WOLKITE UNIVERSITY

COLLEGE OF AGRICULTURE AND NATURAL RESOURCE

DEPARTEMENT OF PLANT SCIENCE

THE EFFECT OF COLD WATER TREATMENT TIME ON SEED GERMINATION OF HARICOT BEANS (PHASEOLUS VULGARIS L.)

RESEARCH REPORT SUBMITTED TO THE DEPARTMENT OF PLANT SCIENCE COLLEGE OF AGRICULTURE AND NATURAL RESOURCE WOLKITE UNIVERSITY FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENT OF BSC IN PLANT SCIENCE

BY

SERKALEM ALTAYE ID: AGS/04/2010

DEMEKE SEYFU ID: AGS/040/2010

MITIKU KERO ID: AGS/089/2010

PETROS GERPUWA ID: AGS/097/2010

TARIKUA MEKONNEN ID: AGS/119/2010

TENAWORK GEZAHAGN ID: AGS/122/2010

WASIHUN MEKONEN ID: AGS/133/2010

WONDEWOSENTEKA ID: AGS/135/2010

ADVISOR:WONDIMU BEKELE (MSc)

MAY, 2022

WOLKITE, ETHIOPIA

Table of Contents

LIST OF TABLES iv

LIST OF FIGURE v

LIST OF ABBREVIATIONS vi

ACKNOWLEDGEMENT vii

ABSTRACT viii

1. INTRODUCTION 1

1.1 Background and Justification 1

1.2. Objective of the review 1

2. REVIEW OF LITERATURE AND DISCUSSIONS 2

2.1. Definition of terms 2

2.2 The effect of seed priming in improving seed germination and seedling growth 2

2.3. Recent achievement of seed priming in adverse environmental conditions 3

2.4 Effectiveness of Seed Priming 4

3. MATERIALS AND METHODS 8

3.1 Description of the study area 8

3.2 Experimental Design 8

3.3 Sowing of seeds 8

3.4 Data collection 8

3.5 Data analysis 9

4. RESULTS AND DISCUSSION 10

4.1 Cold Water Treatment Hours on Germination Parameters of Haricot Bean (*PhaseolusVulgaris L.*) 10

4.1.1 Germination percentage in day one measure 10

4.1.2. Germination percentage in day two 11

4.1.3. Germination percentage in day three 12

4.1.4. Germination percentage in day four measure 13

4.1.5. TOTAL GERMINATION PERCENTAGE 14

4.1.6 MET (Day) of haricot bean under the influence of cold water soaking time 15

4.1.7. Shoot Length of haricot bean 16

4.1.8. Root Length of haricot bean under the influence of cold water soaking time 17
4.2 Discussion 18
5. CONCLUSION AND RECOMMENDATIONS 19
5.1 Conclusion 19
5.2 Recommendations 19
REFERENCES 20
APPENDIX 24

LIST OF TABLES

Table 1: Germination percentage in day one measure of haricot bean under the influence of cold water soaking time

Table 2: Results of second day measures of haricot bean seed germination in non-primed seed and after priming as influenced by priming treatment

Table 3: Germination percentage in day three measure of haricot bean under the influence of cold water soaking time

Table 4: Germination percentage in day FOUR measure of haricot bean under the influence of cold water soaking time

Table 5: Total germination percentage of haricot bean under the influence of cold water soaking time

Table 6: MET (Day) of haricot bean under the influence of cold water soaking time

Table 7: Shoot Length of haricot bean under the influence of cold water soaking time

Table 8: Root Length of haricot bean under the influence of cold water soaking time

LIST OF FIGURE

Figure 1: Germination percentage in day one measure of haricot bean under the influence of cold water soaking time

Figure 2: Germination percentage in day two measure of haricot bean under the influence of cold water soaking time

Figure3: Germination percentage in day three measure of haricot bean under the influence of cold water soaking time

Figure 4: Germination percentage in day four measure of haricot bean under the influence of cold water soaking time

Figure 5: Total germination percentage of haricot bean under the influence of cold water soaking time

Figure 6: MET (Day) of haricot bean under the influence of cold water soaking time

Figure 7: Shoot Length of haricot bean under the influence of cold water soaking time

Figure 8: Root Length of haricot bean under the influence of cold water soaking time

LIST OF ABBREVIATIONS

CSA-Central Statistical Agency

FAO-Food Agricultural Organization

RCBD- Randomized Complete Block design

DAP- Diammonium Phosphate

ANOVA- Analysis of Variance

LSD- List Significance Difference

ACKNOWLEDGEMENT

First and most we would like to thanks our almighty GOD to accomplish and come up with this research report. Next we would like to express our sincere thanks to our advisor Mr. Wondimu B. (MSC) for his advice, comments, suggestion and in general for his professional contribution to the completion of this paper.

ABSTRACT

Haricot beans (Phaseolus vulgaris L.) differ in their low temperature tolerance regarding growth and yield. Varieties tolerant to low temperature during germination and carriers of the seed quality standards are needed for the success of the crop. Generally the present study was done on Haricot Bean (P. vulgaris L.) with the general objective to evaluate the effect of different cold water treatment time on haricot bean seed germination. This study was conducted at Wolkite University in the Plant science Laboratory class. A complete block design (CRD) four treatments with three replications was used. Twenty seeds of haricot bean were sown in sterile petri dish containers with moist under continues white light and dark temperature. Twenty seeds were placed per petridish uniformly and covered with lid to prevent loss of moisture through evaporation. The seeds were allowed to germinate for 10 days at room temperature. The germination percentage was recorded on the 10th day. Germination was considered to have occurred when radicles attained a length of 2 mm. The result showed that the time of cold water socking decreased mean germination time, increased shoot length and root length of haricot bean (P. vulgaris L.). Longer socking duration time in cold water has high influential power on the germination parameters of this crop.

Key words: Bean, controlled, cold water, germination, shoot and root

1. INTRODUCTION

1.1 Background and Justification

Haricot bean (Phaseolusvulgaris L.) is the second most important grain legume cultivated as cash crop in Ethiopia (CSA, 2011) and is mainly produced in the rift valley area of the country characterized by high industrialization and urbanization. The Haricot bean (Phaseolusvulgaris L.) is native to the Americas where two major domestication centers and gene pools have been described, Andean and Mesoamerican, which differ in their adaptation to different climatic and eco-geographic conditions. Differences in the seed type and size are clear between both genetic pools (Singh et al., 1991; Santalla et al., 2001), having the Andean varieties larger seeds than the Mesoamerican ones. Early breeding efforts primarily focused on improved disease resistance and adaptation to local environments, with later efforts focused on improved seed quality, improved plant architecture, and breeding for yield (Duc et al., 2015). Seed germination and seedling emergence in the small seeded Mesoamerican genotypes is generally faster than that in the Andean ones, and this phenotypic trait has been used to distinguish between the two genetic pools (White and Montes, 1993). Faster emergence may reflect both genetic variation for adaptation to specific environments and effects of seed size in emergence. Seed size has been recognized as a factor affecting bean germination (Hanley et al., 2003; Kaya et al., 2008) and is probably related to water uptake, a key process in seedling emergence (Bewley, 1997). Seed germination is the process that commences with uptake of water by the dry seed—i.e., imbibition, and terminates with emergence of the seedling.

Thus, the process involves two temporal stages, namely the germination stage and the emergence, or seedling-growth stage (Bewley and Black, 1985; Bewley, 1997). Fast seed germination is considered an important adaptive trait marking a quick transition to the growth phase in the lifecycle of a plant. The time taken for the germination process to be completed is one of the important parameters of seed quality (Copeland and McDonald, 1995; Dutt and Geneve, 2007). The purpose of germination testing is to provide information on the comparative and foreseeable field planting value of different seed samples. The objectives of this study were to compare the germination of bean seedlings under controlled environment.

The seedlings emerging from primed seeds showed early and uniform germination. Moreover, the overall growth of seedling and plant is improved due to the seed-priming treatments, so that farmers or any of crop producers should be practice and apply the science of seed priming for maximizing crop yield in any of adverse environmental conditions

1.2. Objective of the review

To evaluate the effects of cold water soaking time on haricot bean seeds germination and seedling growth.

2. REVIEW OF LITERATURE AND DISCUSSIONS

2.1. Definition of terms

Seed priming: Seed priming could be defined as controlling the hydration level within seeds so that the metabolic activity necessary for germination can occur but radical emergence is prevented. Different physiological activities within the seed occur at different moisture levels (Taylor et al., 1998).

Seed germination: Seed germination commonly implies three distinct phases; consisting in Phase I: seed hydration process relate d to passive imbibitions of dry tissues associated with water movement first occurring in the apoplastic spaces; Phase II: activation phase associated with the reestablishment of metabolic activities and repairing processes at the cell level; and Phase III: initiation of growing processes associated to cell elongation and leading t o radical protrusion. Phases I and III both involve an increase in t he water content while hydration remains stable during Phase II. It is commonly considered that before the end of Phase II, germination remains a reversible process (Lutts et al., 2016).

2.2 The effect of seed priming in improving seed germination and seedling growth

Doing well crop establishment and high seedling vigor are well thought-out as decisive factors for the success of most field crops, as these parameters contribute to uniform plant growth and maturity, better struggle with weeds, and high productivity. Therefore, improving seed vigor is the primary objective of the industry of crop production to enhance the critical and yield increasing stage of crop establishment. However, low vigor of seeds or ad verse environmental conditions after sowing may cause slow see d germination and uneven seedling emergence under field conditions (Christos et al., 2019). Therefore, seed treatment before sowing is the foundation for activation of seed resources that in combination with external ingredients could contribute to the efficient plant growth and high yield.

Various physiological and non physiological techniques are available for enhancing seed performance as well as to combat environmental constraints. The physiologic al treatments for improving seed germination and stand establishment are composed of seed hydration techniques such as humidification, wetting, and presoaking. The other techniques for promoting germination are comprised of chemical treatments, seed inoculation with beneficial microbes, and seed coating. Seed quality can determine the chance of producing healthy seedlings and sufficient plant population for achieving high yield. In practice, seed quality loosely react the overall of seed performance. Under field conditions, poor seed quality can delay the onset of germination, adversely affects seedling vigor, and reduce the final crop stand. Establishing rate affects crop entity and competitiveness of the crop stand, tillering or branching, and yield and thus is of great importance. Vigorous plants have high ability to capture resources, can better tolerate pests and plant pathogens, struggle with weeds, and are expected to be more tolerant to applied herbicides (Christos et al., 2019).

Seed priming is a physiological method of controlled hydration and drying to enhance sufficient pre-germinative metabolic process for rapid germination (Dawoo d, 2018). It has been reported that primed crop seeds emerged faster and grew more vigorously. They also flowered and matured earlier and gave higher yields, which is very important for adverse environmental condition such as drought prone area (Mamun et al., 2018). More to the point th e benefit of priming is to increase the rate of germination at any particular temperature. On a practical level, primed seeds emerge from the soil faster and often more uniformly than non-primed seeds because of limited adverse environmental exposure. Priming accomplishes this important development by shortening the lag or metabolic phase in the germination process. The metabolic phase occurs just after seeds are fully imbibed and just prior to radical emergence. Since seeds have already gone through this phase during priming, germination times in the field can be reduced by approximately 50% upon sub sequent rehydration. Not only this, priming has been commercially used to eliminate or greatly reduce the amount of seed borne fungi and bacteria. In this framework, we have mentioned the following priming techniques on farm priming, Hardening, Hydro priming, Osmo hardening, Vitamin C (Ascorbate) priming (Mamun et al., 2018). Moreover, seed priming has been shown to improve seed performance under cold environmental conditions (Lin and Sung, 2001).

On the other hand, globally, soil salinization is one of the most important ecological issues of dry land agriculture and has become a major hindrance for the yield of field crops. Besides that, salinization is dispersal in irrigated lands because of inappropriate management of irrigation and farming (Abraha and Yohannes, 201 3). Salinity causes a series of biochemical, physiological and metabolic changes in plants, so that different seed priming techniques has to develop for facing the challenges behind soil salinization and other abiotic crop stress o n seed germination seedling vigor as well as crop productivity (Ibrahim, 2016). Therefore reviewing and gathering information based on the previous research finding, as well as identifying the knowledge and research gaps o the recent achievement of seed priming in improving seed germination and seedling growth in adverse environmental conditions i s very important.

2.3. Recent achievement of seed priming in adverse environmental conditions

Adverse environmental conditions significantly reduce seed germination plant growth, yield, and the marketable produce quality, with annually considerable economic losses as a consequence. Most of the adverse environmental conditions, including drought, salinity, extreme high and low temperature, high light, and pollutants, such as heavy metals or pesticides, result in oxidative stress that is an increase in the cellular levels of reactive oxygen species. Adverse environmental condition stresses not only retard plant growth and diminish yield in cases of crop plants, but also severe stress can trigger programmed cell death (Petrov et al., 2015).

According to Yan Dai et al. (2017) Seed priming treatments, including hydro priming and comprehensive seed priming, can mitigate the effects of soda saline-alkali stress in soybean seedlings. Moreover, seed priming with ZnSO4, CaCl, betaine hydrochloride and GA32 was

found to be more effective than hydro priming to flourish under soda saline alkali stress. The better growth performance of soybean seedlings from comprehensive seed priming was related with a stronger osmotic adjustment, higher activities of the antioxidant defense system, more photosynthetic pigment contents, better membrane integrity and more added starch accumulation under soda saline alkali stress. Sorghum seeds responded well to priming treatments consisting of N, Zn solutions or water.

Priming sorghum seeds with Zn solution for 10hour duration dramatically improved the germination percent, germination index, and reduced mean germination time compared to the unprimed seeds. Priming seeds with Zn for 10 h had also a profound effect on vigor and seedling growth parameters and increased the seedling height, seedling dry weight, and seedling vigor index-1 and 2 as compared to unprimed seeds. Among sorghum varieties studied, varieties Melkam followed by Dekeba and Teshale with 10 h priming duration performed better than the other varieties with respect to the majority of the traits studied. Priming of sorghum seeds for 10h is an optimum priming duration for most of the varieties. Moreover, the use of limiting nutrients such as Zn as seed priming agent would be an excellent option to improve germination, seedling growth, and its vigor. However, in the absence of Zn or urea, farmers have the option to use water to prime their seeds to enhance seedling germination and stand establishment (Wondemu et al., 2018).

In addition this Reduction and delay in the germination of spring maize (Zeamays L.) may be a problem due to low soil temperatures and low water potentials. However, seed priming is used to improve rate and speed of germination, and expand temperature limits for germination under normal and stressful conditions. Maize seeds were subjected to hydro priming, hormonal priming with 100 mg L or IAA solutions and halo priming with 50m M CaCl2 or 50 mg L acerbate (ASA) for 24h. All pre sowing seed treatments resulted i n a higher germination percentage and germination index, lower mean germination time and mean emergence time. All seed treatments resulted in higher seedling fresh and dry weight compared with that of control with maximum dry weight recorded for seeds subjected to ASA, CaCl2 and GA. Performance was better in seeds subjected to CaCl be ascribed to the effect of lower ASA followed by GA 503 3-1 GA for 24 h than for the other priming agents. This could, higher reduce and total sugars as well as higher a-amylase activity (Afzal et al., 2008).

2.4 Effectiveness of Seed Priming

Singh et al. (2014) declared that primed corn seeds under adverse environmental condition had a better chance for producing a good crop with higher economic yield. Moreover seed priming has practical implications in improving performance of vegetable crops under stressed environmentalconditions such as salinity, drought, low and higher temperature (Piri, 2009). Furthermore, the chitosan priming increased the chilling tolerance of maize seedlings confirmed by improving germination speed, and shoots and root growth and maintaining membrane

integrity and higher activities of ant oxidative enzymes. The 0.50% chitosan seems to be a suitable concentration for seed priming (Guan et al., 2009).

According to Afzal et al.(2012), Seed priming was effective in inducing chilling tolerance in maize through modulation of vigor associated attributes such as hydrolytic enzyme activities, chlorophyll and carbohydrate metabolism. Among priming agents, priming with Moraga leaf extract seems more practical being less expensive, non-toxic and the most effective in increasing the ability of maize plants to withstand low temperature. Hence, seed priming with Moraga leaf extract can be recommended after extensive field appraisal across a wide range of environments and genotypes to improve the performance of early spring planted maize. Seed priming treatments such as selenium and salicylic acid priming were tested in field and growth chamber experiments and the results revealed that under chilling stress, seed priming increased the rice seed germination by 20.96 up to 26.31 %. The length and weight of shoots and roots were also significantly increased. The improved germination and seedling growth of primed seeds under chilling stress were strongly linked with higher a amylase activity and total soluble sugar content (Wang et al., 2016).

Similarly, twenty-four diverse maize inbred lines were primed using a synthetic solid matrix and then exposed to 10°C soil conditions. Chilling substantially reduced total emergence for two of 24 genotypes evaluated. For these genotypes, priming provided protection allowing nearly full emergence. Priming significantly reduced mean emergence time and increased the emergence uniformity of chilling sensitive genotypes. The results suggest that the cold sensitive genotypes may benefit from priming pretreatment (Hacisalihoglu et al., 2018). Jafar et al. (2012) concluded that, among the different priming agents used, osmo-priming with CaCl and ascorbate priming was the most effective in alleviating salt stress effects on grain yield irrespective of wheat varieties. Physiologically, the beneficial effect of this priming treatment can be attributed to increased accumulation of soluble proteins, phenolics, soluble sugars and K 2+ with simultaneous decrease in Na+ uptake. These treatments can therefore be employed to improve the performance of wheat under saline conditions. Seed priming with Se or SA, was found to be more effective under chilling stress. The better germination and vigorous growth of primed rice seedlings was associated with; higher starch metabolism, enhanced respiration rate, better membrane integrity, higher metabolite synthesis, and increased activities of antioxidants in these seedlings (Hussain et al., 2016). Priming of maize hybrid seeds with in 50, 100 and 150 ppm (mg l) aerated solutions of SA for 24 h and were dried back. Treated and untreated seeds were sown at 270C (optimal temperature) and at 15 0C (chilling stress) under controlled conditions. Performance of maize seedlings was hampered under chilling stress. But seed priming with SA improved the seedling emergence, root and shoots length, seedling fresh and dry weights, and leaf and root score considerably compared with control both at optimal and chilling temperatures. However, priming in 50 mg l)1 SA solution was more effective, followed by priming in 100 mg l. Seed priming with SA improved the chilling tolerance in hybrid maize mainly by the activation of antioxidants. Moreover, maintenance of high tissue water contents

and reduced membrane permeability also contributed towards chilling tolerance (Farooq et al., 2008).

According to Christos et al. (2019) the effects of hydro-priming for 0,8,16,24,36, and 48 hours on final germination percentage, germination speed, germination index, mean germination time, mean daily germination, synchronization index, and seedling vigor index were studied. Average over priming duration treatments, hydro priming improved germination speed by 16.2%, germination synchrony by 20.7% and seedling vigor index by 13.4%, but did not affect significantlyfinal germination percentage and mean daily germination compared with non-primed seeds. In field trials, seed priming for 8 h resulted in higher fresh weight at anthesis by 22.3% and 8.6% in the first and the second year than the non-primed control. Similarly, seed priming for 8 h provided higher seed yield by 12.0% in the first year and by 5.9% in the second year compared with nonprimed control. Overall, seed hydro-priming accelerated faba bean germination and seedling emergence in the field, but the magnitude of the response was associated with the environment and was more evident under limited soil moisture after sowing, whereas the beneficial effect of priming was masked when rainfall followed sowing.

Similarly, the beneficial effect of priming on seed yield was more pronounced with limited soil moisture after anthesis. As hydropriming is a simple technique, evaluating the efficiency of this priming method in deferent environmental conditions is essential to optimize our chosen priming technique. Molecular priming is evolving as an efficient technology to counteract the adverse effects of abiotic stresses. It is environmentally friendly and applicable to a wide variety of species. The priming agents can be applied only at critical times, for instance just before an upcoming stress, such as chilling or drought, and at discrete developmental stages susceptible to stress (such as fruit set), to maximize their efficiency. In contrast, this flexibility poses certain challenges. An accurate weather forecast and readiness to apply priming agents as environmental conditions change would be crucial for the exploitation of their full potential (Kerchev et al., 2020).

During the early spring or late autumn cultivation periods, the delaying effect of low temperature on germination of lettuce seeds can be significantly compensated with a short period of priming with aqueous solution of vitamin U.Vitality of the photosynthetic apparatus, which converts incident light energy into chemically stored energy, as well as uptake, and assimilation of carbon dioxide by leaves are less inhibited by cold stress if hydroponically grown lettuce plants are primed with mill molar amounts of vitamin U dissolved in the nutrient medium. Leaves of lettuce plants primed with 0.25-2 mM vitamin U have higher vitamin C content and less reduced carotenoid content upon growth at low temperature, thus they have a higher quality as health promoting food source (Fodorpataki, 2019).

Similarly, Priming can improve cold tolerance of lettuce in its early developmental stages, resulting in more efficient cultivation and better quality for human consumption. The first reports on the beneficial influence of priming with vitamin U on photochemical efficiency of

photosynthesis, on carbon dioxide assimilation, on carotenoid pigment and ascorbic acid content of lettuce leaves exposed to cold stress. The fact that effects of priming are not proportional with concentration suggests that vita min U may act as a signaling molecule to boost physiological performances through improvements in anti stress pathways that involve sulfur containing compounds. Further investigations are needed to elucidate the mechanism of action vitamin U in plants exposed to different environmental stresses taking into account that scientific information regarding the role of this biologically active natural metabolite is very scarce (Fodorpataki, 2019).

Seed priming with CaCl2, GA3, and H improved I. indigotica Fort. Seed germination and seedling growth under salt stress. The optimal concentrations were 15 g/L for CaCl priming, 0.2 g/L for GA for H2O23 priming, and 40 mM priming. Seed priming treatments greatly promoted SOD, POD, and CAT activities and alleviated the oxidative damage induced by salt stress in I. indigotic.Therefore, it may be concluded that seed priming is a promising approach to accelerating I. indigotic growth under salt conditions (Jiang et al., 2020).

Pretreatment of Arabidopsis plants with A. nodosum extracts activates partial closure of stomata and modifies ABA signaling and antioxidant systems. These preactivation results in enhanced protection when a secondary drought stress stimulus is detected (Santaniello et al., 2017). Arabidopsis, tomato, and pepper plants primed with an extract from A. nodosum are completely protected against paraquat-induced oxidative stress. At the molecular level, the seaweed extract treatment can modulate micro RNAs, which are key regulators of gene expression, and enhance tolerance to salt stress (Shukla et al., 2018).

Different structurally unrelated endogenous and synthetic organic molecules have been shown to prime plants against stress. The external application of acetic acid, for example, improved drought tolerance in Arabidopsis, rapeseed, maize, rice and wheat plants. This evolutionarily conserved priming mechanism relies on the promotion of jasmonic acid (JA) synthesis and enrichment of histone H4 acetylation that serves as an epigenetic switch by which plants adapt to drought (Kim et al., 2017). Generally and more to the point primed seed can increase their germination characteristics as compared to the unprimed seeds. The highest germination characteristics were obtained from priming by halo and hydro priming which was performed in control conditions. Besides that Priming can improve seed reserve utilization such as: weight of utilized seed reserve, seed reserve utilization efficiency and seedling growth in millet seeds under salinity stress (AghbolaghI and Sedghi et al., 2014). Abiotic stress protection in plants could be mediated by stimulating memory responses at the genetic level (De Palma et al., 2019) but could also be improved by pretreatment with several non pathogenic bacterial strains. Thus the belowground interactions between these beneficial bacterial strains and plants have the capacity to prime and activate defense response (Mhlongo et al., 2018).

3. MATERIALS AND METHODS

3.1 Description of the study area

This study was conducted at Wolkite University in the Plant science cluster campus which is located on 150 Km from Addis Ababa to south on the road of Jimma.

3.2 Experimental Design

A complete block design (CRD) four treatments with three replications was used. Sowing of seeds was carried out on the 20 of May 2022 between 4 and 5pm. Two seeds were sown for each polythene pot; the sowing depth was 1cm. Twenty seeds of haricot bean were sown in containers with moist under continues white light and dark temperature. Monitoring of emergence was carried out every day. Seedlings with a hypocotyls radicle were considered as emerged. Proportions of germination (% of sown seeds) were measured.

3.3 Sowing of seeds

Twelve petri dishes were washed with detergent using hot water as protective measure against pathogens. Haricot bean seeds were also cleaned manually after physical purity is checked. Then the seeds were soaked in cold water for 8, 12 and 24 hours. Twenty seeds of haricot bean were sown with a 3 cm diameter in each petri dish. The petri dishes were kept at room temperature (30oC±4) throughout the study and both treated and control petri dishes were kept moist continuously by adding water for treatment and control, whenever needed.

3.4 Data collection

On the 10th day, 10 seedlings in each Petri plate were sampled to measure the root length and shoot length using a scale. The total number of germinated seedlings after sowing in each expressed as a percentage.

1. Germination percentage: Each petri dish was checked and recorded daily for days to germination after sowing to 3rd day.

2. Number of germinated seed: Numbers of germinated seeds were counted on the 9th day after sowing.

3. Root length: Each of the length of the main root of the control and treated plants was measured in unit of centimeter.

4. Shoot length: Each of the length of the shoot of control and treated plants was measured in unit of centimeter.

5. Germination percentage:The germinated seeds were counted daily to calculate germination rateat the end of the germination period.

6. Mean emergence time (MET):

Three replicates of 180 seeds were placed between layers of paper. The number of germinated seeds was counted every day and the mean emergence time (MET) was calculated using the following formula (Demir et al., 2008):

$$MET = \sum nD / \sum n$$

Where

n is the number of seeds newly germinated on day D from the beginning of the germination test and $\sum n =$ Total number of seeds germinated.

3.5 Data analysis

The experimental layout was completely randomized design with three replications at p=0.05, to get the best treatment time for each of the pre- treatments. Untreated seeds were taken as control. Data collected in the experiment were analyzed using the Gomez and Gomez (1984) ANOVA procedure and significant differences are further sorted out using the LSD.

4. RESULTS AND DISCUSSION

4.1 Cold Water Treatment Hours on Germination Parameters of Haricot Bean (*PhaseolusVulgaris L.*)

The following Table 1 shows the whole analysis of variances where there were significance differences between different cold water treatment hours on germination parameters of haricot bean (Phaseolusvulgaris L.). Germination day, root length and shoot length were significantly influenced duration of cold water soaking time.

As it has been shown in the Table below, germination day decreases with the increasing of the time of the cold water soaking period. It was decreased from control to treatment fourwhich indicates that high level of time soaking of seeds in cold water affect the germination day by shifting it downward when compared to the control.

Shootlength and root lengthwere increased with the increasing of cold water soaking time.All treatments were increased with their root length and shoot length.

4.1.1 Germination percentage in day one measure

Table 1: Germination percentage in day one measure of haricot bean under the influence of cold water soaking time

Treatment	Mean	N	Std. Deviation	Std. Error of Mean	Variance
Control	.3333	3	.57735	.33333	.333
8hours cold water treated	1.3333	3	.57735	.33333	.333
12hours cold water treated	5.6667	3	2.08167	1.20185	4.333
24hours cold water treated	9.6667	3	.57735	.33333	.333
Total	4.2500	12	4.00284	1.15552	16.023

LSD: 0.173

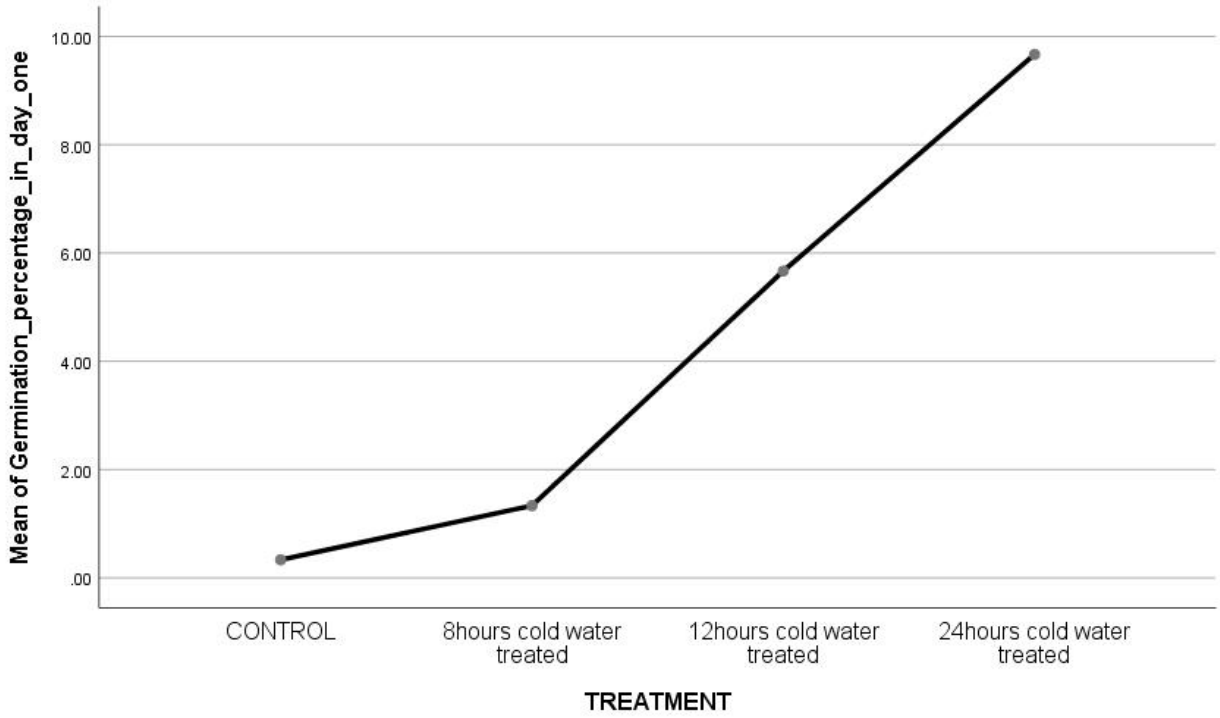

Figure 1: Germination percentage in day one measure of haricot bean under the influence of cold water soaking time

4.1.2. Germination percentage in day two

Table 2:Results of second day measures of haricot bean seed germination in non-primed seed and after priming as influenced by priming treatment

Treatment	Mean	N	Std. Deviation	Std. Error of Mean	Variance
Control	1.3333	3	.57735	.33333	.333
8hours cold water treated	3.6667	3	1.15470	.66667	1.333
12hours cold water treated	5.6667	3	.57735	.33333	.333
24hours cold water treated	6.3333	3	.57735	.33333	.333
Total	4.2500	12	2.13733	.61699	4.568

LSD: 0.114

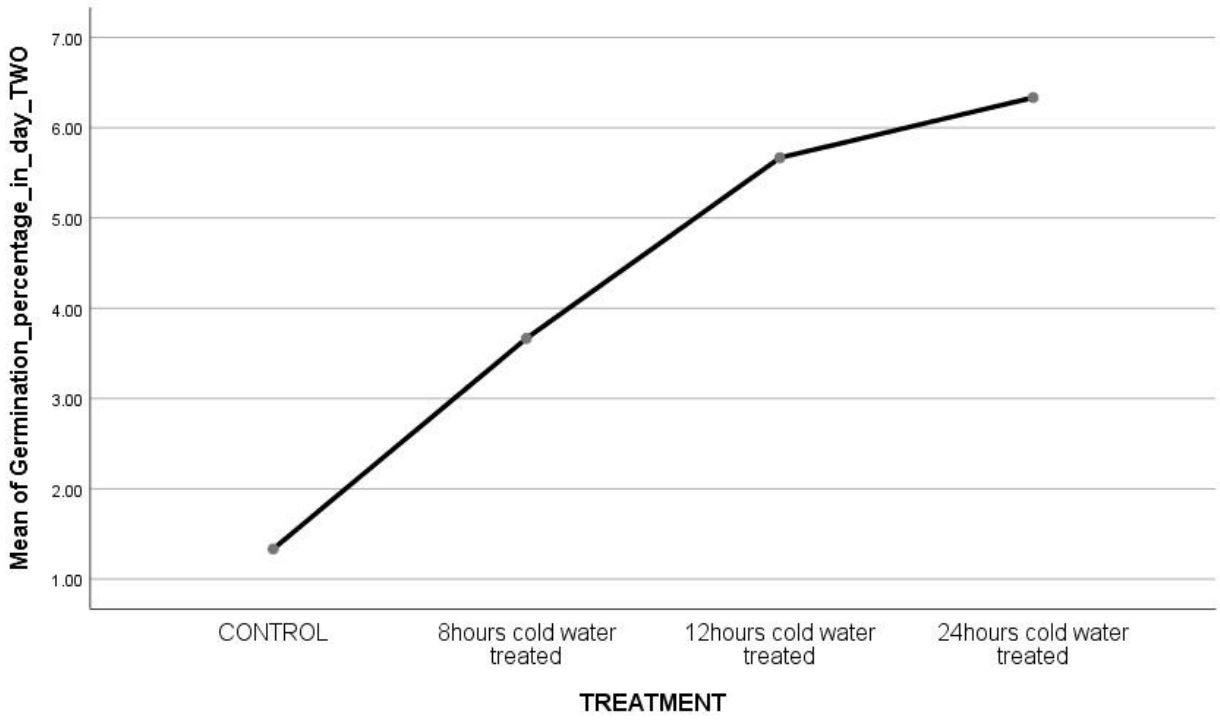

Figure 2: Germination percentage in day two measure of haricot bean under the influence of cold water soaking time

4.1.3. Germination percentage in day three

Table 3: Germination percentage in day three measure of haricot bean under the influence of cold water soaking time

TREATMENT	Mean	N	Std. Deviation	Variance	Std. Error of Mean
CONTROL	5.3333	3	1.52753	2.333	.88192
8hours treated	6.3333	3	1.15470	1.333	.66667
12hours treated	5.0000	3	1.73205	3.000	1.00000
24hours treated	3.0000	3	1.00000	1.000	.57735
Total	4.9167	12	1.72986	2.992	.49937

LSD: 0.207

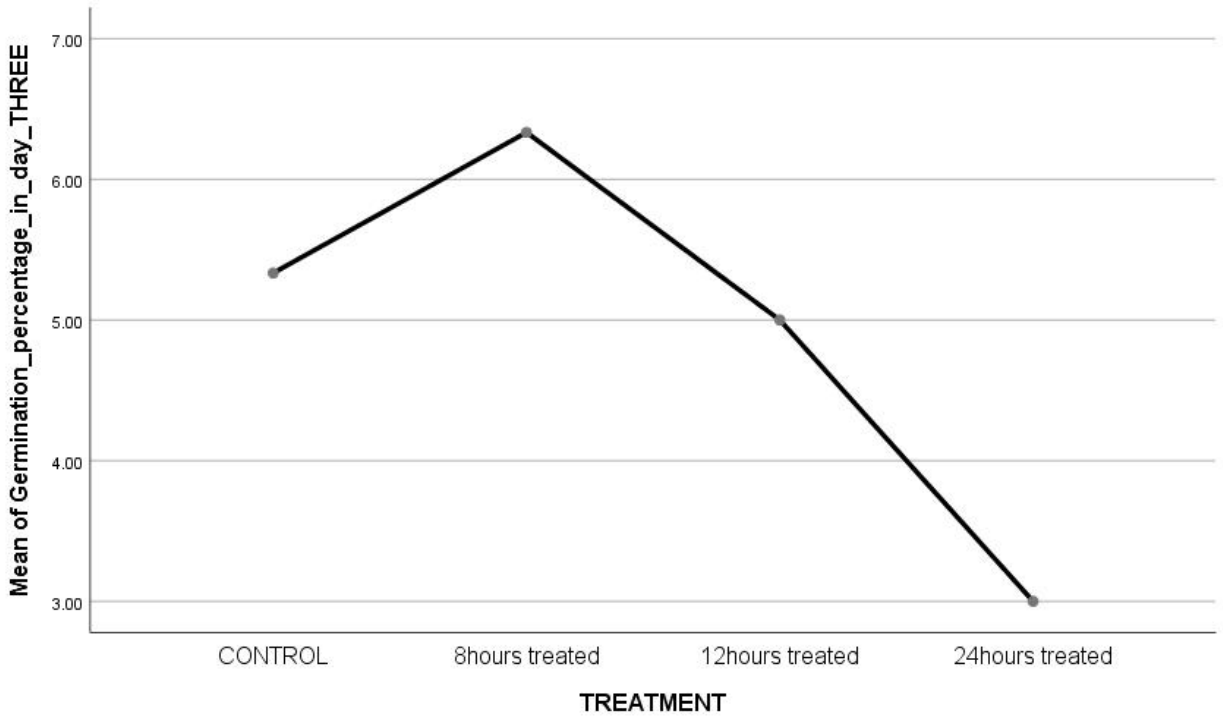

Figure3: Germination percentage in day three measure of haricot bean under the influence of cold water soaking time

4.1.4. Germination percentage in day four measure

Table 4: Germination percentage in day four measure of haricot bean under the influence of cold water soaking time

TREATMENT	Mean	N	Std. Deviation	Variance	Std. Error of Mean
CONTROL	7.0000	3	1.00000	1.000	.57735
8hours treated	5.6667	3	1.15470	1.333	.66667
12hours treated	2.6667	3	1.52753	2.333	.88192
24hours treated	.6667	3	.57735	.333	.33333
Total	4.0000	12	2.76340	7.636	.79772

LSD: 0.167

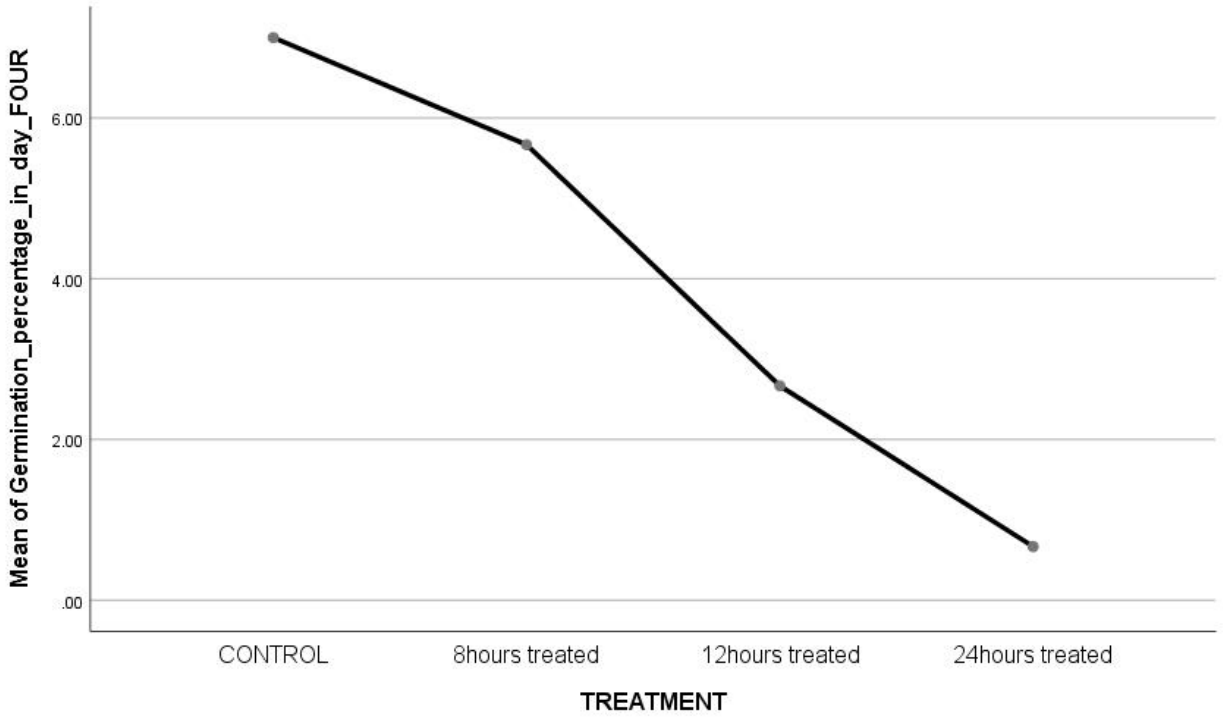

Figure 4: Germination percentage in day four measure of haricot bean under the influence of cold water soaking time

4.1.5. TOTAL GERMINATION PERCENTAGE

Table 5: Total germination percentage of haricot bean under the influence of cold water soaking time

TREATMENT	Mean	N	Std. Deviation	Variance	Std. Error of Mean
CONTROL	19.4433	3	4.19281	17.580	2.42072
8hours treated	29.9967	3	2.88675	8.333	1.66667
12hours treated	31.1133	3	.96417	.930	.55667
24hours treated	33.3300	3	5.00000	25.000	2.88675
Total	28.4708	12	6.37424	40.631	1.84008

LSD: 0.539

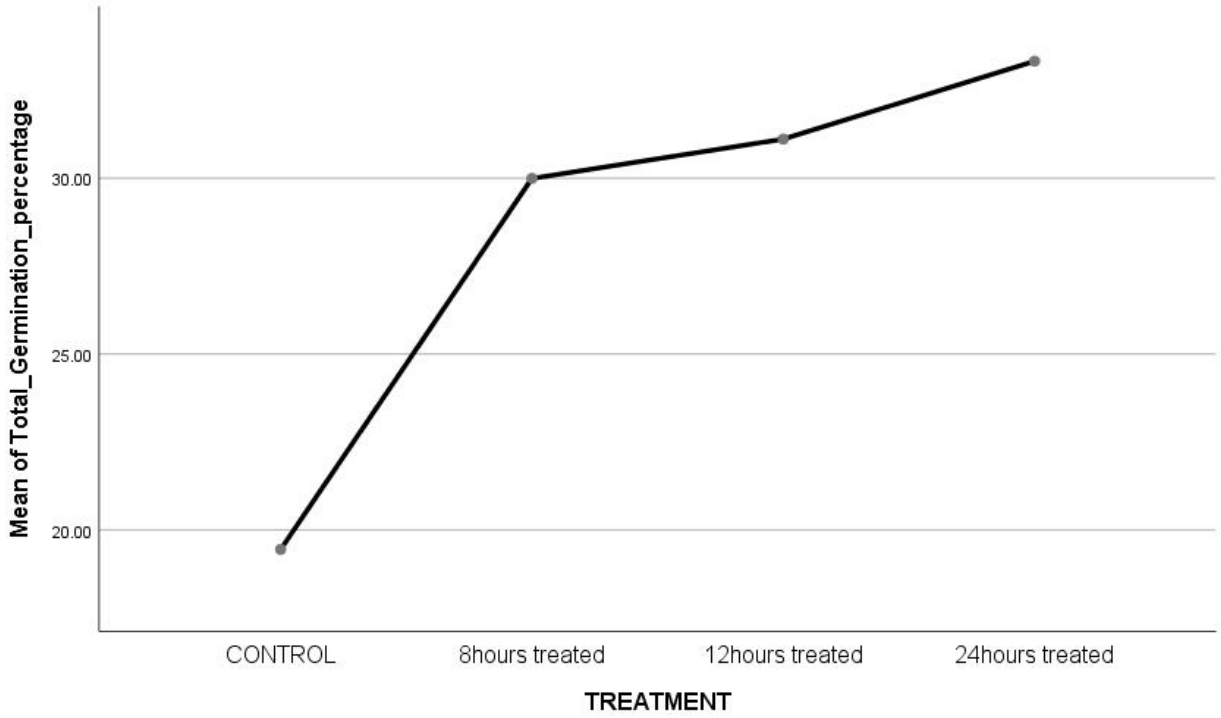

Figure 5: Total germination percentage of haricot bean under the influence of cold water soaking time

4.1.6 MET (Day) of haricot bean under the influence of cold water soaking time

Table 6: MET (Day) of haricot bean under the influence of cold water soaking time

Treatment	Mean	N	Std. Deviation	Variance	Std. Error of Mean
Control	1.7860	3	.23800	.057	.13741
8hours treated	1.5883	3	.01950	.000	.01126
12hours treated	1.4737	3	.16720	.028	.09653
24hours treated	1.2427	3	.03539	.001	.02043
Total	1.5227	12	.24042	.058	.06940

LSD: 0.022

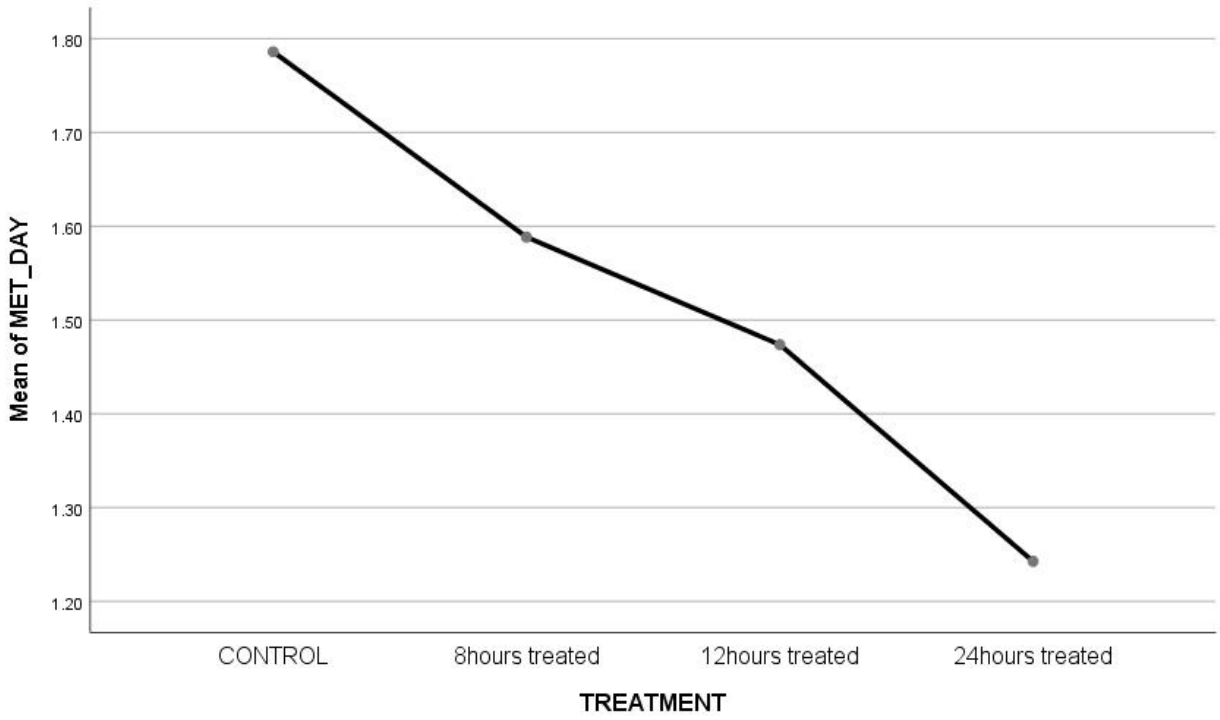

Figure 6: MET (Day) of haricot bean under the influence of cold water soaking time

4.1.7. Shoot Length of haricot bean

Table 7: Shoot Length of haricot bean under the influence of cold water soaking time

Treatment	Mean	N	Std. Deviation	Variance	Std. Error of Mean
Control	3.5833	3	.07638	.006	.04410
8hours treated	4.0167	3	.10017	.010	.05783
12hours treated	4.3200	3	.07550	.006	.04359
24hours treated	4.5500	3	.05000	.002	.02887
Total	4.1175	12	.38362	.147	.11074

LSD: 0.012

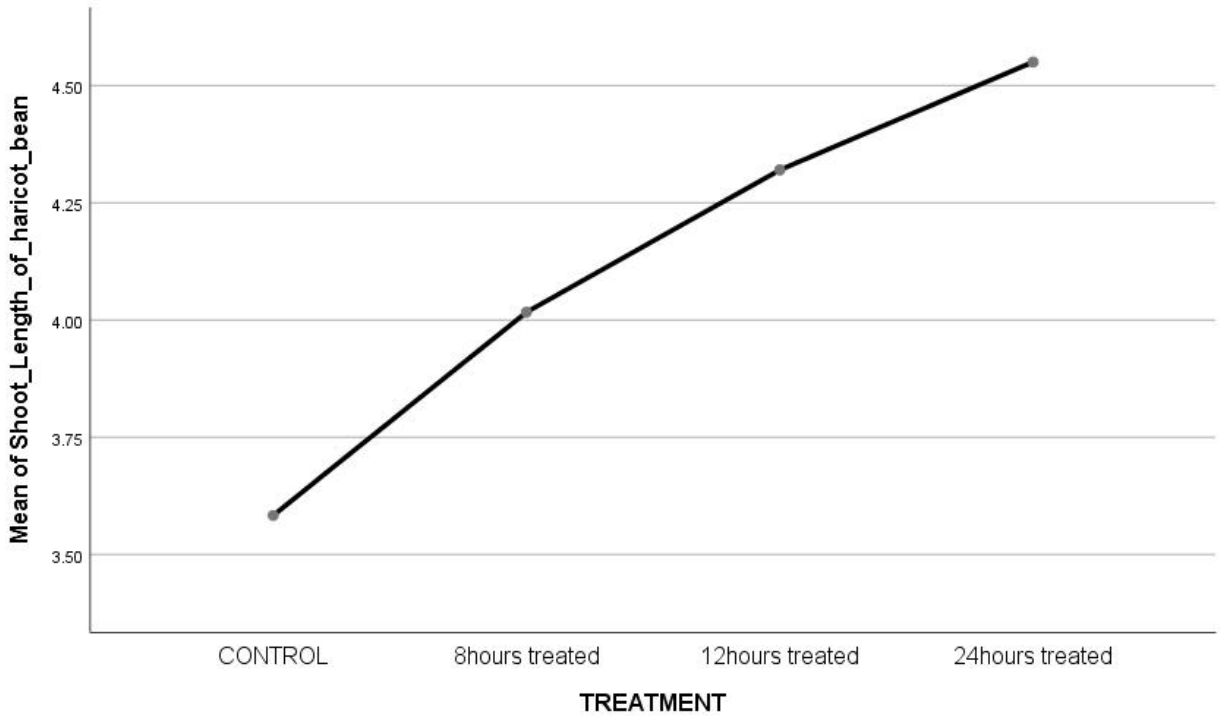

Figure 7: Shoot Length of haricot bean under the influence of cold water soaking time

4.1.8. Root Length of haricot bean under the influence of cold water soaking time

Table 8: Root Length of haricot bean under the influence of cold water soaking time

TREATMENT	Mean	N	Std. Deviation	Variance	Std. Error of Mean
CONTROL	6.5400	3	.09644	.009	.05568
8hours treated	6.8633	3	.05132	.003	.02963
12hours treated	7.0167	3	.07638	.006	.04410
24hours treated	7.2267	3	.07506	.006	.04333
Total	6.9117	12	.26951	.073	.07780

LSD: 0.012

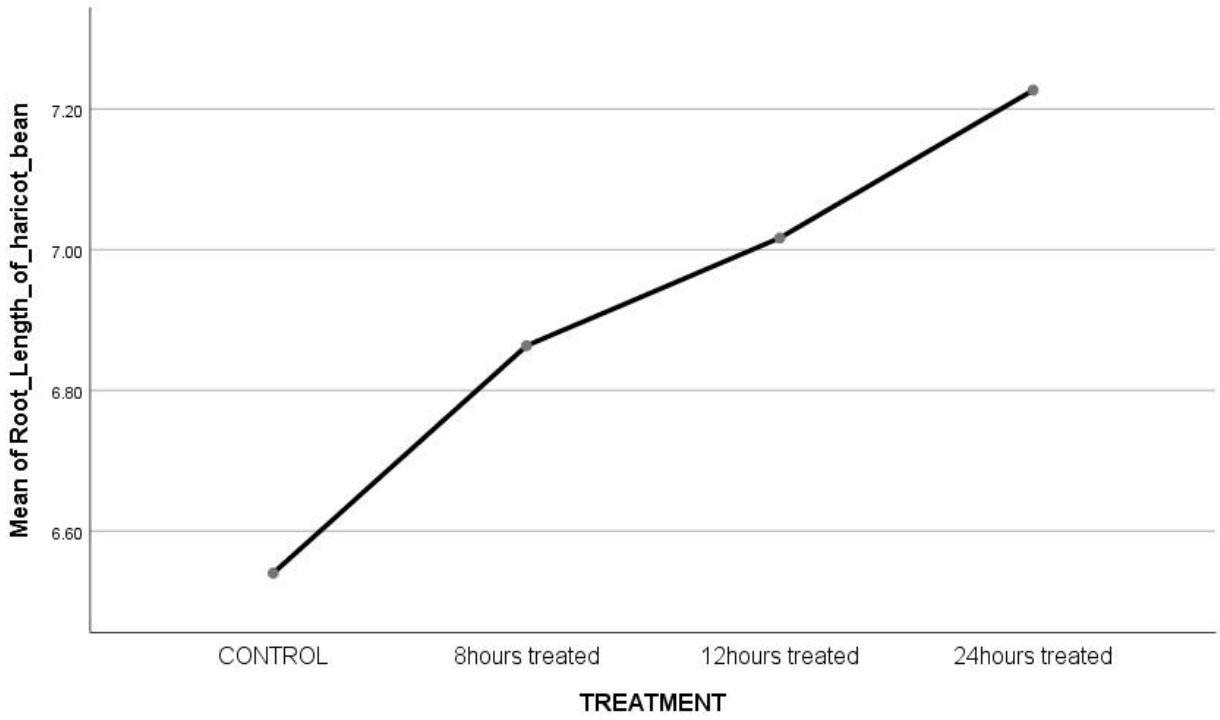

Figure 8: Root Length of haricot bean under the influence of cold water soaking time

4.2 Discussion

Germination day decrease with the increasing of the cold water soaking time, which indicates that high amount of cold water soaking time affect the germination day and percentage by shifting it dawnward when compared to the control as in Table 5.

Germination percentage increase with the increasing of the cold water soaking time, which indicates that high amount of cold water soaking time affect the germination day and percentage by shifting it downwards when compared to the control as in Table --.

Increment of root length and shoot length with the increasing of cold water soaking time were indicating that they were highly influenced the haricot bean seeds.

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Generally the present study was done on Haricot Bean (*P. vulgaris* L.) with the general objective to evaluate the effect of different cold watertreatment time on haricot bean seed germination. The result showed that the time of cold water soaking decreased mean germination time, increased shoot length and root length of haricot bean (*P. vulgaris* L.). Longer socking duration time in cold water has high influential power on the germination parameters of this crop.

5.2 Recommendations

Keeping the above in view, it can be recommended that seeds of haricot bean (*P. vulgaris L.*) should not be planted without cold water treatment due to adverse less tolerance of effects of the environment on their growth and also since this experiment was carried out only in petridish and cold water, another study which can show detail cold water time of socking influence on haricot bean (*P. vulgaris L.)* is kept back.

REFERENCES

Abraha, B. and Yohannes, G. (2013). The role of seed priming in improving seedling growth of maize (Zeamays L.) under salt stress at field conditions. Agricultural Sciences 4:666-672.

Afzal, I., Hussain, B., Basra, S.M.A. and Rehman, H. (2012), Priming with moringa leaf extract reduces imbibitional chilling injury in spring maize; Seed Sci. & Technol., 40, 271-276.

Afzal, I.,Basra, S.M.A., Shahid, M., Farooq, M. and Saleem M. (2008), Priming enhances germination of spring maize (Zeamays L.) under cool conditions Seed Sci. & Technol., 36, 497-503.

Awal M. A., Ikeda T. (2002). Effects of changes in soil temperature on seedling emergence and phenological development in field-grown stands of peanut (Arachishypogaea). Environ. Exp. Bot. 47, 101–113. 10.1016/S0098-8472(01)00113-7

Bewley J. D. (1997). Seed germination and plant dormancy. Plant Cell 9, 1055–1066. 10.1105/tpc.9.7.1055

Bewley J. D., Black M. (1985). Seeds: Physiology of Development and Germination. New York, NY: Plenum Press.

Christos A. Damalas, Spyridon D. Koutroubas and Sideris Fotiadis.(2019). Hydro-Priming Effects on Seed Germination and Field Performance of Faba Bean in Spring Sowing; Journal of Agriculture, 9, 201.

Copeland O. L., McDonald B. M. (1995). Principles of Seed Science and Technology.3rd Edn. New York, NY: Chapman and Hall.

CSA (2011). Central Statistics Authority, Abstract. Addis Ababa. Ethiopia

Covell S., Ellis R. H., Roberts E. H., Summerfield R. J. (1986). The influence of temperature on seed germination rate in grain legumes. I. Acomparison of chickpea, lentil, soybean and cowpea at constant temperatures. J. Exp. Bot. 37, 705–715. 10.1093/jxb/37.5.705

Craufurd P. Q., Ellis R. H., Summerfield R. J., Menin L. (1996). Development in cowpea (Vignaunguiculata). 1. The influence of temperature on seed germination and seedling emergence. Exp. Agric. 32, 1–12. 10.1017/S0014479700025801

Dawood MG (2018) Stimulating plant tolerance against abiotic stress through seed priming. In:Advances in seed priming. Springer, Singapore, pp 147– 183.

De Palma, M., Salzano, M., Villano, C., Aversano, R., Lorito, M., Ruocco, M., Docimo, T.,Piccinelli, A.L., D'Agostino, N., Tucci, M., 2019. Transcriptome reprogramming, epigenetic modifications and alternative splicing orchestrate the tomato root response to the beneficial fungus Trichodermaharzianum. Hortic.Res. 6, 5.

Dickson M. H. (1971). Breeding beans, Phaseolusvulgaris L., for improved germination under unfavorable low temperature conditions. Crop Sci. 11, 848–850. 10.2135/cropsci1971.0011183X001100060024x

Duc G., Agrama H., Bao S., Berger J., Bourion V., Burstin J., et al. (2015). Breeding annual legumes for adaptation to low input cropping systems and new areas: methods to approach more complex traits and target new variety ideotypes. Crit. Rev. Plant Sci. 34, 381–411. 10.1080/07352689.2014.898469

Dutt M., Geneve R. L. (2007). Time to radicle protrusion does not correlate with early seedling growth in individual seeds of impatiens and petunia. J. Am. Soc. Hortic. Sci. 132, 423–428.

Ellis R. H., Covell S., Roberts E. H., Summerfield R. J. (1986). The influence of temperature on seed germination rate in grain legumes. II. Interspecific variation in chickpea (Cicerarietinum L.) at constant temperature. J. Exp. Bot. 37, 1503–1515. 10.1093/jxb/37.10.1503

Hacisalihoglu G, Kantanka S, Miller N, Gustin JL, Settles AM. (2018)Modulation of early maize seedling performance via priming under sub optimal temperatures.PLoS ONE 13 (11): e0206861. https://doi.org/10.1371/journal. pone.0206861. [8] Ibrahim, E.A. 2016. Seed priming to alleviate salinity stress in germinating seeds. Journal of Plant Physiology 192:38-46.

Hanley M. E., Unna J. E., Darvill B. (2003). Seed size and germination response: a relationship for fire-following plant species exposed to thermal shock. Oecologia 134, 18–22. 10.1007/s00442002-1094-2

Kaya M., Kaya G., Kaya M. D., Atak M., Saglam S., Khawar K. M., et al. . (2008). Interaction between seed size and NaCl on germination and early seedling growth of some Torkish cultivars of chickpea (Cicerarietinum L.). J. Zhejiang Univ. Sci. B 9, 371–377. 10.1631/jzus.B0720268

Kim, J.-M., To, T.K., Matsui, A., Tanoi, K., Kobayashi, N.I., Matsuda, F., Habu, Y., Ogawa, D., Sakamoto, T., Matsunaga, S., Bashir, K., Rasheed, S., Ando, M., Takeda, H.,Kawaura, K., Kusano, M., Fukushima, A., Takaho, A., Kuromori, T., Ishida, J., Morosawa, T., Tanaka, M., Torii, C., Takebayashi, Y., Sakakibara, H., Ogihara, Y., Saito, K., Shinozaki, K., Devoto, A., Seki, M., 2017. Erratum: Acetate-mediated novel survival strategy against drought in plants. Nat. Plants 3, 17119.

Laszlo Fodorpataki, Katalin Molnar, BernatTompa Sebastian R.C. PLUGARU.2019. Priming with Vitamin U Enhances Cold Tolerance Of Lettuce (Lactuca sativa L.), Not BotHortiAgrobo, 47(3):592-598. DOI:10.15835/nbha47311433.

Lin, J.M., Sung, J.M., 2001. Pre-sowing treatments for improving emergence of bitter gourd seedlings under optima and sub-optimal temperatures. Seed Science and Technology, 29 (1):39-50.

Ling Yan Dai, Hong De Zhu, Kui De Yin, Ji Dao Du,Yu Xian Zhang.(2017). Seed priming mitigates the effects of saline-alkali stress in soybean seedlings, Chilean Journal of Agricultura Research 77(2).

M. Farooq, T. Aziz, S. M. A. Basra, M. A. Cheema and H. Rehman. 2016. Chilling Tolerance in Hybrid Maize Induced by Seed Priming with Salicylic Acid; Journal of Agronomy & Crop Science, ISSN 0931-2250.

M. Piri, M. B. Mahdieh, J. A. Olfati, and Gh. Peyvast International Journal of Vegetable Science, 15:285–292, 2009 Copyright © Taylor & Francis Group, LLC ISSN: 1931-5260 print / 1931-5279.

M. Z. Jafar& M. Shahid M. Farooq M. A. CheemaI.Afzal S. M.A. Basra M. A. Wahid, T. Aziz & M. Shahid.(2012). Improving the Performance of Wheat by Seed Priming Under Saline ConditionsJ.Agronomy & Crop Science, ISSN 0931-2250.

M.A. Aghbolaghi And M. Sedghi.2014.The Effect Of Halo And Hydro Priming On Germination Characteristics Of Millet Seeds Under Salinity Stress; Vol. Xlvii , No. 2 (158).

Mhlongo, M.I., Piater, L.A., Madala, N.E., Labuschagne, N., Dubery, I.A., 2018. The chemistry of plant-microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. Front. Plant Sci. 9, 112.

Mohamed H. A., Clark J. A., Ong C. K. (1988). Genotypic differences in the temperature responses of tropical crops. I. Germination characteristics of groundnut (Arachishypogaea L.) and pearlmillet (Pennisetumtyphoides S & L). J. Exp. Bot. 39, 1121–1128. 10.1093/jxb/39.8.1121

Muhammad QudratUllahFarooqi, JuKyong Lee. 2016. Cold Stress Evaluation among Maize (Zeamays L.) Inbred Lines in Different Temperature Conditions; Plant Breed.Biotech. 4(3):352~361.

PavelKerchev,Tomvander Meer, NeerakkalSujeeth, Arno Verlee , Christian V. Stevens, Frank Van Breusegem, Tsanko Gechev.2020. Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants; Biotechnology advances, 40 issue 107503.

Petrov,V., Hille, J., Mueller Roeber, B., Gechev, T.S., 2015. ROS mediated abiotic stressinduced programmed cell death in plants. Front. Plant Sci. 6, 69.

Powell A. A., Oliveira D.e, M. A, Matthews S. (1986). The role of imbibition damage in determining the vigour of white and coloured seed lots of dwarf French beans. J. Exp. Bot. 37, 716–722. 10.1093/jxb/37.5.716

Rodríguez VM, Romay MC, Ordás A, Revilla P. 2010. Evaluation of European maize (Zeamays L.) germplasm under cold conditions.Genet.Resour. Crop Evol. 57: 329-335.

Santalla M., De Ron A. M., Voysest O. (2001). European bean market classes, in Catalogue of Bean Genetic Resources, edsAmurrio M., Santalla M., De Ron A. M, editors. (Pontevedra: PHASELIEU FAIR 3463-MBG-CSIC. Fundación Pedro Barrié de la Maza;), 77–94.

Santaniello, A., Scartazza, A., Gresta, F., Loreti, E., Biasone, A., Di Tommaso, D., Piaggesi, A., Perata, P., 2017.Ascophyllumnodosum seaweed extract alleviates drought stress in Arabidopsis by affecting photosynthetic performance and related gene expression. Front. Plant Sci. 8, 1362.

Singh S. P., Gepts P., Debouck D. G. (1991). Races of common bean (PhaseolusvulgarisFabaceae). Econ. Bot. 45, 379–396. 10.1007/BF02887079

Shukla, P.S., Borza, T., Critchley, A.T., Hiltz, D., Norrie, J., Prithiviraj, B., 2018.Ascophyllumnodosum extract mitigates salinity stress in Arabidopsis thaliana by modulating the expression of miRNA involved in stress tolerance and nutrient acquisition. PLoS ONE 13, e0206221.

Stanley Lutts, Paolo Benincasa, Lukasz Wojtyla,, SzymonKubala S, Roberta Pace KatzarinaLechowska, Muriel Quinet and MalgorzataGarnczarska.(2016). Seed Priming: New Comprehensive Approaches for an Old Empirical Technique; http://dx.doi.org/10.5772/64420.

Taylor A.G., Allen P.S., Bennett M. A., Bradford K. J., Burrisand J. S. and Misra M. K. 1998.Seed enhancements. Seed Sci. Res. 8: 245-256.

Wang W,Peng S, Chen Q, Mei J, Dong H, Nie L. 2016. Effects of pre-sowing seed treatments on establishment of dry direct-seeded early rice under chilling stress. AoB PLANTS 8: plw074; doi:10.1093/aobpla/plw074.

White J. W., Montes C. (1993). The influence of temperature on seed germination in cultivars of common bean. J. Exp. Bot. 44, 1795–1800. 10.1093/jxb/44.12.1795

WondemuTessome, TamadoTana, NigussaDechessa and T. N. Singh. (2018). Effect of Seed Priming on Germination and Seedling Growth of Grain Sorghum (Sorghum bicolor L. Moench) Varieties; East African Journal of Sciences, Volume 12 (1) 51-60;

Xu-Wen Jiang, Cheng-Ran Zhang, Wei-Hua Wang, Guang-Hai Xu, and HaiYan Zhang.(2020). Seed Priming Improves Seed Germination and Seedling Growth of Isatisindigotica Fort. Under Salt StressHORTSCIENCE 55(5):647 650. https://doi.org/10.21273/HORTSCI14854-20.

Ya-jing GUAN Jin HU, Xian-ju WANG and Chen-xia SHAO. (2009). Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress; Journal of Zhejiang University SCIENCE; 10(6):427-433.

APPENDIX

Appendix 1: Germination percentage in day one ResultsANOVA

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		165.583	3	55.194	41.396	.000
	Linear Term	Contrast	156.817	1	156.817	117.613	.000
		Deviation	8.767	2	4.383	3.287	.091
Within Groups			10.667	8	1.333		
Total			176.250	11			

Appendix 2: Germination percentage in day two Results ANOVA

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		45.583	3	15.194	26.048	.000
	Linear Term	Contrast	43.350	1	43.350	74.314	.000
		Deviation	2.233	2	1.117	1.914	.209
Within Groups			4.667	8	.583		
Total			50.250	11			

Appendix 3: Germination percentage in day three of haricot bean ANOVA Table

			Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	(Combined)		17.583	3	5.861	3.058	.092
	Linear Term	Contrast	10.417	1	10.417	5.435	.048
		Deviation	7.167	2	3.583	1.870	.216
Within Groups			15.333	8	1.917		
Total			32.917	11			

Appendix 4: Germination percentage in day four of haricot bean ANOVA Table

			Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	(Combined)		74.000	3	24.667	19.733	.000
	Linear Term	Contrast	72.600	1	72.600	58.080	.000
		Deviation	1.400	2	.700	.560	.592
Within Groups			10.000	8	1.250		
Total			84.000	11			

Appendix 5: Total germination percentage of haricot bean ANOVA Table

			Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	(Combined)		343.255	3	114.418	8.828	.006
	Linear Term	Contrast	274.476	1	274.476	21.178	.002
		Deviation	68.778	2	34.389	2.653	.131
Within Groups			103.685	8	12.961		
Total			446.940	11			

Appendix 6: MET day of haricot bean ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		.463	3	.154	7.165	.012
	Linear Term	Contrast	.457	1	.457	21.179	.002
		Deviation	.007	2	.003	.158	.857
Within Groups			.172	8	.022		
Total			.636	11			

Appendix 7: Shoot Length of haricot bean ANOVA Table

			Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	(Combined)		1.571	3	.524	87.019	.000
	Linear Term	Contrast	1.539	1	1.539	255.823	.000
		Deviation	.031	2	.016	2.617	.134
Within Groups			.048	8	.006		
Total			1.619	11			

Appendix 8: Root Length of haricot bean ANOVA Table

			Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	(Combined)		.752	3	.251	42.858	.000
	Linear Term	Contrast	.735	1	.735	125.611	.000
		Deviation	.017	2	.009	1.482	.283
Within Groups			.047	8	.006		
Total			.799	11			