



## Effect of Water Stress during Seed Production and Storage Time on Germination and Seedling Growth of Cowpea Grown in the Dry Zone of Sri Lanka

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### ABSTRACT

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The water stress during the flowering stage of cowpea reduces the seed yield of the same season, but its effect on seed germination and seedling growth in the following season remains poorly understood. This study was carried out to determine the effect of soil water stress maintained during the onset of flowering stage and storage period of seeds on seed germination, vigor index, and seed physical characteristics of five cowpea varieties (*Waruni*, *Dhawala*, *MI 35*, *ANKCP 01* and *Bombay*) in the following season in the dry zone of Sri Lanka. Seed production under well-watered and water-stressed conditions was done at the Field Crops Research and Development Institute (FCRDI), Mahailuppallama, in a Randomized Complete Block Design. The germination study was conducted in a polytunnel at the Faculty of Agriculture, Rajarata University of Sri Lanka, Anuradhapura, following the Completely Randomized Design. Seeds of five cowpea varieties produced after exposing the plants to two soil moisture levels (water stress: -50 kPa and well-watered: -30 kPa) were used in a series of germination trials conducted after 1, 1½, 2, and 2½ months of storage. Three hundred seeds from each genotype, randomly sub-divided into three replicates of 100 seeds each, were sown in sterilized sand media. Germination percentage was recorded on the 4<sup>th</sup> and 6<sup>th</sup> days after sowing. The seedling characters were assessed on the 6<sup>th</sup> day after sowing, using five randomly-selected seedlings from each replicate. The results revealed that the storage time significantly affected germination ( $p=0.0133$ ). However, the soil water status maintained during seed production did not affect the germination percentage of any variety in the following season. The majority of the varieties showed an increase in germination percentage with storage time. Although the vigor index did not change with either the well-watered or water-stressed treatments in all five varieties, the vigor index of all varieties declined with storage period irrespective of the water treatment ( $p<0.0001$ ). The findings suggest that moderate water deficits during cowpea seed production do not compromise seed germination and seedling vigor in the following season while emphasizing the importance of utilizing the seeds for subsequent field cultivation in the following season before their vigor is decreased.

## INTRODUCTION

Water availability is among the most critical environmental factors affecting crop productivity worldwide, and lack of adequate water in the soil stresses plant growth, physiology, and yield formation (Dodd and Ryan, 2016; Sahitya et al., 2018). The responses of plants to water stress could be depicted through complex mechanisms inducing various morphological, biochemical, physiological, and molecular aspects depending on drought avoidance, drought escape, or drought tolerance strategies (Goufo et al., 2017; Sahitya et al., 2018). These responses are determined by the severity of water limitation and the crop growth stage at which the water stress is experienced (Khakwani et al., 2011).

The time at which water stress is imposed determines the total yield and germination characteristics of crop plants. It is particularly important for crops, of which seeds are used for consumption and propagation, such as legumes. Soil water stress during the reproductive stage would reduce the final crop yield (Shouse et al., 1981; Hayatu et al., 2014). Soybean yield lost up to 35-41% when soil water stress is imposed during the seed filling stage in a greenhouse experiment but does not affect germination (Vieira et al., 1991). The causative factors of yield reduction may include the abscission of the reproductive structures and stunting of plants, which lead to a significant reduction in the number of harvested pods per plant under water stress. Water stress has a similar effect even on the number of days taken to maturity, such that some varieties of water-stressed cowpea mature fast. For example, early flowering is observed in some cowpea (*Vigna unguiculata* (L.) Walp.) varieties grown under water-stressed conditions than those grown under well-watered conditions (Ahmed and Suliman, 2010). However, according to Dadson et al. (2005), water stress delays the maturity of certain cowpea varieties.

Seeds are the primary propagation materials of many kinds of cereal and pulse crops. Therefore, seed quality is very important for farmers involved in commercial and subsistence seed production as planting

materials. Multiplication of improved seeds in sufficient amounts to meet the growers' demands (Pervez et al., 2009) and storing them without losing viability until the next growing season are two critical challenges faced by such seed-producing farmers (Bewley and Black, 1994). Seed quality, which is affected by soil water stress (Pervez et al., 2009), is primarily described by germination ability and viability. Seed germination represents an important initial phase in the life cycle of plants (Kuriakose and Prasad, 2008), which begins with the imbibition of water into dry seeds and is completed when the radicle extends to penetrate the growing media (Bewley, 1997). A successful germination event should be preceded by growing into a seedling with high vigor, closely associated with the viability and the vigor of seeds.

Both viability and vigor of seeds are either reduced or lost during storage. This could be faster for seeds produced under soil water stress than those produced under well-watered conditions. In light of climate change, it is important to evaluate how seeds produced under moisture-stressed conditions perform in the following cultivation seasons. Despite the common consensus of some pulses, such as cowpea being drought-tolerant (Dadson et al., 2005; Hayatu et al., 2014), the performance of seeds produced under water-stressed conditions have not been evaluated adequately.

We conducted this study to test whether the soil water stress maintained during seed production and storage time of seeds has a significant effect on seed germination and seedling growth of the following season in five cowpea varieties (*Waruni*, *Dhawala*, *Bombay*, *ANKCP 01*, and *MI 35*) commonly grown in Sri Lanka. The hypotheses tested in the experiment included: (i) cowpea seeds produced under well-watered conditions show a higher germination percentage and seedling vigor than those produced under water-stressed conditions in five cowpea varieties, (ii) the germination percentage of the seeds of five cowpea varieties produced under water-stressed conditions decreases with storage time (1, 1½, 2 and 2½ months after harvesting) than the seeds produced

under well-watered conditions, and (iii) cowpea seeds produced under well-watered conditions possess superior physical characters such as seed length, seed width, and thickness when compared to seeds produced under water-stressed conditions.

## METHODOLOGY

### Seed Production under Water Stress

Experiments were conducted using five cowpea varieties; *Waruni*, *Dhawala*, *MI 35*,

*ANKCP 01*, and *Bombay*, recommended varieties by the Department of Agriculture (see Table 1 for details). First, seeds of selected cowpea varieties were produced during July - November 2020 inside a rain shelter under two soil moisture levels at the Field Crops Research and Development Institute (FCRDI), Mahailuppallama, Sri Lanka (DL1b agro-ecological region).

**Table 1: Details of the five cowpea varieties used in the experiment.**

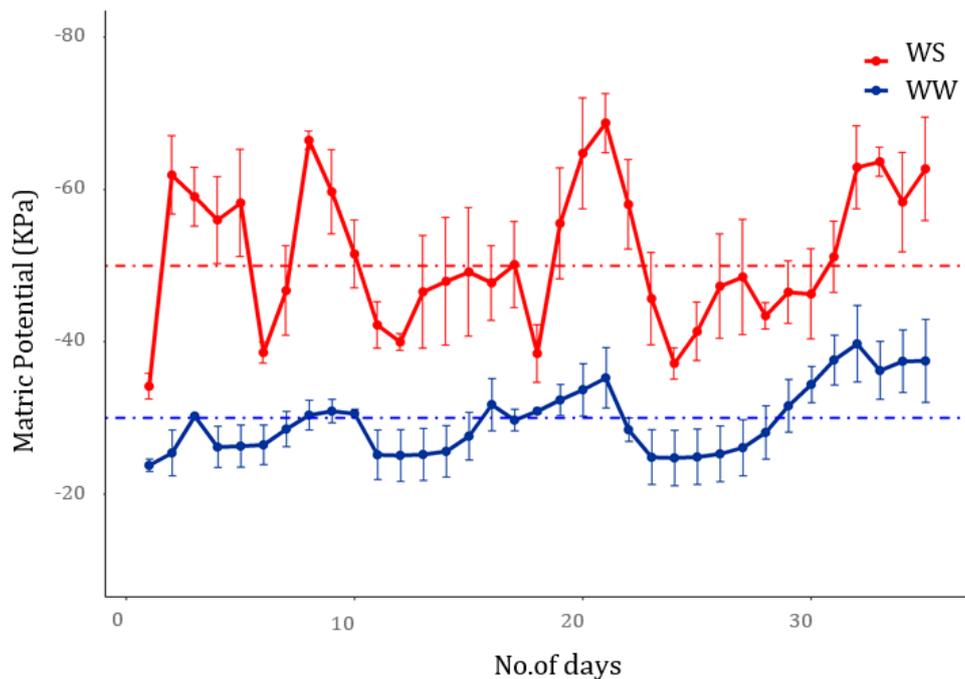
Variety	Released year	Seed colour	Seed size	Flower colour	Days for harvesting	Yield (kg/ha)
Bombay	1960	Speckled grey brown	Large	Purple	75-90	1400
MI 35	1969	Cream	Small	White	60-70	1500
Waruni	1990	Reddish brown	Moderate	Bluish purple	55-65	1600
Dhawala	1997	White	Large	White	60-70	1500
ANKCP 01	2014	Light brown	Large	White	60-65	1500

The site received a mean annual rainfall of 1000-1500 mm, while the mean annual temperature was  $\sim 27$  °C (Department of Agriculture, 2021). Two blocks were maintained inside the rain shelter as well-watered and water-stressed. The plots were arranged in the Randomized Complete Block Design as three replicates of each variety per each water treatment.

Until the onset of flowering, the soil moisture level of all plots was maintained at the field capacity (-30 kPa). At the commencement of flowering, each variety was subjected to two watering levels; 1. well-watered: -30 MPa and 2. water-stressed: -50 MPa. We considered a -50 MPa matric potential to mimic the moderate water stress condition, which is more likely to be experienced in dry zone rain-fed cropping systems. The two water levels were maintained using a manually-operated drip irrigation system with regular

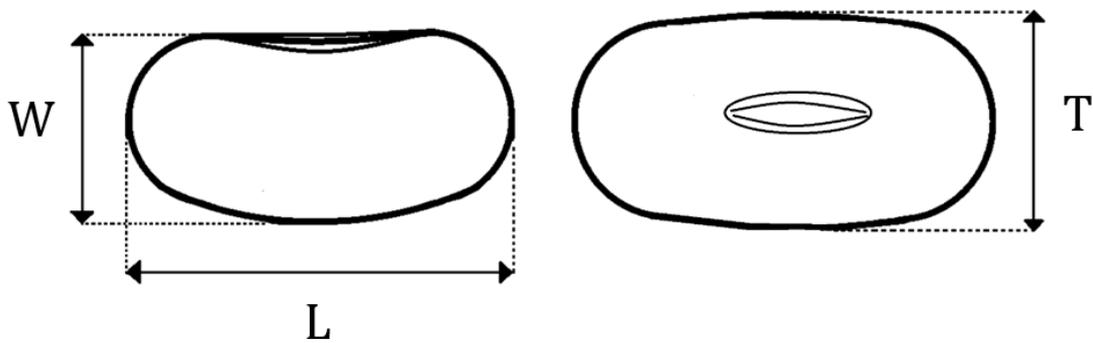
observation of soil matric water potential (Figure 1.) using ceramic-based matric potential sensors (TEROS 21, METER Group, Inc., USA). At 75 days after planting, fully matured pods were harvested manually and sun-dried for three consecutive days to reduce seed moisture up to 12%. The seed moisture content was verified by weighing a sub-sample of seeds before and after oven drying. The seeds were stored inside black polythene bags, placed at  $\sim 30$  °C average ambient temperature until the germination trials as outlined in Figure 1.

For testing the effect of soil water stress maintained during seed production and storage time on seed germination and seedling growth in the following season, a second experiment was conducted in a semi-circular roof type polytunnel with a top vent, at the Faculty of Agriculture, Rajarata University of Sri Lanka, from December 2020 to January 2021.



**Figure 1: Variation of matric potential with time in two water levels: well-watered (WW) and water-stressed (WS) conditions in the experimental site. The average values of -30 kPa and -50 kPa were considered well-watered and water-stressed moisture levels, respectively**

**Seed Germination and Seedling Growth of Seeds Produced under Soil Water stress**



**Figure 2: A diagrammatic representation of the seed physical characteristics measured: seed length (L), seed width (W), and seed thickness (T).**

The mean annual rainfall of the study site is 1640 mm, while the mean annual temperature is ~28 °C (Department of Meteorology, 2022). For evaluating the stored seeds for their germination, emergence, and seedling growth, germination trials were conducted after 1, 1½, 2, and 2½ months of storage. Before the germination test at one month of storage, physical characteristics *viz.* seed length, seed width, and seed thickness were measured

(Figure 2) using a 15 mm Vernier caliper (Jainsons, India). Each germination test consisted of 300 seeds per genotype drawn from the stored seed samples and then randomly sub-divided into three replicates of 100 seeds each. Seeds were placed in plastic trays (35 cm × 30 cm × 5.5 cm) filled with sterilized sand free of weed seeds as the germination media. The experiment was set up in a Completely Randomized Design.

Sufficient moisture was maintained throughout the experimental period by manual watering. Black color polythene was lined to cover all trays to provide complete darkness for three days from the sowing of seeds.

### Measurements

The germination ability was assessed by counting the germinated seeds on the 4th and 6th days after sowing. Seeds were considered germinated when either cotyledons or plumule emerged above the upper surface of the sand media (Pulok *et al.*, 2014). The vigour index was calculated using the following formula (Pulok *et al.*, 2014; Pradeep, 2018):

$$\text{Vigor Index (VI)} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

Shoot length, main root length, and the number of lateral roots of seedlings were

assessed on the 6th day after sowing, using five seedlings randomly chosen from each replicate. The shoot length (from the collar region to the tip of the primary leaf) and the most extended root length of the seedlings were measured using a graduated ruler (mm). The sum of shoot length and main root length was taken as the seedling length. The shoot and root length values were calculated by dividing the sum of the lengths by the number of seedlings evaluated. Similarly, the mean values of the number of lateral roots were also calculated. The fresh weight of shoots and roots of seedlings were measured by separating the shoots and roots (See Table 2 for details of parameters). Weights were measured using a three decimal place balance (Sartorius Electronic Weighing Balance, BSA 224S-CW).

**Table 2: The list of parameters taken with their units, time of obtaining data, and the number of seeds or plants taken for measurements. Seedlings/ seeds were randomly chosen from each replicate.**

Parameter	Units	Time of data collection	No. seedlings/ seeds taken for measurements per replicate
Germination percentage	%	On the 4 <sup>th</sup> and the 6 <sup>th</sup> day after sowing	100
Shoot length	cm	On the 6 <sup>th</sup> day after sowing	5
Main root length	cm	On the 6 <sup>th</sup> day after sowing	5
No. lateral roots of seedlings	-	On the 6 <sup>th</sup> day after sowing	5
Vigor index	-	Calculated after obtaining germination percentage and seedling length	100
Fresh weight of shoots	g	On the 6 <sup>th</sup> day after sowing	5
Fresh weight of roots	g	On the 6 <sup>th</sup> day after sowing	5
Seed length	cm	Before commencement of the germination trial	30
Seed width	cm	Before commencement of the germination trial	30
Seed thickness	cm	Before commencement of the germination trial	30

### Data Analysis

All statistical analyses were conducted in R version 1.1.453 (RStudio Team, 2016). Linear mixed effect models (lmer) in the lme4 package were used to evaluate whether water treatment and storage time were affected by germination and vigor index, using water treatment and storage time as fixed factors and the variety as a random factor. The mean comparison was done using the glht function in the Multcomp package, while the correlation tests determined the relationship between seed physical properties and germination and vigor index. Linear regression lines were fitted to determine the overall trends in the variation of germination percentage and vigor index with storage time.

### RESULTS AND DISCUSSION

The storage period showed a significant effect on the germination of these five varieties ( $p=0.01339$ ). However, contrary to our expectation, soil water stress did not affect the seed germination and seedling vigor in all five varieties of cowpea evaluated in the following season. The responses of germination percentage and vigor index to watering

treatments could also have been affected by the magnitude of the water stress imposed during the reproductive phase. For cowpea, the threshold soil matric suction for initiating the moisture stress conditions is -50 kPa (Bastos et al., 2011). In an experiment by Anyia and Herzog (2004), a matric potential of -75 kPa was maintained to induce water stress for cowpea plants. However, soil matric potential in our experiment was about -50 -50 kPa. Therefore, it can be expected that the plants in our study experienced moderate soil water stress.

### Effect of Water Regime on the Cowpea Seed Germination

We fitted linear mixed effect models to test whether the soil moisture significantly affected seed production on the subsequent seed germination process (Table 3). We hypothesized that seeds produced under well-watered conditions show higher germination than those produced under water-stressed conditions in five cowpea varieties. However, none of the five cowpea varieties supported the above hypothesis.

**Table 3: Model evaluation parameters for the effects of soil moisture level, storage period, and their interaction on the germination percentage and the vigor index (vigor index = germination percentage × seedling length) of five cowpea varieties: MI 35, Waruni, Dhawala, Bombay, and ANKCP 01. The seeds were produced under two watering treatments applied during their early reproductive phase (See Figure 1 for details).**

Response variable	Factors	$\chi^2$	$P^*$	AIC
Germination percentage	Storage time	17.70	<b>0.013</b>	679.47
	Water regime	1.25	0.26	697.75
	Water regime + storage time	16.45	<b>0.01</b>	679.47
Vigor index	Storage time	43.74	<b>&lt; 0.0001</b>	1460.38
	Water regime	0.22	0.67	1547.36
	Water regime + storage time	43.51	<b>&lt; 0.0001</b>	1460.38

\* Responses depicted in bold are significant at  $p < 0.05$ .

Cowpea is known to tolerate a certain degree of drought, and the moderate water stress (-50 kPa) provided in this study appears to be insufficient to cause a reduction in germination percentage. According to Ahmed and Suliman (2010), the reproductive stage of

cowpea is the most sensitive to drought, with a yield reduction of 40 to 50%. Although the total yield reduced when cowpea was subjected to water stress (Shouse et al., 1981; Dadson et al., 2005; Hamidou et al., 2007; Ahmed and Suliman, 2010; Bastos et al., 2011;

Hayatu *et al.*, 2014; Karim *et al.*, 2018), a considerable number of cowpea seeds were produced by the harvesting time. In particular, Shouse *et al.* (1981) report 44.5% and 39% seed yield decrease when the cowpea plant is water-stressed at flowering and pod-filling stages, respectively. Despite a considerable reduction in photosynthetic rate, the grain yield was maintained when cowpea is subjected to soil water stress during grain filling by the translocating photo-assimilates from source organs (Egashira *et al.*, 2020). However, the authors observed above by exposing plants to higher water stress conditions (>75 kPa) which makes them different from the conditions provided in our experiment.

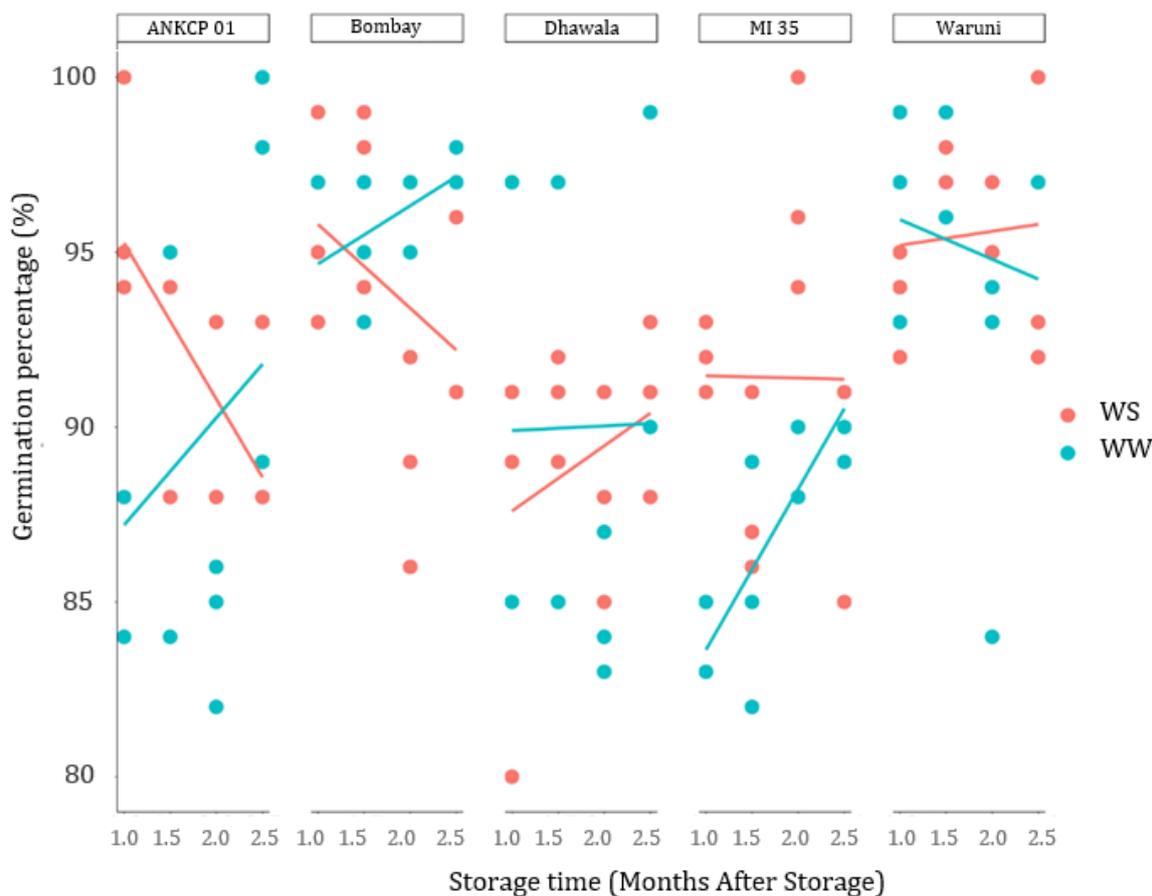
Seed viability (*i.e.* the presence of a viable embryo) is the requirement for successful germination, and then the quantity and quality of storage material determine the seedling vigor. If the magnitude of the biotic or abiotic stresses experienced during the seed production process does not harm the viability of the embryo inside, germination is less likely to be affected. For example, sorghum seeds exposed to mild water stress show significantly higher germination earlier in the maturation period than those grown under ambient conditions (Arnold *et al.*, 1991). Further, water stress imposed during seed development in soybean reduces the overall seed yield; however, germination and vigor were not affected (Vieira *et al.*, 1992). Even though severe soil moisture stress can significantly reduce seed yield and the number of seeds, individual seed weight, germination, and dry weight of the seedling axis can be marginally reduced (Dornbos and Mullen, 1981, Wijayaraja *et al.*, unpublished data). Therefore, we suggest that the moderate soil water stress imposed during the onset of flowering does not cause considerable damage to the seed embryo. Accordingly, seeds produced under moderate soil water stress can effectively be utilized in seed cowpea production programs intended for propagation purposes.

The seeds per pod and seed size were reduced under the water stress treatments (Ahmed

and Suliman, 2010). However, a study aimed at finding the effect of water stress imposed after podding on yield and the nutrient content in chickpea seeds showed that although terminal water stress decreases the total plant dry mass and seed yield, it did not affect the mass of individual pods and seeds. Although increased pod abortion and decreased pod formation were visible as deleterious effects of water stress, seed abortion was not affected (Behboudian *et al.*, 2001). Vieira *et al.* (1991) reported a loss of up to 35-41% yield when drought stress was imposed during seed filling in greenhouse experiments, but the germination was not affected. Low seed germination and vigor are seen only in small, flat, shrivelled, and underdeveloped seeds that occur due to soil water stress. However, these seeds represent a small portion of the seed lot. These data suggest that soil water stress would not affect seed germination or vigor unless the stress was severe enough to produce shrivelled, flat, and underdeveloped seeds. However, in our experiment, water stress was imposed during the onset of the flowering stage. The flowering and pod filling stages of cowpea are more sensitive to water stress (Turk *et al.*, 1980). Therefore, it is suggested that the drought-tolerant nature of cowpea may enable it to maintain the germination percentage and vigor index without being affected by the moderate water stress imposed during the onset of flowering. However, when stress was high enough to reduce the number of seeds and the average weight of seeds, then significant reductions in germination and vigor were possible (Dornbos Jr. and Mullen, 1991). Therefore, it is required to provide higher water stress than the stress imposed during our experiment to determine whether the response of cowpea in terms of germination percentage, vigor index, and seed physical characteristics differ from those obtained through the study.

### **Effect of Storage Period on the Germination Process**

There was a significant difference in germination with changing storage period ( $p=0.0133$ , Table 3).



**Figure 3: The effect of seed storage period (1, 1½, 2 and 2½ MAS – Months After Storage) on seed germination of five cowpea varieties MI 35, Waruni, Dhawala, Bombay, and ANKCP 01. The seeds were produced under two watering treatments (well-watered-WW (-30 kPa) and water-stressed-WS (-50 kPa) applied during their early reproductive phase**

ANKCP 01, Bombay, Dhawala, and MI 35 showed increasing trends in germination with storage time (Figure 3). However, Waruni showed a decreasing germination ability with time. Although a storage period of up to 2½ months could not reduce the germination abilities of varieties, ANKCP 01, Bombay, Dhawala, and MI 35, the rates of the germination ability increments varied. The increase of germination was the highest in MI 35, followed by ANKCP 01 and Bombay. However, these trends were indistinguishable between seeds produced under well-watered and water-stressed soil moisture conditions.

Trends in germination percentages at different storage periods were variety-dependent. Except for the variety Waruni, germination trends increased to 2½ months. It suggests that cowpea seeds from these five

varieties could be stored until 2½ months after harvest without causing a significant loss of germination percentage. Generally, poor vigor is caused by seeds produced under stress conditions like water stress, nutrient deficiency, and extreme temperatures. However, it is more likely that seed mass, germination, and vigor tend to be preserved despite sizable yield reductions due to soil water stress. This is supported by a study conducted with a closely related legume soybean where water-stressed soybean plants produced fewer vigorous seeds than equal numbers of seeds with poor vigor (Dornbos et al., 1989).

Further, the viability of the seeds could reduce when they are stored in adverse conditions such as high levels of temperature, oxygen, and water. Based on the current urge to

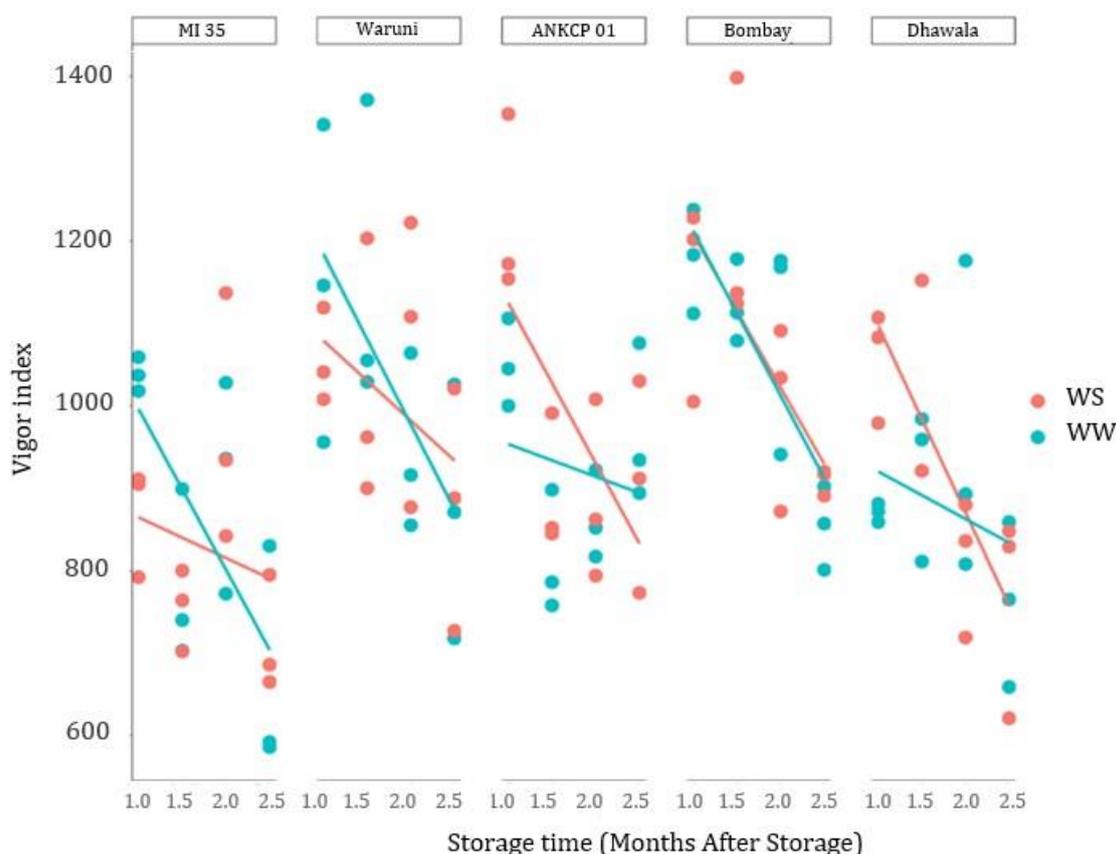
overcome issues related to seed storage parameters, seed preservation of agriculturally important crops from one season to another is crucial (Chhabra *et al.*, 2019). It is essential to utilize the seeds for the

### Variation of the Seedling Vigor Index with Water Regime and Storage Period

According to Pulok *et al.* (2014) and Pradeep (2018), the vigor index was calculated as the product of germination percentage and seedling length. There was a significant difference in vigor index with the storage period ( $p < 0.0001$ ) (Table 3). The vigor index

subsequent cultivation before their germination percentages decrease. However, further research is vital to predicting the threshold storage periods of different varieties.

of all varieties declined with storage period irrespective of the water treatment. In *MI 35*, *Waruni*, and *Bombay*, the decrease in vigor index was almost similar and the highest. *ANKCP 01* and *Dhawala* also displayed similar decreasing trends in vigor index with storage time, but their rates were lower than the above three varieties (Figure 4).



**Figure 4:** The effect of storage period (1, 1½, 2, and 2½ MAS - Months After Storage) on seedling vigor index (vigor index = germination percentage seedling length) of five cowpea varieties *MI 35*, *Waruni*, *Dhawala*, *Bombay*, and *ANKCP 01*. The seeds were produced under two watering treatments (well-watered-WW (-30 kPa) and water-stressed-WS (-50 kPa)) applied during their early reproductive phase.

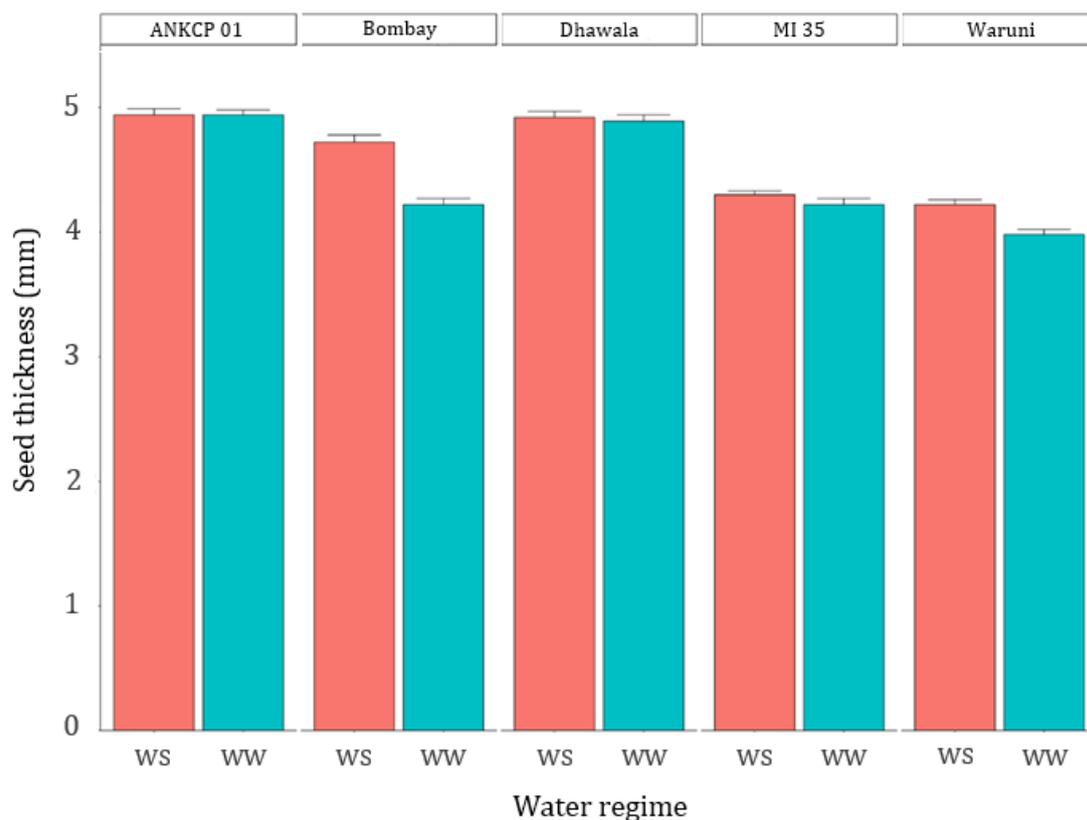
Similar to germination percentage, vigor index also did not vary between the two soil moisture levels. We suggest the inadequacy of the provided stress and the drought tolerance

of cowpea as the underlying reasons for this phenomenon. However, the vigor index declined with storage time. The vigor index is a function of germination percentage and

seedling length (Pradeep, 2018). Therefore, the apparent decrease in vigor index might be mainly attributed to the decrease in seedling length because germination increased with storage time in all other varieties except *Waruni*. Although germination takes place when a viable embryo is available, the subsequent growth of the seedling can vary with time, mainly owing to the quality and

quantity of storage material in the seeds. Some of these materials may be respired with time, reducing the amounts available for seedling growth (Dias *et al.*, 2016). Genetic and environmental factors may also influence seedling vigor (size, health, and growth rate). Even though the seed germinated, the height of the seedlings of long-stored seeds can be reduced.

### Effect of Water Regime on Seed Physical Characteristics



**Figure 5: Effect of watering treatment (well-watered –WW and water-stressed – WS) on the seed thickness of five cowpea varieties; MI 35, Waruni, Dhawala, Bombay, and ANKCP 01. The seeds were produced under two watering treatments applied during their early reproductive phase. Error bars represent the positive standard error of the mean.**

Correlation tests were used to evaluate the relationship between water treatment and seed length, seed width, and seed thickness. There was a slight negative correlation between water regime and seed thickness ( $p < 0.0001$ ,  $r = -0.17$ ), implying that the seeds increased their seed thickness when subjected to water-stressed conditions (Figure 5). It was visible in *Bombay*, *Dhawala*, *MI 35*, and *Waruni*. Neither seed length nor seed width showed a similar relationship to the above. These results suggest that changes in the seed

morphological characteristics are associated with the soil water stress incurred during the reproductive stage of cowpea.

We also evaluated whether the water levels correlated with the hundred seed weight of these cowpea varieties. However, none of the five varieties showed a significant relationship between the water treatment and a hundred seed weight. This implies that the water stress provided at the onset of flowering does not

cause significant reductions in the hundred seed weight.

### **The Relationship of Seed Physical Characteristics with Germination and Vigor Index**

After each storage period, correlation tests were carried out to determine relationships between seed length, width, and thickness on the germination percentage, and vigor index of the five varieties. In *ANKCP 01*, there were positive correlations between seed length and germination percentage of seeds stored for 1½ months, in water-stressed treatment ( $p=0.0295$ ,  $r=0.99$ ), between seed width and germination percentage of 2 months stored seeds, in well-watered treatment ( $p=0.0440$ ,  $r=0.99$ ), between seed width and germination percentage of 2½ months stored seeds, in well-watered treatment ( $p=0.0012$ ,  $r=0.99$ ). In *MI 35*, a negative correlation was found between seed width and germination percentage of seeds stored for 2½ months, in water-stressed treatment ( $p=0.0216$ ,  $r=-0.99$ ). A positive correlation was found between seed width and germination percentage of 2½ months-stored seeds in water-stressed treatment in genotype *Bombay* ( $p<2.2e-16$ ,  $r=1$ ). *Waruni* showed a positive correlation between seed length and germination percentage of 2½ months stored seeds in well-watered treatment ( $p=0.0259$ ,  $r=0.99$ ).

Seed length, width, and thickness did not closely correlate with the vigor index values. In genotype *Waruni*, there was a positive correlation between seed length and the vigor index of 1½-months stored seeds in the well-watered treatment and also a negative correlation between seed length and the germination percentage of 2½ months stored seeds in the water-stressed treatment ( $p=0.0027$ ,  $r=-0.99$ ). *Dhawala* showed a negative correlation between seed thickness and vigor index of seeds stored for 1½ months in water-stressed treatment ( $p=0.0417$ ,  $r=-0.99$ ).

The above correlation test results showed mixed effects of seed length and width on germination percentage. However, the seed length and width affected the germination percentages in storage periods following 1½

months. All the correlations were positive except for the *MI 35* genotype. These relationships imply that seed length or width could impact subsequent seed germination of cowpea, but only after 1½ months storage. However, in the case of vigor index, it was affected by seed length and thickness only in *Waruni* and *Dhawala*. These relationships were also reported in vigor indices of seeds stored for more than 1½ months. Through this, we can suggest that the vigor index is more likely to be affected only in seeds stored for more than 1½ months in these two varieties, probably due to time-dependent seed physiological processes. The hundred seed weight affected the germination percentage of all five varieties. It may be because the seeds were viable despite slight differences among the hundred seed weights of these five varieties. The effect of the initial hundred seed weight was tested on the pooled germination percentages of all five varieties.

This study suggests that cowpea can maintain its germination percentage, vigor index, and seed physical characteristics significantly unchanged over the provision of moderate soil moisture stress (-50 kPa) during the onset of flowering. Seed storage should be considered because it can negatively affect the germination percentage and vigor index. However, further research is necessary to find the maximum threshold levels of water stress that these varieties could withstand without causing significant reductions in germination, vigor, and other seed physical characteristics.

### **CONCLUSION**

The consensus that cowpea is a drought-tolerant crop is supported to a certain extent by this experiment. We suggest that the moderate soil moisture deficits exposed during seed production of cowpea do not affect seed germination and seedling vigor in the following season. However, germination success and seedling vigor were reduced when the seeds were stored for a longer period. This emphasizes using seeds within the most suitable storage period to assure proper seedling establishment in the field. However, we recommend further research to examine the effects of severe soil water stress

experienced at the reproductive stage on seed germination and seedling vigor of cowpea.

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## Author Contributions Statement

Authors of this work contributed for conceptualization: DAUD, MP, IW, NG, LW, DK, WM, Data curation: MP, IW, DAUD, NG, LW, DK, Formal analysis: MP, IW, NG, DAUD, LW, DK, Funding acquisition: NG, DAUD, LW, DK, DMD, Investigation: MP, IW, DAUD, NG, WM, Methodology: MP, IW, DAUD, NG, WM, Project administration: NG, DAUD, Supervision: DAUD, NG, DMD, WM, Visualization: MP, IW, NG, DAUD, LW, DK, Original draft: MP, IW, NG, DAUD, LW, DK, and Review & editing: NG, DAUD, LW, DK, DMD, WM.

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