Phenological coding of *Crambe abyssinica* Hochst based on the BBCH system

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Abstract

Crambe (*Crambe abyssinica* Hochst) is an oilseed crop domesticated in the Mediterranean region and has become increasingly important worldwide. Crambe is now considered an alternative to bioenergy crops and oleochemicals, because of its acclimatisation capacity under inhospitable conditions. Despite the interest in the agronomical characteristics and applications of this crop, investigations on crambe are still scarce and have only focused on production, without taking into account the phenological stages of this species. Thus, a single criterion to quantify the species' phenology can be a useful tool for both researchers and growers. In the present study, a proposed scale of the phenological growth stages of crambe under the BBCH (*Biologische Bundesanstalt Bundessortenamt und Chemische Industrie*) coding system was applied. Growth stages were described using the one- and two-digit decimal coding of the BBCH system and corresponded to, Stage 0: germination; Stage 1: leaf development; Stage 2: lateral sprout formation; Stage 5: inflorescence emergence; Stage 6: flowering; Stage 7: fruit formation; Stage 8: fruit maturation; Stage 9: senescence. Growth stages 3 and 4 (main stem elongation and development of collectable vegetative parts, respectively) were omitted. Figures were included to illustrate the most pertinent stages, and vegetal growth was represented by means of a technical botanical illustration. The BBCH system was efficacious in providing crambe Phenological data, allowing for better growing management practices amid varying climatic conditions, as well as contributing to the standardisation of research methods with this species.

Introduction

Crambe (*Crambe abyssinica* Hochst), also known as Abyssinian kale, belongs to the family *Brassicaceae*. This annual herbaceous plant originates in Ethiopia (Eastern Africa) and has been domesticated in Mediterranean and Turkish-Iranian regions (Leppik and White 1975; Desai 2004). The cultivation of this species has expanded from the Northern Hemisphere (North America and Europe) to the Southern Hemisphere (South America and Asia), generating phenotypic development due to plasticity among genotypes (Falasca et al. 2010).

Global interest in crambe has grown extensively thanks to biomass production and the physical-chemical and technical-functional quality of its oil, considering its potential for use in animal feed, bioenergy production, and, more recently, its value in the oleochemical industry (Oplinger et al. 1991; Ecocrop 2007; Falasca et al. 2010; Guan et al. 2015). Crambe seeds have a spherical shape that is covered by a tegumentary structure called the pericarp. The oil content of crambe seeds is between 26% and 38%, containing approximately 60% erucic acid, which is a monounsaturated omega-9 fatty acid that can be used similar to mineral oils, but with the added benefit of being biodegradable (Wang et al. 2000; Desai 2004; Jasper et al. 2010; Pitol et al. 2010; Ruas et al. 2010; Zulfiqar et al. 2011; Lalas et al. 2012; Qi et al. 2016).

Crambe seeds, pie, and bran can be used as protein supplements for ruminants because they contain between 25% and 35% protein when siliques are attached to the fruits, and between 46% and 58% when

they are removed (Oplinger et al. 1991). On the other hand, crambe bran is not recommended for swine and poultry (Ecocrop 2007). Oil extracted from crambe seeds can be employed as a corrosion inhibitor, industrial lubricant, or as an ingredient for the manufacture of synthetic bladders. In addition, crambe seed oil can be used in the production of adhesives, plastic films, electrical insulators, nylon, and plasticisers. It is from this oil that “erucamide” is obtained, a substance used for making cosmetics, among other industrial uses (Falasca et al. 2010; Uyaroğlu et al. 2018).

Crambe grows at altitudes of up to 2500 m, being cultivated as a spring crop in the European continent and as a winter crop in Mediterranean climates (Falasca et al. 2010). Crambe adapts to environments with 350 to 1200 mm annual rainfall, but the optimal precipitation range in order to reach production aimed at the extraction of its oil is 800 to 1500 mm per year. Because of its efficient radicular system, this species is resistant to and can survive periods of drought, but yield and oil content can be seriously impeded by water scarcity if the drought occurs in the first stages of maturity or during flowering (Falasca et al. 2010; Castleman et al. 1999).

Crambe grows where annual mean temperatures are between 5.7°C and 16.2°C. The seedlings are relatively tolerant to frosts and can survive between − 4°C and − 6°C. In the stages subsequent to growth, tolerance to frost is reduced and temperatures around − 1°C can lead to the death of the plant (Ecocrop 2007). This species is also known to be sensitive to hail, but can recover (Duke 1983). Moreover, this plant has the capacity to grow in marginalised soils with high salinity, environments with low hydric availability, and environments propitious to thermic variations. Crambe grows in substrates in the pH 5.0 to 7.8 range. However, it is not well adapted to shallow, rocky, or flooded soils (Duke 1983; Ionov et al. 2013; de Vasconcelos et al. 2015). Crambe seeds are moderately tolerant to saline soils during germination, when the soil temperature is moderate (10–30°C), but when the temperature remains around 10°C in saline soils, germination is delayed (Falasca et al. 2010).

In the last two decades, several studies have been carried out with crambe, mainly genetic engineering experiments aiming to manipulate the composition of its oil by reducing the linoleic and linolenic acid contents and, simultaneously, increasing the erucic acid content. However, the efficacy of such programmes has been inhibited by the low availability of germplasm banks (Tallent 1972; Leppik and White 1975; Vollmann and Ruckenbauer 1993; Mietkiewska et al. 2008; Li et al. 2010; Li et al. 2012; Lara-Fioreze et al. 2013; Li et al. 2013; Cheng et al. 2015; Guan et al. 2015; Qi et al. 2015; Miklaszewska et al. 2017; Qi et al. 2018).

Despite the agronomical potential of crambe, research focused on the culture characteristics of this crop is still lacking (Leão-Araújo et al. 2017). In particular, knowledge of the phenological cycle of this species would help inform cultivation management and allow agriculturalists to accurately implement requisite management practices, such as those relating to plague, pathogen and weed plant control, as well as fertilisation. Moreover, understanding the phenology of crambe would assist in crop estimation and improvement programmes, and would be indispensable for achieving high productivity (Valentini et al. 2001; Morais et al. 2008).

Although defining phenology is important for developing consistent information, there is still no international consensus on how to quantify it in crambe. Attempts to cultivate this crop have been made during different growing seasons. In this sense, the BBCH (*Biologische Bundesanstalt Bundessortenamt und Chemische Industrie*) scale system can assist in the establishment of a standard scale to quantify plant pheenology, serving as a tool for both researchers and growers (Sosa-Zuniga et al. 2017). The BBCH scale was proposed by Zadoks et al. (1974) and is based on decimal coding, which divides plant growth into principal (0–9) and secondary (0–9) stages. Phenological scales using this widespread methodology have been described for plant species such as natives, cereals, ornamentals, vegetables, fruit trees, and medicinals (Bleiholder et al. 1989; Hack et al. 1992; Meier 2009).

The categories were based on morphological characteristics that were easily identifiable and allowed for descriptions of phenological cycles according to the development of the species or variant. Thus, the present study aimed to apply the BBCH phenological scale standardisation system to a culture of crambe (*C. abyssinica* Hochst).

Materials And Methods

Crambe (*C. abyssinica* Hoechst) seeds of the FMS Brilhante variety, which is the first and only cultivar developed in Brazil, were provided by the Fundação MS Investigations Centre. Plant material remained under controlled conditions in an incubator greenhouse (BOD) until sowing. The experiment was installed at the Farm School of the Dom Bosco Catholic University, Brazil (20° 26' ‘S, 54° 38’ 'W; 532 m).

Sowing was carried out on May 8, 2018, under field conditions, which corresponds to the best period for cultivation in the region (Pitol et al. 2010). The installation consisted of a cultivation area of 16 m^2, with seeds spaced 0.45 x 0.10 m apart, according to the cultivation procedure of Pitol et al. (2010). Eutrophic Red Latosol had a pH of 6.26 and a base saturation of 74.62% (V%). Neither soil fertility correction nor application of nutrients during cultivation was carried out. For invasive plant control, mechanical weeding was implemented 30 days after sowing. The thematic climatic data for this area were obtained with an automatic surface meteorological station (Hanna® HL 141), and showed a mean temperature of 22.7°C, with a maximum of 32°C and a minimum of 4°C.

Assessments and descriptions of the results were based on the BBCH extended scale system (Meier et al. 2009). Phenological events were associated with the main stem and lateral branches of the plants, and were attributed to a decimal scale of 01 and 02 digits. The growth stages of the were as follows: Stage 0: germination; stage 1: leaf development; stage 2: lateral sprout formation; stage 3: elongation; stage 5: inflorescence emergence; stage 6: flowering; stage 7: fruit formation; stage 8: fruit maturation; stage 9: senescence. Growth stage 4 (development of collectable vegetative parts) was omitted because it is not applicable to this species. BBCH stages 5, 6, 7, and 8 were evaluated at the plant level, because inflorescences in the plant are not synchronous.

The plants were observed daily (at 10:00 h) and the date on which each development stage occurred was recorded, defined as when at least 50% of the plants presented observable changes relating to the phenological stage. For each sampling date and during the main Phenological stages, photographs of the plants were registered, and the most representative photo for each phenological stage was selected from the image bank to describe the Phenological events.

Because of the lack of pertinent literature, the present paper describes, in addition to growth stages, the phenotypic characterisation; for example, the colour changes of the structures.

Results And Discussion

A description of the principal growth stages for crambe based on the two-digit BBCH system is displayed in Table 1. Stalk elongation (stage 3) occurred simultaneous to leaf development (stage 1), lateral sprout formation (stage 2), inflorescence emergence (stage 5), and flowering (stage 6), and for that reason, was omitted from the study. In addition, the proposal has already been carried out for other oleaginous species, such as *Gossypium hirsutum* and *Camelina sativa* (Munger et al. 1998; Martinelli and Galasso 2011). The Phenological events were coded and documented using photographs and technical drawings (Figs. 1 and 2).

Crambe plants can be up to 2.0 m high and their stem may or may not branch near the soil surface, often developing thirty or more lateral branches, which themselves compose tertiary branches. Their leaves are oval and asymmetric, approximately 10 cm long and 8 cm wide, and the pubescent petiole is approximately 20 cm long (Oplinger et al. 1991; Desai et al. 2004; Falasca et al. 2010).

Table 1
Description of the phenological growth stages of *Crambe abyssinica* (Hochst), based on the extended BBCH scale.

BBCH coding – field experiment installation		**Figure**
2 digits	**Description**	
Principal growth stage 0: Germination		
00	Dry seed	1B
01	Start of imbibition	–
03	Completion of imbibition	–
05	Rootlet emergence	–
07	Epicotyl emergence	1B
08	Epicotyl with cotyledons growing toward the soil surface	–
09	Cotyledons emergence above the soil	1B ; 2
Principal growth stage 1: Leaf development		
10	Fully unfolded cotyledons	–
11	First pair of visible leaves	1C ; 2
12	Second pair of visible leaves	1C
1.	Coding continues with the same scheme until stage 19	1C
19	Nine or more expanded leaves	1A and 1C ; 2
Principal growth stage 2: Lateral sprout formation		
20	Visible lateral sprouts or expanded leaves without lateral stems	1A and 1D
21	One visible lateral sprout	–
22	Two visible lateral sprouts	–
2.	Coding continues with the same scheme until stage 29	–
29	Nine or more visible lateral sprouts	2
Principal growth stage 3: Elongation		
30	Stem elongation	2
33	30% stem elongation	1A; 2
35	50% stem elongation	1D; 2

BBCH coding – field experiment installation		Figure
39	End of stem elongation	1J; 2
Principal growth stage 4: Development of collectable vegetative parts (omitted)		
Principal growth stage 5: Inflorescence emergence		
50	Inflorescence present but still enclosed by leaves	–
51	Leaves around the separate inflorescence; inflorescence is visible from above	1E
59	Visible inflorescence, but all flowers are still closed	–
Principal growth stage 6: Flowering		
60	First open flowers: visible anthers	–
63	Complete flowering: 30% flowering with visible anthers (anthesis)	1F ; 2
65	Complete flowering: 50% flowering with visible anthers (anthesis)	1D ; 2
67	Floral finish: 70% senescence of anthers	–
69	End of flowering: set of visible fruits	–
Principal growth stage 7: Fruit development		
70	Set of fruits: thickening of ovaries and first visible siliques on the main stem	–
Principal growth stage 8: Ripening		
81	Sensitive silique, liquid content, easily crushed with nails, green pericarp	1H e 1L
82	Sensitive silique, pasty content, easily crushed with nails, green pericarp	1I e 1L
85	Thick silique, pasty content, easily crushed with nails, beige pericarp	1G, 1J e 1L; 2
87	Mature silique, dry content, beige coloration outside	1L
89	Mature silique, dry content, brown coloration, ready for harvest.	1L ; 2
Principal growth stage 9: Senescence		
91	Only basal leaves are dry	–
93	Leaves of the first half of the plant, from the base upwards, are dead	1I
95	All leaves are dead; the colour of the stalk changes from yellow to brown	1J e 1K ; 2
97	Dead and dry plant	–
99	Product ready for harvest	2

Principal growth stage 0: Germination

The principal growth stage BBCH 0 describes seed germination (Table 1; Fig. 1B). Subsequent secondary stages correspond to the start of seed imbibition (BBCH stage 01). Water absorption by seeds is concluded at BBCH stage 03 and rootlet emergence at BBCH stage 05. These initial stages are not directly observable, as they occur below the soil surface. Crambe germination is epigaeal, and epicotyl and cotyledons appear toward the soil surface (BBCH stage 08). The germination stage ends with the emergence of cotyledons above the soil surface (BBCH stage 09; Fig. 2).

Principal growth stage 1: Leaf development

The principal growth stage BBCH 1 describes the emergence of active photosynthetic leaves when they are completely open (Table 1). The stage starts when cotyledons are fully allocated to the soil surface (BBCH stage 10). Photosynthetic leaves then appear on the main aerial part, occurring in pairs (Fig. 2) and determining the secondary growth stage. The formation of the first pair of leaves was codified as BBCH stage 11 (Fig. 2), followed by stage 12, and successively until BBCH 19 (Figs. 1A and 2). Figure 2 displays the sequence of leaves emitted for the species, in which a morphological difference in relation to vegetative growth was observed. Emission of leaves with winged petioles occurred from BBCH stage 13.

Principal growth stage 2: Lateral sprout formation

The principal growth stage BBCH 2 describes lateral sprout formation (Table 1). The formation of the lateral part can be included together with the subsequent principal growth stages using a diagonal trace. There have been no reports on the appearance of lateral sprouts in crambe before or after Anthesis; however, in the present study, we observed that there was continuity in their post-flowering emergence. A lateral formation is considered visible when its length is greater than 1 cm. The first lateral formation is codified as BBCH stage 21, followed by the second lateral formation (BBCH stage 22), and so on, until finalisation at BBCH 29.

Principal growth stage 5: Inflorescence emergence

The principal growth stage BBCH 5 qualitatively describes the inflorescence development (Table 1). Inflorescence emerges at the end of the development stage BBCH 1, with the inflorescence shoot forming initially while still covered by young leaves (BBCH stage 50). Subsequently, the leaves separate and the inflorescence becomes visible from above, corresponding to the BBCH stage 51 (Fig. 1E). After this, the stage at which individual flower buds are visible but still closed occurs (BBCH stage 55). The principal growth stage 5 ends when the inflorescence is visible, but all flowers are still closed (BBCH 59).

Flowers on racemes possess a beige or white colour, with the flowering period varying from 12 to 15 days (Falasca et al. 2010). After fertilisation, the flowers produce encapsulated small fruits, i.e., siliquae, which are round and indehiscent, of a green or greenish-brown colour, and contain a single spherical seed. This seed is orthodox, albuminous, and has a curved embryonic axis, which presents the plumulae enclosed by a mucilaginous thin covering. The seed diameter varies between 0.8 and 2.6 mm, being directly

influenced by the number of seeds per plant, precipitation, and soil fertility (Ellis et al. 1985; Desai et al. 2004).

Principal growth stage 6: Flowering

The principal growth stage BBCH 6 describes the development of flowers within the inflorescence (Table 1). The flowering stage begins when the first flowers are open and the anthers are visible (BBCH 60). When 30% of the floral stand presents exposed anthers, it is classified as BBCH stage 63 (Fig. 1F). Anthesis is defined as when 50% of the stand of flowers presents exposed anthers, which corresponds to BBCH stage 65 (Fig. 1D). The end of flowering starts with the senescence of the anthers (BBCH stage 67) and ends with a set of visible fruits (BBCH stage 69).

Principal growth stage 7: Fruit development

The principal growth stage 7 describes fruit development on the main stem (Table 1). Fruit development starts with fructification (BBCH stage 70), which is defined as the thickening of the ovary and the presentation of the first visible grains (BBCH 79).

Principal growth stage 8: Ripening

The principal growth stage BBCH 8 describes the maturation of siliques (Table 1). During the ripening process, the water content varies, modifying the texture and colour of the silique, which goes from green to beige, and at the end of the cycle, to brown (Fig. 1L). To quantify maturation, we used a combination of methods, employing those adopted by Sosa-Zuniga et al. (2017) and Martinelli and Galasso (2011) for *Chenopodium quinoa* and *Camelina sativa*. Thus, BBCH stage 81 was considered to be sensitive to the siliquae, liquid content, and green pericarp (Figs. 1H and L). BBCH 82, in turn, presented sensitive siliquae, pasty content, and a green pericarp (Figs. 1I and L). At BBCH 85, the siliques were already thick and presented pasty content and a beige pericarp (Figs. 1G, J, and L). The end of the process was established and characterised by a mature silique, dry content, brown colour, and as being ready for harvest (BBCH 89; Figs. 1L and 2).

Principal growth stage 9: Senescence

The principal growth stage BBCH 9 describes plant senescence and occurs during fruit ripening (Table 1). Senescence starts in the basal leaves (BBCH stage 91), continuing upward, but the stem remains green, as per Fig. 1L (BBCH stage 93). Subsequently, the remaining leaves dry and die, and the stem becomes yellow to brown in colour, as shown in Figs. 1J and K (BBCH stage 95). Finally, the whole plant is dead and dry (BBCH stage 97) and the product is harvested, as can be seen in Fig. 2 (BBCH stage 99; 103). Crambe seed yields vary widely between 1.0 and 5.0 tons of seeds per hectare, depending on the environmental conditions and geographical location (Jasper et al. 2010; Falasca et al. 2010).

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Declarations

This manuscript has not been published and is not under consideration for publication in another journal. We also have no conflicts of interest.

Figures

Figure 1

Morphological characteristics of crambe (*Crambe abyssinica* Hochst) Phenological growth, based on the BBCH scale.

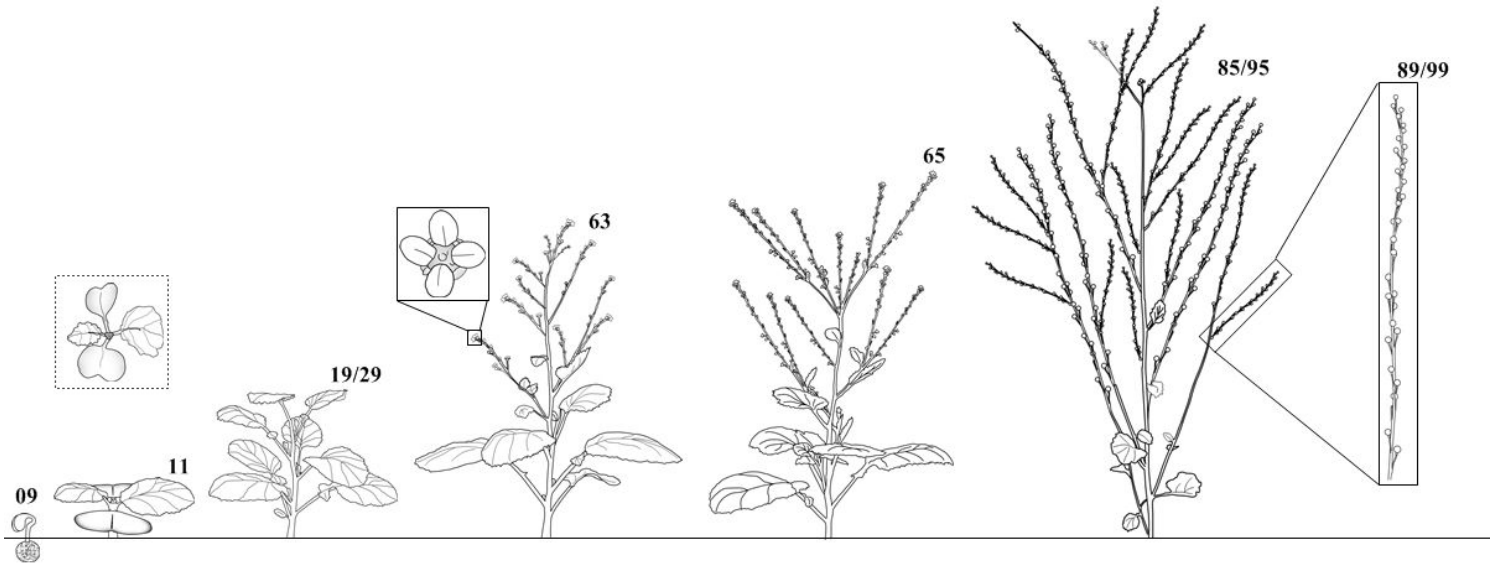

Figure 2

Sequence of crambe (*Crambe abyssinica* Hochst) Phenological growth stages, based on the BBCH Scale.