# Effect of Different Techniques on Germination Efficacy and Antioxidant Capacity of Indigenous Legumes of Pakistan

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**Abstract:** The present study investigated five different strategies for germination, utilizing distinctive substrata like jute bag, separating funnel, muslin cloth, filter paper and aluminum foil followed by evaluation of percent germination, radicle size, weight gain, total phenols and antioxidant activity of eleven indigenous legumes. The results revealed that jute bag displayed the most elevated percent germination in all legumes (84-96) % with the exception of kabuli chick pea, desi chick pea, garbanzo bean and cow pea which demonstrated improved percent germination when filter paper was utilized as substrata. The longest root length (3.1cm) was seen in cow pea when filter paper was used as substrata. It was additionally observed that jute bag demonstrated the highest increment in total phenolic compounds after germination in soy bean i.e. 6.3 mg gallic acid/gram. Among all germinated legumes, cowpea demonstrated the most elevated amount of total antioxidant activity (98.1%) when either filter paper or separating funnel was utilized. The results revealed that every bean requires optimum sprouting technique/conditions inorder to enhance its antioxidant capacity to maximum extent.

**Keywords:** Legumes, germination methods, total phenolic compounds, antioxidant activity.

## 1. INTRODUCTION

Legumes are considered as an economical source of protein, minerals, vitamins and carbohydrates [1]. They are rich in phytochemicals and consist of polyphenols which provides good health effects and protect against ischemia, anaemia, asthma, arthritis, inflammation etc [2]. However along with the nutritional compounds, they also contain antinutritional factors like inositol hexaphosphatase (IP6) known as phytic acid and phytates that affect the bioavailability of minerals and trace elements [3]. They furthermore consist of enzyme inhibitors (trypsin, chymotrypsin), oxalates, saponins, tannins and protease inhibitors, lectins, alpha amylase inhibitors, phenolics, phytates and alpha galactosides [4]. To overcome these unhealthy factors, legumes are either cooked or germinated.

Germination is the simplest, inexpensive and effective treatment compared to dehulling, soaking, cooking, boiling, autoclaving and microwave cooking to reduce antinutrtional properties in legumes [5, 6]. Prior this process, seeds are soaked in water for optimum time so that water soluble abscisic acid which is growth inhibitor hormone could be removed from the seed. Then the seeds are kept in substrata to initiate germination. This process undergoes in three phases. In first phase 'imbibition', dormant seeds uptake water thus increasing in volume and enzymatic activity. These enzymes are synthesized by the hormone called giberellic acid. While in second phase that is preparatory period, metabolic activity increases, protein synthesizes and transports nutrients to all parts of the seed whereas in last phase, radicle emerges from the seed coat providing completely nutritious seed [7]. During this procedure, cell constituents are converted into new ones, affecting biochemical properties [8]. It improves the quality of legumes as it trigger enzymatic activity of sprouting seeds and converts protein, carbohydrates and lipids into simpler forms [9].

The objective of the present study was to compare the effectiveness of five different methods of germination incorporating different substrata. Efficacy of germination was measured in terms of weight gain, radicle size and percent germination. Moreover, this study also compared the effect of different germination techniques on legume phenolic compounds and their antioxidant activity.

## 2. MATERIALS AND METHODS

### 2.1. Legumes

Eleven varieties of legumes namely namelydesi chickpea (Cicer arietinum), kabuli chickpea (Cicer arietinum) and garbanzo bean (garbanzo bean belongs to the family Cicer arietinum), green gram (Vigna radiate), black gram (Vigna mungo), cow pea (Vigna unguiculata), lentils (Lens culinaris), soy bean (Glycine max), adzuki bean (Vigna angularis), pinto bean (Phaseolus vulgaris) and kidney bean (Phaseolus vulgaris) from a single cultivar were obtained from PARC (Pakistan Agriculture and Research Council), Karachi, Pakistan.

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ISSN: 1814-8085 / E-ISSN: 1927-5129/15 

### 2.2. Germination Methods

#### *2.2.1. Jute Bag*

Hundred grams of seeds were soaked in distilled water for 12 h at 25 ˚C and were then spread on a clean water soaked jute bag for 24 h to germinate. The seeds were rinsed after every 6 h intervals in running tap water for 10 min to reduce fungal contamination [10].

#### *2.2.2. Separating Funnel*

Hundred grams of seeds were washed with 0.7% (w/v) sodium hypochlorite solution followed by soaking in distilled water for 6 h. The seeds were rinsed after every 30 minutes. The water was then drained off, and the seeds were transferred to a separating funnel and kept at 25 ˚C for 24 hours. Seeds were sprayed with distilled water after every 4 hours to maintain adequate hydration level [11].

#### *2.2.3. Muslin Cloth*

Hundred grams of seeds were soaked in distilled water for 10 h at room temperature. The hydrated seeds were spread between two moist muslin cloths over a metal tray and were allowed to germinate for 24 h at 25 ˚C [12].

#### *2.2.4. Filter Paper*

Hundred grams of seeds were immersed for 30 minutes in 0.07% (w/v) sodium hypochlorite solution in dark in order to remove surface micro flora of seeds. Seeds were then washed with distilled water until neutral pH. The liquid was removed and seeds were kept for 5.5 h in distilled water. After decantation, seeds were placed in plastic discs with the bottom covered with wet filter paper. Germination was carried out at 25 ˚C for 24 h and seeds were sprayed with water after every 2 h [13].

#### *2.2.5. Aluminium Foil*

Seeds were washed in cold running water and soaked in tap water for 8 h at room temperature. The hydrated seeds were spread on trays lined with previously sterilized moist muslin sheets and covered with aluminium foil. Germination was done at 25 ˚C for 24 hours [14].

### 2.3. Determination of Percent Germination

Percent germination was recorded after 24h and was calculated by using the formula.

$$\text{Percent germination} = \frac{\text{Germinated seeds}}{\text{Total Seed}} \times 100$$

### 2.4. Determination of Root Length and Weight Gained

Radicle length and weight gained was evaluated using the method of Perveen, *et al*. [15].

### 2.5. Preparation of Extract of Germinated Legumes

Seeds were dried at 40 °C for 3 days. Dried beans were ground to a fine powder (particle size 0.5mm) using hammer mill. Five gram sprout powder was suspended in 80% (v/v) methanol solution (100mL) and extracted at 60 °C in a water bath with continuous shaking for 2 hours. The extracted solvent was filtered through whatmann no. 541 and the subsequent filtrate was used for the determination of total phenols and radical scavenging activity.

### 2.6. Determination of Total Phenols

The extracted sample (20µL) was diluted in water (1580µL) followed by addition of 100µL Folin – Ciocalteau (FC) reagent. The sample was mixed thoroughly followed by incubation for 8 min. Sodium carbonate solution (300 µL) was added and the solution was incubated for 2 hours at room temperature. The sample absorbance was measured at 765nm using UV-visible spectrophotometer JASCO Model V670 (JASCO Corporation, Tokyo, Japan). Quantification was performed with respect to the standard curve of gallic acid. The results were expressed as milligram of gallic acid equivalent per gram as described by Waterhouse [16].

### 2.7. Determination of Free Radicals Scavenging Activity by DPPH

The 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay was done according to the method of Marinova and Batchvarov. [17].

### 2.8. Statistical Analysis

Analyses were performed in triplicate. The data was analyzed by (Analysis of Variance) ANOVA using SPSS (Version 17.0. Inc, Chicago, USA) statistical program. Duncan's multiple range tests were carried out to test any significant differences among the treatments employed. Significant levels were defined using $P \leq 0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1. Effect of Different Techniques on Percent Germination and Percent Inhibition Scavenging Activity

Eleven legumes were treated with five different methods individually for determining the most effective method of sprouting. The results of the effect of different germination methods on percent germination and percent inhibition scavenging activity of sprouted legumes are presented in Figure **1**. The results showed that percent scavenging activity increases with the increase in percent germination. According to our study jute bag was the most effective of the five germination methods for black gram, green gram, cow pea, and kidney bean. It possess the highest water retaining capacity, therefore seeds remained hydrated for a longer period of time. Moreover, air could easily be circulated through pores. Similarly, filter paper also showed improved percent germination and percent inhibition scavenging activity for desi chickpea, kabuli chickpea, garbanzo bean, adzuki bean, soy bean and pinto bean owing to high water absorption capacity which prevented drying of seeds during germination. Also pores it allowed the exchange of air with environment. Since both water and air are essential components for germination, therefore both proved to improve the efficacy of germination.

In separating funnel, there are two holes for inlet and outlet of air. However, seeds are stacked which reduced the passage of air circulation and remained wet for prolonged period of time as water could not evaporate through glass. This treatment showed effective result in cow pea which is insignificant from jute bag while muslin cloth dried seeds earlier due to higher porosity of the cloth. In aluminum foil method the seeds were first placed in wet muslin cloth and were then covered with aluminum foil. Since foil acted as a barrier for the escape of water, therefore it delayed the drying of seeds during the course of germination. Both these methods showed 100% germination and highest percent inhibition scavenging activity in green gram which was insignificantly different from other methods. Germination of kidney bean, soy bean and adzuki beans was not as efficient as observed for all legumes. But they showed the highest percent germination in jute bag. It could be attributed to the fact that the aforementioned beans required more soaking and germination time as their seed coats were hard enough to allow ease of water penetration [18]. The results revealed that a single technique could not be suitable for all legumes rather different treatment times and conditions were required for sprouting different legumes. As previous studies showed that black gram, green gram, lentils and cow pea showed efficient germination in 24 hours [19, 20]. However, three to five days were required for chick pea to achieve improved sprouting, whereas pinto bean, adzuki bean, kidney bean and soy bean required three to six days for efficient sprouting [5, 18, 21, 22]. Thus, our results corroborates with the aforementioned studies. The

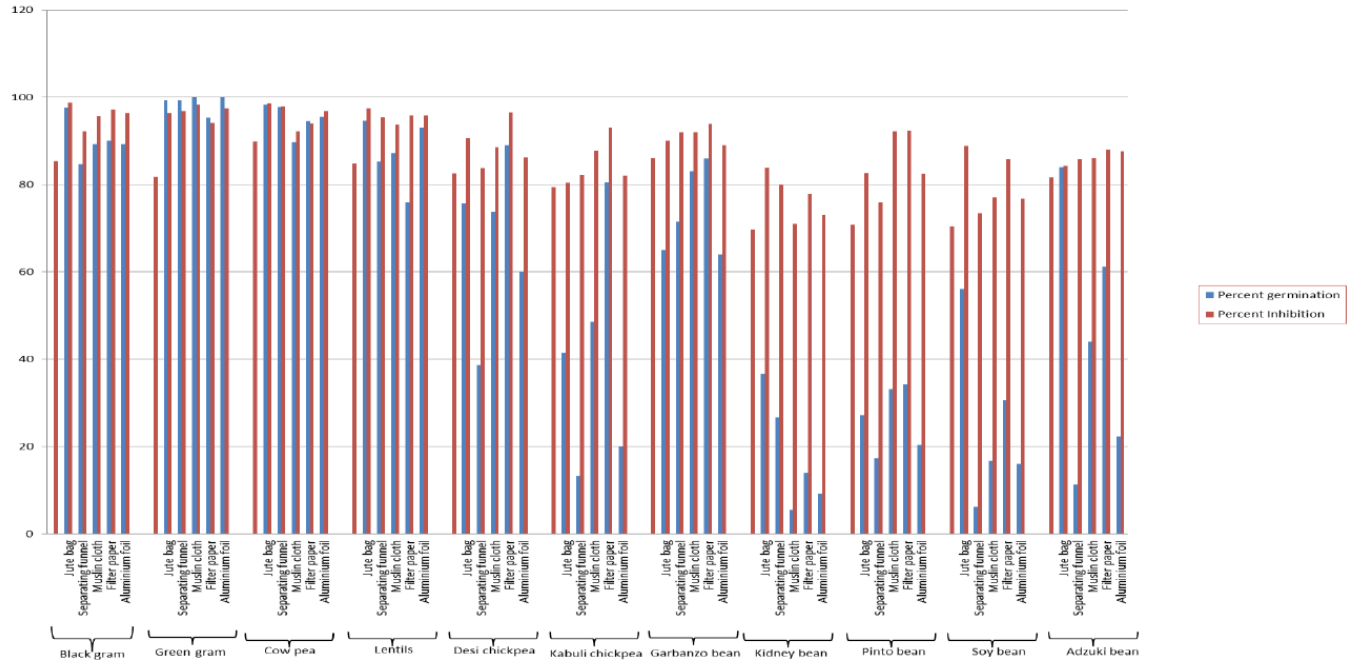


**Figure 1:** Effect of different substrata on percent germination and percent inhibition scavenging activity.

seeds differ in their germination condition due to differences in their seed coats.

### 3.2. Effect of Different Techniques of Germination on Radicle Size and Weight Gained

As in Table **1** green gram and lentils showed similar radicle length irrespective of the method used for sprouting, thus indicating higher germination capacity. However, radicle lengths of lentils were less than green gram. Black gram showed increased radicle length by method jute bag and separating funnel. All legumes except cow pea showed increased radicle length by jute bag whereas cow pea showed the best root length of 3.1cm when using filter paper as substrata. In Table **2**, weight gain was found to be significantly high when method Aluminium foil was employed for legumes except desi chick pea and kidney bean. Increase in moisture content in seeds without any passage of air or moisture to escape allows higher weight gain. Similarly, black gram seeds in muslin cloth which were covered with two wet muslin cloths demonstrated the highest gain of weight.

### 3.3. Effect of Different Germination Methods on Total Phenolic Content (PC) of Legumes

Eleven legume's extract from different germination techniques differed significantly ($p<0.05$) in their total phenolic content (TPC). Total phenolic content of the germinated legumes are presented in Table **3**. The total phenolic content of germinated black gram, green gram, lentils and cow pea was irrespective of the methods used. Using jute bag, soy bean showed significantly higher phenolic content in contrast to beans germinated by others. Higher amount of phenolic content is due to the fact that when jute bag was used as substrata, percent germination was found to be the highest. Thus, improvement in efficiency of germination improved the phenolic content also. Pinto bean had shown insignificant increase in phenolic compounds as compared to control due to its lower tendency to germinate. The phenolic content of garbanzo bean, kabuli chick pea and desi chick pea had not shown significant difference among various methods used. The results suggested that, legumes with dark seed coats showed insignificant increase in phenolic compounds during germination. This is in agreement with the results reported by Lin and Lai [23]. Every legume showed different concentration of phenolic compounds that are irregularly distributed in legume seed coat.

## 4. CONCLUSION

The present study revealed that legumes have different germination capabilities and these capacities

**Table 1: Effect of Different Methods on Radicle Length of Germinated Legumes***

| Methods | Black gram | Green gram | Cow pea | Lentils | Desi chickpea | Kabuli chickpea | Garbanzo beans | Kidney bean | Pinto bean | Soy bean | Adzuki bean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Jute bag | 1.1c | 2.5a | 1.5a | 0.6a | 1.0c | 0.7a | 1.3d | 1.4b | 0.6c | 1.4b | 0.9d |
| Separating funnel | 1.6bc | 1.9a | 1.7ab | 0.4a | 0.2a | 1.0a | 0.2a | 1.7c | 0.4ab | 0.3a | 0.1a |
| Muslin cloth | 1.4d | 2.3a | 2.2b | 0.5a | 0.7b | 0.5a | 1.0c | 0.3a | 0.3ab | 0.4a | 0.7cd |
| Filter paper | 1.1a | 2.1a | 3.1c | 0.5a | 0.7b | 0.6a | 1.2cd | 1.3b | 0.4bc | 0.3a | 0.4bc |
| Aluminium foil | 1.1a | 2.8a | 1.7ab | 0.4a | 0.9c | 0.6a | 0.7b | 1.8b | 0.2a | 0.3a | 0.3ab |

*Values are the mean of three different replicates. Different alphabets within each column are significantly different at $p\leq0.05$.

**Table 2: Effect of Different Methods on Weight Gained of Germinated Legumes***

| Methods | Black gram | Green gram | Cow pea | Lentils | Desi chickpea | Kabuli chickpea | Garbanzo beans | Kidney bean | Pinto bean | Soy bean | Adzuki bean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Jute bag | 216.4b | 239.4b | 228.3d | 197.5ab | 208.5c | 195.3b | 234.8d | 152.2b | 210.6c | 210.3c | 203.9b |
| Separating funnel | 206.7b | 207.7ab | 219.8b | 202.90b | 220.8e | 202.8c | 223.2b | 227.3d | 217.1d | 224.9d | 212.7c |
| Muslin cloth | 508.4b | 242.1bc | 214.4a | 175.2a | 183.1b | 192.0b | 224.4c | 116.3a | 202.8b | 184.2a | 203.4b |
| Filter paper | 169.5a | 192.4a | 224.3c | 182.5ab | 176.3a | 181.7a | 216.0a | 112.1a | 197.8a | 197.2b | 184.7a |
| Aluminum foil | 213.5b | 262.2c | 230.3d | 208.5b | 210.5d | 202.9c | 239.1e | 209.5c | 217.8d | 219.0cd | 210.3c |

*Values are the mean of three different replications. Different alphabets within each row are significantly different at $p\leq0.05$.

**Table 3: Effect of Different Germination Methods on Total Phenols Expressed as mg gallic acid/gram of Germinated Legumes***

| Methods (mg/g) | Black gram | Green gram | Cow pea | Lentils | Desi chickpea | Kabuli chickpea | Garbanzo beans | Kidney bean | Pinto bean | Soy bean | Adzuki bean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 1.5a | 1.3a | 0.8a | 1.2a | 1.5a | 1.4a | 1.6a | 1.4a | 1.6a | 2.3a | 1.7a |
| Jute bag | 2.8b | 1.8a | 1.7b | 1.6b | 1.8b | 2.0b | 2.0b | 1.8d | 1.5a | 6.3d | 1.9b |
| Separating funnel | 2.4b | 1.7a | 1.9b | 1.7bc | 1.8ab | 1.8ab | 1.9ab | 1.7bd | 1.9a | 3.7b | 1.7ab |
| Muslin cloth | 2.4b | 2.4a | 1.7b | 2.4d | 1.8ab | 1.8ab | 2.0b | 1.6bc | 1.7a | 4.2c | 1.8abc |
| Filter paper | 2.6b | 1.6a | 1.9b | 1.8c | 1.8b | 1.8ab | 2.0b | 1.6bcd | 1.7a | 3.7b | 2.0c |
| Aluminum foil | 3.0b | 2.0a | 1.7b | 1.9c | 1.7a | 1.9b | 1.8ab | 1.5ab | 1.9a | 3.6b | 1.9bc |

*Values are the mean of three different replicates. Different alphabets within each column are significantly different at p≤0.05

could be optimized by employing different sprouting procedures or changing the substrata. As efficient germination depends on both intrinsic properties such as seed hardness, seed color, nutritional composition as well on extrinsic properties like soaking time, substrata, temperature and air circulation. The results revealed that a single method could not be applied for all legumes rather different germination conditions were required for sprouting different legumes. Thus, improvement in efficiency of germination simultaneously improved the phenolic content. Therefore, optimization of germination condition of legumes improves its antioxidant capacity. The present study was an effort to suggest the best possible sprouting method for eleven legumes.

## ACKNOWLEDGEMENTS

We would like to acknowledge Dean Faculty of Science, University of Karachi for funding this project.

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Received on 04-03-2015 Accepted on 06-05-2015 Published on 27-05-2015

http://dx.doi.org/10.6000/1927-5129.2015.11.50