Effect of moisture stress on seed germination and early seedling growth of pulse crops

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Abstract
In vitro research was conducted to determine the influence of moisture stress on germination of pulse crops in Agronomy Department, Sindh Agriculture University, Tandojam, during 2014. Four pulse crops viz: Mung bean, field pea, lentil and cow pea were tested for germination traits under three moisture stress conditions. All the crops were kept at room temperature and in germinator at 30 °C. The moisture stress significantly reduced germination and early growth parameters. The germination and growth parameter were recorded after 4 and 12 days, respectively. Root shoot ratio was calculated for growth parameters. The maximum seed germination (87.33 %), shoot length (326.0 mm), root length (145.7 mm) were recorded in field pea. Fresh shoot weight (22640 mg), dry shoot weight (2513 mg) and dry root weight (1897 mg) were recorded in cowpea. The minimum seed germination (67.33 %), fresh shoot weight (2227 mg), fresh root weight (1877 mg), were recorded in mungbean. Whereas minimum shoot length (185.3 mm) and root length (85.00 mm) were observed in cowpea and lentil, respectively. The maximum seed germination (86.42%), root length (112.3 mm), fresh shoot weight and dry root weight were recorded under daily moisture conditions. Results reflected that daily and alternate day moisture application proved better germination and seedling growth traits while two days after moisture application showed stress as the result of poor growth of early plant traits. The best seed germination and growth traits were recorded in field pea while cow pea ranked second, lentil third and mung bean fourth.

Keywords: Cow pea; Field pea; Germination; Lentil; Moisture stress; Mung bean

Introduction
Food legumes are crops grown for their edible seeds belong to family Fabaceae. Their dehulled seeds are called pulses. After cereals, grain legumes are alternative source of proteins [1]. The major pulses are Chickpea, Field pea, Cow pea, Mung bean, Lentil, Red gram and Black gram. Lentil is relatively drought tolerant. Potential losses in yield can range between 6-54%. Field pea is also sensitive to drought and yield losses varies from 21- 54%. Phenology of field pea shows its ability to escape drought as compare to other pulses. Abiotic stress like drought is one of the most important stress that intensely limits plant growth leading to decline in productivity [7]. At sowing inadequate soil moisture affects
seed germination and unsynchronized seedling emergence, affecting the establishment of a stand, with negative effects on the yield [2, 3]. Water deficit not only affects seed germination, but also increase germination time [4]. Factors negatively influencing seed germination may include sensitivity to drought stress [5]. Successful crop production is dependent upon the healthy seedling establishment resulting from a critical stage of seed germination [6].

Cowpeas (Vigna spp.) are cultivated for dry seeds and as vegetables to feed the world population. Cowpeas bears highly efficient stress resistant traits against drought and can recover rapidly during vegetative growth stage by re-watering due to their efficiency in using soil water. Drought stress damaged the function of stomata, which limits water loss and the influx of CO₂. Sank CO₂ influx indicates a reduction in carbon fixation in the Calvin cycle and to a decrease in oxidized NADP+ to assist as an electron acceptor in photosynthesis. As a result, electrons flow to the substitute electron acceptor, O₂, synthesize superoxide radical. [8].

Drought marks every phase of plant growth and metabolism. The germination phase regulates the standard establishment and hence the final yield of the crop. Drought may marks in delayed and may reduce or stop germination. The seed germination is reduced at a small negative osmotic potential [9, 10]. The initiation of cell elongation during germination is differentially sensitive to drought. The root system is reflected as primary sensor of drought stress and plays an important role in drought avoidance by deep penetration into soil [11].

Field pea (Pisum sativum L.) is a main frost-resistant and cool-season leguminous vegetable that is broadly cultivated all over the world. Its consist of (25%) protein, (12%) amino acids, sugars carbohydrate, vitamins A and C, calcium and phosphorus, apart from having a slight quantity of iron. Peas being big source of proteins for human diet are valuable for vegetable purposes. Among the pulse crops, mung bean (Vigna radiata L.) has a special importance of intensive crop production due to its short growth period [12]. The crop cultivated in arid circumstance, encounters drought stress at different growth stages. In a symbiotic relationship with the soil bacteria, mung bean roots can fix atmospheric nitrogen and thus increase soil fertility [13]. Water stress disrupts several physiological processes connected with growth, development, and economic yield of a crop [14]. Water scarcity interrupts normal turgor pressure, and the loss of cell turgidity may stop cell enlargement that effects plant growth negatively [15].

The lentil (Lens culinaris L.) is an annually grain legume crop requiring low to medium rainfall and it has tolerance against water stress [16]. Lentils are a chief source of protein for people in the Mediterranean, Africa, Middle East, Southern Asia and South America. Lentil has been playing a significant role in human nutrition. Its seed is a good source of dietary protein. Lentil is commonly used in India, Southwest Asia, and the Mediterranean areas in the form of split lentil (dhal) and it is still an important source of dietary protein in these areas. Lentil contains approximately 22% protein while the lentil straw can be used as a source of organic material for soil improvement. Objectives of study were as: 1) to assesse pulse crop seedlings behavior under moisture stress conditions, 2) to find out appropriate moisture interval to early seedling growth of pulse crops.

Materials and methods

In vitro study was conducted to determine the effect of moisture stress on germination and early seedling growth traits of pulse crops in the Department of Agronomy, Sindh Agriculture University, Tandojam, during 2014. The experiment was conducted in Complete Randomized Design (CRD) in three replications with two Factors i.e., pulse crops and moisture stress. Four pulse crops i.e. P<sub>1</sub> (Mung
bean), P₂ (Field pea), P₃ (Lentil) and P₄ (Cow pea) and three moisture stress levels viz: S₁ (Daily moisture), S₂ (Alternate day moisture) and S₃ (Two days after moisture). Treatment combinations were: T₁=P₁S₁, T₂=P₁S₂, T₃=P₁S₃, T₄=P₂S₁, T₅=P₂S₂, T₆=P₂S₃, T₇=P₃S₁, T₈=P₃S₂, T₉=P₃S₃, T₁₀=P₄S₁, T₁₁=P₄S₂ and T₁₂=P₄S₃. The increase in moisture stress significantly decline germination and related traits. All the pulse crops kept at room temperature and in germinator (Model-PL3) at 30 °C. The seeds were moisturized as scheduled whenever necessary. After 48 hrs data for seed germination was recorded. The length, fresh and dry weights of shoot and root, were recorded after 12 days.

The seed germination percentage was recorded using the following formula:

\[
\text{Germination} \% = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}}
\]

The shoot and root length in mm of all seedlings in a petri dish of each treatment was taken with the help of foot scale and then mean was calculated by using the following formula:

\[
\text{Shoot/Root length} = \frac{\text{Summation of length of shoot or root of all seedlings}}{\text{Total number of seedlings}}
\]

Shoots and roots were per treatment weighted in mg separately on digital electronic balance. The oven dry weight in mg at 50 °C for 48 hours of roots and shoots of each treatment were recorded on digital electronic balance. The data was analyzed using M STAT C Computer Software. The mean were compared by Least Significant Difference (LSD) at 5% probability level [17].

**Results and discussion**

**Percentage of seed germination**

The data for germination of mung bean were significant affected by moisture stress, pulse crops and their interaction (Table 1). The results showed that maximum seed germination (86.42 and 85.00 %) was recorded in treatments where daily moisture and alternate day moisture application made while the minimum seed germination (65.50 %) was recorded where moisture application was made after two days. In case of crops the maximum seed germination of 87 % was recorded in field pea, followed by lentil with 83 % and cow pea with 78 % whereas as the lower seed germination of 67 % was observed in mung bean. The interaction for seed germination was non-significant. Water deficit is in adequate moisture for normal plant growth and to complete the life cycle [18]. Moisture plays vital role in early growth and yield traits of crops. Drought marks every phase of plant growth and metabolism. The germination phase is of main importance in the growth cycle of plants as it determines the standard establishment and final yield of the crop. Water stress may results in delayed and reduced seed germination or may stop germination completely [9]. The early growth traits of pulse were significantly influenced by moisture stress levels. It seems from results that seed germination percentage showed optimum growth at sufficient soil moisture level as alternate day moisture application and it is observed that crop Mung bean is very sensitive to moisture stress at seed germination stage than other pulse crops. Similar results were found as drought stress severely reduced germination and seedling stand [19].

**Shoot length (mm)**

The data for shoot length of pulse crops affected by different moisture stress levels, pulse crops and their interaction was significant presented in (Table 2). The results showed that maximum shoot length (258.8 mm) was recorded at alternate day moisture application; whereas the minimum shoot length (200.3 mm) was observed at after two days moisture application; in case of mean for crops the maximum shoot length (326.0 mm) was recorded in field pea and followed by (201.4 mm) in lentil whereas as the lower shoot length (185.3 mm) was observed in cowpea. Interaction results showed that maximum shoot length (360.0 and 340.0 mm) was observed at daily and alternate day moisture application × crop field pea
and the minimum interaction value (160.0 mm) was recorded after two days moisture application × crop cow pea.

**Root length (mm)**

The data for root length of pulse crops affected by different moisture stress levels, pulse crops and their interaction presented in (Table 3) was significant. The results showed that maximum root length of 112.3 and 111.9 mm was recorded at daily moisture and alternate day moisture application; whereas the minimum root length (87.25 mm) was observed at after two days moisture application. In case of mean for crops the maximum root length (145.7 mm) was recorded in field pea and the lower root length (85.00 and 85.89 mm) was observed in lentil and cowpea, respectively. Interaction results showed that maximum root length (165.0 and 149.0 mm) was observed at daily and alternate day moisture application × crop field pea and the minimum interaction value (67.00 mm) was observed at after two days moisture application × crop lentil.

In the shoot length and rooth length (mm) results in this study are same as of by [20, 21] as water stress leaded to decrease in root length and shoot length. The crop cowpea is significantly affected by after two days moisture application and field pea is more moisture stress tolerant than other crops at alternate day moisture application and there is variation in case of root length that field pea showed better performance than cow pea. The response of a crop to drought fluctuates with crop species, crop growth stage, soil type, environment and season. Drought stress causes sequences of physiological, biochemical and morphological responses of crops, which finally results in low yield of green gram [22], this statement also supports our results that every pulse crop plants have different water requirements.

**Shoot fresh weight (mg)**

The data for shoot fresh weight of pulse crops affected by different moisture stress levels was significant for moisture stress, pulse crops and their interaction presented high-significant in (Table 4). The results showed that maximum shoot fresh weight (16500 and 15970 mg) was recorded at daily moisture and alternate day moisture application, respectively, whereas the minimum shoot fresh weight (11950 mg) was observed at after two days moisture application. In case of mean for crops the maximum shoot fresh weight (22640 mg) was recorded in cowpea and (18650 mg) field pea, followed by (15710 mg) in lentil whereas the lower shoot fresh weight (2227 mg) was observed in mung bean. Interaction results showed that maximum shoot fresh weight (25400 and 24330 mg) was observed at daily and alternate day moisture application × crop cow pea, respectively and the minimum interaction value (1820 mg) was observed at after two days moisture application × crop mung bean.

| Moisture stress levels | Winter pulse crops | Summer pulse crops | Mean |
|------------------------|--------------------|--------------------|------|
|                        | Field pea | Lentil | Cow pea | Mung bean |          |
| M₁                     | 94.00     | 92.00  | 87.67   | 72.00     | 86.42 A  |
| M₂                     | 92.00     | 90.00  | 84.00   | 74.00     | 85.00 A  |
| M₃                     | 76.00     | 68.00  | 62.00   | 56.00     | 65.50 B  |
| Mean                   | 87.33 A   | 83.33 AB | 77.89 B | 67.33 C   |

Where, M₁ = Daily Moisture application, M₂ = Alternate day moisture application and M₃ = After 2 days moisture application
Table 2. Effect of moisture stress levels on shoot length (mm) of different pulses

| Moisture stress levels | Winter pulse crops | Summer pulse crops | Mean |
|------------------------|--------------------|--------------------|------|
|                        | Field pea          | Lentil             | Cow pea | Mung bean |
| M1                     | 340.0 a            | 214.3 de           | 195.0 e | 241.7 c | 247.8 B |
| M2                     | 360.0 a            | 220.0 d            | 201.0 de | 254.0 c | 258.8 A |
| M3                     | 278.0 b            | 170.0 f            | 160.0 f | 193.0 e | 200.3 C |
| Mean                   | 326.0 A            | 201.4 C            | 185.3 D | 229.6 B |

Where, M1 = Daily Moisture application, M2 = Alternate day moisture application and M3 = After 2 days moisture application.

Table 3. Effect of moisture stress levels on root length (mm) of different pulses

| Moisture stress levels | Winter pulse crops | Summer pulse crops | Mean |
|------------------------|--------------------|--------------------|------|
|                        | Field pea          | Lentil             | Cow pea | Mung bean |
| M1                     | 165.0 a            | 92.00 cde          | 86.67 def | 104.0 bc | 111.9 A |
| M2                     | 149.0 a            | 96.00 cde          | 93.00 de | 111.0 bc | 112.3 A |
| M3                     | 123.0 b            | 67.00 f            | 78.00 ef | 81.00 ef | 87.25 B |
| Mean                   | 145.7 A            | 85.00 C            | 85.89 C | 98.67 B |

Where, M1 = Daily Moisture application, M2 = Alternate day moisture application and M3 = After 2 days moisture application.

Table 4. Effect of moisture stress on shoot fresh weight (mg) of different pulses

| Moisture stress levels | Winter pulse crops | Summer pulse crops | Mean |
|------------------------|--------------------|--------------------|------|
|                        | Field pea          | Lentil             | Cow pea | Mung bean |
| M1                     | 19330 c            | 16820 de           | 25400 a | 2340 g | 15970 A |
| M2                     | 21250 b            | 17900 cd           | 24330 a | 2520 g | 16500 A |
| M3                     | 15360 e            | 12420 f            | 18200 b | 1820 g | 11950 B |
| Mean                   | 18650 B            | 15710 C            | 22640 A | 2227 D |

Where, M1 = Daily Moisture application, M2 = Alternate day moisture application and M3 = After 2 days moisture application.

Root fresh weight (mg)

The data for root fresh weight of pulse crops affected by different moisture stress levels was significant for moisture stress, pulse crops and their interaction presented high-significant in (Table 5). The results showed that maximum root fresh weight (13420 mg) was recorded at alternate day moisture application; whereas the minimum root fresh weight (9173 mg) was observed after two days moisture application. In case of mean for crops the maximum root fresh weight (17110 mg) was recorded in cow pea and (14610 mg) in field pea followed by (12300 mg) in lentil whereas the lower root fresh weight (1877 mg) was observed in mung bean. Interaction results showed that maximum root fresh weight (19100 mg) was observed at alternate day moisture application × crop cow pea and the minimum interaction value (1510 mg) was observed at after two days moisture application × crop mung bean.

Shoot dry weight (mg)

The data for shoot dry weight of pulse crops affected by different moisture stress levels was significant for moisture stress, pulse crops and their interaction presented high-significant in (Table 6). The results showed that maximum shoot dry weight (1970 and 2050 mg) was recorded at daily moisture and alternate daily moisture application, whereas the minimum shoot dry weight (1455 mg) was observed at after two days moisture application. In case of mean for crops the maximum shoot dry weight (2513 mg) was recorded in cow pea and (2070 mg) in field pea followed by (1744 mg) in lentil whereas the lower shoot dry weight (246.7 mg) was observed in mung bean.
Interaction results showed that maximum shoot dry weight (2834 mg) was observed at alternate day moisture application × crop field pea and the minimum interaction value (200.0 mg) was observed after two days moisture application × crop mung bean.

**Root dry weight (mg)**
The data for root dry weight of pulse crops affected by different moisture stress levels was significant for moisture stress, pulse crops and their interaction presented high-significant in (Table 7). The results showed that maximum root dry weight (1430 mg) was recorded at daily moisture application, whereas the minimum root dry weight (1069 and 1105 mg) was observed at alternate day moisture and two days after moisture application. In case of mean for crops the maximum root dry weight (1897 mg) was recorded in cow pea and (1363 mg) in field pea whereas as the lower root dry weight (203.6 mg) was observed in mung bean. Interaction results showed that maximum root dry weight (2150 mg) was observed at daily moisture application × crop field pea and the minimum interaction value (160.0 mg) was observed at after two days moisture application × crop mung bean.

Subbarao and his fallows concluded that shoot and root traits play an important role in regulating water use by crop plant. Our results showed that high moisture stress decreased the shoot fresh weight (mg) of mung bean after two days moisture application and shoot fresh weight were observed increased in field pea at alternate day moisture application [23]. In case of root fresh weight cowpea showed optimum growth at daily moisture application whereas lentil and mung bean showed decreased in shoot fresh weight and root fresh weight. Anbessa and Bejiga [24] also found significant decreased in dry root weight, root volume and rooting depth under low moisture stress. This statement also favors results that if the shoot and root does not shows optimum growth then the plants cannot perform their physiological process as well as early growth traits. The shoot dry weight and root dry weight (mg) were significantly affected in mung bean after two days moisture application whereas the shoot dry weight and root dry weights were found increased in cow pea at alternate day moisture application and daily moisture application, respectively.

**Table 5. Effect of moisture stress levels on root fresh weight (mg) of different pulses**

| Moisture stress levels | Winter pulse crops | Summer pulse crops | Mean |
|------------------------|--------------------|--------------------|------|
|                        | Field pea          | Lentil             | Cow pea | Mung bean |      |
| M₁                    | 15100 c            | 12360 d            | 17900 b | 2020 f    | 11850 B |
| M₂                    | 17330 b            | 15150 c            | 19100 a | 2100 f    | 13420 A |
| M₃                    | 11450 d            | 9400 e             | 14330 c | 1510 f    | 9173  C |
| Mean                  | 14630 B            | 12300 C            | 17110. A | 1877      |      |

Where, M₁ =Daily Moisture application, M₂ = Alternate day moisture application and M₃ =After 2 days moisture application

**Table 6. Effect of moisture stress levels on shoot dry weight (mg) of different pulses**

| Moisture stress levels | Winter pulse crops | Summer pulse crops | Mean |
|------------------------|--------------------|--------------------|------|
|                        | Field pea          | Lentil             | Cow pea | Mung bean |      |
| M₁                    | 2564 bc            | 2235 de            | 2820 ab | 260.0 h   | 1970 A |
| M₂                    | 2834 a             | 2385 cd            | 2700 ac | 280.0 h   | 2050 A |
| M₃                    | 1994 ef            | 1605 g             | 2020 f  | 200.0 h   | 1455 B |
| Mean                  | 2070 B             | 1745 C             | 2513 A  | 246.7 D   |      |

Where, M₁ =Daily Moisture application, M₂ = Alternate day moisture application and M₃ = After 2 days moisture application
Table 7. Effect of moisture stress levels on root dry weight (mg) of different pulses

| Moisture stress levels | Winter pulse crops | Summer pulse crops | Mean |
|------------------------|--------------------|--------------------|------|
|                        | Field pea | Lentil | Cow pea | Mung bean |          |
| M₁                     | 2150 a    | 1370 c  | 1980 a  | 220.7 e    | 1430 A   |
| M₂                     | 245.7 e   | 1680 b  | 2120 a  | 230.0 e    | 1069 B   |
| M₃                     | 1630 b    | 1040 c  | 1590 b  | 160.0 e    | 1105 B   |
| Mean                   | 1342 B    | 1363 B  | 1897 A  | 203.6 C    |          |

Where, M₁ = Daily Moisture application, M₂ = Alternate day moisture application and M₃ = After 2 days moisture application

Conclusion
It is concluded from the results that daily and alternate day moisture application resulted better seed germination, establishment and development of early seedling plant growth traits and two days after moisture application showed stress as the result of poor growth of early plant traits. It is further derived from the data in winter pulse crop field pea resulted the best seed germination and early plant growth traits than lentil while in summer pulse crops cowpea showed better performance in terms of seed germination and early establishment attributes. On the basis of these preliminary experimentation, the farming community is advised to prefer those crops in winter as well as in summer which are more resistant against moisture stress in view of diminishing water resources in the country.

Authors’ contributions
Conceived and designed the experiments: Z Ullah & S Ahmad, Performed the experiments: S Ahmad, Analyzed the data: Z Ullah, MR Siddiqui & ZU Haq, Contributed materials/ analysis/ tools: S Latif, Wrote the paper: Z Ullah.

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