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Effect of Imbibition on Germination of *Vigna Radiata* Seed

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Abstract

Vigna radiata (green gram) are staple food in India. The imbibition of the seed is never correlated with germination and vigour of the seeds. In the present experiment we imbibed the seed in 100 ml sterile distilled water for 0 to 24h. The imbibed seeds were allowed to germinate and data were recorded with respect to normal and abnormal germination, fungal infection, non-germinated seed and mean plant length after 7 day of sowing. The 8h period imbibition is found to be best with respect to vigour. The water imbibed after imbibition for a period of 8h was 0.912±0.018 g/g dry weight seed. The normal germination (%), abnormal germination (%), non-germinated (%) and plant length for the same were 61.001.15, 121.15, 23.671.76, 14.30.3 cm respectively.

Keywords: Vigna Radiata; Imbibition; Germination; Vigour; Seed.

Introduction

Seed imbibition is reported to be correlated with even DNA repair and telomere maintenance (Balestrazzi et al., 2015). Water imbibition is one of the first important stages for germination of a seed. Role of imbibition on *Vigna radiata* germination is reported by Qian et al. (2003). They reported that imbibition also determines the desiccation tolerance of the future plant (Qian et al., 2003). Imbibition is reported to be negatively correlated in hard *V. radiata* seeds (Promila et al., 2000). They also reported leakage of nutrients during early phase of *V. radiata* seed imbibition (Promila et al., 2000; Krishnappa et al., 2017). Seed showing fast imbibition are less vigorous compared to slow one (Abdullah et al., 1991). The imbibition period reported by Krishnappa et al. (2017) is 12 h. The time period (12h) is arbitrarily decided by the researcher. Similarly, Castellanos-Barriga et al. (2017) reported that on 6h imbibition *V. radiata* seed absorbs water equal to its dry weight. Therefore, they might follow the same time period of imbibition in their experiment. Truong et al. (2017) reported that anaerobic imbibition has prominent effect on γ-aminobutyric acid (GABA) accumulation on *V. radiata* seed. In their research seeds were imbibed inside water for 8h and corresponding GABA accumulation is 27 times of normal imbibitions period. After aerobic imbibitions up to 18h sugar present in the cotyledons are primary energy source for growth (Promila & Kumar, 2000). After 18h the energy source is cotyledon starch (Promila & Kumar, 2000). In the method followed by Promila & Kumar (2000), imbibition starts with sowing on tissue paper with 10ml water. The leached sugar in this type of aerobic imbibitions might accumulate in high concentration due to less volume of water (Krishnappa et al., 2017). These high sugar accumulations might stimulate abscisic acid production leading to delay and drop in germination (Rodríguez-Gacio et al., 2009; Xiong & Zhu, 2003). *V. radiata* seeds are reported for imbibing 0.222 to 0.078 g water per g seed dry weight on exposure to relative humidity of 30% and 80% respectively (Murthy et al., 2003). With increasing internal water content sugar accumulation increases

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in *V. radiata* seeds during storage (Murthy et al., 2003). In the light of the above information it might be concluded that imbibing *V. radiata* seed in large volume of water might help in countering the role of abscisic acid based dormancy induction in embryo. On the other hand, imbibing on tissue paper might not be helpful for the same. Benvenuti & Macchia (1995) reported secondary dormancy due to anaerobic germination. Tian et al. (2016) reported high difference of desiccation tolerance among 6h and 18h imbibed *V. radiata* seeds. The difference of water imbibed on 6h and 18h imbibitions is not significant. Therefore, it might be suggested that prolong anaerobic imbibitions is not suitable for optimum germination of *V. radiata* seed.

In the light of above information to answer the optimum water imbibitions time period for *V. radiata* seed, an experiment has been designed. In the present research hour wise imbibitions of V. radiata seeds were performed and data related to amount of water imbibed, germination (%), average length of germinated plants, abnormal germination and fungal contamination during germination were recorded for analysis.

Materials and Methods

V. radiata seeds were purchased from local market. Seeds were washed and surface sterilized by soaking in 70% ethanol for 5 min. The sterilized seeds were washed three times using sterile distilled water to remove traces of ethanol.

To find out the imbibed water 5.3 g (approximately 100 seeds) of dry *V. radiata* seeds were weighed. The lot is now surface sterilized using method described above. The seeds were imbibed in 100 ml sterile distilled water for 0 to 24 h. The data for 15h is not their as the seeds were lost during experiment. The post imbibition weight of seeds was measured. The weight of water imbibed per gram of seed was calculated and recorded.

Three sets of 100 seeds are used for every imbibed time period (0h to 24h) except for 22h were a set is lost due to accidental falloff. Germination, abnormal germinations, non-germinated seed, fungal contamination percentage were recorded for all three lots. The recorded data were processed to find out mean and standard error. The mean plantlet length was calculated from all normal germinations out of 300 seeds for each imbibition time period. All total of 7,100 seeds were used in the present experiment. The vigour index was calculated by multiplying normal germination (%) and mean plant length (cm). Statistical analysis of the data is performed using one way ANOVA analysis at p=0.05.

Results and Discussions

As presented in Figure 1, the water imbibed (g) per g of dry weight of seed increases with increasing period of imbibition in hours. Compared to earlier report of 6h imbibition for 1g water/g dry weight of *V. radiata* seed, in the present study it is found to be 5h (Castellanos-Barriga et al., 2017). In seeds imbibed for 6, 7 and 8h period there is a drop in imbibed water (Figure1). This might be due to presence of more hard seeds (Promila et al., 2000).

The rate of imbibition from 9h onwards goes down because of saturation of imbibition (Table1). The rate of imbibition for the *V. radiata* seeds imbibed for 8h was found to be slower compared to 5-9h group (Table1). This slow imbibition rate might be responsible for high vigour (Figure4) for seeds imbibed for 8h period (Abdullah et al., 1991). Imbibition for 19h period shown a huge variation in imbibed water (Figure 1) and lower vigour compared to nearby time periods (Figure 4).

The vigour loss of 19h imbibed *V. radiata* was contributed by drop in case of germination (%) (Figure 2) and not plant length (Figure 3). As presented in Figure 2 the germination (%) increases with increasing imbibition period. The germination (%) was found to be high for V. radiata seeds imbibed for 7, 10, 11 and 13h. The germination (%) before and after these gradually drops (Figure 2). The same trend is found to in non-germinated (%) with 7 and 13h imbibed *V. radiata* seeds show lowest non-germination (%). It should also noted that germination (%) and non-germinated (%) for every time period of imbibition corresponds to each other (Figure 2). Therefore, it might be suggested that number of abnormal seed did not vary significantly with respect to imbibition time periods (Figure 3). As presented in Figure 3, the plant length was found to be highest in 8h imbibed seeds. Abnormal germination was found highest for 3h imbibed seeds (Figure 3). It should also be noted that more than 5h imbibition did not show any fungal contamination and with increasing h of imbibition fungal contamination decreases (Figure 3).

Abnormal germination (%) is also following a trend of curve with bottom at 12h imbibition and approximately following the desiccation tolerance trend of 6-18h imbibed seed as referred by Tian et al. (2016). The plant length is found to be highest for 8h imbibed *V. radiata* seed. The low standard error of mean plant length suggests synchronized germination

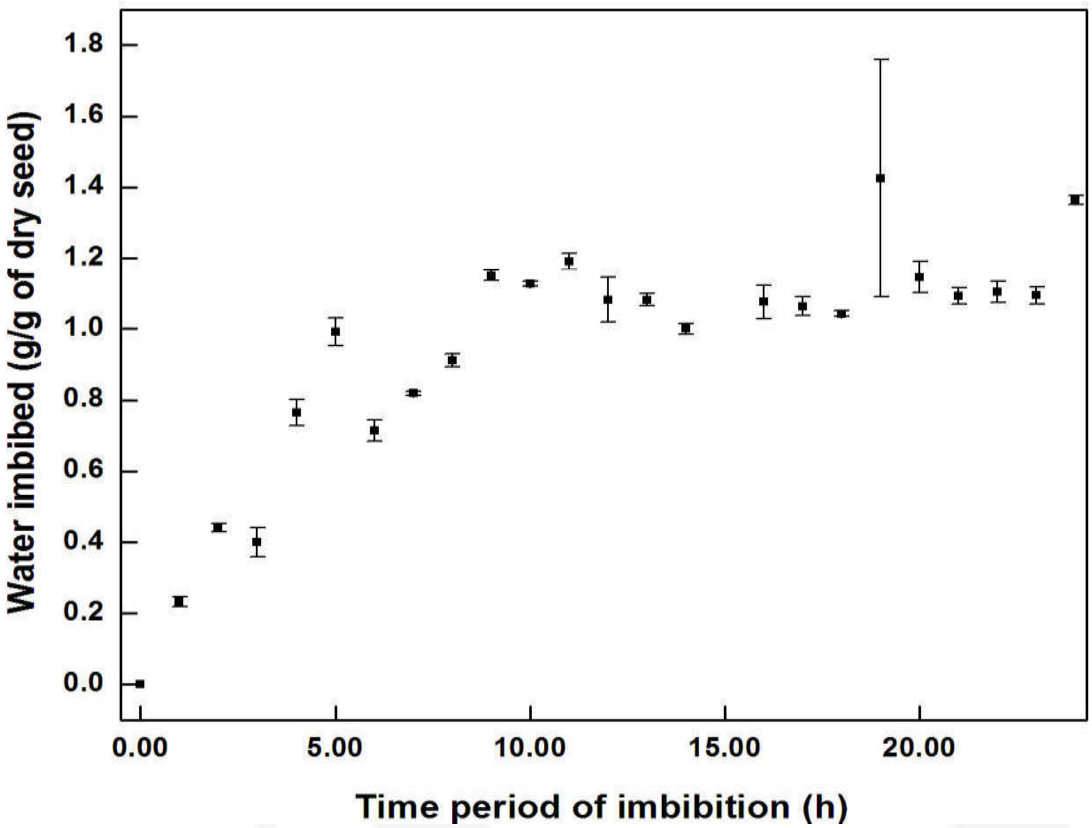

Fig. 1: Water imbibition graph

Table 1: Different parameter of germination with respect to 24 different imbibition time periods

time (H)	Water (G) Per G dry seed		Germinatono (%)		Abnormal germination (%)		Fungal contamination (%)		Non germinated seed (%)		plan length (cm)		vigour index	Rate of water imbibition (g water/g dry wt.h)
	mean	standard error	mean	standard error	mean	standard error	mean	standard error	mean	standard error	mean	standard error		
0.00	0.000	0.000	2.67	0.67	17.33	4.63	3.67	0.33	76.33	4.19	6.7	0.6	17.97	NA
1.00	0.233	0.014	2.67	0.67	16.33	4.06	4.00	0.58	77.00	4.93	7.9	0.5	20.93	0.233
2.00	0.441	0.011	12.33	2.19	18.33	4.91	3.33	0.67	62.00	9.45	7.1	0.7	87.63	0.220
3.00	0.400	0.041	26.67	3.53	23.67	1.45	0.67	0.67	46.33	2.60	13.6	0.5	362.53	0.133
4.00	0.764	0.037	35.33	4.63	14.67	2.33	0.00	0.00	45.67	0.88	12.8	0.4	453.82	0.191
5.00	0.992	0.039	49.33	9.82	17.33	2.67	0.33	0.33	33.67	7.67	12.1	0.3	598.71	0.198
6.00	0.714	0.031	52.33	3.53	19.00	3.21	0.00	0.33	35.33	6.84	9.3	0.2	484.08	0.119
7.00	0.819	0.005	69.00	5.29	13.00	3.00	0.00	0.00	18.00	2.65	9.5	0.2	652.88	0.117
8.00	0.912	0.018	61.00	1.15	12.00	1.15	0.00	0.00	23.67	1.76	14.3	0.3	873.00	0.114
9.00	1.150	0.015	59.67	2.73	12.33	1.20	0.00	0.00	31.33	6.74	12.4	0.3	741.33	0.128
10.00	1.127	0.007	69.33	7.13	7.33	1.45	0.00	0.00	23.00	8.08	11.2	0.3	776.93	0.113
11.00	1.190	0.023	70.00	2.00	10.67	1.86	0.00	0.00	19.33	0.33	11.7	0.3	818.33	0.108
12.00	1.082	0.063	59.67	2.40	6.33	1.76	0.00	0.00	30.67	5.46	12.1	0.3	723.00	0.090
13.00	1.082	0.017	74.00	7.09	9.33	1.33	0.00	0.00	16.67	6.33	11.2	0.2	827.06	0.083
14.00	1.000	0.015	63.33	6.17	13.00	3.51	0.00	0.00	23.67	8.57	12.9	0.3	815.77	0.071
16.00	1.076	0.047	59.67	0.88	7.00	0.58	0.00	0.00	33.33	1.45	9.4	0.3	560.2	0.067
17.00	1.063	0.026	62.00	0.58	16.00	4.58	0.00	0.00	22.00	4.16	12.1	0.3	748.54	0.063
18.00	1.043	0.009	59.33	2.67	16.33	6.74	0.00	0.00	21.00	6.66	12.2	0.4	725.15	0.058
19.00	1.425	0.335	39.67	1.67	8.67	1.20	0.00	0.00	47.00	7.51	12.2	0.5	483.51	0.075
20.00	1.146	0.045	61.67	3.84	13.00	1.00	0.00	0.00	28.67	3.38	12.5	0.4	770.16	0.057
21.00	1.093	0.023	57.00	3.79	10.00	2.31	0.00	0.00	33.00	6.08	11.6	0.3	661.54	0.052
22.00	1.104	0.029	53.00	1.00	17.50	10.50	0.00	0.00	34.50	14.5	10.9	0.3	5.77.80	0.050
23.00	1.094	0.024	57.33	5.36	15.33	4.70	0.00	0.00	27.33	0.67	11.5	0.3	659.17	0.048
24.00	1.364	0.012	43.00	3.06	19.00	5.20	0.00	0.00	38.00	5.51	12.1	0.4	519.64	0.057

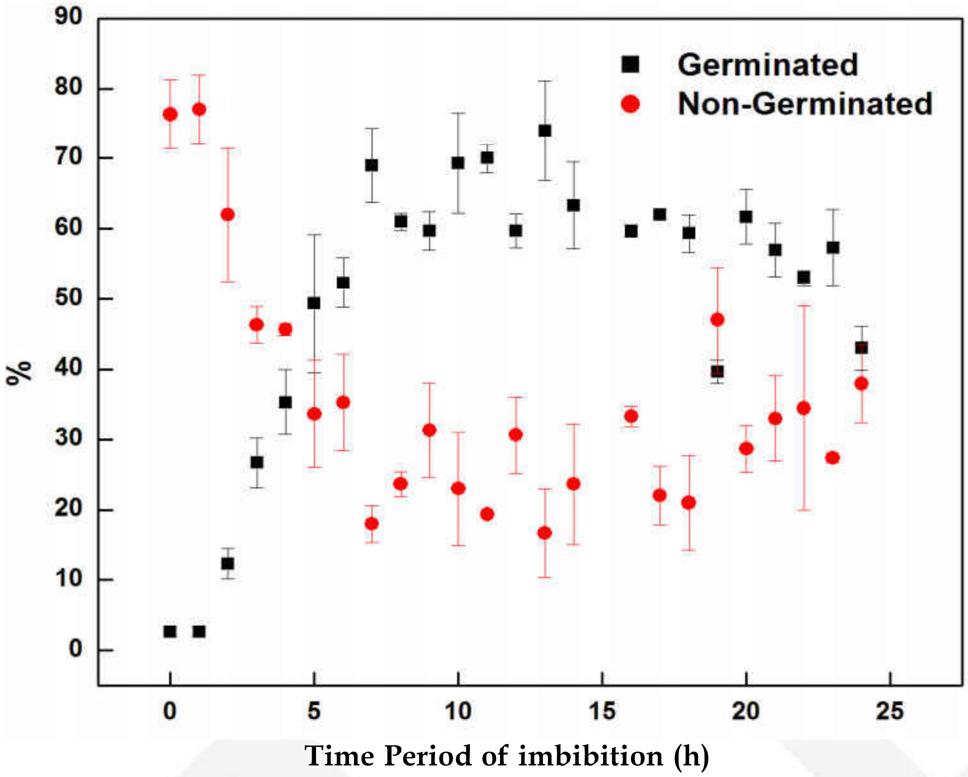

Fig. 2: Germination%

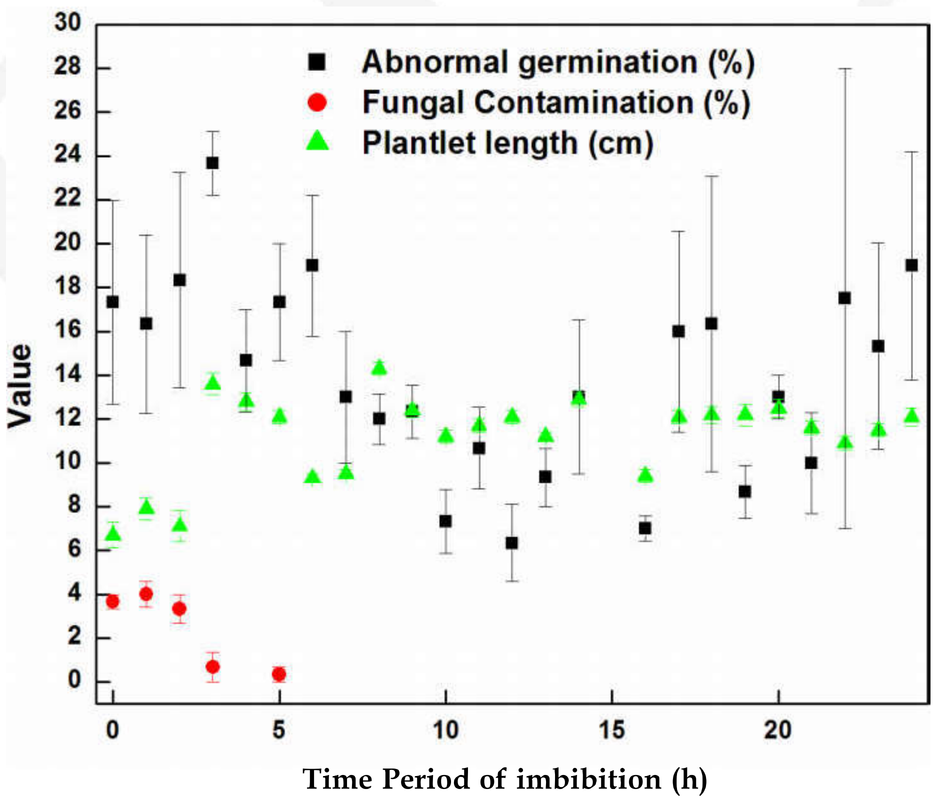

Fig. 3: Abnormgermgraph

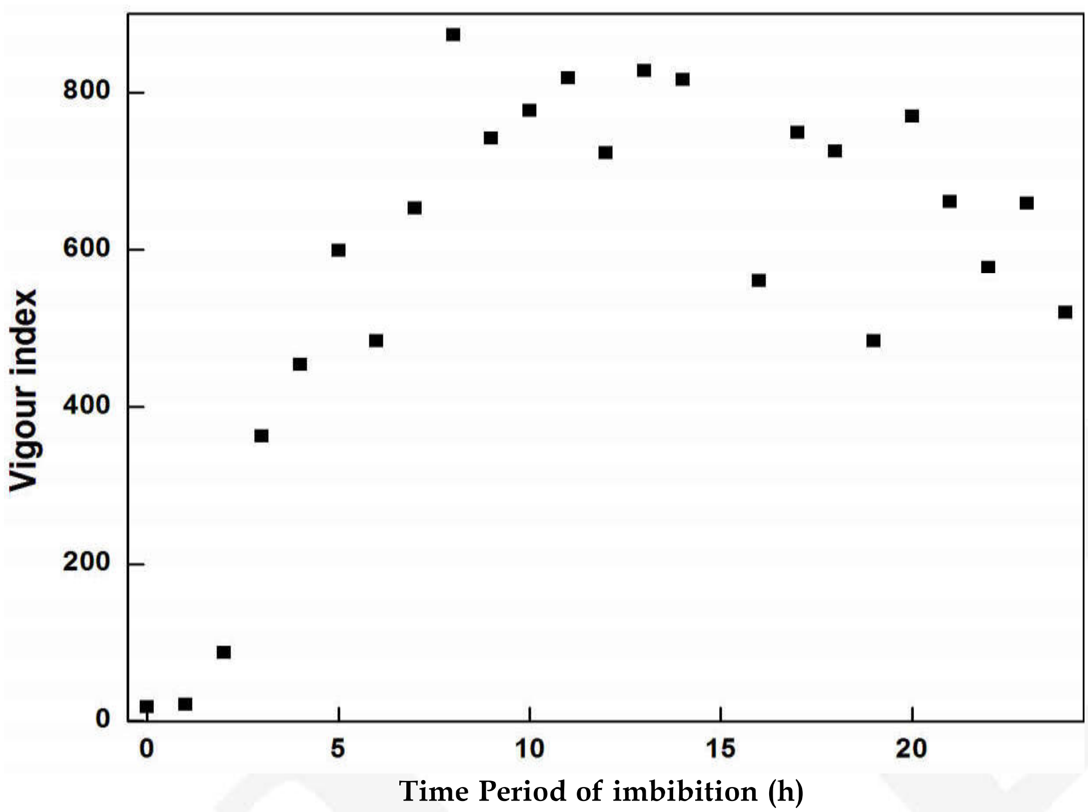

Fig. 4: Vigour index

(Figure 3). It is the synchronized germination which helps farmer with respect to pesticide, fertilizer application and harvesting. The vigour of the plant is also found to following a bell shaped curve with 8-16 h as top of the bell with few exceptions.

Conclusion

From the present research it might be concluded that 8h of imbibition is good for germinating *V. radiata* seed.

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