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Effects of Different Levels of Proline Amino Acid on Yield and Related-Yield of Wheat

SAYED ALI EMAMI[1*], ALI SOLEYMANI[2]
1-PhD Student of Islamic Azad University of Khorasgan, Iran.
2- Deprtment of Agronomy and Plant Breeding, Institute of Agriculture, Water, Food and Nutraceuticals, Isf. C., Islamic Azad University, Isfahan, Iran.

*Corresponding author's E-mail: dr.s.a.emami110@gmail.com

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ABSTRACT

Proline, as an essential amino acid, contributes to the structure of proteins while also acting as an osmoprotectant during periods of environmental stress. Wheat stands as an essential agricultural crop that plays a vital role in ensuring global food security. This research explores the influence of foliar spraying with the amino acid proline at two specific concentrations, 45% and 75%, across two successive growing seasons (2016-2018) at an experimental farm in Barsian village, Ziar, using the wheat cultivar M7318. The aim is to assess how these concentrations affect various yield and grain-related attributes, such as total grain yield, 1000-grain weight, grain weight per individual plant, grain weight per spike, seed count per square meter, and seed count per spike. Also, the study seeks to determine the varying impacts of these proline levels on wheat performance and productivity over the observed period. The results demonstrated a dose-dependent improvement in all measured parameters, with the 75% proline treatment inducing the most significant enhancements. Findings included an increase in all traits compared to the 45%. These improvements stem from proline's dual function as an osmoprotectant and metabolic enhancer, supporting photosynthesis and nitrogen assimilation during grain filling. The 75% proline application resulted in a remarkable yield, highlighting its potential as an effective biostimulant. Revealed strong positive correlations between grain yield and critical yield components, including grain weight per spike and number of seeds per spike, confirming proline's role in improving reproductive efficiency. The study concludes that 75% proline foliar application significantly enhances wheat yield through integrated physiological, agronomic, and molecular mechanisms, offering a sustainable strategy for cereal production.

Keywords: Foliar application, Proline, Yield components, Wheat yield

INTRODUCTION

Wheat (*Triticum aestivum* L.) is recognized as one of the most important global agricultural crops and is studied for its significant contribution to food security due to its carbohydrates and calories. Wheat enriched with proteins, minerals (include iron and zinc), and vitamins (such as B vitamins) can be of great value in maintaining human diets worldwide (Shewry and Hey, 2015; Gupta *et al*., 2024). Despite significant strides by researchers and plant breeders in enhancing wheat yield through genetic improvement and conventional agronomic practices, the escalating global population continues to outpace current production capacities (Hafeez *et al.,* 2023; Sabar *et al*., 2024). To bridge this gap and mitigate reliance on chemical fertilizers and pesticides, innovative approaches such as foliar spraying of amino acids, particularly proline, have gained attention as a sustainable strategy to boost wheat productivity and nutritional quality (Albahri *et al.*, 2023). Research has revealed that amino acids have an important role, both direct and indirect, in regulating physiological processes, as well as plant growth and development. Studies on amino acid-based biostimulants demonstrate their positive impact, improving both quantitative and qualitative growth metrics in a wide range of plant species (Trovato *et al.*, 2021).

Application of the amino acid proline as a foliar spray activates multifaceted mechanisms in wheat, ultimately increasing wheat yield. Proline is an osmotic protector that accumulates under stress conditions, stabilizes cellular structures, and reduces oxidative damage by scavenging free radicals (Ghosh *et al.,* 2022). The amino acid also promotes osmotic regulation in the plant, improving water retention and photosynthetic efficiency, which are important for wheat growth under drought or salinity stress. In addition, proline acts as a precursor for the synthesis of proteins and secondary metabolites, facilitating metabolic adjustments that enhance plant resilience and yield potential. These physiological adaptations highlight the role of proline in optimizing wheat responses to environmental challenges, making it a promising biostimulant for crop management (Hayat *et al.,* 2012).

The effect of foliar spraying of amino acid proline on the morphological characteristics of wheat and its grain is an area of growing interest (Asghar *et al*., 2021). Studies indicate that proline application enhances plant height, increases the number of tillers per square meter, and boosts spike length, grain number per spike, and 1000-grain weight, all of which contribute to elevated grain yield (Raza *et al.,* 2023; Hosseinifard *et al*., 2022). Additionally, proline improves straw yield, protein content, and carbohydrate accumulation in grains, reflecting its influence on both quantitative and qualitative traits. These morphological improvements are attributed to proline's role in enhancing nutrient uptake and photosynthetic activity, yet the extent of these effects varies with wheat genotype, application timing, and environmental conditions (Wang *et al*., 2023).

Despite the promising effects of proline foliar application, there is a significant knowledge gap regarding its application to wheat leaves and its specific impact on wheat grain morphological traits. The current scientific literature lacks comprehensive data on the effects of foliar application of the amino acid proline on wheat. This deficiency highlights the need for targeted studies to clarify these relationships and ensure that proline-based interventions can be effectively integrated into wheat cropping practices to enhance yield sustainability, nutritional quality, and global food security. This paper addresses this gap by investigating the effectiveness of proline foliar application on wheat performance traits and grain morphology.

MATERIALS AND METHODS

This research was conducted during two consecutive cropping seasons (2016–2017 and 2017–2018) in an experimental field located in Barsian Village, Ziar County, Isfahan Province. The site's geographical coordinates, recorded using the World Geodetic System (WGS84), are 51°83'E longitude and 32°43'N latitude, with an elevation of 1570 meters above sea level. The region has a semi-arid climate, receiving approximately 120 mm of annual rainfall and an average yearly temperature of 16°C. The region has a semi-arid climate, with an average annual rainfall of approximately 120 mm and a mean annual temperature of 16°C (Figure 1).

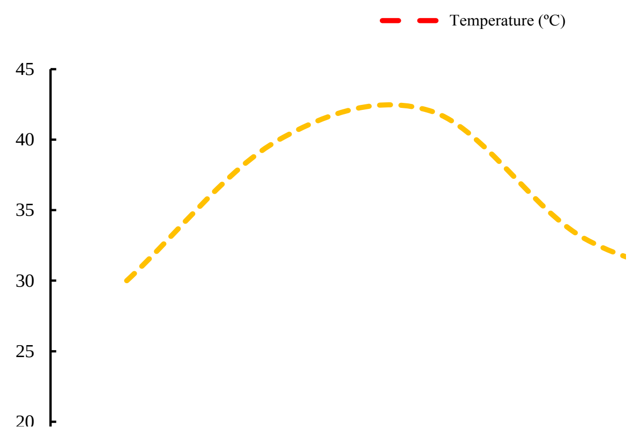

Figure 1.Mean temperature, humidity and precipitation for the two crop years 2016-2017 and 2017-2018

The experiment followed a randomized complete block design with three replications over two growing seasons. In this study, the amino acid proline with high purity (≥99%) was used, which was purchased from Roti Green. The solutions used for foliar spraying were prepared in two concentrations of 45 and 75% (w/v). For preparation, the required amount of proline powder was first dissolved in a specific volume of distilled water. Since proline is completely soluble in water, no additional solvent or cosolvent was required. Finally, the final volume of each solution was brought to a final volume of one liter by adding distilled water to obtain the exact concentrations of 45 and 75 grams per liter (g/L). The pH of the solutions was in the neutral range (~7), and no pH adjustment was performed. These solutions were dissolved in distilled water and modified with 0.1% Tween 20 to ensure uniform distribution and better adhesion to the leaf surface. Treatments involved foliar application of proline at two concentrations (45% and 75%), timed to coincide with critical growth stages according to the Zadoks-Tattman scale (Weaver, 1972): late tillering, flag leaf emergence, and full flowering/pollination using a hand sprayer equipped with a fine nozzle at a rate of 100 liters per hectare was doing. The study utilized the winter wheat cultivar M7318, selected for its high yield potential, stress adaptability, and broad suitability across Iranian agroclimatic zones. This cultivar originated from a targeted hybridization program between the elite Azadi variety and high-performance international wheat lines, followed by a three-year evaluation process prior to its official release and registration. In this study, wheat was sown on November 20th, a date selected based on the climatic conditions of Isfahan County, the growth cycle of the wheat cultivar, and its thermal needs. Prior to planting, the field was prepared through deep plowing (25–30 cm depth) and subsequent disking to break up soil clods and ensure a uniform seedbed. The experimental layout consisted of 320 sowing rows per plot, with each treatment replicated five times across rows spaced 15 cm apart and measuring 4 meters in length. Pre-planting soil analyses were performed for both years of the experiment.

Prior to sowing, soil analysis guided the application of 150 kg/ha of potassium fertilizer as potassium sulfate (K_2SO_2, 50% K_2O) and 100 kg/ha of phosphorus fertilizer as triple superphosphate ($Ca(H_2PO_2)_2$, 46% P_2O_2). These fertilizers were uniformly spread and incorporated into the top 10–15 cm of soil through light tillage. Additionally, well-decomposed farmyard manure was applied during pre-planting plowing to improve soil organic matter and structure. Nitrogen fertilization followed a split-dose approach, with applications timed to critical growth stages: tillering, stem elongation, and flowering. Initial irrigation was performed using flood irrigation to establish adequate soil moisture. For weed control, Pumasuper and Granstar were applied to manage broadleaf and grassy weeds, respectively, during the crop cycle. The traits in this research included important yield parameters, such as grain yield (expressed in grams per square meter) and specific grain-related components, such as 1000-grain weight, grain weight per plant, grain weight per spike, number of seeds per square meter, and number of seeds per spike. To determine 1000-grain weight, a random sample of 1000 grains was selected, weighed using a precision scale, and recorded in grams, a metric that reflects grain size and quality. Additionally, grain weight per plant was assessed by harvesting mature plants individually and measuring their total grain output in grams, providing insight into the productivity of each plant. To measure the grain weight per spike, mature wheat spikes were randomly sampled at physiological maturity, and the grain weight per spike was weighed using a digital scale, and the results were recorded in grams. To measure the number of grains per square meter, one square meter of the field was marked, then all spikes in this area were harvested, and the total number of grains was counted and expressed per square meter. To determine the number of grains per spike, healthy spikes were randomly collected at maturity, threshed individually, and the grains in each spike were counted.

Prior to statistical analysis, the key assumptions of analysis of variance (ANOVA) were carefully assessed, including data normality, residual distribution normality, and the additive effects of blocks and treatments. The experimental design followed a basic randomized block layout with three replicates per treatment, incorporating a compound time-based analysis. For mean comparisons, the Least Significant Difference (LSD) test (α = 0.05) was applied using SAS 9.4 to determine statistically significant differences between treatment groups.

RESULTS

Evaluation and assessment of yield variability and associated agronomic traits

Table 1 reveals the outcomes of the analysis of variance (ANOVA) for various yield-related traits and grain yield, including grain yield, 1000-grain weight, grain weight per plant, grain weight per spike, number of seeds per square meter, and number of seeds per spike, in response to different concentrations of proline amino acid applied as a foliar spray. The findings reveal that the application of amino acids significantly influenced all assessed traits ($P < 0.01$). Based on the results, the foliar application of amino acids markedly increases yield and grain yield-related traits in wheat, suggesting its potential as an effective agronomic practice to boost productivity and improve grain characteristics.

Table 1. Analysis of variance regarding the effects of foliar spraying of proline amino acid at concentrations of 45 and 75% on grain yield and traits related to grain yield in wheat cultivar M7318.

Source of variation	df	MS					
		Grain yield	1000-grain weight	Grain weight per plant	Grain weight per spike	Number of seeds per square meter	Number of seeds per spike
Year	1	0.27^{ns}	0.08^{ns}	8.84^{ns}	0.009^{ns}	520000^{ns}	0.77^{ns}
Rep(Year)	4	0.44	0.81	0.91	0.001	61189	0.57
Proline amino acid	1	7193.20^{**}	190.40^{**}	226.20^{**}	1.920^{**}	151425^{**}	644.45^{**}
Year × Proline amino acid	1	29.45^{ns}	0.75^{ns}	0.52^{ns}	0.030^{*}	2406656^{*}	1.09^{ns}
Error	4	0.73	1.35	1.99	0.002	143735	0.34
CV (%)		14.38	11.24	9.34	6.52	10.07	9.92

**, * and ns : Significant at the 0.01 and 0.05 probability level and non-significant
CV: coefficient of variation, df: degrees of freedom, MS: mean square, Rep: replication

The impact of the proline amino acid on the grain yield and traits related to grain yield of wheat

The results of the mean values for study traits, including grain yield, 1000-grain weight, grain weight per plant, grain weight per spike, number of seeds per square meter, and number of seeds per spike, regarding the effects of foliar spraying of proline amino acid at concentrations of 45 and 75% are presented in Table 2. The results indicated that applying 75% foliar spray of proline amino acid significantly enhanced functional traits compared to a 45% spray.

Table 2. Mean grain yield and traits related to grain yield regarding the effects of foliar spraying of proline amino acid at concentrations of 45 and 75% in wheat cultivar M7318

Proline amino acid	Grain yield (g/plant)	1000-grain weight (g)	Grain weight per plant (g)	Grain weight per spike (g)	Number of seeds per square meter	Number of seeds per spike
45%	573±15b	40.3±0.01b	46.7±0.41b	1.45±0.84b	16011±0.84b	30.5±0.02b
75%	622±10a	48.2±0.01a	55.4±0.48a	2.25±1.01a	18258±0.67a	45.2±0.01a
LSD (0.05)	39.81	6.35	5.29	1.01	1523	7.66

In each common means followed by a same letter are not significantly different according to the LSD test at an alpha level of *0.05*

The assessment of two different proline amino acid concentrations, 45% and 75%, demonstrated significant impacts on grain yield. The findings reveal a consistent trend of enhanced grain yield with higher proline concentrations, with the most substantial increase measured at 75%, yielding 622 g/plant. This suggests that proline application, particularly at elevated levels, effectively boosts productivity, likely due to its role in improving plant stress tolerance and metabolic efficiency. The results indicated that applying 75% foliar spraying of the proline amino acid on wheat leaves, compared to 45%, led to 16% increase in 1000-grain weight and 15% increase in grain weight per plant. The results demonstrated a statistically significant improvement in measured parameters, including grain weight per spike, number of seeds per spike, and seed density per square meter. Notably, increasing the proline concentration from 45% to 75% led to a marked enhancement in grain yield, with the highest concentration (75%) producing the most substantial gains. Specifically, the 75% proline treatment increased the number of seeds per square meter by 12% and the number of seeds per spike by 32%, highlighting its efficacy in optimizing reproductive growth and yield formation (Table 2).

Association between traits affected by foliar application of proline amino acid

The relationships between grain yield and associated functional traits in wheat, as presented in Table 3, revealed both direct and indirect associations among agronomic parameters. The correlation analysis demonstrated that grain yield exhibited a strong positive and highly significant ($p < 0.01$) relationship with 1000-grain weight, grain weight per plant, grain weight per spike, and number of seeds per spike, indicating that these traits are major contributors to wheat productivity. Notably, 1000-grain weight, a critical determinant of grain quality and yield potential, showed a strong positive correlation with grain weight per plant (72%) and number of seeds per square meter (64%), suggesting that foliar proline application enhances both individual seed mass and overall seed set efficiency. Furthermore, grain weight per plant displayed significant positive correlations ($p < 0.01$ and $p < 0.05$) with all examined traits, reinforcing its role as a central yield-influencing factor. A particularly strong association was observed between grain weight per spike and number of grains per spike ($r = 0.90$), highlighting that spike fertility and grain filling efficiency are closely linked under proline supplementation. Additionally, the number of grains per square meter was significantly correlated ($p < 0.01$) with the number of grains per spike ($r = 0.72$), suggesting that proline-induced improvements in spikelet development directly contribute to higher seed density at the canopy level.

Table 3. Correlation between yield and traits related to grain yield in wheat cultivar M7318 regarding the effects of foliar spraying of amino acid at concentrations of 45 and 75%.

Traits	Grain yield	1000-grain weight	Grain weight per plant	Grain weight per spike	Number of seeds per square meter	Number of seeds per spike
Grain yield	1					
1000-grain weight	0.59^{**}	1				
Grain weight per plant	0.61^{**}	0.72^{**}	1			
Grain weight per spike	0.67^{**}	0.21^{ns}	0.76^{**}	1		
Number of seeds per square meter	0.28^{ns}	0.64^{**}	0.45^{*}	0.50^{**}	1	
Number of seeds per spike	0.69^{**}	0.33^{ns}	0.79^{**}	0.90^{**}	0.72^{**}	1

**, * and ns : Significant at the 0.01 and 0.05 probability level and non-significant

DISCUSSION AND CONCLUSION

The study evaluated the effects of foliar-applied proline at two concentrations (45% and 75%) on wheat's functional and yield-related traits. The results indicated a statistically significant enhancement in all measured traits, with grain yield showing a dose-dependent increase. The observed enhancement in functional and yield-related traits of wheat following foliar spraying with proline amino acid at concentrations of 45% and 75%, with an increase at 75%, underscores the potential of this biostimulant in optimizing cereal crop productivity. Proline accumulation in plants under stress conditions, such as drought or salinity, serves as an osmoprotectant, stabilizing cellular structures and mitigating oxidative damage (Szabados & Savouré, 2010). The 16% increase in 1000-grain weight and 15% rise in grain weight per plant at the 75% concentration likely reflect proline's role in enhancing photosynthesis and nitrogen metabolism, which are critical for seed development and filling. These findings align with studies by Ashraf and Foolad (2007), who demonstrated that proline enhances photosynthetic efficiency and protects enzymatic activity, thereby supporting grain yield under adverse conditions.

The significant improvements in yield parameters such as a 12% increase in seeds per square meter and a 32% increase in seeds per spike at 75% proline highlight the efficacy of foliar application as an agronomic practice. This is consistent with the work of Haider *et al.* (2021), who reported that foliar amino acid treatments significantly boosted wheat yield components, including tiller number and grain number per spike, by improving nutrient uptake and plant vigor. The strong positive correlations observed between grain yield and traits like grain weight per spike and number of seeds per spike further suggest that proline enhances reproductive efficiency and spike fertility, a finding supported by Ramazanova et al. (2024), who noted yield increases of 0.27–0.40 t/ha with amino acid sprays at the booting stage. The 622 g/plant yield at 75% proline indicates a robust response, likely due to improved and resource allocation, which could be integrated into precision agriculture systems. Proline's influence extends to gene expression and stress-related pathways. The marked enhancement in grain yield and seed density per square meter may be linked to proline-induced upregulation of genes involved in osmoprotection and antioxidant defense, as reported by Verbruggen and Hermans (2008). This molecular adaptation likely contributes to the observed 72% correlation between 1000-grain weight and grain weight per plant, reflecting improved seed mass and metabolic efficiency. The indirect associations between traits, such as the 64% correlation with seeds per square meter, suggest that proline modulates hormonal signaling, potentially increasing cytokinin levels to promote spikelet development, a mechanism explored by Iqbal *et al.* (2022). These molecular insights provide a foundation for further genetic studies to optimize proline application. In conclusion, the significant improvements in wheat yield and functional traits following 75% proline foliar spraying, as evidenced by increased grain weight, seed number, and yield stability, highlight its potential as a sustainable agronomic tool. These findings, corroborated by physiological enhancements in photosynthesis, agronomic yield boosts, and molecular adaptations, suggest that proline can address productivity challenges in wheat cultivation. However, the lack of comprehensive data on optimal application genotype-specific responses necessitates further research to refine these practices and maximize their impact on global wheat production.

CONCLUSION

The investigation into the effects of foliar-applied proline at 45% and 75% concentrations on wheat demonstrates a clear and statistically significant improvement in both functional and yield-related traits, with the 75% concentration yielding the most pronounced benefits. The observed increases, including a rise in 1000-grain weight, an increase in grain weight per plant, a boost in seeds per square meter, and an enhancement in seeds per spike, alongside a peak grain yield, underscore proline's effectiveness as a biostimulant. This enhancement likely stems from proline's role in stabilizing cellular structures, boosting photosynthesis, and improving nitrogen metabolism, which collectively support seed development and reproductive efficiency. The strong correlations between grain yield and key traits, such as grain weight per spike and seed number, further highlight proline's ability to optimize spike fertility and overall productivity.

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