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Influence of NPK Fertilization on Physiology of Rose

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Authors' contributions

This work was carried out in collaboration among all authors. Author PB designed the study and performed the investigation in field. Authors SM, KD and BH formulated the design of the study and managed the analyses of the study. Author BS wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The current study was carried out to assess the influence of different concentrations of nitrogen (N), phosphorus (P), and potassium (K) and their interaction on important physiological parameters—chlorophyll content, stability index of chlorophyll (CSI), and leaf area duration (LAD)—of rose (Rosa spp.) cv. 'Top Secret' under polyhouse conditions. The experiment was laid out in a factorial randomized block design with three replications at the Experimental Farm, Assam Agricultural University, Jorhat during 2022–2023. Treatment included three levels each of N, P_2O_5 , and K_2O (10, 20, and 30 g/m²). Result indicated that increased levels of nitrogen (N₃) increased chlorophyll content significantly (2.47 mg/g), CSI (26.22%), and LAD (54.93 days). Phosphorus and potassium levels also had an effect on these characters, where P_2 and K_2 were most superior in enhancing chlorophyll content and LAD, but K_3 recorded the highest CSI. Among interaction treatments, $N_2P_1K_3$ had the maximum CSI (30.48%) and LAD (59.70 days), reflecting enhanced physiological efficiency and drought tolerance. The results emphasize the significance of balanced

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NPK fertilization to maximize growth and stress tolerance in rose culture. The findings form a platform for further optimization of nutrient management schemes to promote floricultural performance, particularly in controlled environments.

Keywords: Rose; chlorophyll content; chlorophyll stability index; leaf area duration; NPK fertilization.

1. INTRODUCTION

Rose (Rosa spp.), commonly thought of as the "Queen of Flowers," is among the most economically valuable and extensively grown ornamental crops worldwide. It occupies an important place in cut flower industry. Rose has occupied a prominent place among the various flower crops due to its different sizes, various attractive colours, longer stalks and intriguing fragrance. Besides cut flower, rose can also be used as loose flower. Garland making and extraction of essential oil are some of the major uses of loose rose flowers. Whereas, cut flowers are mainly used in bouquet and for table decoration in vases. Miniature roses are excellent as pot plants.

With its beauty, aroma, and market worth in floriculture, rose production demands delicate nutrient management to provide maximum growth, flowering, and post-harvest quality. Of major plant nutrients, nitrogen (N), phosphorus (P), and potassium (K) are all vital in physiological and biochemical functions like chlorophyll production, leaf growth, and stress resistance. Nitrogen, being an important constituent of amino acids. proteins, and chlorophyll, plays a crucial role in vegetative growth as well as photosynthesis. Phosphorus is involved in energy transfer and root growth, while potassium controls the functioning of stomata, water relations, and enzyme activation. A proper ratio of N, P, and K not only promotes plant vigor

but also enhances photosynthetic efficiency and resistance to stress.

Chlorophyll content, chlorophyll stability index (CSI), and leaf area duration (LAD) are important physiological traits that directly correlate with productivity in plants. CSI is especially significant since it is regarded as an indicator of drought resistance, showing the plant's resistance to chlorophyll loss under stress conditions. LAD shows the functional life of leaves, resulting in extended photosynthesis and potential yield.

This current research seeks to examine the individual and interactional effects of varying levels of nitrogen, phosphorus, and potassium on chlorophyll content, chlorophyll stability index, and leaf area duration in rose. These findings can shed light on optimal nutrient ratios for better physiological efficiency and plant performance.

2. MATERIAL AND METHODS

The present experiment was conducted at naturally ventilated polyhouse in the Experimental Farm, Department of Horticulture, Assam Agricultural University, Jorhat (26°47′ N latitude, 94°12′E longitude and 86.8 m above the mean sea-level) during the period of 2022-2023.

2.1 Design and Layout

The experiment was laid out in factorial RBD with 3 replications. Total experimental area was 167 square metre and the individual plot size was 1 m x 1 m.

2.2 Treatment Details

Table 1. The treatments involve different combinations of N, P and K at 3 levels each (10, 20, 30 g/m²)

Levels of N	Levels of P ₂ O ₅	Levels of K₂O	
$N_1 = 10 \text{ g N/m}^2$	$P_1 = 10 \text{ g } P_2O_5/m^2$	$K_1 = 10 \text{ g } K_2 \text{O/m}^2$	
$N_2 = 20 \text{ g N/m}^2$	$P_2 = 20 \text{ g } P_2 O_5 / \text{m}^2$	$K_2 = 20 \text{ g } K_2 \text{O/m}^2$	
$N_3 = 30 \text{ g N/m}^2$	$P_3 = 30 \text{ g } P_2O_5/m^2$	$K_3 = 30 \text{ g } K_2 \text{O/m}^2$	

The treatment combinations were as: $T_1=N_1P_1K_1=10:10:10\ g/m^2$, $T_2=N_1P_1K_2=10:10:20\ g/m^2$, $T_3=N_1P_1K_3=10:10:30\ g/m^2$, $T_4=N_1P_2K_1=10:20:10\ g/m^2$, $T_5=N_1P_2K_2=10:20:20\ g/m^2$, $T_6=N_1P_2K_3=10:20:30\ g/m^2$, $T_7=N_1P_3K_1=10:30:10\ g/m^2$, $T_8=N_1P_3K_2=10:30:20\ g/m^2$, $T_9=N_1P_3K_3=10:30:30\ g/m^2$, $T_{10}=N_2P_1K_1=20:10:10\ g/m^2$, $T_{11}=N_2P_1K_2=20:10:20\ g/m^2$, $T_{12}=N_2P_1K_3=20:10:30\ g/m^2$, $T_{13}=N_2P_2K_1=20:20:10\ g/m^2$, $T_{14}=N_2P_2K_2=20:20:20\ g/m^2$, $T_{15}=N_2P_2K_3=20:20:30\ g/m^2$, $T_{16}=N_2P_3K_1=20:30:10\ g/m^2$, $T_{17}=N_2P_3K_2=20:30:20\ g/m^2$, $T_{18}=N_2P_3K_3=20:30:30\ g/m^2$, $T_{19}=N_3P_1K_1=30:10:10\ g/m^2$, $T_{20}=N_3P_1K_2=30:10:20\ g/m^2$, $T_{21}=N_3P_1K_3=30:10:30\ g/m^2$, $T_{22}=N_3P_2K_1=30:20:10\ g/m^2$, $T_{23}=N_3P_2K_2=30:20:20\ g/m^2$, $T_{24}=N_3P_2K_3=30:20:30\ g/m^2$, $T_{25}=N_3P_3K_1=30:30:10\ g/m^2$, $T_{26}=N_3P_3K_2=30:30:20\ g/m^2$, $T_{27}=N_3P_3K_3=30:30:30\ g/m^2$, and $T_{28}=N_0P_0K_0\ (Control)$

2.3 Materials Used

The rose variety "Top Secret" was selected for the experiment. Top Secret is a high yielding variety, which produces deep red colour flowers with longer stems. It is a type of Hybrid tea rose. The flower size ranges from 5.5 – 7.0 cm and produces flowers upto 170 number per plant per year. It also has a long self and vase life.

Urea, Single Super Phosphate (SSP) and Muriate of Potash (MOP) were used as the source of N, P and K, respectively. Half dose of urea and full doses of SSP and MOP were applied as basal during the time of final land preparation. Remaining half urea for all the treatments were applied after 30 days of planting.

2.4 Observations

2.4.1 Chlorophyll content (mg/g)

Fresh leaf samples were used for estimation of chlorophyll. The absorbance of the extract was measured at 645 and 663 nm wavelength filter in UV-V in spectrophotometer for the determination of the total chlorophyll content following the procedure of Holden (1965).

2.4.2 Chlorophyll stability index (%)

The Chlorophyll Stability Index was measured by following the method of Chetty *et al.* (2002).

2.4.3 Leaf area duration (days)

The formula of Power *et al.* (1967) was used to calculate the Leaf Area Duration (LAD).

$$LAD (days) = \frac{(LAI2 + LAI1)}{2}(t2 - t1)$$

Where, LAI1 = Leaf Area Index at the time t1
LAI2 = Leaf Area Index at the time t2
t1 = Initial time of observation
t2 = Final time of observation

2.5 Statistical Analysis

The data recorded were statistically analyzed by adopting the procedure of ANOVA given by Panse and Sukhatme (1978).

3. RESULTS AND DISCUSSION

3.1 Chlorophyll Content

Chlorophyll pigment present in leaves or other plant tissues are referred to as chlorophyll content. It is a very good parameter to study as this will help to correlate conversion of chemical energy to biological energy. More chlorophyll

content signifies more photosynthetic ability, which in turn indicates more potential yield.

Chlorophyll content as affected by N, P, K and their interaction are presented in Table 2 and 3. Among the various nitrogen level N2 registered the highest chlorophyll content 2.47 mg/g and 2.21 mg/g both at flower formation and post flowering stages followed by N₃ which registered 2.42 mg/g and 2.19 mg/g. This might be due to the fact that nitrogen is a structural element of chlorophyll (each chlorophyll molecule contains four nitrogen atoms) and protein molecules and thereby affects the formation of chloroplasts and accumulation of chlorophyll in leaves (Tucker, 2004 and Wellburn, 1994). Therefore, sufficient supply of nitrogen is of pivotal importance for sufficient synthesis of chlorophyll molecules in leaves. Nitrogen plays crucial role in formation of chlorophyll molecules in leaves. Hokmalipour and Darbandi (2011) critically witnessed that increasing nitrogen levels in rose growing directly led to a significant increase in the content of chlorophyll.

Again, among the various phosphorus levels, P_2 registered the highest chlorophyll content of 2.42 mg/g and 2.21 mg/g both prior to flowering, subsequently followed by P_3 which had 2.41 mg/g prior to flowering and P_1 which had 2.19 mg/g which proved to be non-significant. Though phosphorus is not a component of chlorophyll molecule, it plays vital indirect role in energy transfer processes necessary for the functioning of chlorophylls in leaves. Phosphorus plays key role in helping in the process of photosynthesis. Phosphorus is a key molecule in adenosine triphosphate (ATP) and several other energy transfer molecules.

Among various potassium levels, K2 had the highest chlorophyll content prior to and subsequent to flowering i.e. 2.43 mg/g and 2.23 mg/g followed by K₁ (2.41 mg/g) prior to flowering and K₃ (2.19 mg/g) subsequent to flowering. The property of potassium through which it aids carbohydrate metabolism might be the reason behind this. This finding is in similar line with the finding of Chaudhary et al. (2018) in rose. Experimentation with several ornamental plants exhibited that the higher potassium levels resulted in superior vegetative growth and flower count (Nabatia, 2022), which might be due to better photosynthetic abilities in those plants supported by potassium. Stomatal functions can be impaired in the deficiency of potassium limiting the availability of CO₂ for photosynthesis.

Table 2. Chlorophyll content (mg/g) before flowering

N level		P level		K level	
N1	2.34	P1	2.39	K1	2.41
N2	2.47	P2	2.42	K2	2.43
N3	2.42	P3	2.41	K3	2.38
S.Ed±	0.01		0.01		0.01
CD0.05	0.03		NS		0.03

	P1	P2	P3		K1	K2	K3		K1	K2	K3
N1	2.34	2.35	2.33	N1	2.34	2.36	2.33	P1	2.37	2.41	2.39
N2	2.42	2.45	2.38	N2	2.46	2.44	2.35	P2	2.45	2.47	2.35
N3	2.41	2.46	2.53	N3	2.45	2.49	2.46	P3	2.42	2.41	2.41
S.Ed:	± 0.03			S.E	Ed± 0.0)3		S.Ec	t± 0.03	3	
CD0.	05 0.06	CE	0.05 NS	3		CD0	.05 0.06	3			

		N1			N2			N3		
	P1	P2	P3	P1	P2	P3	P1	P2	P3	
K1	2.34	2.34	2.33	2.43	2.55	2.38	2.35	2.45	2.54	
K2	2.35	2.40	2.32	2.46	2.47	2.40	2.43	2.53	2.52	
K3	2.36	2.31	2.34	2.37	2.34	2.36	2.46	2.41	2.53	
	± 0.05		2.54	2.31	2.34	2.30	2.40	2.41		
CD0	.05 NS									

Table 3. Chlorophyll content (mg/g) after flowering

N level		P leve	l	K leve	el
N1	2.19	P1	2.19	K1	2.16
N2	2.21	P2	2.21	K2	2.23
N3	2.19	P3	2.18	K3	2.19
S.Ed±	0.01		0.01		0.01
CD0.05	NS		NS		0.03

	P1	P2	P3		K1	K2	K3		K1	K2	K3
N1	2.15	2.22	2.20	N1	2.16	2.21	2.19	P1	2.17	2.19	2.21
N2	2.24	2.22	2.10	N2	2.17	2.23	2.16	P2	2.18	2.25	2.20
N3	2.19	2.19	2.25	N3	2.15	2.24	2.22	P3	2.14	2.24	2.17
S.Ed:	± 0.03			S.Ed	d± 0.03	}		S.Ed	d± 0.03	3	
CD0.	05 0.05			CD0	.05 NS			CDC	0.05 NS		

		N1			N2			N3			
	P1	P2	P3	P1	P2	P3	P1	P2	P3		
K1	2.10	2.20	2.18	2.24	2.22	2.05	2.17	2.11	2.18		
K2	2.16	2.25	2.22	2.23	2.24	2.22	2.20	2.26	2.28		
K3	2.19	2.19	2.21	2.25	2.21	2.04	2.21	2.20	2.27		
S.E	d± 0.05										
CD	0.05	NS									

This may lead to degrade4d photosynthetic ability and in turn chlorophyll stability.

Among the interaction treatments, N_3P_2 reported the highest means of chlorophyll content i.e. 2.46 mg/g. Again, P_2K_2 reported highest chlorophyll content of 2.47 mg/g prior to flowering. Further, in post flowering, the interaction treatment of

N₃P₃ reported the highest chlorophyll content of 2.25 mg/g which was significant. Similar results were obtained by Sharma et al. (2009) in marigold. Jawad and Khalil (2024) also noticed the effect of NPK in leaf chlorophyll content of rose. The interaction treatments of NxPxK were not significant both prior to and post flowering. The application of N, P, and K altogether is vital

for maximizing chlorophyll content and overall plant health in roses. A balanced fertilizer schedule ensures that all necessary components for chlorophyll synthesis and photosynthetic efficiency are available. The effect of all these three macro nutrients is much more in chlorophyll content of leaves than a single macronutrient alone The control treatment showed chlorophyll content of 2.24 mg/g and 1.98 mg/g both before and after flowering.

3.2 Chlorophyll Stability Index

The Chlorophyll Stability Index (CSI) is a extent of a plant's ability to maintain chlorophyll content under stress conditions, particularly drought or salinity. More the CSI, more the ability of the plant to resist adverse stress condition. If the CSI could be increased by any treatment, it can help the researchers to a great extent. The influence of nitrogen, phosphorus and potassium in overall plant health and defense mechanism against stress help in increased Chlorophyll Stability Index.

Chlorophyll stability index due to N, P, K and their interaction are presented in Table 4. Out of various different levels of nitrogen, N₃ with 30 g N/m² revealed highest stability index rate of 26.22% which was significant, different from N₂ reported stability index of 25.26%. Out of various different levels of phosphorus, P2 reported the highest stability rate 25.83% and K₃ reported maximum chlorophyll stability rate of 26.04% followed by P3, registered stability index of 25.26% and K2 registered stability index of 25.53%. It is well known that the Chlorophyll stability index is linearly correlated with the ability of the plant to tolerate drought and phosphorus and potassium can aid to the ability of the plants to tolerate drought. So, increasing levels of levels of phosphorus and potassium exhibited higher chlorophyll stability index. Nitrogen maintain cell membrane stability along with activation of antioxidant defense mechanisms, in turn protect the photosynthetic which apparatus during stress from oxidative damages (Hasanuzzaman et al., 2013). Phosphorus aids in membrane stability and ultimately higher membrane stability leads to higher chlorophyll Stability Index (Ali et al., 2021). Potassium is the most critical element in helping stress tolerance ability of the plants and in turn Chlorophyll Stability Index.

Among the various interactions, N₃P₂ registered the highest means of chlorophyll stability rate of

26.93%. N₃K₃ registered highest means of 27.69% and P₁K₃ registered highest stability 27.00%. which index of were significantly. Among the NxPxK interactions. N₂P₁K₃ registered highest chlorophyll stability rate of 30.48%, which was found to be differed significantly among the treatments. Role of NPK in chlorophyll stability Index was also noticed by Pandya et al. (2023) in wheat. It has been proved that balanced NPK fertilization plays pivotal role in increasing Chlorophyll Stability Index directly leading to better ability of the plants to tolerate stress. All these three major plant nutrients act synergistically in increasing the Chlorophyll Stability Index of rose and other targeted plants.

3.3 Leaf Area Duration

Leaf area duration under the effect of N. P. K and their interactions are presented in the Table 5. Out of the various levels of nitrogen, N₃ (30 g N/m²) registered maximum leaf area duration of 54.93 days followed by N2 that registered 54.41 days. This is because nitrogen contributes to the accumulation of more photosynthates which could have led to greater leaf area. Since leaf area and leaf area duration are both interrelated hence greater nitrogen levels led to greater leaf area duration. Nitrogen plays most important role in increasing leaf area duration. Nitrogen is a component of all proteins, nucleic acids and enzymes which promotes cell division and enlargement. These are the processes which contributes to larger leaf size. Role of nitrogen in chlorophyll synthesis leads to photosynthetically active leaves. Out of the levels of phosphorus, P₂ (20 g P₂O₅/m²) registered maximum leaf area duration of 54.93 days followed by P₁, registered leaf area duration of 54.72 days. Phosphorus improves absorption capacity of roots, which supports sustainable health and growth of the leaves. Hence, the leaf area duration is increased (Saeed & Amin, 2016). Again, K₃ (30 g K₂O/m²) registered highest leaf area duration of 55.61 days followed by K₂, registered 54.36 days, which was proved significant amongst the various levels. Potassium helps in maintaining leaf turgor by regulating water relation and stress tolerance, which in turn extends leaf area duration. Longer leaf area duration in rose due to potassium fertilization was also reported by (Biswas et al., 2024). Chlorophyll degradation and leaf senescence can be restricted by potassium through its action in improving stress tolerance. This protective action ensures more leaf viability, thereby increasing leaf area duration (Ullah et al., 2022).

Table 4. Chlorophyll Stability Index (%)

N lev	el	Р	level	K	level
N1	24.56	P1	24.94	K1	24.46
N2	25.26	P2	25.83	K2	25.53
N3	26.22	P3	25.26	K3	26.04
S.Ed±	0.45		0.45		0.45
CD0.05	0.91		NS		0.91

	P1	P2	P3		K1	K2	K3		K1	K2	K3
N1	22.84	24.72	26.12	N1	23.06	25.46	25.15	P1	23.18	24.65	27.00
N2	26.12	25.83	23.81	N2	25.04	25.45	25.28	P2	25.26	26.61	25.61
N3	25.86	26.93	25.86	N3	25.28	25.69	27.69	P3	24.93	25.34	25.52
S.Ed±	0.78			S.E	S.Ed ± 0.78				d± 0.78	1	
CD0.0	5 1.57			CDC	0.05 NS			CD	0.05 1.57		

		N1			N2			N3	
	P1	P2	P3	P1	P2	P3	P1	P2	P3
K1	20.49	25.30	23.40	23.45	26.22	25.44	25.59	24.28	25.96
K2	25.67	22.74	27.99	24.45	27.77	24.14	23.85	29.33	23.89
K3	22.37	26.11	26.97	30.48	23.52	21.85	28.15	27.19	27.73
S.Ed	d± 1.36								
CDC	0.05 2.73								

Table 5. Leaf Area Duration (Days)

N level		P leve	!	K level				
N1	54.24	P1	54.72	K1	53.61			
N2	54.41	P2	54.93	K2	54.36			
N3	54.93	P3	53.93	K3	55.61			
S.Ed±	0.76		0.76		0.76			
CD0.05	NS		NS		1.53			

	P1	P2	P3		K1	K2	K3		K 1	K2	K3
N1	54.31	54.46	53.94	N1	54.39	53.83	54.49	P1	53.57	53.78	56.81
N2	55.11	54.48	53.65	N2	52.95	53.71	56.57	P2	54.11	54.82	55.85
N3	54.74	55.85	54.21	N3	53.47	55.55	55.77	P3	53.14	54.49	54.16
S.E	d± 1.32			S.Ed	d± 1.32			S.E	d± 1.32		
CDC).05 NS			CDO	0.05 NS			CDO	0.05 NS		

	N1			N2			N3		
	P1	P2	P3	P1	P2	P3	P1	P2	P3
K1	55.26	55.26	52.66	52.86	53.02	52.98	52.60	54.05	53.78
K2	53.40	53.16	54.93	52.76	54.89	53.48	55.19	56.41	55.06
K3	54.27	54.96	54.23	59.70	55.52	54.48	56.45	57.08	53.78
S.Ec	d± 2.30								
CD0.05 NS									

Amongst the interactions, N_3P_2 registered maximum leaf area duration of 55.85 days. In the same way, N_2K_3 and P_1K_3 registered highest leaf area duration of 56.57 days and 56.81 days, respectively which differed non significantly amongst the treatments.

Again, amongst the various interactions of NxPxK, $N_2P_1K_3$ registered highest leaf area

duration of 59.70 days, which proved to be non-significant.

4. CONCLUSION

The results of the research evidently prove the pronounced effect of nitrogen, phosphorus, and potassium contents on chlorophyll content, stability index of chlorophyll, and leaf area

duration in rose. Under different treatments, the maximum chlorophyll content was observed with N₃ (30 g N/m²), P₂ (20 g P₂O₅/m²), and K₂ (20 g K₂O/m²), supporting the positive impact of balanced macronutrient application on photosynthetic pigments.

With respect to chlorophyll stability index, N_3K_3 , P_1K_3 , and $N_2P_1K_3$ exhibited significantly higher Chlorophyll Stability Index values, with the highest Chlorophyll Stability Index of 30.48% in $N_2P_1K_3$, indicating better drought tolerance.

For leaf area duration, optimal responses were seen with N_3 , P_2 , and K_3 treatments alone, whereas $N_2P_1K_3$ registered the highest leaf area duration of 59.70 days, showing longer photosynthetic activity and leaf longevity.

Collectively, the treatment $N_2P_1K_3$ was the best for optimizing physiological efficiency in rose with possible short-term recommendations for nutrient management practices aimed at enhancing stress tolerance and growth performance in rose production.

The results of the research evidently prove the pronounced effect of nitrogen, phosphorus, and potassium contents on chlorophyll content, stability index of chlorophyll, and leaf area duration in rose. Under different treatments, the maximum chlorophyll content was observed with N_3 (30 g N/m²), P_2 (20 g P_2O_5/m^2), and K_2 (20 g K₂O/m²), supporting the positive impact of macronutrient balanced application on photosynthetic pigments. With respect to chlorophyll stability index, N₃K₃, P₁K₃, and N₂P₁K₃ exhibited significantly higher CSI values, with the highest CSI of 30.48% in N₂P₁K₃, indicating better drought tolerance. For leaf area duration, optimal responses were seen with N₃, P_2 , and K_3 treatments alone, whereas $N_2P_1K_3$ registered the highest LAD of 59.70 days, showing longer photosynthetic activity and leaf longevity.

Among all the treatments, the treatment $N_2P_1K_3$ was the best for optimizing physiological efficiency in rose with possible short-term recommendations for nutrient management practices aimed at enhancing stress tolerance and growth performance in rose production.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative Al technologies such as Large Language Models

(ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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