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(Research Article)

A Study on The Determination of The Effects of Salicylic Acid, Ascorbic Acid and Different Salt Concentrations on The Germination of Fodder Pea (*Pisum sativum ssp. arvense* L.) Plants at Various Salt Concentrations

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Abstract

This study aimed to investigate the effects of ascorbic acid (AsA) and salicylic acid (SA) on the germination and early seedling growth of fodder pea (*Pisum sativum* ssp. *arvense* L.) under various salt (NaCl) concentrations. Four concentrations of both SA and AsA (0, 50, 100, and 150 mM) were applied under four different NaCl concentrations (0, 50, 100, and 150 mM). Key parameters such as germination time (days), root length (cm), hypocotyl length (cm), and germination rate (%) were evaluated. The results indicated that increased salt concentrations negatively impacted germination. However, applications of 50 mM and 100 mM AsA and SA demonstrated positive effects on the measured parameters. It was concluded that optimal applications of AsA and SA can positively influence fodder pea germination under saline conditions.

Keywords: *Pisum sativum* ssp. *arvense* L.; Saliyclic acid; Ascorbic acid; NaCl

1. Introduction

A valuable fodder plant from the legume family, the fodder pea (*Pisum sativum* ssp. *arvense* L.) is extremely productive, very adaptable, and capable of fixing nitrogen to the soil. It also leaves a clean field for subsequent plants (Timurağaoğlu ve ark., 2004; Uzun ve ark., 2012; Kadıoğlu ve Tan, 2018; Temel ve Yazıcı, 2021; Dumanoğlu ve ark., 2021). An annual, cool-season forage crop, fodder pea is used for hay, grain feed, silage, pasture, and green manure (Manga et al., 2003; Acikbas et al., 2021). Seed production is carried out to meet both the production material needs and the concentrated feed needs of animals (Sayar, 2021). When harvested at the appropriate time, it is a forage crop with high forage yield and quality. Fodder pea, which grows taller and develops green parts compared to table pea, generally has pink flowers. However, as with table pea, white-flowered varieties are also available.

The environmental stressor known as salinity stress is a member of the chemical stress group that impacts plants under cultivation. Numerous negative effects result from the excessive salt content of the growing environment (Kara et al., 2019). According to Orcutt and Nilsen (2000), these negative effects include oxidative stress, osmotic incompatibility, water intake imbalance, membrane dysfunction, enzyme activation issues, nutritional imbalance, general metabolic process abnormalities, and overall developmental failure.

Some studies have shown that external applications of calcium, potassium, or phosphorus to plants under salt stress compete with sodium in the plant's roots and leaves, reducing its uptake. The plant's capacity to tolerate stress rises as

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its levels of these ions climb to a level where it can do so (Hasegawa and Bressan, 2000; Kaya et al., 2001; Kaya and Higgs, 2003).

Applying salicylic acid has been shown to promote seed germination at both high and low temperatures (Korkmaz, 2005; Özdener and Kutbay, 2008; Ekinci et al. 2011). According to Rivas-San Vicente and Plasencia (2011), salicylic acid is a phenolic molecule that functions as a phytohormone and helps regulate a number of growth and development stages, such as photosynthesis, respiration, flowering, and senescence, of which germination is an essential component. Numerous studies have demonstrated that the effects of salinity on plants can be mitigated by using salicylic acid as a priming therapy (Jini and Joseph, 2017; Anaya et al. 2018).

Ascorbic acid increases the amount of nutrients and water available to plants under salt stress by regulating plant metabolism and development (Torlak 2019). Another strategy to lessen the detrimental effects of salt stress on plants is to use non-enzymatic antioxidant molecules, such as ascorbic acid (Khan et al. 2006).

The purpose of this study was to ascertain the effects of applying salicylic and ascorbic acids on the germination of fodder pea seeds at varying salinity levels.

2. Materials and methods

The study was carried out in 2023 at the Medicinal and Aromatic Plants Laboratory of Balıkesir University Altınoluk Vocational School. Seeds of *Pisum arvense* L. were employed as plant material. In the study, two different priming applications (SA) and (AsA), four distinct concentrations (0, 50, 100, and 150 mM) NaCl and priming application were taken into consideration.

Salt stress was induced using analytical grade NaCl. The seeds were surface sterilized in a 5% sodium hypochlorite solution for ten minutes prior to germination (Uyanık et al., 2014). According to Nazarian (2016), seeds that had been surface sterilized were held in varying concentrations of SA solutions for 12 hours in order to prime them. After that, they were dried on drying papers at room temperature for 24 hours in order to restore their original moisture content (Paşa and Yardan, 2024a).

Following these treatments, the seeds were sown in petri plates at a temperature of 20±1 °C. Seeds were first deemed viable based on ISTA (1996) guidelines. Four-by-twenty-five seeds were germination tested for fourteen days in petri dishes sandwiched between two layers of blotting paper (ISTA, 1996).

The following parameters were measured: average germination time (days), root length (cm), hypocotyl length (cm), germination power (%), germination rate (%), and germination index (%). By dividing the total number of sowed seeds by the number of germinated seeds acquired on the 7th and 14th days, germination power and germination rate were calculated (Akıncı and Çalışkan, 2010; Paşa and Yardan, 2024b).

The TARIST statistical tool was used to statistically examine the experiment's data. The means were compared using the LSD test.

3. Results

3.1. The Effect of Ascorbic Acid on Germination Rate

According to the study, the germination rate of fodder pea seeds in varying concentrations of NaCl solution should be analyzed at the 5 % significance level in order to determine the differences between salt doses, ascorbic acid doses, and the Salt x AsA interaction (Table 1).

When the averages of salt concentrations were examined, the highest germination rate was 98.50 % in 0 mM NaCl, and the lowest germination rate was 82.00 % in 150 mM NaCl.

Upon analyzing the average ascorbic acid dosages, the greatest germination rate was 97.0 % in 50 mM AsA, while the lowest germination rate was 86.75 % in 0 mM AsA. Examining the salt x AsA interaction, the 0 mM x 50 mM, 0 mM x 100 mM, 50 mM x 50 mM, and 50 mM x 100 mM interactions showed the maximum germination rate of 100.00 %, while the 150 mM X 0 mM interaction showed the lowest germination rate of 73.00 %.

Table 1 Effect of ascorbic acid on seed germination rate (%)*

NaCL /AsA	0 mM	50 mM	100 mM	150 mM	Means
0 mM	94.00 c	100.00 a	100.00 a	100.00 a	98.50 a
50 mM	92.00 d	100.00 a	100.00 a	100.00 a	98.00 a
100 mM	88.00 e	97.00 b	93.00 c	91.00 d	92.25 b
150 mM	73.00 i	91.00 d	85.00 f	79.00 g	82.00 c
Means	86.75 d	97.00 a	94.50 b	92.50 c	

LSD $_{salinity}$: 3,152; LSD $_{AsA}$:1,783; LSD $_{Interaction}$: 2,844

*There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

3.2. The Effect of Salicylic Acid on Germination Rate

With regard to the germination rate of fodder pea seeds in varying concentrations of NaCl solution, the study has demonstrated that the differences between salt doses, salicylic acid doses, and the Salt x SA interaction should be investigated at a 5 % significant level (Table 2).

Table 2 Effect of salicylic acid on seed germination rate (%)*

NaCL /SA	0 mM	50 mM	100 mM	150 mM	Means
0 mM	92.00 d	100.00 a	100.00 a	87.00 f	94.75 a
50 mM	90.00 e	98.00 b	96.00 c	81.00 h	91.25 b
100 mM	89.00 e	95.00 c	85.00 g	78.00 i	86.75 c
150 mM	69.00 m	81.00 h	73.00 l	67.00 n	72.50 d
Means	85.00 c	93.50 a	88.50 b	78.25 d	

LSD $_{salinity}$: 2,343; LSD $_{SA}$:3,192; LSD $_{Interaction}$: 1,847; *There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

The germination rate was highest at 94.75 % with 0 mM NaCl and lowest at 72.50% with 150 mM NaCl when the averages of salt concentrations were analyzed. A comparison of the typical salicylic acid dosages revealed that the germination rate was 78.25 % in 150 mM SA and 93.50 % in 50 mM SA. Examining the salt x SA interaction, the 0 mM x 50 mM and 0 mM x 100 mM interactions showed the maximum germination rate of 100.00 %, while the 150 mM x 150 mM interaction showed the lowest germination rate of 67.00 %.

3.3. Effect of Ascorbic Acid on Germination Time

In the study, it has been shown that the difference between Salt doses, Ascorbic acid doses and Salt x AsA interaction in terms of germination time of fodder pea seeds in different concentrations of NaCl solution should be examined at the 5 % significance level (Table 3).

Table 3 Effect of ascorbic acid on seed germination time (days)*

NaCL /AsA	0 mM	50 mM	100 mM	150 mM	Means
0 mM	2.58 c	1.37 f	1.93 e	2.82 b	2.18 c
50 mM	2.64 c	2.02 d	2.11 d	2.70 c	2.37 c
100 mM	3.12 b	2.43cd	2.63 c	2.95 b	2.78 b
150 mM	3.94 a	3.74 a	2.7 5	3.14 b	3.39 a
Means	3.07 a	2.39 c	2.36 c	2.90 b	

LSD $_{salinity}$: 0,221; LSD $_{ASA}$:0, 148; LSD $_{Interaction}$: 0,315; *There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

The fastest germination time 2.18 days was observed at 0 mM NaCl when the average salt concentrations were analyzed, while the slowest germination time 3.39 days was observed at 150 mM NaCl. The earliest germination times in 50 mM AsA and 100 mM AsA were 2.39 and 2.33 days, respectively, when the average ascorbic acid dosages were analyzed. The latest germination time in 0 mM AsA was 3.07 days. The earliest germination time was found to be 1.37 days in the 0 mM x 50 mM interaction when the salt x AsA interaction was investigated. The latest germination times were found to be 3.94 days in the 150 mM x 0 mM interaction and 3.74 days in the 150 mM x 50 mM interaction.

3.4. Effect of Salicylic Acid on Germination Time

The study demonstrated that the germination period of fodder pea seeds in varying concentrations of NaCl solution should be analyzed at a 5 % significance level in order to compare the effects of salt doses, salicylic acid dosages, and the Salt x SA interaction (Table 4).

Table 4 Effect of salicylic acid on seed germination time (days)*

NaCL /SA	0 mM	50 mM	100 mM	150 mM	Means
0 mM	2.62 e	1.56 g	2.14 f	3.74 b	2.52 c
50 mM	2.69 e	2.12 f	2.53 e	3.56 c	2.73 c
100 mM	3.24 d	2.73 e	3.15 d	4.08 a	3.30 b
150 mM	4.11 a	3.97 a	3.86 b	4.03 a	3.99 a
Means	3.17 b	2.60 d	2.92 c	3.85a	

LSD $_{salinity}$: 0,326; LSD $_{SA}$:0, 273; LSD $_{Interaction}$: 0,184; *There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

In terms of germination time, the earliest was 2.52 and 2.73 days at 0 mM NaCl and 50 mM NaCl, and the latest was 3.99 days at 150 mM NaCl, when the averages of salt concentrations were analyzed. The earliest germination period was 2.60 days at 50 mM SA, and the latest germination time was 3.85 days at 150 mM SA, when the average salicylic acid dosages were analyzed. Upon analyzing the salt x SA interaction, the fastest germination time (1.56 days) was recorded in the 0 mM x 50 mM interaction. Conversely, the slowest (latest) germination times were observed under high salinity, such as 4.11 days in the 150 mM x 0 mM interaction and 4.08 days in the 100 mM x 150 mM interaction.

3.5. The Effect of Ascorbic Acid on Root Length

In the study, it has been shown that the difference between Salt doses, Ascorbic acid doses and Salt x AsA interaction in terms of root length of fodder pea seeds in different concentrations of NaCl solution should be examined at a 5% significance level (Table 5). When the average salt concentrations were examined, the highest root length was found with 6.49 cm in 0 mM NaCl, and the lowest root length was found at 4.59 cm in 150 mM NaCl. When the average of ascorbic acid doses were examined, the highest root length was determined as 6.65 cm and 6.41 cm in 100 mM AsA and 50 mM AsA, and the lowest root length was 4.37 cm in 0 mM AsA. When the salt x AsA interaction was examined, the highest root length was determined as 7.40 cm in the 0 mM x 100 mM interaction, and the lowest root length was 3.28 cm in the 150 mM X 0 mM interaction.

Table 5 Effect of ascorbic acid on root length (cm)*

NaCL /AsA	0 mM	50 mM	100 mM	150 mM	Means
0 mM	5.08 f	6.88 b	7.40 a	6.60 c	6.49 a
50 mM	4.74 g	6.56 c	7.32 a	6.13 d	6.19 b
100 mM	4.36 h	6.32 d	6.74 b	5.78 e	5.80 c
150 mM	3.28 l	5.89de	5.12 f	4.08 i	4.59 d
Means	4.37 c	6.41 a	6.65 a	5.65 b	

LSD salinity: 0,336; LSD AsA:0,342; LSD Interaction: 0,248; *There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

3.6. Effect of Salicylic Acid on Root Length

In order to investigate the effects of salt doses, salicylic acid dosages, and the Salt x SA interaction, the study showed that the root length of fodder pea seeds in different concentrations of NaCl solution should be examined at a 5% significant level (Table 6).

Table 6 Effect of salicylic acid on root length (cm)*

NaCL /SA	0 mM	50 mM	100 mM	150 mM	Ortalama
0 mM	4.38d	5.26a	5.31a	5.09b	5.01a
50 mM	4.08e	4.93bc	5.14b	4.80c	4.74b
100 mM	3.24f	4.44d	4.70c	4.38d	4.19c
150 mM	2.86g	3.24f	4.08e	2.92g	3.28d
Ortalama	3.64c	4.47b	4.81a	4.30b	

LSD salinity: 0,261; LSD $_{SA}$:0,288; LSD Interaction: 0,14; *There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

Upon analyzing the average salt concentrations, the longest root length was 5.01 cm in 0 mM NaCl, while the shortest was 3.28 cm in 150 mM NaCl. The greatest root length, 4.81 cm in 100 mM SA, and the smallest, 3.64 cm in 0 mM SA, were found when the average salicylic acid dosages were analyzed. The largest root lengths, 5.31 cm and 5.26 cm in the 0 mM x 100 mM and 0 mM x 50 mM interactions, and the smallest, 2.92 cm and 2.86 cm in the 150 mM x 150 mM and 150 mM x 0 mM interactions, were found when the salt x SA interaction was investigated.

3.7. The Effect of Ascorbic Acid on Hypocotyl Length

In the study, it has been shown that the difference between Salt doses, Ascorbic acid doses and Salt x AsA interaction in terms of hypocotyl length of fodder pea seeds in different concentrations of NaCl solution should be examined at a 5% significance level (Table 7).

Table 7 Effect of ascorbic acid on hypocotyl length (cm)*

NaCL /AsA	0 mM	50 mM	100 mM	150 mM	Ortalama
0 mM	3.33c	3.44b	3.48a	3.53a	3.45a
50 mM	3.14d	3.18d	3.20d	3.27c	3.20b
100 mM	2.73f	2.91e	2.76f	2.86e	2.82c
150 mM	2.31g	2.10h	1.98i	2.35g	2.18d
Ortalama	2.88c	2.91b	2.86c	3.00a	

LSD salinity: 0,188; LSD AsA:0,723; LSD Interaction: 0,839; *There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

When the average salt concentrations were examined, the highest hypocotyl length was found with 3.45 cm in 0 mM NaCl, and the lowest hypocotyl length was found at 2.18 cm in 150 mM NaCl. When the average of ascorbic acid doses were examined, the highest hypocotyl length was determined as 3.00 cm 150 mM AsA, and the lowest hypocotyl length was 2.88 cm in 0 mM AsA. When the salt x AsA interaction was examined, the highest hypocotyl length was determined as 3.48 cm in the 0 mM x 100 mM interaction, and the lowest hypocotyl length was 1.98 cm in the 150 mM X 100 mM interaction.

3.8. Effect of Salicylic Acid on Hypocotyl Length

The study demonstrated that the hypocotyl length of fodder pea seeds in varying concentrations of NaCl solution should be analyzed at a 5 % significance level in order to compare the effects of salt doses, salicylic acid dosages, and the Salt x SA interaction (Table 8).

Table 8 Effect of salicylic acid on hypocotyl length (cm)*

NaCL /SA	0 mM	50 mM	100 mM	150 mM	Ortalama
0 mM	3.86cd	4.23c	4.55b	3.68d	4.08a
50 mM	3.58d	4.38b	5.01a	2.83f	3.95b
100 mM	3.21e	4.11c	4.53b	2.52f	3.59c
150 mM	2.17g	3.33e	3.86cd	2.11g	2.87d
Ortalama	3.21c	4.01b	4.49a	2.79d	

LSD salinity: 0,105; LSD $_{SA}$:0,382; LSD Interaction: 0,373; *There is no statistical (p ‹0.05) differences between values with the same letters in the same colums.

Examining the average salt concentrations, the hypocotyl lengths in 0 mM NaCl and 150 mM NaCl were found to be the highest and lowest, respectively, at 4.08 cm and 2.87 cm. The greatest hypocotyl length, measured at 4.49 cm in 100 mM SA, and the smallest, measured at 2.79 cm in 150 mM SA, were found when the average salicylic acid dosages were analyzed. Examining the salt x SA interaction, the 50 mM x 100 mM interaction had the largest hypocotyl length (5.01 cm), whereas the 150 mM X 150 mM and 150 mM X 0 mM interactions had the lowest hypocotyl lengths (2.11 cm and 2.17 cm, respectively).

4. Discussion

The ASA system functions dynamically in seeds, despite the fact that the processes for generating and using ascorbic acid (ASA) can vary based on the developmental and functional stages of the seed. Tullio and Arrigoni (2007) state that orthodox seeds have a minor amount of dehydroascorbic acid (DHA) and considerable ASA recycling enzyme activity, but neither ASA nor ASA peroxidase at quiescence. This is due to the fact that in orthodox seeds, ASA content and ASA peroxidase activity rise in the early phases of development before falling throughout the desiccation phase. In addition to controlling the physiology of stress in plants, ascorbic acid (AsA) is necessary for the germination phase (Arrigoni et al. 1997; Noctor and Foyer, 1998; Conklin, 2001).

The results of this investigation regarding AsA align with those of Mohsen et al. (2013), Bassuony et al. (2008), and Erkoyuncu and Yorgancılar's (2020) studies.

It was discovered that when increasing SA concentrations were coupled with increasing salt concentrations, the parameters analyzed in this study were negatively affected. Under conditions of extreme salt stress, salicylic acid helps seeds germinate by reducing oxidative damage (Lee et al., 2010). The findings of this study, where SA applications mitigated the negative effects of salinity, align with previous research. For example, similar to the work of El-Tayeb (2005) on barley, our results showed that SA can enhance germination under salt stress. This protective effect is also consistent with findings from studies by Yaver and Paşa (2009), Farahbakhsh (2012), Jam et al. (2012), Soliman et al. 2016), Ramanujam et al. (1998), Mendoza et al. (2002), Tari et al. (2002), Paşa (2022), Paşa and Yardan (2024a), and Paşa and Yardan (2024b).

5. Conclusion

Thus, it was found that increasing NaCl concentrations had a negative effect on the root and hypocotyl lengths of fodder pea seeds, as well as the germination rate and number of days to germination. However, these parameters peaked in treatments with 50 and 100 mM of both SA and AsA. It was found that proper SA and AsA application may have a beneficial effect on the germination of fodder pea plants under saline circumstances.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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