RESPONSE OF CHICKPEA GENOTYPES TO DROUGHT STRESS IN PETRI DISH ENVIRONMENT

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ABSTRACT: Chickpea (*Cicer arietinum* L.), an important leguminous crop has a dwindling crop area everyday due to climate change, and the overall yield has declined. In a controlled condition experiment, four distinct levels of drought were applied to ten different varieties of chickpeas in a petri dish using polyethylene glycol (PEG) @ 0, 10, 20, and 30%. As differentiating factors, germination rate, plumule, and radicle lengths were used. The experiment's results showed that, at 0% PEG, KK-2 and Punjab-2008 demonstrated the highest germination rates (99%), while the lowest (0%) germination rates at (30% PEG) were recorded for Chattan and KK-1 genotypes. At 0% polyethylene glycol (PEG), Chattan and Punjab-2008 had the maximum plumule (0.70 cm) and radicle (7.47 cm) lengths, respectively and with increasing drought they got reduced. Thus, it may be inferred that drought impacted negatively but its impact can be lessened by adopting genotypes that are drought resistant.

Keywords: Chickpea, Drought, Genotypes, PEG, Water.

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INTRODUCTION

Chickpea is a legume and in temperate regions, it is planted as a summer crop, and in the tropical periphery, as a winter crop. The quality and production of chickpeas are greatly influenced by various parameters, including salinity/sodicity, soil moisture content, soil texture and structure, day length, and temperature. It is vulnerable to drought, which has an impact on the chickpea crop at different phases of development. Drought has a big impact on chickpea's seed emergence, biochemical parameters, osmotic balance, and antioxidant production. Farmers in regions where drought problems are the primary cause of production decline and low-quality end products urgently need to analyze the drought-tolerant chickpea variety in all available cultivars. Leguminosae/Fabaceae is the family that includes grain crops (pulses). According to Zander *et al*. (2016), this offers the public a reasonably priced source of dietary plant protein and symbiotically fixes nitrogen to have a significant impact on agricultural ecosystems. Pulses are grown on 5% of Pakistan's total cultivable land (Ullah *et al*., 2020), and the country consumes 4.18 kg of them per person annually, or more than 60% of the crop (Rani *et al*., 2014).

The most severe limitation is posed by drought to chickpea crop, which is part of the climate phenomenon. It is estimated that droughts are responsible for 50% of all agricultural losses (Roy *et al*., 2021). Chickpeas are typically grown as a rotation crop in cereal cropping systems to make the most of the moisture that is left in the soil (Korbu *et al*., 2020). This almost always causes moisture stress towards the end of the growing season, which occurs when the crops are harvested. As a direct consequence of this, the plant is subjected to stress throughout the reproductive season, which results in decreased yields (Mathobo *et al*., 2017).

Around the world, chickpea yields are reduced by between 40-45% because of drought (Devasirvatham and Tan, 2018). Over the course of the last twenty plus years, researchers have been working on breeding chickpea to make it more resistant to drought. Previous studies have established a correlation between drought and a variety of plant characteristics, including early maturity (drought avoidance), root features (drought escape), carbon isotope discrimination, rate of partitioning, shoot biomass, and grain yield. The alterations in physiology and biochemistry that occur in response to drought have been thoroughly described in previous studies (Krishnamurthy *et al*., 2010; Upadhyaya *et al*., 2012; Ramamoorthy *et al*., 2016). These morphological alterations in the plants demonstrated how dryness influences the growth and development of plants (Rathnayaka *et al*., 2020). The detrimental effects of drought can be mitigated by employing a variety of genetic techniques to generate chickpea cultivars that are more resistant to drought and have improved drought tolerance (Kumar *et al*., 2018). The identification of genotypes using straightforward screening procedures is the major objective of the process of producing cultivars with increased resistance. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) discovered that the genotype ICC 4958 is resistant to drought for a shorter period and has a bigger root length and volume (Gaur *et al*., 2008). In the context of drought research, this genotype has been utilized both as a donor parent and as a reference genotype (Bharadwaj *et al*., 2021). On the other hand, it is well recognized that variations in crop length and yield potential influence grain yield notwithstanding the effects of stress (Gaur *et al*., 2019). Under adverse conditions, shorter duration genotypes are more likely to provide higher yields than longer duration genotypes (Varshney *et al*., 2014). Bidinger *et al*. (1987) used the method of multiple regression to get rid of the disparities that existed between the crop phenology and the stress escape. According to this method, the grain yield during a drought is calculated as a function of the crop's production potential and the amount of time until it reaches 50 percent flowering. Using this strategy, out of 211 genotypes, five of the most drought resistant genotypes and twenty of the most drought susceptible genotypes were discovered. In addition, the test determined that ICC 4958 was a genotype with a moderate tolerance to drought. In the outdoor pot experiment that took place at ICRISAT, eight different chickpea genotypes were grown to assess the canopy temperature with an infrared thermometer under conditions of terminal drought. According to the findings, there needs to be a significant number of genotypes to identify differences. As a result, breeding for drought resistance requires an understanding of the crop's developmental stage as well as the intensity and duration of the stress. This is because plants can continue growing even when their available water supply is limited.

MATERIALS AND METHODS

The experiment was conducted at Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar Pakistan. The experiment was carried out in Petri Dishes and ten verities were selected for screening of drought stress at three levels.

Selection and Collection of seeds: A total of 10 local varieties of chickpea (Table 1) were selected for the experiment in a petri dish environment. These varieties were collected from the Grain Research Station, Ahmadwala, Karak, Pakistan Agricultural Research Council, Arid Zone *Research Centre*, Dera Ismail Khan, Pakistan, Arid Zone *Research Institute*, *Bhakkar, Pakistan and Ayub Agriculture Research Institute, Faisalabad, Pakistan.*

Petri Dish Drought Experiment: This experiment contained 10 different chickpea varieties and four drought levels in a petri dish environment. Polyethylene glycol (PEG) at various doses causes drought stress, i.e., 0, 10, 20, and 30 %. Same precautions were followed for cleanliness, temperature, and humidity. PEG solution of desired concentration was applied to each treatment at a regular interval of 2 days. The number of sprouted seeds was observed with two days interval and repeated up to the 14th day.

Germination Rate: The germination percentage is the number of seeds germinated compared to the total number of seeds in a petri dish (09 in our case), to be multiplied by 100. The following formula was adopted as described by Desalegne (1996).

Germination rate (GR):= $\frac{NT3+NT6+NT9+NT12}{Total\ number\ of\ seeds\ germinated}$

Where: NTn=number of seeds germinated while N=days (3, 6, 9, 12)

Plumule and Radicle length (cm): Plumule length (cm) was measured by the stem and embryo length. The radicle length (cm) was measured from the point of first cotyledons' node to the tip of the lengthiest root. Five diverse drought tolerant/susceptible varieties were selected for pot experiments.

Statistical Analysis: These experiments were performed under a complete randomized design (CRD) with a factorial arrangement. The difference between percentage and length was subjected to statistical analysis by using Statistix 8.1 to perform ANOVA (Analysis of Variance). The means significance ($P \leq .05$) for F-test were further subjected to mean separation using DMRT (Duncan's Multiple Range Test).

RESULTS

The rate of Germination (%): The rate of germination (%) of chickpea varieties as affected by different drought levels is showed in Fig. 1. The germination rate depicted maximum germination in control treatment in all genotypes where no drought stress was applied; the trend was as: KK-2 (99%), Bittle-98 (98%), CM-98 (98%), KK-1 (97%) Chattan (96%), Indus (96%), Fakhr-e-Thal (95%), Bhakkar-2011 (95%), Punjab-2008 (95%), KK-3 (93%). Data showed that minimum germination rate was recorded in the highest (30% PEG) drought conditions, while some genotypes at such a higher drought condition showed no germination like Chattan and KK-1. It was also noted that at the highest level of drought, Fakhr-e-Thal showed very low germination compared to all other genotypes at that level which shows germination.

Plumule length (cm): The plumule length (cm) of chickpea genotypes against drought stress is showed in Fig 2. In Chattan genotype, the maximum plumule length (0.70 cm) showed at 0% polyethylene glycol (PEG). Simultaneously, no germination occurred at the highest levels of drought (20% and 30% PEG). In the KK-1 genotype, there was some significant data showed at 0% and 10% PEG, while no plumule length was showed at 20% and 30% PEG levels. In case of KK-2, plumule length was as in following order 0% > 10% > 20% > 30%. Similar growth in plumule length was also observed in KK-3, i.e., at the highest level of drought. In the Fakhr-e-Thal genotype, the highest Plumule Length was showed in the control. The trend was as follows Bhakkar-2011 > Punjab-2008 > Bittle-98 > CM-98 > KK-2 > Indus > KK-3 > Fakhr-e-Thal > Chattan > KK-1.

Radicle Length (cm): The data for radicle length (cm) of chickpea genotypes against drought stress in petri dish condition are showed in Fig 3. Data showed significant difference among the treatments. Results depicted that maximum radicle length (cm) was recorded in Punjab-2008 (7.47 cm) and Bhakkar-2011 (6.13 cm) among all treatments. The radicle length decreased when the extent of drought increased among all genotypes. The trend was as follows Punjab-200 > Bhakkar-2011 > CM-98 > Bittle-98 > KK-2 > Fakhr-e-Thal > KK-3 > Indus > KK-1 > Chattan > at 0% PEG. The sequence of radicle length at 10% PEG is showed as Bhakkar-2011 > Punjab-2008 > CM-98 > Bittle-98 > Indus > KK-3 > Fakhr-e-Thal > KK-2 > KK-1 > Chattan. The trend for radicle length at 20% PEG treatment was as Punjab-2008 > Bhakkar-2011 > CM-98 > Bittle-98 > Fakhr-e-Thal > KK-3 > KK-2 > Chattan >Indus > KK-1. While, trend at the highest drought stress i.e. 30% PEG was as Bhakkar-2011 > KK-2 > Punjab-2008 > CM-98 > Bittle-98 > KK-1 > Indus > Chattan > KK-3 > Fakhr-e-Thal.

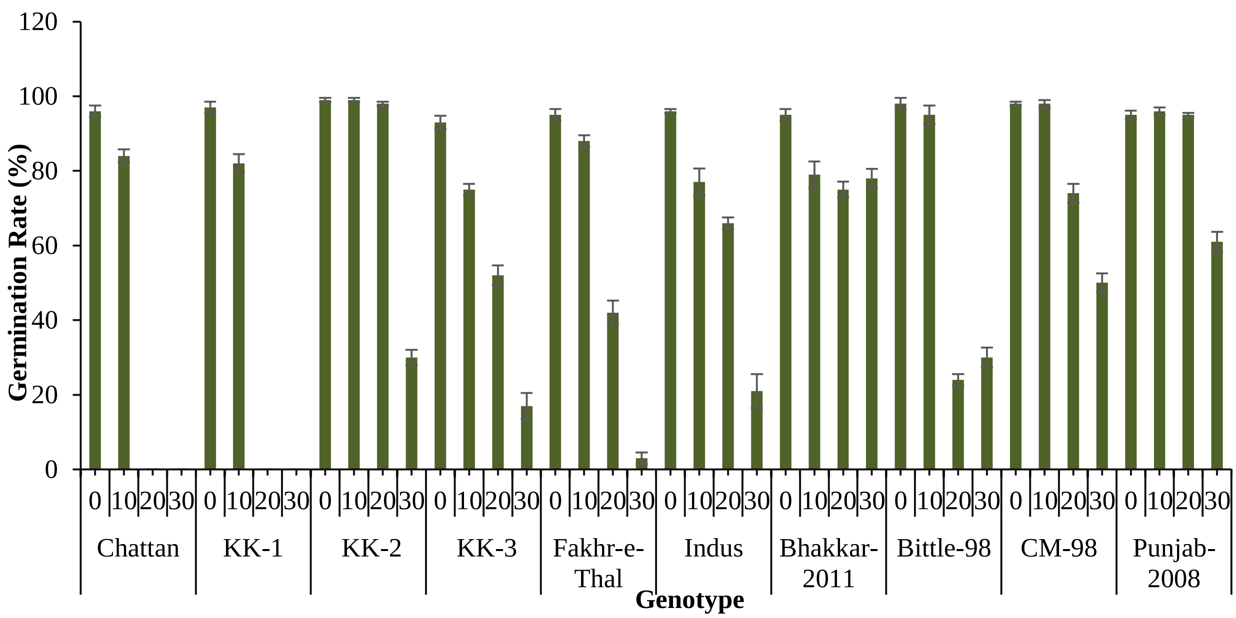

Fig 1. The effect of different drought levels on the Germination rate (%) of chickpea genotypes

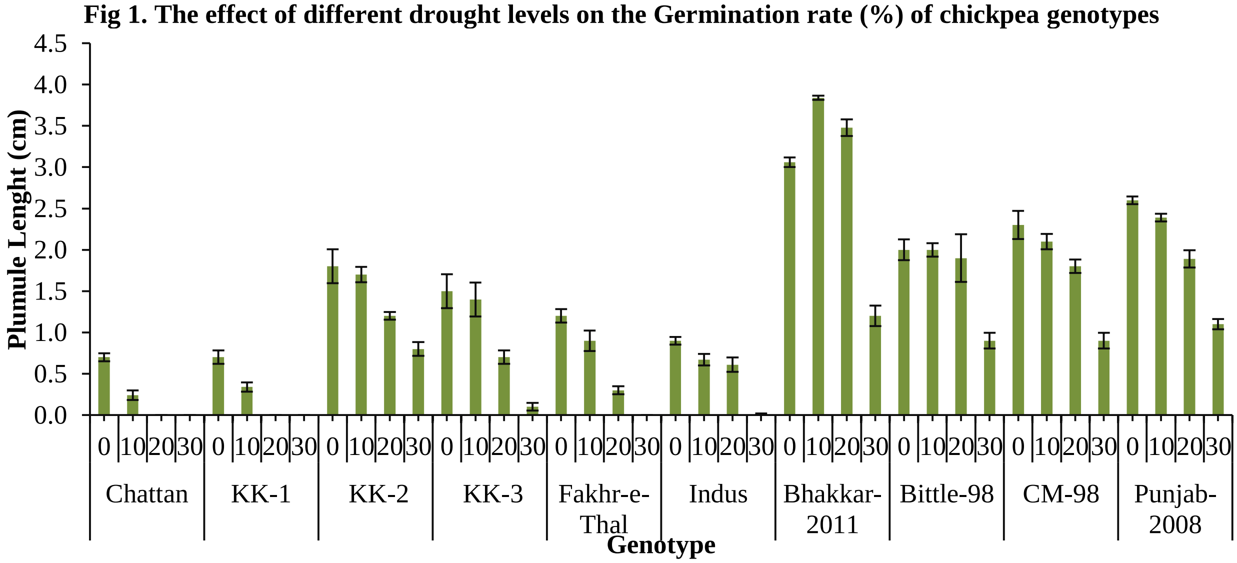

Fig 2. The Effect of different drought levels on the Plumule Length (cm) of chickpea genotypes

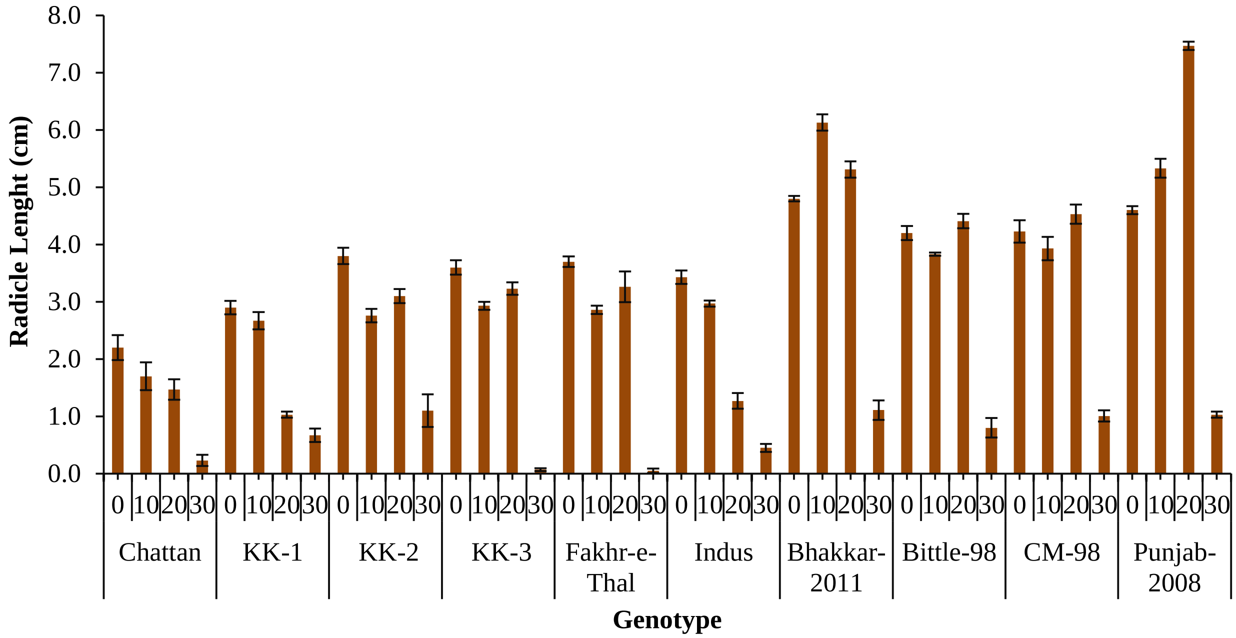

Fig 3. Effect of different drought levels on the Radicle Length (cm) of chickpea genotypes

Table 1 Details of collected chickpea varieties.

Sr. No.	Variety Name
01	KK-1
02	KK-2
03	KK-3
04	CM-98
05	Punjab-2008
06	Indus-2016
07	Bittle-98
08	Bhakkar-2011
09	Chattan
10	Fakhr-e- Thal

DISCUSSION

In majority of crop species around the world, drought is a significant abiotic factor limiting plant growth, development, and yield. Recent estimates based on climate projections foresee a worsening of the water shortage, with around one-third of agricultural regions already experiencing it (Ceccareli *et al*., 2010).

The identification of characteristics associated to drought resistance and water intake is becoming more crucial considering the obvious need to focus on breeding for drought tolerance (Maazou *et al*., 2016; Mwadzingeni *et al.*, 2016; Langridge and Reynolds, 2015). Germination has been suggested as a useful criterion for screening drought resistance since it is one of the most important phases in plant growth and is correlated with seedling establishment and early growth (Boureima *et al.*, 2016). In this context, the goal of this study was to ascertain how ten chickpea genotypes responded to PEG-induced drought stress during the germination phase as a method of selecting genotypes that are tolerant to drought. Our findings, which support previous research on the subject, show that the potential for germination is severely impacted by drought, with the severity of the effects of stress increasing with stress levels (Reza Yousefi *et al.*, 2020; Nadeem *et al.*, 2019; Mickky and Aldesuquy, 2017). Since water availability controls the processes of seedling tissue synthesis and radicle elongation by regulating the production of hydrolytic enzymes, water availability influences germination potential (Muscolo *et al.*, 2014). Stress due to drought directly affects radicle and plumule length (Muscolo *et al*., 2014). The germination rate (%) of chickpea varieties is affected by different drought levels as showed in Fig 1. The data showed that maximum germination rate was showed by KK-2 and Punjab-2008 (99%) followed by Bittle-98 and CM-98 (98%) at control. It was noted that as the drought levels increased, the germination rate (%) decreased gradually. In general, as osmotic water stress increased, the germination rate significantly dropped (Sun *et al*., 2012).

Conclusion: The most important takeaways from this research are that chickpea genotypes were selected for their tolerance and sensitivity to the drought stresses, and the results showed that some chickpea varieties were found to be extremely resistant to these stresses, while other varieties were found to be extremely vulnerable. Due to the fact that its defense mechanisms have not yet fully developed, the plant is in its very early stages very susceptible to the effects of stressors. As a result, the genotypes that are resistant at that stage will likely be defensive once they are introduced into the field. Therefore, it is advised that these be put through more testing on a larger scale before they are put forth as a solution for severe environmental changes and drought stress.

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