

## The effect of Hydro-priming on Germination of Mustard Seeds under Draught Stress Conditions

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**Abstract:** In order to investigate the effect of seed priming beside draught stress on mustard plant, an experiment was conducted in Agriculture Faculty Research Laboratory of Takestan University as a 4-replication Split-factorial layout within completely randomized plan in laboratory conditions in 2012. This experiment was composed of two seed priming levels (priming and no priming) and 5 drought stress levels (0.00, -1.50, -3.00, -4.50 and -6.00 bar). To create these stress levels, crystalline polyethylene glycol 6000 was used as solute. The results showed that seed priming has a meaningful effect on germination percentage, germination rate and seedling vigor index in 1% significance level. Also they indicated that drought stress has a significant effect on germination percentage, germination rate, seedling vigor index, coleoptile length, radicle length and seedling dry weight. Mutual effect of priming and drought stress on germination percentage, seedling vigor index, radicle length and seedling dry weight was significant such that maximum radicle length (92.75mm), seedling dry weight (0.53gr), germination percentage (83%), germination rate (8.35d) and seedling vigor index (14.91) was obtained. The results showed that the stress could decrease measured parameters but in drought stress conditions, seed priming could increase germination percentage, germination rate, seedling vigor index, radicle length and seedling dry weight.

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### 1. Introduction

With white color (like yogurt), low branch, conical and straight roots, White Mustard plant (*Brassia alba Hook f. & Th. Or Sinapsis alba Linn*) belongs to *Cruciferae* family. The stem is straight and cylindrical full of more or less fine fluffs. The leaves have long petioles distributed and located alternatively along the stem and additionally notches are more or less deep full of fine fluffs on surface. The flowers are clustered, integrated and appeared at the end of stems and sub-stems. These flowers are composed of four green color sepals and four yellow color, rather circular, petals. Sepals and petals are located alternatively. The Silik type fruit is a bright green, sword shaped, full of fluffs, 3-4mm thick and 2-4cm long mass aligned in approximately 90° angle respect to stem axis in each of which is 3 to 7 more or less spherical, bright brown or opaque white, approximately 2-2.5mm in diameter seeds weighing each 4-7gr. With respect to chemical composition, it has been reported that there are some glycosides, lots of glaze, fixed oils (30%-35%), Sinapine, arachidic, tetracosanoic and 13-docosenoic (toxic) acids and also 9, 12-octadecadienoic acid, linamarase, lactase, myrosinase and phosphatase in white mustard seed. Fixed fatty oil available in white mustard seeds is golden yellow colored with pungent odor and taste, applicable more in industrial uses but white and pure type only consumed in domestic uses. It is nervous stimulant, emetic, carminative and laxative and when is used in external uses as poultice, candle

and rub solution, it acts for rubella, rheumatic and joint pains [1]. Germination is one of the most important stages of plant growth which influences on durability, stability and final function of crops [3]. Generally ground surface is alternately wetted for rather long time and often is rapidly dried without enough time for seed germination or establishment of young seedling. Therefore, ground water storage is one of the most important environmental factors to control germination and seedling establishment. Base water potential is among parameters which should be determined for seeds of each plant in order to identify germination components under drought stresses. It is minimum water potential that let complete germination process. The relationship between water potential and germination rate is linear, the higher the water potential from the base, the higher is germination rate [2].

Seed germination and seedling establishment are most sensitive stages of plant growth in pastures and crops under environmental stresses (drought, salinity, heat and cold conditions). Using any technique able to improve these stages of growth under mentioned conditions is important. Using seed priming (germination stimulation before planting) technique has been taken into consideration by many researchers to confront environmental stresses and to increase establishment efficacy in crops and pastures.

Researchers are to increase seedling establishment under stress conditions and seed priming is proposed as a strategy to increase plant establishment

especially in adverse conditions. Seed priming, in fact, is a type of treatment before seed germination and the seeds are kept in water potential.

Generally, seed priming aims to pre-activate two initial phases of seed germination, imbibition and digestion, before planting. Seed priming advantages are increasing establishment and germination percentage and rate especially under different environmental stresses, increasing seedling vigor and uniform emergence, better competition with weeds, higher growth rate and finally higher efficacy.

Seed priming causes to stimulate germination and increase its rate, remove phenol compositions from seed covers and increase moisture absorption by the seed, abscisic acid hydrolysis, remove seed dormancy, supplement seed feeds, increase seed vigor, seed enrichment, enhance seedling initial power, more green cover, less needs to gap filling, better competition with weeds and finally higher efficacy [16]. Also, it has been effective in rapid root extension, optimal use of moisture available in the ground, increase germination rate in low temperatures and lack of moisture and subsequently accelerated greening of crop species on the farm. This technique results in uniform establishment of commercial vegetable seedlings and flower seeds [14 and 15]. It reduces the time between seed culture and seedling emergence and causes to uniform greening. Successful increase of water absorption during seed priming which is useful to activate enzymes especially hydrolase is obtained by osmoharding [12]. Beweley and Black (1978) and Khan (1992) reported that greening duration on the farm can be decreased to 50% by priming. Before soil crusted and being damaging, seed priming helps to exit seedling from the ground.

Fujikura et al. (1993) studies showed that using hydro priming technique caused to increase rate, range and percentage of seed germination under drought stress. In this technique, the seed is given chances to absorb some water and proceeds with until second stage of hydroseeding but enters third stage and radicle emergence from seed, not at all [10]. This study was conducted to investigate the effect of hydro priming on improving germination components of mustard seeds under drought stresses.

## 2. Methods and materials

This experiment was conducted in Agriculture Faculty of Islamic Azad University of Takestan in 2012 as a 4-replication Split-factorial layout within completely randomized plan. The treatments included seed priming in two levels (priming and no priming) and 5 drought stress level (0.00, -1.50, -3.00, -4.50 and -6.00 bar). Drought potentials were calculated by Michel-Kaufman equation and using polyethylene glycol 6000. Distilled water was used for drought level zero. In order to treat hydro primed mustard seeds,

Toselli and Casenave method was implemented. In the latter method, mustard seeds were maintained in water at 25°C for 8hr and then removed from water and dried in laboratory conditions. The seeds were disinfected by 2.5% sodium hypo chloride for 1min and for each replication, 25 seeds, healthy and randomly selected, placed inside a Petri dish (9cm opening diameter) on filter paper and sterile Whatman 1 cellulose. Then, 20ml of provided concentrations were added each specimen and finally Petri dishes were transferred to germinator at 25±2°C and complete darkness. The germinated seeds were daily counted at certain hours. Germination criterion was 2ml emergence of radicle from the seed. At the end of 8<sup>th</sup> day when germination was fixed, radicle length, coleoptile length and seedling dry weight of germinated seeds were measured. Germination rate, percentage and seedling vigor rate were calculated from equations 1, respectively:

$$\text{Seedling Vigor Index} = \text{Germination (\%)} \times \text{Seedling Length (cm)}, \quad (1)$$

where GP is germination percentage; S the number of germinated seeds counted at 8<sup>th</sup> day; T the number of seeds inside Petri dish; Rs germination rate; si the number of germinated seeds at i<sup>th</sup> day; Ni days to i<sup>th</sup> count down. Percentage data were converted to angular data. Data analysis was conducted by SAS software and LSD test at 5% probability level was used to compare means.

## 3. Discussion and results

The results obtained from variance analysis indicated that seed priming has effect on germination percentage at 1% significance level. Maximum germination percentage was provided by 50% priming. Seed priming causes to germination stimulation and increasing its rate, removing phenol compositions, covering seeds, increasing moisture absorbed by seed, abscisic acid hydrolysis, removing remove seed dormancy, supplement seed feeds, increase seed vigor, seed enrichment, enhance seedling initial power. Also, it has been effective in rapid root extension, optimal use of moisture available in the ground, increase germination rate in low temperatures and lack of moisture and subsequently accelerated greening of crop species on the farm. Maximum was obtained at 4.67% priming level. This result was confirmed with Tyler et al. results. Priming also affected seedling vigor index at 1% significance level such that highest seedling vigor index was achieved by 6.69 priming.

The results of variance analysis showed that drought stress has affected germination percentage at 1% significance level. Maximum germination percentage was achieved by 0.78% zero drought stress. Higher the drought stress, lower was the seed germination percentage (decreasing germination process resulted from drought stress can be related to decrease water absorbed by the seeds. If water

absorption by the seed is impaired or water is absorbed slowly, then the time to emerge radicle from the seed increases and as a result germination rate will be decreased.

Drought stress has an effect on coleoptile at 1% significance level such that maximum coleoptile length 65.88mm was resulted from zero drought stress. Increasing polyethylene glycol consumption, coleoptile length was decreased such that at -6.00 drought stress level, the seeds had no germination power. The results were confirmed by Nantiyal and Uniyal (1998) studies on a type of *ougeinia dalbergioides Benth.* The results indicated that stress had an effect on radicle length at 1% significance level. Maximum radicle length 89.25mm was resulted from zero drought stress. Often, the root is less influenced by water stress than aerial organs. This may because of the mechanism responsible for accumulation of soluble materials, especially amino acid Proline, and turgor maintenance some times after the stress (Coyuela et al. 1996). Growth of coleoptile and radicle organs is interdependent and decreasing growth of each one will resulted in increasing the other growth. First stage in seed germination process is water absorption and seed inflation and the final is cell division and cells getting larger which causes to seed radicle and coleoptile emergence. Having decreased water absorbable by the seed because of increasing osmosis potential of the solution around the seed, cell division is reduced and seedling growth is impaired. Whereas radicle emerges from seed shell before coleoptile, radicle process initiates earlier and if there is shortage, then coleoptile process will be delayed further [5]. The results demonstrate much lower coleoptile growth, if increase osmosis potential, than radicle [4].

Drought stress had an effect on seedling dry weight at 1% significance level such that maximum 0.52gr was resulted from zero drought stress. Having increases stress intensity, seedling dry weight was decreased drastically. Also, drought stress has an influence on germination rate at 1% significance level. Maximum germination rate 6.18 was related to zero stress level. The results showed that drought stress has an effect on seedling vigor index at 1% significance

level such that maximum seedling vigor rate 13.32 was resulted from zero stress level.

The results of variance analysis showed that mutual effect of seed priming and drought stress on germination percentage is at 1% significant level such that maximum germination percentage 0.83 was resulted from seed priming besides zero drought stress. Fujikura et al. (1993) researches showed that using hydro priming method caused to increase range, percentage and rate of seed germinations under drought stress conditions. In this technique, the seed is given chances to absorb some water and proceeds with until second stage of hydroseeding but enters third stage and radicle emergence from seed, not at all.

The results showed that mutual effect of seed priming and drought stress on radicle length is at 5% significance level such that maximum radicle length 92.75mm was resulted from seed priming beside zero drought stress which was classified beside 85.72mm in similar statistical group. Mutual effect of seed priming and drought stress on seedling dry weight was at 1% significance level. Maximum seedling dry weight 0.53gr was achieved by seed priming besides zero drought stress which was classified beside 0.52gr in similar statistical group. Mutual effect of seed priming and drought stress on germination rate was at 1% significance level. Maximum germination rate 8.35 was resulted from seed priming beside zero drought stress. The results showed that mutual effect of seed priming and drought stress on seedling vigor index was at 1% significance level such that maximum seedling vigor index 14.91 was achieved by seed priming beside zero drought stress.

#### 4. Conclusions

With regard to the results, it can be inferred that under low water stress conditions, germination percentage and germination rate of the plant will be reduced intensely such that at -6.00bar drought stress level, no germination occurred and seed priming will also cause to increase germination percentage and rate. Therefore it can be said that under low water stress conditions, seed priming action will cause to increase germination percentage, germination rate, seedling vigor index, radicle length and seedling dry weight.

**Table 1:** Variance analysis and priming and drought stress with investigated characteristics related to mustard herb

variation sources	DOF	G%	Coleoptile length	Radicle length	Seedling dry weight	Germination rate	Seedling vigor index
Priming	1	0.15**	5.62	67.60	0.0001	32.41**	39.78**
Drought stress	4	0.75**	5902.15**	9549.60**	0.33**	61.48**	227.25**
Priming x drought stress	4	0.013**	18.37	81.85*	0.002**	2.97**	3.20**
error	30	0.003	10.47	28.30	0/001	0/13	0/001
Coefficient of variations		%23.12	%12.68	%10.70	%8/80	%9/65	%4/97

\*, \*\* and \*\*\* indicate very meaningful ( $p \leq 0.01$ ), meaningful ( $p \leq 0.05$ ) and no meaningful differences, respectively.

**Table 2:** Comparing mean simple effect of priming and drought stress on studied properties in mustard herb

	treatments		Coleoptile length		Radicle length		Seedling dry weight		Germination rate		Seedling vigor	
	G%		Mm		Mm		Gr		day		index	
No priming	a	1403	ab	18/88	a	2/31	a	31/80	a	2/32	a	239/1
priming	b	823/9	ab	14/41	b	1/98	a	29/73	a	2/20	a	203/6
No use	a	0/78	a	65/88	a	89/25	a	0/52	a	6/81	a	13/32
-1.50 bar	b	0/62	b	40/25	b	68/75	b	0/32	b	5/71	b	8/65
-3.00 bar	c	0/51	c	13/75	c	58/25	c	0/19	c	4/33	c	4/37
-4.50 bar	d	0/28	d	7/75	d	32/25	d	0/11	d	1/98	d	2/15
-6.00 bar	e	0	e	0	e	0	e	0	e	0	e	0

**Table 3:** Comparing mean mutual effect of priming and drought stress on studied properties in mustard herb

	Priming	Drought stress	Coleoptile length	Radicle length	Seedling dry weight	Germination rate	Seedling vigor rate
priming	No use	0/73 ab	65 a	92/75 a	0/53 a	5/27 cd	11/73 b
	-1.50 bar	0/52 cd	41 b	64/75 b	0/33 b	4/57 d	7/70 d
	-3.00 bar	0/42 de	11 cd	54 c	0/15 d	3/10 e	2/88 g
	-4.50 bar	0/21 f	8/75 d	30/50 d	0/10 d	1/40 f	1/20 h
	-6.00 bar	0 g	0 e	0 e	0 e	0 g	0 i
No priming	No use	0/83 a	66/75 a	85/75 a	0/52 a	8/35 a	14/91 a
	-1.50 bar	0/72 b	39/50 b	72/75 b	0/31 b	6/85 b	9/60 c
	-3.00 bar	0/61 c	16/50 c	62/50 bc	0/22 c	5/57 c	5/87 e
	-4.50 bar	0/35 e	6/75 d	34 d	0/11 d	2/57 e	3/10 f
	-6.00 bar	0 g	0 e	0 e	0 e	0 g	0 i

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