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Assessing the Early Establishment and Adaptability of Italian Ryegrass (*Lolium multiflorum*) in Irrigated and Rainfed Conditions of Punjab, Pakistan

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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

In Punjab, Pakistan, more than 60% expenditure incurred on the feeding of livestock and offered low quality forage thus affecting the productivity of milk and meat. The less production of livestock oriented products often results malnutrition in the society. An effort was made to evaluate the Italian Ryegrass (Lolium multiflorum) early growth and its adaptation to rainfed and irrigated environments. The main objective of this study is to provide important new information for sustainable fodder production systems. The experiment used a Randomized Complete Block Design with seeds obtained from Korea and was carried out in the winter of 2023 in both rainfed Chakwal and irrigated Sargodha zone of Punjab. The plant's response to varying soil conditions and water availability was evaluated by measuring a range of growth parameters, such as plant height, population density, root and shoot biomass, root length, and the root-shoot ratio. The results show that there are significant differences between rainfed and irrigated locations as established by the statistical analysis. Italian Ryegrass in irrigated plots showed higher mean plant height (43.75 cm), higher plant population (317.50 plants/m²), higher shoot biomass (7.425 g/plant), greater length of roots (5.950 cm), and a lower root-shoot ratio (0.1135) than in rainfed areas. Additionally, the study provides data on soil profiles that show differences between the two locations in terms of pH, electrical conductivity, nitrogen, phosphorus, and potassium. The findings provide important information for enhancing Italian Ryegrass cultivation in Punjab's various agro-ecological zones, given the critical role fodder feed plays in supporting livestock and agriculture. The study provides the basis for well-informed farming practices by highlighting the importance of water management strategies to improve fodder production during scarcity period and, by consequently, animal nutrition in the area.

Keywords: Adaptability; biomass; fodder; irrigation and rainfed zone; Lolium multiflorum; Punjab; Pakistan.

1. INTRODUCTION

Pakistan's livestock sector is critical to the national economy, contributing significantly to GDP and rural livelihoods. Forage availability is still a significant barrier, though, especially in dry and semi-arid areas like Punjab [1]. The productivity and sustainability of traditional fodder crops like maize and sorghum are threatened by temperature swings and a growing lack of water (Kumar et al., 2023). This makes it necessary to investigate durable, alternate forage choices such as temperate grasses that can yield high-quality feed all year round. Punjab, Pakistan's goal of sustainable agriculture necessitates the development of resilient forage crops that may flourish under a variety of agroclimatic circumstances [2]. Using its quick growth and high nutritional content, Italian ryegrass (*Lolium multiflorum*) is a viable way to increase forage availability. This cool-season grass species is a focus of agro ecological research because of its widespread recognition for adaptability and appropriateness as animal feed [3].

Italian ryegrass (*Lolium multiflorum*) is an important forage crop that may grow and adapt well in Punjab, Pakistan's rainfed and irrigated regions. The purpose of this study is to evaluate Italian ryegrass's development potential and adaptation in these various agricultural zones. Due to its good nutritious content, high herbage yield, favorable performance, and capacity for regrowth, Italian ryegrass can be harvested numerous times during its life cycle [4]. Furthermore, it has been discovered that the biomass of Italian ryegrass degrades slowly; nine weeks after planting (WAP), almost two-thirds of the plant waste was still present [5]. Additionally, it has been determined that Italian ryegrass is a significant feed crop grown in South Africa, where it is cultivated under irrigated condition for intensive production of dairy, lamb, and cattle [6]. The adaptability of Italian ryegrass to poorly drained circumstances makes it a viable winter cover crop for rotation with irrigated rice, as noted in some agricultural practices [7]. Additionally, research on the morphological and physiological variations between diploid and tetraploid cultivars under salinity stress conditions has been done on Italian ryegrass in relation to its response to salinity stress [8]. For the purpose of evaluating Italian ryegrass's capacity for growth in a variety of agricultural environments, these studies offer insightful information about how adaptable the plant is to various environmental stresses [9]. Italian ryegrass has also been studied for its allelopathic potential, especially in regard to its effect on other crops' germination. Studies have shown that various winter cover crop shoot extracts, such as Italian ryegrass, affect different crops' seed germination rates, suggesting that these extracts may have allelopathic effects on nearby plants. For the purpose of evaluating Italian ryegrass's adaptability and possible influence on agroecosystems, it is imperative to comprehend its allelopathic interactions (Villela et al., 20210).

Further research on Italian ryegrass has examined its involvement in the cycling of nutrients and possible environmental effects in the context of nitrogen removal and nitrate leaching in pasture systems [10]. These studies add to the thorough evaluation of the adaptability and growth capacity of Italian ryegrass in various agricultural systems. The importance of Italian ryegrass is derived from its agronomic qualities. Because of its quick establishment, fast growth, and nutritious content, it's a desirable choice for raising animal productivity. Furthermore, because of its robust root structure, it helps to prevent erosion, which emphasizes its potential importance in maintaining agricultural landscapes [11,12].

The agricultural landscape of Punjab is divided into areas with different patterns of water availability. Sargodha serves as an example of the irrigated zones, which gain from managed water supplies that guarantee steady crop development [13]. Rainfed zones, like Chakwal, on the otherhand, depends on the erratic moisture which affects agricultural output [14]. The potential of Italian-rye grass has been acknowledged, but there still exists a crucial study gap concerning a thorough assessment of its development dynamics in these agro-ecological zones which need to be explored, particularly in places that receive rain and artificial irrigation [15]. In order to measure the Italian rye grass potential, this study measures the growth performance of Italian ryegrass at an early stage, specifically 50days after seeding. The precise goals include calculating the biomass weights of the roots and shoots, measuring plant height, estimating plant population density, and assessing root length in both rainfed and irrigated zones.

The chosen parameters are important indicators to assess the initial growth and adaptation of Italian ryegrass [12]. Plant height signifies growing vigor, but plant population density represents establishment success in plants. The biomass weights of the roots and shoots indicate the distribution of resources, while the length of the roots indicates the plant's capacity to pull moisture and nutrients from the soil, which is essential for adaptation in a variety of agro-ecological zones. The purpose of this study is to determine the growth potential and adaptability of Italian ryegrass under different moisture regimes by comparing zones that are irrigated and those that are rainfed. This research attempts to provide insights into the crop's response to various environmental situations by carefully measuring and observing these parameters, thereby guiding forage production of Italian rye grass in Punjab-Pakistan.

2. METHODOLOGY

The aim of this research is to contribute the prospective integration of Italian ryegrass into fodder production systems in a range of Punjab province ecosystems by offering useful data on its growth and development under various water regimes and soil conditions. An experiment was carried out in the winter of 2023 in two locations: Sargodha (irrigated) and Chakwal (rainfed) in order to assess the growth potential and adaptability of Italian ryegrass (*Lolium multiflorum*) indisparate agro-ecological zones of Punjab, Pakistan. At each location, a Randomized Complete Block Design with three replications was used for each plot. There were a total of four plots, each measuring 505 m^2 (1 kanal). The seeds were arranged from OAK Café Company, Korea. Korean-sourced seeds were sown by drilled method at a rate of 5kg/ha. In Sargodha, two irrigations were applied 1st just after sowing and 2nd after 40 days of 1stirrigation. While no artificial irrigation applied in Chakwal. Different fertilizer applications were made: Chakwal and Sargodha received one bag of NPK 20:20:20 per 4 kanals (2023m^2).

2.1 Plant Population (m^2)

To determine the average plant population for a given plot, the number of plants inside a 1 square meter quadrat in several sample sections of each plot was counted.

2.2 Height of Plant (cm)

Measured the height of ten randomly chosen plants in each plot using a measuring tape or ruler, starting from the base of the stem and ending at the tip of the tallest leaf. Next, the average height for every plot was calculated.

Table 1. Comparison of soil characteristics between irrigated (Sargodha) and rainfed (Chakwal) zones

Parameter	Sargodha (Irrigated)	Chakwal (Rainfed)	Units
Electrical Conductivity (EC)	< 4 dS m^{-1}	< 4 dS m^{-1}	dS m^{-1}
pH	8.4	8.2	-
Nitrogen (N)	134 mg kg^{-1}	96 mg kg^{-1}	mg kg^{-1}
Phosphorus (P)	7.1 mg kg^{-1}	4.0 mg kg^{-1}	mg kg^{-1}
Potassium (K)	104 mg kg^{-1}	84 mg kg^{-1}	mg kg^{-1}

2.3 Root and Shoot Biomass Per Plant

Ten plants, selected at random from each plot, should be carefully uprooted at the specified sampling time. After carefully washing the roots in order to remove the soil, the roots and shoots were separated. To ensure complete drying, the samples were placed in an oven set to the optimal temperature of 105°C for a suggested 48-hour period. The dry weight of root and shoot samples was measured in grams using a balance after they had fully dried. The biomass of the roots and shoots was calculated using formulas 1 and 2, which were used to derive the water-holding ability of the roots and shoots. This standard operating procedure was conducted at the central lab of PMAS Arid Agriculture University in Rawalpindi.

Root biomass=Weight of dried roots (g)−Weight of fresh roots (g)………. eq.1 [16]

Shoot biomass=Weight of dried shoots (g)−Weight of fresh shoots (g)……. eq. 2 [16]

2.4 Root Length

Using a measuring tape, calculate the average root length for each of the ten plants sampled in each plot. For each plot, record the average root length.

2.5 Root-Shoot Ratio

The root-shoot ratio was calculated using the obtained biomass data by eq. 3:

Root-Shoot Ratio = Root Biomass/Shoot Biomass……. eq 3

This ratio served as a measure to determine the way resources were distributed between the root and shoot systems. Following the drying process, the measured biomass of the root and shoot components was used to calculate the amount. At PMAS Arid Agriculture University, Rawalpindi.

2.6 Soil Profile

The soil profile data collection utilized sensors from the Precision Agriculture Lab of Arid Agriculture University. Five samples were gathered from diverse locations, and readings were meticulously recorded and analyzed for key parameters such as electrical conductivity (EC), pH levels, nitrogen (N), phosphorus (P), and potassium (K). Average values for each parameter were computed for both sites.

2.7 Data Analysis

The growth statistics in the rainfed and irrigated treatments had mean values determined by statistical analysis ANOVA or t-tests were employed to identify significant differences among treatments. Relationships between variables were investigated using regression and correlation analysis. To ensure reliable conclusions, LSD was also used to identify significant differences between treatment means.

3. RESULTS AND DISCUSSION

The evaluation of Italian Ryegrass (*Lolium multiflorum*) in rainfed and irrigated conditions in Punjab, Pakistan, showed significant variations in various type of growth parameters.

3.1 Plant Height (cm)

There were significant differences in the average plant height of Italian Ryegrass (*Lolium multiflorum*) between the two treatment methods rainfed and irrigated condition. The mean plant height measured in the irrigated plots was 43.75 cm (Table 2), which was notably more than the 13.75 cm observed in the rainfed plots. This notable 30-cm variance emphasizes that water supply has a significant impact on the vertical growth of the grass species. By evaluating the accuracy of these measures using standard deviation (SD) values, the consistency of each treatment is underscored. The standard deviation of plant height in the irrigated areas was 1.70, while it showed a same variation in the rainfed plots at 1.70 (Table 2). This indicates that, despite the significant variation in mean plant height, there was a very uniform growth pattern within each treatment. The relative variability within each treatment is represented by the coefficient of variation (CV), which is calculated as the ratio of SD to the mean. The plant height CV under irrigating conditions was found to be 3.90%, but it was significantly greater (12.43%) under rainfed conditions. This increased CV (Table 3), under rainfed conditions suggests that there is more variability because of the limited supply of water, as evidenced by the comparatively wider distribution of data points about the mean plant height. The 30 cm difference in plant height between rainfed and irrigated environments shows the significant impact of water availability on upward development. Increase in Plant height in irrigated plots highlights the need for sufficient water resources for ideal growth, which is essential for optimizing fodder yield [17].

3.2 Plant Population or Plant Density (m^2)

The evaluation of the population of Italian ryegrass (*Lolium multiflorum*) plants under various circumstances showed distinct trends. The mean plant population in the irrigated plots was determined to be 317.50 plants/m², whereas in rainfed conditions, the mean population decreased to 240.0 plants/m² (Table 2). The difference indicates that the establishment and density of Italian Ryegrass are significantly influenced by the availability of water. The variety within the populations was further highlighted by the standard deviation (SD) figures. The rainfed plots displayed a little larger SD of 21.602 than the irrigated plots (Table 2), which displayed an SD of 17.078, respectively. There are more noticeable differences in plant density between the plots as a result of the increased fluctuation in rainfed conditions, which highlights how susceptible plant populations are to water stress. Coefficient of variation (CV) calculations supported these results, with the rainfed condition having an approximate higher CV of 9.00% and the irrigated condition showing a CV of 5.38%. This increased CV in the rainfed condition indicates a comparatively larger distribution of plant populations, suggesting that Italian Ryegrass is more susceptible to variations in water availability. P-value statistical analysis further supported the substantial difference ($P < 0.05$) in plant population between the two treatments. When everything is considered, the mean values, along with SD, CV, and statistical analysis, highlight that the populations of Italian Ryegrass plants under irrigated and rainfed environments differ significantly. The important effect that water availability has on Italian Ryegrass growth is one of the main findings [18]. Under irrigated conditions, the plant showed larger mean plant heights, higher population densities, and more biomass in the roots and shoots when water was readily accessible. A more consistent and resilient growth pattern was the outcome of careful irrigation management in Sargodha, highlighting the beneficial relationship between plant development and water availability [19]. Italian Ryegrass is sensitive to water stress, which affects its establishment and abundance. This is demonstrated by the observable decrease in plant population under rainfed conditions [20]. This result highlights how important it is to have an adequate water supply that promotes larger plant densities, which are necessary for strong fodder production [21].

3.3 Root And Shoot Biomass Weight (g/plant)

A critical indicator of the Italian Ryegrass's (*Lolium multiflorum*) reaction to various conditions involves the plant's total weight of roots produced. The mean root biomass weight in the irrigated plots was 0.84 g/plant (Table 2), indicating an effective root system under ideal water availability. A low standard deviation of 0.059 (Table 2), which denotes a significant level of consistency in the data, supported this. The irrigated treatment exhibited a comparatively low degree of variability, as indicated by the coefficient of variation (Cv) of 7.01%. The rainfed plots, on the other, showed a lower mean root biomass weight of 0.44 g per plant, highlighting the water stress shown negative effect on the production of biomass. In comparison to the irrigated plots, the rainfed treatment's standard deviation of 0.059 indicates a marginally higher variability. There is a moderate amount of variability within the rainfed treatment, as indicated by the computed coefficient of variation of 13.36% (Table 3). The statistical significance ($p < 0.05$) of the observed difference between the irrigated and rainfed treatments highlights the impact of water availability on the root biomass of

Italian Ryegrass. The increased mean root biomass in the irrigated condition highlights the significance of water management in maximizing biomass production in forage crops, as well as the beneficial effects of irrigation on below-ground growth.

In the research of Italian Ryegrass (*Lolium multiflorum*) in Punjab, Pakistan, there were significant differences between the irrigated and rainfed conditions in terms of the Shoot Biomass Weight, a critical indication of the productivity of above-ground plants. The mean Shoot Biomass Weight per plant in the irrigated plots was 7.42 g, indicating a strong above-ground biomass output under optimum water availability (Table 2). The rainfed plots, on the other hand, showed a significantly reduced mean shoot biomass weight per plant (2.97 g), indicating the effect of water constraint on the ability of the species to divide resources for shoot development. These observations were confirmed by statistical analysis. While the standard deviation under rainfed conditions was slightly higher at 0.3862 g/plant, suggesting greater variability in shoot biomass due to water stress, the standard deviation in irrigated conditions was 0.59 g/plant, indicating generally consistent biomass output across plots (Table 2). This pattern was further shown by the Coefficient of Variation (CV), which showed that in irrigated conditions the CV was 7.96% but under rainfed conditions it was 12.96% (Table 3). A paired t-test was used to evaluate the significance of the observed differences, and the results showed a p-value that was less than the specified significance limit ($\alpha = 0.05$). This lends weight to the idea that there are statistically significant differences in Shoot Biomass Weight between the rainfed and irrigated conditions. In conclusion, the Shoot Biomass Weight parameter reflects the substantial impact of irrigation on the above-ground biomass production of Italian Ryegrass. The significant difference in biomass weights of roots and shoots between rainfed and irrigated conditions highlights the reliance biomass production is on water availability [22]. Increased biomass in irrigated plots is an indication that water is essential for root and shoot development below ground, which is essential for improving feed quantity and quality [23].

3.4 Root Length (cm)

The investigation of root length in Italian ryegrass (*Lolium multiflorum*), under rainfed and irrigated conditions, revealed significant variations. The average root length in the supplying water plots was found to be 5.95 cm(Table 2). This number, in addition to the low standard deviation of 0.4203, indicates the growth trend across the four plots under evaluation was mostly uniform and steady. This measure's stability is further supported by the coefficient of variation (CV), which has a low value of 7.06%. The lowest degree of variability implied by the low CV supports the validity of the mean as a representative indicator. In contrast, the mean root length of 3.95 cm and the slightly greater standard deviation of 0.4203 were observed in the rainfed plots. The computed coefficient of variation (CV) of 10.63% indicates a little higher level of variability in comparison to the irrigated situations (Table 3). The statistical significance of the observed difference in mean root length between irrigated and rainfed treatments is shown by a p-value below the specified significance level, such as 0.05. This lends credibility to the hypothesis that irrigation significantly affects Italian Ryegrass root length. The findings demonstrate the consistency and dependability of these measurements in addition to the quantitative difference in root length between the treatments. Experiments that were rainfed had shorter roots (3.950 cm) and more variability in root length than irrigated plots, which had an average of 5.950 cm. This shows that the availability of water has an evident impact on root length [24].

3.5 Root Shoot Ratio

Italian ryegrass (*Lolium multiflorum*) development under various conditions was studied in relation to the Root Shoot Ratio, a crucial measure of the distribution of resources in plants. This ratio demonstrates how the biomass of the plant system divides between above-ground (shoot) and below-ground (root). The average Root Shoot Ratio under irrigation was found to be 0.11(Table 2), with a standard deviation (SD) of 0.001(Table 2) [25,26]. This suggests that resources were divided fairly evenly between roots and shoots throughout the plots. On the other hand, the mean Root Shoot Ratio in rainfed conditions was 0.1487, with a standard deviation of 0.001, showing a larger variability It also means that, in the absence of irrigation, there may be more variation in the distribution of resources between roots and shoots, which indicates the plant's reaction to water stress. The relative variability of the Root Shoot Ratio within each treatment is indicated by the Coefficient of Variation [27,28]. In comparison to the rainfed plots, where the CV was greater at 12.20%, the irrigated plots had a significantly lower CV at 9.26% (Table 3), suggesting a more uniform distribution of resources. The Root Shoot Ratio of the irrigated and rainfed conditions differed significantly ($P < 0.05$) according to statistical analysis using the relevant procedures (e.g., t-test or ANOVA) [29-31]. This importance highlights that water availability affects the way that roots and shoots are distributed in Italian ryegrass. The Root Shoot Ratio also showed a substantial difference between rainfed and irrigated conditions, highlighting the impact of water on the distribution of resources within the plant [32] These results highlight that water influences the growth of Italian ryegrass roots and the distribution of resources [24,32].

Table 2. Effects of irrigated and rainfed ecology on growth parameters of Italian rye grass (*Lolium multiflorum*)

Treatment	Plant Height (cm)	Plant Population (plants/m²)	Root Biomass Weight (g/plant)	Shoot Biomass Weight (g/plant)	Root Length (cm)	Root Shoot Ratio
Irrigated	43.75 ± 1.708 (a)	317.50 ± 17.079 (a)	0.8425 ± 0.0590 (a)	7.425 ± 0.590 (a)	5.950 ± 0.420 (a)	0.1135 ± 0.0010 (a)
Rainfed	13.75 ± 1.708 (b)	240.00 ± 21.60 (b)	0.4425 ± 0.0590 (b)	2.975 ± 0.386 (b)	3.950 ± 0.420 (b)	0.1487 ± 0.0018(b)

Table 3. Performance metrics and coefficient of variation for Italian rye grass under different conditions

Treatment	Plant Height (cm)	Plant Population (plants/m²)	Root Biomass Weight (g/plant)	Shoot Biomass Weight (g/plant)	Root Length (cm)	Root Shoot Ratio
Irrigated	3.90%	5.38%	7.01%	7.96%	7.06%	0.93%
Rainfed	12.43%	9.00%	13.36%	12.96%	10.63%	1.22%
Total	**56.03%**	**16.19%**	**34.32%**	**46.58%**	**23.00%**	**14.36%**

Table 4. Results of ANOVA

			Sum of Squares	df	Mean Square	F	Sig.
Plant Height (cm) * Treatment	Between Groups	(Combined)	1800.000	1	1800.000	617.143	.000
	Within Groups		17.500	6	2.917		
	Total		**1817.500**	**7**			
Plant Population (plants/m²) * Treatment	Between Groups	(Combined)	12012.500	1	12012.500	31.681	.001
	Within Groups		2275.000	6	379.167		
	Total		**14287.500**	**7**			
Root Biomass Weight (g/plant) * Treatment	Between Groups	(Combined)	.320	1	.320	91.647	.000
	Within Groups		.021	6	.003		
	Total		**.341**	**7**			
Shoot Biomass Weight (g/plant) * Treatment	Between Groups	(Combined)	39.605	1	39.605	158.950	.000
	Within Groups		1.495	6	.249		
	Total		41.100	7			
Root Length (cm) *	Between Groups	(Combined)	8.000	1	8.000	45.283	.001

			Sum of Squares	df	Mean Square	F	Sig.
Treatment	Within Groups		1.060	6	.177		
	Total		**9.060**	**7**			
Root Shoot Ratio * Treatment	Between Groups	(Combined)	.002	1	.002	1122.375	.000
	Within Groups		.000	6	.000		
	Total		**.002**	**7**			

4. CONCLUSION

In conclusion, there were significant differences in the growth parameters of Italian Ryegrass (Lolium multiflorum) grown in Punjab, Pakistan, under rainfed and irrigated conditions. The grass indicated increased plant height, densities, biomass in the roots and shoots, and more even distribution of resources when it was irrigated. Rainfed conditions, on the other hand, resulted in altered distribution of resources, greater variability, and decreased growth. These results highlight the important influence that water availability has on the productivity and adaptation of Italian Ryegrass. It becomes clear that efficient water management is essential for maximizing the yields of fodder crops in areas like Punjab. For the region to support sustainable agriculture and animal feed, it is critical to understand the role that irrigation contributes in ensuring constant development and biomass production. This will assist in ensuring a steady and considerable availability of fodder.

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 manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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