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Effect of Soaking Condition and Temperature on Imbibition Rate of Maize and Chickpea Seeds

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ABSTRACT

This study was conducted to determine the effect of priming temperature and soaking condition on water absorption pattern of maize (variety BARI hybrid maize-5) and chickpea (BARI chola-5) seeds. Two soaking conditions viz., (1) aerobic and (2) anaerobic and three priming temperature levels viz., (1) 15 (2) 25 and (3) 31°C (ambient) were used in the experiment. The experiment was laid out in a completely randomized design with three replications. BARI hybrid maize-5 and BARI chola-5 seeds absorbed water very rapidly for up to 6 h both in aerobic and anaerobic conditions at all the levels of temperatures. Thereafter a little change in absorption rate was found for up to 30 h in maize and 24 h in chickpea seeds while a slow increase was further noticed for up to 70 h for both the seeds under all the environmental conditions with a little higher rate at aerobic environment than the anaerobic environment. It is observed that visible germination occurred in maize during 30 to 40 h and in chickpea during 24 to 30 h of imbibition. The present study revealed that imbibition period for both maize and chickpea seed increased with increasing temperature and the rate of water absorption was always higher in anaerobic condition than the aerobic condition. The present study concludes that optimum duration of soaking for maize and chickpea seeds at 31, 25 and 15°C of soaking temperature could be 6, 9 and 18 h, respectively.

Key words: Hydropriming, aerobic, anaerobic, water absorption, priming duration, hydration

INTRODUCTION

Seed priming prior to planting enhances germination and seedling growth by controlling the imbibition conditions and reducing vagaries of adverse weather and soil conditions (McDonald, 1999). Seed priming is a process of hydrating and dehydrating the seeds following various protocols which results in improvement in seed vigour, increased germination rate and more uniform emergence under a wider range of field environments (Modi, 2005). Hydropriming (hydration of seed with water only) is the simplest approach to increase the percent and rate of germination and increase the uniformity of stand establishment under stress conditions especially in dry areas (Clark *et al.*, 2001; Mavi *et al.*, 2006; Berchie *et al.*, 2010). During hydropriming the seeds are hydrated in some way to at a moisture level sufficient to initiate the early events of germination but not sufficient to permit radicle protrusion (Ashraf and Foolad, 2005). The water

imbibed by the seed activates enzymes and facilitates metabolism of the stored starch and protein in seed (Kikuchi *et al.*, 2006) and thus, water absorption (imbibition) is the most important event for ensuring nutrient supply to the germinating embryo and to generate energy for the commencement of active germination and seedling growth (Abebe and Modi, 2009). During the process of water uptake the cell wall enlarges and seed coat becomes softened allowing oxygen diffusion in seed respiration. The amount of water to be imbibed for seed germination depends on variety/species. The water needed for soybean and maize may be around 50% and around 34%, respectively (McDonald *et al.*, 2006). The rate of imbibitions increases with increase of temperature in many crop seeds such as sorghum (Kader and Jutzi, 2002), amaranth grain (Resio *et al.*, 2006) and cowpea (Captso *et al.*, 2008).

Seeds germinate after absorbing sufficient amount of water from its surrounding soil under field condition. If drought prevails, seed germination becomes uncertain. In drought-prone areas of India, Nepal, Pakistan and Zimbabwe farmers usually used pre-soaked seeds of different crops to ensure seedling emergence for obtaining good crop stand (Harris *et al.*, 2001a). A huge area in the North-West part of Bangladesh remains fallow after during winter season due to lack of rainfall and irrigation facilities. Farmers of this region usually try to grow some crops such as chickpea (*Cicer arietinum*) and maize (*Zea mays*) after amon harvest using residual soil moisture by sowing of pre-soaked seeds in the well prepared soil (Musa *et al.*, 2001). The time window between harvesting of amon rice and planting of rabi crop is very narrow and therefore, farmers get very minimum time for crop establishment. Seed priming is the simple technique that could help quick and uniform stand establishment under this situation (Sharifzadeh *et al.*, 2006; Ghassemi-Golezani *et al.*, 2008). Many farmers fail to get desired plant establishment in Bangladesh even after sowing of primed seed, probably because of faulty priming practices. Priming is done by soaking of seed in water for a certain period (Harris *et al.*, 2001b). But the lengths of soaking time for maize and chickpea seeds under variable temperature and soaking conditions have not yet been established. Therefore, it is essential to know the duration of priming in relation to temperature to devise good priming protocol for successful establishment of maize and chickpea in the drought-prone areas of Bangladesh. The present study was therefore, undertaken with a view to determine the soaking duration of maize seed (BARI hybrid maize-5) and chickpea seed (BARI chola-5) under variable soaking conditions and temperatures.

MATERIALS AND METHODS

The experiment was conducted at Seed Laboratory of Department of Agronomy, Bangladesh Agricultural University, Mymensingh during August-September 2008. Two soaking conditions viz., (1) aerobic and (2) anaerobic and three temperature levels viz., (1) 15 (2) 25 and (3) 31°C (ambient) were used in the experiment. The trial was laid out in completely randomized design with three replications. Breeder's seeds of BARI hybrid maize-5 and BARI chola-5 were collected from Bangladesh Agricultural Research Institute, Joydebpur, Gazipur. The initial moisture content of the seed was measured to 13 and 10% of BARI hybrid maize-5 and BARI chola-5, respectively (dry basis). Seeds were placed on bashundhara kitchen towel in a petri dish under aerobic condition. Boshundhara kitchen towel was saturated with water so that seed can easily absorb water from it. Water was added to the towel frequently to keep it saturated with sufficient moisture. At an anaerobic condition the seeds were placed in a plastic pot and then submerged with water. Two chambers of a germinator was used for 15 and 25°C temperature respectively while the ambient condition created 31°C temperature level. The seeds for both aerobic and anaerobic conditions were

placed under three temperature levels-15, 25 and 31°C. For each treatment level 200 seeds in three replications were placed in petri dishes/plastic pots for water absorption. The seeds were allowed to imbibe water up to 70 h. Seeds were taken after 1, 2, 3, 6, 9, 12, 18, 24, 30, 40, 50, 60 and 70 h interval from the petri dishes to measure seed moisture. The amount of moisture absorbed by seed was measured by taking 5 seeds randomly. The seed moisture was measured following high temperature constant oven dry method by placing the seeds in an electric oven (Memmert, Germany) at 130°C temperature for 4 and 3 h for maize and chickpea, respectively (ISTA, 2003).

The whole process was repeated three times and all the data were pooled. Data analysis was done statistically using Analysis of Variance (ANOVA) technique with MSTAT-C program and mean separation was done by DMRT at 5% level.

RESULTS

Soaking condition: Water absorption rate of maize and chickpea seeds were influenced significantly by soaking condition (Table 1). The amount of water absorption was significantly higher in anaerobic condition than the aerobic condition at each time of measurement for both in maize and chickpea. The water absorption by maize seed in aerobic condition at 1, 2, 3, 6, 9, 12, 18, 24, 30, 40, 50, 60 and 70 h were 20.14, 23.64, 29.14, 36.90, 39.40, 40.88, 42.74, 44.23, 45.37, 47.41, 49.84, 53.35 and 57.81%, respectively while those values at anaerobic condition were 25.58, 33.99, 38.38, 44.67, 52.07, 53.90, 55.00, 56.86, 58.44, 59.91 and 61.50%, respectively (Table 1). The water absorption percentage in chickpea seed under anaerobic condition was higher than aerobic condition. The result showed BARI chola-5 absorbed 70.29 and 131.20% at 1 and 70 h under anaerobic condition and the corresponding values for aerobic conditions were 58.13 and 146.33%, respectively (Table 1). Table 1 showed that difference in water absorption between aerobic and anaerobic condition for maize seed was higher than chickpea seeds. It was also found that the moisture absorption difference between the two conditions became narrow with duration of soaking time in maize seed and became very close at 70 h. On the other hand, moisture difference between aerobic and anaerobic condition in chickpea remained very close up to 60 h while the difference became wide at 70 h where the seed at aerobic condition absorbed more water than anaerobic condition (Table 1).

Table 1: Effect of soaking condition on water absorption of BARI hybrid maize-5 and BARI chola-5 at different time intervals

Soaking condition	Water absorption (%)												
	1 h	2 h	3 h	6 h	9 h	12 h	18 h	24 h	30 h	40 h	50 h	60 h	70 h
BARI Hybrid maize-5													
Aerobic	20.14b	23.64b	29.14b	36.90b	39.40b	40.88b	42.74b	44.23b	45.37b	47.41b	49.84b	53.35b	57.80b
Anaerobic	25.58a	33.99a	38.38a	44.67a	47.67a	49.57a	52.07a	53.90a	55.00a	56.84a	58.44a	59.91a	61.50a
S x	0.2337	0.2822	0.2924	0.2202	0.2449	0.2276	0.2212	0.1717	0.2146	0.2011	0.2050	0.1622	0.1907
Level of significane	**	**	**	**	**	**	**	**	**	**	**	**	**
CV (%)	3.07	2.94	2.60	1.62	1.69	1.51	1.40	1.05	1.28	1.16	1.14	0.86	0.96
BARI chola-5													
Aerobic	58.13b	68.20b	78.14b	100.16b	108.97b	113.27b	116.66b	118.10b	119.57b	122.81b	127.83b	135.17a	146.33a
Anaerobic	70.29a	82.29a	90.17a	105.53a	111.97a	116.40a	120.80a	123.90a	125.63a	127.57a	128.95a	129.93b	131.20b
S x	0.331	0.308	0.182	0.268	0.336	0.322	0.270	0.222	0.229	0.223	0.153	0.193	0.170
Level of significane	**	**	**	**	**	**	**	**	**	**	**	**	**
CV (%)	1.55	1.23	0.65	0.78	0.91	0.84	0.68	0.55	0.56	0.53	0.36	0.44	0.37

Values with common letters with in column do not differ significantly as per DMRT at 5% level. **Significant at 1% level

Temperature: Different temperature level showed significant effect on water absorption of BARI hybrid maize-5 and BARI chola-5 at every hour interval (Table 2). At every point of observation, it was found that water absorption was the highest with 31°C and lowest with 15°C for both maize and chickpea seeds. For instance, at 15°C water absorption percent of BARI hybrid maize-5 at 1, 2, 3, 6, 9, 12, 18, 24, 30, 40, 50, 60 and 70 h were 21.81, 26.36, 30.46, 36.70, 38.86, 40.46, 42.21, 43.67, 44.65, 46.11, 47.75, 49.41 and 51.80, respectively whereas it was higher at 25°C which were 22.33, 28.17, 32.86, 40.51, 43.31, 44.90, 47.31, 48.99, 50.11, 52.21, 54.21, 57.02 and 60.05 for the given duration and at 31°C (ambient condition) water absorption percent was highest which were 24.45, 31.92, 37.96, 45.15, 48.45, 50.30, 52.70, 54.55, 55.81, 58.06, 60.45, 63.46 and 67.11 at each time of measurement (Table 1). It was noted from Table 2 that water absorption rate increased rapidly up to 6 h for maize and chickpea and then it increased slowly up to 70 h at all levels of temperature (Table 1).

Interaction effect: Interaction effect of soaking condition and priming temperature showed significant effect on imbibition at every interval of time for both crops except at 1 h for maize (Table 3). Water absorption percent of BARI hybrid maize-5 at aerobic condition at 31°C were 22.32, 28.40, 34.98, 42.30, 44.90, 46.60, 48.80, 50.70, 52.00, 54.61, 57.80, 62.01 and 67.51 at 1, 2, 3, 6, 9, 12, 18, 24, 30, 40, 50, 60 and 70 h, respectively which were higher than the water absorption at 25 and 15°C level of temperature (Table 3). Similar result was found at anaerobic condition (Table 2). Among all interaction effect the treatment anaerobic × 31°C showed highest amount of water absorption percent which were 26.58, 35.44, 40.95, 48.00, 52.00, 54.00, 56.60, 58.40, 59.61, 61.50, 63.10, 64.91 and 66.71 at 1, 2, 3, 6, 9, 12, 18, 24, 30, 40, 50, 60 and 70 h respectively whereas aerobic × 5°C showed lowest absorption at each level of measurement. Similar result was observed in BARI chola-5. It was noted that under aerobic condition visible germination was observed in BARI hybrid maize-5 during 30 to 40 h and in BARI chola-5 during 24 to 30 h while no visible germination was found for seed kept at anaerobic condition.

Table 2: Effect of temperature on water absorption of BARI hybrid maize-5 and BARI chola-5 at different time intervals

Temperature levels	Water absorption (%)												
	1 h	2 h	3 h	6 h	9 h	12 h	18 h	24 h	30 h	40 h	50 h	60 h	70 h
BARI hybrid maize-5													
15°C	21.81b	26.36c	30.46c	36.70c	38.86c	40.46c	42.21c	43.67c	44.65c	46.11c	47.75c	49.41c	51.80c
25°C	22.33b	28.17b	32.86b	40.51b	43.31b	44.90b	47.31b	48.99b	50.11b	52.21b	54.21b	57.02b	60.05b
31°C	24.45a	31.92a	37.96a	45.15a	48.45a	50.30a	52.70a	54.55a	55.81a	58.06a	60.45a	63.46a	67.11a
S x	0.2862	0.3456	0.3581	0.2697	0.2999	0.2787	0.2710	0.2103	0.2629	0.2463	0.2510	0.1987	0.2336
Level of significance	**	**	**	**	**	**	**	**	**	**	**	**	**
CV (%)	3.07	2.94	2.60	1.62	1.69	1.51	1.40	1.05	1.28	1.16	1.14	0.86	0.96
BARI chola-5													
15°C	55.22c	65.21c	77.15c	94.48c	102.85c	107.56c	110.80c	113.15c	114.40c	115.91c	117.75c	121.99c	128.39c
25°C	66.13b	76.30b	83.55b	103.41b	111.09b	115.80b	120.21b	122.56b	124.15b	127.36b	131.68b	135.20b	141.25b
31°C	71.28a	84.22a	91.75a	110.66a	117.45a	121.15a	125.19a	127.30a	129.25a	132.30a	135.75a	140.46a	146.65a
S x	0.405	0.377	0.223	0.329	0.411	0.395	0.331	0.271	0.280	0.273	0.187	0.236	0.208
Level of significance	**	**	**	**	**	**	**	**	**	**	**	**	**
CV (%)	1.55	1.23	0.65	0.78	0.91	0.84	0.68	0.55	0.56	0.53	0.36	0.44	0.37

Values with common letter with in column do not differ significantly as per DMRT at 5% level. **Significant at 1% level

Table 3: Interaction effect of soaking condition and temperature on water absorption of BARI hybrid maize-5 and BARI chola-5 at different time intervals

Soaking type × Temperature levels	Water absorption (%)												
	1 h	2 h	3 h	6 h	9 h	12 h	18 h	24 h	30 h	40 h	50 h	60 h	70 h
BARI hybrid maize-5													
Aerobic×15°C	18.70	20.11e	24.73e	31.69e	33.91e	35.22e	36.42e	37.72f	38.70f	40.02f	42.01e	44.01e	47.29e
Aerobic×25°C	19.41	22.41d	27.72d	36.71d	39.41d	40.80d	43.01d	44.29e	45.41e	47.61e	49.71d	54.02d	58.60c
Aerobic×31°C	22.32	28.40c	34.98c	42.30c	44.90c	46.60c	48.80c	50.70c	52.00c	54.61c	57.80b	62.01b	67.51a
Anaerob.×15°C	24.91	32.61b	36.19c	41.70c	43.81c	45.70c	48.00c	49.61d	50.60d	52.20d	53.50c	54.81d	56.30d
Anaerob.×25°C	25.25	33.93b	38.00b	44.31b	47.20b	49.00b	51.60b	53.70b	54.80b	56.81b	58.71b	60.01c	61.50b
Anaerob.×31°C	26.58	35.44a	40.95a	48.00a	52.00a	54.00a	56.60a	58.40a	59.61a	61.50a	63.10a	64.91a	66.71a
S x	0.4048	0.4887	0.5065	0.3814	0.4242	0.3942	0.3832	0.2974	0.3718	0.3483	0.3550	0.2810	0.3303
Level of significance	NS	**	**	**	**	**	**	**	**	**	**	**	**
CV (%)	3.07	2.94	2.60	1.62	1.69	1.51	1.40	1.05	1.28	1.16	1.14	0.86	0.96
BARI chola-5													
Aerobic×15°C	51.14e	59.21f	72.00f	93.60e	102.40e	106.90d	109.61e	111.81f	112.80f	114.31e	116.50e	124.00d	135.29d
Aerobic×25°C	59.29d	69.00e	77.41e	100.51c	109.69d	114.30c	118.01c	119.11d	120.30d	124.71c	132.00c	138.00b	149.10b
Aerobic×31°C	63.97c	76.39c	85.00c	106.38b	114.80b	118.60b	122.37b	123.40c	125.60c	129.40b	135.00b	143.51a	154.60a
Anaerob.×15°C	59.30d	71.20d	82.30d	95.36d	103.29e	108.21d	112.00d	114.50e	116.00e	117.50d	119.00d	120.00e	121.50f
Anaerob.×25°C	72.96b	83.60b	89.70b	106.30b	112.50c	117.30b	122.40b	126.00b	128.00b	130.00b	131.36c	132.40c	133.40e
Anaerob.×31°C	78.60a	92.05a	98.50a	114.94a	120.10a	123.70a	128.00a	131.20a	132.89a	135.20a	136.50a	137.40b	138.70c
S x	0.573	0.533	0.315	0.465	0.582	0.558	0.468	0.384	0.396	0.386	0.264	0.334	0.294
Level of significance	**	**	**	**	**	**	**	**	**	**	**	**	**
CV (%)	1.55	1.23	0.65	0.78	0.91	0.84	0.68	0.55	0.56	0.53	0.36	0.44	0.37

Values with common with in column do not differ significantly as per DMRT at 5% level. **Significant at 1% level, NS = Not significant

DISCUSSION

The rate of water uptake was higher under anaerobic condition than the aerobic conditions at every point of observation during the whole period of soaking. The higher water absorption under anaerobic condition might be related to the fact that the seeds under this condition was kept under water which allowed the water to come in contact with the embryo and seed surface and thus facilitated the rapid water entry into the seed. On the other hand, in the present study under aerobic condition seed was placed on the kitchen towel saturated with water. This system allowed moisture entry into the seed through the portion of seed that was in touch with the kitchen towel. McDonald *et al.* (1994) reported that when seeds were positioned to one side the maize seed absorbed water rapidly through embryo than the endosperm when it comes to the close contact of moisture. Thus the position of seed that remains in contact with water is very important for absorption of water. Since, the seed absorbs water only through the area that is only in contact with water which might have reduced the water entry in to the seed under aerobic condition than the anaerobic condition. Therefore, seeds should be imbibed in such a way that whole seed surface can intake water to reduce the imbibition duration.

The present study showed that water absorption increased with increase in soaking temperature. Similar increase of water absorption with increase of temperature was reported in different legumes by Seyhan-Gurtas *et al.* (2001). They found that legume seeds reached the maximum water absorption level within 7 h against that required 18 h at 15°C. The higher temperature accelerates chemical change that controls the rates of water absorption through a semi permeable membrane. Cold water is composed of complex molecule having at least several H₂O groups combined in to a single molecule. With the rise in temperature these more complex molecules are supposed to break down into simpler groups and the water become less viscous and is able to

penetrate the semi permeable coats of seed more rapidly (Kader and Jutzi, 2002). The lower temperature requires longer lag period of water uptake because of lower water diffusivity which is substantially reduced or even disappeared at higher temperatures.

The water uptake was rapid for up to 6 h followed by a slower uptake that continued up to 70 h at anaerobic condition for both chickpea and maize seeds. On the other hand, under the aerobic condition, after the rapid uptake of water for up to 6 h, the water uptake continued at a slow rate for up to 30 and 40 h, respectively for BARI hybrid maize-5 and BARI chola-5 seeds. The moisture absorption of maize seed under anaerobic condition was higher than aerobic condition during the whole period of soaking and the difference started to become narrower after 40 h of soaking in maize seed although did not reach at the same level until 70 h. In contrast, chickpea seed showed higher moisture content in anaerobic condition than aerobic condition for up to 40 h. Further increase of soaking duration the moisture absorption increased in aerobic condition and became same at 50 h of soaking. After 50 h, the moisture percentage under the aerobic condition became higher than under anaerobic condition. The increase in moisture absorption under aerobic condition after certain period of soaking was probably due to start active germination of seed (Manz *et al.*, 2005).

Water uptake under aerobic condition showed a triphasic pattern in both the seeds with a marked increase during the initial phase of imbibitions (phase I) and then a slow increase (phase II) followed by a second substantial increase (Phase III). Under anaerobic condition, only phase I and phase II were evident in both the seeds. In the present study, for BARI chola-5, rapid water uptake was occurred up to 6 h both under aerobic and anaerobic conditions at different levels of temperature which may be considered as phase I. This phase was followed by a slow water uptake phase which continued up to the end of soaking period (70 h) under anaerobic condition but up to 24 h for aerobic condition. Then again an increase of water uptake was evident in both the seeds in aerobic condition which corresponds to the phase III. The initial rapid uptake of water in a short period of time is related to matric potential of seed tissue which is similar in both the dead and live seeds (Jeller *et al.*, 2003). In the phase II the water uptake is slower and less intense than the previous one although the water percentage reached the highest level. During phase I, the glycolytic and oxidative pentose pathways both resume and the Krebs cycle enzymes become activated. Phase II includes preparatory metabolic events preceding the emission of primary roots which marks the establishment of the phase III, which is characterized by renewal of absorption and the growth of embryonic axis and radical emergence (Mei and Song, 2008). The second substantial increase of water absorption was evident in the aerobic condition in the present study with visible emergence of radical due to growth of embryo.

Present study revealed that the water absorption reached to a substantial level sufficient for seed germination in 6-18 h under anaerobic soaking condition while this duration was required 9-24 and 18-70 h, respectively for chickpea and maize seed under aerobic conditions depending on soaking temperature (Table 3). The amount of water absorbed during this period is sufficient for seed germination (McDonald *et al.*, 2006). The result also showed that the moisture absorption is rapid when seeds are kept under water and the soaking temperature is higher. The visible emergence of seed germination is the end of phase II and start of phase III. During phase II some very important physiological and biochemical events such as DNA and RNA synthesis are accomplished and hence the primed seeds become physiologically more close to germination comparing with unprimed seed (McDonald, 1999). Therefore, the end of phase II could be considered as the appropriate duration for seed priming. The phase I was completed for both maize

and chickpea seed at about 6 h of soaking under water at 31°C and therefore, 6 hours soaking is sufficient for both the species. Present result could be supported by Mei and Song (2008), who reported that within 6 h of imbibitions in maize seed completed phase I and the mobilization of the stored reserves occurred. The visible emergence was found in 24 to 30 h for chickpea and in 30 to 40 h in maize which coincide with the start of phase III. Olisa et al. (2010) reported that the phase I and II of imbibitions lasted for 4 and 18 h in pigeon pea and 6 and 24 h in African yam bean, respectively. The priming should be ended up before the end of phase II as because no visible emergence is expected to ensure good priming (Abebe and Modi, 2009). Therefore, soaking and priming would require only 9 and 18 hours respectively for chickpea and maize seeds. The present study indicates that soaking of chickpea and maize seed for 6 h at 31°C temperature is sufficient to gain moisture required for activation of germination process. The imbibition duration could be extended at lower soaking temperatures but long time immersing of seed in water may cause injury to seeds and thus prolonged seed imbibitions under anaerobic condition could result loss of seed germination (Quan *et al.*, 2004). Therefore, optimum imbibition period for BARI hybrid maize-5 and BARI chola-5 seed may be 6 h at soaking temperature of 31°C. The visible emergence was found at 30 and 40 hours after placing in soaking in aerobic condition. The basic biochemical changes to initiate seed germination process should be completed before the start of phase III, therefore, further research is necessary to find out the duration of priming chickpea and maize seeds after imbibition.

CONCLUSION

Imbibition period for both maize and chickpea seed increased with increasing temperature and the rate of water absorption was always higher in anaerobic condition than the aerobic condition.

The present study concludes that optimum duration of soaking for maize and chickpea seeds at 31, 25 and 15°C of soaking temperature could be 6, 9 and 18 h, respectively.

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