# MAGIC POPULATION: AN APPROACH TO GENERATE VARIABILITY

## Abstract

Crop populations generated from experimental crossings promote modern plant breeding and allow for the genetic analysis of complex features. Multi-parental populations now take the lead among these. A new generation of mapping populations is emerging for the study of plant genetics, one of which is the multiparent advanced generation inter-cross (MAGIC) population. Typically, they are produced by crossing many founder lines over a number of generations by utilizing the advantages of both natural and artificial recombination, the MAGIC populations provide an alternative to conventional linkage or association mapping populations by improving the precision of quantitative trait loci (QTL) mapping resolution and analysis of gene–trait correlation. MAGIC hold enormous promise for analyzing genetic organization and enhancing breeding populations. They have significant genetic diversity, high power and resolution for mapping quantitative trait loci (QTL) and little population structure for instance. Crop species have created several multi-parent populations and these populations inbred germplasm and related phenotypic and genotypic data serve as permanent resources. Their use has expanded from being a tool for mapping quantitative trait loci to a way to supply breeding programmes with germplasm. The range of research and breeding applications for multi-parent populations has increased thanks to genomic techniques, such as *de novo* genome assemblies and gene annotations for the population founders, which permitted the imputation of rich sequence information into the descendent population. Here, we discuss recent achievements in crop multi-parent

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populations. In order to maximize the utilization of multi-parent populations as powerful communal resources for agricultural research development and breeding, we also suggest an ideal genotypic, phenotypic and germplasm "package" that should be present in these populations.

**Keywords:** Genotype, Germplasm, MAGIC population, Multiparent population, Phenotype, QTL

## I. INTRODUCTION

Multi-parent Advanced Generation Inter Crosses (MAGIC) is a population generated by producing recombinant inbred lines by intercrossing numerous parental lines known to as founder lines and self-crossing the offspring for several generations (RILs). As an enhancement of the advanced intercrossing (AIC) approach, it was originally used in animals i.e., in mice [1]. The first MAGIC plant population was designed in *Arabidopsis thaliana*, where [2] first introduced the concept. Multiple allele introgression, higher recombination and improved mapping resolution can all be achieved using the multi-parent advanced generation intercross (MAGIC) method. It is an successful method for fine-mapping numerous (QTL) or different phenotypes in the same individual by increasing the precision of genetic markers connected to the quantitative trait loci (QTL).

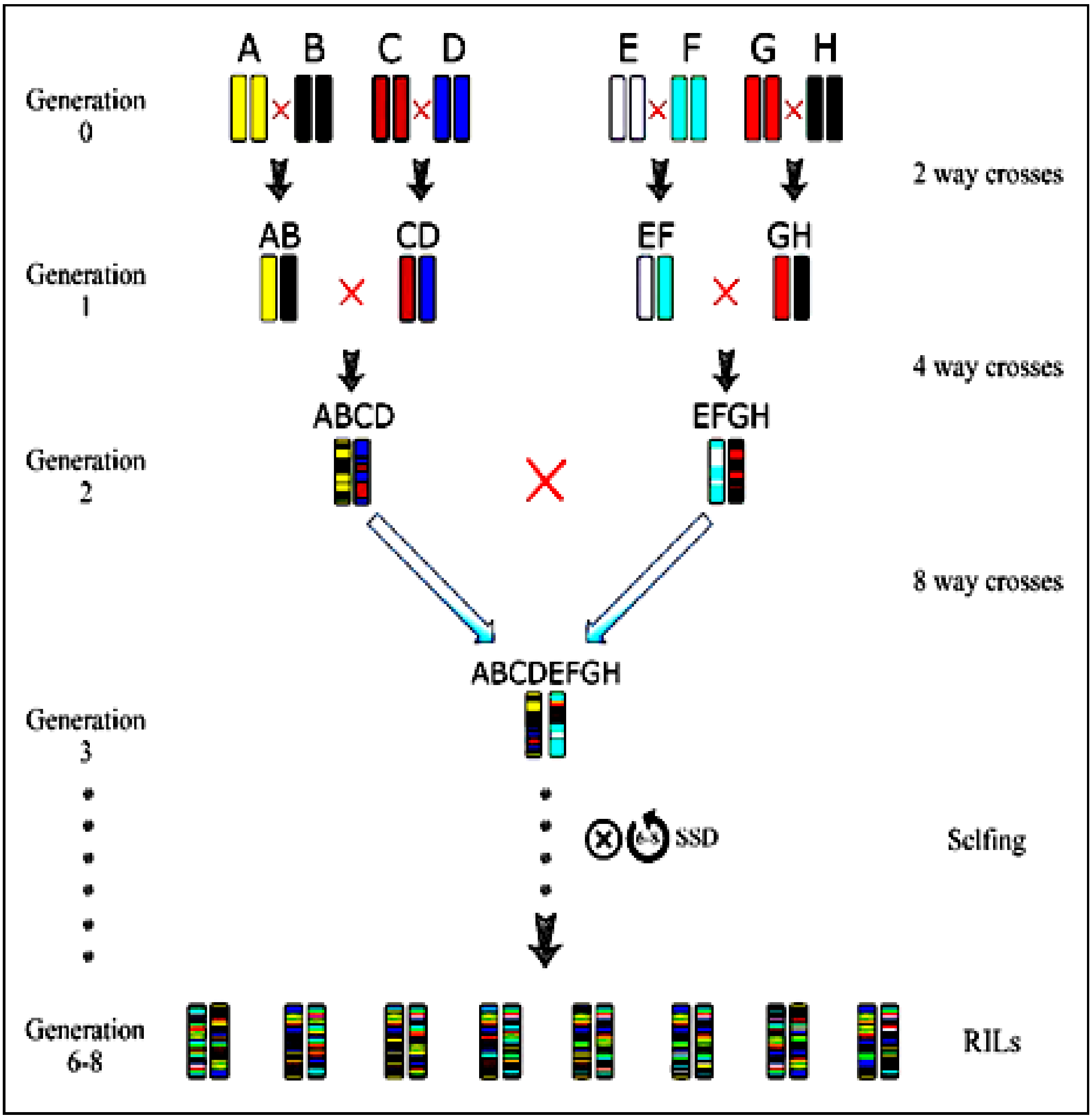

**Figure 1: Cross-Designs of a 4-way MAGIC Population.**

Due to the simple availability of cutting-edge molecular facilities like high-throughput SNP genotyping devices and other bioinformatics tools to determine data from such a big population, multi-parent populations have now turned into an attractive tool for plant breeders. Numerous crops have formerely developed MAGIC populations, including *Arabidopsis thaliana* [3], wheat [4; 5], rice [6], chickpeas [7], sorghum [8], maize [9], and tomato [10]. In MAGIC designs, multiple inbred founders are intercrossed several times in a well-defined order to combine the genetic material of all the founders in a single line [11]. This leads to highly diverse genotypes each with a unique mosaic of founder alleles. Advanced intercrossed lines (AILs) are generated by intercrossing a population that is initially derived through crosses between pairs of inbred lines known as "founder lines," which are initially chosen randomly and sequentially. Founder lines may be solely rely on genetic and/or phenotypic diversity, either in a limited collection of material (such as elite cultivars or geographical adaption) or material with a wider range of ancestry (worldwide germplasm collections, distant relatives). To develop a broad genetic foundation, eight parents are typically intercrossed. Next, the inbred founders are paired off and intermated in a specific order for each line, a process called a funnel. The mixing stage will take ‘n’ generations if each recombinant inbred line (RIL) is the consequence of a 2 ‘n’-way cross.

A set of lines with genomes made up of contributions from each of the founder parents emerged from this stage. These crossed genotypes are then randomly mated throughout several generations. As linkage disequilibrium (LD) is less severe with each generation, QTL may be mapped more precisely. The final step involves practicing inbreeding on the homozygous individuals developed by the advanced intercrossing stage.

## II. WHY MAGIC?

A mapping population is required for the discovery of gene-character relationships for complex (multi-genic) characteristics. Biparental crosses and association mapping, also known as linkage disequilibrium mapping, are the two main techniques that have been used for a very long time to identify gene-character relationships.

1. **Biparental crosses:** To find the parts of the genome that affect the characteristics, traditional experimental populations integrate the genomes of two parents with diverse phenotypes. Reference no. [12&13] developed the idea of biparental mating design. In this method, plants are chosen at random from an $F_2$ or later generation of a cross, and the chosen plants are crossed (intermated) according to a predetermined plan. In other words, "Biparental mating" is the term for random intercross mating between $F_2$ individuals or later generations, and the offspring born as result are known as biparental progenies (BIPs). Biparental progenies are based on the fundamental idea that uncommon recombinants that are confined due to linkage disequilibrium are quickly produced by forced recombination and become available for selection in early segregating generations ($F_3$/$F_4$). The additive (2A) and dominance (2D) components of genetic variance as well as the average dominance level can be accurately estimated using BIPs. Biparental crossings, which result in full-sibling and half-sibling offspring, are predicated on the following presumptions. 1. The genotype distribution is random. 2. The plants that mated are chosen at random. 3. Regular segregation of diploids. 4. Lack of maternal effect, linkage and epistasis 5. The absence of several alleles and 6. All genotypes survive equally.

**Advantages**

- Biparental mating enables analysis of segregating populations of an individual cross made between two inbred lines ($F_2$ or later generation).
- It gives information on additive and dominant components of genetic variance.
- Biparental mating is particularly successful at severing harmful links. Recombination between related genes is more likely to occur when randomly selected plants in a segregating population are mated.
- By generating heterozygosity, biparental mating also increases genetic variation in a population.
- This method can be used to enhance both self-pollinated and cross-pollinated crops.
- Using this method makes it easier to select the best breeding strategy for genetically enhancing different polygenic genotypes.

**Limitations**

- Only two alleles with genetic recombination in the populations are analysed.
- It is only possible to discover those genomic areas which differ between two founders in bi-parental crosses.
- Due to their narrow genetic base, which limits the resolution for QTL detection and genetic mapping.

2. **Association mapping/ Linkage disequilibrium mapping:** The high-resolution method of association mapping, which is based on the theory of linkage disequilibrium, has considerable potential for the analysis of complex genetic characteristics [14]. It is a potent technique for the analysis of complex agronomic traits and for the discovery of alleles that may improve a target trait. The size of the experimental population affects the power of association studies. The required statistical significance level, the density of the markers utilized the rate of LD decay between the marker and the target allele, inaccuracies in the phenotyping and genotyping data and the magnitude of the target allele effect [15].

**Advantages**

- It gives elevated mapping resolution.
- It gives significant allele number and wide reference population.
- It helps in possibility of manifesting historically measured character data.
- It takes lesser time for establishing an association.

**Limitations**

- It requires very large samples to have sufficient power to discover genomic regions of interest and may have trouble detecting uncommon alleles of significance.
- It is mostly influenced by unknown population structure, resulting inaspurious association.

- This new sort of complicated experimental design called the MAGIC population is intermediate to biparental and association mapping designs in terms of power, variety, and resolution as a result of the shortcomings of existing designs.

**MAGIC populations are now adapted by researchers due to**

- Presence of marker systems that allow genotyping of the populations by providing a high-throughput SNP (Single Nucleotide Polymorphism) genotyping platform.
- Advances in statistical methods i.e., requirements for a better mapping population, genetic variability for target phenotype, reproducible quantitative genotyping.

## III. STEPS OF GENERATING MAGIC POPULATION

**There are four steps involved in the development of the MAGIC population**

1. Founder selection
2. Mixing of parents
3. Advanced intercrossing
4. Inbreeding

**Founder selection:** Before initiating population development, founder lines must be chosen. This could be based on genetic or phenotypic diversity, either in a restricted collection of material (such as elite cultivars or geographical adaptation) or material with a wider range of ancestries (worldwide germplasm collections, distant relatives). It is difficult to get an ideal degree of genetic variation. While using landraces as founders may increase variety, it will also make it harder to generalize to existing breeding populations. In some species, genetic incompatibility can significantly reduce the number of offspring that may result from particular crosses. Gross chromosomal changes that are variety-specific, such as rearrangements or alien/wild introgressions, may also have an impact on how the final population is produced and used for genetic mapping.

However, limited genetic variety might make it difficult to calculate founder probabilities and restrict researchers from making the most of their populations. To create a resource that is also useful, the phenotypic variety must be carefully maintained in addition to the genetic diversity. It is possible to prevent unwanted values from being segregated in the progeny by taking into account founder traits like flowering duration. This will not only help with phenotypic evaluation in the future but will also make cross-breeding easier. In the end, choosing the founders will be one of the most crucial design factors and largely rely on the population's objectives. However, founders selected solely on relevance to a breeding programme for specific traits may result in a MAGIC population that more quickly translates into superior breeding lines. More diversified founder sets may provide biological knowledge into a wide variety of attributes. In breeding, it may be particularly advantageous to concentrate on capturing the allelic variety existing within the programme across market categories and making sure the population size is sufficient to produce sufficient progeny predicted to meet each market group.

**Mixing of parents:** Multiple parents crossed each other during the earliest phase of population development to create a large genetic base. This was prompted by the heterogeneous stock (HS), which produces an outbred population produced from numerous parents and was first proposed by [16] and implemented by [17]. During this stage, called to it a funnel, the inbred founders are paired off and interbred in a specific order for each line. The mixing stage will take n generations if each recombinant inbred line (RIL) is a result of a 2n-way cross. A group of lines with genomes made up of contributions from each of the founders are the end product of this stage.

The design and choice of the funnels involved in the cross will have an effect on the final genetic make-up of the population, as reference no. [18] shows that the composition of these contributions depends on the funnel structure. Although it would demand more investment in terms of money and time, more variation in the funnel types developed will assure more robustness in accounting for issues like maternal effects, population structure and segregation distortion. More information on balancing these aspects could be obtained through simulation. The degree of relatedness resulting from common recombination events is a further issue when producing a small number of funnels. Genetic diversity will be decreased if there are fewer individuals in any generation since those who are derived from them will be associated to each other as well. If properly taken into account, this relatedness can also lead to population structure, which will bias assessments like linkage map building and QTL mapping.

**Advanced intercrossing:** As in the advanced intercross (AIC) proposed by [19], the mixed lines from several funnels are progressively and randomly intercrossed in the second stage. This intercrossing's primary aim is to enhance the population's recombination rate. Reference no. [20] Conducted a simulation study to examine the impact of various intercrossing generations on the genomic structure. They came to the conclusion that significant increases in QTL mapping power required at least six cycles of intercrossing. Advanced intercrossing lines of different generations may be combined in the study, but it's important to look for evidence of population structure [21] to make sure the intercrossing hasn't caused changes in structure between the subsets of the population.

**Inbreeding:** The third step involves progressing the individuals from the advanced intercrossing stage to produce homozygous individuals. In plants, single seed descent [22; 23 and 24] or two-fold haploid production can result in RILs [25; 26]. Although numerous generations of selfing will result in extra recombination, it will be less than during the mixing and advanced intercrossing stages, even if doubled haploid production is frequently faster. Except for double haploid lines, the population's offspring won't be entirely inbred in practice.

This remaining heterozygosity has both potential benefits and drawbacks. Due to the inability to differentiate heterozygotes for some markers in genotyping, particularly for polyploids and genotyping by sequencing (GBS) methods, there may be problems [27]. It should preferably be addressed as in reference no. [28], which estimates genotype probabilities for people in intermediate generations with high heterozygosity, as it may present problems in data analysis by violating the simple condition of full inbreeding [18]. Plants have been self-pollinating in many of the populations covered here for five or more generations, therefore the expected amount of genome heterozygosity is less than 3%. In general, it takes at least 8 crop seasons to mature MAGIC populations to the S5 generation.

## IV. IMPORTANCE OF MAGIC POPULATION IN CROP IMPROVEMENT

1. MAGIC populations can be used to create varieties with a number of agronomically advantageous features.
2. MAGIC can aid in the growth of diversity that can be adapted to a range of different locations of the world and suited for a variety of different climatic circumstances.
3. For the direct extraction and creation of breeding lines and varieties, MAGIC populations can be exploited as a source of the vast genetic diverse plant breeding material.
4. MAGIC can offer solutions for a variety of production-related problems (biotic and abiotic).
5. MAGIC may accelerate reproduction and improve its effectiveness.
6. MAGIC will have a significant impact on agricultural output and have the potential to alter how scientists find the genes that affect quality and disease resistance.
7. It can be used to develop a new variety.

## V. LIMITATIONS OF MAGIC POPULATION

1. It needs skilled labour for crossing.
2. Large-scale of segregation occurred, and it can be difficult to handle.
3. For the recovery of recombinants with all the desired features, a large population size is necessary.
4. The resource population development takes more time.
5. For a certain attribute, large-scale phenotyping is necessary.
6. It needs more input.
7. A significant decrease in the number of offspring might result from parental incompatibility.
8. Better marker system is necessary to detect QTL.
9. If less diverse parents are selected then it’s difficult to get desirable recombination for the focused study.

## VI. APPLICATIONS OF MAGIC POPULATION

1. **Multiline variety:** “Reference [29]” had examined the 12-parent MAGIC population which has been used to develop the multiline variety Duo-Ji-Xin 3, which is being commercialized in China [30]. IRRI breeders have investigated the *indica* MAGIC population for targeting irrigated and rainfed conditions as well as for direct and indirect application in variety breeding [6].

2. **Precise QTLs mapping:** For accurate QTL mapping, the MAGIC populations serve as permanent mapping populations [6]. Utilizing the complex qualities of plant height and hectoliter weight in wheat, the resource MAGIC population was used to map quantitative trait loci [4]. Characterizing the entire MAGIC maize population will improve the power and definition of QTL mapping and establish the groundwork for a better comprehension of maize phenotypes, including heterosis [9]. 8-way populations are more effective than MAGIC populations for identifying QTLs [31]. Quantitative trait loci (QTL) in the Wheat Snn1 locus [32] and MAGIC lines have been fine-mapped [3].

3. **Linkage map construction:** The development of a linkage map using a MAGIC population was created for the first time [7]. In comparison to biparental populations, the linkage map revealed an 87 per cent increase in recombination frequencies in tomato [10]. Recently, in wheat high-density, a MAGIC linkage map was created [33].

4. **Genome introgressions:** The MAGIC populations also offer chances to examine how chromosomal recombination and genome introgression interact [6]. This resource's applications include researching the wheat genome [4].

## VII. INSTITUTES INVOLVED IN MAGIC POPULATION

| Sr. No. | Crop | Institute |
|---|---|---|
| 1. | Bread wheat | NIAB, Cambridge, United Kingdom |
| 2. | Durum wheat | University of Bologna, Italy |
| 3. | Rice | IRRI, Philippines |
| 4. | Oats | IBERS, Aberystwyth, United Kingdom |
| 5. | Soybean | IISR, Indore, India |
| 6. | Sorghum | ICRISAT, Hyderabad, India |
| 7. | Cowpea | IITA, Ibadan, Nigeria |
| 8. | Groundnut | ICRISAT, Hyderabad, India |

## VIII. CURRENT STATUS OF MAGIC POPULATION IN CROPS

| Crop | Design | Progeny | Genotyping | Phenotyping | Analyses | References |
|---|---|---|---|---|---|---|
| Rice | Eight indica cultivars crossed in half-diallel to produce 35 funnels: indica MAGIC (AI0RIL) MAGIC PLUS 9AI2RIL) AGIC PLUS DH (A12DH) | (May 2014) 1831 S6:8, 2206 s6:8, 144 S8, 48 S4:6, 76 DH | GBS-17,000 SNP | Yield in multiple-environment trials, drought and salinity tolerance, disease resistance, grain quality | QTL, GWAS | [6] |
| Rice | Eight japonica cultivars crossed in half-diallel to produce 35 funnels: japonica MAGIC(AI0RIL, in progress) | (May 2014) 400 S4:6 | - | - | - | [6] |
| Rice | Eight indica + eight japonica cultivars, each crossed in half-diallel with 35 funnels, then intercrossed: MAGIC GLOBAL (AI0RIL) | (May 2014)1402 S7 | - | - | - | [6] |
| Wheat | Four spring wheat cultivars, 1 funnel, AI0RIL | 1579 F6 | **8**26 DArTs, 283 SNPs and 53 SSRs: 9K SNP array: 90K SNP array | Plant height, hectoliter weight, coleoptiles length | LMC, QTL | [4]; [34]; [35] |
| Wheat | Eight spring wheat cultivars crossed in half-diallel with 315 funnels: AI0RIL, AI2RIL, AI3RIL | 2099 F6,367 F6, 473 F6 | 9K SNP array: 90K SNP array: GBS | ---- | LMC, QTL | CSIRO (unpublished) |
| Wheat | Eight winter wheat cultivars, crossed in half-diallel with 315 funnels, AI0RIL | 1091 F7 | 90K SNP array | Yield, flowering time, plant height, yellow rust, fusarium, mildew, awn presence | QTL, GWAS | [5]: [36] |
| Wheat | 16 spring wheat (in progress),125 funnels, Ai0RIL | 600 RILs | ------ | ----- | ---- | CSIRO (unpublished) |
| Wheat | 16 winter wheat, crossed in half-diallel with 15 funnels, AI0RIL | 800 RILs | -------- | -------- | ------- | NIAB (unpublished) |
| Wheat | 60 parents randomly intercrossed, AI12RIL | 1000 F3 | 9KSNP Array | Heading date | LDA, GWAS | [37] |

| | | | | | | |
|---|---|---|---|---|---|---|
| Chickpea | Eight *desi* (complete) | 1000 F6 | Resequencing-1000 lines in progress | -------- | -------- | [7] |
| Chickpea | Eight *Kabuli* (in progress) | -------- | -------- | --------- | ------- | ICRISAT (Unpublished) |
| Pigeon pea | EIGHT (IN PROGRESS), 7 FUNNELS | At S1 stage | ------- | ------- | ---------- | ICRISAT (Unpublished) |
| Peanut | Eight (in progress), 14 funnels | At S1 stage | -------- | ---------- | ------------ | ICRISAT (Unpublished) |
| Maize | Eight parents crossed in half-diallel with 35 funnels, AI0RIL | >1300 F6 | 50K SNP chip: GBS | Plant height, ear height, yield, flowering time | ------- | [38] |
| Barley | Seven landraces, one modern variety, 1 funnel, AI0 DH | ~5000AI0 DH: 534 genotyped and phenotyped | 9K SNP chip | Flowering time | LMC, QTL, GWAS | [39] |
| Tomato | Four normal tomato, four cherry tomato, 1 funnel, AI0RIL | 397 S3 | 1536 Custom SNP chip | Fruit weight | LMC, QTL, GWAS | [10] |
| Cotton | 12-way, funnel | cultivars | 1500F7 | Fibre yield and resistance genes | ------ | [40] |
| Cotton | 11-way, diallel | Cultivars and a breeding line | >550S6 | Fibre quality | TASSEL, GAPIT | [41] |
| Rapeseed | 8-way | Elite cultivars | 680 f6 | Disease resistance, yield, plant architecture | ------ | [42] |
| Chinese mustard | 8-way, diallel | Breeding lines | 408 F6 | Quality traits (glucosinolate) | TASSEL | [43] |

## IX. FUTURE THRUST

Additional QTL those are responsible for stress resistance and other significant features used in the MAGIC population are found and utilized. Multiparent populations of all kinds are still in their infancy; it is too soon to assess their usefulness and potential based on their capacity to produce solutions and grasp genetics. This approach can be successfully used in identifying plant features that are of economic significance to us. Crops may be investigated and their MAGIC populations developed, and so on, but these issues can all be resolved.

## X. CONCLUSION

By improving mapping precision MAGIC populations can provide an alternative to conventional mapping populations. The many founder lines intercrossed over several generations to generate the MAGIC populations, which resulted in more recombination events being accumulated over time. Genetic diversity was in great supply in the MAGIC population. The production of multiline varieties, the creation of linkage maps, the investigation of gene-trait associations, the exact mapping of quantitative traits and other tasks benefit from the use of MAGIC populations.

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