



Effect of Exogenous IAA on Single and Combined Salt and Drought Stress Responses in Tall Fescue Cultivars with Different Tolerance Levels

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Abstract

The increasing occurrence of combined drought and salinity stress poses a significant threat to plant growth and productivity. As a fundamental auxin, indole-3-acetic acid (IAA) plays a crucial role in plant stress responses by regulating growth and physiological mechanisms. This study investigated the effects of exogenous IAA treatment on germination and seedling growth characteristics under single or combined salinity and drought stresses in three *Festuca arundinacea* (tall fescue) cultivars ('Grande', 'Starlet', and 'Titan RX'), with varying levels of tolerance to these stresses. In the study, the efficacy of IAA under stress conditions was determined by evaluating germination rate, radicle and plumule length, radicle and plumule dry weight. Exogenous IAA treatment led to a slight improvement in salinity and drought tolerance, particularly in the more stress-sensitive cultivars. The most pronounced IAA-related enhancement was observed in 'Starlet', whereas the response of 'Titan RX' remained limited under the tested conditions. The results of the study suggest that exogenous IAA treatment can be used to enhance early developmental stage tolerance in tall fescue plants showing high and moderate sensitivity to salinity and drought, under areas having these stress conditions.

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1. Introduction

As the impacts of climate change become more pronounced, the intensity and prevalence of abiotic stress factors have also increased (Chaudhry and Sidhu, 2022). Recent studies indicate that plants are frequently exposed to multiple abiotic stresses simultaneously, leading to more complex and distinct physiological responses compared to single stress conditions (Demirkol and Yilmaz, 2023). Consequently, investigating the interactions and combined effects of multiple stress factors has become increasingly critical in plant stress physiology research. In recent years, salt and drought stresses have been among the most significant abiotic factors causing yield and quality losses in plants (Ibrahim et al., 2019; Kurgan and Temel, 2024). Research indicates that these stresses frequently co-occur in various regions, exacerbating their detrimental effects on plant growth and productivity (Abobatta, 2020). *Festuca arundinacea* (tall fescue), a perennial cool-season grass, is a widely utilized forage and turf species due to its strong adaptability (Sato, 2022). However, salinization and drought conditions pose a significant constraint on the growth of tall fescue, adversely impacting turf persistence, forage yield and quality (Kaplan et al., 2017; Chen et al., 2022). Notably, it exhibits substantial intra-population variability in tolerance to salinity and drought stresses (Esmailpournoghadam et al., 2024). Therefore, it is essential to reveal the differences in mechanisms between susceptible and resistant genotypes in tall fescue. Indole-3-acetic acid (IAA) is a key auxin that regulates plant growth and development, particularly by influencing cell elongation, root architecture, and stress responses (Gomes and Scortecci, 2021). Exogenous treatment of IAA has been found to enhance plant tolerance to osmotic stress by modulating antioxidant defense systems, improving water and nutrient uptake, and maintaining ion homeostasis (Amombo et al., 2017). However, research on the effects of IAA under salinity and drought stress conditions in tall fescue plants remains limited. The aim of this study was to investigate the

effect of IAA on germination and seedling growth characteristics under single or combined salt and drought stress conditions in tall fescue cultivars with varying levels of tolerance to these stresses.

2. Material and Methods

2.1. Material

The experiment was conducted using seeds from three tall fescue cultivars ('Grande', 'Starlet', and 'Titan RX'). The seeds were surface-sterilized by immersing them in a 70% (v/v) ethanol solution with constant shaking for 1 minute, followed by three washes with sterile distilled water.

2.2. Growth and stress conditions

The experiment was conducted using a Completely Randomized Factorial Design with four replications. Each replication consisted of 50 seeds placed in a 120-mm Petri dish, a size commonly used for stress-related germination studies (Kurgan and Temel, 2024). Seeds were placed between filter papers inside petri dishes. The petri dishes were divided into two main groups: one group received 10 mL indole-3-acetic acid (IAA; 5 mg L⁻¹) treatment, while the other group served as the control without IAA treatment (10 mL distilled water). Within each main group, nine different treatment combinations were applied: (1) PEG- NaCl-, (2) PEG₁ NaCl-, (3) PEG₁ NaCl₁, (4) PEG₂ NaCl-, (5) PEG₂ NaCl₁, (6) PEG₂ NaCl₂, (7) PEG- NaCl₁, (8) PEG- NaCl₂, and (9) PEG₁ NaCl₂. In this context, PEG- indicates no drought stress, while PEG₁ and PEG₂ correspond to drought stress levels of -5 and -10 bar, respectively. Similarly, NaCl- indicates no salinity stress, whereas NaCl₁ and NaCl₂ represent salinity levels of 100 mM and 200 mM NaCl, respectively. PEG solutions were prepared using PEG-6000 in distilled water following Michel and Kaufmann (1973) to achieve the desired osmotic potentials, while NaCl solutions were prepared by dissolving analytical-grade NaCl in distilled water at the specified molarities. At the beginning of the experiment, 10 mL of the respective treatment solution were applied to each Petri dish to ensure proper moistening of the filter papers.

Subsequently, 5 mL of pure water were added as required to maintain adequate moisture (Kurgan and Temel, 2024). The petri dishes were placed in an incubator at a temperature of 24 ± 1 °C, which was determined as the optimal germination temperature for the tall fescue cultivars used in this study (data not shown). Throughout the experiment, the seeds were monitored daily, and those that developed a radicle of at least 2 mm in length were considered germinated (Kurgan and Temel, 2024). Samples were collected on the 14th day, which was considered sufficient to evaluate the effects of stress treatments, as seedlings exposed to the highest stress levels showed pronounced dehydration and growth reduction by that time. The germination rate (%), the lengths (cm) and fresh weights of the roots and shoots in tall fescue cultivars were measured. Then the samples were kept in an oven at 60 °C for 72 h (the samples reached a constant weight within this time frame). Afterwards, dry weights (g plant^{-1}) of the roots and shoots were determined.

2.3. Statistical analyses

The experimental data were analyzed using a Completely Randomized Factorial Design. Statistical analyses were conducted with the Minitab software package, and results were interpreted at a 5% significance level following two-way ANOVA. Data comparisons were performed using Tukey's multiple comparison test. In this study, IAA treatment and stress factors were considered as two independent factors.

3. Results and Discussion

The analysis of variance conducted in the study revealed that the treatments differed significantly in all evaluated traits (Table 1-5). The study demonstrated that increasing stress

levels adversely affected all measured traits in the tested cultivars, with differences in the degree of response reflecting their relative tolerance tendencies. Grande and Starlet showed more pronounced growth reductions, whereas Titan RX maintained relatively stable performance, suggesting variation in inherent tolerance capacity rather than a direct statistical comparison among cultivars. In Titan RX, which displayed stable growth under stress, IAA treatment did not result in a statistically significant change compared to the control. In the study, Grande cultivar plants treated with IAA exhibited higher germination rates under NaCl_2 , PEG-, and combined NaCl_2 PEG₂ stress conditions, compared to non-treated plants (Table 1). A similar effect was observed in the Starlet cultivar under NaCl_1 PEG₂, NaCl_2 PEG-, NaCl_2 PEG₁, and NaCl_2 PEG₂ stress conditions (Table 1). Similar findings in IAA treatment were observed in plumule length in three cultivars (Table 3). In the study, Grande cultivar plants treated with IAA exhibited longer radicle lengths under NaCl_1 PEG₂ and NaCl_2 PEG- stress conditions, compared to non-treated plants. A similar response was observed in the Starlet cultivar under NaCl_1 PEG₁, NaCl_1 PEG₂, NaCl_2 PEG-, NaCl_2 PEG₁, and NaCl_2 PEG₂ conditions (Table 2). In the study, IAA-treated Grande cultivar plants exhibited higher radicle dry weight under NaCl_1 PEG₁ and NaCl_1 PEG₂ stress conditions, compared to non-treated plants. A similar effect was observed in the Starlet cultivar under NaCl_1 PEG₂ and NaCl_2 PEG₂ conditions (Table 4). In the study, Grande cultivar plants treated with IAA showed higher shoot dry weight under NaCl_2 PEG- stress conditions, compared to non-treated plants. A similar effect was observed in the Starlet cultivar under NaCl_1 PEG₁, NaCl_1 PEG₂, NaCl_2 PEG-, and NaCl_2 PEG₁ conditions (Table 5).

Table 1. The effect of stress treatments on germination rate (%) in tall fescue cultivars

	Grande		Starlet		Titan RX	
	IAA (-)	IAA (+)	IAA (-)	IAA (+)	IAA (-)	IAA (+)
NaCl (-) PEG (-)	95.1±5.1 Aa	93.7±6.0 Aa	96.7±4.0 Aa	93.8±6.4 Aa	93.9±4.5 Aa	94.2±4.2 Aa
NaCl (-) PEG (1)	76.2±3.4 Ab	74.6±2.8 Ab	89.8±5.4 Aa	88.0±3.5 Aa	90.7±3.6 Aa	91.2±5.8 Aa
NaCl (-) PEG (2)	41.2±1.7 Ac	48.7±3.4 Ac	61.7±3.6 Ac	59.7±2.9 Ab	80.2±4.4 Ab	85.5±6.1 Aab
NaCl (1) PEG (-)	65.3±2.8 Ab	59.6±1.8 Ac	81.7±2.3 Ab	86.4±6.7 Aa	89.4±2.8 Aa	83.4±5.4 Aab
NaCl (1) PEG (1)	38.2±2.6 Ac	44.7±2.4 Ac	58.2±1.6 Ac	65.8±3.7 Ab	79.6±5.4 Ab	82.4±3.8 Aab
NaCl (1) PEG (2)	11.7±0.8 Ad	14.0±1.0 Ade	37.3±2.8 Bd	59.7±2.1 Ab	77.5±5.9 Ab	80.3±4.1 Aab
NaCl (2) PEG (-)	10.1±0.5 Bd	19.1±1.1 Ad	43.4±1.9 Bd	64.6±2.0 Ab	77.9±3.8 Ab	78.7±2.8 Ab
NaCl (2) PEG (1)	10.8±0.7 Ad	13.0±0.8 Ade	35.0±2.2 Bd	51.2±3.4 Abc	68.2±4.0 Abc	64.5±3.9 Ac
NaCl (2) PEG (2)	3.5±0.2 Be	10.6±0.5 Ae	11.2±0.7 Be	45.7±1.8 Ac	59.0±3.5 Ac	62.4±4.4 Ac

The values in the table represent the mean ± standard error. Within the same cultivar and stress treatment, differences between IAA treatment means that do not share a common uppercase letter are statistically significant ($p < 0.05$). Likewise, within the same cultivar and IAA treatment, differences between stress treatment means that do not share a common lowercase letter are statistically significant ($p < 0.05$).

Table 2. The effect of stress treatments on radicle length (mm) in tall fescue cultivars

	Grande		Starlet		Titan RX	
	IAA (-)	IAA (+)	IAA (-)	IAA (+)	IAA (-)	IAA (+)
NaCl (-) PEG (-)	52.3±3.4 Aa	50.4±2.9 Aa	47.2±2.7 Aa	48.6±2.8 Aa	55.9±4.3 Aa	56.1±2.7 Aa
NaCl (-) PEG (1)	44.3±3.2 Ab	46.8±2.4 Aa	45.9±2.9 Aa	46.5±2.9 Aa	57.3±2.8 Aa	59.2±2.9 Aa
NaCl (-) PEG (2)	32.1±1.8 Ac	35.0±2.9 Ab	46.2±3.0 Aa	44.9±3.5 Aa	57.6±2.9 Aa	57.1±3.2 Aa
NaCl (1) PEG (-)	34.5±2.0 Ac	32.8±2.0 Ab	45.1±1.8 Aa	46.3±3.2 Aa	53.9±3.5 Aa	55.5±2.1 Aa
NaCl (1) PEG (1)	30.6±1.1 Ac	33.1±1.5 Ab	36.7±1.9 Bb	43.2±4.0 Aab	52.9±4.1 Aa	54.7±4.2 Aa
NaCl (1) PEG (2)	25.3±1.5 Bcd	30.9±1.8 Abc	26.1±2.1 Bc	40.8±2.2 Aab	45.0±2.7 Ab	49.2±3.3 Aab
NaCl (2) PEG (-)	20.7±1.3 Bd	27.1±1.6 Ac	27.8±2.2 Bc	38.6±1.7 Ab	51.9±1.9 Aa	53.3±2.8 Aa
NaCl (2) PEG (1)	13.6±0.8 Ae	12.1±0.6 Ad	25.9±1.8 Bc	34.4±1.9 Ab	44.7±4.2 Ab	43.4±1.9 Ab
NaCl (2) PEG (2)	7.5±0.5 Af	8.8±0.5 Ae	12.6±0.8 Bd	18.7±1.1 Ac	32.6±2.4 Ac	35.1±1.2 Ac

The values in the table represent the mean ± standard error. Within the same cultivar and stress treatment, differences between IAA treatment means that do not share a common uppercase letter are statistically significant ($p < 0.05$). Likewise, within the same cultivar and IAA treatment, differences between stress treatment means that do not share a common lowercase letter are statistically significant ($p < 0.05$).

Table 3. The effect of stress treatments on plumule length (mm) in tall fescue cultivars

	Grande		Starlet		Titan RX	
	IAA (-)	IAA (+)	IAA (-)	IAA (+)	IAA (-)	IAA (+)
NaCl (-) PEG (-)	83.2±3.2 Aa	88.9±4.5 Aa	70.5±5.2 Aa	68.7±2.1 Aa	64.3±3.4 Aa	66.2±2.4 Aa
NaCl (-) PEG (1)	60.5±2.5 Ab	62.4±3.9 Aab	61.2±4.1 Ab	66.4±2.8 Aa	67.1±2.8 Aa	68.9±3.5 Aa
NaCl (-) PEG (2)	49.2±2.9 Ac	54.3±2.7 Ab	52.3±3.8 Ac	58.0±3.5 Ab	61.8±3.9 Aa	64.0±4.1 Aa
NaCl (1) PEG (-)	63.6±4.0 Ab	59.7±3.1 Aab	59.7±2.9 Ab	56.5±2.9 Ab	64.3±4.5 Aa	62.7±2.9 Aa
NaCl (1) PEG (1)	52.6±4.8 Ac	55.1±3.2 Ab	52.8±4.8 Ac	57.8±4.2 Ab	49.5±3.4 Ab	52.1±2.8 Ab
NaCl (1) PEG (2)	37.5±2.7 Ad	41.0±2.6 Ac	48.9±2.5 Bc	59.1±3.8 Ab	47.5±2.9 Ab	48.6±3.7 Ab
NaCl (2) PEG (-)	44.7±3.0 Bcd	56.1±2.1 Ab	50.5±1.9 Bc	58.9±4.1 Ab	46.8±3.6 Ab	49.8±1.8 Ab
NaCl (2) PEG (1)	46.5±1.9 Acd	43.4±3.7 Ac	41.6±3.7 Bd	50.5±3.7 Ac	47.9±4.1 Ab	50.4±2.9 Ab
NaCl (2) PEG (2)	14.8±1.0 Be	21.0±1.4 Ad	26.9±2.0 Be	46.8±3.0 Ad	45.6±2.8 Ab	51.0±4.6 Ab

The values in the table represent the mean ± standard error. Within the same cultivar and stress treatment, differences between IAA treatment means that do not share a common uppercase letter are statistically significant ($p < 0.05$). Likewise, within the same cultivar and IAA treatment, differences between stress treatment means that do not share a common lowercase letter are statistically significant ($p < 0.05$).

Table 4. The effect of stress treatments on radicle dry weight (g seedling⁻¹) in tall fescue cultivars

	Grande		Starlet		Titan RX	
	IAA (-)	IAA (+)	IAA (-)	IAA (+)	IAA (-)	IAA (+)
NaCl (-) PEG (-)	0.025±0.002 Aa	0.023±0.001 Aa	0.020±0.001 Aa	0.021±0.002 Aa	0.030±0.001 Aa	0.029±0.002 Aa
NaCl (-) PEG (1)	0.018±0.001 Ab	0.018±0.001 Ab	0.021±0.002 Aa	0.019±0.001 Aa	0.032±0.002 Aa	0.033±0.003 Aa
NaCl (-) PEG (2)	0.015±0.001 Ab	0.017±0.000 Ab	0.019±0.001 Aa	0.019±0.000 Aa	0.029±0.002 Aa	0.030±0.002 Aa
NaCl (1) PEG (-)	0.016±0.000 Ab	0.018±0.001 Ab	0.016±0.001 Ab	0.017±0.001 Aa	0.030±0.002 Aa	0.029±0.002 Aa
NaCl (1) PEG (1)	0.013±0.001 Bbc	0.018±0.001 Ab	0.015±0.001 Ab	0.017±0.001 Aa	0.028±0.002 Aa	0.030±0.003 Aa
NaCl (1) PEG (2)	0.010±0.002 Bc	0.015±0.001 Abc	0.014±0.000 Bb	0.018±0.001 Aa	0.028±0.001 Aa	0.029±0.002 Aa
NaCl (2) PEG (-)	0.011±0.001 Ac	0.013±0.001 Ac	0.014±0.001 Ab	0.014±0.001 Aab	0.021±0.002 Ab	0.024±0.001 Ab
NaCl (2) PEG (1)	0.006±0.000 Ad	0.005±0.000 Ad	0.009±0.000 Ac	0.011±0.000 Ab	0.019±0.001 Ab	0.022±0.001 Ab
NaCl (2) PEG (2)	0.005±0.000 Ad	0.006±0.000 Ad	0.005±0.000 d	0.008±0.000 Ac	0.018±0.001 Ab	0.017±0.001 Ac

The values in the table represent the mean ± standard error. Within the same cultivar and stress treatment, differences between IAA treatment means that do not share a common uppercase letter are statistically significant ($p < 0.05$). Likewise, within the same cultivar and IAA treatment, differences between stress treatment means that do not share a common lowercase letter are statistically significant ($p < 0.05$).

The observed improvement in studied traits following IAA treatment, particularly in the more stress-sensitive cultivars, highlights the potential of exogenous IAA to partially alleviate the adverse effects of salinity and drought stress during early developmental stages. The stimulatory effect of IAA in these genotypes suggests that hormonal priming may be particularly effective in enhancing stress resilience where the plant's intrinsic tolerance mechanisms are limited or moderately functional. These genotype-specific responses

indicate that auxin-mediated modulation of early growth traits is not uniform across cultivars but depends on their physiological capacity to perceive and respond to stress-related hormonal signals. Moreover, the relatively stable performance observed in Titan RX suggests that cultivars with higher inherent tolerance may already maintain optimal auxin homeostasis and antioxidant regulation, thereby reducing the apparent impact of external IAA application.

Table 5. The effect of stress treatments on plumule dry weight (g seedling⁻¹) in tall fescue cultivars

	Grande		Starlet		Titan RX	
	IAA (-)	IAA (+)	IAA (-)	IAA (+)	IAA (-)	IAA (+)
NaCl (-) PEG (-)	0.050±0.003 Aa	0.048±0.003 Aa	0.042±0.002 Aa	0.044±0.003 Aa	0.039±0.003 Aa	0.036±0.002 Aa
NaCl (-) PEG (1)	0.041±0.003 Ab	0.041±0.004 Ab	0.034±0.003 Bb	0.043±0.02 Aa	0.042±0.002 Aa	0.039±0.003 Aa
NaCl (-) PEG (2)	0.036±0.001 Ab	0.039±0.003 Ab	0.028±0.001 Ac	0.030±0.002 Ab	0.041±0.004 Aa	0.040±0.004 Aa
NaCl (1) PEG (-)	0.038±0.002 Ab	0.037±0.002 Ab	0.029±0.003 Ac	0.028±0.002 Ab	0.025±0.001 Ab	0.028±0.002 Ab
NaCl (1) PEG (1)	0.035±0.003 Ab	0.037±0.003 Ab	0.025±0.001 Bc	0.027±0.002 Ab	0.023±0.001 Ab	0.024±0.002 Ab
NaCl (1) PEG (2)	0.026±0.001 Ac	0.024±0.001 Ac	0.026±0.002 Bc	0.027±0.001 Ab	0.022±0.001 Ab	0.024±0.003 Ab
NaCl (2) PEG (-)	0.017±0.001 Bd	0.025±0.001 Ac	0.018±0.000 Bd	0.016±0.001 Ac	0.024±0.001 Ab	0.022±0.002 Ab
NaCl (2) PEG (1)	0.014±0.000 Ad	0.015±0.000 Ad	0.012±0.001 Be	0.014±0.000 Ac	0.023±0.002 Ab	0.025±0.003 Ab
NaCl (2) PEG (2)	0.008±0.000 Ae	0.010±0.000 Ae	0.010±0.000 Ae	0.09±0.000 Ad	0.016±0.000 Ac	0.014±0.001 Ac

The values in the table represent the mean ± standard error. Within the same cultivar and stress treatment, differences between IAA treatment means that do not share a common uppercase letter are statistically significant ($p < 0.05$). Likewise, within the same cultivar and IAA treatment, differences between stress treatment means that do not share a common lowercase letter are statistically significant ($p < 0.05$).

Although previous studies have reported that IAA treatment can enhance stress tolerance against salinity or drought individually in several plant species (Alam et al., 2020; Zhang et al., 2020; Kang et al., 2023), to the best of our knowledge, this is the first study to investigate the effectiveness of IAA across genotypes with differing sensitivity levels. Unlike earlier reports focusing on a single stress factor, this research provides a broader perspective by revealing that the combined NaCl × PEG stress exerts a more pronounced inhibitory effect, and that IAA can only partly counteract this suppression in genotypes with limited tolerance potential. Earlier studies have reported that the likely mode of action in IAA treatment may involve improved water uptake, stabilization of cell membranes, or activation of stress-responsive pathways modulated by auxin signaling (El Sabagh et al., 2022; Ali et al., 2025). Our findings support these assumptions and further suggest that the magnitude of IAA-induced enhancement is genotype-dependent, reflecting the interplay between stress perception, hormonal cross-talk, and metabolic adjustment capacity. However, these findings were interpreted as cultivar-specific tendencies rather than direct statistical comparisons, in line with the experimental design. This observation

underlines that exogenous IAA application may be more effective as a compensatory mechanism in genotypes with lower inherent resilience rather than as a universal stress mitigation strategy. These findings emphasize the importance of tailoring stress mitigation strategies to the tolerance level of the cultivar and suggest that IAA application could be a valuable tool for enhancing seed performance in stress-prone environments, particularly for vulnerable or moderately resilient genotypes. The data also point to a potential limitation of low-dose applications, which may be insufficient to overcome the complex constraints imposed by abiotic stresses, especially in sensitive genotypes. Therefore, tailored IAA treatments, adjusted for stress intensity and plant genotype, may be necessary to maximize their agronomic value.

4. Conclusion

This study demonstrates that exogenous IAA application can enhance early-stage tolerance to salinity and drought stress in tall fescue cultivars, particularly those with high sensitivity. The positive effects observed under severe or combined stress conditions indicate that the response to IAA is both dose- and stress-level-dependent. These findings highlight the potential of IAA as a targeted

priming agent to improve seedling establishment and vigor in challenging environments, especially when genotype-specific sensitivity and appropriate application strategies are carefully considered.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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