



## Priming and drought stress memory: Adaptive bet-hedging strategy of pea (*Pisum sativum*) and chickpea (*Cicer arietinum*) seeds

FAHIMA NABI<sup>1\*</sup>, HAMIDA SADJI-AIT KACI<sup>2</sup>, CHAKER HADDADJ ASSIA<sup>2</sup> and CHEBAANI MERIEM<sup>3</sup>

Yahia Fares Medea University, Ouzera 26100, Medea 26100, Algeria

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### ABSTRACT

In agriculture, interference between environmental constraints and plant life processes is a determining factor of crop productivity. Conservation of adapted phylogenetic resources is a tool to preserve biodiversity and ensure food security. The present experiment was conducted during 2022 and 2023 at Yahia Fares University of Medea, Algeria to study the response strategy of two indigenous legumes, pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.) genotypes to drought stress explored at seeds emergence and seedlings establishment stages. The experiment was laid out in a factorial completely randomized design (F-CRD). The seeds of both species were primed with different H<sub>2</sub>O<sub>2</sub> concentrations (0, 2, 5, 10 and 12 mM) and germinated under water stress (5%, 10%, 15% of PEG) along with unprimed control (0%) using distilled water. Drought stress had significantly reduced germination in pea seeds than chickpea seeds. Primed chickpea showed high germination rates (+85%) at (15% of PEG), speed germination (SG) and seedling vigour (SVI) (+38%) and (+40%), respectively at (10% of PEG) compared to pea seeds. Maximum growth parameters: radical length, hypocotyls length, epicotyls length and whole plant increased about 38%, 30%, 50% and 10% respectively for pea seedlings grown under all treatments. Fresh and dry weights were significantly improved in pea plants than chickpea. Statistical analysis indicated significant differences in the combined impacts of the studied species, the primer concentrations and the drought stress intensity at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ . Our findings are beneficial to understanding the response mechanisms to drought stress for breeding programme of grain legumes.

**Keywords:** Chickpea, H<sub>2</sub>O<sub>2</sub>, Pea, Stress memory, Tolerance, Water stress

Pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.) are two legumes species highly consumed in the world. India is the first producer of chickpea with 70% of global production (Nedumaran *et al.* 2024). Pea and chickpea seeds are rich in nutritional compounds such as protein, fiber, vitamins and minerals due to their ability to induce a symbiotic nitrogen fixation with *Rhizobia* (Reginah *et al.* 2024). In Algeria, the introduced genotypes of pea and chickpea record notable loss due to drought stress in arid and sub-arid areas. The germination stage is the beginning of the development cycle of plants. Nevertheless, the seed germination and seedlings establishment are very susceptible to limited water availability. The inadequate moisture induces irregular germination of seeds and asynchronization of plants establishment. It also alters the metabolic and physiological mechanisms, cell division, cell osmotic pressure and enzymatic activities, thereby

reducing the number of pods, grain filing and crop yield. Priming technique is technique whose principle is based on storage in seeds and plants of information from a previously applied stress as a stress memory once the stress stopped and the memory is retrieved during a subsequent “triggering stress” to improve stress tolerance (Leuendorf *et al.* 2020, Nabi *et al.* 2020). According to Bailly *et al.* (2008), the H<sub>2</sub>O<sub>2</sub> priming of seeds acts as the perception of the “oxidative window for germination”. H<sub>2</sub>O<sub>2</sub> is reactive oxygen species (ROS) regulating the genes expression for enhancing antioxidant response, reducing lipid peroxidation, ensuring membrane integrity and relative water contents in seeds and plants. Very few works focuses on comparative response of two legumes species grown in water stress × priming conditions and to evaluate the regulatory potential between the stages of development of plant. In current study, we assessed a comparative response between germination stage and seedling stage submitted to a progressive water stress intensity; to determine the impact of H<sub>2</sub>O<sub>2</sub> priming on seed dormancy, redox balance, seedling vigour and finally to explore; the impact of H<sub>2</sub>O<sub>2</sub> priming combined with drought stress on memory potential of cells at the germination stage and its impact on seedling

<sup>1</sup>Dr Yahia Fares Medea University, Ouzera, Medea, Algeria; <sup>2</sup>Houari Boumediene University, Algiers, Algeria; <sup>3</sup>Higher Normal School of Kouba, Echeikh Mohamed Elbachir Elibrahimi, Vieux-Koub, Algiers, Algeria. \*Corresponding author email: nabi.fahima@univ-medea.dz

performance and survival of chickpea and pea. Hence the study was conducted to explore a new criterion for selection of species and varieties for cultivating them in arid regions where water stress is severe and soils are degraded.

## MATERIALS AND METHODS

**Seeds material and surface sterilization:** The present experiment was conducted during 2022 and 2023 at Yahia Fares University of Medea, Algeria. Collection of two indigenous legumes seeds, viz. chickpea (*Cicer arietinum* L. var HmsM) and pea (*Pisum sativum* L. var JlbM) were obtained from a new harvest (1 year harvest for each experimental year) of the Cereals and Pulses Cooperative (CCLS). To homogenate the seed emergence, the uniform and healthy seeds of both legumes were selected, sterilized using a 1% sodium hypochlorite for 5 min, and then rinsed four times with sterilized water. Germination capacity test revealed no abnormal germination of seeds.

**H<sub>2</sub>O<sub>2</sub> priming and water stress preparation:** Drought stress was applied using the solutions of distilled water and polyethylene glycol at different concentrations (PEG-6000: 0, 5, 10 and 15%). Use of PEG on seeds and plants generates osmotic stress like drought stress without having any toxic effects in order to study the tolerance of species (Guo *et al.* 2024). For priming design, seeds were soaked in H<sub>2</sub>O<sub>2</sub> solutions at different concentrations (0, 2, 5, 10 and 12 mM) for 4 h at 25°C. The ratio of seed weight to solution was 1:5 (g/ml). After the priming period, seeds were rinsed three times with distilled water and then dried at 25 ± 2° C up to original weight for two days (Mahmoudi *et al.* 2012). Unprimed and non stressed seeds were considered as control.

**Experiment setup and stress application:** An aliquot of 10 ml of the respective PEG-6000 solution was added in 9 cm petri dishes containing two layers of filter papers, and then 20 primed and no-primed chickpea and pea seeds were placed on moistened filter paper. Study was performed in triplicate in factorial experimental based on a completely randomized design (CRD), and kept for germination testing in germinator in the dark at 25 ± 0.5 °C and 80 ± 1% of relative humidity. Petri dishes were tightly sealed to prevent evaporation for minimizing changes in concentration of PEG solutions. Seeds were recorded as germinated when the radicle protruded (1 mm) through the coat of the seed, and seedlings were estimated established when the radicle length achieved seed length (Peng *et al.* 2022). The germinated seeds were counted daily until no new seed germination was recorded in three successive days, thus the count lasted 7 days (Sabah-Taher *et al.* 2022).

**Determination of germination performance:** Germination percentage (%) was calculated by using the following formula (Yemm and Willis 1954):

$$GP = n/N \times 100$$

Where n, Number of germinated seeds; N, Total number of seeds.

**Seed emergence parameters:** Various parameters were

evaluated for each treatment such as final germination rate (FGP) (Chen *et al.* 2021), germination speed (SG) (Shahba *et al.* 2008), and seedlings vigour index (SVI) (Memon *et al.* 2013) according to formulae given below:

$$FGP = N_g/N_t \times 100$$

Whereas N<sub>g</sub>, Number of the germinated seeds at 7<sup>th</sup> day and N<sub>t</sub>, Total seeds.

$$SG = \sum_i [g_i - g_{(i-1)}] / i$$

Where i, Incubation day; g<sub>i</sub>, Total germination percentage on incubation day i; g<sub>(i-1)</sub>, Total germination percentage on previous day.

$$SVI = [\text{Seedling length (cm)} \times FGP]$$

Where FGP, Final germination rate.

**Seedling growth feature:** To evaluate the impact of (water stress × H<sub>2</sub>O<sub>2</sub> priming) combination on seedling growth, 10 seedlings were randomly selected, kept whole or cut into roots, hypocotyls, epicotyls for measuring the lengths (cm). Furthermore, the fresh weight of each part of seedlings was evaluated and dry weight was evaluated after oven drying at 70°C for 48 h.

**Statistical analysis:** Statistical study of pooled data from two years of experiment 2021–22 and 2022–23 were performed by ANOVA and means were analysed by the least significant difference (LSD) at P ≤ 0.05 using IBM SPSS 25.0 statistical software.

## RESULTS AND DISCUSSION

Underscoring of germination parameters such as final germination rate (FGP), seedling vigour index, speed germination (SG) and germination percentage is important to identify the tolerant genotypes adapted to limited water availability and selection of drought resilient crop varieties (Kim and Lee 2023). The development of agriculture in aride and sub-arid areas is an approach based on use of legumes genotypes with robust germination ability under stressful conditions. In this work, the descriptive statistics results pointed out significant variability in germination and seedling related traits to studied pea and chickpea varieties. Among the treatments, without priming, the chickpea seeds have increased their germination capacity regardless of the intensity of stress while the high seed emergence in unprimed pea scored are (100%) and (68%) at 5% and 10% of water stress, respectively. The germination percentage of primed chickpea seeds exhibited a mean score of 93% and 63% at moderate and severe water stress respectively while the germination of primed pea was highly affected and recorded average value of 18% under severe drought stress (Table 1). According to Nayban *et al.* (2017), the priming of seeds had stimulated the hydration and boosted the metabolic processes resulting in amelioration of germination uniformity, seeds germination, and growth of seedlings in both normal and stress conditions (Nayban *et al.* 2017, Rana and Sathiyarayanan 2024). The difference in response between pea and chickpea seed could be

Table 1 Effects of drought stress of PEG-6000 and H<sub>2</sub>O<sub>2</sub> priming interactions on final germination percentage of pea and chickpea seeds during 7 days

Drought stress levels	Priming H <sub>2</sub> O <sub>2</sub> (mM)	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
		Pea	Chickpea	Pea	Chickpea	Pea	Chickpea	Pea	Chickpea	Pea	Chickpea	Pea	Chickpea	Pea	Chickpea
0	Unprimed	0 ± 0	0 ± 0	23.33 ± 25,17a	3.33 ± 5,77a	100 ± 0a	20 ± 10a	23.33 ± 11,55a	100 ± 0a	100 ± 0a	23.33 ± 11,55a	100 ± 0a	23.33 ± 11,55a	100 ± 0a	23.33 ± 11,55a
	2 mM	0 ± 0	0 ± 0	0 ± 0a	50 ± 45,83ab	63.33 ± 25,17a	76.66 ± 11,55b	86.67 ± 15,28b	93.33 ± 5,77a	100 ± 0a	90 ± 10b	100 ± 0a	90 ± 10b	100 ± 0a	90 ± 10b
	5 mM	0 ± 0	0 ± 0	0 ± 0a	70 ± 17,32b	60 ± 10 a	73.33 ± 15,28b	73.33 ± 15,28b	93.33 ± 5,77a	100 ± 0a	80 ± 20b	100 ± 0a	80 ± 20b	100 ± 0a	80 ± 20b
	10 mM	0 ± 0	0 ± 0	0 ± 0a	73.33 ± 15,28b	70 ± 17,32a	76.66 ± 11,55b	76.67 ± 11,55b	96.67 ± 5,77a	96.67 ± 5,77a	80 ± 10b	96.67 ± 5,77a	80 ± 10b	96.67 ± 5,77a	80 ± 10b
	12 mM	0 ± 0	0 ± 0	0 ± 0a	56.67 ± 11,55ab	60 ± 10a	66.66 ± 15,28b	73.33 ± 20,82b	100 ± 0a	100 ± 0a	73.33 ± 20,82b	100 ± 0a	73.33 ± 20,82b	100 ± 0a	80 ± 17,32b
Mean	0 ± 0	0 ± 0	3.88 ± 5,034a	50,67 ± 22,56	72.77 ± 15,62c	62,67 ± 12,73d	66,67 ± 14,96	96,67 ± 5,77c	99,33 ± 5,77c	62,22 ± 14,47b	99,33 ± 5,77b	69,33 ± 14,47b	99,33 ± 5,77b	70,67 ± 13,77b	
0.05	Unprimed	0 ± 0	0 ± 0	0 ± 0	70 ± 10a	30 ± 10a	70 ± 10a	90 ± 17,32a	100 ± 0a	100 ± 0	96.67 ± 5,77a	100 ± 0	96.67 ± 5,77a	100 ± 0	96.67 ± 5,77a
	2 mM	0 ± 0	0 ± 0	0 ± 0	46.66 ± 15,28a	6.66 ± 5,77a	53.33 ± 20,82a	96.67 ± 5,77a	90 ± 10a	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	5 mM	0 ± 0	0 ± 0	0 ± 0	30 ± 20a	20 ± 20a	83.33 ± 5,77a	86.67 ± 23,09a	90 ± 10a	100 ± 0	93.33 ± 11,55a	100 ± 0	93.33 ± 11,55a	100 ± 0	93.33 ± 11,55a
	10 mM	0 ± 0	0 ± 0	0 ± 0	53.33 ± 28,87a	23.33 ± 20,82a	76.66 ± 15,28a	83.33 ± 5,77a	96.67 ± 5,77a	100 ± 0	86.67 ± 5,77a	100 ± 0	90 ± 10a	100 ± 0	90 ± 10a
	12 mM	0 ± 0	0 ± 0	0 ± 0	23.33 ± 20,82a	20 ± 17,32a	76.66 ± 15,28a	83.33 ± 5,77a	90 ± 10a	100 ± 0	86.67 ± 5,77a	100 ± 0	90 ± 10a	100 ± 0	90 ± 10a
Mean	0 ± 0	0 ± 0	44,67 ± 18,99b	18,33 ± 14,78b	72 ± 13,43c	92,67 ± 12,22c	100 ± 0c	90 ± 9,77c	95,33 ± 7,7c	100 ± 0b	96 ± 9,11c	100 ± 0b	96 ± 9,11c	96 ± 9,11c	
0.1	Unprimed	0 ± 0	0 ± 0	0 ± 0	0 ± 0a	0 ± 0a	30 ± 17,32ab	76.67 ± 25,17a	76.67 ± 25,17a	93.33 ± 5,77a	80 ± 20a	96.67 ± 5,77a	80 ± 20a	96.67 ± 5,77a	80 ± 20a
	2 mM	0 ± 0	0 ± 0	0 ± 0	6.67 ± 11,55a	0 ± 0a	20 ± 10a	60 ± 26,46a	60 ± 11,55a	90 ± 10a	90 ± 10a	93.33 ± 11,55a	90 ± 10a	93.33 ± 11,55a	90 ± 10a

Contd.

Table 1 (Concluded)

Drought stress levels	Priming H <sub>2</sub> O <sub>2</sub> (mM)	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
		Pea	Chickpea	Pea	Chickpea	Pea	Chickpea	Pea	Chickpea	Pea	Chickpea	Pea	Chickpea	Pea	Chickpea
0.15	5 mM	0 ± 0	0 ± 0	0 ± 0	10 ± 10a	13.33 ± 23,09a	56.66 ± 11,55bc	53.33 ± 50,33a	90 ± 17,32a	73.33 ± 46,19a	83.33 ± 5,77a	96.67 ± 28,87a	96.67 ± 5,77a	93.33 ± 11,55a	96.67 ± 5,77a
	10 mM	0 ± 0	0 ± 0	0 ± 0	46.66 ± 5,77b	0 ± 0a	60 ± 0c	50 ± 30a	93.33 ± 11,55a	90 ± 10a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a
	12 mM	0 ± 0	0 ± 0	0 ± 0	3.33 ± 5,77a	0 ± 0a	46.66 ± 5,77abc	43.33 ± 30,55a	66.67 ± 11,55a	60 ± 36,06a	93.33 ± 5,77a	83.67 ± 15,28a	96.67 ± 5,77a	86.67 ± 15,28a	96.67 ± 5,77a
	Mean	0 ± 0	0 ± 0	13.33 ± 8,27a	2.22 ± 23,09a	42.67 ± 11,16b	56.67 ± 28,62b	78 ± 15,43b	81.33 ± 21,6b	92 ± 10,39bc	92 ± 15,376	92.67 ± 10,39bc	94 ± 11,04b	93.67 ± 10,39bc	93.67 ± 10,39bc
	Unprimed	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3.33 ± 5,77a	6.67 ± 11,55a	23.33 ± 40,41a	23.33 ± 40,41a	50 ± 20a	26.67 ± 46,19a	56.67 ± 20,82a	26.67 ± 46,19a	60 ± 20a	60 ± 20a
	2 mM	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0a	26.67 ± 11,55a	6.67 ± 5,77a	50 ± 26,46a	13.33 ± 15,28a	56.67 ± 20,82a	13.33 ± 15,28a	60 ± 17,32a	60 ± 17,32a
	5 mM	0 ± 0	0 ± 0	0 ± 0	0 ± 0	13.33 ± 11,55a	0 ± 0a	43.33 ± 5,77a	6.67 ± 11,55a	76.67 ± 23,09a	6.67 ± 11,55a	76.67 ± 23,09a	10 ± 10a	80 ± 17,32a	80 ± 17,32a
	10 mM	0 ± 0	0 ± 0	0 ± 0	0 ± 0	13.33 ± 5,77a	6.67 ± 11,55a	40 ± 26,46a	13.33 ± 23,09a	46.67 ± 35,12a	13.33 ± 23,09a	46.67 ± 35,12a	13.33 ± 23,09a	46.67 ± 35,12a	46.67 ± 35,12a
	12 mM	0 ± 0	0 ± 0	0 ± 0	0 ± 0	10 ± 10a	10 ± 10a	40 ± 17,32a	16.67 ± 15,28a	66.67 ± 32,15a	26.67 ± 25,17a	70 ± 26,46a	30 ± 30a	70 ± 26,46a	70 ± 26,46a
	Mean	0 ± 0	0 ± 0	0 ± 0	0 ± 0a	8 ± 8,27a	4.67 ± 11,03a	43.99 ± 13,37a	13.33 ± 13,33a	58 ± 27,36a	17.33 ± 24,25a	61.33 ± 25,26a	18.67 ± 24,91a	63.33 ± 23,24a	63.33 ± 23,24a

The values in a column with the same alphabetical letter are not significantly different at LSD test ( $P \leq 0.05$ ) among priming for each PEG treatments.

attributed to genotypic diversity of two legume species. Similar observations were reported in faba bean (Chaker-Haddadj *et al.* 2014), cowpea (Nabi *et al.* 2017, 2024) and wheat (Karjule *et al.* 2019). The pooled analysis of results during (2021–2023) showed that a behavioural diversity of two studied legume species which might be attributed to genotypic diversity, seed size, seed nutrient content highly correlated with seed emergence capacity (Nyasulu *et al.* 2024). In addition, the seeds control had markedly accelerated their emergence at 2<sup>nd</sup> day against the 3<sup>rd</sup> and 4<sup>th</sup> day for stressed seeds (Fig. 1). PEG-6000 has altered the germination traits by increasing the osmotic potential, reducing the water absorption, disturbing the metabolic mechanisms and generating an oxidative stress. An oxidative stress generates the reactive oxygen species called ROS (O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub>, OH<sup>\*</sup>) which provoke oxidative damage, alter molecular structures such as proteins, lipids, DNA and the membrane integrity (Lotfi *et al.* 2019). Remarkable superior of germination performance of chickpea was noted. This result reflects the efficiency of use of absorbed water of cells of chickpea seeds and this parameter is positively correlated with accumulation of osmolytes such as proline, proteins LEA, soluble carbohydrates, thereby protecting enzymes functioning (Sadji-Ait Kaci *et al.* 2017, 2018). Even more, H<sub>2</sub>O<sub>2</sub> priming triggers an anti-oxidative response during the germination through activation of gene to facilitate the transition from the seed dormant state to germinated seeds (Kambona *et al.* 2023). Besides, the priming (2% and 5% of H<sub>2</sub>O<sub>2</sub>) significantly improved seedling vigour (SVI) in chickpea (Fig. 1). The high seed vigour is an indicator of faster germination capacity and breaking dormancy under drought stress due to priming application. Similar results were found in rice (Jira-Anunkul and Pattanagul 2021) and lettuce (Silva *et al.* 2023). Biological activity of H<sub>2</sub>O<sub>2</sub> is to reduce the free radicals release by a quenching strategy and/or elimination of ROS produced by a scavenging strategy using enzymatic and non-enzymatic mechanisms. Under water stress, the H<sub>2</sub>O<sub>2</sub> priming improved the fresh and dry weight in pea and chickpea about 10% and 30%, respectively compared to control (Fig. 2). These findings were confirmed by significant differences recorded in the combined impacts of the studied species, the primer concentrations and the different intensity of drought stress at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  on radicals, hypocotyls and epicotyls growth (Table 2). The low and moderate water stress (5%, 10% PEG) increased radical length (+30%) but severe drought stress decreased it (-77%) for both unprimed pea and chickpea seedlings (Table 3). Under moderate and severe water stress, the H<sub>2</sub>O<sub>2</sub> priming significantly reduced the epicotyls growth (-75%) in chickpea seedlings compared to pea seedlings which recorded positive response to limited water availability. According to Hussain *et al.* (2017), the priming regulates the number of mitochondria and the protein pool during cell division to rapidly preserve the stressed cells. Our results indicated two different adaptive strategies based on priority given to the germination stage than growth phase and vice versa. Using the H<sub>2</sub>O<sub>2</sub> priming method, the

Table 2 ANOVA analysis of the effects of drought stress of PEG-6000 and H<sub>2</sub>O<sub>2</sub> priming combinations on speed of germination, final germination percentage, total seedling length and seedling vigour index of pea and chickpea seeds

Source of variation	Df	Speed of germination			Final germination percentage (%)			Total seedling length (cm)			Seedling vigour index		
		Mean square	F value	P level	Mean square	F value	P level	Mean square	F value	P level	Mean square	F value	P level
Drought	3	327455	135798	0,000***	20460	88317	0,000***	247791	10804	0,000***	3,2E+08	93973	0,000***
Genotype	1	150521	62422	0,000***	213333	921	0,340ns	9684	4222	0,000***	481333	141	0,239ns
Priming	4	55657	2308	0,005**	32625	1408	0,239ns	5165	225	0,000***	471495	1381	0,000***
Drought *Genotype	3	210561	8732	0,000***	701556	30283	0,000***	11123	485	0,004**	8410790	24642	0,000***
Drought *Priming	12	61613	2555	0,007**	413472	1785	0,065ns	5356	233	0,013*	601983	1764	0,001**
Genotype *Priming	4	181017	7507	0,000***	694583	2998	0,023*	5539	241	0,056ns	1209366	3543	0,01*
Drought *Genotype *Priming	12	101832	4223	0,000***	310694	1341	0,213ns	4687	204	0,031*	450273	1319	0,224ns
Error	80	327455			231667			2293			341315		
Total	120												

ns, Non significant; \*, Statistically significant at  $P < 0.05$ ; \*\*, Statistically significant at  $P < 0.01$  and \*\*\*, Statistically significant at  $P < 0.001$ .

Table 3 Effects of drought stress of PEG-6000 and H<sub>2</sub>O<sub>2</sub> priming combinations on radical length, epicotyl length, hypocotyl length and total seedling length

PEG%	Radical length (cm)				Epicotyl length (cm)				Hypocotyl length (cm)				Total seedling length (cm)			
	Control	0.05	0.1	0.15	Control	0.05	0.1	0.15	Control	0.05	0.1	0.15	Control	0.05	0.1	0.15
Pea	Unprimed	4,32 ± 0,8a	4,49 ± 0,25a	3,18 ± 0,59ab	0,97 ± 1,67a	0,91 ± 0,1a	0,44 ± 0,22	0,08 ± 0,13a	0,77 ± 0,1a	1,01 ± 0,2a	0,53 ± 0,12a	0,12 ± 0,21a	6,89 ± 1,53a	6,41 ± 0,3a	4,16 ± 0,87ab	1,17 ± 1,82a
	H <sub>2</sub> O <sub>2</sub> @2 mM	4,99 ± 0,73a	3,78 ± 0,27a	5,21 ± 1,29b	0,24 ± 0,21a	0,73 ± 0,15a	0,42 ± 0,1a	0 ± 0a	0,57 ± 0,09a	0,86 ± 0,25a	0,64 ± 0,05a	0 ± 0a	6,93 ± 0,81a	5,37 ± 0,53a	6,28 ± 1,37b	0,24 ± 0,21a
	H <sub>2</sub> O <sub>2</sub> @5 mM	4,64 ± 0,77a	4,4 ± 1,07a	2,26 ± 0,57a	0,28 ± 0,25a	2,16 ± 2,45a	0,12 ± 0,07a	0 ± 0a	0,74 ± 0,34a	0,76 ± 0,16a	0,26 ± 0,17a	0 ± 0a	6,81 ± 1,02a	7,31 ± 2,63a	2,63 ± 0,8a	0,28 ± 0,25a
	H <sub>2</sub> O <sub>2</sub> @10 mM	5,7 ± 0,55a	5,08 ± 0,22a	3,51 ± 1,44ab	0,28 ± 0,48a	0,93 ± 0,15a	0,56 ± 0,24a	0 ± 0a	0,53 ± 0,07a	0,82 ± 0,53a	0,84 ± 0,53a	0 ± 0a	7,8 ± 0,91a	6,83 ± 0,34a	4,91 ± 1,89ab	0,28 ± 0,48a
	H <sub>2</sub> O <sub>2</sub> @12 mM	5,56 ± 0,97a	4,93 ± 0,15a	3,01 ± 0,45ab	0,77 ± 0,8a	1,3 ± 0,13a	0,23 ± 0,17a	0,02 ± 0,04a	0,79 ± 0,24a	1,13 ± 0,06a	0,54 ± 0,31a	0,61 ± 0,65a	7,91 ± 1,07a	7,37 ± 0,03a	3,79 ± 0,77ab	1,4 ± 1,49a
Mean	5,04 ± 0,76c	4,54 ± 0,39c	3,43 ± 0,87b	0,51 ± 0,68a	1,21 ± 0,60b	0,35 ± 0,16a	0,02 ± 0,03a	0,68 ± 0,17bc	0,92 ± 0,15c	0,56 ± 0,24b	0,15 ± 0,17a	7,27 ± 1,07c	6,66 ± 0,77c	4,35 ± 1,14b	0,67 ± 0,85a	
Chickpea	Unprimed	1,56 ± 1,1a	7,26 ± 1,18a	4,3 ± 0,38a	1,68 ± 0,56a	1,89 ± 0,27a	0,62 ± 0,16a	0,26 ± 0,11a	0,37 ± 0,27a	0,92 ± 0,08a	0,72 ± 0,2a	0,34 ± 0,15ab	2,67 ± 1,89a	10,07 ± 1,36a	5,64 ± 0,39a	2,28 ± 0,77a
	H <sub>2</sub> O <sub>2</sub> @2 mM	3,92 ± 2,04a	6,56 ± 2,08a	5,43 ± 1,78a	1,63 ± 0,91a	1,6 ± 0,5a	0,67 ± 0,19a	0,11 ± 0,19a	0,86 ± 0,15ab	0,94 ± 0,1a	0,84 ± 0,08a	0,23 ± 0,29a	7,14 ± 2,7ab	9,1 ± 2,68a	6,94 ± 1,83a	1,98 ± 1,38a
	H <sub>2</sub> O <sub>2</sub> @5 mM	6,82 ± 1,85a	6,04 ± 1,41a	6,19 ± 1,41a	1,38 ± 0,41a	1,47 ± 0,22a	1,31 ± 0,22b	0,38 ± 0,34a	1,29 ± 0,41b	0,86 ± 0,25a	0,84 ± 0,13a	0,84 ± 0,36ab	10,46 ± 1,92b	8,37 ± 1,66a	8,34 ± 1,55a	2,6 ± 0,62a
	H <sub>2</sub> O <sub>2</sub> @10 mM	5,01 ± 1,75a	6,21 ± 0,67a	5,49 ± 0,89a	1,46 ± 0,92a	1,82 ± 0,45a	1,3 ± 0,15b	0,07 ± 0,07a	1,08 ± 0,14b	0,89 ± 0,13a	0,78 ± 0,13a	0,27 ± 0,19a	8,27 ± 1,71ab	8,92 ± 1,14a	7,57 ± 0,7a	1,79 ± 1,15a
	H <sub>2</sub> O <sub>2</sub> @12 mM	4,84 ± 3,11a	6,92 ± 2,24a	4,34 ± 0,45a	2,36 ± 1,01a	2,17 ± 0,73a	1,34 ± 0,25b	0,16 ± 0,17a	0,92 ± 0,1ab	1,01 ± 0,28a	0,86 ± 0,15a	1,24 ± 0,59b	8,17 ± 3,04ab	10,1 ± 2,87a	6,54 ± 0,75a	3,76 ± 1,32a
Mean	4,43 ± 1,97b	6,60 ± 1,51c	5,15 ± 0,98bc	1,70 ± 0,76a	1,79 ± 0,43c	1,05 ± 0,19b	0,20 ± 0,18a	0,90 ± 0,21b	0,92 ± 0,17b	0,81 ± 0,14ab	0,58 ± 0,32a	7,34 ± 2,37c	9,31 ± 1,94b	7,01 ± 1,04	2,48 ± 1,05a	

The values in a column with the same alphabetical letter are not significantly different at LSD test ( $P \leq 0.05$ ) among priming for each PEG treatments.

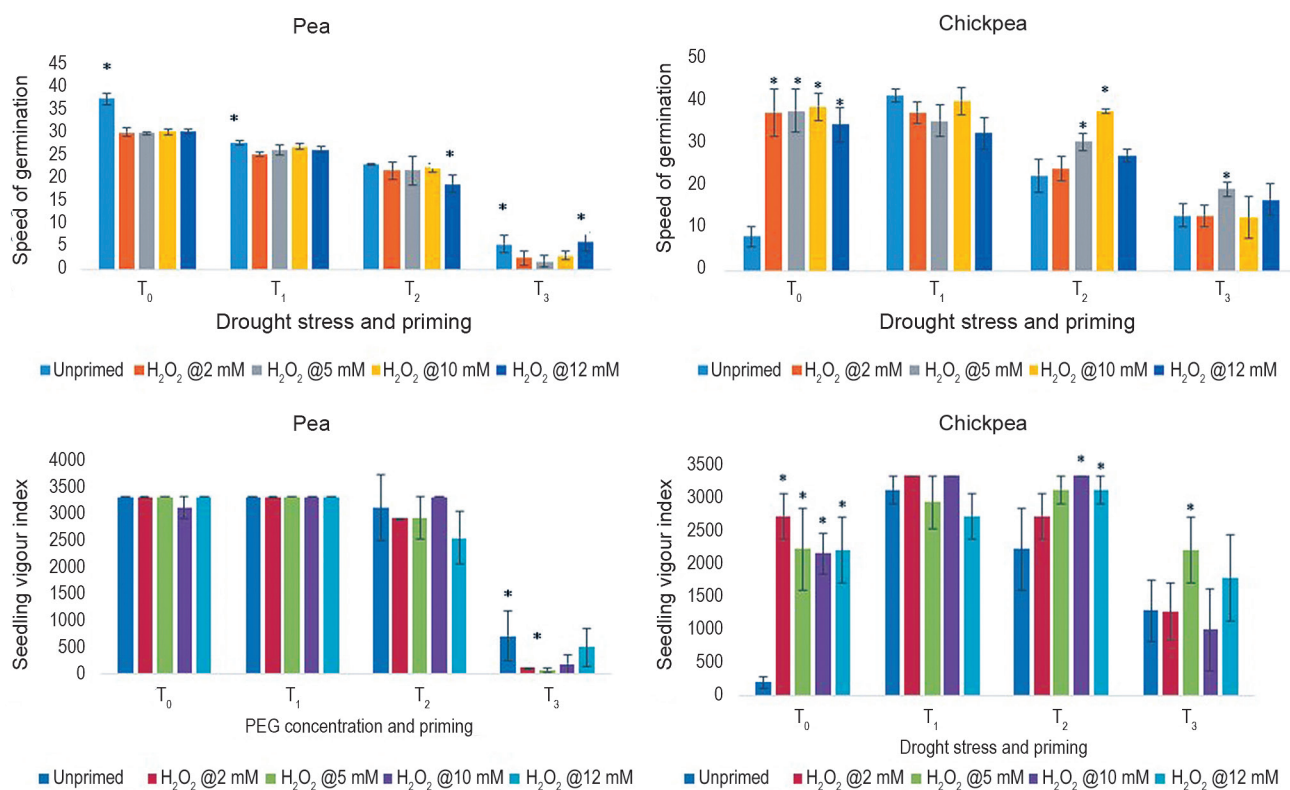


Fig. 1 Interactive effects of drought stress of PEG-6000 and  $H_2O_2$  priming on germination speed and seedling vigour index of pea and chickpea seeds.

\*, Significant differences at LSD test  $P \leq 0.05$ . Values represent the mean of three replicates and error bars represent standard error.  $T_0$ , PEG-6000 @0;  $T_1$ , PEG-6000 @5%;  $T_2$ , PEG-6000 @10% and  $T_3$ , PEG-6000 @15%.

germination stage of pea seeds was more sensitive to drought stress than that of chickpea seeds confirmed by reduction of germination parameters. Conversely, in the growth stage, pea seedlings revealed more remarkable positive responses with optimal growth of radicals, hypocotyls and epicotyls than chickpea seedlings where aerial part was completely inhibited. It could be a response defense against the stressful conditions. Similar findings have showed that drought stress had severe impact on shoots compared to roots (Kundururu *et al.* 2024). This difference could be explained by severity of stress that impairs nutrient uptake in chickpea plants at the growth stage, as demonstrated by Ali *et al.* (2018) and Sadjji-Aitkaci *et al.* (2022). They showed that mineral absorption such as potassium, calcium, iron, magnesium contents was significantly reduced under stress. It could also be explained by exhaustion of the cell energy by metabolic processes during germination stage followed by loss of seedlings and their ability to ensure an optimal growth under applied treatments. According to Gumus *et al.* (2023), the differences observed in both pea and chickpea species could be attributed to seed memory involving the ability of plants to recall earlier stress after priming with stressor factors linked to epigenetic mechanisms linked to DNA methylation, accumulation of signaling molecules and modifications of histones by deacetylases. Additionally, the priming provokes an abiotic stress on seeds that slows down the germination but accelerates responses to stress

by increasing the accumulation of proteins called late embryogenesis abundant (LEA) and their mRNAs in plant tissue, seed maturation for protection of the cell membranes and seed adaptation to stress (Kambona *et al.* 2023). In buckwheat, Yalamalle *et al.* (2024) demonstrated that seeds treatment with  $H_2O_2$  improved germination by enhancing the  $\alpha$ -amylase content. In rapeseed, the seed priming combined with drought stress have ameliorated the CAT, SOD, GSH, POD and ascorbic acid activity (Hussain *et al.* 2019). Application of  $H_2O_2$  priming to stressed maize seeds have regulated the enzyme ( $\Delta 1$ -pyrroline-5-carboxylate synthetase: P5CS) of proline synthesis implied in cell osmoregulation, anti-oxidant response and protection of proteins against denaturation induced by the stress (Xiong *et al.* 2012). Our findings revealed major genetic variability during seed emergence and seedling stages under water-stressed conditions, highlighting important results for improving water stress tolerance in legume species and cultivars for targeted breeding programmes.

The study showed a diversity of responses between two stressed grain legumes pea and chickpea primed or unprimed with hydrogen peroxide. Although primed chickpea showed a higher germination performance under drought stress, the primed pea seeds were more tolerant to water stress at growth stage as indicated by improvement of growth parameters proportionally with the severity of applied drought stress.

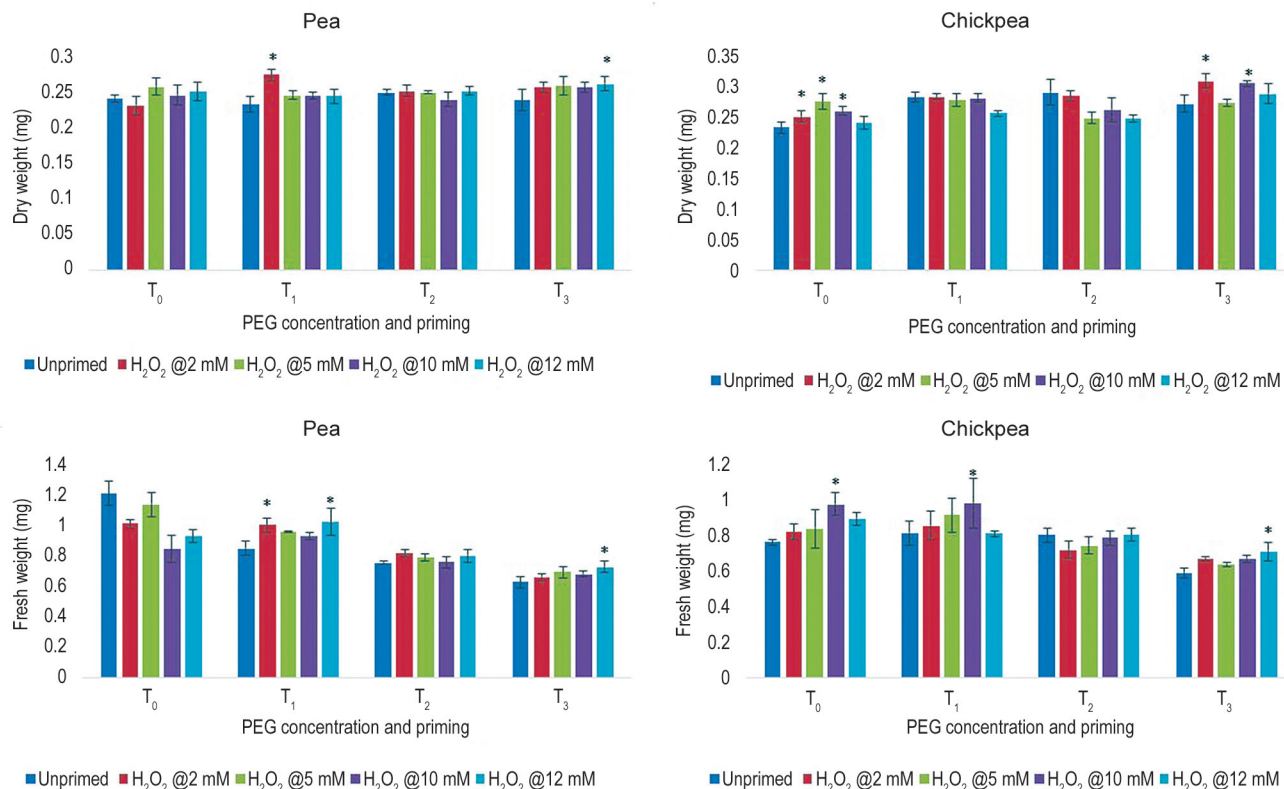


Fig. 2 Interactive effects of drought stress of PEG-6000 and  $H_2O_2$  priming on fresh weight and dry weight of pea and chickpea seeds. \*, Significant differences at LSD test  $P < 0.05$ . Values represent the mean of three replicates and error bars represent standard error.  $T_0$ , PEG-6000 @0;  $T_1$ , PEG-6000 @5%;  $T_2$ , PEG-6000 @10% and  $T_3$ , PEG-6000 @15%.

## REFERENCES

- Ali Q, Javed M T, Noman A, Haider M Z, Waseem M, Iqbal N and Perveen R. 2018. Assessment of drought tolerance in mung bean cultivars/lines as depicted by the activities of germination enzymes, seedling's antioxidative potential and nutrient acquisition. *Archives of Agronomy and Soil Science* **64**: 84–102.
- Bailly C, El-Maarouf-Bouteau H and Corbineau F. 2008. From intracellular signaling networks to cell death: The dual role of reactive oxygen species in seed physiology. *Comptes Rendus Biologies* **331**(10): 806–14.
- Chaker-Haddadj A, Nabi F, Ait Kaci-Sadji H and Ounane SM. 2014. Effects of salt stress on growth, nodulation and nitrogen fixation of faba bean (*Vicia faba* L. Minor) cultivated in Algeria. *International Journal of Biotechnology and Research* **4**: 1–10.
- Chen X, Zhang R, Xing Y, Jiang B, Li B, Xu X and Zhou Y. 2021. The efficacy of different seed priming agents for promoting sorghum germination under salt stress. *PLoS One* **16**: 1–14.
- Dietz K J, Zorb C and Geilfus C M. 2021. Drought and crop yield. *Plant Biology* **23**(6): 881–93.
- Gumus T, Meric S, Ayan A and Atak C T. 2023. Plant abiotic stress factors: Current challenges of last decades and future threats. *Plant Abiotic Stress Responses and Tolerance Mechanisms*, pp.1–27. IntechOpen, London.
- Guo M, Zong J, Zhang J, Wei L, Wei W, Fan R, Zhang T, Tang Z and Zhang G. 2024. Effects of temperature and drought stress on the seed germination of a peatland lily (*Lilium concolor* var. *megalanthum*). *Frontiers in Plant Science* **15**: 1462655.
- Hussain A, Rizwan M, Qasim A and Shafaqat A. 2019. Seed priming with silicon nanoparticles improved the biomass and yield while reduced the oxidative stress and cadmium concentration in wheat grains. *Environmental Science and Pollution Research* **26**: 7579–88.
- Hussain M, Farooq M and Lee D J. 2017. Evaluating the role of seed priming in improving drought tolerance of pigmented and non-pigmented rice. *Journal of Agronomy and Crop Science* **203**: 269–76.
- Jira-anunkul W and Pattanagul W. 2021. Effects of hydrogen peroxide application on agronomic traits of rice (*Oryza sativa* L.) under drought stress. *CAAS Agricultural Journals* **67**(4): 221–29.
- Kambona C M, Koua P A, Leon J and Ballvora A. 2023. Stress memory and its regulation in plants experiencing recurrent drought conditions. *Theoretical and Applied Genetics* **136**(26): 2–21.
- Karjule A, Kalyanrao P, Sasidharan N and Patel D A. 2019. Effect of different seed priming treatments on yield attributes of wheat (*Triticum aestivum* L.). *The Indian Journal of Agricultural Sciences* **89**(10): 1649–53.
- Kim K and Lee B. 2023. Effects of climate change and drought tolerance on maize growth. *Plants* **12**(20): 1–21.
- Kunduru S, Chaudhary A, Kamra A, Bana R S, Kumar S N and Yalamalle V R. 2024. Seed priming with *Aloe vera* and *Trichoderma asperellum* improves germination in chickpea under osmotic and temperature stress. *Seed Science and Technology* **52**(3): 265–81.
- Leuendorf J E, Frank M and Schumling T. 2020. Acclimation, priming and memory in the response of *Arabidopsis thaliana*

- seedlings to cold stress. *Scientific Reports* **10**: 689.
- Lotfi N, Soleimani A, Vahdati K and Cakmakci R. 2019. Comprehensive biochemical insights into the seed germination of walnut under drought stress. *Scientia Horticulturae* **250**: 329–43.
- Mahmoudi H, Ben Massoud R, Baatour O, Tarchoune I, Ben Saleh I, Nasri N, Abidi W, Kaddour R, Hannoufa A, Lachaal M and Ouerghi Z. 2012. Influence of different seed priming methods for improving salt stress tolerance in lettuce plants. *Journal of Plant Nutrition* **35**: 1910–22.
- Memon N, Gandahi M B, Pahoja V M and Sharif N. 2013. Response of seeds priming with boron on germination and seedling sprouts of broccoli. *International Journal of Agricultural Science and Research* **3**(2): 183–94.
- Midhul Rana A and Sathiyarayanan G. 2024. Standardization of various chemical seed priming on seed quality parameters in blackgram (*Vigna mungo*). *Environment and Ecology* **42**(4): 1570–76.
- Nabi F, Chaker-Haddadj A, Tellah S, Ghalem A, Ounane G, Ghalmi N, Djebbar R and Ounane S M. 2017. Evaluation of Algerian cowpea genotypes for salt tolerance at germination stage. *Advances in Environmental Biology* **11**(5): 79–88.
- Nabi F, Chaker-Haddadj A, Chebaani M, Ghalem A, Mebdoua S and Ounane S M. 2020. Influence of seed priming on early stages growth of cowpea [*Vigna unguiculata* (L.) Walp.] grown under salt stress conditions. *Legume Research* **43**(5): 665–71.
- Nabi F, Sadji-Ait Kaci H, Chebaani M, Chaker Haddadj A and Ounane S M. 2024. Growth, leaf water potential, photosynthetic pigments, compatible solutes and yield components in cowpea landraces under salinity. *Natural Resources and Sustainable Development* **14**(2): 227–44.
- Nayban G, Mandal A K and De B K. 2017. Seed priming: A low-cost climate-resilient tool for improving germination, growth and productivity of mungbean. *SATSA Mukhaptra Annual Technical Issue* **21**: 162–72.
- Nedumaran S, Sharma D K, Bhatia A, Shrivastava M, Shivay Y S, Mohan D, Dinesh G K, Murugesan K and Mahadeva M S. 2024. Interactive effect of ambient and elevated levels of tropospheric ozone, nutrition, and PGPR on growth and yield of chickpea (*Cicer arietinum*). *The Indian Journal of Agricultural Sciences* **94**(5): 507–11.
- Nyasulu M, Zhong Q, Li X, Liu X, Wang Z, Chen L, He H and Bian J. 2024. Uncovering novel genes for drought stress in rice at germination stage using genome wide association study. *Frontiers in Plant Science* **15**: 1421267.
- Peng L, Sun S, Yang B, Zhao J, Li W, Huang Z, Li Z, He Y and Wang Z. 2022. Genome-wide association study reveals that the cupin domain protein OsCDP3.10 regulates seed vigour in rice. *Plant Biotechnology Journal* **20**: 485–98.
- Reginah P, Singh K N, Rajesh T, Mayurakshee M, Samuthirapandi S, Lyngdoh A A and Chamroy T. 2024. Genetic variability and association study of SSR markers for yield and powdery mildew disease in pea (*Pisum sativum*). *The Indian Journal of Agricultural Sciences* **94**(3): 258–62.
- Sabah-TaHER M, Anead-Alamrani H, Auuad- Hassn I, Khalaf-Anead I and Alaa- Kadem B. 2022. The influence of gamma rays and electric shock on seed germination and seedling growth in burdock plants. *Revis Bionatura* **7**(1): 30.
- Sadji-AitKaci H, Chaker-Haddadj A and Aid F. 2017. Interactive effects of salinity and two phosphorus fertilizers on growth and grain yield of *Cicer arietinum* (L.). *Acta Agriculturae Scandinavica, Section B Soil and Plant Science* **67**(3): 208–16.
- Sadji-Ait Kaci H, Chaker-Haddadj A and Aid F. 2018. Enhancing of symbiotic efficiency and salinity tolerance of chickpea by phosphorus supply. *Acta Agriculturae Scandinavica Section B Soil and Plant Science* **68**(6): 534–40.
- Sadji-Ait Kaci H, Chaker-Haddadj A, Nedir-Kichou A and Aid F. 2022. Act of phosphorus on cell hydraulic state, K<sup>+</sup> use efficiency and induction of positive correlations between yield and vegetative traits in chickpea. *Acta Agriculturae Scandinavica Section B Soil and Plant Science* **72**(1): 3325–32.
- Shahba M A, Qian Y L and Lair K D. 2008. Improving seed germination of salt grass under saline conditions. *Crop Science* **48**(2): 756–62.
- Silva S, Dias M C and Silva A M S. 2023. Potential of MgO and MgCO<sub>3</sub> nanoparticles in modulating lettuce physiology to drought. *Acta Physiologiae Plantarum* **45**(31): 1–9.
- Xiong J, Zhang L, Fu G, Yang Y, Zhu C and Tao L. 2012. Drought-induced proline accumulation is uninvolved with increased nitric oxide, which alleviates drought stress by decreasing transpiration in rice. *Journal of Plant Research* **125**: 155–64.
- Yalamalle V R, Dunna V, Chawla G, Mishra G P, Vijayakumar H P, Ahmad D, Lal S K, Joshi D C and Meena R P. 2024. Overcoming physiological dormancy in common buck wheat (*Fagopyrum esculentum* Moench). *Genetic Resources and Crop Evolution* **71**(5): 1659–72.
- Yemm E W and Willis A. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal* **57**(3): 508–14.