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Impact of Osmotic Stress on Seed Germination and Seedling Growth in Chickpea (*Cicer arietinum* L.)

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ABSTRACT

Background: Osmotic stress is one of the major constraints in the global production of economically important crops by adversely affecting their yield, quality and energy costs. *Cicer arietinum* L. commonly known as chickpea is an important pulse crop worldwide due to its high protein content and agronomic significance. Chickpea is susceptible to osmotic stress and effects its productivity on a large scale. The objective of this study was to investigate the impact of osmotic stress on seed germination and seedling growth.

Methods: Osmotic stress was induced using three different concentrations of PEG-6000 (5%, 10% and 15%). Distilled water treatment was used as control. Percentage of seed germination, seedling growth, enzymatic activity of amylase and protease and protein content of chickpea was evaluated after a week.

Result: Osmotic stress considerably affected seed germination as well as all other allied traits. Studies showed that increase in osmotic stress decreased the percentage of seed germination. Seeds treated with 5% PEG considerably improved the length of the plumule and radicle, fresh and dry weight when compared to control in *Cicer arietinum*. The enzymatic activity of protease increased with increasing osmotic gradients. Seeds treated with 15% PEG showed maximum protein content. This study contributes to current knowledge of the physiological responses of chickpea to osmotic stress and may assist in the development of strategies to enhance crop resilience and productivity under adverse environment conditions.

Key words: Chickpea, Germination, Osmotic stress, PEG.

INTRODUCTION

Legume seeds, due to their high nutrient content, are a valuable source of food, feed and industrial products. It is through the act of germination that a seed, typically from a plant, grows into a seedling under favorable environmental conditions. It involves the activation of the embryo within the seed, resulting in the emergence of a radicle or embryonic root, followed by the growth of the radicle or plumule (Nonogaki, 2019). However, their germination and growth can be negatively impacted by abiotic stresses, including osmotic stress (Karimizadeh *et al*., 2021; Kaur *et al.,* 2021). The process of germination is crucial for the establishment and propagation of plant species and is influenced by a variety of environmental factors such as water, temperature, light and nutrient availability (Bewley and Black, 1994). Osmotic stress can affect the initial stages of seed germination by altering the water potential gradient across the seed coat, which can inhibit or promote water uptake by the seed. Different legume seeds respond differently to osmotic stress.

Chickpea (*Cicer arietinum* L.) is a pulse crop cultivated in several countries mainly due to its high protein content (Arriagada *et al.,* 2022). The Indian subcontinent (India, Pakistan, Myanmar, Bangladesh and Nepal) is the major chickpea producer, contributing almost 70% of the world's production (Bar-El Dadon *et al.,* 2017). It is used for human consumption as well as for feeding animals in the dry and semi-arid regions of the world. Its ability to fix atmospheric nitrogen significantly contributes to the reduction of fertilizer application. Changed climate and environmental stresses has a negative impact on growth and development in plants. Biotic and abiotic stresses are known to limit the production of chickpea globally. Drought stress exerts a major impact in terms of growth inhibition and yield losses accounting for about 50% globally (Koskosidis *et al.,* 2020; Gaur *et al.,* 2012).

Legume seeds when exposed to osmotic stress shows reduced water uptake, which could lead to reduced germination and seedling growth. The osmotic stress might damage proteins and cell membranes, which can affect the metabolic processes required for germination (Rangani and Parikh, 2017). Osmotic stress frequently causes the upregulation of genes that produce abscisic acid (ABA), a plant hormone that controls seed dormancy and stress responses. Osmotic stress can affect the enzymatic activity of enzymes involved in carbohydrate metabolism and

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other nutrients required for germination. For example, alpha-amylase, an enzyme required for the breakdown of starch into glucose, is often inhibited by osmotic stress. This can lead to reduced availability of glucose for energy production and growth. Soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*) are reported to exhibit more tolerance to osmotic stress while chickpea (*Cicer arietinum*) and lentil (*Lens culinaris)* are more susceptible (Kaur *et al*., 2021 and Farooq *et al*., 2012). PEG is often used as an osmoticum that induces osmotic potential in plants by reducing the growing medium's water potential. When PEG is added to the growth medium it creates a solute gradient that causes exosmosis from the plant tissues and into the surrounding medium, resulting in dehydration stress. The current study aims to introduce different concentrations of PEG 6000 treatment on *Cicer arietinum* as osmotic agent and study its impact on germination and seedling growth.

MATERIALS AND METHODS

The experiment was conducted during 2022-23 at the Department of Life Sciences, Kristu Jayanti College, Autonomous, Bengaluru. *Cicer arietinum* seeds were acquired from the local market. Osmotic stress was induced using three different concentrations of PEG-6000 (5%, 10% and 15%). Chickpea seeds were disinfected with 0.1% $HgCl_2$ for 3 minutes and repeatedly washed with distilled water. The seeds were then placed in petri dishes containing moistened cotton soaked in 5 ml of appropriate PEG solution (5%, 10% and 15%) and distilled water for test and control. Petri dishes were wrapped and sealed with parafilm to prevent evaporation of moisture (Emmerich and Hardegree, 1991). The petri dishes were incubated in a dark room for 7 days with constant moistening and monitoring.

Each treatment was analyzed with three replicates, consisting of fifteen seeds in each treatment. The growth parameters like germination percentage, length of plumule and radicle, fresh and dry weight of the germinated seeds was evaluated on the seventh day.

Estimation of amylase activity

5 gm of young germinated seeds with their radicle removed were ground to a paste with ice cold buffer of pH 7. The mixture was made up to 50 ml with phosphate buffer and then centrifuged at 5000 rpm for 10 minutes. The supernatant was used as the test sample. To 0.5 ml of test samples taken in separate test tubes, 1 ml buffer solution and 1 ml (1%) starch solution was added and incubated at room temperature for 5 minutes. 1 ml DNS reagent was then added. The test tubes were kept in a bath of boiling water for 20 minutes. The solution turned orange yellow. 2.5 ml of distilled water was then added into all the test tubes and optical density measured at 540 nm (Arnal *et al*., 2023; Keharom *et al.,* 2016). A standard maltose graph was plotted and amylase activity was calculated.

Estimation of protease activity

Protease activity was analyzed by sigma's non-specific protease assay method described by Akhtaruzzaman *et al.* (2012) and Anson (1938) with some modifications. 5 gm of sprouted seeds were ground with small amount of chilled acetone and followed by 10 mM Tris- HCl at pH 8 and chilled for 3 hours. This extract was further centrifuged at 10,000 rpm for 10 minutes. The supernatant obtained was used for the estimation of protease activity. The reaction mixture contained the supernatant along with substrate of 250 µl of enzyme solution and 1 ml of 2% casein solution. After a brief period of incubation at 37°C for 30 minutes, the reaction was terminated by the addition of 500 µl of 10% trichloroacetic acid (TCA). It was then centrifuged at 10,000 rpm for 10 minutes. 300 µl of the supernatant was collected, to which 2.5 ml of alkaline copper solution was added and incubated for 15 minutes at room temperature. 250 µl of diluted Folin's reagent was then added to the mixture. The optical density was calorimetrically observed at 660 nm. Catalytic activity of protease was calculated using tyrosine as the standard.

Estimation of proteins

Protein content was estimated using bovine serum albumin (BSA) as the standard protein. The amount of protein was calculated from the standard curve as mg of protein per ml of test sample. (Lowry *et al*., (1951).

Data were analyzed using one-way analysis of variance (ANOVA) and differences between means were compared by the Least Significant Difference (LSD) test. Results were expressed as mean of three replicates ± standard deviation. Differences were considered significant according to Student t test at $p<0.05$.

RESULTS AND DISCUSSION

The germination percentage progressively diminished with increasing concentrations of osmotic stress induced by PEG when compared to control. 93.3% seed germination was observed in the control set (Table 1) while 77.30% of germination was observed in 15% PEG treated seeds, thus indicating that PEG induced osmotic stress decreases the rate of seed germination and growth of seedling as well (Surbhaiyya *et al*., 2018; Hossain *et al*., 2024). Osmotic stress is known to reduce the water potential gradient between seeds and their environment, thus causing seed germination to decrease. Metabolic disorders during stress conditions may also decrease the percentage of seed germination (Ali *et al*., 2017). Similar kind of results were observed in the wheat varieties when exposed to PEG treatment (Ahmed *et al.,* 2019; Mansour and Indoush, 2020; Mahpara *et al*., 2022).

Maximum length of plumule (5.63±0.42 cm) and radicle (10.7±0.45 cm) was recorded in the seeds treated with 5% PEG. As the concentration of PEG increased to 10% and 15%, the length of shoot and root were seen to decrease. A study by Elahi *et al*. (2023) showed similar results where the seeds subjected to 5% PEG, produced noticeably longer

shoot and root lengths than the other treatments tested (10% and 15%). The deficiency in the growth of the plumule and radicle was earlier recorded in other plants under conditions of osmotic stress (Ahmed *et al*., 2019; Abro *et al*., 2020; Majid *et al*., 2022). The lack of transfer of nutrients from the storage tissues of the seeds into the embryo may result in decreased growth. Shoot and root meristems are probably affected, disrupting the cell division and elongation process (Mohammadi and Mojaddam, 2014; Chachar *et al*., 2016). Higher concentration of PEG not only prevents germination properties but also obstructs the growth of stretching of seedlings (Rana *et al*., 2017).

The fresh weight (1.65±0.10 g) and dry weight (1.01± 0.12 g) measured in 5% PEG treated seeds was reported higher than the other treatments including the control and gradually reduced with increasing the concentration of PEG treatment. The fresh and dry weight of the seedlings are negatively affected by higher drought levels (Mansour and Indoush, 2020). 5% PEG treatment might have stimulated plant growth by enhancing nutrient uptake and photosynthesis. A low concentration of PEG treatment might provide a moderate level of stress that stimulates the plant's adaptive responses without causing severe damage. Osmopriming with PEG improved the fresh and dry weights of the plumule in few plants (Elahi *et al*., 2023; Mirmazloum *et al*., 2020). A significant increase in pigment content and stomatal density was observed in explant cells of *Cotinus coggygria* when cultivated on PEG 4.0-6.0% (Zholobova *et al*., 2024). In contrast, higher concentrations of PEG may be too stressful for the plants to cope with, resulting in reduced growth and yield.

10% PEG-treated seeds showed the highest level of amylase activity (3.00±0.42) (Table 2). As the concentration increased to 15% the amylase activity decreased. A similar effect of PEG on amylase activity was recorded earlier in chickpea seeds (Munir *et al*., 2018; Srivastava *et al.,* 2018). This may be due to the fact that high concentrations of PEG can cause severe osmotic stress, leading to damage or disruption of cellular structures and processes, including the synthesis and activity of amylase. An also possible explanation for why the amylase activity decreased at high doses of PEG was the environment's capacity for water was reduced, which could decrease the availability of water for the hydrolysis of starch by amylase (Choudhary *et al*., 2005, Farooq *et al*., 2012).

When subjected to osmotic stress, the protease activity levels were seen to increase with an increase in PEG concentration. Seeds treated with 15% PEG showed the maximum (0.0067±0.0009 U/mg) proteolytic activity while the control set showed the least activity. With increasing concentrations of PEG (10% and 15%), the protease enzyme activity increased in the chickpea seedling. Plant proteases help counteract the process of ROS (reactive oxygen species) as a biological reaction to stress in plants. By destroying broken, denatured and aggregated proteins, plant proteases reduce this process which could be a possible explanation for the increase in the protease activity in the seedlings affected by drought (D'Ippólito *et al*., 2021). Osmotic stress induced by PEG was significant in increasing the protein content of the seedlings. The control seedlings showed the least protein content (0.17±0.03 mg.) while seeds treated with 15% PEG showed the highest protein content (0.29±0.01 mg). Priming the seeds with 5% PEG showed in increase in protein content in Canola crops (Elahi *et al*., 2023. When polyethylene glycol added to a solution, their water potential is reduced, which causes water to move out of the seedling cells. As a result, the concentration of solutes inside the cells rises, including proteins. There are a number of causes for the rise in protein concentration. The reduced water potential causes an influx of ions, such as calcium, which can activate genes implicated in protein synthesis (Chugh and Kaur, 2017). Increased crop production due to PEG treatment was reported in *Lens culinaris* (Eesha *et al*., 2024).

Table 1: Effect of osmotic stress on seed germination and seedling growth.

Treatments	Germination (%)	Length of plumule (cm)	Length of radicle (cm)	Fresh weight of seedling (g)	Dry weight of seedling (g)
Control set	93.30	4.16±0.58^{b}	7.7±0.75	1.56±0.17^{b}	0.91±0.13^{c}
5% PEG	88.60	5.63±0.42^{a}	10.7±0.45	1.65±0.10^{a}	1.01±0.12^{a}
10% PEG	82.00	1.46±0.23^{c}	5.47±1.10	1.39±0.07^{c}	0.97±0.03^{b}
15% PEG	77.30	0.89±0.11^{d}	2.63±0.54	1.18±0.02^{d}	0.8±0.03^{d}

Alphabets significant at 0.05 level, Values represent Mean ± SD.

Table 2: Effect of osmotic stress on enzymes and proteins.

	Amylase (IU)	Protease (U/mg)	Protein (mg/ml)
Control	1.89±0.14^{c}	0.0021±0.001	0.17±0.03^{c}
5% PEG	2.59±0.16^{a}	0.0042±0.0002	0.25±0.002^{b}
10% PEG	3.00±0.42^{a}	0.0047±0.002	0.25±0.004^{b}
15% PEG	2.11±0.22^{b}	0.0067±0.0009	0.29±0.01^{a}

Alphabets significant at 0.05 level, Values represent Mean ± SD.

CONCLUSION

The present study focused on the influence of osmotic stress on seed germination and seedling growth in *Cicer arietinum*. Treatment with PEG can help improve the overall health of plants' by maintaining appropriate hydration, promoting cell division and growth. In the current study, although enhanced PEG concentration delayed the percentage germination of seeds, low concentrations of PEG treatments are known to enhance the morphological growth of seedlings. Osmotic stress caused by PEG and can lead to changes in the activity of enzymes that are crucial for plant metabolism and defense systems, like amylase and protease. Seeds primed with PEG initiate the physiological state of plants thus triggering the plants' defense mechanism. Improvements in the defense system shields the plants from diseases and stresses. Hence, seed priming is a promising technique in stress management. Overall, understanding the physiological effects of osmotic stress brought on by PEG and metabolism can provide valuable insights into drought stress plant adaptability and devising effective stress mitigation strategies. As chickpea is an important pulse crop, investigating its response to osmotic stress can contribute to sustainable agricultural practices.

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Conflict of interest

All authors declare that they have no conflict of interest.

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