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ASSESSING TOLERANCE IN CHICKPEA (*CICER ARIETINUM*) VIA SUCROSE-INDUCED DROUGHT STRESS

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Abstract: Seed germination is a critical stage for crop establishment and is highly sensitive to osmotic stress, which limits water uptake and can disturb the plant's internal balance. This study evaluates the way 22 different chickpea (*Cicer arietinum* L.) genotypes, a crop with increasing importance for the food industry and agriculture in Bulgaria, respond to sucrose-induced osmotic stress. Seeds were treated with sucrose concentrations of 50, 150, and 250 mM, that correspond to osmotic potentials of -0.12, -0.37, and -0.61 MPa. The results indicate that sucrose-induced stress restricts seedling development with the increment of concentration; however, the degree varied significantly by genotype. Kabuli-type accessions (Variants 11 and 12) proved to be highly susceptible to the stressor and showed a complete lack of germination at -0.61 MPa. On the other hand, Desi-type genotypes (Variants 14 and 15) demonstrated very high resilience, sustaining germination rates above 90%. The resilience in Desi-types hypotheses of its superior capacity for maintaining redox homeostasis through mitigating the oxidative burst triggered by osmotic imbalance. This indicates that vigor inhibition is linked to disrupted metabolic signaling and antioxidant defenses rather than mere dehydration. Our findings validate sucrose-based assays as rapid, cost-effective tools for identifying promising genotypes for breeding programs in water-scarce regions.

Keywords: germination, germ length, germ weight, inhibition, seed vigor index.

INTRODUCTION

Seed germination is a critical stage in the plant life cycle that determines crop establishment and productivity (Lamichhane et al., 2018). It is highly sensitive to environmental factors that alter water availability,such as drought, high salinity, since they exert osmotic stress on plant cells. Osmotic stress is characterized by a decrease in the osmotic pressure inside the cell (to match the decreased external pressure), a decrease in the cell turgor and volume, sometimes to the point of the so-called „incipient plasmolysis"(Oparka, 1994; Yu et al., 2024).

After the incipient plasmolysis the cell volume very rapidly decreases. This usually becomes the „point of no return" for the cell, even without disruption of the cell membrane, since additional harmful processes can occur. First, the rapid decrease of the cell volume leads to an increased concentration of the same number of molecules inside cells. Due to the decreased water content of the cell the proteins, and most importantly, the enzymes lose their hydration shells, which subsequently leads to their denaturation (Xiong and Zhu, 2002). Both the increased concentration of metal ions and the denatured proteins cause rapid accumulation of ROS and oxidative stress. During early germination, the resumption of metabolic activity naturally additionally leads to an increase in mitochondrial ROS production, and maintaining this redox balance is essential for avoiding oxidative damage and ensuring normal seedling process (Bailly et al., 2008). At the same time, ROS also act as important signaling molecules involved in termination of dormancy and the mobilization of stored reserves (Bailly, 2019). Moreover, osmotic stress can further disturb cellular homeostasis besides its effect on water uptake, leading to increased ROS accumulation during germination (Bailly et al., 2008; Khadraji et al., 2017). The management of ROS has an mportant role as a fundamental factor in a plant's capacity to survive and withstand drought (Sarangi et al., 2023). Seed vigor often declines under osmotic stress as the equilibrium is disrupted (Khajeh-Hosseini et al., 2003).

In maize, for instance, high ROS levels triggered by salt and osmotic pressure have been connected to metabolic interference and poor germination (Pencheva et al., 2022). These findings suggest that osmotic shifts frequently manifest as oxidative stress, which in turn hampers the germination process.

The specific type of osmotic agent used also plays a role in how a seed responds. Stress induced by PEG or NaCl usually decreases germination rates and inhibits seedling development in various crops (Irving and Zhang, 2021; Vu et al., 2018). On one hand, NaCl restricts water uptake and also introduces ion-specific toxicity, so the buildup of Na^+ and Cl^- can be toxic to the seed quite apart from the osmotic pressure itself (Pencheva et al., 2022). In contrast, PEG is a non-ionic molecule that does not penetrate the seed. Interestingly enough, it often suppresses germination and early growth more severely than its osmotic potential would suggest. This heightened inhibition is likely caused by high viscosity of PEG solutions, that slows down water movement and limits oxygen diffusion, alongside potential trace impurities (El-Katony et al., 2024).

Stomatal closure is regulated by complex multicomponent signaling pathways in which phytohormone signaling, particularly abscisic acid in "cross-talk" with other signaling networks, plays a crucial role. Drought stress fundamentally interrupts plant ionic, redox, and metabolite homeostasis (Kaur et al., 2016). In the present study, we hypothesize that

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exogenous sucrose application creates a low osmotic potential (reaching -0.61 MPa), acting as a primary stress signal (Ishibashi et al., 2017) that triggers germination.

The cells of plant seeds in nature could be usually found in a very dehydrated state (5-10% of water). To achieve this, seeds accumulate large amounts of abscisic acid (ABA), the dormancy phytohormone, during maturation (Chen et al. 2019). ABA slowly decreases their physiological activity and protects them from premature germination (Kremode, 2005). Subsequently, during germination, the specific conditions lead to fast decrease of the ABA content, which in turn activates metabolism (Millar et al., 2006). Therefore, any stress occurring during germination could lead to irreversible damage. Moreover, metabolism reactivation alters ABA regulation, disrupting its effects on stress mitigation (Gianinetti and Vernieri, 2007). As a conclusion, seeds must rely on already acquired mechanisms for stress mitigation, one of which are the antioxidants, and more specifically, the polyphenols.

Given the well-established links between sugar signaling and mitochondrial activity, these sucrose-induced shifts likely provoke an oxidative imbalance that leads to turgor loss in guard cells and restricted gas exchange. Even though the measurement of direct biochemical markers was outside of the scope of ths study, the observed reductions in seed germination, germ length, and seed vigor index (SVI) provide an oxidative context for interpreting seedling performance. This suggests that the inhibition of biomass accumulation in sensitive genotypes is not just a physical dehydration effect but part of a broader framework in which osmotic stress triggers physiological drought and metabolic inhibition during early germination.

MATERIALS AND METHODS

For this experiment, seeds from 22 chickpea (Cicer arietinum L.) genotypes, harvested in 2024, were used. Germination tests and early seedling growth assessment were performed using 20 seeds per genotype, arranged in three replicates. Seeds were placed in 9 cm Petri dishes lined with filter paper in a growth chamber at 22±1 °C for seven days. Each sample received 5 mL of distilled water (control) or 5 mL sucrose solution at one of the following concentrations: 50 mM, 150 mM, or 250 mM. To minimize the impact of micro-environmental fluctuations, Petri dishes were arranged in a completely randomized design, and their positions were rotated daily during the experiment. This way, each genotype and treatment replication experienced uniform light and temperature exposure throughout the experiment.

The osmotic potential of the solutions was calculated based on the Van't Hoff equation (Ford, 2015):

$\Psi s = -CiRT$ where: C is the molar concentration of the solutes (molarity = moles/L), i is the osmotic coefficient (the value of i is 1 for molecules that do not dissociate in solution (sucrose) and can be 2 or more for molecules that completely dissociate (NaCl), R is the gas constant (0.008314 L·MPa / (mol·K)), and T is the absolute temperature (K). The calculated values for 50-, 150-, and 250-mM sucrose were -0.12, -0.37, and -0.61 MPa, respectively.

Every two days, an additional 5 mL of the corresponding solution was added to maintain moisture and osmotic conditions. After 7 days, the length (cm) and the weight (g) of the germs were measured.

The methodology followed the general principles described by El-Abady et al. (2015).

Germination percentage was calculated using the formula: Germination (%) = Number of germinated seeds /Total number of seeds × 100.

Seed vigor was quantified according to Abdul-Baki and Anderson (1973) using the formula: SVI = Germ length (cm) × Germination (%).

This index integrates germination capacity with seedling growth and provides a composite measure of early vigor under osmotic stress.

Experimental data for seed germination (%), seedling length (cm), and fresh weight (g) were expressed as the mean of three independent replicates ± standard error (SE). The SE was calculated from the number of successfully germinated seeds per replicate.

To assess the influence of different saccharose concentrations (50, 150, and 250 mM) relative to control (0 mM), a Student's t-test for unequal variances was performed for pairwise comparisons. Statistical significance was defined at * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. This specific approach was prioritized to account for the high genotypic variability among the 22 tested variants. By testing the significance of sucrose-induced inhibition relative to the specific baseline of each independent genotype, the analysis minimized potential bias from inherent physiological differences among chickpea genotypes. The data processing and statistical analyses were conducted using Microsoft Excel.

RESULTS AND DISCUSSION

Sucrose-induced osmotic stress (ranging from -0.12 to -0.61 MPa) produced clear differences among the 22 chickpea genotypes tested. Germination percentage varied widely, reflecting strong genotypic influence on stress response. As expected, different cultivars exhibited variations in their physiology and biochemistry in response to stress (Kaur et al., 2020).

The two Kabuli-type accessions (variants 12 and 1, Table 1) showed the lowest germination even under control conditions (8.33% and 36.67%) and failed to germinate at 250 mM sucrose. Similar strong sensitivity of Kabuli chickpeas to osmotic restriction has been reported in recent studies on filter paper (Mbarek & Boubaker, 2013). In contrast, Desi type variants 14 and 15 (Table 1) maintained exceptionally high germination, reaching 100% in the control and remaining above 85% under 150 mM sucrose concentration. Variant 9 (pea-type, brown seeds, Table 1) also showed consistently high germination (>90%) across all treatments, supporting earlier findings that some pea-type chickpeas possess moderate osmotic tolerance due to their phenolic content (Farooq, 2018).

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The polyphenols comprise very large groups of secondary metabolites with various functions in plants (Sharma et al. 2019). Most polyphenols possess very high antioxidant capacity; thus they are one of the main antioxidant groups (Stagos 2020; Zagoskina et al. 2023). It could be hypothesized about the function of polyphenols for germination under high osmotic stress. However, without conclusive evidence about the markers of oxidative stress, this only remains an assumption. The most probable suggestion would be that polyphenols act as quenchers of the ROS, thus preventing the oxidative stress at higher osmotic pressures (Sharma et al., 2019).

Table 1.

Germination (%) of 22 variants of chickpeas (*Cicer arietinum*) at different concentrations of saccharose (0 mM, 50 mM, 150 mM and 250 mM) Be = beige; RBr = reddish brown; W = white; Br = brown; B = black

Variant	Control	50 mM	150 mM	250 mM	Shape/ color
1. Alfa	85.00 ±10.41	88.33 ±6.01	43.33 ±8.82*	55.00 ±2.89*	Be pealike
2. Beta	88.33 ±6.67	55.00 ±5.00*	40.00 ±10.00*	71.67 ±7.26	Be pealike
3. Businsky	73.33 ±6.01	81.67 ±4.41	50.00 ±10.41	81.67 ±1.67	Be pealike
4. Irenka	75.00 ±12.58	73.33 ±9.28	43.33 ±17.40	73.33 ±13.02	RBr Desi
5. Krajova z Kralovej	91.67 ±4.41	86.67±4.41	41.67 ±12.02*	45.00 ±8.66**	Be pealike
6. Maskovsky Bagovec	71.67 ±7.26	68.33 ±6.67	60 ±8.66	76.67 ±11.67	Be pealike
7. Slovak	80.00 ±2.89	86.67 ±4.41	33.33 ±15.90*	76.67 ±6.01	Be pealike
8. FLIP 84-149 C	76.67 ±1.67	65.00 ±2.89*	43.33 ±10.93*	16.67±4.41***	Be pealike
9. VSP 11	96.67 ±3.33	95.00 ±2.89	90.00 ±10.00	100	Br pealike
10. Прогрес	90.00 ± 2.89	70.00 ±11.55	68.33 ±6.67*	76.67 ±4.41	Be pealike
11. Балкан (st)	76.67 ±1.67	65.00 ±7.64	43.33 ±11.67*	23.33±10.93**	W pealike
12. Garbanzo 1	8.33 ±4.41	3.33±1.67	5.00 ±2.89	0.00	W Kabuli
13. Blanco Lechoso	36.67±14.81	30.00±15	16.67 ±6.01	0.00	Br Kabuli
14. Caqui	100.00	100.00	86.67 ±7.26	31.67 ±31.67	B Desi
15. Garbanzo negro	100.00	100.00	93.33 ±4.41	30.00 ±30	B Desi
16. Tadzikskij 10	93.33 ±4.41	98.33 ±1.67	93.33 ±4.41	21.67 ±21.67*	Br Desi
17. Sovhoznyj 14	60.00 ±18.93	78.33 ±19.22	98.33 ±1.67	28.33 ±28.33	Be Desi
18. Dneprovskij 1	15.00 ±2.89	20.00 ±2.88	8.33 ±6.01	1.67±1.67*	Be pealike
19. Garbanzo 2	88.33 ±3.33	78.33 ±12.02	40.00 ±10.41**	10±10***	Be pealike
20. Garbanzo 3	18.33 ±1.67	18.33 ±1.67	8.33 ±6.01	0.00	Be pealike
21. Garbanzo 4	55.00 ±20.00	73.33 ±8.82	26.67 ±10.93	0.00	Be pealike
22. Пловдив 8	63.33 ±10.14	61.67 ±8.33	33.30 ±10.93	1.67 ±1.67***	Be pealike

* significant at $p < 0.05$; **significant at $p < 0.01$; ***significant at $p < 0.001$ compared to the control (0 mM).

Germ length followed a similar pattern. Under control conditions, several genotypes—including variants 9 and 14 produced long seedlings, whereas germ length declined substantially under higher sucrose concentrations (Table 2). In all tested chickpea variants, no matter the length of the control, at a saccharose concentration of 250 mM, the length of the germ was under 1 cm. Even at 150 mM, many Kabuli and pea-type accessions produced very short germs, indicating delayed or arrested early development. In contrast, several Desi genotypes (variants 14, 15, 16, and 1) retained measurable germ growth even at 250 mM sucrose, and their germs were the longest from all the tested variants under this concentration. This is consistent with other reports that tolerant chickpeas maintain turgor and metabolic activity longer during osmotic stress (Table 2, Amede et al., 2003).

Table 2.

Germ length (cm) of 22 variants of chickpeas (*Cicer arietinum*) at different concentrations of saccharose (0 mM, 50 mM, 150 mM, and 250 mM)

Variants	Control	50 mM	150 mM	250 mM
1. Alfa	6.06 ±0.36	3.95 ±0.19***	2.16 ±0.15***	0.68 ±0.08***
2. Beta	6.18±0.34	2.89±0.2***	2.16±0.21***	0.18±0.05***
3. Businsky	4.54±0.35	4.41±0.23	2.00±0.12***	0.3±0.11***
4. Irenka	5.47±0.42	3.40±0.2***	1.83±0.23***	0.58±0.12***
5. Krajova z Kralovej	6.58±0.38	3.90±0.22***	1.61±0.18***	0.58±0.16***
6. Maskovsky Bagovec	3.45±0.24	3.48±0.2	2.04±0.15***	0.22±0.07***
7. Slovak	4.66±0.32	5.48±0.28*	1.87±0.14***	0.32±0.08***
8. FLIP 84-149 C	5.74±0.34	4.47±0.3**	2.22±0.19***	0.42±0.19***
9. VSP 11	9.07±0.38	7.11±0.34***	3.86±0.23***	0.49±0.12***

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Variants	Control	50 mM	150 mM	250 mM
10. Прогрес	4.79±0.28	4.71±0.29	2.21±0.1***	0.28±0.1***
11. Балкан (st)	7.01±2.94	3.22±0.35***	2.11±0.85***	0.27±0.17***
12. Garbanzo 1	0.67±0.33	1.03±0.45	1.58±0.93	0.00
13. Blanco Lechoso	2.04±0.12	1.56±0.24	1.5±0.31	0.00
14. Caqui	6.83±0.3	6.69±0.16	5.6±0.28**	0.95±0.18***
15. Garbanzo negro	. 6.3±0.21	5.16±0.16***	4.64±0.17***	0.76±0.14***
16. Tadzikskij 10	5.54±0.3	4.04±0.23***	4.57±0.15***	0.89±0.21***
17. Sovhoznyj 14	6.36±0.24	5.16±0.22***	4.65±0.16***	0.78±0.18***
18. Dneprovskij 1	2.49±0.32	1.68±0.32	0.69±0.04**	0.33±0.5
19. Garbanzo 2	6.01±0.22	3.08±0.16***	2.89±0.37***	0.51±0.34***
20. Garbanzo 3	2.87±0.62	1.26±0.16*	1.51±0.67	0.00
21. Garbanzo 4	4.17±0.35	3.62±0.26***	2.55±0.34***	0.00
22. Пловдив 8	5.43±0.33	3.81±0.3***	3.44±.0.5**	0.33±0.5**

* significant at p < 0.05; **significant at p < 0.01; ***significant at p < 0.001 compared to the control (0 mM).

Germ weight measurements supported these trends. Increased sucrose concentration strongly reduced biomass accumulation in most genotypes. Sensitive genotypes reached very low biomass at 250 mM, whereas several Desi lines maintained detectable fresh mass (Table 3), indicating a stronger ability to sustain metabolic processes under water restriction, which is consistent with observations by Nayyar et al. (2006).

Table 3.

Seedling fresh weight (g) of 22 variants of chickpeas (*Cicer arietinum*) at different concentrations of saccharose (0 mM, 50 mM, 150 mM and 250 mM)

Variant	Control	50 mM	150 mM	250 mM
1. Alfa	0.14	0.09±0.01***	0.08±0.01***	0.05±0.02***
2. Beta	0.14±0.01	0.08±0.01***	0.07±0.03	0.01***
3. Businsky	0.09	0.13***	0.07*	0.01***
4. Irenka	0.14±0.01	0.07±0.01***	0.05***	0.01***
5. Krajova z Kralovej	0.14±0.01	0.08±0.01***	0.07±0.01***	0.01***
6. Maskovsky Bagovec	0.07±0.01	0.10±0.01***	0.08±0.01	0.01***
7. Slovak	0.14±0.01	0.15±0.01	0.09±0.01***	0.01***
8. FLIP 84-149 C	0.13±0.01	0.07±0.01***	0.07±0.01***	0.02***
9. VSP 11	0.17±0.01	0.15±0.01	0.08***	0.01***
10. Прогрес	0.12±0.01	0.13±0.01	0.09±0.01***	0.01***
11. Балкан (st)	0.15±0.02	0.10±0.01***	0.06±0.01***	0.01±0.01***
12. Garbanzo 1	0.01	0.05±0.03	0.05±0.05	0.00
13. Blanco Lechoso	0.06±0.01	0.03±0.01	0.04±0.02	0.00
14. Caqui	0.1±0.01	0.10±0.01	0.13±0.01***	0.03±0.01***
15. Garbanzo negro	0.08	0.09	0.12±0.01***	0.01***
16. Tadzikskij 10	0.08	0.08	0.11***	0.06±0.01*
17. Sovhoznyj 14	0.07	0.06	0.10±0.01**	0.02±0.01
18. Dneprovskij 1	0.05±0.01	0.04±0.02	0.01***	0.00
19. Garbanzo 2	0.08	0.07±0.01	0.05±0.01***	0.01±0.02*
20. Garbanzo 3	0.03±0.01	0.02	0.02±0.01	0.00
21. Garbanzo 4	0.04	0.07±0.01**	0.06±0.01	0.00
22. Пловдив 8	0.07	0.09±0.01***	0.10±0.01*	0.01±0.01***

* significant at p < 0.05; **significant at p < 0.01; ***significant at p < 0.001 compared to the control (0 mm).

The SVI, combining germination and growth, provided the clearest differentiation among genotypes. Desi-type cultivars showed consistently high vigor under control and moderate stress conditions, whereas Kabuli and pea-type accessions showed strong declines, with many reaching near-zero SVI at 250 mM (Table 4).

Beyond the classical physiological interpretation, these patterns align with oxidative mechanisms known to regulate early germination. The transition from dry seed to active growth naturally involves a controlled increase in ROS, which mediates dormancy release and signals metabolic activation (Bailly, 2008). Under osmotic restriction, however, ROS accumulation may exceed the physiological range, leading to oxidative imbalance that disrupts membrane integrity, impairs reserve mobilization and inhibits cell expansion.

Overall, the results indicate that sucrose-induced osmotic stress suppresses germination and early seedling development primarily through limited hydration and secondarily through oxidative disturbance.

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Table 4.
Seed Vigor Index of 22 variants of chickpeas (*Cicer arietinum*) at different concentrations of saccharose (0 mM, 50 mM, 150 mM and 250 mM)

Variant	Control	50 mM	150 mM	250 mM
1. Alfa	514.98	349.09	93.71	37.54
2. Beta	545.74	158.80	86.33	13.25
3. Businsky	333.12	360.35	100.08	24.36
4. Irenka	410.40	249.45	79.40	42.57
5. Krajova z Kralovej	602.92	337.92	67.14	26.00
6. Maskovsky Bagovec	247.20	237.57	122.38	15.93
7. Slovak	402.00	461.5	77.95	33.37
8. FLIP 84-149 C	440.31	290.57	96.27	6.94
9. VSP 11	877.00	675.01	347.06	49.00
10. Прогрес	431.30	329.82	150.69	21.80
11. Балкан (st)	537.72	209.50	91.34	6.31
12. Garbanzo 1	5.56	3.44	7.92	0.00
13. Blanco Lechoso	74.73	46.75	24.97	0.00
14. Caqui	683.17	669.17	485.40	29.94
15. Garbanzo negro	630.17	515.67	432.70	22.67
16. Tadzikskij 10	517.18	397.75	426.50	19.39
17. Sovhoznyj 14	381.83	404.50	457.07	66.5
18. Dneprovskij 1	37.33	33.66	5.76	0.56
19. Garbanzo 2	530.48	241.61	115.72	5.11
20. Garbanzo 3	52.61	23.07	12.57	0.00
21. Garbanzo 4	229.53	265.58	67.98	0.00
22. Пловдив 8	344.10	234.72	33.33	1.67

Despite the clear genotypic differentiation, this study has certain limitations. The reliance on phenotypic markers (germination and vigor) provides a broader view of stress tolerance but does not capture the underlying molecular and metabolic shifts. Due to this, future research incorporating biochemical profiling of ROS scavengers and phytohormone dynamics would further validate the link between osmotic signaling and redox homeostasis in these genotypes.

CONCLUSION

Our results suggest that stress tolerance during early stages of plant development includes a set of mechanisms that are genotype-related. The resilience of Desi-type chickpeas is in sharp contrast to the sensitivity of Kabuli-type and pea-type accessions, which struggled once sucrose levels reached 150 mM. Therefore, sucrose-based osmotic assays provide a reliable and informative approach for identifying chickpea germplasm with enhanced capacity to withstand osmotic and possibly oxidative stress, offering valuable guidance for breeding programs targeting drought-prone environments.

AUTHORS CONTRIBUTIONS

Conceptualization, N.G. and V. K.; methodology. I.V. and M. K.; data collection, S.N.; data validation, M.K, data processing, I.V, S.N., N.G., V.K.; writing – original draft preparation: N.G. and V. K.; writing – review and editing, I.V. and M.K.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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