

# Crambe, seakale

Klaus Ammann, TU Delft

For feedbacks email [klaus.ammann@ips.unibe.ch](mailto:klaus.ammann@ips.unibe.ch)

## Taxonomy

(Francisco-Ortega et al., 1999) note: *Crambe* L. (Brassicaceae) is an OldWorld genus with a disjunct distribution among four major centers of species diversity. A phylogenetic analysis of nucleotide sequences of the internal transcribed spacers (ITS) of the nuclear ribosomal repeat was conducted with 27 species of *Crambe* and 18 related genera. Cladistic analyses using weighted and unweighted parsimony support *Crambe* as a monophyletic genus with three major lineages. The first comprises those taxa endemic to the Macaronesian archipelagos. Taxa with a predominant Mediterranean distribution form the second assemblage, and a disjunction between east Africa (*C. abyssinica*) and the Mediterranean (*C. hispanica*) occurs in this clade. The third lineage includes all Eurosiberian–Asian taxa and *C. kilimandscharica*, a species from the highlands of east Africa. A basal biogeographic split between east Africa and Eurasia is present in the third clade. The patterns of relationships in the ITS tree are concordant with known climatic events in northern Africa and southwestern Asia since the middle Miocene. The ITS trees are congruent with the current sectional classification except for a few members of sections *Crambe*, *Leptocrambe*, and *Orientecrambe* (*C. cordifolia*, *C. endentula*, *C. kilimandscharica*, and *C. kotschyana*). Low levels of support in the basal branches do not allow resolution of which genera of the subtribes Raphaniae or Brassicinae are sister to *Crambe*. Both subtribes appear to be highly polyphyletic in the ITS trees.

### *Monophyly and Origin of Crambe*

Brassica, *Crambe*, *Diplotaxis*, and *Erucastrum* are the only genera of the Brassiceae with more than 10 species. Among these genera, *Crambe* is the only genus that has been shown to be monophyletic using molecular data (Warwick & Black, 1991, 1997b; Warwick et al., 1992). Chloroplast DNA restriction site data provided only weak support (30% bootstrap value) for the monophyly of *Crambe* (Warwick & Black, 1997b). Our analysis of ITS variation provides stronger support for the monophyly of the genus (bootstrap values ranged between 63 and 71%).

Both cpDNA restriction site and morphological data suggest that *Crambe* may represent a distinct subtribe within the Brassiceae (Gomez-Campo, 1980) (Warwick & Black, 1997b). The ITS data do not resolve this issue because relationships among genera of the Brassiceae are weakly supported in most cases. No single genus is sister to *Crambe* in the ITS trees. Previous suggestions of a close phylogenetic relationship between *Crambe* and *Calepina* (Clemente & Hernandez-Bermejo, 1978a, b, 1980a, b) or *Hemicrambe* (Gomez-Campo & Tortosa, 1974) are not supported by the ITS data. Our phylogeny agrees with other hypotheses (Gomez-Campo, 1980) (Warwick & Black, 1997a), which suggested that *Calepina* should not be considered part of the Brassiceae.

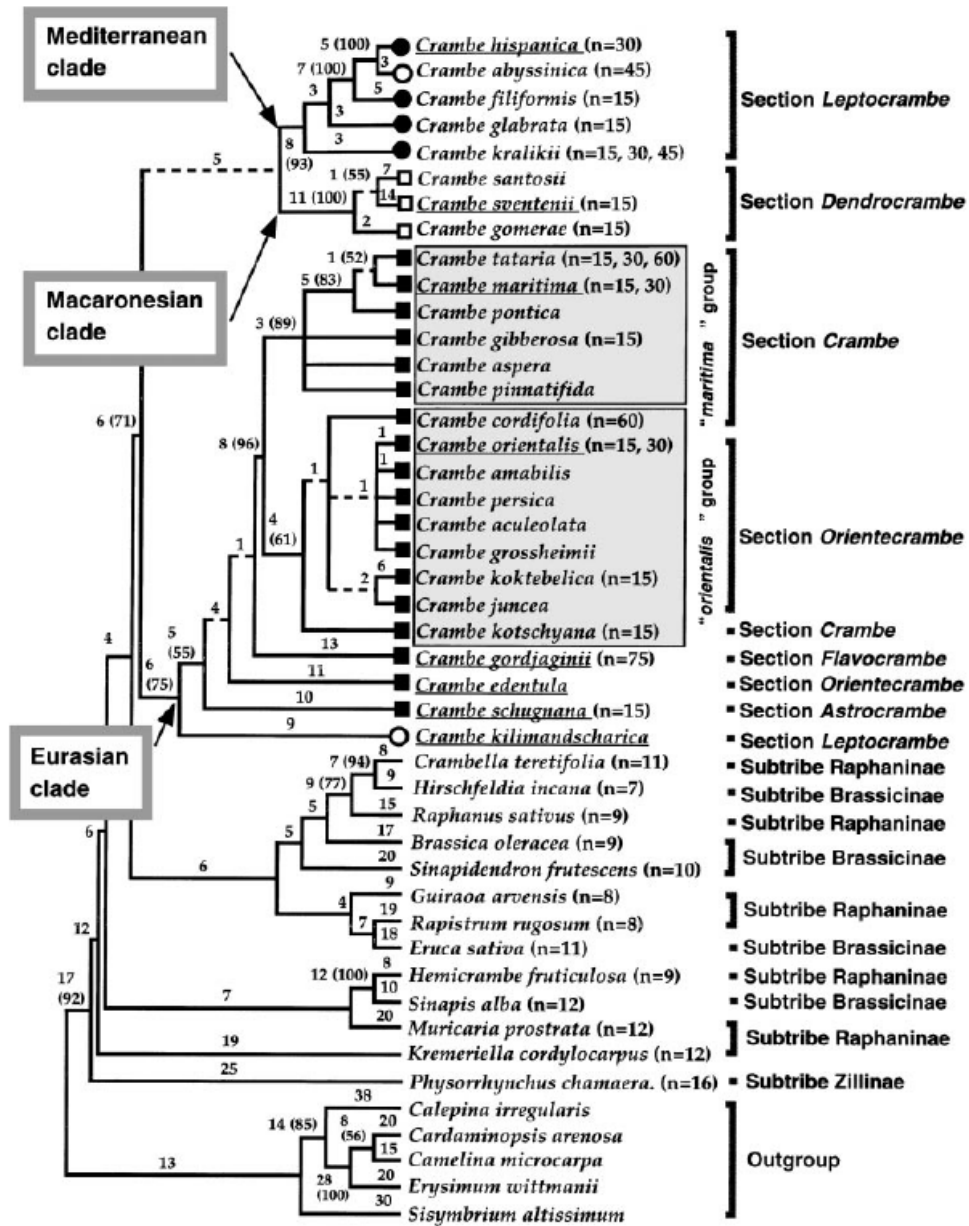


Fig. 1 One of the 1491 shortest trees from the unweighted parsimony analysis that treated indels as missing data (637 steps; CI 5 0.478, without autapomorphies; RI 5 0.681). Branches which collapse in the strict consensus tree are indicated by dashed lines. Number of changes are indicated along each branch. Bootstrap values higher than 50% are indicated in parenthesis. Distribution of *Crambe* species are: closed circles, Mediterranean; open circles, east African; open squares, Macaronesian; closed squares, Eurasian. Haploid chromosome numbers are indicated in parentheses when known. Species examined to test the molecular clock hypothesis are underlined. From (Francisco-Ortega et al., 1999).

### Sectional Classification

The main incongruence between the ITS phylogeny and the current sectional classification concerns *C. kilimandscharica*. This annual species from east Africa has been traditionally considered part of sect. *Leptocrambe*. In addition, this species has small, rugose fruits that are virtually identical to most of the Macaronesian species. *C.*

*kilimandscharica*, however, is the basal member of a clade which includes the four Eurosiberian–Asian sections of Khalilov (1991a,b). This placement may indicate that the morphological similarities between *C. kilimandscharica* and sect. *Leptocrambe* may be homologous and plesiomorphic for the Eurasian clade.

Another disagreement between the ITS trees and the current sectional classification concerns the monophyly of the four Eurosiberian–Asian sections (Khalilov 1991a,b). These sections are clearly not monophyletic in the ITS tree (Figs. 1–3). However, the ITS phylogeny is congruent with the earlier classification of Schulz (1919), which recognized one section for all taxa of the Eurasian clade with the exception of *C. kilimandscharica*. Within the Eurasian clade, the ITS trees identify two major lineages, here designated “*maritima*” and “*orientalis*”. These two groups are also geographically distinct. All species in the “*maritima*” group have European distributions and do not occur east of the Caucasus region. In contrast, species in the “*orientalis*” group have a predominant Asian distribution. The major disagreement between all previous treatments of the Eurasian taxa and the ITS trees concerns *C. edentula*. This species has been suggested to be closely related to *C. orientalis* by (Khalilov, 1991a, b). However, the ITS phylogenies do not place it in the “*orientalis*” group.

The ITS trees and previous taxonomic treatments (Candolle, 1821, 1824) (Schulz, 1936) (Gomez-Campo, 1980) (Khalilov, 1991a, b) concur that the Macaronesian taxa are monophyletic. They also indicate a close phylogenetic relationship among the Mediterranean species (including *C. abyssinica*). The sister relationship of *C. hispanica* and *C. abyssinica* has strong support, confirming previous hypotheses of the taxonomic proximity of these species (White, 1975) (Jonsell, 1982). See also (Inaba & Nishio, 2002)

(Prina, 2000) published a recent taxonomic revision of the genus *Crambe*, sect. *Leptocrambe*, Brassicaceae:

As part of a revision of the genus *Crambe* based on the morphological study of herbaria and cultivated material, the systematics of sect. *Leptocrambe* DC. is presented here. Section *Leptocrambe* is considered to comprise five species: *C. kilimandscharica* O. E. Schulz, *C. sinuatodentata* Hochst. ex Petri, *C. hispanica* L., *C. filiformis* Jacq. and *C. kralikii* Coss. *C. hispanica* includes three subspecies, subsp. *hispanica*, subsp. *glabrata* (DC.) Cout. and subsp. *abyssinica* (Hochst. ex R. E. Fr.) stat. nov. which includes var. *abyssinica* and var. *meyeri* (O. E. Schulz) comb. nov. *C. kralikii* includes two subspecies, subsp. *kralikii* and subsp. *garamas* (Maire) Podlech.

*Crambe* as a genus has been created by Linné *Species Plantarum* vol.2, 1753, the Type is not designated. (International Plant Names Index, 2004).

(Bond et al., 2005) used genetic analysis for the tracing of seed dispersal and population genetics: The main aims of this research were to investigate in *C. maritima* if population size influenced genetic diversity, if populations showed isolation by distance and if ocean currents could explain patterns of genetic differentiation. They have found no relationship between population size and genetic diversity and no relationship between geographic and genetic distance. However, patterns of genetic differentiation are related to ocean current in the region. In *C. maritima*, high levels of gene flow between

populations in the English Channel and North Sea appear to maintain genetic variation. In contrast, populations on the Biscay coast have less opportunity for exchanging genes. The authors expect the patterns of population differentiation revealed here will be reflected in other coastal species whose seeds tolerate immersion in salt water for long periods of time. By using a genetic approach they have shown that it may not be accurate to view gene flow between coastal populations as strictly linear, especially when the seeds of the species examined tolerate immersion in seawater. Seed viability experiments do not prove that long distance seed dispersal actually happens; only that it is possible. Investigating population structuring using genetic techniques verifies if gene flow is, or has been, occurring between isolated populations. They have shown that detailed information about seed dispersal can be obtained using simple universal markers. In species with seeds dispersed by the sea, populations that are geographically close may be isolated because of currents, whilst geographically distant populations may be linked by currents. In light of our findings, it is important that the conservation of coastal species takes into consideration the possibility that gene flow may not occur between geographically adjacent populations. Our findings imply that gene flow between populations on either side of the English Channel can occur through the exchange of salt-water tolerant seeds.

(Briard et al., 2002): Seakale is a Brassicaceae, native to the coastal sands of Northwestern Europe. To bring this species closer to commercialisation and thereby enhance the diversification of vegetable crops, a breeding program was initiated in 1992. A systematic search for wild populations was undertaken in France, from Quiberon (south Brittany) to Dunkerque (north France near Belgium) to enlarge its genetic basis. Many sites previously described in the literature have disappeared, while five large sites, not previously described, were found. Morphological descriptors and molecular markers (RAPD) were used to study the phenotypic and phenetic variability of the collected plants. A great variability for leaf and leaf-stalk colour, limb, flowers and siliques sizes, was observed. Among the wild collected plants, molecular similarity varied from 25 to 85%. The mean distance from all the wild genotypes to the breeding material already in collection was large (50%). Even if no clear correlation was found between morphological assessment and molecular data except for the leaf-stalk descriptor, the collecting trip was a success. A real enlargement of the variability was obtained.

(Fontana et al., 1998): The use of oil with a high level of erucic acid in some industrial sectors appears to offer excellent prospects from a technological and environmental point of view. This study was carried out to determine the potential of *Crambe abyssinica* for producing high erucic acid oil seeds in the Po Valley environment (North Italy). The productive yield of 3-year trials using six different genotypes was generally satisfactory, even though significant differences were found between the years. The different weather patterns recorded during the trials showed how emergence, flowering and seed-filling stages are particularly important phases for obtaining good yields. The grain production in these years ranged between 2.3 and 3.2 t ha<sup>-1</sup> and were similar to that of other spring oilseed crops in our environment. The seed had an oil concentration of between 320 and 370 g kg<sup>-1</sup>, with a fatty acid composition regularly characterized by a level of erucic acid higher than 53%. This paper also reports and discusses some other seed

characteristics (protein content, weight per hectolitre, thousand kernel weight, hull/seed ratio and number of seed per plant). Amongst the genotypes tested, Mario gave the highest seed and oil yields followed by Belann and C-29, whereas Mejer and Belenzian gave lower yields, especially as a result of an insufficient emergence.

(Wang et al., 2000): *Crambe abyssinica* is an annual herb. After introduction and cultivation in China, the oil content of its whole seed reaches 34.48%, of which 62.50% is erucic acid. Some available characters, such as short growing season, potentiality of high yield and good resistance to diseases were found. In the Chengdu area, the experiment of sowing date and density indicated that the sowing date of *C. abyssinica* could be preliminarily determined: from the beginning of October to mid-October and 150 000 plants:ha was more suitable. After single-plant selection, two new strains named N01 and B07, respectively, were obtained. Multi-locational evaluation showed that N01 and B07 were superior to the original both in yield and stress resistance. Therefore, *C. abyssinica* shows itself a promising oil crop, which can be used in industry in China but further studies are needed.

## Biosafety considerations

There are only a few publications available on gene flow:

(Beck et al., 1975): A dominant marker gene controlling leaf pubescence has been used to measure outcrossing in crambe (*Crambe abyssinica* Hochst. Ex. R. E. Fries), a cruciferous crop species producing a seed oil with high content of erucic acid. In experimental plots, outcrossing rates within the range of 4.8 to 10.7 per cent have been determined in different types of plots between dominant and recessive genotypes. Overall outcrossing rates of 9 to 14.3 per cent considering both inter and intragenotypic cross-pollination, have been calculated.

(Beck et al., 1975) studied inheritance of pubescence and its use in outcrossing measurements between a *Crambe-hispanica* type and *Crambe-abyssinica* Hochst Ex Fries.

There are no experiments published with the aim of determining gene flow for biosafety assessments. In this situation it will be advisable to rely on the vast experience with other Brassicaceae.

## Some remarks about production, related to biodiversity

(Wang et al., 2000): Increasing the yield of Crambe could be carried out by two ways, i.e. raising the planting density or the effective branch number. If the planting density was too high, the plant couldn't grow well and the primary branch number and the seed number per plant would decrease. Table 6 shows that the yield of 150 000 plants:ha was the highest, yield of 180 000 plants: ha was the second, and yield of 120 000 plants:ha was the lowest. Through LSR-method analysis the authors found that the difference between the yield of 150 000 plants:ha and that of 120 000 and 180 000 plants:ha was

significant at 5% level, however, the difference between 120 000 and 180 000 plants:ha was not significant. Therefore, we suggested that the density of 150 000 plants:ha in the Chengdu area was more suitable.

(Wang et al., 2000):

Crambe oil is a good source of long-chain fatty acids and contains 10–15% more erucic acid than industrial rapeseed oil. Moreover, Crambe is a main self-pollination plant and it traverses cross pollination occurring between industrial rapeseed and canola or edible oil rapeseed in adjacent fields, which the result is an oil with intermediate erucic acid content of low value for industrial purposes and unfit for human consumption. Crambe introduced in China is an annual herb with higher seed yield and erucic acid content, and lower glucosinolate content. Therefore, Crambe shows promise of becoming a new oil crop with a high erucic acid content, which can be used in industry and an additional protein source as well in China. But problems with Crambe are weaker resistance to diseases and lack of required genetic diversity for improving it through breeding. More recently, by the treatment of seed with different concentration and dose of EMS and <sup>60</sup>Co, the single-plant selection of M<sub>1</sub> generation was carried out.

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