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In-vitro regeneration studies of red banana (*Musa Acuminata* L.) in response to plant growth regulators

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Abstract

Red Banana (*Musa acuminata* L.) is an economically important banana cultivar valued for its attractive fruit colour, nutritional quality and market preference. However, large-scale cultivation is constrained by the limited availability of healthy and disease-free planting material through conventional sucker propagation. Therefore, the present investigation was undertaken to standardize an efficient *in vitro* regeneration protocol for Red Banana using shoot tip explants. The study was conducted at the Centre of Excellence in Plant Biotechnology, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during 2024-25 and 2025-26.

The different combinations of plant growth regulators were evaluated for shoot establishment, shoot multiplication and root induction. Among the media combinations tested, MS medium supplemented with 4.0 mg L⁻¹ BAP and 0.5 mg L⁻¹ NAA recorded the highest shoot establishment (86.67%), maximum number of multiple shoots per explant (7.63) and minimum time for shoot establishment (24.18 days). For rooting, MS medium supplemented with 1.5 mg L⁻¹ IBA, 10 mg L⁻¹ ascorbic acid and 0.5% activated charcoal recorded the minimum time for root induction (12.03 days) and the highest rooting percentage (93.33%).

The results demonstrated that plant growth regulator concentration and combination influenced regeneration and rooting responses in Red Banana. The optimized media combinations provide a useful basis for rapid multiplication and production of healthy, uniform and disease-free planting material.

Keywords: Red Banana; *Musa acuminata*; shoot tip culture; *in vitro* regeneration; micropropagation; BAP; NAA; IBA.

Introduction

Banana (*Musa* spp.) is one of the most important fruit crops cultivated throughout the tropical and subtropical regions of the world. It belongs to the family Musaceae and is believed to have originated in Southeast Asia (Simmonds and Shepherd, 1955) [13]. Owing to its year-round availability, affordability, nutritional value and wide adaptability, banana plays a significant role in food security and livelihood generation in many developing countries. India is the largest producer of banana in the world and contributes substantially to global production (FAO, 2023). The crop occupies a prominent position among fruit crops in terms of area and production and is cultivated extensively in states such as Maharashtra, Andhra Pradesh, Gujarat, Tamil Nadu and Karnataka (NHB, 2021-22; FAO, 2023). Among the diverse banana cultivars grown in India, Red Banana (*Musa acuminata* L.) has gained considerable importance due to its attractive fruit colour, consumer preference and premium market value. The cultivar is recognized for its unique organoleptic qualities and nutritional richness, making it increasingly popular among growers and consumers.

Conventionally, banana is propagated through suckers; however, this method is associated with several limitations. The multiplication rate is relatively low, and the planting material often serves as a source of transmission of soil-borne pathogens and viral diseases, particularly Banana Bunchy Top Virus (BBTV). Furthermore, sucker-derived plantations frequently exhibit non-uniform growth and development, which adversely affects crop management and productivity. The limited availability of healthy and disease-free suckers also restricts rapid expansion of banana cultivation (Vuylsteke and De Langhe, 1985) [15]. Therefore, the availability of quality planting material remains one of the major constraints in the commercial cultivation of banana. Plant tissue culture has emerged as an efficient

biotechnological approach for rapid multiplication of elite banana genotypes and production of genetically uniform, disease-free planting material throughout the year. Micropropagation enables large-scale multiplication under controlled conditions and has been successfully utilized for commercial production of several banana cultivars (Cronauer and Krikorian, 1984; Vuylsteke and Ortiz, 1996)^[2]. The success of *in vitro* propagation largely depends on the optimization of culture media and plant growth regulators used during different stages of regeneration. Among the various cytokinins employed in banana tissue culture, 6-benzylaminopurine (BAP) has been widely reported to be effective in promoting shoot induction and multiplication. Likewise, auxins such as naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA) play important roles in shoot development, root induction and establishment of regenerated plantlets. Previous studies have demonstrated that the response of banana explants to plant growth regulators varies considerably among cultivars, emphasizing the need for genotype-specific protocol standardization (Hegde *et al.*, 2018; Rajoriya *et al.*, 2018)^[5, 10]. Although considerable progress has been made in the micropropagation of banana, cultivar-specific optimization of plant growth regulator combinations remains important for efficient regeneration of Red Banana. The response of shoot tip explants depends on the type and concentration of cytokinins and auxins used during shoot establishment, multiplication and rooting. Therefore, the present investigation was undertaken to standardize an efficient *in vitro* regeneration protocol for Red Banana (*Musa acuminata* L.) using shoot tip explants by evaluating different combinations of BAP and NAA for shoot establishment and multiplication and different concentrations of IBA for root induction under *in vitro* conditions.

Materials and Methods

The experiment was conducted at the Centre of Excellence for Plant Biotechnology, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, during the academic years 2024-25 and 2025-26. Healthy sword suckers of Red Banana (*Musa acuminata* L.) obtained from the mother block nursery maintained at the Centre of Excellence for Plant Biotechnology were used as the source of shoot tip explants. Murashige and Skoog (MS) basal medium was used throughout the investigation. The medium was supplemented with 3% sucrose and 0.8% agar, and the pH was adjusted to 5.8 before sterilization. Stock solutions of the basal MS medium constituents were prepared using triple-distilled water and stored under refrigerated conditions until use.

Autoclaving

The prepared culture media were sterilized by autoclaving at 121°C and 15 psi pressure for 20 min. Sterilized culture vessels and other required materials were maintained under aseptic conditions until use.

Culture room sterilization and transfer area

All aseptic operations were carried out in a laminar airflow cabinet. The working surface was cleaned with 70% rectified spirit and exposed to ultraviolet (UV) light before use. Petri plates and instruments used for inoculation were

sterilized with rectified spirit and flame-sterilized before and during culture operations.

Sterilization of explant

The healthy sword suckers were thoroughly washed under running tap water and trimmed to obtain suitable shoot tip explants. The explants were subjected to the standardized surface-sterilization procedure using Bavistin, Tween-20, citric acid, ascorbic acid, sodium hypochlorite and mercuric chloride under aseptic conditions. The sterilized explants were subsequently washed thoroughly with sterile distilled water and used for inoculation onto MS medium.

Inoculation of explants

The explants were transferred aseptically to sterile Petri plates using sterilized forceps. The explants were inoculated vertically into culture vessels containing autoclaved MS medium supplemented with the respective plant growth regulators. Care was taken to maintain the shoot tip or nodal portion above the surface of the culture medium. After inoculation, the mouth of each culture vessel was quickly flamed and sealed with a sterilized cap.

Effect of media on shoot establishment and multiplication

The effect of different combinations of BAP and NAA on shoot establishment and multiplication of Red Banana shoot tip explants was evaluated using MS medium. Four combinations of 6-benzylaminopurine (BAP) and naphthalene acetic acid (NAA), viz., 2.0 mg L⁻¹ BAP + 0.5 mg L⁻¹ NAA, 3.0 mg L⁻¹ BAP + 1.0 mg L⁻¹ NAA, 4.0 mg L⁻¹ BAP + 0.5 mg L⁻¹ NAA and 6.0 mg L⁻¹ BAP + 1.0 mg L⁻¹ NAA, were evaluated. Hormone-free MS medium was maintained as the control treatment. Observations were recorded on shoot establishment percentage, days required for shoot establishment and number of multiple shoots produced per explant.

Effect of rooting media on root induction

The well-developed shoots obtained during the multiplication phase were transferred to rooting media for root induction. The rooting medium consisted of MS medium supplemented with 10 mg L⁻¹ ascorbic acid and 0.5% activated charcoal. IBA was incorporated at concentrations of 0.5, 1.0, 1.5 and 2.0 mg L⁻¹, along with a hormone-free control treatment. Observations were recorded on the days required for root induction and rooting percentage.

Observations recorded

The observations recorded during the investigation included shoot establishment percentage, days required for shoot establishment, number of multiple shoots per explant, days required for root induction and rooting percentage.

Experimental design and statistical analysis

The experiment was laid out in a Completely Randomized Design (CRD) with three replications and 10 explants per treatment in each replication. The recorded data were subjected to analysis of variance (ANOVA), and treatment differences were tested at the 5% level of probability following the procedure described by Snedecor and Cochran (1980)^[14].

Result and Discussion

Effect of media combinations on shoot establishment (%)

The data pertaining to the effect of different media combinations on shoot establishment of Red Banana are presented in Table 1. Significant differences were observed among the treatments with respect to shoot establishment percentage. Among the media combinations evaluated, treatment T4 (MS + 4.0 mg L⁻¹ BAP + 0.5 mg L⁻¹ NAA) recorded the highest shoot establishment (86.67%) and was found significantly superior to the remaining treatments. This was followed by T3 (76.67%), T5 (63.33%) and T2 (46.67%). The lowest shoot establishment (33.33%) was recorded in the control treatment (T1).

The results indicated that shoot establishment in Red Banana was markedly influenced by the concentration and combination of plant growth regulators present in the culture medium. An increasing trend in shoot establishment was observed with increasing concentration of BAP up to 4.0 mg L⁻¹ in combination with 0.5 mg L⁻¹ NAA. The superior response recorded in treatment T4 indicates that the concentration of BAP and NAA used in this treatment was optimum for successful establishment and growth of cultured explants. However, a slight decline in shoot establishment was observed in treatment T5, suggesting that higher concentrations of BAP may not further improve establishment response under *in vitro* conditions.

The present findings are in agreement with those reported by Hegde *et al.* (2018) [5], who observed enhanced establishment of Red Banana explants on MS medium supplemented with optimum concentrations of BAP. Similar observations were recorded by Rajoriya *et al.* (2018) [10], and Keskar *et al.* (2018) [7], who reported improved culture establishment under suitable cytokinin concentrations in banana. Kadam *et al.* (2023) [6], also reported that balanced concentrations of plant growth regulators significantly improved explant establishment and regeneration efficiency. Similar findings were reported by Patil *et al.* (2025) [9], who recorded maximum shoot establishment in Red Banana

cultured on MS medium supplemented with suitable concentrations of BAP and growth-promoting additives. Thus, MS medium supplemented with 4.0 mg L⁻¹ BAP and 0.5 mg L⁻¹ NAA was found most suitable for shoot establishment of Red Banana under *in vitro* conditions.

Table 1: Effect of different media combinations on shoot establishment (%) in Red Banana

Treatment	Media combination	Shoot establishment (%)
T1	MS control media	33.33 (35.26) ^e
T2	MS + 2.00 BAP + 0.5 NAA	46.67 (43.09) ^d
T3	MS + 3.00 BAP + 1.00 NAA	76.67 (61.12) ^b
T4	MS + 4.00 BAP + 0.5 NAA	86.67 (68.59) ^a
T5	MS + 6.00 BAP + 1.0 NAA	63.33 (52.73) ^c
S.E.m. ±		2.24
C.D. at 5%		7.07
C.V. (%)		7.63

(Figures in parentheses indicate arcsine transformed values.)

Note

- Treatment means having common superscript are statistically non-significant.
- Treatment means having different superscript are statistically significant.



Plate 1: Surface Sterilization and Inoculation of Red Banana Shoot Tip Explants on MS Medium



Plate 2: (a,b,c). Initiation of Explants on MS Medium Supplemented with Plant Growth Regulators

Effect of media combinations on days required for shoot establishment

The days required for shoot establishment varied among the different media combinations evaluated (Table 2). Treatment T4 (MS + 4.0 mg L⁻¹ BAP + 0.5 mg L⁻¹ NAA) recorded the minimum time required for shoot establishment

(24.18 days), followed by T5 (25.47 days), T3 (26.73 days) and T2 (28.56 days). The maximum time required for shoot establishment (30.84 days) was recorded in the control treatment (T1). The results indicated that the concentration and combination of BAP and NAA influenced the time required for shoot establishment. The faster response in T4

may be attributed to the favourable hormonal balance provided by BAP and NAA, which supported early shoot initiation and establishment. The slightly longer establishment period observed in T5 compared with T4

suggests that the higher PGR combination used in T5 was less favourable for rapid shoot establishment under the present conditions.

Table 2: Effect of BAP and NAA on Days Required for Shoot Establishment

Treatment	Media combination	Days required for shoot establishment
T1	MS control medium	30.84 ^e
T2	MS + 2.00 mg L ⁻¹ BAP + 0.5 mg L ⁻¹ NAA	28.56 ^d
T3	MS + 3.00 mg L ⁻¹ BAP + 1.00 mg L ⁻¹ NAA	26.73 ^c
T4	MS + 4.00 mg L ⁻¹ BAP + 0.5 mg L ⁻¹ NAA	24.18 ^a
T5	MS + 6.00 mg L ⁻¹ BAP + 1.00 mg L ⁻¹ NAA	25.47 ^b
	S.E. (m.±)	0.36
	C.D. at 5%	1.13

Note

- Treatment means having common super script are statistically non-significant.
- Treatment means having different super script are statistically significant.

Effect of media combinations on multiple shoots per explant

The data pertaining to the effect of different media combinations on multiple shoot induction in Red Banana are presented in Table 3. Significant differences were observed among the treatments with respect to the number of shoots produced per explant. Among the media combinations evaluated, treatment T4 (MS + 4.0 mg L⁻¹ BAP + 0.5 mg L⁻¹ NAA) recorded the highest number of shoots per explant (7.63) and was found significantly superior to all other treatments. This was followed by T5 (6.63 shoots), T3 (4.63 shoots) and T2 (3.20 shoots). The minimum number of shoots per explant (1.53) was recorded in the control treatment (T1).

The results clearly indicated that shoot multiplication in Red Banana was significantly influenced by the concentration and combination of plant growth regulators used in the culture medium. A progressive increase in the number of shoots per explant was observed with increasing concentration of BAP up to 4.0 mg L⁻¹ in combination with 0.5 mg L⁻¹ NAA. The superior response recorded in treatment T4 suggests that the concentration of BAP and

NAA used in this treatment was optimum for shoot multiplication and proliferation of cultured explants. However, a decline in shoot production was observed in treatment T5, indicating that increasing BAP concentration beyond the optimum level did not further improve the multiplication response.

The present findings are in agreement with those reported by Hegde *et al.* (2018) ^[5], who observed maximum shoot multiplication in Red Banana cultured on MS medium supplemented with optimum concentrations of BAP. Rajoriya *et al.* (2018) ^[10], also reported enhanced shoot proliferation in banana under suitable cytokinin concentrations. Similar observations were recorded by Keskar *et al.* (2018) ^[7], who obtained increased shoot multiplication in Red Banana on growth regulator supplemented media. Kadam *et al.* (2023) ^[6], further reported that optimum concentrations of plant growth regulators significantly improved shoot proliferation and regeneration efficiency in banana cultures. Comparable results were obtained by Patil *et al.* (2025) ^[9], who recorded maximum multiple shoot induction in Red Banana cultured on optimized media combinations.

The results of the present investigation indicate that MS medium supplemented with 4.0 mg L⁻¹ BAP and 0.5 mg L⁻¹ NAA was the most effective treatment for achieving higher shoot multiplication in Red Banana under *in vitro* conditions.

Table 3: Effect of different media combinations on multiple shoots per explant in Red Banana

Treatment	Media combination	Multiple shoots per explant
T1	MS control media	1.53 (1.426) ^e
T2	MS + 2.00 BAP +0.5 NAA	3.20 (1.923) ^d
T3	MS + 3.00 BAP + 1.00 NAA	4.63 (2.266) ^c
T4	MS + 4.00 BAP + 0.5 NAA	7.63 (2.852) ^a
T5	MS + 6.00 BAP+1.0 NAA	6.63 (2.671) ^b
	S.Em. ±	0.023
	C.D. at 5%	0.072
	C.V. (%)	1.78

(Figures in parentheses indicate square root transformed values.)

Note

- Treatment means having common superscript are statistically non-significant.

- Treatment means having different superscript are statistically significant.

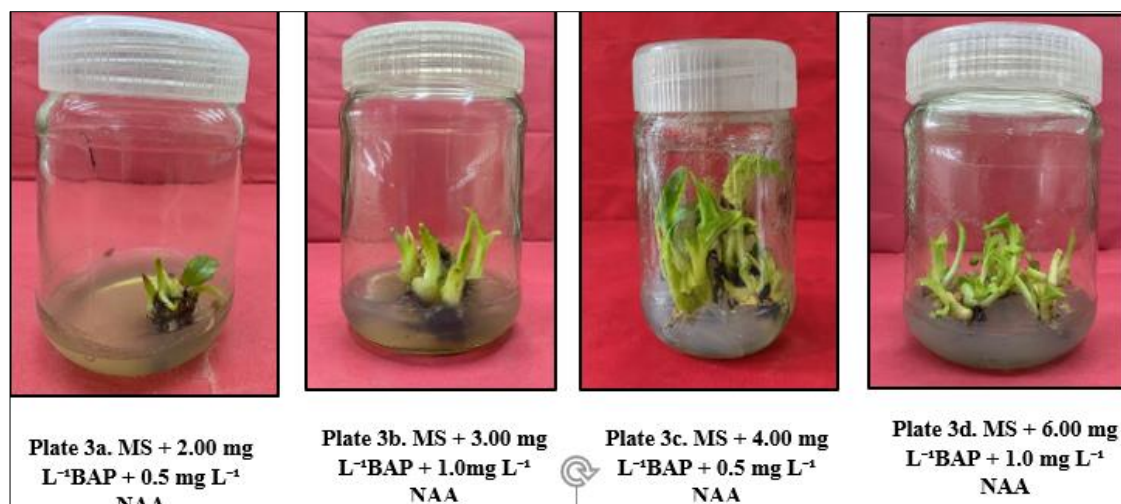


Plate 3: (a, b, c, d). Effect of BAP and Supplement Combinations on Multiple Shoot Induction

Effect of media combinations on days required for root induction

The data pertaining to the effect of different media combinations on days required for root induction in Red Banana are presented in Table 4. Significant differences were observed among the treatments. Treatment T4 (MS +

1.5 mg L⁻¹ IBA + 10 mg L⁻¹ ascorbic acid + 0.5% activated charcoal) recorded the minimum time required for root induction (12.03 days), followed by T5 (14.50 days), T3 (18.07 days) and T2 (23.03 days), while the maximum time was recorded in the control treatment T1 (29.40 days).

Table 4: Effect of different media combinations on days required for root induction in Red Banana

Treatment	Media combination	Days required for root induction
T1	MS control media	29.40 ^c
T2	MS + 0.5 mg L ⁻¹ IBA + 10 mg/l Ascorbic acid + 0.5% A. Charcoal	23.03 ^d
T3	MS + 1.0mg/l IBA + 10 mg/l Ascorbic acid + 0.5% A. Charcoal	18.07 ^c
T4	MS + 1.5mg/l IBA + 10 mg/l Ascorbic acid + 0.5% A. Charcoal	12.03 ^a
T5	MS + 2.0mg/l IBA + 10 mg/l Ascorbic acid + 0.5% A. Charcoal	14.50 ^b
	S.Em. ±	0.04
	C.D. at 5%	0.12
	C.V. (%)	0.36

Note

- Treatment means having common superscript are statistically non-significant.
- Treatment means having different superscript are statistically significant.

The results indicated that increasing IBA concentration up to 1.5 mg L⁻¹ progressively reduced the time required for root induction, with T4 showing the earliest root initiation. However, a slight increase in the number of days was observed in T5, indicating that increasing IBA concentration beyond the optimum level did not further accelerate root induction. The present findings are in agreement with Ali *et al.* (2011) ^[1], and Govindaraju *et al.* (2012) ^[4], who reported improved root initiation and reduced rooting time in banana under suitable auxin concentrations.

Effect of media combinations on rooting percentage in red banana (%)

The data pertaining to the effect of different media combinations on rooting percentage in Red Banana are presented in Table 5. Significant differences were observed

among the treatments with respect to rooting percentage. Treatment T4 (MS + 1.5 mg L⁻¹ IBA + 10 mg L⁻¹ ascorbic acid + 0.5% activated charcoal) recorded the highest rooting percentage (93.33%) and was statistically at par with treatments T3 (83.33%) and T5 (86.67%). Treatment T3 recorded 83.33% rooting, followed by T2 (66.67%), whereas the lowest rooting percentage (43.33%) was observed in the control treatment (T1).

The results indicated that rooting response in Red Banana was influenced by the concentration of IBA in the culture medium. Rooting percentage increased with increasing IBA concentration up to 1.5 mg L⁻¹, with T4 showing the highest response. However, a slight reduction in rooting percentage was observed at 2.0 mg L⁻¹ IBA (T5), indicating that increasing IBA concentration beyond the optimum level did not further improve rooting efficiency.

The present findings are in agreement with Ali *et al.* (2011) ^[1], and Govindaraju *et al.* (2012) ^[4], who reported improved rooting responses in banana under suitable auxin concentrations. Similar observations were also reported by Kadam *et al.* (2023) ^[6] and Patil *et al.* (2025) ^[9], for Red Banana under optimized rooting conditions.

Table 5: Effect of different media combinations on rooting percentage (%) in Red Banana

Treatment	Media combination	Rooting percentage (%)
T1	MS control media	43.33 (41.14) ^c
T2	MS + 0.5 mg L ⁻¹ IBA + 10 mg/l Ascorbic acid + 0.5% A. Charcoal	66.67 (54.76) ^b
T3	MS + 1.0mg/l IBA + 10 mg/l Ascorbic acid + 0.5% A. Charcoal	83.33 (66.11) ^a
T4	MS + 1.5mg/l IBA + 10 mg/l Ascorbic acid + 0.5% A. Charcoal	93.33 (77.69) ^a
T5	MS + 2.0mg/l IBA + 10 mg/l Ascorbic acid + 0.5% A. Charcoal	86.67 (68.82) ^a
S.Em. ±		3.47
C.D. at 5%		10.94
C.V. (%)		9.75

(Figures in parentheses indicate arcsine transformed values.)

Note

i) Treatment means having common superscript are statistically non-significant.

ii) Treatment means having different superscript are statistically significant.

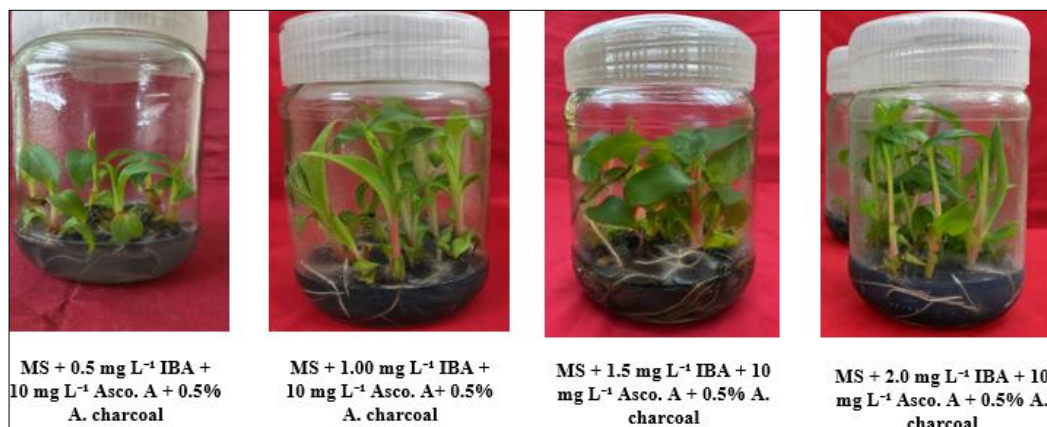


Plate 4: (a, b, c, d). Effect of Different Plant Growth Regulator Combinations on Rooting

Conclusion

The present investigation demonstrated that the regeneration response of Red Banana (*Musa acuminata* L.) was strongly influenced by the concentration and combination of plant growth regulators used in the culture media. MS medium supplemented with 4.0 mg L⁻¹ BAP and 0.5 mg L⁻¹ NAA was the most effective treatment for shoot establishment and multiplication, recording the highest shoot establishment (86.67%), the minimum time required for shoot establishment (24.18 days) and the maximum number of multiple shoots per explant (7.63). For root induction, MS medium supplemented with 1.5 mg L⁻¹ IBA, 10 mg L⁻¹ ascorbic acid and 0.5% activated charcoal resulted in the earliest root induction (12.03 days) and the highest rooting percentage (93.33%). Thus, the optimized combinations of BAP, NAA and IBA identified in the present study provide a useful basis for efficient *in vitro* regeneration and multiplication of Red Banana.

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