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Development of Improved Mutants for Grain Yield and Related Traits in Finger Millet (*Eleusine coracana* **L Gaertn.) Through Gamma Mutagenesis**

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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

Recombination breeding is limited in finger millet due to tiny florets and irregular anthesis. Induced mutagenesis using gamma irradiation was attempted to improve grain yield and related traits in three elite cultivars (GPU28, GPU 67 and MR 6). Mean performance and estimates of BLUP and BLUE of 61 mutants showed variability among the mutants for grain yield in comparison to parental genotypes. Grain yield being a complex trait and influenced by high $G \times E$ interaction recorded low heritability estimate sand therefore BLUP provided more reliable estimates for grain yield. Stability analysis was conducted using GGE biplot techniques and also by plotting the trait means with their respective coefficient of variation values(CVs). From mean performance and stability analysis, the mutants GPU28-2212, GPU28-2086, GPU28-2082 derived from GPU 28 variety, GPU67-3358, GPU67-3359, GPU67-2094 from GPU67 variety and MR6-3393 from MR6 variety were superior for grain yield and related traits. Combined association analysis revealed tillers per plant as highly correlated trait with grain yield indicating key trait for grain yield improvement in finger millet. Mutants with improved finger traits and blast resistance are also discussed.

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1. INTRODUCTION

Finger millet [*Eleusine coracana* (L.) Gaertn.] popularly known as *ragi*, in Southern India is a highly valued as nutritious food and fodder crop. Among the different millet crops grown worldwide, finger millet occupies around 10% of the total cultivated area [1]. In India the crop is cultivated on an area of about 1.2 million ha, with production of 1.8 million tones and with average productivity of 1610 kg/ha. Karnataka occupies about 60% of the area grown followed by other states Uttarakhand, Maharashtra, Tamil Nadu, Odisha, Andhra Pradesh and Gujarat. Finger millet grain is nutritious, digestible and can be consumed cooked or ground to make flour for porridge or used to make cakes. It has the highest calcium content (300–350 mg/100 g) among the small millets which is 15-20 times higher than that of any cereal and therefore it is promising to be used as weaning foods and also for growing children for development of bones and teeth.

Genetic potential for grain yield in finger millet has increased from 500 kg/ha during 1913 to 4000-4500 kg/ha and this has been taken place in five phases. Phase-III of yield improvement marks the remarkable phase in the history of finger millet improvement which has brought quantum jump in finger millet productivity by crossing Indian and African types which were otherwise isolated gene pools. Subsequent improvement in finger millet was aimed at improving blast resistance which is the major constraint in major finger millet growing regions. In finger millet, most of cultivars released during last decade were through germplasm selections and during the current decade recombination breeding are being used as an major breeding method to develop improved cultivars. However, recombination breeding has not been exploited to its fullest potential compared to other major cereals, due to small size florets leading to difficulties in hybridization [2] [3].

Mutation breeding is one of the alternative approaches for creation of new variation and development of improved cultivars and has been successfully demonstrated in many crop species especially in groundnut and pulses. Development of improved varieties with stable yield and disease resistance is one of the major objectives in crop breeding programs. For stability analysis, methods like coefficient of variation, linear regression, additive main effects and multiplicative interaction (AMMI) model and Genotype plus GE (GGE) biplot analysis [4] were used to evaluate the test data and identify stable genotypes. AMMI and GGE methods were adopted in multi-environment (MET) two-way data matrices. In AMMI, GE interaction has been only taken into model and genotype effects were ignored while evaluating genotypes. Therefore, GGE biplot model is considered as one of the effective method for stability of genotypes [5]. Researchers have used GGE biplot widely for evaluating mean performance, stability of cultivars [6] and discrimination of test sites [7]. Selection in crop breeding is mostly practiced on simple arithmetic mean. Recently BLUE (Best linear unbiased estimate) based on fixed genotype effects and BLUP (Best linear unbiased prediction) which defines genotypes as random effect are being used for making more precise selections in plant breeding. With these background, the present study aimed at multilocational evaluation of the gamma irradiated finger millet mutants for grain yield and related traits and also identification of stable mutants using BLUP, BLUE and biplot techniques.

2. MATERIALS AND METHODS

2.1 Material Description and Growing Conditions

Three hundred grams seeds of three popular varieties GPU 28, GPU 67 and MR 6 were irradiated with gamma rays (400 Gy to 600Gy) and M¹ generation was raised during *kharif*, 2015. In M¹ generation, chlorophyll mutants were observed. About 750-1000 panicles from populations obtained from 500Gy and 600Gy gamma irradiation from each of the three mutant population (GPU 28, GPU 67 and MR 6) were harvested separately and were raised in panicle to row progeny in M² generation along with parental genotype. Selections were done during $M₂$ and $M₃$ generation as release of variability continued up to M³ generation. Selected lines were advanced till M₇ generation. During *kharif* 2019 and 2020, 61 promising mutants along the parental genotypes were conducted in replicated field trials in 4 environments: during 2019 and 2020 at Indian Institute of Millets Research, Hyderabad, Telangana, and during 2020 at Agricultural Research Station, Vizianagaram in Andhra Pradesh and AICRP Millets Project Coordinating Unit, Bengaluru in Karnataka. The trial was laid out in Randomized complete block design with two replications during the finger millet growing season (June–October). Standard crop management practices were used to raise the crop.

2.2 Data Collection and Data Analysis

Observations related to grain yield and its attributing traits such as plant height (ground level to tip of panicle in cm), days to 50% flowering (days from sowing to flowering in 50% of the plants), number of tillers, number of fingers (number of spikes counted in each panicles), finger length (average of finger length in cm excluding thumb finger)were recorded on five randomly selected plants in each replication. Blast score was recorded as per the procedure given in [18] Das et al. 2021. The grain yield was recorded on plot basis and were converted to kg/ha. Stability analysis was performed using GGE biplot implemented in GEA-R version 4.1 software [8] with the model equation: Yijμ+Gi+Ej+Σλkαikγjk+ eij Where Yij is the yield of ith genotype in the jth environment; Gi and Ej represent the genotype and environment deviations from the grand mean, respectively; μ denotes the grand mean λk is the eigenvalue of the PCA axis k; αik and γjk indicate the genotype and environment PC scores, respectively, for the axis k and eij denotes the error term. Mean against stability'' is based on averageenvironment coordination (AEC) view of the GGE biplot [9]. The linear models are used to calculate BLUEs and BLUPs and estimate the variance components implemented in the META software [10]. When calculating the BLUEs, genotypes are considered as fixed effects, whereas all other terms are declared random effects; for calculating the BLUPs, all effects are considered random. The model is Yijkl = μ + Envi + Repj (Envi) + Genl + Envi × Genl + εijkl. where Yijkis the trait of interest, μ is the mean effect, Repj is the effect of the jth replicate within the ith Environment, Genl is the effect of the lth genotype, εijkl is the error associated. Heritability for combined environment is calculated as $h2 = σ2g/ {σ2g + σ2ge/nEnvs +}$ σ2e /(nEnvs × nreps)}, where the new term σge is now the genotype by environment interaction variance component and nEnvs is the number of environments in the analysis.

3. RESULTS

3.1 Estimation of BLUE, BLUP and Genetic Components

Significant variation was observed among the mutants for grain yield and other yield attributing traits. With regard to proportion of variance explained across locations, finger length, plant height, number of tillers per plant followed by number of fingers recorded high genotypic variance indicating role of genetic component in expression of traits. Grain yield recorded lowest genotypic variance and was influenced by high genotype × location variance followed by days to flowering indicating effecting of location in genetic expression of traits. Heritability which determines the proportion of heritable proportion of variation was estimated. Finger length (0.96) followed by plant height (0.95), number of tillers per plant (0.69) and days to flowering (0.6) recorded highest heritability. Low heritability was recorded by grain yield per hectare (0.38). BLUPs and BLUEs which determines more precise estimation of genetic values were also estimated (Table 1). BLUP gives more precise estimate of genetic values for traits with low heritability especially in a multilocation trial. The BLUP values for grain yield ranged from 2.59 to 3.41 kg/ha while the BLUE ranged between 1.85 to 4.01 kg/ha. Likewise BLUP and BLUE values for different yield attributing traits are also estimated. For grain yield, GPU28_2122 followed by GPU67_3358, GPU67_3359 recorded higher BLUP values from combined analysis over four locations. Likewise the BLUP and BLUE values for other traits are given in Table 1.

3.2 Stability Analysis

Stability of mutant genotypes for grain yield and its related traits were graphically visualized using GGE biplot technique (Fig 1a) also by plotting the trait mean values of the mutant genotypes with their respective coefficient of variation (CVs) (Fig 1b). For grain yield, the first two principal components (PC1 and PC2) explained 79% of the total variation indicating greater proportion of the variability explained by the biplot. Stability analysis using GGE biplot was constructed using the average environment coordination (AEC) method. In the Fig 1a the line with the arrow head is the AEC abscissa. AEC passes through the origin of the biplot and marks the average environment and it points towards the higher mean for the respective trait. The perpendicular line passing through the biplot origin is referred as AEC ordinate. The length of the dotted line between the genotype and its orthogonal axis onto the biplot axis is the measure of the genotypic stability. The greater the length of the dotted line, the less stable the genotype is. Short lines indicate greater stability. For grain yield (Fig 1a), the mutant genotypes GPU28_2122 followed by GPU28_3359, GPU28_3394 pointing towards the average environment (arrow head) indicating higher grain yield. Also these genotypes were found to be stable as indicated by shorter vector length. Mutants GPU67_2135 and MR6_2130 stably recorded lowest yields. For better understanding of the stability of the genotypes, the combined means of the genotypes were plotted with their respective coefficient of variation (CV). Genotypes having high mean performance with low CV are considered to be stable. For grain yield,

GPU28_3394 and GPU67_3360 recorded high mean with low CV therefore considered as stable mutants.

Biplot technique was used to work out the correlation among grain yield and its attributing traits from combining data across four locations (Fig 2). The relationship or association between variables is positive if the angle between the two vectors is acute (<90°c) and negative in the case of an obtuse angle $>90^{\circ}$ c). The 1st two principal components accounted for 78% of the total variation explained by different traits. Number of tillers per plant showed very close assocation with grain yield. Days to flowering showed close relationship with plant height and finger length which indicates late maturing mutants had long fingers and also taller plant height. Number of fingers showed negative association with grain yield.

Fig. 1. Stability biplot for grain yield (1a. Mean Vs Stability biplot 1b. Mean vs CV biplot)

Fig. 2. Combined genetic correlation among grain yield and its related traits (YD – Grain yield, TIL- Number of tillers, PHT- Plant height, DF- Days to 50% flowering, FL- Finger length, FINnumber of fingers)

Mutants	BLUP-Days to	BLUE-Days to	BLUP-	BLUE-	BLUP-Finger	BLUE-Finger	BLUP-Grain	BLUE-Grain	
	50% flowering	50% flowering	Fingers	Fingers	length	length	yield	yield	
GPU28_2035	74.26	73.88	7.66	7.85	9.5	9.56	3.2	3.47	
GPU28 2036	74.48	74.25	7.24	7.15	8.65	8.67	3.24	3.58	
GPU28_2037	74.63	74.5	7.56	7.69	8.22	8.23	3.2	3.45	
GPU28 2038	78.53	81	7.84	8.16	10.25	10.34	2.92	2.74	
GPU28_2040	76.5	77.63	7.64	7.81	9.63	9.69	2.76	2.29	
GPU28_2076	76.06	76.88	7.4	7.41	8.12	8.13	3.23	3.55	
GPU28_2077	74.56	74.38	7.45	7.5	8.41	8.43	2.69	2.11	
GPU28_2078	74.63	74.5	7.09	6.89	8.51	8.53	3.07	3.13	
GPU28_2082	75.76	76.38	7.22	7.11	8.25	8.26	3.22	3.51	
GPU28_2084	75.38	75.75	7.27	7.2	8.25	8.26	3.1	3.2	
GPU28_2085	73.73	73	7.39	7.4	8.13	8.13	2.95	2.81	
GPU28- Check	75.76	76.38	7.39	7.4	7.81	7.8	3.14	3.3	
GPU28_2086	77.03	78.5	7.57	7.7	7.92	7.91	3.24	3.57	
GPU28 2092	73.88	73.25	7.33	7.29	8.85	8.88	3.21	3.49	
GPU28_2122	74.63	74.5	7.21	7.09	7.89	7.88	3.41	4.01	
GPU28_2123	74.56	74.38	7.39	7.4	8.81	8.84	2.97	2.86	
GPU28_2131	74.78	74.75	7.31	7.26	10.23	10.31	3.06	3.09	
GPU28_3361	75.38	75.75	7.48	7.55	8.17	8.18	2.94	2.78	
GPU28_3364	75.01	75.13	7.11	6.93	7.99	7.99	3.07	3.12	
GPU28_3368	74.93	75	7.24	7.15	9.83	9.91	3.09	3.16	
GPU28 3369	75.53	76	7.19	7.06	8.23	8.24	3.3	3.72	
GPU28_3370	73.81	73.13	7.59	7.73	7.83	7.82	2.99	2.91	
GPU28_3394	75.08	75.25	7.59	7.73	8.38	8.39	3.25	3.6	
GPU67_2047	78.15	80.38	7.79	8.06	9.44	9.49	2.88	2.62	
GPU67_2048	73.96	73.38	7.64	7.81	6.55	6.49	2.78	2.35	
GPU67_2051	74.18	73.75	7.04	6.81	6	5.93	3.12	3.26	
GPU67 2054	73.51	72.63	7.79	8.06	7.62	7.6	3.05	3.08	

Table 1. BLUE and BLUP for grain yield and its attributing traits

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Traits	Mutants	Mutants								Checks			\overline{CV}	$\overline{\mathsf{LS}}$
		GPU ₂	GPU ₂	GPU28	GPU ₂	GPU ₂	GPU28_2	GPU6	GPU67	GPU28-	GPU67	MR6-		D
		8_203	8 208	2084	8 212	8_208	094	$7 - 335$	3366	Check		Check		(5%
		8	$\overline{\mathbf{2}}$		2			8			Check			
	Hyd19	93.5	80.0	81.5	80.0	87.0	83.5	81.5	83.5	81.5	80.0	90.5	2.0	3.3
Days to	Hyd20	93.0	79.5	81.5	79.5	86.5	83.5	81.0	83.5	82.5	80.0	89.5	1.8	2.9
50%	Blore ₂₀	75.0	70.0	70.0	70.0	85.0	70.0	70.0	70.0	70.0	70.0	80.0	2.1	2.9
flowering	Vizag20	62.5	76.0	70.0	68.5	72.0	68.5	73.5	66.0	71.5	70.0	71.0	2.3	3.0
	Combined	81.0	76.4	75.8	74.5	82.6	76.4	76.5	75.8	76.4	75.0	82.8	2.1	3.0
	Hyd19	8.3	7.0	7.5	7.0	7.0	6.0	7.5	9.0	7.0	8.0	7.0	11.0	1.6
Number	Hyd20	8.3	7.0	7.3	6.8	6.8	6.3	7.5	9.0	6.8	8.0	7.3	6.3	0.9
of fingers	Blore20	8.3	7.4	7.2	7.5	8.1	7.5	7.5	8.1	8.1	7.2	8.1	3.3	0.5
	Vizag20	7.9	7.1	6.9	7.1	7.8	7.2	7.2	7.8	7.8	6.9	7.8	3.4	0.5
	Combined	8.2	7.1	7.2	7.1	7.4	6.7	7.4	8.5	7.4	7.5	7.5	13.5	2.2
	Hyd19	10.3	8.3	8.2	7.8	10.3	10.0	7.0	8.3	8.0	7.5	10.3	9.7	1.6
Finger	Hyd20	11.3	8.8	9.0	8.5	11.8	11.8	6.8	8.3	7.5	6.5	10.5	9.1	1.6
length	Blore20	10.1	8.2	8.1	7.8	10.6	10.0	5.5	8.5	8.0	6.2	9.5	6.3	1.0
(cm)	Vizag20	9.8	7.9	7.8	7.6	10.4	9.8	5.3	8.4	7.8	6.0	9.3	10.2	1.5
	Combined	10.3	8.3	8.3	7.9	10.7	10.4	6.1	8.3	7.8	6.6	9.9	17.74	2.4
	Hyd19	2.8	3.4	2.7	3.9	2.7	2.7	3.4	2.9	2.5	2.6	3.3	7.6	0.4
Grain	Hyd20	2.7	1.8	1.5	4.1	2.2	3.4	3.5	2.5	2.7	3.6	3.6	5.9	0.3
yield	Blore20	2.4	3.1	3.3	3.0	3.2	3.4	3.0	2.0	3.3	2.7	2.5	9.8	0.5
(kg/ha)	Vizag20	3.1	5.7	5.4	5.1	5.7	5.3	5.8	3.7	4.5	5.0	4.3	9.6	0.4
	Combined	2.7	3.5	3.2	4.0	3.4	3.7	3.9	2.8	3.3	3.3	3.4	32.9	2.2
	Hyd19	6.3	3.0	4.5	4.0	5.0	4.3	6.3	6.0	4.3	6.3	5.3	5.5	0.6
Neck	Hyd20	3.8	3.5	4.5	3.3	3.5	3.5	4.0	5.3	4.0	4.0	4.0	11.0	0.9
Blast (G)	Blore20	2.5	2.8	3.3	2.5	2.8	2.5	3.3	3.3	3.3	3.8	2.5	17.4	1.1
	Vizag20	6.5	3.5	5.5	5.0	6.5	5.8	7.0	7.0	4.8	7.3	5.0	7.4	1.0
	Combined	4.9	3.2	4.4	3.7	4.3	3.9	5.1	5.4	4.4	6.1	4.2	15.1	1.1
	Hyd19	6.3	3.0	3.3	4.0	4.0	4.0	4.5	5.0	4.0	5.0	5.0	7.0	0.8
Finger	Hyd20	4.8	4.3	4.3	4.0	4.3	4.3	4.8	5.5	4.0	3.8	5.0	11.6	1.1
blast (G)	Blore ₂₀	2.5	2.5	2.0	3.3	3.8	3.5	3.5	4.0	2.0	3.3	4.0	20.3	1.4
	Vizag20	7.3	3.5	4.0	5.0	5.5	5.5	5.5	6.0	4.8	6.0	6.0	6.5	0.8
	means	5.3	3.3	3.4	4.1	4.3	4.2	4.4	5.1	4.3	5.4	5.0	15.8	1.2

Table 2. Improved finger millet mutants for finger traits, grain yield and blast resistance

Table 2. provided details of the mutants with improved finger traits, grain yield and blast resistance. GPU28_2081, GPU28_2094 and GPU28_2038 recorded highest finger length (>10 cm) as compared to its original parent variety GPU 28 which recorded 7.8 cm. Mutant GPU28_2038 also showed superiority for number of fingers. GPU67_3366 recorded highest number of fingers (8.5) as compared to its original parents GPU 67 which recorded 7.5 fingers. For grain yield, the mutant GPU28_2122 recorded high grain yield followed by GPU28_2094 mutant as compared to GPU 28 original variety. Mutant GPU28_2082 recorded significant superiority for Neck blast and Finger blast in addition to grain yield. These mutants identified can be best donors for genetic improvement of finger traits, grain yield as well as blast resistance.

4. DISCUSSION AND CONCLUSION

In small grained cereals especially finger millet where the florets are tiny and irregular anthesis is common, emasculation and hybridization is very difficult and induced mutagenesis is one of the alternate approaches for genetic improvement. In the present study, the traits envisaged for improvement of productivity of finger millet were the grain yield components such as early flowering and maturity, tillers per plant, fingers per ear, finger length, and blast disease resistance.

From induced mutagenesis of three elite cultivars (GPU 28, GPU 67 and MR6) belonging to medium (105-115) days to late (115-125 days) maturity group, 61 mutants were developed following selection from M_2 onwards till M_7 generation. Evaluation of the 61 selected mutant progenies of M_8 generation indicated that most of the progenies differed significantly from the parent variety in one or more traits. Muduli and Misra [11] reported high frequency of desirable mutations for test weight, finger length and fingers per ear derived from VR 708 variety and fingers/ear and finger length from GPU 26 variety. They also observed plant characters changes with mutagens, their doses and variety. In our study, mutation doses 500-600 Gy was more effective in inducing more desirable mutations and therefore populations of these doses were carried forward for identifying desirable mutants. Ambavane et al. [12] identified two early maturing mutants and three high yielding mutants isolated from 500 Gy dose and 600 Gy dose derived from Dapoli-1 variety.

Stability in performance for grain yield and related traits is one the important aspect considering during varietal release. GPU 28 is medium maturing (115-120 days) mega cultivar previously occupied on an area about 40% in major growing state Karnataka. From present study, mutants GPU28_2122, GPU28_3369, GPU28_2122, GPU28_2086, GPU28_2036, GPU28_2082, GPU28_3394 recorded higher grain yield compared to its parental genotype GPU28. The superiority of performance of these mutant genotypes is also evident from BLUP and BLUE values. The high grain yield recorded in G15 is due to the high number of tillers (4.8) recorded compared to its parental genotype as compared to parental genotype which recorded 4.2 tillers. From correlation biplot it was observed that number of tillers per plant showed strong association with grain yield. Similar studies showing strong positive correlation of grain yield with tillers per plant was reported by Nandini *et al* [13] and Owere et al. [14]. Mutation breeding of GPU 67 variety aimed at identifying mutants with blast resistance, non-shattering type and with all other traits similar to its parental type.GPU67 mutants GPU67_3358 and GPU67_2094 recorded average 8-12% superiority for grain yield in comparison to parental genotype GPU 67 while GPU67_3394, GPU67_3359, GPU67_3360 and GPU67_2067 were observed on par performance for grain yield. GPU67_2094, GPU67_3359 and GPU67_3360 were stable across environments for grain yield. Compared to parental genotype, all the mutants showed slightly lesser number of tillers except GPU67 3359 which recorded marginal increase in number of tillers. Induced mutagenesis of MR6 variety aimed at identification of lines with early maturity with blast resistance as preferred traits. G61 recorded good superiority over its parental genotype and could be due to high number of tillers and G61 were early maturing by 7 days. MR6_3458 showed *on a par* performance for grain yield but recorded early maturity by 10 days. No mutant showed superiority for blast resistance.

During selections in varietal breeding programs, pooled mean over locations are generally used to understand its genetic yielding potential. Recently mixed model techniques such and BLUP and BLUE are used for precise estimate of grain yield potential. In our study, we observed high correlation between arithmetic means, BLUP and BLUE. But we observed that BLUP gave a much conservative estimate of the means. Piepho and Mohring [15] suggested to use BLUP rather than by BLUE for analysis of unbalanced data having large number of genotypes. Literature suggests BLUP based selection method predicts genetic effects more accurately than the BLUE based methods [16] [17]. The increase in accuracy of BLUP over BLUE based methods are partly from the shrinkage property [15] which means that the mean values of above average individual will be shrunken towards the general mean whereas the average values of below average individuals will be shrunken upwards towards the general mean and the degree of shrinkage depends on the environmental variation. The shrinkage property of BLUP anticipates the regression to the mean observed in the selected progeny and is advantageous during taking decisions of individuals with extreme values as a need for caution [18]. Also BLUP are more reliable for selection considering traits with low heritability. In our study, grain yield being a complex trait and influenced by high $G \times E$
interaction and recorded low heritability interaction and recorded low heritability estimates. Therefore BLUP would be more reliable for identifying genotypes with high grain yield.

5. CONCLUSION

Mutation breeding is of immense value in generating new variability in small grained cereals especially small millets where hybridization is difficult task and recombination breeding is not exploited to its fullest extent. The present study could identify promising genotypes for grain yield and its related traits. Apart from aiming at yield improvement, mutation breeding has several other applications. The genetic male-sterile line INFM 95001 was also developed using EMS mutagenesis of germplasm line IE 3318 [19]. A partial sterile line (PS 1) was also isolated through EMS mutagenesis of GPU 28 [20]. The partial male sterile line PS 1 is genetically controlled by single gene with completely fertile dominant over partial sterility. The line is being utilized by research stations at India to develop improved crop breeding materials which helps in overcoming the barriers in recombination breeding.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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