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Enhancing Morphological Diversity in Onion (*Allium cepa L.*) Genotypes using Iron Oxide Nanoparticle

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Abstracts

Iron oxide nanoparticles are gaining attention for their potential for enhancing plant growth and productivity. This study investigated the effects of iron oxide nanoparticle on five onion (*Allium cepa L.*) genotypes at varying concentrations (0 rpm, 20 rpm, 40 rpm, 60 rpm, 80 rpm). This experiment was laid in Randomized Complete Block Design (RCBD), with three replication and data were analysed using two-way ANOVA and means were separated using LSD. key morphological parameters such as establishment count, plant height, number of leaves, final stand count, bulk biomass, fresh plant weight, bulb weight, bulb horizontal and vertical diameter and dry weight were investigated. Results showed that moderate concentrations (20 rpm and 40 rpm) of nanoparticles generally enhanced establishment, plant height, and biomass accumulation. Super Yali and Prema exhibited improvements not significantly different in leaf number and bulb development at 80 rpm. Significant correlations were observed between establishment count and final stand count (r = 0.977), while fresh plant weight had a negative correlation with leaf number (r = -0.876). Principal component analysis (PCA) revealed that the first principal component accounted for 57.7% of the variance, dominated by traits such as establishment count, plant height, and leaf number. The study concluded that iron oxide nanoparticle can effectively enhance onion growth and development, with optimal nanoparticle concentrations varying across genotypes.

Keywords: Correlation, Genotypes, Iron oxide nanoparticles, Morphological, Principal Component Analysis, Onion,

Introduction

Onions (*Allium cepa L.*), belonging to the genus *Allium* in the family Alliaceae, are one of the oldest cultivated vegetables. They come in a variety of colourful cultivars and are widely used worldwide (Sidhu *et al*., 2019). After tomatoes, onions are the second most extensively utilized vegetable, prized not only for their culinary applications but also for their historical significance (FAO, 2012). Onion is originated in Central Asia, particularly between Turkmenistan and Afghanistan. Wild relatives of the onion still thrive in these regions. Over time, onions spread to the Near East and areas surrounding the Mediterranean Sea, where they further developed and adapted to varying environments (Ochar and Kim, 2023).

Onion productivity varies significantly across agro-climatic conditions and among different cultivars, even within the same environment. This variability is primarily influenced by the interplay between a cultivar's genetic composition and environmental factors (Sirajo and Namo, 2019). Such differences necessitate targeted studies to identify optimal genotypes for specific regions.

Krantz et al. (2008) reported that progress in plant growth modelling has been achieved through the integration of biological knowledge with mathematical, computational, and advanced technological approaches, thereby facilitating precise analysis of trait–environment interactions and enhancing strategies for crop improvement and management.

A recent study by Husen *et al*. (2021) showed the potential for underutilized onion varieties to outperform commonly cultivated ones in specific environments. For instance, research conducted in the highland areas of West Hararghe, Ethiopia, demonstrated that newer varieties such as Nafis, achieved superior performance in terms of bulb weight, marketable yield, and total yield compared to established varieties. This suggests that strategic evaluation of onion genotypes can identify varieties better suited to particular conditions, enhancing commercial production.

On the other hand, the emergence of nanotechnology using nanoparticles in crop improvement is so imperative in addressing the escalating food demand of our growing global population, utilizing both natural and synthetic resources (Shang *et al.,* 2019). Biologically synthesized nanoparticles are preferred over chemically synthesized nanoparticles due to their less toxicity and environment-friendly nature. Plant contains a variety of terpenoids, polysaccharides, phenols, and flavonoids which act as reducing agents and facilitate the formation of stable metal nanoparticles (Chatterjee *et al.,* 2021; Sarkar *et al*., 2021).

This study investigated the variability in the morphological attributes among some onion genotypes exposed to iron oxide nanoparticles. By understanding the variability of these traits in relation to iron oxide treatments, it may be possible to establish the distinctiveness of individual genotypes and their response to iron oxide nanoparticles to optimize their cultivation and to meet increasing market demands.

Materials and Methods

The experiment was conducted at the Research and Experimental Garden of the Department of Plant Science and Biotechnology of the Federal University of Lafia, Lafia Nasarawa State located on latitude 8°28'N, longitude 8°52'E and elevation of 158meter above sea level.

Five onion genotypes were sourced from the International Institute of Tropical Agriculture (IITA) Kano. These genotypes include: Three local (Wade, Mara kara and Me kara) and two improved genotypes (Prema and Super yali) were used for this study.

Green synthesized iron oxide nanoparticle was extracted from avocardo leaves. The process was conducted at the Chemistry Department of Federal University of Lafia. Iron oxide nanoparticles were synthesized using the modified protocol from the previous studies of Jabbar *et al,* (2022). A typical procedure, 0.01 M $Fe(NO_3)_3 \cdot 9H_2O$ solution was prepared using Ethanol as solvent. Avocado leaves were soaked in the ethanol in the volume ratio of 1:1. The reduction reaction occured immediately after the addition of $Fe(NO_3)_3 \cdot 9H_2O$. The formation of nanoparticles was evidenced by the appearance of black colour in the solution. The mixture was later air dried to get iron oxide nanoparticles in solid form.

Preparation of Iron oxide nanoparticle solutions and treatment of onion seeds: Five different solutions of iron oxide nanoparticle were prepared to different concentrations of 0rpm (control), 20rpm (0.002g iron oxide nanoparticle dissolved in 1000mls of distilled water), similar procedure was repeated for 40rpm, 60rpm and 80rmp in that order (Raliya *et al.,*2021). Each of these solutions were subjected to treat the five genotypes of onions for 24hrs and dried afterward in the laboratory.

All land preparation and clearing were done manually. The onion seedlings were raised for 8 weeks and subsequently transplanted and raised for 12weeks. The seedlings are typically sown at a depth 1 to 2 inches and 2 to 3 inches apart, then thinning them to 3 to 4 inches apart as they grow (Utah State University Extension,), and an inter plots spacing of 0.5 meter.

The experiment was laid out 5 x 5 factorial experiment layout in a Randomized Complete Block Design (RCBD) with three replications. A total of 75 plots was represented the three replicates (25 treatment combinations in three replicates).

Data were collected at 4weeks, 8weeks and 12weeks after transplanting. Morphological parameters observed are Establishment counts, Final stand count, Plant height, Number of leaves per Plant, Neck diameter, Fresh plant weight, Bulk biomass, Horizontal and vertical bulb diameter, Fresh and dry weight of bulbs.

Data obtained were subjected to two-way ANOVA using the GENSTAT statistical tool (Genstat 23 version). Means were separated using LSD.

Results and Discussion

The impact of iron oxide nanoparticle treatments on onion genotypes, as presented in Tables 1 to 6, demonstrates a variable influence on growth, morphology, and biomass accumulation, influenced by both the treatment concentration and genotype though most of the parameters studied were not significantly different. Previous studies have highlighted that nanoparticles can interact with plants in genotype specific manners, influencing growth and physiological responses under varying environmental conditions (Salama *et al*., 2019; Goler and Kwon- Ndung., 2019 ; Kwon-Ndung *et al., 2019;*Adil *et al*., 2022).

The combined analysis of variance revealed that genotypic differences significantly influenced establishment count, final stand count, and bulb biomass (Tables 1 and 2). This underscores the strong role of genetic background in determining both early growth vigor and yield formation in onion. Similar findings were reported by Pérez-Ortolá *et al*. (2015), who observed significant genotype-dependent variability in onion growth and yield across environments.

The effect of iron oxide nanoparticle treatments was significant for plant height and horizontal bulb diameter, suggesting that nanoparticles selectively enhance vegetative growth and certain bulb traits. This agrees with previous reports that nanoparticle applications can promote cell elongation and organ expansion through improved nutrient uptake and physiological stimulation (Prakash *et al*., 2022; Nair *et al.,* 2010). However, traits such as leaf number, neck thickness, and fresh plant weight were not significantly affected, indicating that the growth-promoting effect of nanoparticles may be trait-specific.

Importantly, the genotype × treatment interactions were significant for establishment count, plant height, and bulb biomass, implying that the response to nanoparticle treatments was not uniform across onion genotypes. Some genotypes showed improved establishment and biomass under nanoparticle exposure, while others exhibited limited or no response. Such interactions have been reported in similar studies on nanoparticle–crop relationships, where varietal differences strongly conditioned the effectiveness of treatments (Sirajo *et al*., 2024; Raliya *et al*., 2016).

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Table 1: ANOVA table for growth parameters of Onion Genotypes Exposed to Iron Oxide Nanoparticle Treatments

Source of variation		Establishment Count		Plant Height		Number of Leaves		Neck Thickness		Final Stand Count		Fresh Plant Weight	
	df	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F
Genotype	4	1281.2	5.70*	211.57	2.15^{ns}	22.99	0.43^{ns}	2.51	0.97^{ns}	202.67	7.96*	1434.1	2.34^{ns}
Treatment	4	461.33	2.05^{ns}	592.57	6.03*	44.45	0.83^{ns}	0.4	0.15^{ns}	38.49	1.51^{ns}	1088.7	1.78^{ns}
Genotype x Treatment	16	2872.1	3.19*	1097.9	2.80*	78.61	0.37^{ns}	9.83	0.95^{ns}	180.75	1.77^{ns}	1899.7	0.78^{ns}
Residual	50	2810		1227.4		671.33		32.49		318.33		7657.7	

Note: df = Degree of freedom, SS = Sum of square. "*" significant at p value < 0.05, ns = not significant.

Table 2: ANOVA table for yield parameters of Onion Genotypes Exposed to Iron Oxide Nanoparticle Treatments

Source of variation	df	Bulb Biomass		Bulb Weight		Horizontal Bulb Diameter		Vertical Bulb Diameter		Dry Weight	
		SS	F	SS	F	SS	F	SS	F	SS	F
Genotype	4	1083.9	5.19*	240.56	2.88^{ns}	11.91	2.48^{ns}	12.52	0.50^{ns}	74.25	1.74^{ns}
Treatment	4	256.86	1.23^{ns}	230.21	2.76^{ns}	13.33	2.78*	7.4	0.30^{ns}	104.28	2.45^{ns}
Genotype x Treatment	16	2161.3	2.58*	569.58	1.71^{ns}	20.01	1.04^{ns}	47.19	0.48^{ns}	143.71	0.84^{ns}
Residual	50	2612.9		1042.4		59.98		310.19		533.03	

Note: df = Degree of freedom, SS = Sum of square. "*" significant at p value < 0.05, ns = not significant.

The results (Tables 3 and 4) confirm that genotype strongly determines onion performance under iron oxide nanoparticle treatment, especially for establishment, stand count, and bulb biomass. Me Kara consistently showed superior growth (plant height, stand count, fresh weight) and yield (bulb biomass, bulb weight, dry matter), suggesting it is the most responsive genotype to nanoparticle application. Super Yali also performed well in yield traits, while Wade lagged across most parameters.

This aligns with earlier findings (Tables 1 and 2), where genotype effects were dominant, and genotype × treatment interactions influenced key traits. Similar genotype-dependent responses have been documented in onions and other crops under nanoparticle exposure (Prakash *et al*., 2022; Raliya *et al*., 2016; Sirajo *et al.,* 2024). These findings emphasize the need for genotype-specific optimization of nanoparticle treatments for maximum productivity.

Table 3: Onion Genotype performance for growth parameters treated with Oxide Nanoparticle

Genotype	Establishment Count	Plant Height	Number of Leaves	Neck Thickness	Final Stand Count	Fresh Plant Weight
Mara kara	42.60 ± 8.06	23.86 ± 10.25	7.93 ± 2.94	2.54 ± 0.80	9.81 ± 2.62	27.16 ± 13.65
Me kara	41.60 ± 10.02	28.09 ± 4.88	8.20 ± 2.91	2.89 ± 0.60	14.07 ± 2.87	33.90 ± 14.52
Prema	33.47 ± 9.28	24.81 ± 5.18	7.53 ± 2.39	2.33 ± 0.48	12.67 ± 2.44	27.22 ± 10.66
Super yali	33.40 ± 11.84	23.89 ± 6.45	8.07 ± 3.47	2.67 ± 0.79	14.00 ± 3.21	30.24 ± 14.06
Wade	41.27 ± 6.87	26.79 ± 3.35	9.20 ± 4.68	2.64 ± 1.10	14.07 ± 2.66	20.61 ± 7.22
LSD	6.82*	4.71^{ns}	2.45^{ns}	0.57^{ns}	2.02*	8.98^{ns}

Note: Values represent mean ± standard deviation. LSD = Least significant difference, * = significant at p value < 0.05, ns = not significant.

Table 4: Onion Genotype performance for yield parameters treated with Oxide Nanoparticle

Genotype	Bulb Biomass	Bulb Weight	Horizontal Bulb Diameter	Vertical Bulb Diameter	Dry Weight
Mara kara	21.78 ± 9.45	10.57 ± 5.02	4.46 ± 0.99	10.06 ± 2.06	5.98 ± 3.58
Me kara	28.27 ± 9.30	12.71 ± 4.48	3.91 ± 0.83	9.14 ± 2.56	6.81 ± 4.51
Prema	20.47 ± 7.50	10.11 ± 7.81	3.80 ± 1.48	9.28 ± 2.39	5.11 ± 3.40
Super yali	26.69 ± 7.57	10.37 ± 3.95	3.22 ± 0.84	9.74 ± 2.28	5.53 ± 2.86
Wade	18.20 ± 8.37	7.11 ± 3.10	3.69 ± 1.45	8.95 ± 2.09	3.81 ± 1.70
LSD	6.17*	3.74^{ns}	0.84^{ns}	1.66^{ns}	2.43^{ns}

Note: Values represent mean ± standard deviation. LSD = Least significant difference, * = significant at p value < 0.05, ns = not significant.

Table 5 and 6 results indicate a dose dependent effect of iron oxide nanoparticles on onion growth and yield. While survival traits (establishment and stand count) remained largely unaffected, vegetative and yield traits improved progressively at higher nanoparticle concentrations, with 80 rpm showing consistent superiority. This trend aligns with nanoparticle studies in onions and other crops, where higher but controlled dosages enhanced plant metabolism, nutrient assimilation, and photosynthetic efficiency (Ahmed *et al*., 2023; El-Saadony *et al.*, 2021; Farooqui *et al.,* 2019; Rajput *et al*., 2018).

However, the non-significance of several traits (fresh weight, bulb weight, dry weight) indicates that nanoparticle effects are trait-specific and strongly influenced by genotype × treatment interactions, as seen in earlier tables. This suggests that both genotype selection and nanoparticle optimization are crucial for achieving yield gains.

Table 5: Iron Oxide Nanoparticle Treatments Effect for growth parameters of Onion Genotypes

Treatment	Establishment Count	Plant Height	Number of Leaves	Neck Thickness	Final Stand Count	Fresh Plant Weight
0rpm	35.93 ± 11.67	22.47 ± 6.01	6.93 ± 2.66	2.62 ± 0.91	12.60 ± 2.29	24.42 ± 12.88
20rpm	35.67 ± 14.25	24.16 ± 4.37	8.73 ± 5.16	2.72 ± 0.91	14.03 ± 4.21	24.77 ± 10.76
40rpm	42.20 ± 4.86	26.30 ± 3.85	8.13 ± 2.53	2.66 ± 0.61	12.75 ± 3.47	25.51 ± 9.69
60rpm	40.07 ± 7.07	23.96 ± 8.09	7.93 ± 2.58	2.51 ± 0.86	11.90 ± 3.09	30.27 ± 12.49
80rpm	38.47 ± 9.20	30.55 ± 6.75	9.20 ± 2.91	2.58 ± 0.67	13.33 ± 2.35	34.17 ± 15.92
LSD	16.23^{ns}	9.80*	5.41^{ns}	1.30^{ns}	5.16^{ns}	20.40^{ns}

Note: Values represent mean ± standard deviation. LSD = Least significant difference, * = significant at p value < 0.05, ns = not significant.

Table 6: Iron Oxide Nanoparticle Treatments Effect for yield parameters of Onion Genotypes

Treatment	Bulb Biomass	Bulb Weight	Horizontal Bulb Diameter	Vertical Bulb Diameter	Dry Weight
0rpm	21.69 ± 8.11	10.82 ± 3.83	3.80 ± 0.93	9.95 ± 2.21	5.53 ± 2.40
20rpm	22.03 ± 8.39	9.23 ± 4.52	3.60 ± 1.18	9.27 ± 2.24	4.91 ± 3.78
40rpm	21.15 ± 6.77	8.02 ± 4.79	3.27 ± 0.97	9.41 ± 2.25	4.03 ± 1.65
60rpm	25.90 ± 9.86	9.62 ± 2.85	3.85 ± 1.11	9.00 ± 2.58	5.16 ± 2.24
80rpm	24.64 ± 11.80	13.19 ± 8.14	4.55 ± 1.46	9.53 ± 2.20	7.59 ± 5.09
LSD	14.90^{ns}	8.38^{ns}	1.87*	3.74^{ns}	5.34^{ns}

Note: Values represent mean ± standard deviation. LSD = Least significant difference, * = significant at p value < 0.05, ns = not significant.

The correlation results (Table 7) highlighted a complex interaction between iron oxide nanoparticle treatments and genotype-specific responses in onions. Positive correlations among traits like establishment count. Plant height, and dry weight suggest that nanoparticles can enhance vegetative growth in certain genotypes. For instance, there was correlations between establishment count and plant height (r = 0.854) as well as Establishment count and Dry Weight (r = 0.947*) (Table 12) this indicate that better establishment improves growth outcomes. Prakash *et al*. (2022) suggest that taller plants with well-established stands are more likely to achieve better biomass accumulation.

However, trade-offs were evident between vegetative and reproductive traits. For example, Fresh Plant Weight exhibited strong negative correlations with number of leaves (r = -0.876) and Bulb Weight (BW) (r = -0.908*) (Table 12), suggesting resource reallocation between leaf growth and bulb development. This is consistent with studies showing that nanoparticles may differentially influence plant physiology based on treatment concentrations and genotypic traits (Raliya *et al*., 2015).

The Principal Component Analysis (PCA) results (Table 8) reinforce these findings by identifying key traits that drive variability among genotypes. The first principal component axis, PC1, which explained 57.7% of the variation, was primarily influenced by Establishment count, Number of leaves per plant, and Final Stand Count, suggesting that establishment and vegetative growth traits dominate genotype responses. This emphasizes the importance of these traits in distinguishing genotype performance under nanoparticle treatments. In contrast, PC2 (27.2% variance) was influenced by bulb-related traits such as FPW, Bulb Base (BB), and BW, indicating a secondary but critical role of reproductive traits in response differentiation.

The dendrogram (Figure 1) further illustrates these genotype-specific responses. Genotypes like "wade" and "super yali" clustered closely, indicating similar adaptive strategies under iron oxide nanoparticle treatments. This clustering suggests that these genotypes may share traits linked to efficient resource allocation, making them promising candidates for further evaluation. On the other hand, genotypes like "mara kara" and "me kara" formed separate branches, reflecting variability in adaptability, which could guide targeted breeding programs or agronomic interventions.

Table 7: Correlation amongst the Morphological Attributes of some Genotypes Exposed to Iron Oxide Nanoparticle Treatments in Lafia

	EC	*PH*	*NL*	*NT*	*FSC*	*FPW*	*BB*	*BW*	*VBD*	*HBD*	*DBW*
EC	1										
PH	0.854	1.000									
NL	0.455	0.384	1.000								
NT	0.606	0.754	0.703	1.000							
FSC	0.977	0.920*	0.409	0.707	1.000						
FPW	-0.400	-0.115	-0.876	-0.518	-0.318	1.000					
BB	-0.205	0.145	-0.758	-0.279	-0.093	0.963*	1.000				
BW	-0.260	-0.021	-0.908*	-0.430	-0.163	0.968*	0.954*	1.000			
VBD	-0.855	-0.462	-0.401	-0.319	-0.758	0.588	0.511	0.433	1.000		
HBD	-0.746	-0.724	-0.646	-0.457	-0.672	0.373	0.213	0.410	0.517	1.000	
DBW	0.947*	0.718	0.190	0.367	0.907*	-0.245	-0.101	-0.074	-0.896*	-0.579	1

EC-establishment count, PH- plant height, NL-number of leaves, FSC-final stand count, BB-bulk biomass, FPW-fresh plant weight, BW-bulb weight, HBD- horizontal bulb and VBD-vertical bulb diameter and DBW-dry bulb weight

Table 8: Principal Component Analysis the Morphological Attributes of some Genotypes Exposed to Iron Oxide Nanoparticle Treatments in Lafi

Eigenvalue	6.3426	2.9916	1.1239	0.5419	0.0000	0.0000	-0.0000	-0.0000
Proportion	0.577	0.272	0.102	0.049	0.000	0.000	-0.000	-0.000
Cumulative	0.577	0.849	0.951	1.000	1.000	1.000	1.000	1.000
Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
EC	0.360	0.232	0.126	-0.003	0.281	0.040	0.462	0.125
PH	0.292	0.336	-0.327	0.010	0.049	-0.320	-0.649	0.114
NL	0.310	-0.301	-0.309	-0.157	0.258	0.504	0.075	0.431
NT	0.294	0.014	-0.522	0.517	0.024	0.122	0.031	-0.222
FSC	0.343	0.279	-0.005	0.184	-0.595	-0.181	0.418	0.114
FPW	-0.289	0.389	-0.080	-0.151	0.093	-0.197	0.147	0.475
BB	-0.217	0.463	-0.223	-0.086	0.125	0.204	0.011	0.218
BW	-0.251	0.446	0.016	0.103	-0.151	0.635	-0.061	-0.329
VBD	-0.326	-0.053	-0.531	-0.073	0.274	-0.319	0.398	-0.355
HBD	-0.308	-0.095	0.171	0.791	0.214	-0.055	-0.019	0.347
DBW	0.301	0.297	0.377	0.050	0.574	-0.084	0.003	-0.308

EC-establishment count, PH- plant height, NL-number of leaves, FSC-final stand count, BB-bulk biomass, FPW-fresh plant weight, BW-bulb weight, HBD- horizontal bulb and VBD-vertical bulb diameter and DBW-dry bulb weight

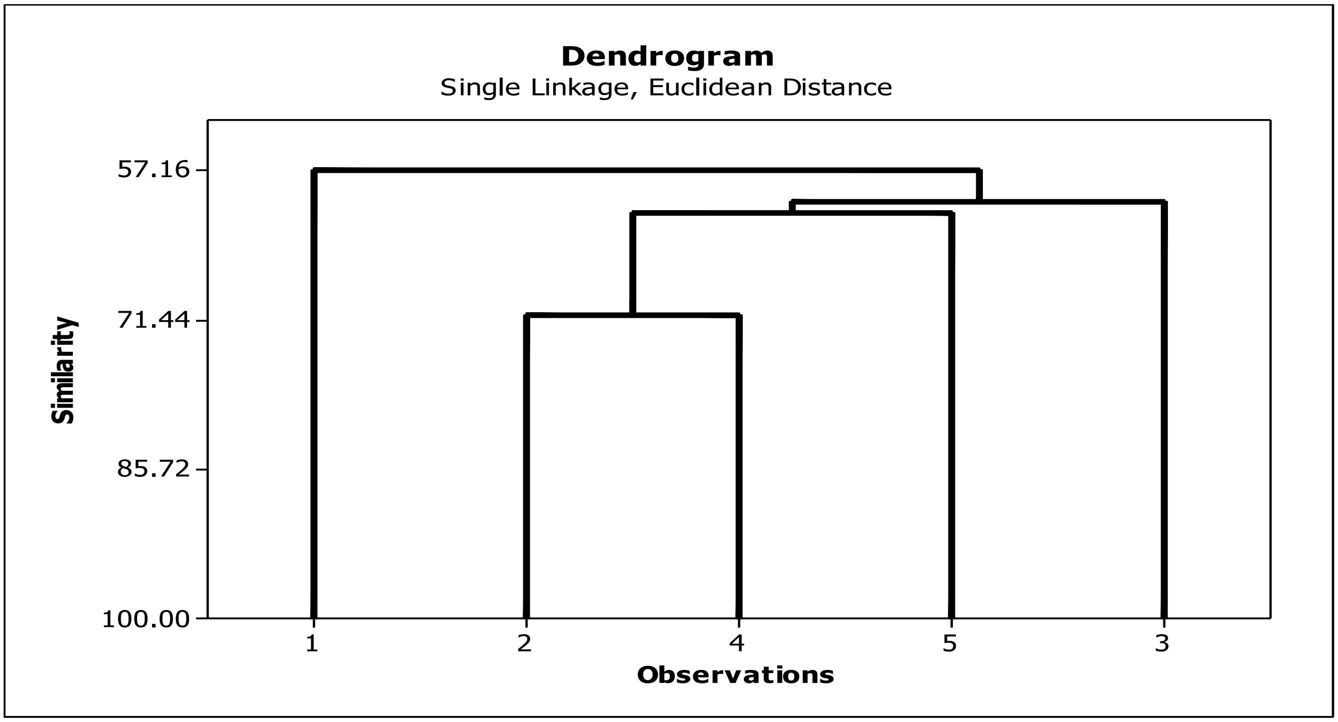

Figure 1: Dendrogram showing the Phylogenetic Relationship amongst 5 Onion Genotypes treated with Iron Oxide Nanoparticles in Lafia.

Keys

1-wade, 2-prema, 3-mara kara,4-super yali, 5-me kara

Conclusion

This study revealed that the effects of iron oxide nanoparticles on onion genotypes in Lafia are strongly influenced by both concentration and genotype. Genotypes like Me Kara and Super Yali exhibited positive responses in terms of plant height, leaf count, and bulb weight at optimal nanoparticle concentrations, while other genotypes showed varying results, despite some yield traits not showing significant statistical differences, the trends observed suggest that nanoparticle application can enhance onion productivity when integrated with genotype-specific management. Positive correlations between traits like establishment count, plant height, and dry bulb weight suggest that nanoparticles can enhance vegetative growth, but trade-offs were observed between vegetative and reproductive traits, such as fresh plant weight and bulb weight. The Principal Component Analysis revealed that vegetative traits played a dominant role in genotype responses, while reproductive traits were secondary but still significant. The dendrogram further illustrated genotype clustering based on their adaptive responses to the treatments. The potential of iron oxide nanoparticles can improve onion growth, but the effectiveness of the treatment is dependent on genotype and concentration.

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