Medicinal and Aromatic Plants collecting missions in Portugal

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Abstract: Medicinal and aromatic plants (MAP) are used in all civilizations and cultures and have always played a key role in health care systems worldwide. These plants constitute a major part of the flora, which provide raw materials that are primarily used for therapeutic, aromatic and/or culinary purposes, as components of medicinal and cosmetics products and other natural health products. They are also the starting materials for value-added processed natural ingredients such as essential oils, dry and liquid extracts and oleoresins. Portugal has given rise to very diverse environmental habitats, is cradle to a rich flora, comprising 3,995 described taxa, 500 of which are of aromatic and/or medicinal potential. Some of these species are endemic, sometimes with very vulnerable ecological niches. Plant genetic resources' collecting is a fundamental activity, widely used in *ex situ* conservation. Between 1990 and 2014, the Banco Português de Germoplasma Vegetal (BPGV), carried out national and international MAP collecting missions, which resulted in the *ex situ* conservation of 1,606 accessions. The main focus of this communication is to demonstrate the valuable contribution of those collecting missions in MAP genetic diversity conservation and evaluation.

Keywords: collecting missions, Genebank, medicinal and aromatic plants, Portugal

Introduction:

The Mediterranean region is one of the world's major centres of plant diversity, where nearly 25,000 species occur, half of which are endemic to the region (Heywood & Skoula, 1999).

The Mediterranean flora is very rich in medicinal and aromatic plants (MAP) species, especially from the Labiatae, Umbelliferae and Compositae families, and the region is also regarded as one of the main centres of diversity of medicinal and aromatic plants (Bettencourt, 1998; Heywood, 1998).

Allied with human and geographical factors, Portugal has given rise to very diverse environmental habitats, with a rich flora (Bettencourt & Gusmão, 1995; Barata *et al*., 2011c),

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comprising 3,995 described taxa (Sequeira *et al*., 2011) of which 500 are of aromatic and/or medicinal potential (Barata *et al*., 2011c; ICN, 1998). These taxa are distributed mainly by the families Apiaceae, Asteraceae, Compositae, Cupressaceae, Hypericaceae, Labiatae, Lamiaceae, Lauraceae, Leguminosae, Liliaceae, Malvaceae, Myrtaceae, Oleaceae, Pinaceae, Rosaceae, Rutaceae and Umbeliferae (Barata *et al*., 2011c; Figueiredo *et al*., 2006). Some of these taxa are endemic, sometimes with very vulnerable ecological niches. Many of these taxa are the subject of monographs in European and Portuguese pharmacopoeias (Barata *et al*., 2011c).

For many decades' conservation of genetic resources of MAP in the world was neglected (Rao, 2016). TRAFFIC (2016) reports that a large majority of the world's MAP species are collected from the wild and are considered threatened pointing out that one in five of the world's plant species is estimated to be threatened with extinction in the wild, being the unsustainable harvest the major factor. So, there is an important responsibility to collect, conserve and protect these species within their territory.

Since the 1980s, several International organizations have been carrying out systematic and coordinated efforts for MAP conservation and sustainable use (CBD, 2002; Kathe, 2006; Rao, 2016).

Since 1990, *in situ* and *ex situ* (seed banks, field collections, *in vitro* collections and cryopreservation), as complementary conservation strategies, are being implemented in Europe and other continents for plant genetic resources in general, and MAP species in particular (Barata *et al*., 2016).

Between 1990 and 2014, with the objective of MAP conservation and sustainable use, the Banco Português de Germoplasma Vegetal (BPGV), the national genebank in Portugal, carried out national and international systematic collecting missions.

Collecting missions are a fundamental activity, widely used to support *ex situ* conservation in order to preserve and protect genetic diversity.

Materials and methods:

The target species were selected according to the following priorities:

- Need to conserve landraces of MAP at risk of being replaced by commercial varieties (e.g. *Coriandrum sativum* L, *Petroselinum crispum* (Mill.) Fuss);
- Economic and social importance;

- Respond to the needs of wild MAP germplasm clearly expressed by national and international users;
- Increase the genetic diversity of existing collections of MAP in *ex situ* conservation.

Until 2005, the preparatory steps included the survey of Flora de Portugal (Coutinho, 1939), Nova Flora de Portugal (Continente e Açores) (Franco, 1971, 1984; Franco & Afonso, 1994, 1998, 2003), Flora Iberica (Castroviejo *et al*., 1986-2001) and Flora Europaea (Tutin *et al*., 1964-1980) in order to find detailed descriptions and information on how to differentiate between related taxa. Then, herbaria were visited that provided the precise location and phenological (flowering / seed maturation time) data of the target species. (ENSCONET, 2009).

The survey of sources of information on existing germplasm collections was also an asset (Bettencourt, 1998; Bettencourt & Gusmão, 1998; Bettencourt & Gusmão, 1999; Bettencourt, 2011). Bibliographic survey, ecogeographic studies and the definition of collecting strategies were preliminary steps to set up the logistics and define a plan of action for the collecting missions (Farias & Bettencourt, 2006).

After 2005, with the publishing of digital floras available in the web such as the Flora Digital de Portugal, (2005), Flora Iberica (2005) and Flora-On (2014) they have been used as the main sources of information.

The expedition route was established with the help of road maps (1:350,000) and, more recently, using a Geographical Positioning System (GPS). The timing of collecting missions was defined based on the seed maturation time of the target species.

The itinerary of collecting missions was based on the places previously selected based on the information collected in herbaria or in public websites on the flora or identified during the preparatory missions.

The distance between the collecting sites was at least 10 km, as recommended by ENSCONET (2009), Hawkes, 1980 cited by Astley (1991), Hawkes *et al*. (2000) and yet, when the altitude varied from 100 m, as mentioned by Hawkes *et al*. (2000).

According to Marshall & Brown (1975, 1983), the optimum sampling strategy should enable the collector to obtain, with 95% certainty, all the alleles occurring in the population at a frequency greater than 5% (ENSCONET, 2009; Ford-Lloyd & Jackson, 1986; Hawkes, 1983; Marshall & Brown, 1975; Vieira, 1996). To achieve this, whenever possible, seeds were collected randomly from at least 50 plants per population (ENSCONET, 2009).

In the case of small populations, the recommendations of Yonezawa (1985) were followed, which advises collecting ten plants per site, since the number of visited sites is maximized. This approach is also supported by Porceddu & Damania (1992), Damania (1996) cited by Ford-Lloyd & Jackson (1986), Hawkes (1983), Hawkes *et al*. (2000) and Walter & Cavalcanti (1996). Astley (1991), Walter *et al.* (1996) and Vieira (1996) report that it is more important to sample the maximum number of sites (sites / populations) than to sample the theoretically ideal number of plants per location, because covering a larger area can capture greater diversity associated with adaptation to geographical and micro geographical differences in environmental factors. Brown & Marshall (1995) recommend sampling more sites in areas where the species is more abundant or where more variability for certain specific characteristics is clear. Taking into account these recommendations, as many places as possible were sampled, only restricted by: duration of collecting missions; relative abundance of target species; and available resources (Hawkes *et al*., 2000).

During the collection missions, special care was taken to limit the seed collected to no more than 20% of the total mature seeds available, on the day of collection, in order to minimize risks of genetic erosion and ensure the future survival of the plant populations (ENSCONET, 2009).

Sampling of natural populations of wild species was the most random possible (Brown & Marshall, 1995; ENSCONET, 2009). Where large populations in a uniform landscape were found, it was used systematic sampling at regular intervals along transects (ENSCONET, 2009).

Wherever possible, enough seeds were collected in order to maintain a collection without the need for immediate seed multiplication, avoiding problems of genetic drift, additional costs and loss (Brown & Marshall, 1995; ENSCONET, 2009). In general, for most wild populations, more than 5,000 seeds were collected as recommended by ENSCONET (2009). However, in some populations, the number of seeds which could be collected was lower, but according to Vieira (1996), is preferable in most cases to sample a small amount of seed of a population, which does not necessarily represent its " genetic pool ", than to ignore their availability due to the fact of not having a theoretically perfect sampling.

The seed samples were introduced in cloth or paper bags, duly identified.

For each collected sample complete passport data was recorded, according to the internationally accepted list of descriptors proposed by "Bioversity International".

Results and discussion

Between 1990 and 2014, BPGV carried out a total of sixty-one (61) MAP collecting missions in Portugal (figure 1). Seeds and vegetative material of MAP cultivated and wild species were collected (Barata, *et al*., 2009; Barata, *et al*., 2011a; Barata *et al*., 2011b; Farias, 2002a; Farias, 2002b; Lopes *et al*., 2015; Marcelino, 2002; Mota *et al*, 2005; Rocha, 2000; Rocha, 2005; Rocha *et al*., 2010a; Rocha *et al*., 2013; Rocha *et al*., 2014).

The Key point dates of collecting missions:

- **1990 to 1993 -** seed samples of medicinal, aromatic plants found in farms, such as *Coriandrum sativum* L. and *Petroselinum crispum* (Mill.) Fuss, started to be included in the national and international collecting missions;
- **1994** - Systematic prospection and collecting missions for genus *Allium*;
- **1997 to 1999** - Systematic prospection and collecting for wild hop;
- **2000 to 2014** - Systematic prospection and collecting missions for several medicinal and aromatic plants.

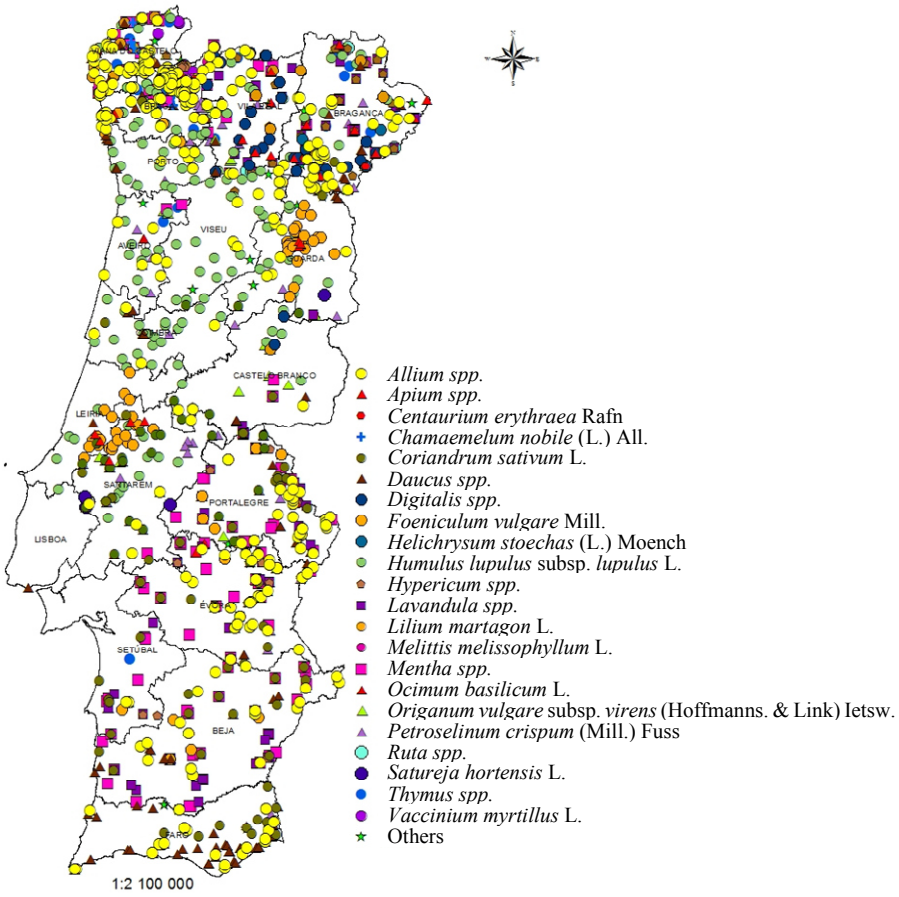

Figure 1. Collecting sites in Portugal by species

The MAP collection has a wide distribution throughout the country, with more samples collected in the North region, followed by Alentejo region and Central region and less in Lisbon & the Tagus Valley and in the Algarve region (figure 2).

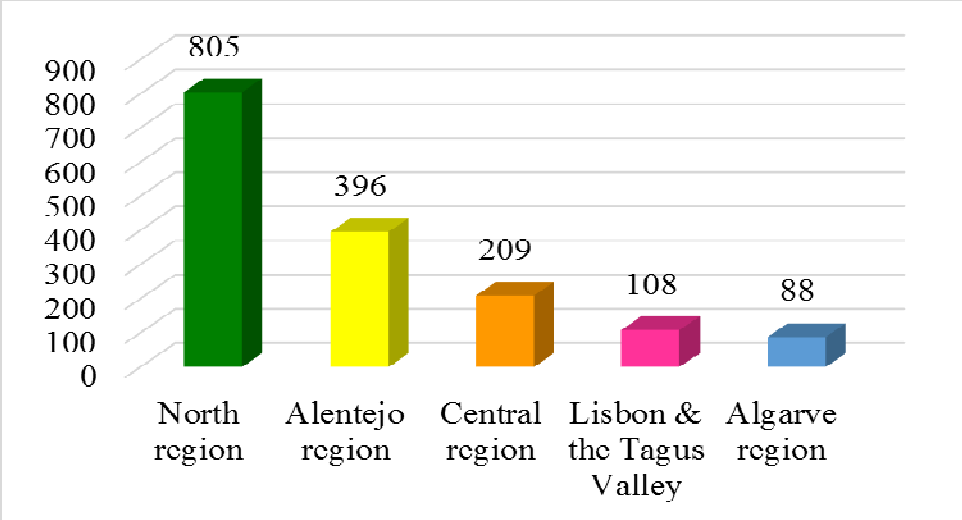

Figure 2. Number of collected samples by Portugal's regions

The most represented families in terms of number of accessions are Amaryllidaceae, Apiaceae, Lamiaceae followed by Cannabaceae and Hypericaceae (figure 3).

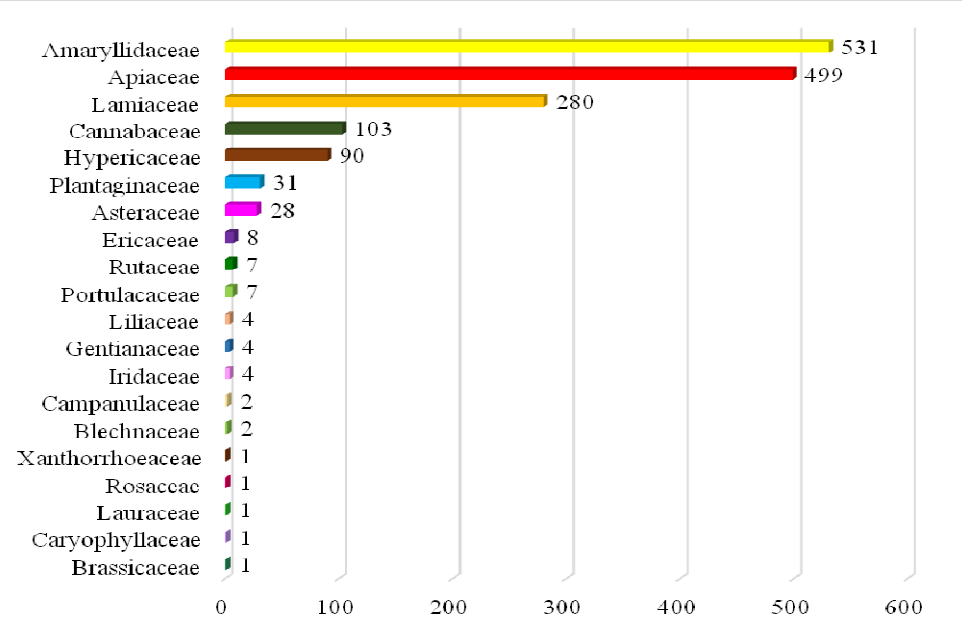

Figure 3. Number of collected samples by botanical families

The most represented genera in terms of number of accessions are *Allium* (528), *Daucus* (131), *Coriandrum* (126) followed by *Humulus* (103), *Foeniculum* (101), *Petroselinum* (100)

Mentha (92), *Lavandula* (91), *Hypericum* (90), *Origanum* (42), *Apium* (41) and *Thymus* (41). The distribution of species collected by botanical genus is represented in figure 4.

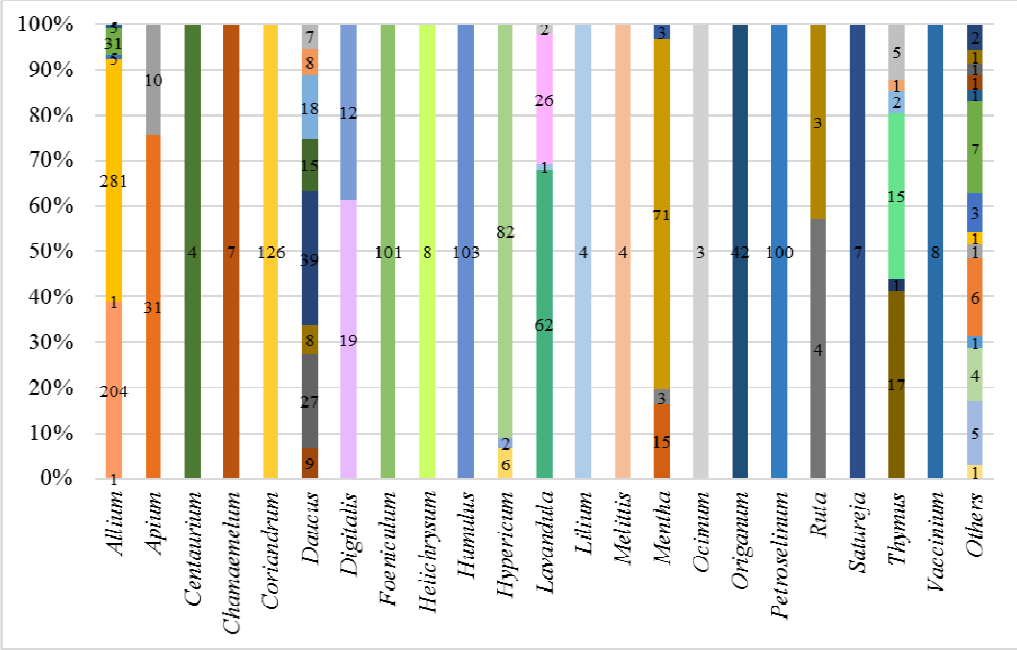

Figure 4. Distribution of species collected by botanical genus

In terms of the biological status, the most represented are Wild (54 %) followed by Landraces (46%) (figure 5).

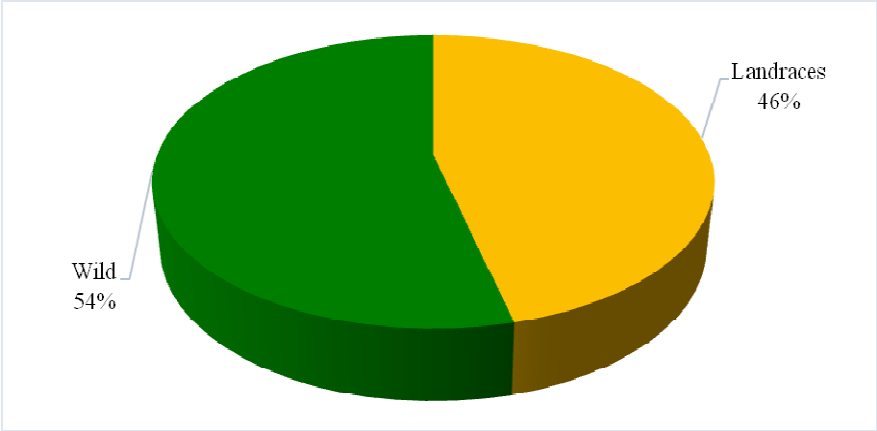

Figure 5. Biological status of MAP collections, as a percent of total number of accessions

The collected landraces are: *Allium ampeloprasum* L. (14), *Allium ascalonicum* L. (5), *Allium cepa* L. (204), *Allium sativum* L. (281), *Carthamus tinctorius* L. (1), *Coriandrum sativum* L. (126), *Ocimum basilicum* L. (3), *Petroselinum crispum* (Mill.) Fuss (100), *Satureja hortensis* L. (7) and *Sinapis alba* L. (1).

The collected wild species are mainly represented by: *Humulus lupulus* subsp. *lupulus* L. (103), *Foeniculum vulgare* Mill. (101), *Hypericum* spp. (90), *Mentha pulegium* L. (71), *Lavandula pedunculata* (Mill.) Cav. (62) and *Origanum vulgare* subsp. *virens* (Hoffmanns. & Link) Ietsw. (42).

In terms of the acquisition source, most of the MAPs were collected from wild habitats. The landraces were mostly collected in farm stores, home gardens, farmlands, and also in local markets (figure 6).

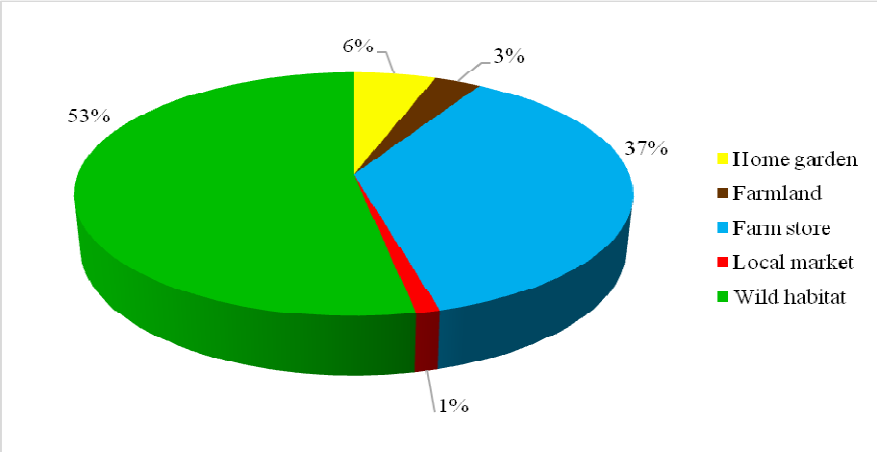

Figure 6. Acquisition source of MAP collections, as a percent of total number of accessions

The selection of the collecting locations, considers, not only the distance between the sites, but also the altitude. This collecting approach has the advantage of ensuring differences between sampling sites, which are likely to be relevant for conservation and to future use (Table 1).

Table 1. Distribution of botanical genus collected by altitude classes

Genus	Number of samples by Altitude Classes (m)												
	4-100	100-200	200-300	300-400	400-500	500-600	600-700	700-800	800-900	900-1000	1000-1100	1100-1200	1200-1295
Allium	86	102	104	73	68	41	21	20	10	4			
Daucus	60	23	31	6	3	3	3	1	1				
Coriandrum	28	39	25	13	8	8	3	2					
Humulus	37	31	7	8	11	5	2	1	1				
Foeniculum	14	17	16	13	13	20	5	3					
Petroselinum	15	29	16	12	13	7	3	2		2			
Mentha	26	16	17	7	6	7	8	3	1	1			
Lavandula	4	17	21	14	7	5	9	7	3	4			
Hypericum	7	12	14	8	13	5	9	11	5	6			
Origanum	3	6	8	7	7	4	3	3					1
Apium	14	4	6	3	1	6	2	3	2				
Thymus	3	4	5	3	4		6	8	3	3	1		1
Digitalis		1	1	2	1	3	8	8	3	3		1	
Others	7	13	7	9	6	8	13	8	6	7	2	2	1
Total	**304**	**314**	**278**	**178**	**161**	**122**	**95**	**80**	**35**	**30**	**3**	**3**	**3**

According to these results, most of the accessions were collected in areas with altitude up to 500 m (76.9%).

The collection site with lower altitude (4 m) is located in Cabanas, Tavira and the collection site with highest altitude (1,295 m) is located in Armedas, Ponte da Barca.

All the passport data were documented and now are available in the Grin-Global database platform (http://bpgv.iniav.pt).

Conclusions:

MAP populations were sampled from diverse ecological biotopes (habitats, altitude and different soil and climatic conditions).

As a result of collecting missions carried out from 1990 to 2014, 1,606 accessions are maintained in *ex situ* conditions – seed, clonal collections and *in vitro* conservation, representing 67 species from 37 Genera and 20 botanical families.

This MAP collection is representative of most of the genetic variability of MAP in the country and is safely maintained for the benefit of present and future generations.

Gap analysis for some genera and species should be done to inform future gap filling collecting mission in order to ensure the conservation of the largest variability possible.

Competing interests

The authors declare that they have no competing interests.

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