Phenological growth stages of pepino (*Solanum muricatum*) according to the BBCH scale

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ABSTRACT

The pepino (*Solanum muricatum*) is a solanaceous vegetatively propagated fruit crop of Andean origin. We provide a detailed description of phenological stages because it is of interest for pepino crop management and research. Given the increasing prominence of this crop, and the fact that it morphologically and developmentally variable, and different from other major solanaceous crops, we have developed a pepino specific BBCH (Biologische Bundesanstalt, Bundessortenamt, CHemische Industrie) numerical scale. Nine principal stages are described for germination/rooting, leaf development, formation of side shoots, main shoot elongation, inflorescence emergence, flowering, development of fruit, ripening of fruit and seed, and senescence. Secondary stages (two-digit scale) have been identified for all principal stages. Complementary descriptions using mesostages (three-digit scale) have been developed for leaf development, formation of side shoots, inflorescence emergence, and flowering phenological stages. A description of all phenological stages combined with illustrations is provided. The utility of the BBCH scale has been validated by comparing several traits of agronomic interest at specific developmental stages in a collection of pepino local varieties, modern cultivars and wild relatives. The BBCH scale developed provides uniform criteria for the description, identification and selection of phenological stages of the pepino and will facilitate the management, breeding and conservation of genetic resources of this crop.

Keywords: characterization, development stages, phenological scale, Solanaceae, varietal differences

1. Introduction

The pepino (*Solanum muricatum* Aiton) is an herbaceous crop domesticated in the northern Andes, where its closest wild relatives, from *Solanum* section *Basarthrum* also thrive (Anderson et al., 1996; Blanca et al., 2007). The pepino can be very variable in shape and colour, and is mostly consumed when fully ripe as a fresh fruit. At maturity, it has a characteristic mild sweet flavour and intense fruity aroma, which has some resemblance to that of melon (Prohens et al., 2005). In the last few decades demand for pepinos in commercial exotic fruit markets has grown, which has increased the interest and production of this crop not only in its region of origin but also in other temperate regions of the world (Rodríguez-Burruezo et al., 2011). As for other emerging crops, there is little information on production statistics, but the production in Ecuador is estimated at around 400 ha (Hidalgo, 2006).

The pepino has a number of specific features that distinguish it from major solanaceaous fruit crops such as tomato (*S. lycopersicum* L.), pepper (*Capsicum annuum* L.) or eggplant (*S. melongena* L.) (Rodríguez-Burruezo et al., 2011). These include vegetative propagation; in agricultural practice, the pepino is usually propagated by cuttings which root easily when placed in a wet substrate. An alternative way of clonal propagation is the use of *in vitro* micropropagation, which allows the production of disease free plants (Cavusoglu and Sulusoglu, 2013). Also, the pepino grows luxuriantly, and such vegetative growth may compete with fruit set, so the highest yields are obtained when the lateral side-shoots are removed, nitrogen fertilization is controlled to avoid excessive vegetative growth, and the plants are trained with vertical strings using a one or two main shoot system (Kowalczyk and Kobryn, 2003). Another difference with major solanaceous fruit crops is that many pepino cultivars display a strong tendency to parthenocarpy, with some cultivars obligately parthenocarpic (Prohens et al., 2005). In addition, the pepino fruit needs a long time (up to 70 days) to fully ripen since. Finally, fruit quality, especially sugar concentration, may be influenced by temperature during ripening; high temperatures result in a lower sugar content and in the development of an off-flavour (Rodriguez-Burruezo et al*.*, 2011).

The development of characterization tools for the precise and standardized description of the pepino plants and fruits is essential for an increased efficiency and effectiveness of research experiments, breeding programmes, conservation of germplasm and for the comparison of experimental data (Gotor et al., 2008; Meier et al., 2009). As a result, we produced a list of standarized descriptors (IPGRI and COMAV, 2004). Although this list is useful for the description of characteristics of pepino varieties and wild relatives and for the study of the morphological variation in collections and segregating generations, no standardized scales to precisely describe the phenological stage of pepino plants, which would be of great utility for agronomic and botanical research (Meier, 2001), are available. The BBCH (Biologische Bundesantalt, Bundessortenamt und Chemische Industrie) scale for the phenological identification of the growth stages of all species of mono- and dicotyledonous plants (Lancashire et al., 1991). This scale uses 10 principal stages (0-9), each of which is further divided into 10 secondary (0-9) growth stages. An extended BBCH-scale, using mesostages (0-9), was proposed for some crops using a third-digit scale (Meier, 2001). Both scales (simple and extended) have been developed and are widely accepted for many crops (Meier et al., 2009). The development stages of pepino have not yet been defined and described. Given the increasing interest in pepino cultivation and breeding (Rodríguez-Burruezo et al., 2011), we suggest that the development and validation of a phenological BBCH scale for pepino might be of interest for the efficient development of this emerging crop.

2. Material and methods

2.1. Plant material

Phenological observations were made by the authors through a period of more than half a century (initiated C.B. Heiser in the 1960s (Heiser, 1964) and followed up by G.J. Anderson) of pepino research, cultivation, evaluation and breeding of pepinos. These proposals are based on research that has included examination of pepino plants growing in different environments and cultivation conditions in its native home in the Andean region, as well under cultivation outside and in glasshouses in the USA, Spain, and a number of other countries. In addition, the phenological cycle of pepino was specifically studied for the development of the BBCH scale in a characterization trial performed from February to July 2014 in an experimental greenhouse on the campus of the

Universitat Politècnica de València (Valencia, Spain). This latter area has a typical Mediterranean climate, with mild winters and long warm and dry summers. Materials used in this trial included 14 clonal pepino varieties consisting of six local varieties from the Andean region and eight commercial cultivars. In addition eight accessions of the wild species most closely related to the domesticate pepino were studied, including: *S. caripense* Humb. and Bonpl. ex Dun. (four accessions), *S. catilliflorum* G.J. Anderson, Martine, Prohens and Nuez (one accession), *S. perlongistylum* G.J. Anderson, Martine, Prohens and Nuez (one accession), *S. tabanoense* Correll (one accession) and *S. trachycarpum* Bitter and Sodiro (one accession) (Anderson, 1979; Anderson et al., 2006). Pepino materials were vegetatively propagated *in vitro* and after acclimatization were transplanted in the greenhouse in 1 m deep benches filled with silica sand as substrate. Wild relatives were germinated from seed and one individual was clonally propagated *in vitro* for the trial. For each of the pepino varieties and wild relatives, five plants were cultivated and arranged in a completely randomized design. Watering and fertilizers were applied with the drip irrigation system. Plants were cultivated in the winter-spring cycle and trained using vertical strings. For self-incompatible wild relatives, manual pollinations with compatible pollen were carried out to ensure fruit set. In order to validate the BBCH scale for comparison of varieties, several traits of agronomic importance (stem length, fruit length, fruit width, fruit length/width ratio, time from transplant to beginning of ripening, and soluble solids content) were taken at specific BBCH stages. Univariate analyses of variance (ANOVA) were performed for the traits considered. Significance of differences among clones was studied using the Student-Newman-Keuls multiple range test at a significance level of P=0.05.

2.2. Pepino BBCH scale characteristics

Based on the existing extended BBCH-scale (Meier, 2001), the completed growth cycle of pepino was divided into nine principal growth states, including germination (for seed propagation) / rooting (for vegetative propagation) (stage 0), leaf development (stage 1), formation of side shoots (stage 2), main shoot elongation (stage 3), inflorescence emergence (stage 5), flowering (stage 6), development of fruit (stage 7), ripening of fruit and seed (stage 8), and senescence (stage 9). BBCH-scale stage 4 (development of harvestable vegetative plant part or vegetatively propagated organs / booting) is not applicable to pepino. Each principal growth stage was classified into secondary stages, ordered from 0 to 9, which can represent an ordinal number or a percentage (1=10%, 2=20%, etc.) that are used to describe precise time points or shorts intervals of development within each principal stage. The combination of the principal stage number with the secondary stage number results in a two-digit code. For situations in which the growth stages are not defined with sufficient precision with the two-digit code, the inclusion of a mesostage (with a 0 to 9 code) between the principal and secondary stages provides a further subdivision and results in a three-digit scale (Meier, 2001). This results in a three-digit scale, that can be used as an alternative to the regular two-digit scale. For main stages where the mesostage is not applicable, then a 0 is used for the mesostage when the three-digit scale is used. The principal growth stages do not need to proceed in the strict sequence defined, but may occasionally proceed in parallel (Meier, 2001). In this case, if two or more principal stages proceed in parallel, they can be indicated using a diagonal stroke (e.g., 33/61 or 303/601).

3. Results and discussion

Unlike major solanaceous fruit crops, like tomato, pepper, or eggplant, that are propagated by seeds, the pepino is mostly propagated vegetatively in the agricultural practice (Prohens et al., 2005; Cavusoglu and Sulusoglu, 2013). Although potato (*Solanum tuberosum* L.) is also vegetatively propagated, the fact that potato is cultivated for its tubers and pepino for its fruits results in many differences in the phenology of both crops. There are also important morphological and developmental differences in pepino vs. other major solanaceous crops (Prohens et al., 1998; IPGRI and COMAV, 2004; Prohens et al., 2005; Rodríguez-Burruezo et al., 2011); these differences strongly argue for the development of a BBCH scale specifically for the pepino.

The two-digit scale provides a precise definition of most of the phenological growth stages in most crops (Meier, 2001). As a result of our observations we have developed a two-digit BBCH scale for pepino. However, for stages 1 (leaf development), 2 (formation of side shoots), 5 (main shoot elongation), and 6 (flowering) we consider that mesostages appropriate for more precise description in certain circumstances, and therefore, we have also developed the three-digit scale. In Solanaceous crops for which the BBCH scale is available, the use of mesostages is common in particular for stages involving the development of vegetative aerial parts, and flowering, and fruiting (Hack et al., 1993, Feller et al., 1995; Ramírez et al., 2013). Below, we provide a description of the phenological cycle stages for pepino based on our studies. The pepino BBCH phenological stages scale provides a complement to the descriptors for pepino (IPGRI and COMAV, 2004), that we developed for describing the morphological variation of the crop. Furthermore, we have validated the utility of the BBCH scale for the comparison among pepino varieties and wild relatives of several agronomically relevant traits (Prohens et al., 2005; Rodríguez-Burruezo et al., 2011) measured at specific developmental stages.

3.1 Principal growth stage 0: germination / rooting

This stage describes the germination of seed when plants are produced from seed and the rooting of explants when plants are vegetatively propagated (Table 1). Seed propagation in pepino is used in breeding programmes (Rodríguez-Burruezo et al., 2011) and is also the natural reproductive system of pepino wild relatives (Anderson et al., 1979, 2006). Vegetative propagation, either using herbaceous cuttings for rooting in a substrate like peat, perlite, or vermiculite, or through *in vitro* micropropagation, is used in the commercial production of pepino (Cavusoglu and Sulusoglu, 2013). When propagated by seed, this stage begins with the dry seeds (stage 00 or 000), that after being sown in a substrate, in a Petri dish, or in an *in vitro* culture medium, typically take a few days to get fully imbibed (stage 03 or 003). In a period between three and 30 days, the radicle emerges from the seed (stage 05 or 005) and after three days to one week after this stage, the emergence of cotyledons takes place (stage 09 or 009). When vegetatively propagated, the stage begins with the cuttings or explants (stage 00 or 000). After being placed in a wet substrate or in *in vitro* growing medium there is a swelling of the explant part in contact with the substrate or medium (stage 00 or 001) and in a few days root protuberances are evident (stage 03 or 003). On occasions the cuttings already have hardy root protuberances, however we consider that the stage 03 or 003 is reached when these protuberances are swollen and in the process of breaking for developing actively growing adventitious roots (stage 05 or 005). The subsequent stage is when axillary buds begin breaking (stage 07 or 007), which is followed after two to six days by the buds showing green tips (stage 09 or 009).

3.2 Principal growth stage 1: Leaf development

The development of the young plant mostly involves the growth and appearance of new leaves. The number of leaves in the most developed (main) shoot determines the phenological stage code (Table 2). Pepino leaves are alternate and can be simple or pinnate, depending on the variety and on the stage of development of the plant and leaf (IPGRI and COMAV, 2004). For seed propagated plants, this main stage begins with the cotyledons being completely unfolded and, in the case of vegetatively propagated plants, with the dominant axillary bud leaf emerging (stage 10 or 100). The following stages continue with the unfolding of subsequent leaves in the main shoot so that when the first leaf is unfolded the plant is at stage 11 or 101 and end when at least the 9 (two-digit scale) or 19 leaves (three-digit scale) of the main shoot are unfolded (stages 19 and 119, respectively).

3.3. Principal growth stage 2: Formation of side shoots

Pepino plants form side shoots derived from axillary buds of the main shoot, or in the case of vegetatively propagated plants, from buds other than the dominant bud in the cutting or explant. This main stage begins with the first primary apical side shoot being visible (stage 21 or 201) and ends when at least nine or more apical side shoots are visible (stage 29 or 209) (Table 3). Depending on the training system of the plant, side shoots are left to grow (in the case of untrained plants or plants trained in a hedgerow system) or removed in the case of plants trained to one or two main shoots. The appearance and development of side shoots are stimulated by conditions that are favourable for vegetative growth (i.e., high humidity and high soil nitrogen). The production of many side shoots competes directly with fruit set and development of fruits. Because of this, the highest fruit production is obtained in pruned plants where the side shoots have been removed (Kowalczyk and Kobryn, 2003). If side shoots are removed, then this growth stage is not applicable to the pepino crop.

3.3. Principal growth stage 3: Main shoot elongation

The shoots of the pepino plant are indeterminate and the maximum length of the main shoot depends on the training system. The main shoot length may reach more than 200 cm when the plant is trained in greenhouse-cultivated plants. Reciprocally, the main shoots of un-trimmed and untrained plants are much shorter, due to competition (Prohens et al., 1996; Kowalczyk and Kobryn, 2003). The scale begins with the length of the main shoot up to 10 cm long (stage 31 or 301) and ends up when the elongation of the main shoot has ceased (stage 39 or 309) (Table 4). The time required for passing from one stage to the next depends on the cultivation techniques as well as on the environmental conditions. That is, the main shoot grows faster when plants are trained and pruned and it grows slower when plants are not trained nor pruned.

3.4. Principal growth stage 4: Development of harvestable vegetative plant parts or vegetatively propagated organs / booting (main shoot)

The pepino is almost always cultivated for its harvestable fruits, though rarely plants may be grown as an ornamental (Prohens et al., 1996). In consequence, this principal growth stage, which is included in the BBCH scale (Meier, 2001), is not applicable to the pepino.

3.5. Principal growth stage 5: Inflorescence emergence

The number of inflorescences in the most developed (main) shoot determines the phenological stage code (Table 5). And, given that the pepino has indeterminate growth, the number of inflorescences is a matter of time and age; i.e., inflorescences continue to be produced as long as the main shoot continues to grow. Typically, the first inflorescence is visible (stage 51 or 501) after 10-20 leaves (e.g., stage 19 in the two-digit scale, and between stages 110 and 119 in the three-digit scale) have been matured along the main shoot (this is usually when the main shoot has reached between 20 and 70 cm (e.g., between stages 31 and 34). Subsequently, new inflorescences appear each two to four nodes. Depending of the rate of growth, it may take 3 to 8 days to pass from one stage to the subsequent stage (e.g., from stage 51 or 501 to stage 52 or 502).

3.6. Principal growth stage 6: Flowering

The pepino inflorescence is an indeterminate pseudoterminal cymose raceme with one or two axis and 5 - 20 hermaphrodite flowers (Anderson, 1979), which open acropetaly, i.e., from the base towards the tip of an inflorescence. Pepino flowers are white, purple or white marked with purple and have inserted or slighty exserted stigma. This phenological stage is determined by the opening of the first (basal) flower of each of the inflorescence (Table 6). Opening of all flowers of the inflorescence usually takes 3 to 10 days, depending on growth conditions and number of flower buds in the inflorescence. The pepino is self-compatible and mostly autogamous (Mione and Anderson, 1992), although when pollinators are present, a frequent situation in open field cultivation or in greenhouses where bumblebees are used for stimulating pollination, a high degree of outcrossing may occur (Murray et al., 1992). When no pollination occurs, the flower may set parthenocarpic fruits (Prohens et al., 1998). In any case, infructescences usually include 1 to 3 mature fruits.

3.7. Principal growth stage 7: Development of fruit

The pepino fruit is a fleshy berry with two to three locules that follows a sigmoidal growth pattern (Schaffer et al., 1989). The fruit usually weighs between 100 and 400 g, the weight depending both on genetics (the cultivar) and the environment (growth conditions). The shape of the fruit, as well as the colour patterning, also depend on the cultivar (Rodríguez-Burruezo et al., 2011). The fruit usually takes between 30 to 50 days to grow to full size (Prohens and Nuez, 2001). At this time the fruit is physiologically unripe and has a green colour and may be harvested for use in salads in the same way as cucumbers (Prohens et al., 1996). Phenological stage 7 begins when the first fruit of the oldest (lowest) infructescence bears the first mature (in size and colour) fruit (Table 7). Given that it is quite unusual that more than six clusters bearing fruit appear on individual shoots, the scale for this phenological stage begins with the first fruit of the first cluster (stage 71 or 701) and ends with nine or more clusters in the main shoot having the first fruit having reached typical size and shape (stage 79 or 709).

3.8. Principal growth stage 8: Ripening of fruit and seed

The pepino fruit takes between 7 to 25 days after reaching full size until, until it is fully ripe (Prohens and Nuez, 2001). When fully ripe, the fruit has a pale green to golden yellow colour, which may be covered by purple stripes or not. The fruit normally is very aromatic and has a mild flavour, with sugar content ranging between 6% to 10%, and with a low acidity (Rodríguez-Burruezo et al., 2011). The fruit may be parthenocarpic or seeded, and in the latter case it may contain up to 200 small seeds (Anderson, 1979). Seeds are physiologically mature when the fruit evinces the typical fully ripe colour. Phenological stage 8 is determined by the percentage of fruits produced by the plant that have reached the typically fully ripe colour (Table 8). The scale begins with 10% of the fruits showing the typical fully ripe colour (stage 81 or 801) and ends with all fruits having the typical fully ripe colour (stage 89 or 809).

3.9. Principal growth stage 9: Senescence

Like tomato, pepper, eggplant and other solanaceous fruit crops, the plant of pepino is perennial, although it is usually grown as an annual (Prohens et al., 1996; Rodríguez-Burruezo et al., 2011). This is because after six to nine months of production, plants begin to develop symptoms of senescence, especially under intensive cultivation conditions, with oldest leaves getting yellowish and subsequently brownish. This situation may be aggravated when plants are affected by pests or diseases, problems that accelerate senescence. This phenological stage begins with the initiation of leaf yellowing (stage 91 or 901) and ends when all fruits have been harvested (stage 99 or 909) (Table 9).

3.10. Validation of the utility of the BBCH scale

Measurement at a specific developmental stage is of great relevance for comparison of different varieties in characterization and phenomics studies (Fiorani and Schurr, 2013). In our case, measuring traits of agronomic interest at specific BBCH developmental stages in a collection of cultivated pepino accessions and wild relatives has allowed a precise characterization that has resulted in the detection of significant ($P<0.05$) differences among accessions for all traits (Table 10). We have found that wild relatives have, with the exception of *S. trachycarpum* (E-34), a longer stem when the first inflorescence in the main shoot is visible (stage of 51/501). This is probably caused by the fact that selection during the domestication process of the pepino has favoured more compact plants that are better adapted to cultivated environments (Anderson et al., 1996; Prohens et al., 1996; Meyer and

Purugannan, 2013). Many differences have been found in fruit length and width, measured in the first fruit of the first cluster that reaches the typical form and colour (stage 71/701), in the materials evaluated (Table 10). This is in agreement with the high variation and heritability of this trait (Prohens et al., 2005). Also, as expected, cultivated materials have had a fruit size generally larger than those of wild relatives. There are, as well, many differences in fruit shape, measured as fruit length/width ratio at this same stage (71/701),. Differences in cultivated pepino have been much larger than in the wild relatives, which is something expected as artificial selection has yielded materials highly variable for fruit shape (Prohens et al., 1996; Rodríguez-Burruezo et al., 2011). For earliness, measured as time from transplant to beginning of fruit ripening (stage 81/801), few significant differences have been found, the only significant ones being between Col-1 and, Puzol, and E-7 accessions (earlier) and Sweet Long (later) (Table 10). Finally, for soluble solids content, an important trait for fruit quality (Rodríguez-Burruezo et al., 2011), many differences have been found in the materials measured at stage 81/801. Wild relatives have generally had significantly higher levels of soluble solids content than cultivated materials, with several accessions having contents above 10% (Table 10), confirming that wild relatives are sources of variation of interest for pepino quality breeding (Prohens et al., 2005). In pepino, as in other crops (like tomato) in which there is a sequential fruit set, (Aurand et al., 2012), differences may exist among fruit characteristics harvested at different developmental stages and therefore it is important to measure the traits at the same developmental stage in order to have comparable and relevant measures. In summary, the BBCH scale has proved as very useful to compare different pepino varieties at the same developmental stage, which results in information of relevance for horticulturists and breeders.

4. Conclusions

The specific BBCH scale developed for pepino, with its two-digit (simple) and three-digit (extended) versions, allows the precise identification of the phenological stages of this crop. We have shown that the measurement of traits of agronomic interest at specific BBCH developmental stages is important because it allows the proper comparison of varieties, given that there is no bias due to differences in developmental stages. The BBCH scale offers a standardized tool that will help pepino researchers, agronomists, breeders, and germplasm curators in an efficient management, breeding, and conservation of genetic resources of this emerging crop.

5. References

Aurand, R., Faurobert, M., Page, D., Maingonnat, J.F., Brunel, B., Causse, M., Bertin, N. 2012. Anatomical and biochemical trait network underlying genetic variations in tomato fruit texture. Euphytica 187, 99-116.

Anderson, G.J. 1979. Systematic and evolutionary consideration of *Solanum* section *Basarthrum*. In Hawkes, J.G., Lester, R.N., Skelding, A.D. (Eds.), The biology and taxonomy of the Solanaceae. Royal Botanic Gardens Kew and Linnean Society, London, UK, pp. 549-562.

Anderson, G.J., Jansen, R.K., Kim, Y. 1996. The origin and relationships of the "pepino", *Solanum muricatum* (Solanaceae). Economic Botany 50, 369-380.

Anderson, G.J., Martine, C.T., Prohens, J., Nuez, F. 2006. *Solanum perlongystilum* and *S. catilliflorum*, new endemic Peruvian species of *Solanum*, section *Basarthrum*, are close relatives of the domesticated pepino, *S. muricatum*). Novon 16, 161-167.

Blanca, J.M., Prohens, J., Anderson, G.J., Zuriaga, E., Cañizares, J., Nuez, F. 2007. AFLP and DNA sequence variation in an Andean domesticate, pepino (*Solanum muricatum*, Solanaceae): Implications for evolution and domestication. American Journal of Botany 94, 1219-1229.

Cavusoglu, A., Sulusoglu, M. 2013. In vitro propagation and acclimatization of pepino (*Solanum muricatum*). Journal of Food, Agriculture and Environment 11, 410-415.

Feller, C., Bleiholder, H., Buhr, L., Hack, H., Hess, M., Klose, R., Meier, U., Stauss, R., van den Boom, T., Weber, E. 1995. Phänologische Entwicklungsstadien von Gemüsepflanzen: II.

Fruchtgemüse und Hülsenfrüchte. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 47, 217-232.

Fiorani, F., Schurr, U. 2013. Future scenarios for plant phenotyping. Annual Review of Plant Biology 64, 267-291.

Hack, H., Gall, H., Klemke, T., Klose, R., Meier, U., Stauss, R., Witzenberger, A. 1993. Phänologische Entwicklungsstadien der Kartoffel (*Solanum tuberosum* L.). Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 45, 11-19.

Heiser, C.B. 1964. Origin and variability of the pepino (*Solanum muricatum*): A preliminary report. Baileya 12, 151-158.

Hidalgo, A. 2006. Proyecto de exportación de pepino dulce al mercado alemán. Universidad Tecnológica Equinoccial, Quito, Ecuador.

Gotor, E., Alercia, A., Rao, V.R., Watts, J., Caracciolo, F. 2008. The scientific information activity of Bioversity International: the descriptor lists. Genetic Resources and Crop Evolution 55, 757-772.

IPGRI, COMAV. 2004. Descriptors for pepino (*Solanum muricatum*). International Plant Genetic Resources Institute, Rome, Italy.

Kowalczyk, K., Kobryn, J. 2003. Effect of plant training method and hormone treatment of pepino (*Solanum muricatum* Ait.) on the fruit yield. Acta Horticulturae 614, 279-283.

Lancashire, P.D., Bleiholder, H., van der Boom, T., Langeluddeke, P., Stauss, R., Weber, E., and Witzenberger, A. 1991. A uniform decimal code for growth stages of crops and weeds. Annals of Applied Biology 119, 561-601.

Meier, U. 2001. Growth stages of mono-and dicotyledonous plants – BBCH monograph. Federal Biological Research Centre for Agriculture and Forestry. http://www.bba.de/veroeff/bbch/bbcheng.pdf.

Meier, U., Bleiholder, H., Buhr, L., Feller, C., Hack, H., Hess, M., Lancashire, P.d., Schnock, U., Stauss, R., van den Boom, T., Weber, E., Zwerger, P. 2009. The BBCH system to coding the phenological growth stages of plants – history and publications. Journal für Kulturpflanzen 61, 41-52.

Meyer, R.S., Purugannan, M.D. 2013. Evolution of crop species: Genetics of domestication and diversification. Nature Reviews Genetics 14, 840-852.

Mione, T., Anderson, G.J. 1992. Pollen-ovule ratios and breeding system evolution in *Solanum* section *Basarthrum* (Solanaceae). Americal Journal of Botany 79, 279-287.

Murray, B.G., Hammett, K.R.W., Grigg, F.D.W. 1992. Seed set and breeding system in the pepino *Solanum muricatum*, Ait., Solanaceae. Scientia Horticulturae 49, 83-92.

Prohens, J., Nuez, F. 2001. Improvement of mishqui (*Solanum muricatum*) earliness by selection and ethephon applications. Scientia Horticulturae 87:247-259.

Prohens, J., Rodríguez-Burruezo, A., Nuez, F. 2005. Utilization of genetic Resources for the introduction and adaptation of exotic vegetable crops: The case of pepino (*Solanum muricatum*). Euphytica 146, 133-142.

Prohens, J., Ruiz, J.J., Nuez, F. 1996. The pepino (*Solanum muricatum*, Solanaceae): A "new" crop with a history. Economic Botany 50, 355-368.

Prohens, J., Ruiz, J.J., Nuez, F. 1998. The inheritance of parthenocarpy and associated traits in pepino. Journal of the American Society of Horticultural Science 123, 376-380.

Ramírez, F., Fischer, G., Davenport, T.L., Pinzón, J.C.A., Ulrichs, C. 2013. Cape gooseberry (*Physalis peruviana* L.) phenology according to the BBCH phenological scale. Scientia Horticulturae 162, 39-42.

Rodríguez-Burruezo, A., Prohens, J., Fita, A.M. 2011. Breeding strategies for improving the performance and fruit quality of the pepino (*Solanum muricatum*): A model for the enhancement of underutilized exotic fruits. Food Research International 44, 1927-1935.

Schaffer, A.A., Rylski, I., Fogelman, M. 1989. Carbohydrate and sucrose metabolism in developing *Solanum muricatum* fruits. Phytochemistry 28, 737-739.

Table 1

Description of the phenological stages of pepino growth stage 0 (germination / rooting) using a 2-digit and a 3-digit scale BBCH scale.

2-digit code	3-digit code	Description	
		Germination (seed propagation)	Rooting (vegetative propagation)
00	000	Dry seeds	Cuttings
00	001	Beginning of seed imbibition	Swelling of the cutting/explant
03	003	Seed imbibition complete	Root protuberances evident
05	005	Radicle emerges from seed	Adventitious roots developing
07	007	Hypocotyl with cotyledons breaking through seed coat	Beginning of axillary bud breaking
09	009	Emergence: cotyledons break through soil surface	Axillary buds showing green tips

Table 2

Description of the phenological stages of pepino growth stage 1 (leaf development) using a 2-digit and a 3-digit scale BBCH scale.

2-digit code	3-digit code	Description
10	100	Cotyledons completely unfolded (seed propagation) or emerging leaf in dominant axillary bud having grown to 1 cm (vegetative propagation)
11	101	First true leaf on main shoot fully unfolded
12	102	2nd leaf on main shoot unfolded
13	103	3rd leaf on main shoot unfolded
1.	10.	Stages continuous until...
19	109	9 or more leaves on main shoot unfolded (2-digit scale) 9th leaf on main shoot unfolded
-	110	10th leaf on the main shoot unfolded
-	11.	Stages continuous till…
-	119	19 or more leaves on main shoot unfolded

Table 3

Description of the phenological stages of pepino growth stage 2 (formation of side shoots) using a 2-digit and a 3-digit scale BBCH scale.

2-digit code	3-digit code	Description
21	201	First primary apical side shoot visible
22	202	2nd primary apical side shoot visible
2.	20.	Stages continuous till . . .
29	209	9 or more primary apical side shoots visible (two-digit scale) 9th primary apical side shoot visible (three-digit scale)
-	210	10th primary apical side shoot visible
-	21.	Stages continuous till…
-	219	19 or more primary apical side shoots visible

Table 4

Description of the phenological stages of pepino growth stage 3 (main shoot elongation) using a 2-digit and a 3-digit scale BBCH scale.

2-digit code	3-digit code	Description
31	301	Main shoot up to 10 cm long
32	302	Main shoot up to 20 cm long
33	303	Main shoot up to 40 cm long
34	304	Main shoot up to 70 cm long
35	305	Main shoot up to 100 cm long
36	306	Main shoot up to 130 cm long
37	307	Main shoot up to 160 cm long
38	308	Main shoot up to 200 cm long
39	309	Elongation growth of the main shoot is ceased

Table 5

Description of the phenological stages of pepino growth stage 5 (inflorescence emergence) using a 2-digit and a 3-digit scale BBCH scale.

2-digit code	3-digit code	Description
51	501	First inflorescence in the main shoot visible
52	502	2nd inflorescence in the main shoot visible
53	503	3th inflorescence in the main shoot visible
5.	50.	Stages continuous until...
59	509	9 or more inflorescences in the main shoot visible (two-digit scale) 9th inflorescence in the main shoot visible (three-digit scale)
-	510	10th inflorescence in the main shoot visible
-	51.	Stages continuous until...
-	519	19 or more inflorescences in the main shoots visible

Table 6

Description of the phenological stages of pepino growth stage 6 (flowering) using a 2-digit and a 3-digit scale BBCH scale.

2-digit code	3-digit code	Description
61	601	First inflorescence in the main shoot: first flower open
62	602	2nd inflorescence in the main shoot: first flower open
63	603	3th inflorescence in the main shoot: first flower open
6.	60.	Stages continuous until...
69	609	9 or more inflorescence in the main shoot with open flowers (two-digit scale) 9th inflorescence in the main shoot: first flower open (three-digit scale)
-	610	10th inflorescence in the main shoot: first flower open
-	61.	Stages continuous until...
-	619	19th inflorescence in the main shoot: first flower open

Table 7

Description of the phenological stages of pepino growth stage 7 (development of fruit) using a 2-digit and a 3-digit scale BBCH scale.

2-digit code	3-digit code	Description
71	701	First fruit cluster in the main shoot: First fruit reaches typical size and form and colour
72	702	2nd fruit cluster in the main shoot: First fruit reaches typical size and form
73	703	3th fruit cluster in the main shoot: First fruit reaches typical size and form
7.	70.	Stages continuous until...
79	709	9 or more fruit clusters in the main shoot with the first fruit having reached typical size and form

Table 8

Description of the phenological stages of pepino growth stage 8 (ripening of fruit and seed) using a 2-digit and a 3-digit scale BBCH scale.

2-digit code	3-digit code	Description
81	801	10% of fruits show typical fully ripe colour
82	802	20% of fruits show typical fully ripe colour
83	803	30% of fruits show typical fully ripe colour
84	804	40% of fruits show typical fully ripe colour
85	805	50% of fruits show typical fully ripe colour
86	806	60% of fruits show typical fully ripe colour
87	807	70% of fruits show typical fully ripe colour
88	808	80% of fruits show typical fully ripe colour
89	809	Fully ripe: all fruits have typical fully ripe colour

Table 9

Description of the phenological stages of pepino growth stage 9 (senescence) using a 2-digit and a 3-digit scale BBCH scale.

2-digit code	3-digit code	Description
91	901	Beginning of leaf yellowing
95	905	50% of leaves brownish
99	909	All fruits harvested

Table 10

Differences among cultivated pepino (*Solanummuricatum*) varieties and accessions of wild relatives for traits of agronomic interest at specific phenological stages as defined by the BBCH scale. The phenological stage at which each trait was measured in both the two-digit and three-digit scales is indicated within square brackets.

Variety/accession	Stem length (cm) [51/501][a]	Fruit length (cm) [71/701]	Fruit width (cm) [71/701]	Fruit length/width ratio [71/701]	Time from transplant to beginning of ripening (d) [81/801]	Soluble solids content (%) [81/801]
Local varieties of cultivated pepino (*S.muricatum*)						
37-A	64.4 a	7.34 de	4.10 ab	1.79 def	147 ab	5.58 ab
Col-1	53.4 a	7.34 de	7.90 cdef	0.93 ab	143 a	7.22 bcde
CH2-22	50.6 a	7.86 e	7.74 cdef	1.03 ab	153 ab	7.52 cde
OV-8	70.6 a	5.70 cde	5.60 bcd	1.03 ab	153 ab	6.80 abcd
PT-154	46.4 a	7.87 e	11.10 g	0.72 a	150 ab	7.27 bcde
RP-1	52.0 a	5.64 cde	8.14 def	0.71 a	145 ab	5.90 abc
Modern varieties of cultivated pepino (*S.muricatum*)						
El Camino	53.4 a	7.61 e	6.04 bcde	1.27 bc	145 ab	6.92 bcd
Kawi	46.8 a	14.47 g	9.37 f	1.55 cd	150 ab	5.60 ab
Puzol	43.0 a	10.90 f	6.84 cde	1.60 de	143 a	7.56 cde
Quito	60.4 a	6.57 de	6.10 bcde	1.08 ab	149 ab	5.13 a
Sweet Long	44.0 a	10.77 f	5.73 bcde	1.89 ef	161 b	6.40 abcd
Sweet Round	50.2 a	7.17 de	8.30 ef	0.87 ab	145 ab	7.87 def
Turia	47.6 a	15.50 g	7.43 cdef	2.08 fg	150 ab	6.70 abcd
Valencia	44.0 a	11.62 f	5.34 bc	2.21 g	147 ab	7.58 cde
Wild relatives[b]						
BIRM/S 1034 (*S. ca.)*	115.0 b	2.77 ab	2.73 a	1.01 ab	153 ab	9.27 fg
E-7 (*S. ca.)*	143.8 c	3.52 abc	3.62 ab	0.97 ab	144 a	10.28 gh
EC-40 (*S. ca.*)	99.0 b	2.82 ab	2.78 a	1.01 ab	151 ab	8.02 def
QL-013 (*S. ca.*)	104.2 b	3.24 abc	2.94 a	1.10 ab	152 ab	10.38 gh
P-80 (*S. ct.*)	93.7 b	1.16 a	1.80 a	0.94 ab	152 ab	10.30 gh
P-62 (*S. pe.*)	99.2 b	2.15 ab	2.25 a	0.96 ab	147 ab	10.50 gh
E-257 (*S. ta.*)	96.2 b	4.60 bcd	3.67 ab	1.26 bc	154 ab	8.90 efg
E-34 (*S. tr.*)	63.2 a	2.50 ab	2.12 a	1.18 b	150 ab	11.60 h

[a]Means separated by different letters within each column are significantly different at $P<0.05$, according to the Student-Newman-Keuls multiple range test.

[b]The species corresponding to each of the wild accessions is indicated in brackets according to the following code: *S. c.=S. caripense*; *S. ct.=S. catilliflorum*; *S. pe.=S. perlongistylum*; *S. ta.=S. tabanoense*; *S. tr.=S.trachycarpum*).

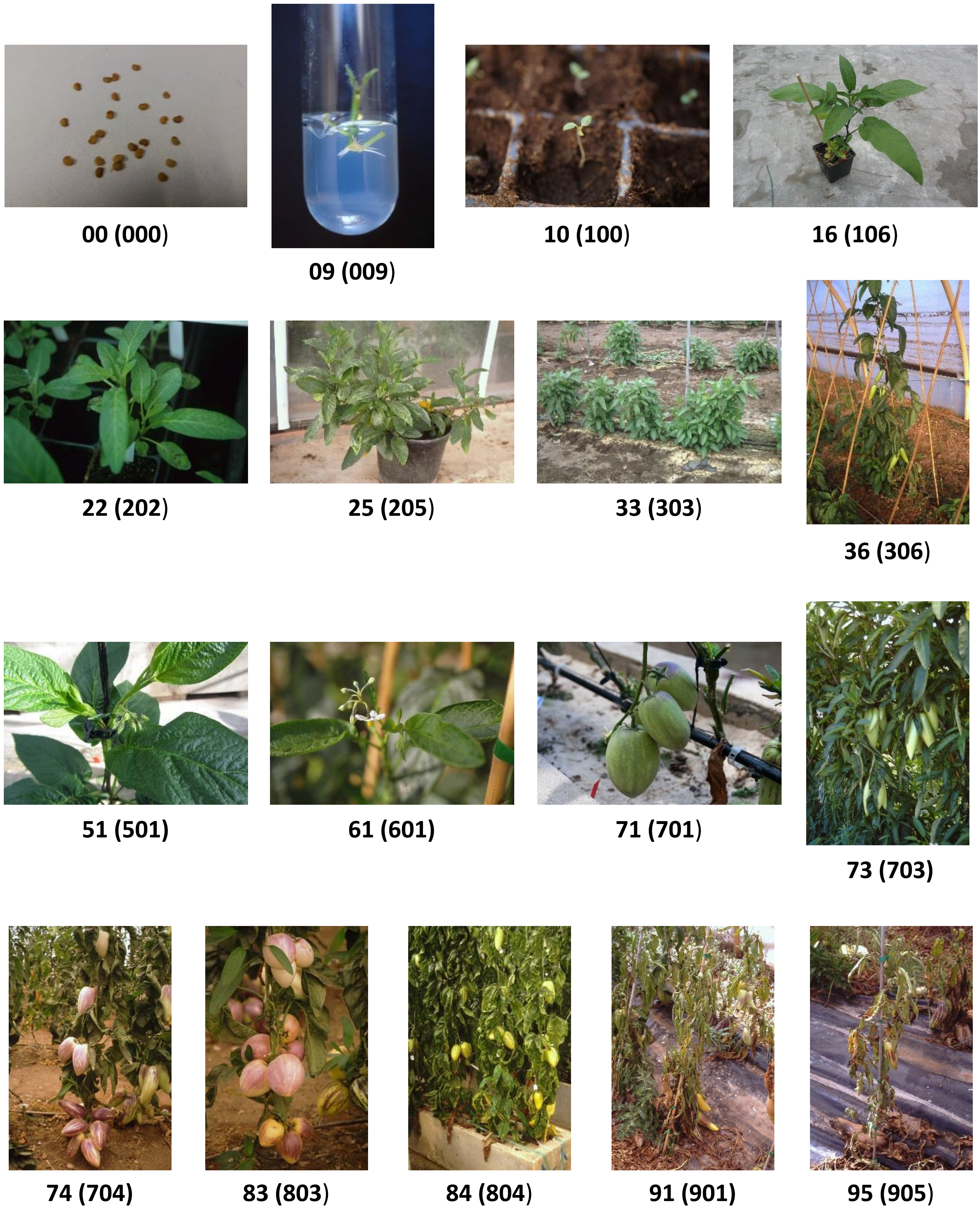

Fig. 1. Illustrations of some of the phenological stages of pepino (*Solanum muricatum*) according to the BBCH scale. Two-digit and three-digit (between brackets) scale codes are indicated. See Tables 1-9 for the description of each of the phenological stage codes.