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## The Impact of Glucose-induced Priming on Nutrients Accumulation and Certain Primary Attributes of *Brassica napus* L. Under the Saline Regimes

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### Abstract

In the present study, the response of primary attributes (seedling growth, percent emergence, seedling fresh and dry biomass, and seedling moisture contents) and nutrient contents (Calcium, Magnesium, Iron, Manganese, Zinc, Copper) of *Brassica napus* L. was evaluated as a function of glucose-induced priming and salinity. The priming comprised 30 minutes, 60 minutes and 90 minutes of seeds soaking in glucose solution (0.50 M) and salinity stress was simulated by the solutions of 0, 15, 18, 21 and 24 milli Molar (mM) NaCl. The results revealed that doses of salinity induced significant changes in the fresh weight of *Brassica napus* L. The response of selected nutrients (except Magnesium) as a function of salinity was also highly significant ( $P \leq 0.05$  %). The salinity doses reduced plants' mineral contents (except Mn) compared to control. The priming of seeds for 90 minutes significantly ( $P \leq 0.05$  %) enhanced certain early growth traits (plumule growth, radical growth, fresh weight and dry weight) of *Brassica napus*. On the other hand, for improving germination (%) and moisture contents (%) of *Brassica napus*, soaking durations of 30 minutes and 60 minutes are more suitable. The pre-soaking of seeds for 60 minutes increased the Calcium, Magnesium and Manganese contents (mg/litre) of *Brassica napus*. The Iron and Zinc contents (mg/litre) showed hype in seedlings raised from seeds primed for 30 minutes. The priming of seeds for 90 minutes was found to be stimulatory for Copper (mg/litre) only. The influence of factors interaction (treatments  $\times$  priming durations) on the initial growth attributes and the studied minerals of *Brassica napus* L. was highly significant ( $P \leq 0.05$  %). From the gathered evidence, the present study concludes glucose as a potent priming agent that can boost oil-yielding plants' performance under saline conditions.

**Keywords:** salt stress, priming amendments, glucose, germination and seedling growth

### Introduction

Salinity is considered the main reason behind low crop productivity worldwide. According to an estimate, up to 20% of agricultural land is under the influence of salinity (Hafeez *et al.*, 2021). Moreover, the world is facing the threat of further salinization due to high evaporation and low precipitation. The soil salinization and alkalization reduced soil productivity and affected the sustainability of the agricultural system. Due to salinity, sodium and chloride ions concentrations increase in the rhizosphere, damaging many cellular metabolic systems (varying activities of enzymes like catalases, peroxidases, and ascorbate peroxidases) and all the biological attributes (Ferreira *et al.*, 2021; Pompelli *et al.*, 2022). Furthermore, over production of reactive oxygen species takes by the stress conditions that changes normal cellular metabolism (e.g. oxidation of biological molecules). Due to excess salt in the rhizosphere, lands cannot sustain vegetation affecting the world's overall economy because of low crop production (Munns and

Tester *et al.*, 2008). Therefore, developing techniques (like priming) to enhance crop production on such lands is immensely important. The selection of salt-resistant cultivars and exploration of new salinity-combating accessions could be the other alternatives for making such barren land cultivable (Pompelli *et al.*, 2022).

Priming techniques are the most common and cheapest tool for combating salinity hazards. The priming amendments involve seeds soaking in solutions of various strength (Ghobadi *et al.*, 2012). The previous workers have confirmed stimulatory tendencies of pre-soaking amendments on various traits of plants like *Triticum* (Iqbal & Ashraf, 2007), *Helianthus* (Kaya *et al.*, 2006), chickpea (Kaur *et al.*, 2002), *Capsicum* (Patade *et al.*, 2011), *Glycine max* (Sadeghi *et al.*, 2011), maize (Foti *et al.*, 2008), cucumber (Ghasemi-Golezani & Esmaeilpour, 2008). The priming tendencies of various chemicals such as Calcium Chloride (Fuller *et al.*, 2012; Taghvaei *et al.*, 2012; Abbas *et al.*, 2018), Sodium Chloride (Jamal *et al.*, 2011; Nejad *et al.*, 2013; Shoor *et al.*, 2014), Zinc

Oxide nano-particles (Al-salama 2022), sorghum water (Huang et al., 2021), Potassium Nitrate (Ahmadvand et al., 2012), aspirin (Ehtaiwesh & Almajdob, 2021), Potassium Permanganate (Hassanpouraghdam et al., 2009; Hassanpouraghdam et al., 2009), vitamin B<sub>12</sub> (Jorjandi & Sirchi, 2012), plant hormones (Sedghi et al., 2010; Salih et al., 2022), PEG-6000 (Aydinoglu et al., 2019; Fuller et al., 2012), Ascorbic Acid, Potassium Silicate, Proline and Spermidine (Feghhenabi et al., 2020) had been already studied. In this study, we have explored the priming tendencies of glucose on a prominent oil-yielding plant, the canola for the first time keeping in view the importance of glucose for initial stages of germination of plants. The second widely used approach in combating salinity is selecting salt-resistant crop cultivars. In the literature, sufficient evidence supports the salt-resistant nature of canola (Khajeh-Hosseini et al., 2003; Kaya et al., 2006). The *Brassica napus* L. (canola) contributes about 13 % of the world's edible oil supply (Hidayati et al., 2011). Pakistan's per hectare canola yield (839 kg ha<sup>-1</sup>) is very low (Minfal et al., 2022), in contrast to developed countries. The low yield of canola is attributed to infertile soils and certain abiotic stresses. Exogenous applications of nutrients (nitrogen and sulfur) are required to obtain a higher yield of canola on such land. The initial growth stages of canola may be affected by salinity (Steppuhn and Volkmar et al., 2001). The priming amendments could boost the germination of canola on such saline lands. The on going study is another effort to alleviate salt stress through priming techniques.

## Materials and Methods

The study was carried out at the Laboratory Department of Botany, Govt. Degree College Tangi, Charsadda, Khyber Pakhtunkhwa, Pakistan. The experiment was arranged on a CRD pattern (Salinity Doses × Priming durations) with three replications. The doses of salinity (0, 15, 18, 21 and 24 milliMolar) were the first factor, while the glucose-induced priming and its durations (30 minutes, 60 minutes, 90 minutes) was the second factor. Seeds of the *Brassica napus* L. were soaked in glucose solution (0.50 M) for the mentioned durations. The pre-soaking amendments were conducted separately following the method given by Basra et al. (2006). The primed seeds were grown in sterilized (Rowid et al., 2007) petri dishes of equal size. Seeds were put in petri dishes on two fold whatman filter paper # 1. The

primed seeds grown in distilled water (10 ml) was treated as control. In the rest of petri dishes the selected doses of salinity were applied. Each treatment was properly replicated. The petri dishes were sterilized at 170 °C in an oven prior to use. Seeds were equidistantly placed in each petri dish.

**The germination percentage (GP) was calculated using the following formula:**

$$GP = \left[ \frac{\text{Total seeds germinated}}{\text{Total number of seeds}} \right] \times 100$$

Mean plumule and radical lengths were measured per replication. The fresh weight of the seedlings was determined. The dry weights of seedlings were taken with the help of an electric balance after drying each replication at 70°C in the oven to get the constant weight (Afzal et al., 2005). Moisture contents of seedlings were calculated following Hussain (1989). The plant sample was finely grounded to determine mineral contents, and one gram of powder was taken in a small beaker. The powder was soaked in concentrated HNO<sub>3</sub> (10 mL) overnight. After 24 hours, the beaker was heated on a hot plate until the production of red NO<sub>2</sub> fumes had ceased. The beaker was cold and 2-4 mL of perchloric acid (70% HClO<sub>4</sub>) was added to the beaker. The beaker was heated again and the extract was evaporated to a small volume. In last, distilled water (50 mL) was added to the extract (Adrian, 1973). The extract was subjected to atomic absorption spectrometry, and data regarding various minerals was recorded. The data were statistically analysed and the means were separated as significant with the help of LSD test using Statistix 8.1 software (2003).

## Results and Discussion

**Statistical Interpretation:** The doses of salinity induced significant variations in fresh seedling weight (g) of *Brassica napus* L. However, salinity failed to induce significant variations in germination (%), dry weight (g), moisture contents (%) and seedling growth (cm) of *Brassica napus* L. On the contrary, the impact of salinity on the nutrient contents (except Magnesium) was highly significant. Similarly, the priming and interaction (treatments × priming durations) significantly influenced the growth attributes and most of the nutrients. However, the glucose-induced priming could not produce significant changes in Manganese contents (Table 1 & 2).

**Table 1.** Mean squares table for plumule growth (cm), radical growth (cm), germination (%), fresh weight (g), dry weight (g) and moisture contents (%).

Factors	DF	PG	RG	Ger.	FW	DW	MC
Treatment	4	0.00802	0.08692	103.333	0.00382	0.00720	11.7152
Priming	2	<b>1.31779</b>	0.47849	326.667	<b>0.03643</b>	<b>0.05154</b>	85.6704
Treatment × Priming Durations	8	0.02780	0.06716	101.667	<b>0.00841</b>	0.01355	27.4714
Error	28	0.01962	0.11410	80.000	0.00250	0.00853	18.6092

DF = Degree of freedom, PG = Plumule growth, RG = Radical growth, Ger. = Germination, FW = Fresh Weight, MC = Moisture contents, Bold values denote Significance, Normal values denotes Non-significance.

**Table 2.** Mean squares for Calcium, Magnesium, Manganese, Iron, Zinc and Copper contents (mg/litre).

Factors	Degree of Freedom	Calcium	Magnesium	Manganese	Iron	Zinc	Copper
Treatment	1	141.905	12.5801	0.01100	0.00186	0.01372	0.00252
Priming	2	6.939	8.44436	8.327E-04	0.00785	2.429E-04	0.01390
Treatment × Priming Durations	2	6.939	8.44436	8.347E-04	0.00770	1.642E-04	0.01390
Error	10	0.424	1.306E-05	4.589E-06	1.100E-06	1.122E-06	8.000E-07

Bold values denote Significance, Normal values denotes Non-significance.

**Effect on Germination (%):** Salinity doses of 15, 21 and 24 mM caused a non-significant increase in germination percent emergence values over the control.

Priming of seeds for 30 minutes (90.667) enhanced percent emergence values over 60 minutes (89.33) and 90 minutes duration (82.000). However, seed primed for 30 minutes (90.667) and 60 minutes (89.33) produced non-significant influence on the percent emergence. On the other hand, priming for 30 minutes (90.667) and 90 minutes (82.000) recorded significant variations for germination percentage (%). Similarly, seedlings raised from seeds primed for 60 minutes (89.33) and 90 minutes (83.000) displayed significant variations for percent emergence.

Interaction study revealed that seed priming for 60 minutes could enhance percent emergence over the control. However, seeds pre-soaking for 90 minutes could reduce percent emergence compared to the control. Seed priming for 30 minutes may either enhance or inhibit percent emergence (Table 3).

Germination occurs through cell division and elongation. The fore-mentioned developmental phases are greatly influenced by salinity (Khajeh-Hosseini *et al.*, 2002). Moreover, the salinity causes damage to the enzymes involved in germination resulting in inhibition or delay in seedling emergence (Ataket *et al.*, 2006; KhoshKholghSima *et al.*, 2013). There is sufficient evidence that priming induces seeds to absorb more water and helps in increasing percent emergence (Atak *et al.*, 2006; Kaya *et al.*, 2006). The reason behind the negative impact of salt stress on

seedling emergence is accredited to the physiological drought it creates or the ion toxicity on the germinated seeds (Aydinoglu *et al.*, 2019).

During pre-soaking amendments, the seeds absorb water and exert pressure on the endosperm. The compression force of the embryo and hydrolytic activities on the endosperm cell walls may deform the tissues that have lost their flexibility upon dehydration, producing free space and facilitating root protrusion after rehydration. Priming seeds with glucose confirmed the same logic by reducing the hazards of salinity. Increase in germination % by priming techniques in canola (Hassanpoughdam *et al.*, 2009; Heshmat *et al.*, 2011; Aboutalebian *et al.*, 2012; Mousavi *et al.*, 2019; Khan *et al.*, 2021; Zhu *et al.*, 2021), calendula (Sedghi *et al.*, 2010), Wheat (Jamal *et al.*, 2011; Michal *et al.*, 2012; Worku *et al.*, 2016; Khan *et al.*, 2018; Abbas *et al.*, 2018; Ehtaiwesh & Almajdor, 2021), alfalfa (Jorjandi & Sirchi, 2012), calotropis (Taghvaei *et al.*, 2012), soyabean (Ahmadvand *et al.*, 2012), Aeleorupus (Nejad *et al.*, 2013), cumin (Shoor *et al.*, 2014), maize (Akter *et al.*, 2018; Shah *et al.*, 2021), Vicia (Aydinoghlu *et al.*, 2019), barley (Tabatabaei & Ansari, 2020), camelina (Huang *et al.*, 2021) confirmed our results regarding enhancement of percent emergence of wheat by pre-sowing seeds treatments. On the contrary, studies on wheat (Afzal *et al.*, 2005; Akbarmoghaddam *et al.*, 2011; Biabani *et al.*, 2013), canola (Bybordi & Tabatabaei, 2009; Mohammadi *et al.*, 2010), and maize (Akter *et al.*, 2018) declared priming techniques as non-efficient in inducing positive effects on germination of plants.

**Table 3.** The germination (%) of *Brassica napus* L. as a function of salinity (milli-Molar) and glucose-induced priming and its durations (minutes).

Treatments (mM)	Priming durations (Minutes)			Treatments means
	30	60	90	
Control	83.33 <sup>bc</sup>	83.33 <sup>bc</sup>	86.67 <sup>abc</sup>	84.444 <sup>a</sup>
15	100.00 <sup>a</sup>	93.33 <sup>abc</sup>	80.00 <sup>bc</sup>	91.111 <sup>a</sup>
18	80.00 <sup>c</sup>	86.67 <sup>abc</sup>	83.33 <sup>bc</sup>	83.333 <sup>a</sup>
21	96.67 <sup>ab</sup>	93.33 <sup>abc</sup>	80.00 <sup>c</sup>	90.000 <sup>a</sup>
24	93.33 <sup>abc</sup>	90.00 <sup>abc</sup>	80.00 <sup>c</sup>	87.778 <sup>a</sup>
Priming means	90.667 <sup>a</sup>	89.333 <sup>a</sup>	82.000 <sup>b</sup>	

Alpha = 0.05 %. The critical value for comparison for treatments = 8.6368, priming = 6.6901, and interaction = 14.959.

**Influence on Plumule Growth (cm):** Glucose-induced priming for 90 minutes (1.5787) enhanced plumule growth significantly over the rest of the

soaking durations (30 and 60 minutes). However, the effect of priming of seeds for 30 minutes (1.0716) and

60 minutes (1.0592) on the plumule growth was highly non-significant.

The interaction (treatments × priming durations) study confirmed the significant effects of seed priming for 90 minutes on plumule growth under the control (1.6533), 15 mM (1.5967), 21 mM (1.6067) and 24 mM (1.6100) salinity levels compared to other priming durations (Table 4).

It is suggested that priming induces stress on seeds that activate certain physiological mechanisms, which are helpful for plants in adaptation to unfavourable environments (Bhanuprakash and Yogeesh, 2016; Saddiq et al., 2019). The significant effect of glucose-induced priming on plumule growth is attributed to the mentioned physiological changes in seeds brought by the pre-sowing treatments.

The previous studies on wheat (Afzal et al., 2005; Abbas et al., 2018; Ehtaiwesh and Almajdor, 2021), canola (Hassanpoughdam et al., 2009; Heshmat et al.,

2011; Aboutalebian et al., 2012; Zhu et al., 2021), *Calendula officinalis* (Sedghi et al., 2010), alfaafa (Jorjandi & Sirchi, 2012), *Calotropis procera* (Taghvaei et al., 2012), soyabean (Ahmadvand et al., 2012), *Aeluropus* (Nejad et al., 2013), cumin (Shoor et al., 2014), maize (Akter et al., 2018; Shah et al., 2021), *Vicia* (Aydinoglu et al., 2019), *Camilina* (Huang et al., 2021) and hargel (Salih et al., 2022) have confirmed stimulatory effects of pre-soaking amendments on plumule lengths under salt stress which fully support our findings. However, some workers have reported a decrease in the plumule growth of plants like wheat (Akbarimoghaddam et al., 2011; Biabani et al., 2013; Khan et al., 2018) and canola (Bybordi and Tabatabaei, 2009; Mohammadi et al., 2010) by priming techniques under salinity. On the contrary, Jamal et al. (2011) deduced non-significant effects of priming on plants negating our findings

**Table 4.** The plumule growth (cm) of *Brassica napus* L. as a function of salinity (milli-Molar) and glucose-induced priming and its durations (minutes).

Treatments (mM)	Priming durations (Minutes)			Treatments means
	30	60	90	
Control	1.0600 <sup>cd</sup>	1.0000 <sup>cd</sup>	1.6533 <sup>a</sup>	1.2378 <sup>a</sup>
15	1.1200 <sup>cd</sup>	0.9877 <sup>cd</sup>	1.5967 <sup>a</sup>	1.2348 <sup>a</sup>
18	0.9380 <sup>d</sup>	1.2000 <sup>bc</sup>	1.4267 <sup>ab</sup>	1.1882 <sup>a</sup>
21	1.1367 <sup>cd</sup>	1.0533 <sup>cd</sup>	1.6067 <sup>a</sup>	1.2656 <sup>a</sup>
24	1.1033 <sup>cd</sup>	1.0550 <sup>cd</sup>	1.6100 <sup>a</sup>	1.2561 <sup>a</sup>
Priming means	1.0716 <sup>b</sup>	1.0592 <sup>b</sup>	1.5787 <sup>a</sup>	

Alpha = 0.05 %. Critical value for comparison for treatments = 0.1352, priming = 0.1048 and interaction = 0.2343.

**Effect on Radical Growth (cm):** Priming of seeds for 90 minutes (3.2680) enhanced radical growth over the rest of the pre-soaking durations. However, differences between radical growth values recorded from 90 minutes (3.2680) and 60 minutes durations (2.1591) were statistically non-significant. Similarly, the effect of priming for 30 minutes and 60 minutes on radical growth was also non-significant. On the other hand, radical growth means recorded from 30 minutes (1.9189) and 90 minutes (3.2680) priming durations were highly significant.

Interaction study revealed that seed priming for 30 minutes reduced radical growth value over control. On the other hand, soaking of seeds for 60 minutes enhanced radical growth over control. Priming of seeds for 90 minutes may increase or decrease radical growth over control (Table 5).

Probably, priming of seeds for 60 minutes and 90 minutes has counteracting effects on cell elongation and division against the osmotic effect created around the radical. Hence, seedling growth is enhanced in high salt-containing environments (Shah et al., 2017; Caruso et al., 2018; Aydinoglu et al., 2019). Moreover, pre-sowing seeds treatment

induced metabolic activities causing higher radical growth (Rafiq et al., 2006; Jamal et al., 2011). Similarly, priming might induce germination metabolites, DNA, RNA and protein synthesis, boosting radical growth under salt stress (Rafiq et al., 2006; Jamal et al., 2011).

Our study regarding the significant effect of priming amendments on radical growth under salinity is similar to the results recorded from plants like wheat (Afzal et al., 2005; Abbas et al., 2018; Ehtaiwesh & Almajdor, 2021), canola (Hassanpoughdam et al., 2009; Heshmat et al., 2011; Aboutalebian et al., 2012; Khan et al., 2021; Zhu et al., 2021), calendula (Sedghi et al., 2010), alfalfa (Jorjandi and Sirchi, 2012), calotropis (Taghvaei et al., 2012), soyabean (Ahmadvand et al., 2012), aeleoropus (Nejad et al., 2013), cumin (Shoor et al., 2014), maize (Akter et al., 2018; Shah et al., 2021), camilina (Huang et al., 2021) and hargel (Salih et al., 2022). Inhibitory effects of priming on plants exposed to salt stress were rarely seen in the literature (Biabani et al., 2013; Bybordi and Tabatabaei, 2009; Mohammadi et al., 2010).

**Table 5.** The radical growth (cm) of *Brassica napus* L. as a function of salinity (milli-Molar) and glucose-induced priming and its durations (minutes).

Treatments (mM)	Priming durations (Minutes)			Treatments means
	30	60	90	
Control	2.0933 <sup>abc</sup>	2.0010 <sup>abc</sup>	2.1933 <sup>abc</sup>	2.0959 <sup>a</sup>
15	1.9800 <sup>bc</sup>	2.3200 <sup>ab</sup>	2.2100 <sup>abc</sup>	2.1700 <sup>a</sup>
18	1.7513 <sup>c</sup>	2.0667 <sup>abc</sup>	2.0467 <sup>abc</sup>	1.9549 <sup>a</sup>
21	1.8167 <sup>c</sup>	2.2907 <sup>abc</sup>	2.3400 <sup>ab</sup>	2.1491 <sup>a</sup>
24	1.9533 <sup>bc</sup>	2.1173 <sup>abc</sup>	2.5500 <sup>a</sup>	2.2069 <sup>a</sup>
Priming means	1.9189 <sup>b</sup>	2.1591 <sup>ab</sup>	3.2680 <sup>a</sup>	

Alpha = 0.05 %. Critical value for comparison for treatments = 0.3262, priming = 0.2527 and interaction = 0.5650.

**Impact on Fresh Weight (g):** Seeds subjected to 18 mM salinity recorded highest fresh weight values. The impact of 0, 15, 18, 21 mM doses on fresh weight values were however, non-significant. On the contrary, seedlings raised in saline solutions of 18 mM and 24 mM concentrations displayed significant variations for the fresh weight.

Seeds primed for 90 minutes (1.9600) recorded highest fresh weight values followed by 60 minutes (1.8907) and 30 minutes (1.8647) soaking durations, respectively. Moreover seeds primed for 90 minutes (1.9600) and 30 minutes (1.8647) exhibited significant variations for fresh weight values. However, the impact of seeds priming for 30 minutes (1.8647) and 60 minutes (1.8907) on the fresh weight was statistically similar at the selected probability level (0.05 %).

Interaction study revealed that priming of seeds for 60 minutes could enhance fresh weight values over the control. Seeds primed for 30 minutes and 90 minutes showed non-significant variations in fresh weight under the influence of 0, 15 and 18 mM salinity. However, priming of seeds for 30 minutes

and 90 minutes is capable to induce significant variations in fresh weight values under 21 and 24 mM salinity doses (Table 6).

Significant effects of priming may be accredited to the fact that seeds pre-soaking enhanced germination and metabolic activities (synthesis of nucleic acids, protein and increasing respiratory activity and energy reserve utilization), resulting in the efficient development of the embryonic axes (Fuller *et al.* 2012; Ibrahim *et al.*, 2016). Priming stimulated cell division of the apical meristem of the seedlings which caused an increase in seedlings' growth and biomass (Farooq *et al.*, 2007).

Studies conducted by Afzal *et al.* (2005), Khan *et al.* (2018), Akter *et al.* (2018), Abbas *et al.* (2018), Zhu *et al.* (2021) and Shah *et al.* (2021) have confirmed the stimulatory effects of priming on fresh biomass of plants like wheat, maize and canola which are in complete accordance to our results. However, Bybordi and Tabatabaei (2009) and Biabani *et al.* (2013) reported inhibitory effects of priming on the fresh-weight of wheat negated our findings.

**Table 6.** The fresh weight (g) of *Brassica napus* L. as a function of salinity (milli-Molar) and glucose-induced priming and its durations (minutes).

Treatments (mM)	Priming durations (Minutes)			Treatments means
	30	60	90	
Control	1.9267 <sup>abc</sup>	1.8367 <sup>de</sup>	1.9767 <sup>a</sup>	1.9133 <sup>ab</sup>
15	1.8300 <sup>de</sup>	1.9467 <sup>ab</sup>	1.8833 <sup>bcde</sup>	1.8867 <sup>ab</sup>
18	1.9233 <sup>abc</sup>	1.9000 <sup>abcd</sup>	1.9800 <sup>a</sup>	1.9344 <sup>a</sup>
21	1.8300 <sup>de</sup>	1.9133 <sup>abcd</sup>	1.9767 <sup>a</sup>	1.9067 <sup>ab</sup>
24	1.8122 <sup>e</sup>	1.8567 <sup>cde</sup>	1.9833 <sup>a</sup>	1.8844 <sup>b</sup>
Priming means	1.8647 <sup>b</sup>	1.8907 <sup>b</sup>	1.9600 <sup>a</sup>	

Alpha = 0.05 %. Critical value for comparison for treatments = 0.0483, priming = 0.0374 and interaction = 0.0836.

**Effect on Dry Weight (g):** Seeds pre-soaked for 90 minutes (1.7673) recorded the highest dry weight values, followed by 30 minutes (1.7340) and 60 minutes durations (1.5635), respectively. However, dry weight values recorded from seeds primed for 30 minutes (1.7340) and 60 minutes (1.5635) were statistically similar. On the other hand, priming of seed for 60 minutes (1.5635) was found to be inhibitory for dry weight values.

The interaction study revealed that priming of seeds for 90 minutes could enhance dry weight values under saline conditions. On the other hand, seeds primed for 30 minutes may show drop in the dry weight compared to the control. The dry weight of

seeds primed for 60 minutes may go in the direction of either increase or decrease (Table 7).

The significant variations in seedling biomass might be due to the reserve mobilization of food material, activation, and re-synthesis of some enzymes during seed priming (Buriro *et al.*, 2011). Improved seedling biomass with priming amendments could be due to increased cell division within the apical meristem of seedling, which caused an increase in plant growth (Farooq *et al.*, 2008).

Recent studies conducted on various field crops (Afzal *et al.*, 2005; Hassanpoughdam *et al.*, 2009; Ahmadvand *et al.*, 2012; Akter *et al.*, 2018; Abbas *et al.*, 2018; Feghhenabai *et al.*, 2020; Ehtaiwesh and

almajdor, 2021; Zhu *et al.*, 2021; Shah *et al.*, 2021) declared that priming techniques are stimulatory for dry weight of plants. Similarly, an increase in the dry weight of some medicinal plants like calotropis (Taghvaei *et al.*, 2012), camelina (Huang *et al.*, 2021)

and hargel (Salih *et al.*, 2022) by pre-sowing seeds treatments are also reported. On the other hand, Mohammadi *et al.*, (2010) and Biabani *et al.* (2013) suggested mix tendencies of increase or decrease in the dry weight of wheat.

**Table 7.** The dry weight (g) of *Brassica napus* L. as a function of salinity (milli-Molar) and glucose-induced priming and its durations (minutes).

Treatments (mM)	Priming durations (Minutes)			Treatments means
	30	60	90	
Control	1.8200 <sup>a</sup>	1.6800 <sup>abc</sup>	1.6900 <sup>abc</sup>	1.7300 <sup>a</sup>
15	1.6867 <sup>abc</sup>	1.6167 <sup>c</sup>	1.7367 <sup>abc</sup>	1.6800 <sup>a</sup>
18	1.8133 <sup>a</sup>	1.6333 <sup>bc</sup>	1.8100 <sup>a</sup>	1.7522 <sup>a</sup>
21	1.6700 <sup>abc</sup>	1.7333 <sup>abc</sup>	1.7833 <sup>ab</sup>	1.7289 <sup>a</sup>
24	1.6800 <sup>abc</sup>	1.6033 <sup>c</sup>	1.8167 <sup>a</sup>	1.7000 <sup>a</sup>
Priming means	1.7340 <sup>a</sup>	1.6533 <sup>b</sup>	1.7673 <sup>a</sup>	

Alpha = 0.05 %. Critical value for comparison for treatments = 0.0892, priming = 0.0691 and interaction = 0.1545.

**Effect on Moisture Contents:** Priming of seeds for 60 minutes (11.514) brought significant increase in the seedlings moisture content over 30 minutes (6.795) durations. However, priming for 60 minutes (11.514) and 90 minutes (9.814) failed to affect seedlings moisture content significantly. Similarly, variations between priming durations of 30 minutes (6.795) and 90 minutes (9.814) were also non-significant.

The interaction study showed that the primed seeds (30 minutes and 60 minutes) under the influence of salinity recorded highest moisture contents. On the other hand, seeds primed for 90 minutes reduced the moisture contents of seedlings under saline conditions (Table. 8).

The huge amount of salt deposit in the solution creates the physiological drought. The gene regulation

is under the control of various stimuli. These environmental changes switch on/off certain genes specialized for stress conditions. Significant variations in moisture contents induced by the priming could be linked to its switch on/off effects on genes related to various channels or pumps, thereby inducing its activities for combating physiological drought (Basra *et al.*, 2006).

The increase in seedling moisture contents by priming are in complete accordance with the findings of Khan *et al.* (2018). Our results are further supported by the studies conducted on numerous plants like calotropis (Taghvaei *et al.*, 2012), camelina (Huang *et al.*, 2021) and hargel (Salih *et al.*, 2022) that conclude stimulatory effects of pre-sowing seeds treatments on various biological attributes.

**Table 8.** The moisture contents (%) of *Brassica napus* L. as a function of salinity (milli-Molar) and glucose-induced priming and its durations (minutes).

Treatments (mM)	Priming durations (Minutes)			Treatments means
	30	60	90	
Control	5.547 <sup>c</sup>	8.537 <sup>bc</sup>	14.557 <sup>ab</sup>	9.547 <sup>a</sup>
15	7.823 <sup>bc</sup>	16.990 <sup>a</sup>	7.760 <sup>bc</sup>	10.858 <sup>a</sup>
18	5.640 <sup>c</sup>	9.057 <sup>bc</sup>	8.580 <sup>bc</sup>	7.759 <sup>a</sup>
21	7.613 <sup>bc</sup>	9.393 <sup>bc</sup>	9.773 <sup>bc</sup>	8.927 <sup>a</sup>
24	7.353 <sup>bc</sup>	13.593 <sup>ab</sup>	8.400 <sup>bc</sup>	9.782 <sup>a</sup>
Priming means	6.795 <sup>b</sup>	11.514 <sup>a</sup>	9.814 <sup>ab</sup>	

Alpha = 0.05 %. Critical value for comparison for treatments = 4.1656, priming = 3.2266 and interaction = 7.2150.

**Effect on Calcium Contents (mg/litre):** The seeds pre-soaked for 30 minutes (18.580) significantly enhanced the Calcium contents of seedlings over 60 minutes (20.350) and 90 minutes (18.407) durations, respectively.

The seeds subjected to 24 mM (16.304) dose of salinity recorded a significant decline in Calcium contents compared to the control (21.920). The factors

interaction (treatments × priming durations) showed that priming of seeds for various durations could boost up Calcium contents of seedling under non-saline conditions. The seeds primed for 60 minutes (20.350) significantly stimulated Calcium contents in contrast to 30 minutes (18.580) and 90 minutes (18.407) durations (Table 9).

**Table. 9.** The Calcium contents (mg/litre) of *Brassica napus* L. as a function of salinity (mM) and glucose-induced priming durations (minutes).

Treatments (mM)	Priming durations (Minutes)			Treatments means
	30	60	90	
Control	21.920 <sup>a</sup>	21.920 <sup>a</sup>	21.920 <sup>a</sup>	21.920 <sup>a</sup>
24	15.240 <sup>c</sup>	18.780 <sup>b</sup>	14.893 <sup>c</sup>	16.304 <sup>b</sup>
Priming means	18.580 <sup>b</sup>	20.350 <sup>a</sup>	18.407 <sup>b</sup>	

Alpha = 0.05 %. Critical value for comparison for treatments = 0.6836, priming = 0.8372 and interaction = 1.1840.

**Effect on Magnesium Contents (mg/litre):** The highest Magnesium contents value (9.8610) was recorded in seedlings raised from seeds primed for 60 minutes. Moreover, variations among the selected priming durations were highly significant for the subject trait.

The factors interaction (treatments × priming durations) study revealed that priming of seeds for 30 minutes and 60 minutes could stimulate Magnesium contents of seedlings under salinity. However, pre-soaking of seeds for 90 minutes is unable to enhance the seedlings Magnesium contents in saline conditions (Table. 10).

**Table. 10.** The Magnesium contents (mg/litre) of *Brassica napus* L. as a function of salinity (milli-Molar) and glucose-induced priming and its durations (minutes).

Treatments (mM)	Priming durations (Minutes)			Treatments means
	30	60	90	
Control	8.189 <sup>c</sup>	8.189 <sup>c</sup>	8.189 <sup>c</sup>	8.1890 <sup>b</sup>
24	9.828 <sup>b</sup>	12.250 <sup>a</sup>	7.505 <sup>d</sup>	9.8610 <sup>a</sup>
Priming means	9.008 <sup>b</sup>	10.220 <sup>a</sup>	7.847 <sup>c</sup>	

Alpha = 0.05 %. Critical value for comparison for treatments = 3.797 E-03, priming = 4.650E-03 and interaction = 6.576 E-03.

**Effect on Iron Contents (mg/litre):** The seedlings that rose from seeds primed for 30 minutes (0.5035) recorded the highest Iron contents, followed by 60 minutes (0.4678) and 90 minutes (0.4312) durations, respectively. Furthermore, the impact of selected priming durations on the Iron contents of seedlings was highly significant.

The factors interaction study confirmed the significant impact of seed priming on the Iron contents of seedlings. Moreover, pre-soaking of seeds for 30 minutes durations could alleviate salt stress (Table. 11).

**Table. 11.** The Iron contents (mg/litre) of *Brassica napus* L. as a function of salinity (milli-Molar) and glucose-induced priming durations (minutes).

Treatments (mM)	Priming durations (Minutes)			Treatments means
	30	60	90	
Control	0.4780 <sup>b</sup>	0.4777 <sup>b</sup>	0.4773 <sup>b</sup>	0.4777 <sup>a</sup>
24	0.5290 <sup>a</sup>	0.4580 <sup>c</sup>	0.3850 <sup>d</sup>	0.4573 <sup>b</sup>
Priming means	0.5035 <sup>a</sup>	0.4678 <sup>b</sup>	0.4312 <sup>c</sup>	

Alpha = 0.05 %. Critical value for comparison for treatments = 1.101 E-03, priming = 1.349 E-03 and interaction = 1.908 E-03.

**Effect on Zinc Contents (mg/litre):** The doses of salinity reduced the Zinc content values (0.2771) of seedlings significantly compared to the control (0.3323).

The impact of glucose induced priming on the Zinc contents of seedlings was also highly significant. The highest Zinc contents values were recorded in

seedling rose from seeds primed for 30 minutes followed by 90 and 60 minutes, respectively.

The factors interaction (Treatment × Priming) negatively affected Zinc contents of seedlings raised from seeds primed for various durations. The priming of seeds for 30 minutes brought a significant increase in the Zinc contents of the seedling as compared to other priming durations (Table 12).

**Table. 12.** The Zinc contents (mg/litre) of *Brassica napus* L. as a function of salinity (milli-Molar) and glucose-induced priming and its durations (minutes).

Treatments (mM)	Priming durations (Minutes)			Treatments means
	30	60	90	
Control	0.3330 <sup>a</sup>	0.3310 <sup>d</sup>	0.3330 <sup>a</sup>	0.3323 <sup>a</sup>
24	0.2860 <sup>c</sup>	0.2640 <sup>e</sup>	0.2813 <sup>d</sup>	0.2771 <sup>b</sup>
Priming means	0.3095 <sup>a</sup>	0.2975 <sup>c</sup>	0.3072 <sup>b</sup>	

Alpha = 0.05 %. Critical value for comparison for treatments = 1.112 E-03, priming = 1.362 E-03 and interaction = 1.927 E-03.

**Effect on Copper Contents (mg/litre):** The seedlings raised in distilled water recorded maximum Copper contents (0.2810). Moreover, variations between the control (0.2810) and 24 mM (0.2573) doses of salinity were highly significant.

The pre-soaking of seeds for 90 minutes was found to be stimulatory for the Copper contents of seedlings only. The seedlings raised from seeds

primed for 30-minutes caused maximum inhibition of Copper contents.

The factors interactions study further confirmed the stimulatory nature of seeds priming for 90 minutes towards Copper contents. The selected priming durations recorded highly significant variations in Copper contents under saline conditions (Table 13).

**Table. 13.** The Copper contents (mg/litre) of *Brassica napus* L. as a function of salinity (milli-Molar) and glucose-induced priming and its durations (minutes).

Treatments (mM)	Priming durations (Minutes)			Treatments means
	30	60	90	
Control	0.2810 <sup>c</sup>	0.2810 <sup>c</sup>	0.2810 <sup>c</sup>	0.2810 <sup>a</sup>
24	0.1500 <sup>d</sup>	0.2860 <sup>b</sup>	0.3360 <sup>a</sup>	0.2573 <sup>b</sup>
Priming means	0.2155 <sup>c</sup>	0.2835 <sup>b</sup>	0.3085 <sup>a</sup>	

Alpha = 0.05 %. Critical value for comparison for treatments = 9.395 E-04, priming = 1.150 E-03 and interaction = 1.627 E-03.

**Effect on Manganese Contents (mg/litre):** The seeds subjected to a 24 mM dose of salinity recorded maximum Manganese contents values compared to the control.

The priming of seeds for 60 minutes brought a significant increase in Manganese contents of seedlings followed by 90 minutes and 30 minutes durations, respectively.

The factors interactions boosted up the Manganese contents of seedlings compared to the control. Priming of seeds for 60 minutes brought the maximum increase in seedling Manganese contents. Furthermore, the selected priming durations induced significant variations in the seedlings Manganese contents (Table 14).

**Table. 14.** The Manganese contents (mg/litre) of *Brassica napus* L. as a function of salinity (milli-Molar) and glucose-induced priming and its durations (minutes).

Treatments (mM)	Priming durations (Minutes)			Treatments means
	30	60	90	
Control	0.1590 <sup>d</sup>	0.1603 <sup>d</sup>	0.1623 <sup>d</sup>	0.1606 <sup>b</sup>
24	0.1940 <sup>c</sup>	0.2370 <sup>a</sup>	0.1990 <sup>b</sup>	0.2100 <sup>a</sup>
Priming means	0.1765 <sup>c</sup>	0.1987 <sup>a</sup>	0.1807 <sup>b</sup>	

Alpha = 0.05 %. Critical value for comparison for treatments = 2.250 E-03, priming = 2.756 E-03 and interaction = 3.897 E-03.

Determining ions content in crops grown in stress conditions is an easy approach for assessing salt tolerance capability (Ashraf and Harris, 2004). Plants accumulate numerous nutrients for various physiological and biochemical processes. Plant Zn contents ensure vigorous seedling growth (Harris *et al.*, 2007). The ions like Potassium have a role in the synthesis of cells (Tester and Davenport, 2003). Certain ions (Mn, Cl etc.) are required for the proper photosynthesis functioning, membrane potential regulation, turgidity and pH. The Magnesium ion is a co-factor of enzymes like kinases that catalyzes the reaction involving the transfer of the phosphate group. Iron is a prosthetic group for several enzymes (like cytochrome, catalase, and peroxidase) and is required for many metabolic processes (DNA synthesis, respiration, photosynthesis). Iron also plays a significant role in the early phases of seedling growth (Balk and Pilon 2011; Rout and Sahoo 2015; Guha *et al.* 2018). The Taibi *et al.* (2012) reported that cations can enter the cells through ion channels. These channels may regulate the transport of cations to the xylem. The priming probably has certain effects on these ion channels or pumps responsible for the

transfer of various ions associated with various biological functions. In other words, priming stabilizes the structure of the cell membrane under stress conditions helping in the absorption of certain ions like Calcium. The Calcium ions regulate the translocation of other ions and act as an activator of many ions (Unno *et al.*, 2002). The review of the literature (Harris *et al.*, 2001; Ajouri *et al.*, 2004; Johnson *et al.*, 2005; Chen *et al.*, 2012) has confirmed the significant impact of seed priming on the mineral contents of plants under stress conditions. The present study fully agrees with previous findings regarding the significant effect of priming on the accumulation of certain ions grown under salinity.

### Conclusion

The glucose-induced priming caused significant variations in the morpho-physiological attributes of *Brassica napus* L. The priming durations of 90 minutes were found to be stimulatory for traits like seedlings growth, fresh and dry weight of *Brassica napus*. On the contrary, soaking durations of 30 minutes and 60 minutes was found most appropriate for enhancing germination and moisture contents of



*Brassica napus*, respectively. Furthermore, the pre-soaking of seeds for 60 minutes increased the Calcium, Magnesium and Manganese contents (mg/litre) of *Brassica napus*. The Iron and Zinc contents (mg/litre) showed hype in seedlings raised from seeds primed for 30 minutes. The priming of seeds for 90 minutes was stimulatory for Copper (mg/litre) only. The selected salinity treatments brought significant variations in the studied nutrients contents. Fresh weight may increase or decrease under the influence of salinity treatments. The influence of factors interaction on the studied parameters was also significant.

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