



Effect of Milk and Hydro-priming on Seed Germination Characteristics of Bitter Gourd (*Momordica charantia* L.)

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ABSTRACT

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To meet the demand for an appropriate soaking duration and priming type, there is a need to embrace cheap, fast, natural, accessible, and adaptable physiological techniques as soaking of seeds in water and fresh cow milk. Fresh cow milk contains hormones that relieve seeds from photo, thermo, physiological and mechanical dormancy. In light of this, this study was conducted to assess the effect of hydration and fresh cow milk on different seed quality attributes like germination percentage, mean germination time, the uncertainty of germination process, and synchrony of germination process of bitter gourd seeds. The study involved two factors namely hydropriming and different concentrations of fresh cow milk (viz. 60%, 80% and 100%), and the other factor was seed soaking duration with 4 levels (i. e., 0, 12, 18 and 24 hours). The experiment was conducted in a Complete Randomized Design (CRD) with three replicates. Results revealed that the percentage germination value of seeds soaked in all concentrations of fresh cow milk was better than hydropriming. A significant germination percentage value of 96% and 93% were recorded for seeds treated for 18 hours in 80% and 100% concentrations of fresh cow milk respectively. Maximum uncertainty of the germination process and minimum synchrony of germination the process was recorded in untreated seed.

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Introduction

Bitter gourd (*Momordica charantia* L.) usually called Karela or balsam pear belongs to family Cucurbitaceae. It is one of the most important summer vegetable crop in Bangladesh. It is a widespread vegetable grown in Asia and other parts of the world. It is usually grown as an annual crop, but it can also perform as a perennial in mild areas and frost-free winters as well. The summer season crop is sown from January to June in the plains (Singh et al., 2006). The optimum temperature of 25-28°C is required for the germination of bitter gourd seeds (Peter et al., 1998). Bitter gourd has tremendous economic and dietic importance. Immature fruit is a good source of Vitamin C, and also contains Vitamin A. Bitter gourd is a blood purifier, activates spleen and liver and is highly beneficial in diabetes (Yibchok-Anun et al., 2006). The bitter gourd is very much helpful in curing diabetes. Studies have shown that bitter melon has anti-carcinogenic properties and can be used as a cytotoxic agent against many types of cancer (Grover and Yadav, 2004). Emergence is always a

problem in bitter gourd even with the seeds of high germinability due to thick seed coats. To overcome this problem, pre-sowing treatments -soaking or priming of seeds can be practice. Pre-germinated seeds proved superior in the emergence and in stand establishment. The additional advantages of this treatment include, (i) lower seed rate, (ii) dead seeds can be discarded before sowing, and (iii) germinating, but low vigor seeds can be removed before sowing. Recent studies demonstrate speedy germination, early emergence, and vigorous seedlings accomplished by seed soaking in water and milk for a while, followed by surface drying prior to sowing, which may lead to higher crop yield (Harris et al., 2000). This practice of soaking is expressed that seed priming is a simple, inexpensive, and less risk process of improving faster seedling establishment and vigorous early crop growth. Each crop cultivar requires a critical soaking duration and it should be less than the safe limit (Harris et al., 2000). Pre-sowing seed treatments resulted in higher

germination and earlier seedling emergence, strong growth, early flowering, maturity, and high yields. Speedily germinating seedlings also produce a deep root system and improved seedling establishment in many crops. Seed soaking is a useful to bitter gourd growers under sub-optimal temperature condition for definite successful seedling establishment (Wang et al., 2002; Lin and Sung, 2001). But the literature in Bangladesh in this context is very little. The present research work was conducted in order to study the effect of milk and hydropriming with different soaking duration on the germination and seedling vigor of bitter gourd.

Materials and Methods

The experiment was laid out at the research laboratory of Seed Technology Division, BARI, Joydebpur, Gazipur, Bangladesh during 2019-2020 in a two factor Complete Randomized Design (CRD) and each treatment was replicated 3 times. The variety of bitter gourd was BARI Korolla-1.

Factor A: Types of priming i.e. hydropriming, and priming with 60% fresh milk, priming with 80% fresh milk and priming with 100% fresh milk of cow.

Factor B: Soaking duration with four levels i.e. 0 h, 12 h, 18 h, and 24 h.

Data were recorded considering the following parameters:

- Germination percentage
- Mean germination time
- Uncertainty of the germination process
- Synchrony of the germination process
- Initial seed weight and seed weight after treatment
- Amount of water absorbed at different treatment

Seed germination was conducted using sand media at room temperature (25°C). Seeds were soaked in water and 60%, 80% and 100% fresh milk for 12 h, 18 h and 24 h. Initial weight of seed was measured and weight was taken after 12 h, 18 h, and 24 h soaking. The difference between initial weight and weight after soaking for 12 h, 18 h, and 24 h provides the amount of absorbed water.

Twenty-five seeds with three replications were used and expressed in percentage. Then the percentage data were transformed in an Arcsine Transformation.

The mechanics of data transformation are greatly facilitated by using a table of the arc sine transformation. The value of 0% was substituted by (1/4n) and the value of 100% by (100 - 1/4n), where n is the number of units upon which the percentage data was based.

The arcsine transformations were used because the percentage of data ranged from 0 to 100%. Before the transformation, all zero values are replaced by [1/4(25)] and all 100 values by (100 - [1/4(25)]}. The transformed data are shown (following the procedure of Chapter 2, Section 2.1.2- Statistical Procedure of Agricultural Research) is in Table 1. For comparisons of all possible pairs of means, the values were first applied to the transformed means and then transferred to the original means.

Mean germination time was calculated by the Equation 1:

$$\bar{t} = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i} \quad (1)$$

Where, t_i : time from the start of the experiment to the i^{th} observation (day for the example); n_i : number of seeds germinated in the i^{th} time (not the accumulated number, but the number correspondent to the i^{th} observation), and k : last time of germination (Labouriau and Valadares, 1976; Labouriau, 1983; Ranal and Santana, 2006). Uncertainty of the germination process was calculated by the expression:

$$U = - \sum_{i=1}^k f_i \log_2 f_i, \text{ being } f_i = \frac{n_i}{\sum_{i=1}^k n_i} \quad (2)$$

Where, n_i : number of seeds germinated in the i^{th} time and k : last time of germination (Labouriau and Valadares, 1976; Labouriau, 1983).

Synchrony of germination was calculated by the expression:

$$Z = \frac{\sum_{i=1}^k C_{n_i,2}}{C_{\sum n_i,2}}, \text{ being } C_{n_i,2} = \frac{n_i(n_i-1)}{2} \quad (3)$$

Where, $C_{n_i,2}$: combination of the seeds germinated in the i^{th} time, two by two; n_i : number of seeds germinated in the i^{th} time (Labouriau and Valadares, 1976; Labouriau, 1983).

Data Analysis

The data collected on the effect of soaking duration and types of priming on germination and related parameters were subjected to two-way analysis of variance (ANOVA) using open-source R software. Separation of significant means was carried out using the Least Significant Difference (LSD). All percent of germination data were arcsine percentage transformation prior to analyses because it is appropriate for the percentage of data covering a wide range (Akindele, 2004; Gomez and Gomez, 2010; Sananse and Maidapwad, 2014).

Results and Discussion

The germination capacity of one seed, based on a binary answer (germinated/non germinated), is one qualitative attribute of the germination process generally converted in a quantitative attribute, commonly in percentage. The results of (Table 1) showed that there are significant differences (at 5% level) between effective treatments on germination characteristics and the different treatments resulted in significant differences among germination properties.

The results of this research showed that the germination percentage of *Momordica charantia* increased due to the application of priming in different duration and concentrations. According to the obtained results, treatment for 80% milk with 18 h soaking duration was the most effective treatments for improvement of seed germination (96%) which was statistically identical with 100% and 60% milk with 18 h soaking duration. This technique can be as a seed treatment that can increase rate, percentage, and uniformity of germination or seedling emergence. Results for zero hours (0h) soaking duration for 60%, 80%, and 100% milk reflect not having any combination of this treatment. In all cases of interaction

effects germination percentage for untreated seeds were minimum (66.33%).

Considering individual and combine effect, germination percentage has increased with soaking duration up to 18 hours, and then it starts declining. Results reveal that soaking duration and priming types have a great influence on germination for the seeds having thick seed coats. Milk has considered better priming agent than that of water.

Fresh cow milk had 0.012 µg mL⁻¹ and 0.006 µg mL⁻¹ for IAA and ABA, respectively. The excellent performance of fresh cow milk in breaking the dormancy in *M. charantia* seeds could be attributed to the presence of hormones such as Indole Acetic Acid (IAA) and Abscisic acid (ABA) (Adelani and Maisamari, 2016). Cow milk (organic or otherwise) has been shown to contain 35 different hormones and 11 growth factors (Djamgoz and Jane, 2015). Hormones are known to speed up the rate of germination of plant seeds. Major plant growth regulators (PGRs) significantly enhanced seed germination rate in black gram and horse gram (Chauhan et al., 2009), floral buds in Jojoba (Prat et al., 2008) and other growth parameters in different plants. Growth regulators are

organic substances besides nutrients, synthesized in plants, causing an alteration in their cellular metabolism (Rastogi et al., 2013).

Considering the interactive effect of soaking duration and fresh cow milk on mean germination time (Table 2), the lowest mean germination time was recorded in hydration for 24 hours (6.667) and the highest was recorded in untreated seed (8.471). In all cases mean germination time has reduced with increasing of soaking duration except for priming with 100% milk. This expression would have a direct interpretation on the sense that low values of mean germination time would be associated with concentrated germination in time.

From Table 2, maximum (2.890) value for the uncertainty of germination process was recorded in untreated seeds and the minimum (1.925) was recorded in 80% milk for 18 h soaking duration. Considering all values (except for 100% milk), it has been evidenced that soaking duration has a great effect on the uncertainty of the germination process then it comes to consider types of priming. The value for priming with 100% milk and 24 h soaking duration (2.823) is almost near to the value of the untreated seed.

Table 1. Interaction effect of soaking duration and type of priming on germination of *M. charantia* seeds

SD	Types of priming							
	Hydro-Priming		60% Milk		80% Milk		100% Milk	
	TM	OM	TM	OM	TM	OM	TM	OM
0 h	52.74 ^g	66.33 ^{gh}	0.57 ^h	0.00 ^h	0.57 ^h	0.00 ^h	0.57 ^h	0.00 ^h
12 h	61.98 ^{def}	77.87 ^{def}	59.88 ^{efg}	74.67 ^{efg}	62.58 ^{def}	78.67 ^{def}	59.01 ^{fg}	73.33 ^{fg}
18 h	68.77 ^{bcd}	86.80 ^{bcd}	71.53 ^{bc}	89.33 ^{bc}	80.48 ^a	96.00 ^a	75.55 ^{ab}	93.33 ^{ab}
24 h	62.25 ^{def}	78.33 ^{def}	64.73 ^{cdef}	81.33 ^{cdef}	66.34 ^{cde}	83.33 ^{cde}	59.18 ^{efg}	73.00 ^{efg}
CV(%)	6.66	7.78	6.66	7.78	6.66	7.78	6.66	7.78

*SD-Soaking duration, TM: Transformed Mean, OM: Original Mean

Table 2. Interactive effect of soaking duration and types of priming on mean germination time, uncertainty of germination process and synchrony of germination process

Soaking duration	Types of priming			
	Mean Germination Time			
	Hydration	60% Milk	80% Milk	100% Milk
0h	8.471	-	-	-
12h	7.947	7.941	7.933	8.053
18h	7.905	7.625	7.609	7.458
24h	6.667	7.333	7.176	7.875
CV (%)	6.45			
LSD	0.67			
Uncertainty of germination process				
0h	2.890	-	-	-
12h	2.629	2.631	2.826	2.580
18h	2.343	2.049	1.925	2.689
24h	2.698	2.398	2.593	2.823
CV (%)	9.39			
LSD	0.67			
Synchrony of germination process				
0h	0.095	-	-	-
12h	0.129	0.125	0.105	0.110
18h	0.133	0.167	0.272	0.233
24h	0.152	0.152	0.150	0.174
CV (%)	1.85			
LSD	-			

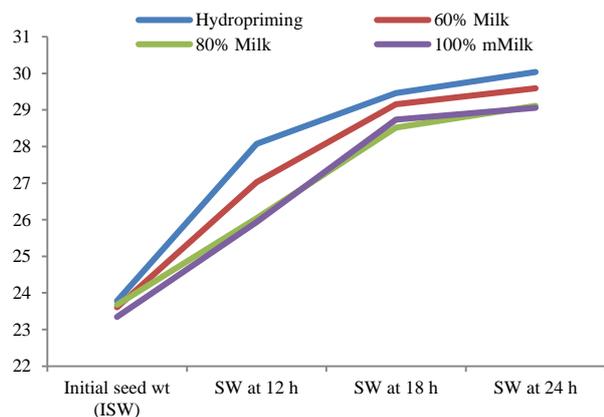


Figure 1. Variation of seed weight at different soaking duration and types of priming

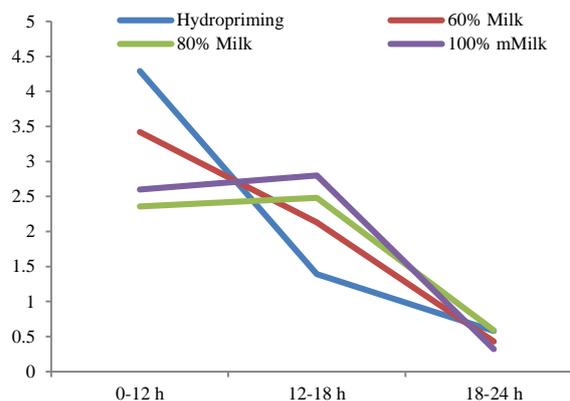


Figure 2. Amount of water absorbed at different Soaking duration and types of priming

From Table 2, maximum (2.272) value for synchronization of the germination process was recorded in 80% milk for 18 h soaking duration and the minimum (0.095) was recorded in untreated seeds. In all cases except hydration, the value for synchronization of the germination process has increased up to 18 hours soaking duration and then it declined. In the case of hydration, all values were increased with time.

Short intervals between two consecutive evaluations make the determination of mean germination time more accurate. It means that the mean germination time and synchrony are estimated using high intervals between observations (Dorneles et al., 2005).

It is possible to quantify the characteristic of synchronization of germination process by means of a measurement named synchrony of the germination process, uncertainty associated with the distribution of the relative frequency of germination (U), or informational entropy. Another important point is related to the interval between two consecutive evaluations. Depending on this interval, seeds could be considered as synchronized and homogeneous.

The result (Figure 1) showed that maximum seed weight (28.07 g, 29.46 g and, 30.04 g respectively) was found when the seeds were soaked in water in all three cases of soaking duration (12 h, 18 h and 24 h) i.e., maximum absorption of water has occurred in that case and the minimum was recorded when the seeds were soaked in 100% milk. The water absorption rate was almost similar in priming with 80% milk and 100% milk. It has also revealed that only water absorption does not effect on seed germination process though 80% milk provide maximum seed germination.

The rate of absorbing water from 0 h to 12 h was very high in hydro-priming and in soaking with 60% milk and then it declined, but in the case of 80% milk and 100% milk water absorption rate was maximum from 12 h to 18 h and then it declined (Figure 2).

Conclusion

Choosing the measurements and the interval of evaluations, according to the objective, it can be suggested that priming with 80% milk for 18 hours is mostly preferable for seed germination and related quality of bitter gourd seed.

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