The Role of Tissue Culture in Banana Disease Research: An Australian Perspective

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Abstract

The Australian banana industry is under constant threat from three important banana diseases: (1) Banana Bunchy Top Virus, (2) Black Sigatoka ***(Mycosphaerella fijiensis)*** *and (3) race 4 Fusarium Wilt* ***(Fusarium oxysporum*** *f.sp.* ***cubense)****. Tissue culture has an important role to play in the rapid propagation of clean planting material. However viruses, such as Banana Bunchy Top Virus and Banana Streak Virus, can be transmitted in tissue cultures and care must be exercised when disseminating micropropagated plants. Tissue culture also has an important role in both providing and developing disease-resistant varieties. The initiatives of the Queensland Department of Primary Industries in applying tissue culture techniques in their black Sigatoka and Fusarium wilt research programs are presented.*

Introduction

Australia's banana industry, estimated to be worth in excess of $250 million per annum, is under increasing pressure from a number of diseases. Two of the most serious of these diseases, Banana Bunchy Top Disease (BBTD) and race 4 Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*), occur in important production areas in northern New South Wales and southern Queensland (Fig. 1). They are kept in check

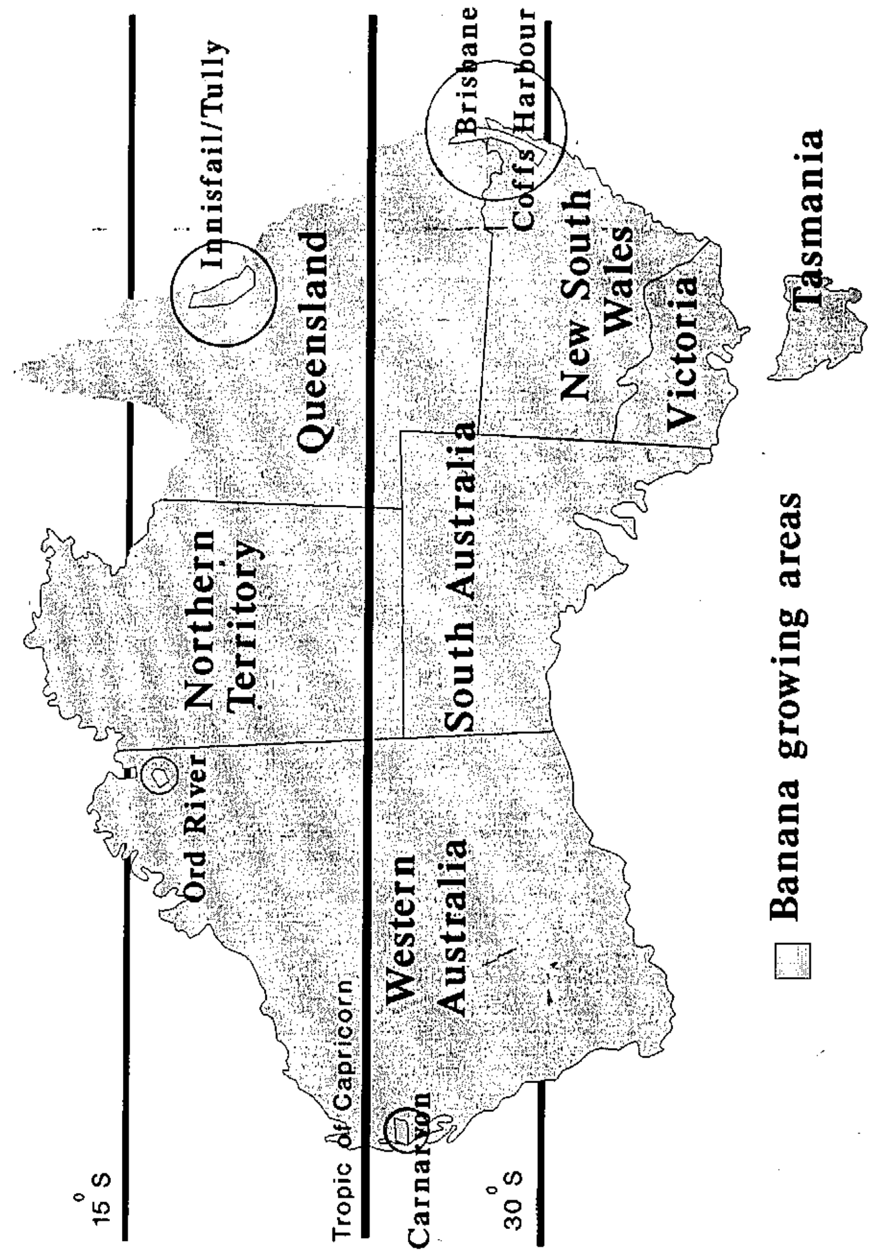

Fig. 1. Banana growing areas in Australia.

by quarantine measures, but are slowly spreading onto new properties. Another major disease, black Sigatoka (*Mycosphaerella fijiensis*), has been found only in isolated communities on the Cape York Peninsula and some of the Torres Strait Islands. A program to replant affected areas with resistant dessert and cooking bananas, to act as a buffer to further southern movement of the disease, is well underway.

Fortunately the major banana production area of Tully-Innisfail in north Queensland, which produces approximately 60% of Australia's banana crop, is presently free of these three serious diseases. These diseases are also not found in the Coffs Harbour area of New South Wales and in the Carnarvon and Ord regions of Western Australia, although there is a recent report of Cavendish being infected by Fusarium Wilt in Carnarvon. Because they attack the Cavendish group of banana cultivars, they are a major threat to our industry and it is most important that these diseases be contained and prevented from moving into areas where they presently do not occur. Tissue culture will have an important role to play in the provision of disease-free planting material. It will also be important in the development of disease-resistant cultivars, not only through rapid clonal propagation of resistant material, but by generating novel plants by in vitro mutation breeding. The initiatives of the Queensland Department of Primary Industries in combating these disease pressures is highlighted with emphasis on the role of tissue culture.

Provision of 'Clean' Planting Material

Banana tissue culture involves the growth of material under sterile conditions and therefore most pests and pathogens are either eliminated during disinfestation of explants prior to culture establishment (Hamill et al., 1993) or are readily apparent in cultures and can be discarded with the contaminated culture. For instance, the culture medium most frequently used for banana micropropagation is a Murashige and Skoog (1962) mineral salt mixture containing sucrose and other growth factors to enhance plant growth and development (Smith and Hamill, 1991). This same medium supports the growth of many saprophytes, but also serious pathogens such as *Fusarium oxysporum* f.sp. *cubense* and *Pseudomonas solanacearum* (M.K. Smith and L. Diatloff, unpublished results). Contaminated cultures can therefore be detected and removed to ensure only clean material is released to the industry. However there is no room for complacency and, if the material is being imported from overseas, the growing of plants in quarantine is recommended to prevent the introduction of unwanted diseases.

In practical terms, as far as the Queensland banana industry is concerned, tissue culture eliminates the threat of introducing nematodes (*Radopholus similis*, *Meloidogyne* spp., *Pratylenchus coffeae*, *Helicotylenchus multicinctus*), banana weevil borer (*Cosmopolites sordidus*) and Fusarium Wilt in planting material. It also eliminates the need for hot water treatments and pesticides to control these organisms. However viruses can still be transmitted through tissue-cultured planting material and of particular concern is Banana Bunchy Top Virus (BBTV).

Drew et al. (1989) were able to demonstrate that BBTV was transmitted in micropropagated bananas. These results demonstrated that cultures could be successfully established from plants showing obvious symptoms of BBTD and that these micropropagated plants could be maintained in culture in an apparently symptomless condition. Furthermore a portion of the plants multiplied from the BBTV infected mother plant did not develop symptoms of the disease when established in the glasshouse and later in the field. Drew et al. (1992) suggested that the virus may have been inadvertently eliminated from some plantlets during the rapid in vitro multiplication of cultures.

Further studies have been conducted on the transmission of BBTV in microprogated bananas using ELISA techniques which provide a rapid, reliable and sensitive technique for verifying the presence of virus in samples (Thomas and Dietzgen, 1991). A total of seven Cavendish (AAA) and four Lady Finger (AAB) plants, with typical field symptoms of BBTD and giving positive ELISA reactions, were established in culture. After four months in culture most lines displayed some chlorotic flecks on the leaves and BBTV was readily detected in all lines (Thomas, 1991). Symptom expression has become progressively milder and, after two years in culture, ten of the lines are producing some symptomless plants. BBTV was readily detected by ELISA even in plantlets with very mild symptoms, but totally symptomless plantlets tested negative and have remained symptomless for six months after planting out.

Multiplication rates for the Williams and Lady Finger cultivars were unaffected by BBTV infection and it would have been possible to multiply tens of thousands of infected plants over the duration of the experiment. One of the worrying aspects is that when the plants are actively multiplying, the infected plants are almost indistinguishable from non-infected control plants grown in vitro. The difficulty associated with detecting BBTV-positive BBTV-negative plants in vitro is illustrated in Figure 2. Samples 7, 10 and 11 were taken from the BBTV trial and compared with plants from known negative controls (8, 9, 12 and 13). ELISA tests revealed that samples 7 and 11 contained detectable levels of virus, while sample 10 tested negative (Figure 2A). However when four individuals familiar with BBTV symptoms were separately asked to rate the samples for disease on morphological characteristics, there was little agreement on what constituted a positive or negative sample (Figure 2B). In fact, only one individual correctly identified both positive samples.

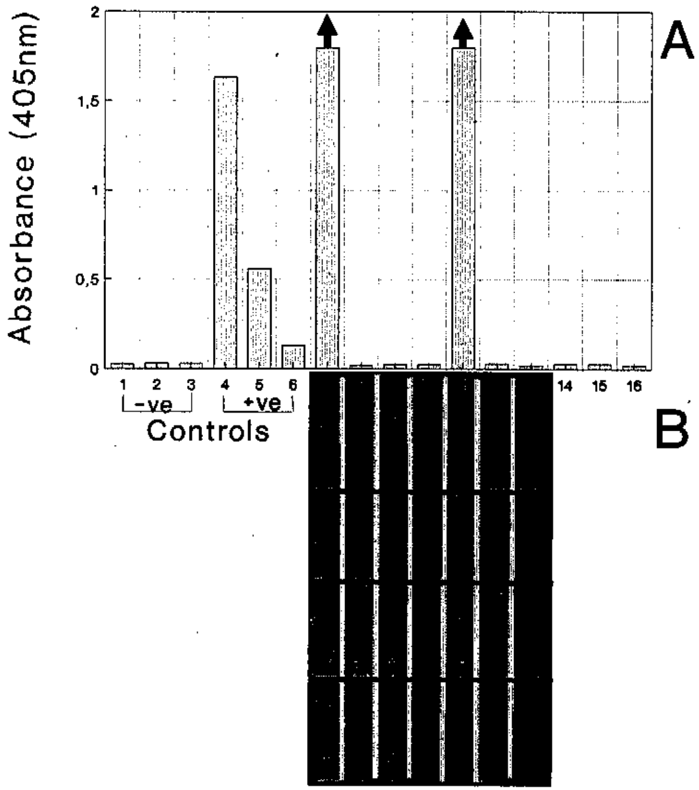

Fig. 2. **An assessment of BBTV expression in micropropagated bananas. Graph A shows absorbance value of samples and were based on ELISA results. Samples 1-3 were negative controls, 4-6 were positive controls diluted 1/4, 1/20 and 1/100, 7-13 were the test sample array and 14-16 were buffer. BBTV was tected by ELISA only in 7 and 11 from the test sample array. Graph B shows four individual's ratings based on the physical appearance of micropropagated plants. Individuals were to determine whether samples 7-13 contained BBTV (+) or whether they were virus-free.**

These results again highlight the danger inherent in transmitting viruses through micropropagated bananas. More recently it has been demonstrated that Banana Streak Virus (BSV), which is believed to be a strain of Sugarcane Bacilliform Badnavirus (SCBV), is transmitted in micropropagated Mysore (AAB). Mysore has a long history of BSV infection (Wardlaw, 1961) and ELISA tests are currently being developed for this virus. Following rapid *in vitro* multiplication of this material the appearance of symptomless plants are again being observed. Both BSV-positive and BSV-negative plants have been established in the field to determine if the virus will have an effect on fruit size, yield and reaction to Fusarium Wilt.

The Australian Banana Industry is concerned about the threat of introducing virus-infected planting material through tissue culture and has developed strict guidelines which must be followed when growers obtain permission to plant. By following these guidelines only clean, virus-tested, planting material would be available for distribution throughout the industry.

Disease Resistance: The Road to Responsible and Sustainable Production

Black Sigatoka

In 1981, black Sigatoka was recorded in Australia for the first time on islands in the Torres Strait and in the Bamaga area on the tip of Cape York Peninsula (Jones and Alcorn, 1982). Many of the aboriginal settlements in this area are dependant on bananas to supplement their diet and bananas have a traditional role in food preparation for ceremonial occasions. However, the presence of black Sigatoka was recognised as a major threat to the Queensland Banana Industry. There was a risk, with more tourists visiting the area and improved road access from the south, that the disease would spread to major production areas in the Innisfail-Tully region of north Queensland.

Although Cavendish varieties are susceptible to Sigatoka leaf diseases, there are banana and plantain varieties available that show resistance to black Sigatoka. Some of these dessert and cooking types are acceptable as home garden varieties and are currently being introduced from our laboratory into the South Pacific and the Cape York Peninsula-Torres Strait area as micropropagated plants. The decision of which cultivars to introduce to the Far North came from trial work in the South Pacific as part of an Australian Centre for International Agricultural Research (ACIAR) project for banana improvement. Black leaf streak or black Sigatoka disease of banana and plantain caused by *Mycosphaerella fijiensis* is found on most islands in the South Pacific (Dingley et al., 1981). It is one of the major factors that has led to a decline in banana export industries in the region.

Field trials have been established in Tonga, Western Samoa and the Cook Islands to evaluate a range of banana germplasm for its reaction to black Sigatoka. Earlier trials established in 1989 consisted of 18 cultivars with a range of genomic backgrounds, and six cultivars were found to have good to moderate resistance to black Sigatoka (Table 1). The rankings were based on the number of leaves with less than ten mature lesions and the number of the leaf with first streak

Table 1. Cultivars ranked as having good to moderate resistance to Black Sigatoka (*Mycosphaerella fijiensis*).

Based on observations from 18 selected cultivars from field trials in Tonga, Western Samoa and the Cook Islands. The rankings were determined on the basis of the number of leaves with less than ten mature lesions and the number of the leaf with first streak symptoms.

Cultivar	Genotype	Origin	Comments
Ducasse	ABB	Local selection from Queensland, imported from Kenya	Dessert fruit, moderate yielding, extremely vigorous
Bluggoe	ABB	Local selections from Queensland, originally from Pacific region	Cooking banana, moderate yielding
Pisang Mas, Amas	AA	Imported into Queensland from Philippines	Dessert fruit, low-moderate yielding
Mysore	AAB	Imported into Queensland from Jamaica and Honduras, originally from Indian sub-continent	Dessert fruit, moderate yielding
T8 (TU8)	AAAA	Tetraploid (61-882) from Jamaican breeding program	Cavendish-like dessert fruit, moderate-high yielding, wind susceptible
SH-3436	AAAA	Tetraploid from FHIA breeding program	Cavendish-like dessert fruit, high yielding, wind susceptible

symptoms. Therefore the more mature the leaf or higher the number of leaf in which symptoms or lesions developed, the better the resistance and hence ranking. Ducasse (ABB) consistently rated higher than the other cultures, gave good yields, and was acceptable as a dessert banana. Mysore (AAB) was also regarded highly as a dessert banana, while Bluggoe (ABB) was acceptable as a cooking-type. The two Cavendish-like tetraploids, T8 (AAAA) and SH-3436 (AAAA), had moderate resistance to black Sigatoka, however there was concern about shelf life and low bunch cycling. It was also of interest to note that the FHIA breeding diploids, SH-3142 and SH-3362, were also found to have good resistance to black Sigatoka in these trials. More recent introductions include Dwarf Kalapua (ABB), Kandrian (ABB/ABBB), Saba (BBB) and SH-3481 (AAAB), which may also prove to have a good level of resistance.

Meanwhile, the Queensland Banana Industry Protection Board initiated a black Sigatoka control program in 1988 because if black Sigatoka reaches the commercial growing areas, initial losses to the Queensland Banana Industry have been estimated to be about A $10-15 million (Jones, 1991). All banana plants at locations where the disease occurred on the Torres Strait and Cape York Peninsula have been progressively destroyed and replanted, after a host-free period, with the banana cultivars T8, Bluggoe, Mysore and Ducasse. Other cultivars will be added as they become available. This program aims to reduce inoculum levels and so reduce the chance of the disease spreading into the commercial growing areas. By replacing susceptible bananas with resistant cultivars the communities in the region are given access to a range of new plants with greater productivity. Micropropagation has enabled this replacement program to proceed swiftly with the minimum of disruption to the communities in the area.

Fusarium Wilt

Fusarium wilt is regarded as the most serious threat to the industry due to inability to control the disease by chemical or cultural practices, and the lack of a commercial variety resistant to race 4 which would replace Cavendish. Suitable replacements for Lady Finger (AAB), which is the only non-Cavendish cultivar with significant commercial production in Australia, are also sought that have resistance to race 1.

Although race 1-resistant varieties that can potentially substitute for Lady Finger are currently being evaluated in south-eastern Queensland (see Moore et al. this proceedings), obtaining a race 4 resistant Cavendish replacement has been much more difficult. One approach has been to develop a technique to generate autotetraploids by the use of colchicine (Hamill et al., 1992). Autotetraploids of SH-3362 that have resistance to race 4 Fusarium wilt have been produced and returned to the FHIA breeding program. Alternatively, to help generate novel varieties, an *in vitro* mutation breeding program was initiated (Smith et al., 1990, Smith and Drew, 1990). The main advantage of mutation induction in a vegetatively propagated crop, such as banana, is the ability to change one or a few characters of an otherwise outstanding cultivar without altering the remaining and often unique part of the genotype. This is not yet possible using conventional breeding approaches to banana improvement.

Over 20,000 plants have now been evaluated in the field at a race 4 screening site and nursery at Wamuran (latitude 27oS) in south-eastern Queensland. Approximately half of these have been gamma-irradiated plantlets and the remainder material collected from both local and overseas germplasm collections and breeding programs. Included in this material is *Musa* germplasm collected from Papua New Guinea (Sharrock, 1990). Currently 368 accessions have been screened and, of these, only 6 accessions have shown resistance to race 4 Fusarium wilt (Table 2). They include two wild species, *Musa ornata* and *M. velutina*; two superior breeding diploids from the FHIA breeding program, SH-3362 and SH-3142; and Dwarf Parfitt (AAA), an extra-dwarf Cavendish variety. Mysore, an AAB cultivar that has small fruit resembling Lady Finger, is susceptible when young but tolerates race 4, as the stool matures. Though all of these plants show good levels of resistance, they are not immune to Fusarium wilt. Under extreme conditions of cold and drought, as existed in south-eastern Queensland in 1991, even some plants from this group became infected and showed symptoms of the disease. In fact, immunity is not a realistic goal to aim for in a Fusarium wilt breeding program in banana because regardless of the pathotype or cultivar used, the fungus is able to penetrate and establish in the vascular system of the root (Pegg and Langdon, 1987). Resistance can breakdown under periods when the plants are stressed and the pathogen is active. However if the level of resistance observed from these accessions can be expressed in a suitable commercial cultivar, then the possibility of serious epidemics of the disease may be considerably reduced. Fusarium wilt could be effectively managed with resistant cultivars.

Our approach to mutation induction has involved two strategies. On the one hand superior Cavendish varieties (eg Williams, New Guinea Cavendish), that have susceptibility to race 4, have been irradiated in the hope of obtaining resistant lines after screening and

Table 2. Musa species and cultivars considered to show good resistance to race 4 Fusarium Wilt (*Fusarium oxysporum* f. sp. *cubense*).

Based on observations from 368 accessions grown on heavily-infested soils at Wamuran in south-eastern Queensland.

Species/Cultivar	Genotype	Origin	Comments
Dwarf Parfitt	AAA	Local selection from New South Wales	Member of Cavendish sub-group. 1m at bunch emergence. No commercial value
SH-3362, SH-3142	AA	Improved diploids from FHIA breeding program	Important as male, diploid parents but of no immediate commercial use
Mysore	AAB	Imported into Queensland from Jamaica and Honduras, originally from Indian sub-continent	Dessert fruit, moderate yielding
Musa ornata, M. velutina		Local selections from New South Wales	Ornamental banana, colourful bracts

selection. On the other hand an agronomically inferior, but race 4 resistant, variety (eg Dwarf Parfitt) has been irradiated in an attempt to improve its agronomic characteristics.

The first strategy has yielded a few promising lines but a markedly improved level of resistance has not been observed. Planting material collected from the surviving plants has been replanted for a second, third, and now, for a fourth cycle of evaluation. Of the few remaining plants, all are characterised by a dwarf, robust stature (average height of 1.9m at bunching). Unfortunately, these plants are also prone to 'choking', a condition when the bunch fails to emerge fully from the crown of the plant.

The second strategy has yielded the most promising material to date. Dwarf Parfitt is the only Cavendish-type banana evaluated that has shown a high level of resistance to race 4. Unfortunately the plants are very small (average height of 1.0 m at bunching) and are extremely prone to 'choking'. Following irradiation of micropropagated plants at a dose of 20 Gy, with a further three subculture cycles before establishment in the field, a population of 35 plants was produced which possessed improved characteristics (Table 3). Plants were larger, they bunched earlier, yield was considerably increased and choking was eliminated. They were also apparently more cold tolerant than standard Cavendish varieties. More importantly they also appear to retain the resistance to race 4 shown by the mother plant, Dwarf Parfitt. Currently we have established a replicated field planting of this improved selection with some well-known standard varieties and will be examining the physiological features of these plants, particularly as they may relate to Fusarium wilt.

Table 3. Preliminary growth and yield data of Dwarf Parfitt and its putative mutant, Giant Parfitt, in south-eastern Queensland.

Values are the averages from approximately 20 plants. The data for Williams was taken from non-infested sites as Williams is susceptible to Race 4 Fusarium Wilt.

	Dwarf Parfitt	Giant Parfitt	Williams
Height at bunching (cm)	104	182	203
Bunch weight (kg)	8.2	24.2	22.1
No. of hands/bunch	9.0	10.3	10.4
Planting to harvest (months)	19.8	16.3	17.9

Conclusions and Future Directions

Of the material so far produced from QDPI's in vitro mutation breeding program, the putative mutants derived from Dwarf Parfitt show the most promise as a race 4 resistant Cavendish replacement under the subtropical conditions existing in Queensland and New South Wales. We are still at an early stage in the evaluation of this material and we wish to determine the genetic stability of this material and make further comments on the nature of the resistance.

Research is also underway to determine photosynthetic efficiency and carbohydrate utilization and how they relate to cold tolerance (see Moore et al., this proceedings). It is interesting that Fusarium wilt of Cavendish is most prevalent in the sub-tropical regions of the world (Ploetz et al., 1990) where periods of cold and drought may place plants under stress. Leaves of our standard Cavendish varieties are typically more yellow than Dwarf Parfitt following winter (photo inhibition-induced chlorosis), and carry fewer photosynthetically-competent leaves. We have speculated that the 'weakened' Cavendish varieties are therefore most susceptible following the winter and unable to prevent invasion of the fungus into the corm during the spring when the disease can reach almost epidemic proportions. By developing a greater understanding of the physiological mechanisms of resistance then there is an opportunity that a reliable screening technique can be employed either in culture or in the nursery to screen the large numbers of plants produced from mutation or conventional breeding programs. The lack of a suitable small plant screening technique continues to be one of the major obstacles in effectively breeding for Fusarium wilt resistance.

Research with BBTV transmission through tissue culture is nearing completion. We have demonstrated that the virus can be transmitted in micropropagated bananas, sometimes in a virtually symptomless condition, and this has important consequences for the dissemination of *Musa* within Australia and internationally. Rapid and sensitive ELISA tests are now available. The mode of BBTV transmission during micropropagation, where both virus-positive and virus-negative plantlets occur together in a population, mean that all micropropagated plants derived from an untested source plant must be indexed for virus before being mass propagated in culture. This provides a safeguard against inadvertently indexing virus-free plants in a population that may still contain virus-infected plants.

Finally screening for black Sigatoka-resistant plants continues in the South Pacific. Although a number of dessert and cooking bananas have been identified with good resistance to the disease, it is hoped that a resistant Cavendish replacement will soon be on the horizon. Tissue culture will continue to play an important role in the multiplication and rapid dissemination of this material.

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