stage

MS	ER	SL	RL	SFW	RFW	SDW	RDW
Mature seeds	82.96a	136.4a	69.6a	154.80a	46.73a	10.24a	5.43a
Immature seeds	13.33b	73.4b	48.6b	61.57b	27.76b	4.14b	2.84b
Semi-mature seeds	5.18c	68.0b	37.4c	60.33b	27.30b	3.97b	2.61b
LSD Value	5.29	6.15	6.65	12.19	5.24	0.78	0.74

MS: maturidy stage, ER: emerence rate (%), SL: seedling length (mm), RL: root length (mm), SFW: seedling fresh weight (mg plant^{-1}), RFW: root fresh weight (mg plant^{-1}), SDW: seedling dry weight (mg plant^{-1}), RDW: root dry weight (mg plant^{-1}).

Storing hemp seeds in a sheltered but uncontrolled environment for 2 years reduces the germination rate to about 70-80% rather quickly and it is not recommended to use these seeds in sowing. In commercial applications, 85-90% of minimum germination of hemp seeds was recommended (Bósca & Karus, 1998; Small & Brookes, 2012). While the germination rate we obtained from mature seeds in our study (84.67%) matched the germination rates recommended for commercial applications, the germination rates obtained from immature or semi-mature seeds were far below these values (14.67% and 6.67%, respectively).

Harvest time significantly affects the thousand seed weight (Gu et al., 2017). It is reported that the thousand seed weight of hemp seeds varies between 12-30 g (İncekara, 1979; Gönen, 2009; Gün, 2019; Dumanoğlu et al., 2021). While the thousand seed weights of the mature seeds we used in this study (17.04 g) are among the values reported for hemp, the thousand seed weights of the immature and semi-mature seeds are below these values (6.89 and 7.01 g, respectively). İncekara (1979) reported that hemp seeds are 4-6 mm in length and 3-3.5 mm in width, while Gün (2019) reported that they are 4 mm long and 3 mm wide. While the sizes of mature (length 4.81 mm, width 3.24 mm) and semi-mature (length 4.47 mm, width 2.74 mm) seeds we used in this study were like the dimensions reported for hemp, the dimensions of immature seeds were behind these values (length 3.42 mm, width 1.97 mm). Although the sizes of the mature and semi-mature seeds we used in this study are similar, there are differences in thousand seed weights. This situation shows that embryo and endosperm development in semi-mature seeds could not reach a sufficient level. According to the results we obtained from study, the values obtained from semi-mature seeds were similar to the values obtained from immature seeds. Seed size is an important factor affecting germination. Nutrients hidden in plant seeds are used for sprouting and also affect the growth and development of the plant (Dunlap & Barnett, 1983; Ambika et al., 2014; Moon et al., 2020). Moon et al., (2020) reported that small-sized hemp seeds are not suitable for germination, so seed screening is important to separate small seeds.

In many cultivars, seeds can germinate a few days after zygote formation. In this case, germination means a protrusion of the primary root, not a normal seedling formation. Because histo-differentiation is incomplete and nutrient reserve accumulation in the seed is still in its beginning at this stage. The germination described here leads to the production of seedlings with low viability (Leishman, 2001). Theoretically, it is possible to state that the germination rate of seeds increases with maturation and germination reaches its maximum when the seeds reach maximum dry weight (Bareke, 2018).

Harvesting seeds at different maturation periods can change germination rates (Miyajima, 1997; Gu et al., 2017; Yağız, 2019). The physiologically mature seeds have a positive effect on storability and germination, and they also perform better in field conditions (Doijode, 1988; Pallais et al., 1989; Rao et al., 2005). Seed maturity affects not only seed germination but also seedling development. In addition, immature seeds are more susceptible to diseases and adverse environmental conditions (Singh & Singh, 1992; Negi & Todaria, 1995).

Rao et al., (2005) reported that seed viability, evaluated based on germination rate and seedling length in tomato plants, was at the highest level in mature seeds compared to immature and over-mature seeds, while the maximum germination rate (86.5 %) was obtained from mature seeds. Zecevic et al., (2006) reported that mature seeds of wheat plants significantly affect shoot and root dry matter content, and the amount of both dry matters increases from the milk maturity stage to the full maturity stage of the seeds. The low amount of dry matter in seedlings obtained from seeds that have not reached full maturity is due to the low nutrient reserves in endosperm and seed weight (Zecevic et al., 2006). In this context, it has been reported that growth is weak in immature seeds with low nutrient content, whereas mature seeds with high nutrient content allow stronger seedling growth and higher dry matter content (Babayan, 1960; Boyd et al., 1971; Pucaric & Ujevic, 1986; Zecevic et al., 2006).

Conclusion

In this study, the germination and emergence performances of hemp seeds at different maturity stages were evaluated, the highest values in terms of all parameters examined were obtained from mature seeds. The use of seeds that are not suitable for hemp cultivation will cause the desired density not to be achieved, thus reducing the yield and quality. In this sense, impurities and seeds that have not reached full maturity should be eliminated from seeds to be used in hemp cultivation. In addition, it is necessary to attach importance to the production of seeds suitable for hemp cultivation, to take necessary steps in this regard and to raise awareness of farmers who turn to hemp farming.

Declarations

Author Contribution Statement

Y.G., Y.C., G.N.D.: Concept; Y.G., Y.C., G.N.D.: Desing; Y.C., G.N.D.: Data Collection or Processing; Y.G., Y.C.: Statistical Analyses; Y.G., Y.C., G.N.D.: Literature Search; Y.G., Y.C., G.N.D.; Writing, Review and Editing.

Fund Statement

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Conflict of Interest

We declare that there is no conflict of interest between us as the article authors.

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