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Original Research Paper

Impact of staggered transplanting of pollen parents and repeated pollination methods in hybrid seed production of marigold (*Tagetes erecta* L.)

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ABSTRACT

Marigold is an important commercial flower crop of India and hybrids are in demand. However, the hybrid seeds are being imported due to the limited number of hybrids. Seed production of F_1 hybrids needs to be enhanced to reduce the dependency on imported seeds. Therefore, the present study was initiated to work out the strategies for maximizing the hybrid seed production in four hybrid combinations. Seed parents exhibited delayed flowering with an extended duration ranging from 104.89 to 114.50 days, in contrast to the early-flowering pollen parents, which had a shorter flowering duration of 65.39 to 72.28 days. This discrepancy could be effectively addressed by staggered planting of pollen parents at multiple intervals, ensuring consistent pollen availability throughout the active flowering period of the seed parents. Irrespective of the hybrid combinations, repeated pollination for three-time registered higher seed setting percentage (49.56%), more number of seeds (112.79) and higher seed weight (250.67 mg) per flower compared to either one or two time pollinations. The study highlighted the potential for enhanced hybrid seed production through staggered planting of pollen parents combined with repeated pollination, ensuring maximum seed set per flower and optimizing seed yield per unit area of production.

Keywords: Marigold, pollination, seed production, staggered transplanting, synchronization

INTRODUCTION

The African marigold (*Tagetes erecta* L.), family Asteraceae, is native to Mexico and Central America. Marigold hybrid flowers are popular for garlands and decorative purposes during religious festivals, weddings and special occasions. The plant attracts insect pests, making it an effective trap crop in vegetables and cereal crops. Additionally, it is used against nematodes due to the presence of nematicidal compounds in its roots (Kumar et al., 2023). Marigold is gaining its popularity for its diverse uses, including a wide range of flower colors and its applications in industries such as oil and carotenoid extraction (Shaik et al., 2023). In India, marigold cultivation spans approximately 87.07 thousand hectares, with an annual production of 952.60 thousand metric tonnes of loose flowers and 13.55 thousand metric tonnes of cut flowers (Anonymous, 2024). In recent years, F_1 hybrids of marigold are in high demand due to their higher yield, early flowering, improved flower shape, longer shelf life, and uniform blooming. However, few hybrids are available, and hybrid seeds remain expensive due to the labor-intensive process of emasculation required to remove multiple disc florets, which bear both anthers and stigmas. Male sterility systems are widely utilized to eliminate the need for emasculation in hybrid seed production of marigold (Tejaswini et al., 2016). Globally, the demand for marigold hybrid seeds is estimated at approximately 10,000 kg annually (Sukwiwat et al., 2023).

In India, flower seed production is practiced on about 600–800 hectares, primarily in regions like Punjab (Sangrur, Patiala, and Ludhiana); Haryana (Panipat, Sirsa); Karnataka (Bengaluru, Ranebennur); Himachal Pradesh (Kullu Valley); Jammu & Kashmir (Sri Nagar Valley); and West Bengal (Kalimpong) (Patil et al., 2023). However, these are mainly open pollinated seeds of annual flower crops meant for landscape and farmers still face challenges in obtaining hybrid seeds for commercial flower production. With the increasing demand for marigold driven by their multiple utility, the seed business is emerging as a growing sector. Despite the growth in the seed sector, India relies heavily on imported marigold hybrid seeds. In India, ICAR-Indian Institute of Horticultural Research is the only public sector organization that has come out with four marigold hybrids. However, the seed production is still challenging in these hybrids and the research to maximize seed production in these four combinations is essential to overcome the dependency on imported seeds.

The importance of high-quality seeds cannot be overlooked as they play a vital role in enhancing profitability of farmers and growers (Tejaswini et al., 2024). F_1 hybrid seed production basically depends on emasculation and efficient pollination. Although the male sterility system in marigolds eliminates the labor requirement for emasculation, hand pollination remains essential for hybrid seed production. Inadequate pollination is one of the primary reasons for low seed set or low hybrid seed quality. Previous studies on repeated hand pollination of sterile marigold plants have reported higher seed set and improved seed yield (Kumar et al., 2004).

A critical factor in achieving higher seed yield and quality is the synchronization of flowering between male and female parents. Staggered transplanting of pollen parents at different intervals is a simple, effective, and economical strategy to synchronize flowering between male and female parents. Studies on staggered sowing to synchronize flowering between female and male parents in cotton and maize hybrids demonstrated that staggered planting leads to maximum synchronization (Doddagoudar et al., 2008; Kumar et al., 2019). Therefore, this study aims to evaluate the effect of staggered planting of pollen parents and repeated pollination on enhancing seed quality, with a particular focus on improving seed setting percentage of marigold hybrid seed production.

MATERIALS AND METHODS

Plant materials and study site

The present study was carried out at ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India (13.13°N; 77.49°E) during 2022-2024. Two male sterile seed parents namely, IIHRMOS-1 (orange) and IIHRMYS-1 (yellow) were used in the study. Each seed parent was crossed with two pollen parents of corresponding flower colours. Altogether, four marigold hybrid combinations *viz.*, IIHRMOS-1 × IIHRMO-12, IIHRMOS-1 × IIHRMO-53, IIHRMYS-1 × IIHRMY2-1 and IIHRMYS-1 × IIHRMY1-4 were experimented in four exclusive naturally ventilated polyhouses. As marigold is an often-cross pollinated crop, the experiments were conducted in polyhouses to avoid out-crossing.

Staggered planting

Multiple intervals of transplanting were followed in pollen parents, while, the seed parents were transplanted only one time. Pollen parents were transplanted at multiple intervals from the date of transplanting male sterile seed parents *viz.*, T_1: 30 days after transplanting of seed parents (1^{st} planting); T_2: 50 days after transplanting of seed parents (2^{nd} planting); and T_3: 65 days after transplanting of seed parents (3^{rd} planting). Observations on plant height, days to first bud initiation, days to 50% flowering and

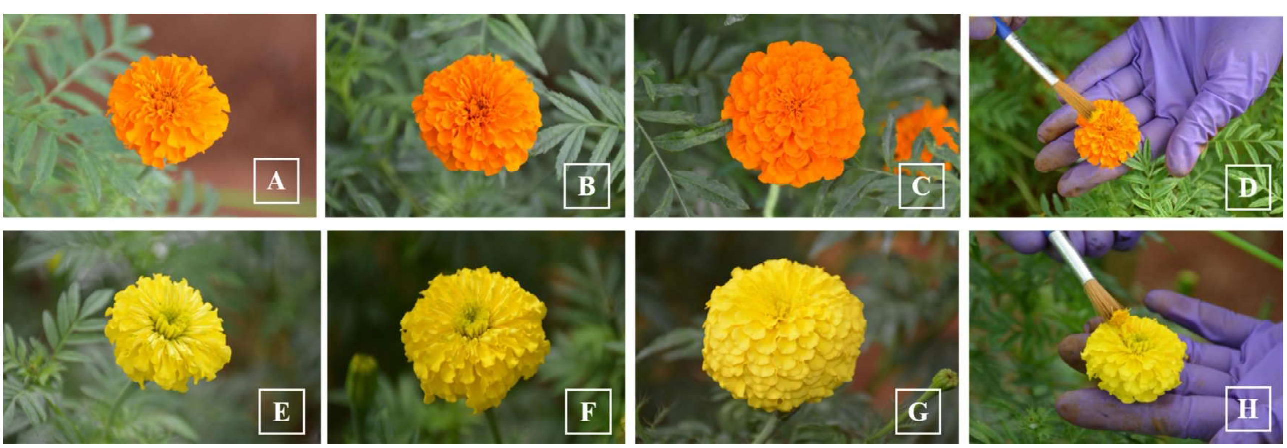

Fig. 1 : Flower opening and hand pollination in marigold
A & E: one third open, B & F: half open, C & G: full open,
D & H: hand pollination in IIHRMOS-1 (orange) and IIHRMYS-1 (yellow)

duration of flowering was recorded at the active growth period of plants. Number of flowers per plant was recorded throughout the flowering period at 15 days intervals.

Repeated pollination

For the repeated pollination experiments, flowers of the seed parents (IIHRMOS-1 and IIHRMYS-1) were repeatedly pollinated by pollen parents of respective hybrid combinations *viz.*, IIHRMOS-1 × IIHRMO-12, IIHRMOS-1 × IIHRMO-53, IIHRMYS-1 × IIHRMY2-1 and IIHRMYS-1 × IIHRMY1-4. Pollination was repeated one, two and three-times for comparison. One-time pollination was attempted at the full flower opening stage; two-time pollination was attempted i) during the half and ii) full flower opening stages; and three-time pollination was done during i) one-third, ii) half and iii) full flower opening stages (Fig. 1). The observation recorded includes the number of seeds per flower, seed weight per flower, seed setting percentage and seed germination.

Statistical analysis

A staggered transplanting experiment was conducted by using completely randomized design with three replications and the results obtained were analyzed by one-way analysis of variance (ANOVA) with $p \leq 0.05$ level of significance. A repeated pollination experiment was conducted by adapting completely randomized design under factorial set up with three replications and the results obtained were analyzed by two-way analysis of variance (ANOVA) with $p \leq 0.05$ level of significance. Analysis was carried out using statistical analysis software (SAS version 9.3).

RESULTS AND DISCUSSION

Staggered planting

Observations on vegetative and flowering parameters were taken to determine the synchronization of flowering in seed and pollen parents. The significant differences were observed in plant height, days to first bud initiation, days to 50% flowering, duration of flowering and number of flowers per plant between the seed and pollen parents (Table 1).

Flowering took more time in seed parents, IIHRMOS-1 (45.94) and IIHRMYS-1 (43.00) for days to bud initiation compared to that of pollen parents IHRMY 2-1 (36.67), IIHRMO 12 (37.83 days), IIHRMO 53 (38.61 days) and IIHRMY 1-4 (40.83 days), suggesting the necessity of early planting of seed parents compared to pollen parents. Flowering duration was longer in both the seed parents namely IHRMYS-1 (114.50 days) and IIHRMOS-1 (104.89 days) compared to their respective pollen parents. As seed parent is propagated through the vegetative cuttings and since the flowers are male sterile, the flowering duration and flowering time is longer in seed parent as compared to pollen parent. For instance, the longest flowering duration was recorded in IIHRMY 1-4 (72.28 days), followed by IIHRMO 53 (70.00 days) and IIHRMO 12

Table 1 : Mean performance of seed and pollen parents for vegetative and flowering characters

Genotype	Plant height (cm)	Days to first flower bud initiation	Days to 50% flowering	Total number of flowers/ plant	Flower diameter (cm)	Flower duration (days)
Seed parent						
IIHRMOS-1	69.58^{c}	45.94^{a}	70.61^{a}	236.09^{b}	4.83^{d}	104.89^{b}
IIHRMYS-1	87.22^{b}	43.00^{b}	67.72^{b}	336.44^{a}	5.95^{a}	114.50^{a}
Pollen parent						
IIHRMO 12	53.61^{d}	37.83^{d}	61.00^{d}	125.32^{c}	4.63^{e}	69.39^{d}
IIHRMO 53	54.72^{d}	38.61^{d}	62.72^{c}	127.61^{c}	4.62^{e}	70.00^{d}
IIHRMY 2-1	92.48^{a}	36.67^{e}	55.78^{e}	104.68^{d}	5.61^{b}	65.39^{e}
IIHRMY 1-4	87.77^{b}	40.83^{c}	69.11^{b}	122.07^{c}	5.10^{c}	72.28^{c}
CD at 5%	3.03	1.3	2.02	2.99	0.22	1.54
CV %	18.11	9	8.64	49.27	10.32	23.77

Values in same column with different letters indicate statistically significant differences at $p \leq 0.05$

(69.39 days) indicating the necessity of multiple planting times to ensure availability of pollen for hybridization throughout the flowering duration of seed parents. Between the seed parents, IIHRMYS1 recorded a higher total number of flowers per plant (336.44) compared to IIHRMOS-1 (236.09). Among the pollen parents, a significantly higher total number of flowers per plant were observed in IIHRMO 53 (127.61) which is at par with IIHRMO 12 (125.32). Among the seed parents flower diameter was maximum in IIHRMYS-1 (5.95 cm), while, IIHRMY2-1 with 92.48 cm was the tallest genotype among the pollen parents. Both seed and pollen parents of orange hybrid combinations were found to be shorter in height and smaller flowers based on flower diameter compared to parents of yellow hybrid combinations.

Availability of flowers at a particular time is more important than total number of flowers; hence it was assessed the availability of flowers across the flowering duration of seed parent with staggered planting of pollen parents (Fig. 2). The number of flowers per plant increased gradually up to 90 days after transplanting (45.00) in pollen parent IIHRMO 12 that was in synchrony with flowering in seed parent IIHRMOS-1. From 90 days onwards, seed parent continued to increase in number of flowers produced, reaching its peak at 105 days (65.50), but pollen parent flowers of 1st planting started reducing. However, 2nd and 3rd staggered planting of pollen parents started reaching their peak synchronizing with flowering of seed parent on the 105th (44.67) and 120th (43.83) days ensuring the availability of pollen for the flowers of the seed parent (Fig 2a). Similar trend was exhibited in pollen parent IIHRMO 53 with seed parent IIHRMOS-1 ensuring the availability of pollen due to different staggered planting (Fig. 2b). For the yellow seed parent IIHRMYS-1 and pollen parents (IIHRMY 2-1, IIHRMY 1-4), synchrony between the number of flowers per plant could not be obtained at any stage of growth (Fig. 2c & 2d). Number of flowers per plant in seed parent IIHRMYS-1 was always higher than that of pollen parents, while, multiple staggered planting ensured the availability of pollen throughout the flowering period of seed parent. The trend observed in the number of flowers per plant available in pollen and seed parents for the entire flowering period suggested the necessity of an enhanced number of pollen parents compared to seed parents. The present findings are in agreement with the reports of Doddagoudar et al. (2008), where the staggered sowing of male and female parents achieved

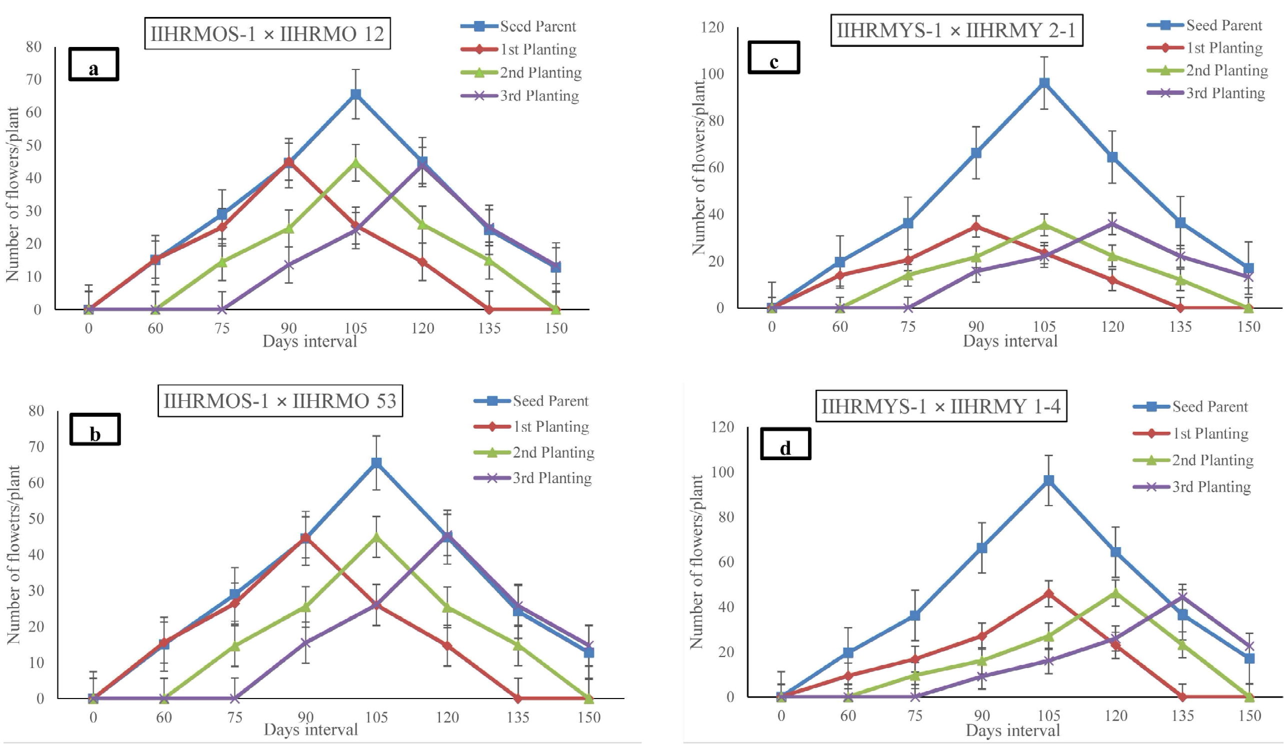

Fig. 2 : Synchronizations of flowering in pollen and seed parent plants of different hybrid combinations with respect to the different staggered planting stages of pollen parents in marigold

Table 2 : Effect of repeated pollination on seed parameters in different hybrid combination of marigold

Hybrid combinations	Number of seeds per flower				Seeds weight per flower (mg)				Seed germination (%)			
	Number of pollinations			Mean	Number of pollinations			Mean	Number of pollinations			Mean
	One time	Two time	Three time		One time	Two time	Three time		One time	Two time	Three time	
IIHRMOS-1 x IIHRMO 12	35.37	44.03	68.53	48.89^{c}	73.89	94.50	141.08	103.06^{c}	86.06	84.60	84.96	85.21^{c}
IIHRMOS-1 x IIHRMO 53	34.10	46.20	63.33	48.30^{c}	66.89	76.89	127.19	90.33^{c}	86.23	85.90	84.76	85.63^{c}
IIHRMYS-1 x IIHRMY2-1	104.80	137.83	177.20	139.94^{a}	217.19	294.40	429.83	313.74^{a}	92.90	91.40	90.86	91.22^{b}
IIHRMYS-1 x IIHRMY1-4	85.87	114.70	142.10	114.22^{b}	188.13	225.82	304.57	239.51^{b}	96.66	92.86	91.93	92.48^{a}
Mean	65.03^{c}	85.69^{b}	112.79^{a}	-	136.41^{c}	172.90^{b}	250.67^{a}	-	90.46^{a}	88.69^{b}	88.13^{b}	-
Comparison	SE	CD at 5%	CV%		SE	CD at 5%	CV%		SE	CD at 5%	CV%	
Hybrid combination (A)	1.08	2.18			6.52	13.10	64.49		0.29	0.59		
No. of repeated pollination (B)	0.94	1.89	59.35		5.64	11.35			0.25	0.51	4.73	
A x B	1.88	3.78			11.29	22.70			0.50	1.02		

Values in same column with different letters indicate statistically significant differences at $p \leq 0.05$

better synchronization of their flowering period for production of cotton hybrids, for efficient utilization of male flowers and for obtaining good quality hybrid seeds. On the contrary, seed yield and quality could not be enhanced by staggered planting in maize (Kumar et al., 2019).

Repeated pollination

Inadequate pollination is one of the main reasons for poor seed set and seed quality, therefore, repeated pollination was attempted to study the impact on quality seed production in marigold. Hybrid combinations had significant impact on all the seed parameters studied (Table 2).

Among the hybrid combinations, IIHRMYS-1 x IIHRMY 2-1 exhibited the significantly higher result with respect to the number of seeds per flower (139.94), seed weight per flower (313.74 mg) and seed setting (56.59%) (Fig. 3), while, IIHRMYS-1 x IIHRMY 1-4 hybrid combination exhibited the highest seed germination percentage (92.48%). In comparison to yellow hybrid combinations, both the orange hybrid combinations, IIHRMOS-1 × IIHRMO 53 and IIHRMOS-1 × IIHRMO 12 exhibited the minimum number of seeds (48.89, 48.30), seed weight per flower (90.33 mg, 103.06 mg), seed setting (24.95%, 25.70%) and seed germination (85.63% and 85.21%).

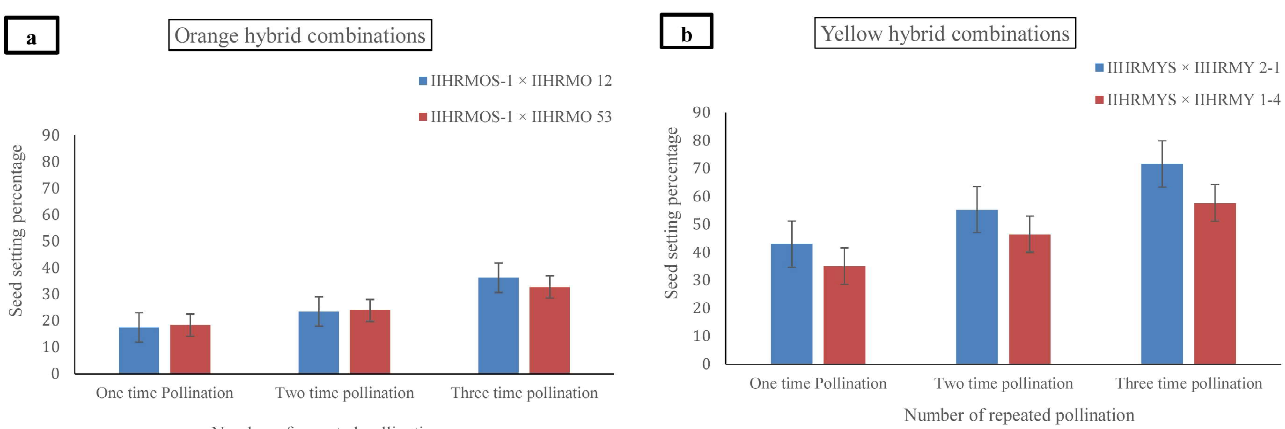

Fig. 3 : Effect of repeated pollination on seed setting percentage in orange (a) and yellow (b) hybrid combinations of marigold

Compared to one and two-time pollination, pollination repeated three times exhibited significantly higher results for seed parameters such as the number of seeds per flower (112.79), seed weight per flower (250.67 mg) and seed setting percentage (49.56%). One-time pollination resulted in a minimum seed weight per flower (136.41 mg) and seed setting (28.41%) (Fig. 3).

Nevertheless, one-time hand pollination exhibited maximum seed germination (90.46%) (Table 2). The interaction between the hybrid combination and number of repeated pollinations had significant impact on all the seed parameters. Hybrid combination of IIHRMYS-1 × IIHRMY 2-1 with three times repeated pollination, exhibited the significantly highest number of seeds per flower (177.20), seed weight per flower (429.83 mg), and seed setting percentage of 71.59% (Fig. 3). However, for seed germination, a hybrid combination of IIHRMYS-1 × IIHRMY 1-4 with one time pollination exhibited significantly higher results (96.66%) (Table 2).

In marigold plants, due to the complex flower capitulum and gradual opening of flower, the stigma receptivity in multiple ray florets within a flower capitulum varies. Availability of viable pollen from pollen parents and receptivity of stigma in seed parents must overlap so that the quality of the seeds as well as seed setting percentage will be good. Therefore, it is crucial to pollinate more than once so as to ensure the pollination to each of the ray florets in order to maximize the seed production per flower. Consequently, three times hand pollination exhibited better results. However, considering labor cost and maximizing quality seed production per unit area, two to three times repeated pollination can be attempted for economical seed production depending upon seed production area. The results are in agreement with Kumar et al. (2004), who studied marigold plants in which the repeated hand pollination of sterile flowers resulted in greater seed set and bold seed yield. Similarly, in hybrid tea rose repeated pollination at three, four and five times at 24 h intervals exhibited a greater number of achenes per fruit, greater fruit weight and weight of achenes, and greater numbers of seeds per pollinated flower (Devrise & Dubois, 1983).

CONCLUSION

The present study revealed the effectiveness of staggered transplanting of pollen parents and repeated pollination on marigold hybrid seed production. Flowering duration of seed parents was significantly longer compared to that of pollen parents. The multiple staggered transplanting of pollen parents could ensure the availability of pollen throughout the flowering duration of seed parents. Repeated pollination enhanced the percentage of seed set and resulted in higher seed yield. Additionally, the results also suggested the necessity of more pollen parents compared to seed parents in marigold hybrid seed production as the number of flowers per plant in seed parents were considerably higher compared to pollen parents at any point of growing time. Maximisation of hybrid seed production can be achieved by staggered and multiple times planting of pollen parents, repeated pollination along with enhancing plant ratio between pollen to seed parent.

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