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# VIGOUR AND VIABILITY OF OSMOPRIMED HARVESTED SEEDS OF WHEAT VARIETIES

Naimat Ullah[1], Asim Muhammad[2], Habib Ullah Marwat[3], Hamayoon Khan[2] and Muhammad Subhan[4]
[1]Department of Biotechnology, University of Science and Technology, Bannu, Pakistan
[2]Department of Agronomy, The University of Agricultural, Peshawar, Pakistan
[3]Department of Agricultural Chemistry, The University of Agricultural, Peshawar, Pakistan
[4]Department of Botany, SBBU, Sheringal Upper Dir, Pakistan
E-Mail: asimmuh@aup.edu.pk

**ABSTRACT**

Laboratory experiment was conducted in the Department of Biotechnology, Faculty of Basic Sciences, University of Science and Technology Bannu, Khyber Pukhtunkhwa, Pakistan. Wheat Seeds of Fakhr-e-Sarhad and Pir Sabaak (2005) was obtained from previously treated with five priming levels i.e. Distilled water (Water soaked), $CaCl_2$ (22.2g $L^{-1}$), KCL (20.7 g $L^{-1}$), $NH_4SO_4$ (20 g $L^{-1}$), NaCl (60 g $L^{-1}$) and control (dry seeds) for 8 h at room temperature. The harvested primed seeds of wheat were used to determine the vigor and viability in the laboratory. Results showed that priming significantly improved germination percentage, growth rate, seedling dry weight, seed vigor index, shoot length, root length, shoot weight, and root weight. It is concluded that calcium chloride treated seeds produced heavier seedlings (9 mg), rapid growth rate (9.6), highest seed vigor index (5092) and heavier roots (4.6mg). Pir sabaak-2005 wheat variety showed maximum germination percentage (65%), fastest growth rate (9), maximum seedling dry weight (8 mg) and heavier shoot weight (4.1 mg), and heavier root weight (3.8 mg). It is concluded that Pir sabbak-2005 have highest vigour and viability and suggested for general cultivation in Bannu region.

**Keywords:** vigor, viability, osmopriming, wheat.

## 1. INTRODUCTION

In Pakistan wheat is grown on an area of 8.9 million hectares having the production of yield of 2.5 metric tons per hectare (Anonymous) [1], which is very low as compared to other wheat producing countries like India, USA and China. There are many factors responsible for decrease in yield. This low yield is mainly due to poor germination and poor stand establishment in the field particularly under rain fed condition.

The resulting poor crop stands leave gaps in the canopy, which are rapidly filled by vigorously growing weeds, compete with the crop plants for light, water and the limiting nutrients Kropft and Laar [2]. Accelerating and homogenizing the germination process is a prerequisite for a good crop establishment process and helps to increase yield eventually (Harris) [3]. Bray *et al*. [4] reported that hydration of seed up to, but not exceeding, the lag phase with priming permits early DNA replication, faster embryo growth (Dahal *et al*.) [5] (Saha *et al.)* [6] and reduced leakage of metabolites (Styer and Cantliffe) [7]. Priming of wheat seed in water may improve germination and emergence (Ashraf and Shakra) [8] and promote vigorous root growth (Carceller and Soriano) [9] under low soul water potential. Priming in water has shown good potential to enhance germination, emergence, growth, and/ or grain yield of wheat. Seed priming technique use to increase wheat germination, stand establishment and yield under rain fed conditions. Seeding priming has been used successfully to improve the germination, stand establishment and yield of most of field crops. Seed priming is a controlled hydration technique in which the quiescent seeds are exposed to an osmoticum which stimulate physiological and biochemical activities, but prevent radical protrusion from the seed.

Seed priming have improved germination particularly under adverse seed bed condition. Seed priming is a low cost and low risk intervention used to overcome poor stand establishment (Harris) [3]. Seed priming comprises the soaking of seed in water and drying back to the storage moisture until use. The soaking induces a range of biochemical changes in the seed that required starting the germination process (breaking of dormancy, hydrolysis or metabolization of inhibitors, imbibitions and enzyme activation). Some or all of these process that precede the germination is triggered by priming persist following the desiccation of the seeds (Asgedom and Becker) [10]. Thus, upon priming, seeds rapidly imbibe and revive the metabolism, resulting in a higher germination rate and reduction in the inherent physiologically heterogeneity in germination (Rowse) [11]. Vigorous early seedling growth has been shown to be associated with higher yield (Harris *et al*.) [12]. The vigor of seed can be improved by techniques generally known as seed priming, which enhances the speed and uniformity of germination (Heydecker *et al*.) [13].

Seed vigor is an important component of seed quality and satisfactory levels are necessary in addition to traditional quality criteria of moisture, purity, germination and seed health to obtain optimum plant stand and high production of crops. . The technology of seed vigor testing has not been perfected so far, due to availability of many methods for testing seed vigour. Therefore research is needed to further refine the current seed vigor test methods and to develop new ones which are more related to specific conditions.

## 2. MATERIAL AND METHODS

### 2.1 Seed materials and priming treatments

Experiment was conducted in the laboratory of the Department of Biotechnology, Faculty of Basic Sciences, University of Science and Technology, Bannu, Khyber Pakhtun Khwa, Pakistan. Seeds of two wheat varieties Fakhr-e-Sarhad and Pir Sabaak-2005 were used. These were pretreated with five priming sources distilled water (water soaked), $CaCl_2$ (22.2g $L^{-1}$), KCL (20.7 g $L^{-1}$), $NH_4SO_4$ (20 g $L^{-1}$) and NaCl (60 g $L^{-1}$) for 8 h at room temperature and the dry seeds were kept as control. Wheat varieties were sown during rabi season, 2010. The harvested primed seeds of wheat were used to determine their vigor and viability in the laboratory during 2011. After harvesting the wheat, clean dry seeds of wheat varieties were subjected to different quality evaluation tests. The following parameters were studied during the experiment.

### 2.2 Standard germination test

Four lots of 100 seeds each from various treatments were drawn, placed on two sheets of standard germination paper and covered with an additional sheet. The sheets were folded from the bottom and rolled loosely in a rubber band. Rolls were placed upright in wire basket covered with plastic, and placed in incubator at 25°C. The first and the last count were made on 7$^{th}$ and 10$^{th}$ day of incubation respectively. Normal seedlings were described, those having a vigorous primary root or a set of secondary roots sufficient to anchor the seedlings in the soil, hypocotyls without open cracks or lesions, at least one attached cotyledon, one primary leaf and an intact terminal bud. Seedlings lacking these qualities were considered abnormal. Averages of the four lots were computed to determine germination percentage (ISTA) [14].

### 2.3 Shoot, root length and shoot, root dry weight

Treated and control seeds were sown in 6 x 4 cm plastic trays (100 in each) having moist blotting paper replicated 3 times and were placed in growth chamber. Root and shoot length and seedling fresh and dry weights were recorded 7 days after sowing (Shaukat) [15].

### 2.4 Growth rate

The germination rate was calculated by dividing the number of germinated seedlings per 100 seeds obtained at each counting. The values obtained at each count were then summed to obtain the germination rate (Kader) [16].

$$GR = \frac{\text{No. of germinatet seed}}{\text{Days to first count}} + \dots\dots\dots + \frac{\text{No of germinated seed}}{\text{Days to final count}}$$

### 2.5 Seedling dry weight test

Four replications of 50 seeds each of various treatments were allowed to grow at 25ºC for 07 days after germination in incubator. The cotyledons were removed and the seedlings were placed in paper bag in oven at 60° C for 24 h for determining seedling dry weight (ISTA) [17].

### 2.6 Seed Vigor Index (SVI)

Seed vigor index was calculated by determining the germination percentage and seedling length of the same seed lot. Fifty seeds each in four replications were germinated in towel papers in germination test. The seedling lengths of five randomly selected seedlings were measured. Seed vigor index was calculated by multiplying germination (%) and seedling length. The seed lot showing the higher seed vigor index is considered to be more vigorous (Dezfuli *et al.*) [18].

### 2.7 Statistical analysis

Data analysis was performed using the MSTAT-C statistical software (Version 6.12). Randomized complete block design was used in this experiment. Mean separations were performed by least significant difference test (LSD) at 5% level.

## 3. RESULTS AND DISCUSSIONS

### 3.1 Germination % age

Germination percentage was significantly affected by priming sources and varieties. Maximum germination (72.5%) was recorded when seeds primed with KCl for 8 hours, while minimum germination percentage (25.6%) was observed in control plots (Table-1). These results were in agreement with Kaur *et al.* [19] who reported that, osmoprimed and hydroprimed seeds of pea possess better emergence and better emergence rate as well as better yield in comparison with control group seeds. Maximum germination percentage (65) was counted in Pir-Sabaak-2005 wheat variety than Fakhr-e-Sarhad (57.6). These results agree with those reported by Bakht *et al.* [20] 2010, who concluded that minimum germination %age was observed in unprimed seeds. While the interaction of priming sources and varieties have no significant effect on germination percentage of wheat varieties (Table-3). However, maximum germination (77.3%) was recorded in Pirsabaak-2005 when primed with NaCl, while minimum germination (17%) was counted in control plots in Fakhr-e-sarhad variety. In pigeon pea (*Cajanus cajan* L.), hydropriming was determined to be very effective in the mobilization of compounds such as proteins, free amino acids, and soluble sugars from storage organs to growing embryonic tissues under salt stress (Jyotsna and Srivastava) [21]. In contrast, lipid composition of Korean black soybean (*Glycine max*) seed, including percentages of neutral fats, glycolipids, and phospholipids, was determined to remain unchanged after soaking in water (Oh *et al.)* [22]. Probably, uncontrolled water uptake, causes seed coat weakening and vital electrolyte may leak which results, in germination interference (Jet *et al.*) [23].

### 3.2 Growth rate

Germination rate possess an important role in the seedling establishment and in the growth improvement as well as crop yield, especially in the form unfavorable conditions (Rouhi *et al.*) [24]. Growth rate was significantly affected by priming sources (Table-1). Rapid growth rate (12.1) was recorded, when seeds primed with NaCl for 8 hours, while minimum growth rate (4.2) was observed in control plots. Similar results were concluded by Neametollahi *et al.* [25] they reported that maximum germination rate and percentage were obtained at NaCl and $Na_2SO_4$ level providing 0 MPa in hydropriming treatments. Delay in growth rate (7.7) was observed in Fakhr-e-Sarhad wheat variety, while rapid growth rate (9.0) was noted in Pir Sabaak-2005. These results are in line with Rahatullah and Khalid [26]; they reported that maximum emergence rate (12) was produced by priming treatments as compared to controlled plots (11). Priming sources and varieties have no significant effect on growth rate of wheat varieties. However, maximum growth rate (14.1) was recorded in Pirsabaak-2005, when primed with NaCl, while minimum growth rate (2.7) was counted in control plots in Fakhr-e-sarhad variety. Basra *et al.* [27] showed that in canola, germination percentage as well as germination rate were increased in response to priming. This is indicated that suitable conditions of the beginning of germination ensure to the seeds quickly emerge.

**3.3 Seedling dry weight (mg)**

Seedling dry weight was significantly affected by priming sources and varieties (Table-1). Maximum seedling dry weight (9.0mg) was recorded when seeds primed with $CaCl_2$ for 8 hours, while minimum seedling dry weight (4.5mg) was observed in control plots (Table-1). Similar results were also reported by Chiu *et al.* [28] who concluded that priming can improve germination, reduced light per-oxidation, enhance anti-oxidative activity and increase seedlings growth. Arif *et al.* [29] reported that maximum seedling dry weight was recorded by seeds treated with 300 g PEG $L^{-1}$ water followed by seeds treated with 200 g $PEGL^{-1}$ water. Minimum seedling dry weight was obtained by seeds treated with 0 and 400 g $PEGL^{-1}$ water. Maximum seedling dry weight was attained from hydroprimed seeds under saline condition, while osmopriming could not improve this character under all condition as compared to control (Janmohammadi *et al.*) [30]. Maximum seedling dry weight (8mg) was recorded in Pir Sabaak-2005, while minimum seedling dry weight (6.7mg) was seen in Fakhr-e-Sarhad variety. P x V have no significant effect on seedling dry weight of wheat varieties. However, maximum seedling dry weight (10.3mg) was recorded in Pirsabaak-2005, when primed with NaCl, while minimum seedling dry weight (3.3mg) was counted in control plots in Fakhr-e-sarhad variety. Pretreatment of soybean seed with 0.25 M $CaCl_2$ for 24 h resulted in plants with increased seed number and reduced number of seedless pods, although it did not affect 100-seed weight (Eleiwa) [31].

**3.4 Seed Vigour Index**

Vigorous seed index (5092.05) was recorded when seeds primed with $CaCl_2$ for 8 hours as compared to control plots (245.32) (Table-1). Hossain *et al.* [32] reported that highest germination value (4.41), germination energy (58.9) and vigour index (5291) was obtained in seeds when soaked in cold water for 24 hours which was significantly different from the control and other treatments. Highest seed vigour index (2799.7) was seen in Fakhr-e-Sarhad while lowest seed vigour index (1433.9) was noted in Pir Sabaak-2005. Priming sources and varieties have no significant effect on seed vigour index of wheat varieties. However, maximum seed vigour index (8250.4) was recorded in Fakhr-e-sarhad variety when primed with $CaCl_2$, while minimum seed vigour index (137.0) was counted in control plots in Fakhr-e-sarhad. According to Rouhi *et al*. [24] analysis of variance showed that, except for seedling dry weight, the other traits include standard germination, germination rate, seedling length and vigor index were significantly influenced by osmopriming.

**3.5 Shoot length (cm)**

Maximum shoot length (4.9cm) was recorded when seeds primed with $CaCl_2$ & KCl for 8 hours, while minimum shoot length (2.5cm) was observed in control plots (Table-2). These results were in line with Janmohammadi *et al.* [30] who reported that both radical and coleoptile length of seeds that were subjected to hydropriming significantly different from those of control. Fakhr-e-Sarhad recorded lengthy shoots (4.3cm) as compared to Pir Sabaak-2005 (3.8cm). Dezfuli *et al.* [18] who concluded that maximum coleoptiles were noted in seeds osmoprimed in urea solution for 96 hours which followed by seeds hydroprimed for 36 hours. Lengthy shoots (5.5cm) was recorded in Fakhr-e-sarhad wheat variety when primed with KCl, while shortest shoot (2.4cm) was counted in control plots in Fakhr-e-sarhad variety. This result was matched with the observation of Jet *et al.* [23] but opposed with the observation of Afzal *et al.* [33].

**3.6 Root length (cm)**

Priming sources and varieties significantly affected root length of wheat. Lengthy roots (6.8cm) was recorded when seeds primed with KCl for 8 hours, while minimum root length (3.5cm) was observed in control plots (Table-2). Different results were concluded by Basra *et al.* [34] that maximum root length was recorded by Auquab-2000 wheat variety when seeds were hardened for 8 hours and minimum root length was observed in controlled treatment. Lengthy roots (5.9cm) were noted in Fakhr-e-Sarhad wheat variety than Pir Sabaak-2005 (5.3cm). The observed improvements in root length of KCl primed seeds may be attributed to various biochemical activities (Sung and Chang) [35]. P x V have no significant effect on root length of wheat varieties. However, maximum root length (7.9cm) was recorded in Fakhr-e-sarhad, when primed with KCl while minimum root length (3.2cm) was counted in control plots. Length of plumule and seedling dry weight showed that some activator treatments were capable of inducing improvements over the control. Hydro-priming remarkably improved the root length of seedlings from one, two and three year old seed lots (Karta *et al.*) [36].

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### 3.7 Shoot weight (mg)

Shoot weight was significantly affected by priming sources. Maximum shoot weight (4.7mg) was recorded when seeds primed with NaCl for 8 hours, while minimum shoot weight (2.2mg) was observed in control plots. Similar results were also reported by *Chiu et al*. [28] who concluded that priming can improve germination, reduced lipid per oxidation, enhance anti-oxidative activity and increase seedling growth. Pir Sabaak-2005 recorded maximum shoot weight (4.1mg) as compared to Fakhr-e-Sarhad (3.9mg). Basra *et al.* [34] concluded that minimum dry weight was shown by control treatments. Priming sources and varieties have no significant effect on shoot weight of wheat varieties. However, heavier shoots (5.0mg) was recorded in Pirsabaak-2005, when primed with KCl while minimum shoot weight (2.0mg) was counted in control plots in Fakhr-e-sarhad (Table-2). Ashraf and Rauf [37] who reported that fresh and dry weights of wheat seeds treated with $CaCI_2.2H_20$ were significantly higher as compared to other salt treatments and control.

### 3.8 Root weight (mg)

Heavier roots (4.6mg) was recorded when seeds primed with $CaCl_2$ for 8 hours as compared to control plots (2.2mg) (Table-2). Hossain *et al.* [32] reported that leaf dry weight and shoot dry weight was highest (2.26 g and 1.53 g respectively) in T4 (soaked in cold water for 48 hours), while root dry weight was maximum (0.9 g) in T5 (soaked in hot water at 80 C° – 100 C° for 2 minutes) and were immediately washed in cold water. Maximum dry root weight (3.8mg) was observed in Pir Sabaak-2005 wheat variety as compared to Fakhr-e-Sarhad (3.4mg). Different results were reported by Ghassemi-Golezanik *et al.* [38] that hydropriming significantly improved germination rate and root weights, compared to other seeds treatments. PxV have no significant effect on root weight of wheat varieties. However, heaviest roots (5.0mg) were recorded in Fakhr-e-sarhad when primed with $CaCl_2$, while lowest roots weight (1.6mg) was counted in control plots. Seeds subjected to halopriming with 50 mM $CaCI_2.2H_20$ had significantly higher shoot length, fresh and dry weight of seedlings than those treated with other salts or control (Afzal *et al*. [39].

## 4. CONCLUSIONS AND RECOMMENDATIONS

### 4.1 Conclusions

It is concluded rapid growth rate (9.6), heavier seedlings (9 mg), vigorous seed index (5092.05), lengthy shoots (4.9 cm) and heavier roots dry weight (4.6 mg), recorded when seeds were primed with calcium chloride for 8 hours. Maximum germination percentage (72.5) and maximum root length (6.8 cm) were observed in those seeds of wheat, when pre-treated with Potassium Chloride. Sodium Chloride treated seeds produced heavier shoots (4.7 mg). Fakhr-e-sarhad wheat variety showed lengthy shoots (4.3 cm), lengthy roots (5.9 cm) and maximum seed vigor index (2799.7). Maximum germination percentage (65%), heavier shoot weight (4.1g), heavier root weight (3.8 mg), fast growth rate (9) and heaviest seedling (8) was produced by Pir sabaak-2005 wheat variety. It is recommended that both potassium chloride and calcium chloride is suitable for better vigour and viability in Pir Sabaak-2005 wheat variety.

**Table-1.** Effect of Priming Sources on germination % age, growth rate, seedling dry weight and seed vigour index.

| Priming sources | Germination %age | | | Growth rate | | | Seedling dry weight (mg) | | | Seed Vigor Index | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | V1 | V2 | Means | V1 | V2 | Means | V1 | V2 | Means | V1 | V2 | Means |
| Control | 17.0 | 34.3 | 25.6 b | 2.7 | 5.7 | 4.2 d | 3.3 | 5.6 | 4.5 c | 137.0 | 353.6 | 245.3 |
| $H_2O$ | 67.3 | 70.6 | 69.0 a | 6.3 | 7.9 | 7.1 c | 7.0 | 7.3 | 7.1 b | 1805.6 | 1330.9 | 1568.2 |
| CaCl2 | 64.6 | 61.3 | 63.0 a | 10.4 | 8.8 | 9.6 b | 8.3 | 9.6 | 9.0 a | 8250.4 | 1933.7 | 5092.0 |
| KCl | 70.3 | 74.6 | 72.5 a | 7.2 | 8.9 | 8.1 bc | 7.3 | 8.0 | 7.6 ab | 3351.9 | 2067.2 | 2709.6 |
| NH4So2 | 68.0 | 72.0 | 70.0 a | 9.5 | 8.5 | 9.0 bc | 7.3 | 7.3 | 7.3 b | 1452.8 | 1184.0 | 1318.4 |
| NaCl | 58.6 | 77.3 | 68.0 a | 10.1 | 14.1 | 12.1 a | 7.0 | 10.3 | 8.6 ab | 1801.0 | 1734.3 | 1767.6 |
| Mean | 57.6 b | 65.0 a | | 7.7 | 9.0 | | 6.7b | 8.0a | | 2799.7 | 1433.9 | |
| LSD | **P** | **V** | **V x P** | **P** | **V** | **V x P** | **P** | **V** | **V x P** | **P** | **V** | **V x P** |
| Values | 16.11 | 11.18 | Ns | 2.183 | Ns | ns | 1.651 | 2.138 | Ns | Ns | Ns | Ns |

V1= Fakhr-e-Sarhad, V2= Pir sabaak-2005, P= Priming, V= Varieties

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**Table-2.** Effect of Priming Sources on shoot length, root length, shoot weight and root weight.

| Priming sources | **Shoot length** (cm) | | | **Root length** (cm) | | | **Shoot weight** (mg) | | | **Root weight** (mg) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **V1** | **V2** | **Means** | **V1** | **V2** | **Means** | **V1** | **V2** | **Means** | **V1** | **V2** | **Means** |
| Control | 2.4 | 2.7 | 2.5 | 3.2 | 3.8 | 3.5 c | 2.0 | 2.3 | 2.2 c | 1.6 | 2.7 | 2.2 |
| $H_2O$ | 4.4 | 3.1 | 3.8 | 6.1 | 5.9 | 6.0 ab | 3.8 | 3.7 | 3.7 b | 3.3 | 3.6 | 3.5 |
| CaCl2 | 4.7 | 5.1 | 4.9 | 6.2 | 6.2 | 6.2 ab | 4.2 | 4.9 | 4.5 a | 5.0 | 4.3 | 4.6 |
| KCl | 5.5 | 4.3 | 4.9 | 7.9 | 5.8 | 6.8 a | 4.2 | 5.0 | 4.6 a | 4.6 | 4.4 | 4.5 |
| NH4So2 | 3.6 | 3.5 | 3.5 | 5.8 | 4.7 | 5.2 b | 4.4 | 4.3 | 4.3 ab | 3.3 | 3.6 | 3.4 |
| NaCl | 5.4 | 4.1 | 4.8 | 6.2 | 5.4 | 5.8 ab | 4.9 | 4.5 | 4.7 a | 3.0 | 4.3 | 3.6 |
| Mean | 4.3 | 3.8 | | 5.9 | 5.3 | | 3.9 | 4.1 | | 3.4 | 3.8 | |
| LSD | **P** | **V** | **V x P** | **P** | **V** | **V x P** | **P** | **V** | **V x P** | **P** | **V** | **V x P** |
| Values | Ns | Ns | Ns | 1.17 | ns | Ns | 0.61 | Ns | ns | ns | ns | Ns |

V1= Fakhr-e-Sarhad, V2= Pir sabaak-2005, P= Priming, V= Varieties

**Table-3.** Analysis of variance for germination %age, growth rate, seedling dry weight, seed vigour index, shoot length, root length, shoot weight, root weight of wheat varieties as affected by different osmopriming sources.

| **S. O. V** | **D. F** | **Germination** | **G.R** | **S.D.W** (mg) | **S.V.I** | **S.L** (cm) | **R.L** (cm) | **S. W** (mg) | **R.W** (mg) |
|---|---|---|---|---|---|---|---|---|---|
| Replication | 2 | 738.861 | 19.103 | 6.194 | 10192867.465 | 0.644 | 15.123 | 0.223 | 1.757 |
| Priming (P) | 5 | 1893.494** | 42.166** | 15.244* | 16519370.109 | 5.559 | 7.931* | 5.481** | 4.756 |
| Error | 10 | 78.461 | 5.761 | 3.294 | 11904875.806 | 1.682 | 1.671 | 0.451 | 2.321 |
| Varieties (V) | 1 | 491.361* | 14.912 | 16.000* | 16789165.054 | 2.454 | 3.484 | 0.321 | 1.174 |
| P x V | 5 | 113.494 | 7.209 | 2.467 | 9212179.504 | 0.992 | 1.350 | 0.352 | 0.899 |
| Error | 12 | 78.972 | 4.794 | 2.889 | 10986350.931 | 0.990 | 1.128 | 0.620 | 2.225 |
| Total | 35 | | | | | | | | |

G.R= Growth rate, S.D.W= Seedling dry weight, S. V. I= Seed vigour index, S.L = Shoot length, R. L= Root length, S.W= Shoot weight, R. W= Root Weight

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