

Efficiency of Arbuscular Mycorrhizal Fungi in Soybean (*Glycine max* (L.) Merr.): An Integrated Field and Controlled-Stress Research Framework

Megha M. Rayate¹, Sanjay K. Shinde²

¹Research Centre, Department of Botany, K.T.H.M. College, Nashik, Maharashtra, India.

²Department of Botany, Lasalgaon College, Lasalgaon, Nashik, Maharashtra, India.

Corresponding Author: Dr. Sanjay K. Shinde

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) form a mutualistic association with plant roots and can improve nutrient acquisition, root exploration, water relations and resilience to abiotic stress. Soybean is a major oilseed legume whose productivity is frequently constrained by phosphorus limitation, episodic drought and salinity. This research framework evaluates native AMF associated with soybean across five sites in Niphad Tehsil, Nashik District, Maharashtra: Vinchur, Takli, Khadakmalegaon, Sarolekhurd and Ranwad. Rhizosphere soil and fine roots are sampled at 30-day intervals. AMF propagules are recovered by wet sieving and decanting with sucrose centrifugation as required, root colonisation is assessed after clearing and staining, and morphotypes are identified using accepted taxonomic keys. Selected isolates are multiplied in trap cultures using suitable host plants and screened in soybean under non-stressed, water-deficit and salinity treatments. Water stress is evaluated using 8-, 16- and 24day stress intervals, while chloride and sulphate salinity are imposed using graded NaCl and Na₂SO₄ treatments. The response matrix includes plant height, root growth, biomass, nodulation, chlorophyll, relative water status, stress

metabolites, mineral nutrition, seed yield, oil content, root colonisation and mycorrhizal dependency. Factorial analysis of variance, post-hoc comparisons, correlation and multivariate analysis are used to identify AMF isolates that combine effective colonisation with improved soybean performance. The framework is intended to support selection and mass multiplication of locally adapted AMF bioinoculants for sustainable soybean production under variable soil and moisture conditions.

Keywords: Arbuscular mycorrhizal fungi; soybean; *Glycine max*; rhizosphere; drought stress; salinity; mycorrhizal dependency; bioinoculant

INTRODUCTION

Mycorrhiza denotes a symbiotic association between soil fungi and plant roots in which the host supplies photosynthetically fixed carbon while the fungal partner extends the effective absorbing surface of the root. Arbuscular mycorrhizal fungi are the most widespread agricultural mycorrhizal group and produce intraradical structures, especially arbuscules, that serve as interfaces for nutrient exchange [1,2]. The classical methods developed by Gerdemann and Nicolson [3], Phillips and Hayman [4], and Giovannetti and Mosse [5] remain

foundational for AMF recovery and colonisation assessment.

Soybean (*Glycine max* (L.) Merr.) is an important grain legume and oilseed crop because of its protein, oil and nutritionally active constituents. Its symbiosis with rhizobia satisfies part of the nitrogen requirement, but phosphorus acquisition, water availability and ionic stress can strongly influence nodulation, photosynthesis and yield. AMF can complement the soybean-rhizobium association by increasing soil exploration beyond the root depletion zone and by modifying root architecture, nutrient uptake and water relations [6-9].

Field evidence indicates that the outcome of AMF inoculation depends on fungal identity, host genotype and soil phosphorus status. In a Haplic Acrisol, AMF inoculation combined with a reduced phosphorus rate improved root colonisation, phosphorus uptake and soybean productivity [7]. Under low-phosphorus field conditions, distinct isolates including *Funneliformis mosseae*, *Rhizophagus intraradices* and *Claroideoglomus etunicatum* produced different effects on soybean growth and grain yield [10]. These results support screening locally adapted isolates instead of assuming that a single commercial inoculum will perform uniformly across soils.

AMF is also relevant to drought and salinity management. Soybean associated with AMF has shown improved water status, photosynthetic performance, biomass, nutrient uptake and stress physiology under water limitation [11-15]. Under salinity, AMF can influence growth, nodulation, nitrogenase activity and ionic balance, although responses remain genotype- and stress-dependent [9]. This variability is especially important in field-oriented work because effective AMF strains must colonise roots, remain functional in local soils and translate symbiosis into agronomic benefit. The present research therefore integrates field survey, AMF isolation, soil analysis, root colonisation, inoculum multiplication and controlled soybean assays. The study focuses on five localities in Niphad Tehsil and addresses the following objectives: (i) isolate and identify AMF associated with soybean; (ii) characterise rhizosphere soil in relation to AMF occurrence; (iii) evaluate soybean responses at different growth stages; (iv) quantify the influence of AMF on water stress, chloride/sulphate salinity, growth and productivity; (v) estimate soybean mycorrhizal dependency; and (vi) identify promising strains for large-scale inoculum production.

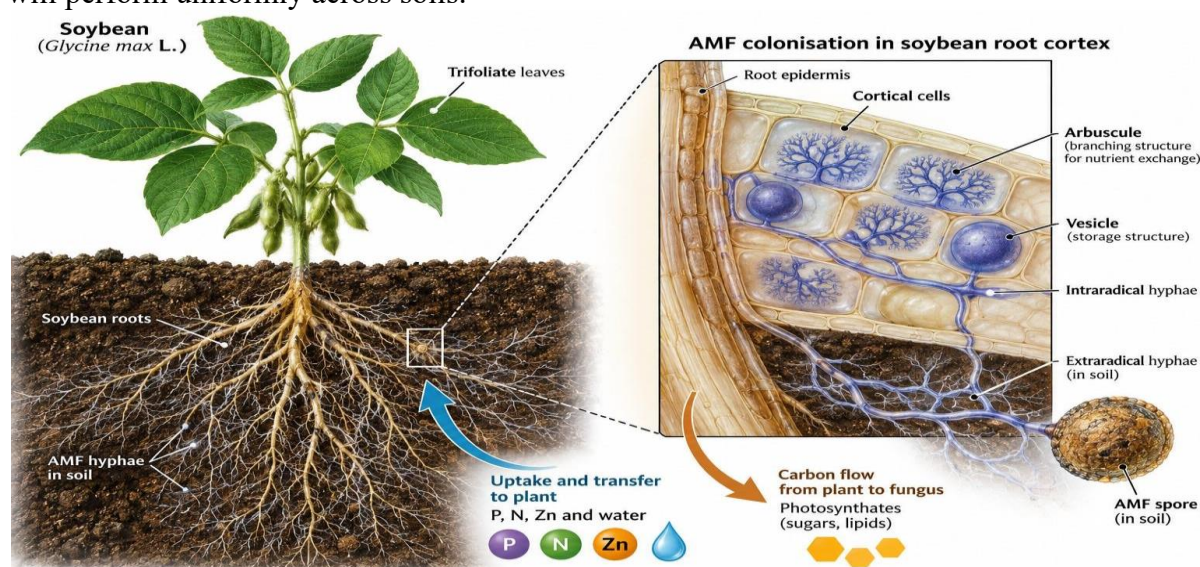


Figure 1. Conceptual illustration of AMF colonisation in soybean roots and the bidirectional exchange of mineral nutrients, water and photosynthates. The illustration is explanatory and is not an experimental micrograph.

MATERIALS & METHODS

Study area and sampling sites

The study area comprises soybean-growing locations in Niphad Tehsil, Nashik District, Maharashtra. Five sites specified for the investigation are Vinchur, Takli,

Khadakmalegaon, Sarolekhurd and Ranwad. Root and rhizosphere-soil sampling is conducted at 30-day intervals so that AMF occurrence and root colonisation can be related to crop development and site conditions.

Table 1. Field sampling framework for the five study locations.

Site	Sampling material	Interval	Primary outputs
Vinchur	Rhizosphere soil + fine roots	30 days	AMF spores/morphotypes, root colonisation, soil properties
Takli	Rhizosphere soil + fine roots	30 days	AMF spores/morphotypes, root colonisation, soil properties
Khadakmalegaon	Rhizosphere soil + fine roots	30 days	AMF spores/morphotypes, root colonisation, soil properties
Sarolekhurd	Rhizosphere soil + fine roots	30 days	AMF spores/morphotypes, root colonisation, soil properties
Ranwad	Rhizosphere soil + fine roots	30 days	AMF spores/morphotypes, root colonisation, soil properties

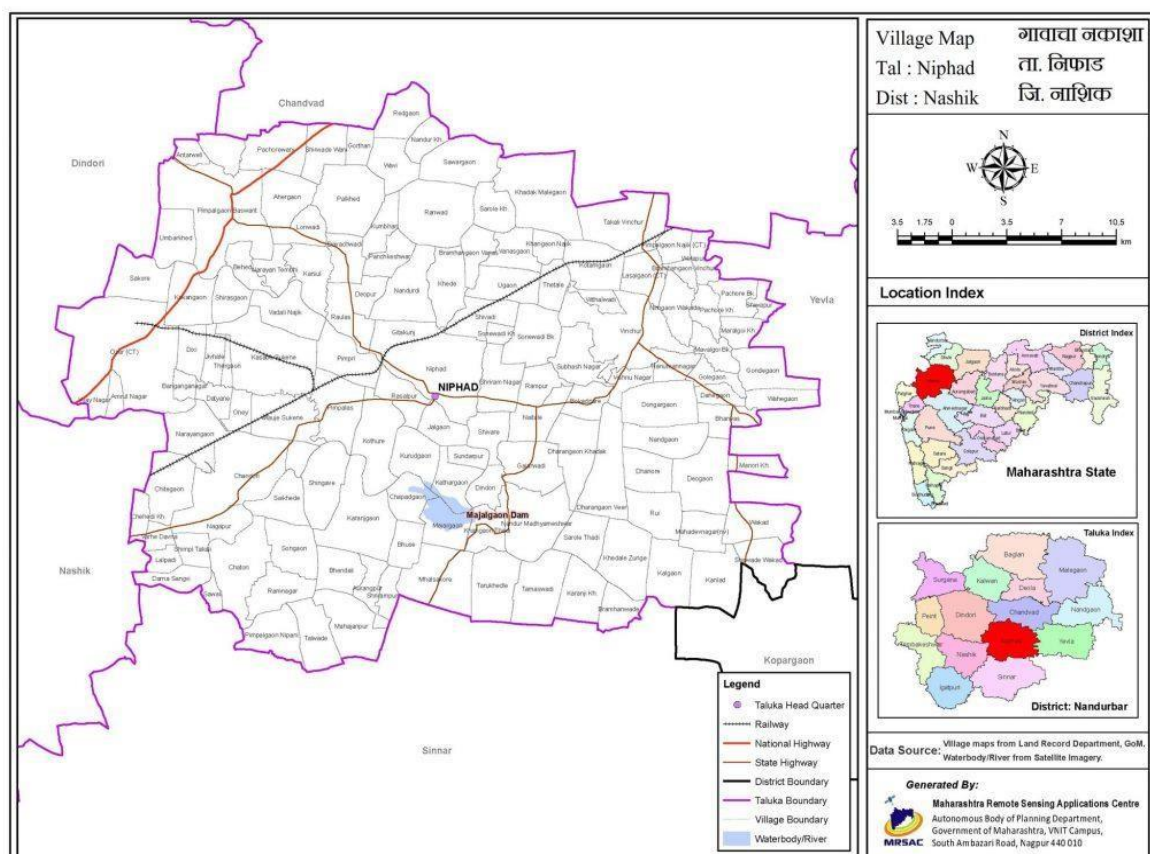


Figure 2. Study-area map of Niphad Tehsil, Nashik District, showing the regional setting used for selection of the five soybean sampling localities.

Soil and root collection

At each visit, composite rhizosphere soil is collected from the active root zone of

representative soybean plants, while fine roots are retained for colonisation assessment. A matched non-rhizosphere soil

sample is included where feasible to distinguish root-associated changes from background soil conditions. Samples are transported in clean labelled containers, with fresh root material processed promptly and soil divided into portions for biological and physicochemical analyses.

Soil physicochemical analysis

Soil analysis follows standard procedures based on Jackson [16]. The core dataset includes pH, electrical conductivity, texture, organic carbon, available nitrogen, available phosphorus and available potassium; additional micronutrients are included where analytical capacity permits. The same parameters are used when comparing rhizosphere and non-rhizosphere soil so that AMF abundance and soybean response can be interpreted against edaphic variation.

Isolation and identification of AMF

AMF propagules are isolated from soil by wet sieving and decanting [3]. When greater purification is needed, the spore fraction is further separated by sucrose centrifugation [17]. Spores are examined under a stereomicroscope and grouped into morphotypes according to colour, size, wall structure, subtending hypha and other diagnostic characters. Identification is performed using current keys, monographs and accepted taxonomic resources. Species-level assignments are retained only when morphological characters are sufficient; otherwise, isolates are reported at genus or morphotype level.

Assessment of root colonisation

Washed roots are cut into short segments, cleared and stained using the Phillips and Hayman method [4]. Colonised segments are

examined microscopically for intraradical hyphae, vesicles and arbuscules. Percentage root colonisation is estimated using a gridline/slide-count approach derived from Giovannetti and Mosse [5]. Colonisation is analysed independently from spore density because the two measures represent different aspects of AMF establishment.

Trap culture and inoculum multiplication

Promising field isolates are multiplied in pot cultures using a suitable host such as maize or jowar (sorghum), as proposed in the study plan. Inoculum consists of colonised root fragments, spores and associated growth substrate. Trap cultures are maintained until adequate colonisation and spore production are achieved. Each candidate inoculum is characterised by viable propagule abundance and host-root colonisation before use in soybean experiments.

Controlled soybean experiment

A factorial controlled experiment is used to distinguish the effects of inoculation, water deficit and salinity. At minimum, each test includes a non-mycorrhizal control and one or more selected AMF isolates or an isolate consortium. Water stress is imposed using 8-, 16- and 24-day stress intervals specified in the research plan. Salinity is evaluated separately using graded sodium chloride (NaCl) and sodium sulphate (Na₂SO₄) treatments established after a preliminary range-finding test so that the stress series remains biologically informative without causing immediate plant death. Treatments are randomised and replicated, and non-mycorrhizal controls receive an equivalent amount of sterilised inoculum carrier where applicable.

Table 2. Experimental factors and principal response variables.

Experimental component	Levels / comparison	Key measurements
AMF inoculation	Non-mycorrhizal control; selected isolate(s); consortium where justified	Root colonisation, spore density, growth, nutrition, nodulation, yield
Water stress	Well-watered control; 8-, 16- and 24-day stress intervals	RWC, chlorophyll, biomass, proline, MDA, nutrient status, yield

Chloride salinity	Control + graded NaCl levels after range-finding	Growth, ion balance, stress markers, colonisation, productivity
Sulphate salinity	Control + graded Na ₂ SO ₄ levels after range-finding	Growth, ion balance, stress markers, colonisation, productivity
Field validation	Selected AMF versus uninoculated soybean	Establishment, nodulation, yield components, seed yield, oil content

Growth, physiological and biochemical measurements

Plant response is evaluated at defined growth stages where facilities are available, catalase and peroxidase using a compact set of complementary endpoints. activities. Mineral analysis focuses on N, P and K, with Na Morphological variables include plant height, root length, and selected micronutrients included in salinity leaf area,

shoot and root dry biomass, number and dry mass experiments. At maturity, pods per plant, seeds per pod, of nodules, and root-to-shoot ratio. Physiological content. Biochemical measurements include proline and malondialdehyde (MDA) as stress-response indicators and, measurements include chlorophyll status and relative water 100-seed mass, seed yield and seed oil content are recorded.

Table 3. Measurement matrix for linking AMF establishment with soybean performance

Domain	Variables	Interpretive role
AMF establishment	Spore density; morphotype richness; % root colonisation; arbuscules/vesicles	Confirms inoculum establishment and field occurrence
Soil	pH; EC; texture; organic C; available N, P, K	Explains site-to-site variation and nutrient constraints
Growth	Plant height; root length; leaf area; shoot/root dry mass	Primary vegetative response to AMF and stress
Symbiotic performance	Nodule number/mass; tissue N and P	Links AMF with legume nutrient economy
Stress physiology	RWC; chlorophyll; proline; MDA; antioxidant enzymes	Quantifies drought/salinity injury and tolerance
Productivity	Pods; seeds; 100-seed mass; seed yield; oil content	Determines agronomic value of inoculation

Mycorrhizal dependency

Relative mycorrhizal dependency is calculated according to the classical concept of Plenchette et al. [18] as: $RMD (\%) = [(M - NM) / M] \times 100$, where M is the total dry biomass of mycorrhizal plants and NM is the total dry biomass of comparable non-mycorrhizal plants. The index is interpreted together with colonisation and yield response because a high percentage of root infection does not necessarily imply a large agronomic benefit.

Statistical analysis

Data are examined for distributional assumptions using graphical diagnostics and

the Shapiro-Wilk test. Site surveys are analysed using analysis of variance or nonparametric alternatives as appropriate. Controlled experiments are analysed by factorial ANOVA with AMF, water regime or salinity level as fixed factors, followed by Tukey-adjusted multiple comparisons. Repeated sampling is handled with repeated-measures or mixed-effects models when the same experimental units are followed through time. Pearson or Spearman correlation is used to relate colonisation, soil variables and plant response, and principal component analysis (PCA) is used to visualise multivariate treatment patterns. Statistical significance is evaluated at $P <$

0.05, with effect sizes and confidence intervals reported alongside P values.

RESULT

The study framework generates four linked result sets: (1) site-wise AMF occurrence and diversity; (2) soybean root colonisation and mycorrhizal dependency; (3) soil-toplant relationships under field conditions; and (4) controlled drought, salinity and productivity responses to selected inocula. The supplied research synopsis defines these experimental components but does not contain completed numerical observations; therefore, no biological effect size is introduced here in place of measured data. The final manuscript should populate the reporting structure

below directly from the experimental dataset.

Site-wise AMF community and soil matrix
For each locality, the final dataset should report spore density per unit soil, morphotype richness, dominant AMF taxa or morphotypes, percentage root colonisation and the associated soil physicochemical variables. The principal comparison is whether AMF abundance and colonisation track available phosphorus, electrical conductivity, organic carbon or other site attributes. Because field colonisation can be high even when sporulation is low, spore and root data should be presented as complementary variables rather than combined into a single index.

Table 4. Recommended final result-reporting structure.

Outcome	Minimum statistics to report	Primary comparison
AMF spore density / richness	Mean $\pm$ SD; range; n; site	Among five field sites
Root colonisation (%)	Mean $\pm$ SD; n; colonisation structures	Site and inoculum treatments
Soil properties	Mean $\pm$ SD by site / sampling period	Rhizosphere vs. non-rhizosphere; site
Growth / biomass	Mean $\pm$ SD; effect size; 95% CI	AMF x water or AMF x salinity
Physiological stress markers	Mean $\pm$ SD; effect size; interaction P	Stress with and without AMF
Yield and oil content	Mean $\pm$ SD; % change vs control; 95% CI	Selected isolate/consortium vs control
Mycorrhizal dependency	RMD (%) with biomass basis stated	Among isolates and stress regimes

Growth and stress-response matrix

The controlled experiment should report treatment means with variability (mean $\pm$ SD or SE), sample size and the AMF x stress interaction. Under water deficit, the most informative outcome is whether inoculated soybean maintains higher biomass, water status, chlorophyll and yield while limiting lipid peroxidation. Under NaCl and Na₂SO₄ stress, plant growth should be interpreted with tissue ion balance and root colonisation so that improved growth is not attributed to AMF without evidence of a functional symbiosis.

DISCUSSION

Importance of local AMF screening

The decision to begin with a multi-site field survey is important because AMF performance is context dependent. Soil phosphorus is a central determinant of the cost-benefit balance of mycorrhizal symbiosis; in P-deficient soils, fungal hyphae can extend P acquisition beyond the root depletion zone, whereas very high P availability can reduce colonisation or reduce the plant response. Soybean studies under field conditions have shown that AMF isolate identity and P supply can interact to modify colonisation, phosphorus use efficiency and grain yield [7,10]. Consequently, isolate selection should be based on both colonisation and plant response in the target soil.

AMF, soybean nodulation and nutrient acquisition

Soybean is particularly suitable for AMF research because N and P acquisition are biologically linked. Rhizobial nodulation has a substantial phosphorus demand, and improved P nutrition can support nodule activity and plant growth. Co-inoculation experiments have shown that AMF and rhizobia can alter root architecture and the availability of N and P to soybean [6]. More recent drought studies also indicate that combined microbial inoculation can maintain growth, chlorophyll, nodulation and grain yield more effectively than uninoculated controls under water limitation [11,14]. These findings justify including nodulation and tissue N together with P uptake rather than treating biomass alone as the endpoint.

Water-deficit response

The proposed 8-, 16- and 24-day stress intervals allow the response to be resolved across progressively longer water limitation. AMF effects under drought can arise from increased root-soil exploration, changes in hydraulic conductance, improved nutrient status and regulation of photosynthetic and antioxidant processes. Thermal and physiological studies in soybean show that AMF can improve water-use traits, biomass and nutrient acquisition under drought [12,13]. Inoculation with *Rhizophagus clarus* has also been associated with better water status and photosynthetic performance

under imposed drought, although cultivar-specific responses remain evident [13]. Therefore, the Niphad study should interpret AMF benefit as a treatment-by-stress interaction rather than as a universal growth-promoting effect.

Salinity response

Salinity can restrict soybean through osmotic stress, Na toxicity, nutrient imbalance and suppression of nodulation. AMF-associated soybean genotypes have shown improved physiological performance under salt stress, but the magnitude of benefit differs with host genotype and fungal compatibility [9]. The planned comparison of chloride and sulphate salts is therefore useful because equal electrical conductivity does not necessarily generate identical ionic effects. In addition to biomass and yield, salinity experiments should report root colonisation and tissue Na/K balance wherever possible.

From isolate screening to inoculum production The final translational step is mass production of effective AMF using suitable hosts such as maize or jowar. Selection for inoculum production should require reproducible colonisation, propagule production and a positive soybean response across more than one soil or stress condition. A consortium should be preferred only when it outperforms its component isolates or provides greater stability across environments. This avoids the common assumption that a more diverse inoculum is automatically more effective.

Table 5. Recent soybean studies that provide benchmarks for interpretation of the proposed experiment.

Study	AMF / treatment context	Main relevance to the present work
Wang et al. (2011) [6]	AMF with rhizobia; contrasting soybean root architecture	Links colonisation, nodulation, N/P availability and root traits
Thioub et al. (2019) [7]	<i>G. mosseae</i> / <i>R. irregularis</i> with P fertilisation	Shows AMF x phosphorus effects on P uptake and grain productivity
Hashem et al. (2019) [9]	Soybean cultivars under NaCl stress	Supports genotype-dependent AMF effects under salinity
Sheteiwy et al. (2021) [11]	AMF + Bradyrhizobium under drought	Supports integrated growth, nodulation, biochemical and yield endpoints
Adeyemi et al. (2021) [10]	Several AMF isolates in P-deficient field soil	Supports isolate screening under low-P field conditions

Ma et al. (2022) [12]	AMF soybean under 40% vs 60% water-holding capacity	Demonstrates effects on water status, biomass and nutrient uptake
Oliveira et al. (2022) [13]	R. clarus with two soybean cultivars under drought	Shows cultivar-specific physiological protection under drought
Revanna et al. (2022) [14]	AMF + rhizobia across moisture levels	Supports stress-tolerance and productivity testing
Ezzati Lotfabadi et al. (2022) [15]	Three AMF species under graded drought	Supports nutrient and seed-quality assessment under drought

Interpretation limits and quality control

Several controls are essential for defensible interpretation. Non-mycorrhizal treatments should receive the same carrier material after sterilisation, and inoculation status must be verified microscopically. Stress intensity should be quantified rather than inferred only from the duration of withholding water or the nominal salt concentration. The final analysis should report biological replication, exact treatment n, effect sizes and confidence intervals. These practices separate true AMF-mediated tolerance from differences caused by initial soil fertility, inoculum nutrients or uneven stress exposure.

CONCLUSION

The proposed study provides an integrated route from field discovery of soybean-associated AMF to controlled validation and inoculum development. Its strength lies in linking five-site rhizosphere sampling with soil chemistry, root colonisation, isolate multiplication, water and salt stress testing, mycorrhizal dependency, yield and oilcontent measurements. Recent soybean research supports the biological plausibility of improvements in P acquisition, nodulation, water relations and stress resilience after compatible AMF inoculation, but also demonstrates strong isolate, genotype and environment dependence. The final value of the Niphad programme will therefore depend on identifying strains that consistently colonise soybean and produce measurable agronomic benefits under local conditions. Such isolates can then be advanced for largescale

pot culture and field validation as locally adapted bioinoculants.

Declaration by Authors

Ethical Approval: Not applicable to the plant and soil study design.

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