



# Response of Seed Priming to Varied Levels of Salinity in Safflower

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

The experiment was conducted in the year 2020-2021 to study the seed priming technologies and their effects on seed quality and seed physiological parameters under salinity stress conditions in safflower were analysed. The experiment was laid out in a factorial complete randomised design consists of seven different seed priming chemicals like control (untreated), hydropriming, KCl, Salicylic acid, Salicylhydroxamic acid, chitosan and Penconazole. And five levels of salt stress

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condition were created using NaCl which includes 0 (control), 5 g L<sup>-1</sup>, 10 g L<sup>-1</sup>, 15 g L<sup>-1</sup> and 20 g L<sup>-1</sup>. The experiment was carried out in two replications and the effect of priming on some physiological responses of safflower seeds was studied. The results of this investigation showed that seed priming caused a significant increase in all the different seed physiological factors viz. germination %, shoot length, root length, germination factor, Seedling vigour index I and Seedling vigour index II of the safflower seeds. And also results revealed that, Seed priming with KCl and salicylic acid improved the first count (%), seed germination %, shoot length, root length, seedling dry weight, seedling vigour index I and seedling vigour index II in both control and salt stress conditions compared to unprimed seeds. Hence, these results have practical implications that the pre-sowing seed treatment with KCl and salicylic acid could enhance the seed germination and other physiological attributes of safflower seeds under salinity condition.

**Keywords:** Salinity levels; seed priming; seed physiology; seed germination; germination factor; seedling factor; seedling length; seedling vigour indices.

## 1. INTRODUCTION

Safflower (*Carthamus tinctorius* L.) has been grown commercially as one of the oldest oilseed crop. This crop grows well on newly reclaimed soils that are hard to produce other oilseed crops (Kandil et al., 2016). Safflower is an underutilized crop but gaining attention due to its oil yield potential and the ability to grow under abiotic stresses like high temperatures, drought and salinity. Safflower is cultivated mainly for its seed, which is used as edible oil, for its flowers, used as a source of dye and medicinal purposes (Ekin, 2005 and Dordas, 2008). It's oil consumption reduces blood cholesterol level and lipoprotein levels, a risk factor for cardio-vascular diseases. In textile industry, flowers are used for their yellow, red, red-purple and olive pigments and in pharmaceutical and cosmetic industry for their many therapeutic properties. Safflower has been investigated by degrees due to its medicinal value and health care properties and is used in herbal medicine in east Asia for the promotion of bone formation and in the treatment of osteoporosis and rheuma-tism (Bessada et. al. 2015). Oil crops are essential ingredients for food supply due to the growing world population. With the human population expected to reach 9 billion by 2050, it has been estimated that 44% of the required additional calories will come from oil crops (Teh et al. 2017).

Salt poses particular challenges to global agriculture as it already affects 20% of cultivated and 33% of irrigated agricultural lands, with some predictions that salinization could impact 50% of arable lands by 2050 (Aftab et al., 2011). A major issue with safflower production in many parts of the world is salinity. Salinity is an environmental factor limiting plant growth and productivity in arid and semiarid regions. Like most of the

cultivated plants, growth and yield of safflower decrease under salinity. Safflower is rated as a moderately salt tolerant plant and it can produce profitable crops on saline soils. Although safflower is thought to be tolerant, high soil salt levels have also been shown to diminish yields (Bassil and Kaffka, 2002). It exhibits resilience to environmental stresses such as drought and salinity. The deep vertical root system of safflower, capable of penetrating up to 2.2 m into the soil, significantly contributes to its high drought tolerance. Due to these characteristics, safflower is particularly well-suited for arid and semi-arid climates, offering considerable potential for optimizing the efficient utilization of limited water resources (Singh et al., 2017). Soil salinization adversely affects sustainability and productivity of cultivable land by altering seed germination, growth and physio-biochemical attributes consequently limiting agricultural output (Sabarni et al. 2023).

Number of strategies can be used to lessen the effect of salinity on crop yields, including the addition of organic matter (Walker and Bernal, 2008), calcium and potassium additions (Tuna et al., 2007), and genetic engineering techniques to increase plant resistance to salt stress (Cushman and Bohnert, 2000). Another alternative is to use the technique of seed priming to increase tolerance and crop productivity under salt stress. A wide range of adaptations and mitigation strategies are required to cope with such impacts. Efficient resource management and crop improvement for evolving better varieties can help to overcome salinity stress. However, these approaches are time-consuming and expensive, simple solutions for managing salinity stress must be developed that are easy to use and effective. To enhance germination, emergence, and stand establishment in salinity

environments, a variety of strategies are employed which is one of the most widely used techniques. Seed priming is a pre-sowing seed enhancement technique that allows controlled hydration of seeds to imbibe water and go through the first stage of germination but does not allow radical protrusion through the seed coat. It consists of soaking seeds in an osmotic solution where the first two phases of germination (imbibition and activation phases) takes place without emergence and growth of the radicle. This technique has proven its effectiveness for good crop establishment in saline soil (Ahraf and Rauf, 2001 and Basra, 2003). Priming techniques have been used in various crops to overcome salinity stress at germination stage. Seed priming is an efficient method for increasing plant growth and improvement of yield in saline condition (Mc Donald, 2000). Soil salinity imposes ion toxicity, osmotic stress, nutrient (N, Ca, K, P, Fe, Zn) deficiency and oxidative stress on plants, and thus limits water uptake from soil. Soil salinity significantly reduces plant phosphorus uptake because phosphate ions precipitate with Ca ions. Some elements, such as sodium, chlorine, and boron, have specific toxic effects on plants (Pooja and Kumar, 2015). Excessive accumulation of sodium in cell walls can rapidly lead to osmotic stress and cell death. Salinity also affects photosynthesis mainly through a reduction in leaf area, chlorophyll content and stomatal conductance (Acosta-Motos *et al.*, 2017).

Priming is not a simple post harvest enhancement technique, but rather it is the process involves prior exposure of seed to an abiotic stress, making a seed more resistant to future exposure that confers a "cross tolerance" on seeds. For this reason, this study attempt to investigate the germination behavior of safflower seed and to highlight the effect of priming on germination and seedling growth under salt stress.

## 2. MATERIALS AND METHODS

The safflower seeds of variety ISF-764 were soaked for seed priming purpose with the respective chemicals at their concentrations like 50ppm of Salicylic acid (50mg in 1000ml of water), 5 % of KCl (5 mg of KCl in 100 ml), 100ppm of Salicylhydroxamic acid (100 mg of Salicylhydroxamic acid in 1000 ml of water), 0.25 % of Chitosan (0.25 mg of chitosan in 100ml of water) and Penconazole at 15 mg in 1000 ml of

water with 1:5 seed to solution ratio (weight by volume) for 10 hours at room temperature ( $25 \pm 2$  °C) for 48 hours and dried back to its original moisture content. After that, the seeds were uniformly spread over the blotter paper and kept for air drying under shade to re-dry to their original moisture level. Primed seeds were used for assessing the seed quality parameters. Germination paper were immersed in five different saline solutions consisted of 0, 5, 10, 15 and 20 g L<sup>-1</sup> concentrations of NaCl for 6 hours to create salinity stress conditions.

### 2.1 Parameters Measured in this Experiment Were

#### 2.1.1 First count (%)

The standard germination test was conducted in four replications of 100 seeds each by following between paper method was incubated in the germinator maintained at  $25 \pm 2$  °C temperature and  $90 \pm 5$  per cent relative humidity. The first count of germinated seeds was taken on 4<sup>th</sup> day of germination test (Anonymous, 2013).

#### 2.1.2 Germination % (%)

The standard germination test was conducted in four replications of 100 seeds each by following between paper method was incubated in the seed germination room maintained at  $25 \pm 2$  °C temperature and  $90 \pm 5$  per cent relative humidity. The final count was taken on 14<sup>th</sup> day. The numbers of normal seedlings from each replication was counted and mean germination was expressed in % (Anonymous, 2013).

### 2.2 Shoot length (cm)

On the day of final count, ten normal seedlings were selected from each treatment in all replications. The shoot length was measured from the base of primary leaf to the base of hypocotyl and mean shoot length was expressed in centimetre (Anonymous, 2013).

### 2.3 Root Length (cm)

The seedlings used for measuring the shoot length were also used for measuring the root length. It was measured from tip of primary root to the base of hypocotyl and the mean root length was expressed in centimetre (13).

### 2.4 Total Seedling Length (cm)

The sum of shoot and root length gives total seedling length and expressed in centimetres (Anonymous, 2013).

## 2.5 Seedling Dry Weight (mg)

The ten normal seedlings which were used for measuring shoot and root length were placed in butter paper after removing the cotyledons and placed in hot air oven maintained at 70 °C temperature for 24 hours for drying. Then, the seedlings placed in butter paper were removed from hot air oven and allowed to cool in desiccators for 20 minutes before weighing in electronic balance. The average weight was calculated and expressed in milligram (Anonymous, 2013).

## 2.6 Seedling Vigour Index - I

The seedling vigour index - I was calculated by multiplying the germination % with total seedling length (cm) and expressed as whole number (Anonymous, 2013).

Seedling vigour index I = Seed germination (%) × Total seedling length (cm)

## 2.7 Seedling Vigour Index - II

The seedling vigour index - II was calculated by multiplying the germination % with seedling dry weight (mg) and expressed as whole number (14).

Seedling vigour index II = Seed germination (%) × Seedling dry weight (mg)

## 2.8 Germination Factor

Germination factor was calculated by dividing germination % by 100

Germination factor =  $\frac{\text{Germination percentage}}{100}$

## 2.9 Seedling Factor

Germination factor was calculated by dividing seedling length by highest seedling length

Seedling factor =  $\frac{\text{Seedling length}}{\text{Highest seedling length}}$

## 2.10 Germination Seedling Factor

Germination seedling factor was calculated by multiplying germination factor with seedling factor

Germination seedling factor = Germination factor × Seedling factor

## 2.11 Statistical Analysis

The data obtained from the experiments were statistically analyzed by adopting appropriate statistical methods as outlined by Panse and Sukhatme (1985). Whenever 'F' test was found significant, the critical difference (CD) values were calculated and treatment mean were compared at 1 per cent for laboratory observation. The data of parameters in per cent was transferred to arc sine values and used for statistical analysis.

## 3. RESULTS AND DISCUSSION

Analysis of variance indicated that both salinity level and priming have significant effects on studied parameters. The increase in salt stress in culture medium causes a significant decrease in germination %, shoot length, root length and seedling length, seedling dry weight and vigor indices in both control and for primed seed. However, the decrease was more significant for non-primed seeds. Salinity levels and seed priming had a significant effect on physiological parameters. Increasing salinity levels had significantly reduced all the physiological parameters. This reduction was more important in control seed when compared with primed seed when exposed to different salinity levels. Similarly, the effect of seed priming was more profound on first count at high salinity level (20 g L<sup>-1</sup>).

### 3.1 First Count (%) and Seed Germination

KCl seed priming seeds has showed the highest first count (%) and seed germination percent in both control and at different salinity stress levels. There is a significant decrease in the seed quality parameters as the salinity stress levels increased in both control and primed seeds. However, this decrease was more pronounced for un-primed seeds than primed seeds.

Among the different seed priming techniques, seed priming with KCl had significantly increased first count of 15.31 percent compared to unprimed seeds and germination % of 8.67 % when exposed to 20 g L<sup>-1</sup> salinity and compared with non-primed treatment followed by seed priming with salicylic acid. Among the different priming treatments, KCl (T<sub>4</sub>) showed highest first count and seed germination % (71.40 and 89.55 respectively) followed by salicylic acid (T<sub>3</sub>; 70.20 and 88.00 respectively). Unprimed seeds (T<sub>1</sub>)

has showed least first count and seed germination (61.30 and 82.30) and folowed by hydropriming (T<sub>2</sub>; 63.40 and 83.00 respectively)

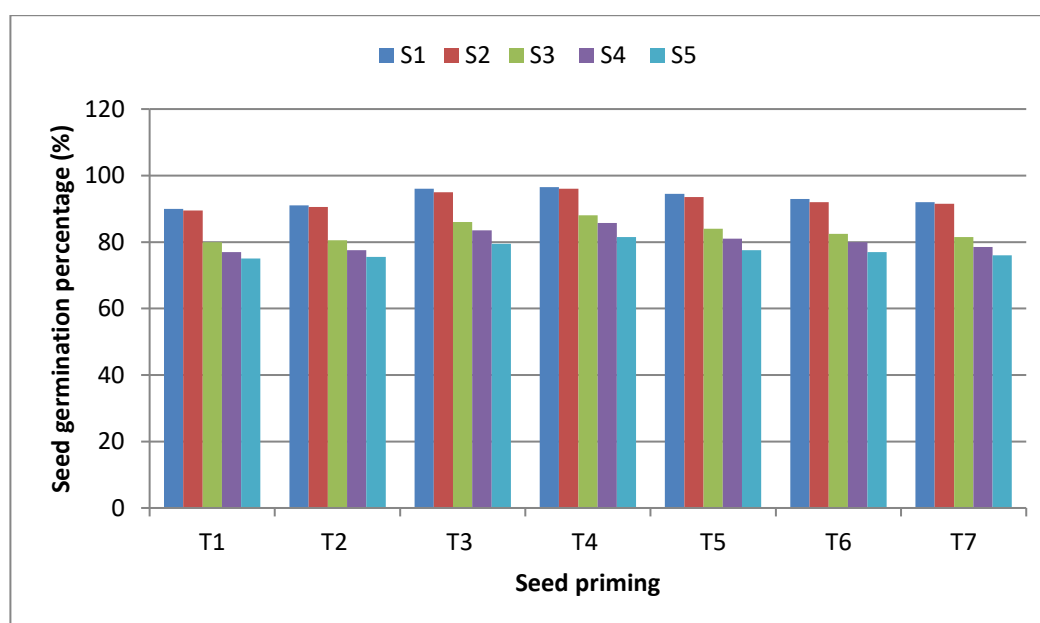
Among the different salinity levels, 0g L<sup>-1</sup> (Control) treatment has recorded highest first count and seed germination % (71.57 and 93.29 respectively) followed by at 5 g L<sup>-1</sup> salinity stress level(70.29 and 92.57 respectively). Lowest first count and seed germination % was recorded in at 20 g/l salinity stress level (59.93 and 77.43 respectively) followed by at 15 g L<sup>-1</sup> salinity stress level (64.43 and 80.46 respectively)

KCl primed seeds showed that highest first count and seed germination in both control and at 20g L<sup>-1</sup> salinity stress level (77.00 and 64.00,96.50 and 81.50 respectively) followed bysalicylic acid in control and at 20g L<sup>-1</sup> salinity stress level (76.00 and 63.50, 96.00 and 79.50 respectively). And the least first count and seed germination% was recorded by unprimed seeds in both control and at 20g L<sup>-1</sup> salinity stress level (65.00 and 90.00, 55.50 and 75.00) followed by hydropriming(68.00 and 91.00, 56.00 and 75.50 respectively) and depicted in Table 1 and Figs. 1 and 2.

**Table 1. Influence of seed priming on first count(%), seed germination %, shoot length(cm), root length (cm) and seedling dry weight (mg) in safflower in salinity stress condition**

Treatments	First count (%)	Germination % (%)	Shoot length (cm)	Root length (cm)	Seedling dry weight (mg)
<b>Factor 1: Priming treatments</b>					
T <sub>1</sub> = Control	61.30	82.30	7.50	14.99	0.12
T <sub>2</sub> = Hydropriming	63.40	83.00	7.62	15.35	0.13
T <sub>3</sub> = Salicylic acid (50 ppm)	70.20	88.00	8.30	16.74	0.18
T <sub>4</sub> = KCl (5 % for 24 h) at 25°C	71.40	89.55	8.50	17.11	0.19
T <sub>5</sub> = Salicylhydroxamic acid (100 ppm)	68.50	86.10	8.13	16.28	0.16
T <sub>6</sub> = Chitosan (0.25 %)	66.90	84.90	7.92	16.04	0.15
T <sub>7</sub> = Penconazole at 15 mg <sup>-1</sup>	64.90	83.90	7.75	15.59	0.14
<b>SEm±</b>	<b>0.27</b>	<b>0.12</b>	<b>0.03</b>	<b>0.09</b>	<b>0.00</b>
<b>CD at 1 %</b>	<b>1.03</b>	<b>0.45</b>	<b>0.13</b>	<b>0.34</b>	<b>0.01</b>
<b>F test</b>	<b>S</b>	<b>S</b>	<b>S</b>	<b>S</b>	<b>S</b>
<b>Factor 2: Salinity stress</b>					
S <sub>1</sub> = Control	71.57	93.29	8.55	16.51	0.20
S <sub>2</sub> = NaCl at 5 g L <sup>-1</sup>	70.29	92.57	8.30	16.30	0.18
S <sub>3</sub> = NaCl at 10 g L <sup>-1</sup>	67.07	83.21	7.93	16.02	0.16
S <sub>4</sub> = NaCl at 15 g L <sup>-1</sup>	64.43	80.46	7.65	15.73	0.13
S <sub>5</sub> = NaCl at 20 g L <sup>-1</sup>	59.93	77.43	7.37	15.50	0.10
<b>SEm±</b>	<b>0.20</b>	<b>0.09</b>	<b>0.02</b>	<b>0.07</b>	<b>0.00</b>
<b>CD at 1 %</b>	<b>0.73</b>	<b>0.32</b>	<b>0.09</b>	<b>0.24</b>	<b>0.01</b>
<b>F test</b>	<b>S</b>	<b>S</b>	<b>S</b>	<b>S</b>	<b>S</b>
S <sub>1</sub> T <sub>1</sub>	65.00	90.00	8.10	15.50	0.17
S <sub>1</sub> T <sub>2</sub>	68.00	91.00	8.25	15.85	0.18
S <sub>1</sub> T <sub>3</sub>	76.00	96.00	8.85	17.20	0.23
S <sub>1</sub> T <sub>4</sub>	77.00	96.50	9.05	17.55	0.24
S <sub>1</sub> T <sub>5</sub>	73.00	94.50	8.74	16.85	0.22
S <sub>1</sub> T <sub>6</sub>	72.00	93.00	8.50	16.52	0.20
S <sub>1</sub> T <sub>7</sub>	70.00	92.00	8.35	16.10	0.19
S <sub>2</sub> T <sub>1</sub>	64.50	89.50	7.85	15.25	0.15
S <sub>2</sub> T <sub>2</sub>	67.00	90.50	7.95	15.72	0.17
S <sub>2</sub> T <sub>3</sub>	74.00	95.00	8.60	17.00	0.20
S <sub>2</sub> T <sub>4</sub>	75.00	96.00	8.85	17.30	0.22
S <sub>2</sub> T <sub>5</sub>	72.00	93.50	8.46	16.65	0.19
S <sub>2</sub> T <sub>6</sub>	71.00	92.00	8.32	16.33	0.18
S <sub>2</sub> T <sub>7</sub>	68.50	91.50	8.10	15.89	0.17
S <sub>3</sub> T <sub>1</sub>	63.00	80.00	7.50	15.00	0.12

Treatments	First count (%)	Germination % (%)	Shoot length (cm)	Root length (cm)	Seedling dry weight (mg)
S <sub>3</sub> T <sub>2</sub>	64.00	80.50	7.65	15.35	0.13
S <sub>3</sub> T <sub>3</sub>	70.00	86.00	8.30	16.85	0.18
S <sub>3</sub> T <sub>4</sub>	72.00	88.00	8.50	17.00	0.19
S <sub>3</sub> T <sub>5</sub>	69.00	84.00	8.00	16.23	0.17
S <sub>3</sub> T <sub>6</sub>	66.50	82.50	7.85	16.10	0.16
S <sub>3</sub> T <sub>7</sub>	65.00	81.50	7.70	15.60	0.14
S <sub>4</sub> T <sub>1</sub>	58.50	77.00	7.15	14.85	0.10
S <sub>4</sub> T <sub>2</sub>	62.00	77.50	7.25	15.00	0.11
S <sub>4</sub> T <sub>3</sub>	67.50	83.50	8.00	16.40	0.16
S <sub>4</sub> T <sub>4</sub>	69.00	85.75	8.28	16.90	0.18
S <sub>4</sub> T <sub>5</sub>	66.50	81.00	7.80	15.95	0.14
S <sub>4</sub> T <sub>6</sub>	65.00	80.00	7.65	15.70	0.13
S <sub>4</sub> T <sub>7</sub>	62.50	78.50	7.40	15.30	0.12
S <sub>5</sub> T <sub>1</sub>	55.50	75.00	6.90	14.33	0.07
S <sub>5</sub> T <sub>2</sub>	56.00	75.50	7.00	14.85	0.08
S <sub>5</sub> T <sub>3</sub>	63.50	79.50	7.75	16.25	0.12
S <sub>5</sub> T <sub>4</sub>	64.00	81.50	7.80	16.79	0.13
S <sub>5</sub> T <sub>5</sub>	62.00	77.50	7.65	15.70	0.11
S <sub>5</sub> T <sub>6</sub>	60.00	77.00	7.30	15.55	0.10
S <sub>5</sub> T <sub>7</sub>	58.50	76.00	7.20	15.05	0.09
<b>SEm±</b>	<b>1.37</b>	<b>0.60</b>	<b>0.17</b>	<b>0.45</b>	<b>0.01</b>
<b>CD at 1 %</b>	<b>5.13</b>	<b>2.26</b>	<b>0.64</b>	<b>1.70</b>	<b>0.05</b>
<b>F test</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>



**Fig. 1. Influence of seed priming on seed germination % in safflower in salinity stress condition**

Data revealed the significant effect of seed priming triggered the safflower germination in salinity stress. This study demonstrated that germination and other quality parameters from primed seeds were significantly higher from unprimed seeds when exposed to different salinity levels. Similar findings were reported by Kaya et

al., 2006. It seem also that, salinity stress affects seed germination via the limitation of seed water absorption, excessive use of nutrient pool and creation of disorders in protein synthesis (Mohamed and Cherif Hannachi, 2012). Seed priming triggers the production of transcription factors which phosphorylates MAPKs and

CDPKs (Cyclin dependent protein kinases) promoting expression of stress-responsive genes (Bukhat et al., 2020). The water absorption efficiency is much better in primed seeds and primed seeds observe more water from growing media, that's why metabolic activities in seed during germination process commence much earlier than radicle and plumule appearance. Generally increased salinity decreases seed germination capacity and this may be due to the toxic effects of Na<sup>+</sup> and Cl<sup>-</sup> in the process of germination (Khajeh-Hosseini et al., 2003).

### 3.2 Shoot and Root Length (cm)

Among the different priming treatments, KCl(T<sub>4</sub>) showed highest shoot and root length (8.50 and 17.11 cm respectively) followed by salicylic acid (T<sub>3</sub>; 8.30 and 16.74 cm respectively). Unprimed seeds (T<sub>1</sub>) has showed least shoot and root length (7.50 and 14.99) and followed by hydropriming (T<sub>2</sub>; 7.62 and 15.35 respectively)

Among the different salinity levels, 0g L<sup>-1</sup> (S<sub>1</sub>; Control) treatment has recorded highest shoot and root length (8.55 and 16.51 cm respectively) followed by at 5 g L<sup>-1</sup> salinity stress level (8.30 and 16.30 cm respectively). Lowest shoot and root length was recorded in at 20 g L<sup>-1</sup> salinity stress level (7.37 and 15.50 cm respectively) followed by at 15 g L<sup>-1</sup> salinity stress level (7.65 and 15.73 respectively).

KCl primed seeds showed that highest shoot and root length in both control and at 20g L<sup>-1</sup> salinity stress level (9.05 and 7.80 cm, 17.55 and 16.79

respectively) followed by salicylic acid in control and at 20g L<sup>-1</sup> salinity stress level (8.85 and 7.75, 17.20 and 16.25 respectively). And the least was recorded by unprimed seeds in both control and at 20g L<sup>-1</sup> salinity stress level (8.10 and 6.90, 15.50 and 14.33) followed by hydropriming (8.25 and 7.00, 15.85 and 14.85 respectively). Under saline environment, shortest seedling length was observed in unprimed seeds because of accumulation of salts which affects the plant ability to access water and nutrients, further impeding overall seedling growth. Reduction in total seedling length under salt stress has been reported in several other species (Achakzai et al., 2010 & Akram et al., 2019). It has also been reported that salinity suppresses the uptake of essential nutrients like potassium and phosphorous (Nasim et al., 2008), which could adversely affect the seedlings growth in turn reduces the total seedling length. KCl seed priming can enhance osmotic adjustment by increasing the osmolyte concentration in seeds, thus improving water uptake and reducing water stress during germination and early seedling growth leads to improvement in overall total seedling length. Reprogramming of antioxidant genes has been documented to protect the primed seedlings from NaCl-induced damages enabling improved growth and development (Thomas and Puthur 2019). The results obtained in the present investigation are in accordance with the findings of Katembe et al., 1998 in Atriplex species, Cicek and Cakirlar, 2002 in maize and Ahmadvand et al., 2012 in soybean.

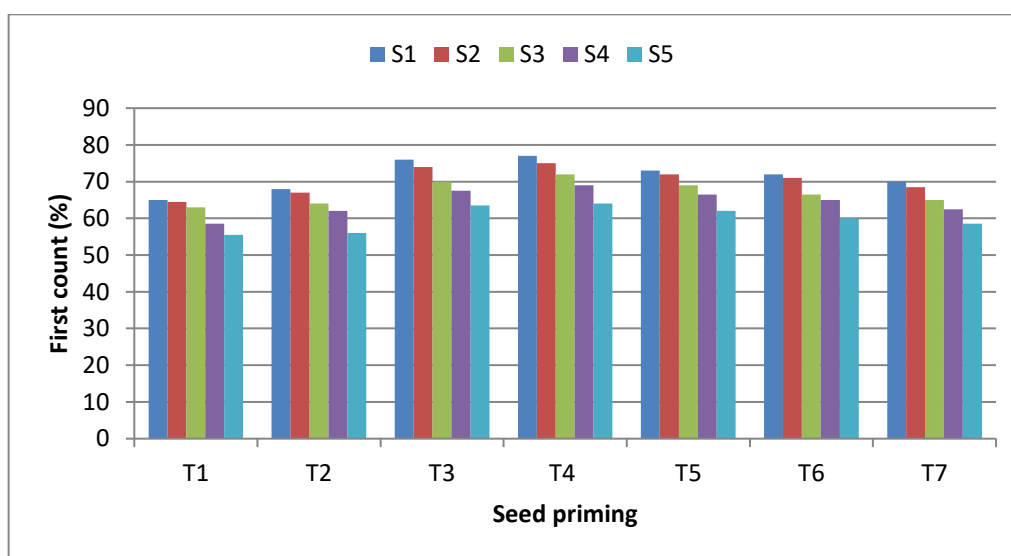


Fig. 2. Influence of seed priming on first count (%) in safflower in salinity stress condition

### 3.3 Seedling Length (cm) and Seedling Dry Weight (mg)

Among the different priming treatments, KCl ( $T_4$ ) showed highest seedling length (cm) and seedling dry weight (25.60 cm and 0.19 mg respectively) followed by salicylic acid ( $T_3$ ; 25.04 cm and 0.18 mg respectively). Unprimed seeds ( $T_1$ ) has showed lowest seedling length and seedling dry weight (22.49 cm and 0.12 mg) and followed by hydropriming ( $T_2$ ; 22.97 cm and 0.13 mg respectively)

Among the different salinity levels, 0g L<sup>-1</sup> ( $S_1$ ; Control) treatment has recorded highest seedling length (cm) and seedling dry weight (25.06 cm and 0.20 mg respectively) followed by at 5 g L<sup>-1</sup> salinity stress level (24.61 cm and 0.18 mg respectively). Lowest seedling length (cm) and seedling dry weight was recorded in at 20 g L<sup>-1</sup> salinity stress level (22.87 cm and 0.10 mg respectively) followed by at 15 g L<sup>-1</sup> salinity stress level (23.37 cm and 0.13 mg respectively)

KCl primed seeds showed that highest seedling length (cm) and seedling dry weight in both control and at 20g L<sup>-1</sup> salinity stress level (26.60 cm and 24.59 cm, 0.17 and 0.13 mg respectively) followed by salicylic acid in control and at 20g L<sup>-1</sup> salinity stress level (26.05 cm and 24.00 cm, 0.23 mg and 0.12 mg respectively). And the least was recorded by unprimed seeds in both control and at 20g L<sup>-1</sup> salinity stress level (23.60 and 21.23 cm, 0.17 and 0.07 mg) followed by hydropriming (24.10 and 21.85 cm, 0.18 and 0.08 mg respectively). Salicylic acids as an internal regulator play an important role in the defense mechanisms against biotic and abiotic stress (Szalai *et al.*, 2000). Hayat *et al.* (2005) also reported that pre-treatment with salicylic acid lead to increase dry and fresh weight of leaves. The results are depicted in Table 1.

### 3.4 Seedling Vigor Indices

Among the different priming treatments, KCl ( $T_4$ ) showed highest (2297) seedling vigour index I and seedling vigour index II (17.18) followed by salicylic acid ( $T_3$ ; 2208 and 15.78 respectively). Unprimed seeds ( $T_1$ ) have showed lowest Seedling vigour index I and Seedling vigour index II (1855 and 10.26) and followed by hydropriming ( $T_2$ ; 1912 and 11.19 respectively)

Among the different salinity levels, 0g L<sup>-1</sup> ( $S_1$ ; Control) treatment have recorded highest

seedling vigour index I and seedling vigour index II (2340 and 18.88 respectively) followed by at 5 g L<sup>-1</sup> salinity stress level (2280 and 16.77 respectively). Lowest seedling vigour index I and seedling vigour index II was recorded in at 20 g/l salinity stress level (1773 and 7.73 respectively) followed by at 15 g L<sup>-1</sup> salinity stress level (1884 and 10.72 respectively)

KCl primed seeds showed that highest seedling vigour index I and seedling vigour index II in both control and at 20g L<sup>-1</sup> salinity stress level (2569 and 22.58, 2004 and 10.60 respectively) followed by salicylic acid in control and at 20g L<sup>-1</sup> salinity stress level (2502 and 21.45, 1908 and 9.54 respectively). The least was recorded by unprimed seeds in both control and at 20g L<sup>-1</sup> salinity stress level (2124 and 15.30, 1592 and 5.25) followed by hydropriming (2192 and 16.38, 1650 and 6.04 respectively).

Results have shown that seedling vigour index-I and seedling vigour index-II have varied significantly among the different seed priming treatments. SVI-I in primed seeds was more than the control (Badar-us-zaman *et al.*, 2012). This improvement in vigour of primed seeds might be due to mobilization of reserve food materials, activation and re-synthesis of some enzymes and DNA and RNA synthesis during osmotic priming (Sadeghi *et al.*, 2011). The repair mechanism caused by seed priming results into rapid embryo growth (Ungar, 1996). Higher vigour index was attributed to higher germination per cent and seedling length. The SVI-I and SVI II increased when the salt concentration decreased, which revealed that increased sodium chloride concentration caused a detrimental effect in the seed. Since, seed priming emphasis on repair mechanism and stress tolerance, KCl primed seeds showed highest seed germination and seedling dry weight and SVI-I and SVI-II are calculated based on seed germination percentage and seedling dry weight. Hence SVI-1 and SVI-11 were found highest in KCl primed seeds. Under elevated salinity stress, both seed germination per cent and total seedling length decreased significantly ultimately, it declined the seedling vigour (Basra *et al.*, 2003).

### 3.5 Germination Factor

Seeds primed with KCl ( $T_4$ ) showed highest germination factor in both control and salinity conditions. In control, KCl ( $T_4$ ) recorded significantly highest germination factor (0.90) followed by salicylic acid ( $T_3$ ; 0.88). And the least



germination factor was recorded in unprimed seeds ( $T_1$ ; control-0.82).

Among the different salinity levels, there was significant difference observed for germination factor. Highest germination factor was noticed in  $S_1$  and  $S_2$  (0.93) followed by  $S_3$  (0.83). least germination factor was recorded in  $S_5$  (0.77) followed by  $S_4$  (0.80).

KCl primed seeds showed that highest germination factor in both control and at  $20\text{g L}^{-1}$  salinity stress level (0.97 and 0.82 respectively) followed by salicylic acid in control and at  $20\text{g L}^{-1}$  salinity stress level (0.96 and 0.80 respectively). And the least was recorded by unprimed seeds in both control and at  $20\text{g L}^{-1}$  salinity stress level (0.90 and 0.75 respectively) followed by hydropriming (0.95 and 0.76 respectively).

Safflower seeds primed with different chemicals germinated faster than unprimed ones as it has been reported by Ashraf and Rauf working with other priming treatments, such as polyethylene glycol (PEG), inorganic salts or even ABA. According to Bewley and Black 1982, seed priming leads to the initiation of primary metabolic processes, so the time required for germination is reduced. This positive effect is probably due to the stimulatory effect of priming on later stages of the germination process through the mediation of cell division in germinated seeds (Sivritepe et al., 2002). Prolonged salt exposure affects photosynthesis and stomatal conductance and increases transpiration rate (Farooq et al., 2015). Argerich and Bradford, 1989, found that the swelling of the embryo inside primed tomato seed may speed up germination by facilitating water absorption.

### 3.6 Seedling Factor

Among the different priming treatments, KCl ( $T_4$ ) showed highest seedling factor (0.93) followed by salicylic acid ( $T_3$ ; 0.91 respectively). Unprimed seeds ( $T_1$ ) has showed lowest seedling factor (0.81) and followed by hydropriming ( $T_2$ ; 0.83)

Among the different salinity levels,  $0\text{ g L}^{-1}$  ( $S_1$ ; Control) and  $5\text{g L}^{-1}$  (NaCl at  $5\text{ g L}^{-1}$ ) treatment has recorded highest seedling factor (0.9.3) followed by at  $10\text{ g L}^{-1}$  salinity stress level (0.83). Lowest seedling factor was recorded in at  $20\text{ g L}^{-1}$  salinity stress level (0.77) followed by at  $15\text{ g L}^{-1}$  salinity stress level (0.80 respectively)

KCl primed seeds showed that highest seedling factor in both control and at  $20\text{g L}^{-1}$  salinity stress level (0.97 and 0.86 respectively) followed by

salicylic acid in control and at  $20\text{g L}^{-1}$  salinity stress level (0.96 and 0.82 respectively). And the least was recorded by unprimed seeds in both control and at  $20\text{g L}^{-1}$  salinity stress level (0.90 and 0.75) followed by hydropriming (0.91 and 0.76 respectively).

### 3.7 Germination Seedling Factor

Seeds primed with KCl ( $T_4$ ) showed highest germination seedling factor in both control and salinity conditions. In control, KCl ( $T_4$ ) recorded significantly highest germination seedling factor (0.83) followed by salicylic acid ( $T_3$ ;0.80). And the least germination factor was recorded in unprimed seeds ( $T_1$ ; control-0.67).

Among the different salinity levels, there was significant difference observed for germination seedling factor. Highest germination seedling factor was noticed in  $S_1$ (0.84) and followed by  $S_2$  (0.82) and least germination seedling factor was recorded in  $S_5$  (0.64) followed by  $S_4$  (0.68).

KCl primed seeds showed that highest germination seedlingfactor in both control and at  $20\text{g L}^{-1}$  salinity stress level (0.92 and 0.72 respectively) followed by salicylic acid in control and at  $20\text{g L}^{-1}$ salinity stress level (0.90 and 0.69 respectively). And the least was recorded by unprimed seeds in both control and at  $20\text{g L}^{-1}$  salinity stress level (0.76 and 0.57 respectively) followed by hydropriming (0.79 and 0.59 respectively). The results are depicted in Table 2.

The similar results are with the findings of Jumsoon et al., 1996 that Indeed, the priming is an effective technique that increases seed vigor and improves germination and seedling growth. Jiang et al. (2016) reported the role of maize seed priming with 0.4, 0.8 and 1.6 mM solutions of melatonin for 24 h at room temperature in alleviation of salinity by decreasing mean emergence time, electrolyte leakage,  $\text{Na}^+$  content and lipid peroxidation. Several studies have shown that seed priming homogenized seed germination in a short period of time (Hopper et al., 1979). Seed Priming with KCl and Salicylhydroxamic acid helps to withstand the stress by repairing the cell damage, protein misfolding caused due to salinity and triggers the protein synthesis, activation of enzymes and antioxidant machinery, formation of new mitochondria and DNA repair and also priming triggers cell division, synthesis of nucleic acids and ATP to increase cellular energy (Devika et al., 2021.)

**Table 2. Influence of seed priming on seedling length and seedling vigour index I and seedling vigour index II of safflower under different salinity stress condition**

Treatments	Seedling length (cm)	SVI – I	SVI – II	Germination factor	Seedling factor	Germination seedling factor
<b>Factor 1: Priming treatments</b>						
T <sub>1</sub> = Control	22.49	1855	10.26	0.82	0.81	0.67
T <sub>2</sub> = Hydropriming	22.97	1912	11.19	0.83	0.83	0.69
T <sub>3</sub> = Salicylic acid (50 ppm)	25.04	2208	15.78	0.88	0.91	0.80
T <sub>4</sub> = KCl (5 % for 24 h) at 25°C	25.60	2297	17.18	0.90	0.93	0.83
T <sub>5</sub> = Salicylhydroxamic acid (100 ppm)	24.40	2107	14.37	0.86	0.88	0.76
T <sub>6</sub> = Chitosan (0.25 %)	23.96	2040	13.22	0.85	0.87	0.74
T <sub>7</sub> = Penconazole at 15 mg <sup>-1</sup>	23.34	1962	11.89	0.84	0.85	0.71
<b>SEm±</b>	<b>0.10</b>	<b>9.57</b>	<b>0.27</b>	<b>0.0012</b>	<b>0.0041</b>	<b>0.0038</b>
<b>CD at 1 %</b>	<b>0.38</b>	<b>35.82</b>	<b>1.01</b>	<b>0.0045</b>	<b>0.0154</b>	<b>0.0141</b>
<b>F test</b>	<b>S</b>	<b>S</b>	<b>S</b>	<b>S</b>	<b>S</b>	<b>S</b>
<b>Factor 2: Salinity stress</b>						
S <sub>1</sub> = Control	25.06	2340	18.88	0.93	0.90	0.847
S <sub>2</sub> = NaCl at 5 g L <sup>-1</sup>	24.61	2280	16.77	0.93	0.89	0.826
S <sub>3</sub> = NaCl at 10 g L <sup>-1</sup>	23.95	1995	12.97	0.83	0.86	0.723
S <sub>4</sub> = NaCl at 15 g L <sup>-1</sup>	23.37	1884	10.72	0.80	0.84	0.682
S <sub>5</sub> = NaCl at 20 g L <sup>-1</sup>	22.87	1773	7.73	0.77	0.82	0.642
<b>SEm±</b>	<b>0.07</b>	<b>6.83</b>	<b>0.19</b>	<b>0.001</b>	<b>0.003</b>	<b>0.003</b>
<b>CD at 1 %</b>	<b>0.27</b>	<b>25.59</b>	<b>0.72</b>	<b>0.003</b>	<b>0.011</b>	<b>0.010</b>
<b>F test</b>	<b>S</b>	<b>S</b>	<b>S</b>	<b>S</b>	<b>S</b>	<b>S</b>
S <sub>1</sub> T <sub>1</sub>	23.60	2124	15.30	0.90	0.85	0.769
S <sub>1</sub> T <sub>2</sub>	24.10	2192	16.38	0.91	0.87	0.793
S <sub>1</sub> T <sub>3</sub>	26.05	2502	21.45	0.96	0.94	0.905
S <sub>1</sub> T <sub>4</sub>	26.60	2569	22.58	0.97	0.96	0.929
S <sub>1</sub> T <sub>5</sub>	25.59	2418	20.34	0.95	0.93	0.876
S <sub>1</sub> T <sub>6</sub>	25.02	2329	18.60	0.93	0.91	0.845
S <sub>1</sub> T <sub>7</sub>	24.45	2244	17.48	0.92	0.89	0.814
S <sub>2</sub> T <sub>1</sub>	23.10	2067	13.43	0.90	0.84	0.749
S <sub>2</sub> T <sub>2</sub>	23.67	2142	14.94	0.91	0.86	0.776
S <sub>2</sub> T <sub>3</sub>	25.60	2431	19.02	0.95	0.93	0.880

Treatments	Seedling length (cm)	SVI – I	SVI – II	Germination factor	Seedling factor	Germination seedling factor
S <sub>2</sub> T <sub>4</sub>	26.15	2510	20.61	0.96	0.95	0.909
S <sub>2</sub> T <sub>5</sub>	25.11	2347	17.77	0.94	0.91	0.850
S <sub>2</sub> T <sub>6</sub>	24.65	2267	16.56	0.92	0.89	0.821
S <sub>2</sub> T <sub>7</sub>	23.99	2195	15.10	0.92	0.87	0.795
S <sub>3</sub> T <sub>1</sub>	22.50	1800	9.60	0.80	0.81	0.652
S <sub>3</sub> T <sub>2</sub>	23.00	1852	10.47	0.81	0.83	0.671
S <sub>3</sub> T <sub>3</sub>	25.15	2163	15.52	0.86	0.91	0.783
S <sub>3</sub> T <sub>4</sub>	25.50	2244	16.69	0.88	0.92	0.812
S <sub>3</sub> T <sub>5</sub>	24.23	2035	13.86	0.84	0.88	0.737
S <sub>3</sub> T <sub>6</sub>	23.95	1976	13.21	0.83	0.87	0.715
S <sub>3</sub> T <sub>7</sub>	23.30	1899	11.41	0.82	0.84	0.688
S <sub>4</sub> T <sub>1</sub>	22.00	1694	7.70	0.77	0.80	0.613
S <sub>4</sub> T <sub>2</sub>	22.25	1724	8.14	0.78	0.81	0.624
S <sub>4</sub> T <sub>3</sub>	24.40	2037	13.37	0.84	0.88	0.738
S <sub>4</sub> T <sub>4</sub>	25.18	2159	15.44	0.86	0.91	0.782
S <sub>4</sub> T <sub>5</sub>	23.75	1924	11.36	0.81	0.86	0.697
S <sub>4</sub> T <sub>6</sub>	23.35	1868	10.00	0.80	0.85	0.676
S <sub>4</sub> T <sub>7</sub>	22.70	1782	9.02	0.79	0.82	0.645
S <sub>5</sub> T <sub>1</sub>	21.23	1592	5.25	0.75	0.77	0.576
S <sub>5</sub> T <sub>2</sub>	21.85	1650	6.04	0.76	0.79	0.597
S <sub>5</sub> T <sub>3</sub>	24.00	1908	9.54	0.80	0.87	0.691
S <sub>5</sub> T <sub>4</sub>	24.59	2004	10.60	0.82	0.89	0.726
S <sub>5</sub> T <sub>5</sub>	23.35	1810	8.53	0.78	0.85	0.655
S <sub>5</sub> T <sub>6</sub>	22.85	1759	7.71	0.77	0.83	0.637
S <sub>5</sub> T <sub>7</sub>	22.25	1691	6.46	0.76	0.81	0.612
<b>SEm±</b>	<b>0.50</b>	<b>47.83</b>	<b>1.34</b>	<b>0.006</b>	<b>0.02</b>	<b>0.018</b>
<b>CD at 1 %</b>	<b>1.89</b>	<b>179.12</b>	<b>5.03</b>	<b>0.022</b>	<b>0.07</b>	<b>0.07</b>
<b>F test</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>

#### 4. CONCLUSION

From the obtained results, it was found that KCl seed priming treatment had significantly improved all the physiological parameters when compared to all other treatments under salt stress. Thus, the priming may be an effective method to meet the demands of farmers during the installation of the culture in the field and especially in conditions of salt stress. Future studies can be conducted at molecular level to analyse the changes occurred in seeds caused due to seed priming treatments, different seed invigoration methods other than chemicals like bio-seed priming to mitigate the salt stress can be studied and studies on use of foliar spray of chemicals to mitigate the salinity stress in safflower can be undertaken.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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