# Whole plant growth stages documentation: 03/08/2005

# Objectives of POC for developing an ontology for whole plant stages:

- 1) To capture the biological concept and transcend the differences in growth stage terminology and arrive at comparable growth stages in different species.
- 2) Standardize plant growth stage nomenclature.
- 3) Integrate monocot and dicot growth stages.
- 4) To enable querying of comparable growth stages across plant species.
- 5) To enable annotation to growth stages of plants from a common platform.

# **Introduction:**

Growth is a complex process with different organs developing, growing and dying in overlapping sequences. Understanding the orderly succession of changes leading from the simple structure of the embryo to the highly complex organization of the mature plant, presents some of the most fascinating and challenging problems of biology today. The succession of changes can be assessed as a series of growth stages to describe what is known about the processes underlying and controlling plant development. Growth stages help to track and demarcate the observable changes that occur during the life cycle of a plant, from germination to fruiting and senescence.

In most plant species, the growth and development is continuous, but humans have repeatedly identified easily recognizable growth stages to help them monitor this development at the whole plant level. The length of each stage is greatly influenced by the environment and variety (Genotype X Environment or GxE). Every crop has its own scale for defining its growth stages and consequently a large volume of literature exists, detailing growth stages of individual plant models. Critical to the system is the *visibility* criteria. In the field, it is convenient to observe things that are visible to the naked eye or with amplification from a small hand lens. Consequently plant morphological features that are visible with magnification less than or equal to 10x are favored as growth stage criteria (ref).

# Plant molecular biology and its relation to growth stages:

Capitalizing on this wealth of knowledge, we attempt to translate the plant's growth staging systems into a genomic tool, for comparative assessment, starting with the reference species Arabidopsis, rice, maize and some of the major cereals like oat, wheat, barley and sorghum. Recognizable morphological landmark events of a growing plant are treated as identifiers to indicate the underlying biology of growth (physiology, major biochemical pathways, genes and gene interactions.). Thus making it possible to accurately and reliably estimate the growth stage of a particular plant model, this allows one to leverage information available from this model to study a similar mechanism in another species. While providing only a coarse level of resolution, agreement on objective criteria for classifying crop growth stages assures that different people evaluating the same or even different plant material/model will recognize the same or equivalent growth stages

Four commonly used scales <u>Zadoks</u>, <u>Feeks</u>, <u>Haun</u> (all for cereal plant models) and BBCH (for both dicot and monocot plant models) are all based on the external appearance of a plant. Common growth terms are used, which act as indirect guides to the actual events of interest.

We understand that unless a comparative study has been done it is very difficult to compare a growth stage from one plant species to that of the other. However, in the present state of research and available literature citations, the most convenient way of making comparisons is based on the similar/equivalent event(s) observed. For example it is difficult to say what are the underlying processes occurring in an Arabidopsis plant when the seedling is at 4 leaf stage compared to that of the maize or rice. Yet for it is useful to make some comparison as adopted by Boyes et al (2001) for Arabidopsis, we can make a statement saying that the plant from each of these three species was at 4 leaf stage. This equivalence is independent of how long (days after germination/days after sowing) it took to reach at this stage.

Here are some of the advantages of using BBCH scales cited by Boyes et al (2001)

- 1. As a model organism for agricultural biotechnology Arabidopsis presents opportunity to provide key insights into gene function that can be used in improving an agronomic trait of commercial importance. A requisite part of gene function determination is the characterization of phenotypes that result when expression levels or the structural components of the gene of interest are altered. The resultant phenotypes may be molecular, biochemical, morphological, developmental or stress related in nature and are often associated with **one or more stages of growth.**
- 2. Plants exhibit widely different developmental timelines and morphologies depending on the environment in which they are grown. Thus comparison of data collected by laboratories in which they are grown under slightly different conditions can be problematic while doing comparative studies. Unlike intra-species, this may become significantly less informative if there are inter-species comparisons.
- 3. This is especially true if data is collected solely with the reference to chronological age alone. In contrast, phenotypic data collected with reference to a **commonly defined series of growth stages** would provide a coherency not otherwise achieved by collection procedures based on chronological age alone defined for each species or in a project experiment with no reference to any other staging system.
- 4. Regarding the BBCH scale, the authors say that it provides a comprehensive growth stage description and can be adapted for most crops and weed species. It was originally designed as a means of communication among agriculturists, adaptation of such a universal scale at the laboratory level would allow for easier data comparison between and within species.

# Advantages of adopting BBCH system over any other scale:

BBCH is the **only** system that has attempted to integrate the growth stages of different crops. In addition it is the same scale to which <u>Boyes et al (2001)</u> have used to draw equivalences from Arabidopsis to cereal plant growth stages for phenotypic evaluation. The same scales were integrated as Arabidopsis growth stage vocabulary developed by TAIR. Thus there is

already a foundation for correlating growth stages across crops and much of the initial work of extracting and integrating ideas from published literature has already been accomplished by this system. The BBCH scale offers a standardized description of plant development in the order of their phenological characteristics and has coded these descriptions for easy comparison across major monocot and dicot plant models. The BBCH scale is based on Zadoks scale, so it brings two systems together. Zadoks scale is the most widely used scale for cereals in the United States by agronomists. Hence the BBCH scale seems most promising for our purpose of integrating, the descriptions of growth stages from the dicot Arabidopsis and the monocots rice, maize and other major cereal plants and come up with a generic structure that can be used by any of these species to define their growth stages.

Gramene database follows the Zadok scale for rice and other cereals

(http://www.gramene.org/db/ontology/search\_term?id=GRO:0007199)

TAIR follows the BBCH system for Arabidopsis

Boyes et al. 2001 Plant Cell. 2001 Jul;13(7):1499-510.

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list\_uids=11449047 Arabidopsis growth stage

http://www.arabidopsis.org/servlet/Search?action=expand&type=tree&tree\_type=keyword&node\_id=13665&id=7

MaizeGDB follows Ritchie's growth staging system (REF).

Gramene replicated the MaizeGDB system for presenting the maize growth stages. <a href="http://www.gramene.org/db/ontology/search\_term?id=GRO:0007002">http://www.gramene.org/db/ontology/search\_term?id=GRO:0007002</a>

Zadok, Feeks and Haun scales

http://weeds.montana.edu/crop/Growth stages book/p22 23.htm

See its application in staging the members of Triticeae
http://weeds.montana.edu/crop/Growth stages book/p2 3 4 5.htm

# Validation of the BBCH model

Sigmoid growth curve of a plant can be used to trace the changes in physiological states of the plant continuously through the growth period. The external growth identifiers or growth stages link to the underlying physiological state and as we move from germination to senescence stage, the distinct or overlapping sequences of all the life-processes can be studied via the growth stage model. As the major physiological processes are studied in molecular detail the growth stage model proposed here provides an overview of the plants biology. And most importantly, it lays the foundation for cross species comparison that integrates specific physiological states and the genes acting therein.

The plant stages that are often overlapping or undergoing concurrent changes as depicted in the following table.

Table-1

Stages	0 germination	1 leaf production	2 formation of axillary shoot	3 rapid growth stage	4 inflorescence visible	5 flowering	6 fruit formation	7 ripening	8 senescence **
0 germination	X								
1 leaf production		X	X	X	X				
2 formation of axillary shoot			X	X	X				
3 rapid growth stage				X	X	X			
4 inflorescence visible					X	X			
F (1)						X	X		
5 flowering									
6 fruit formation							X	X	
<u> </u>							X	X X	X

Cells in yellow represent the higher level growth stages. Cells in gray color represent the intersection and overlap of growth stages. \*\* the stage 8 senescence is given the last order in the temporal scale, however it is understood that as the plant is growing many of its older plants parts are already undergoing senescence.

# **Modified BBCH for POC growth stage ontology:**

The BBCH scale is primarily designed for crop breeders, so changes had to be made to convert it to use in the context of genomic research. The scale was analyzed to understand the scientific basis for comparing monocot and dicot growth stages and it was then adapted with modifications to improve its utility for the POC. The principal stages of the BBCH scale were essentially maintained, with some changes recommended in organizing and numbering. Some stages were 'grouped' (BBCH stage 4 and 5) into a single stage and others (BBCH stage 3) was separated into sub stages. These steps were taken, where biology experts considered some monocot/dicot stages may or may not be comparable.

#### **Methodology:**

The staging system divides the growth stages of plants into 8 principal growth stages, with subdivisions of these stages, designated numerically, x.00 to x.08 correspond to the respective ordinal numbers or percentage values. For example stage x.03 could represent: 3rd leaf, 3rd tiller/axillary branch, 3rd node or 30% of the final length or size typical of the species or 30% of the flowers open. The stage number x.09 usually represents the final sub stage in that section or simply says nine or more or 90% or more, with an exception in POC stages namely, 3 stem elongation (PO:0007089) and 1 leaf production (PO:0007112), where the number series has grown up to x.99 to cover the indeterminacy of the number of laves and internodes.

It is understood from the table-1 that there are overlaps between the growth stages, but no effort has been made to display the same in the POC growth stages as well as in the BBCH. This is done considering the fact that the boundaries for growth stages and their overlaps may not be strictly followed from one plant model to another model. However the search by synonyms for example "late whorl stage" in maize will give you 6 non over lapping stages namely.

PO:0007101 1.09 nine leaves, leaf pairs or whorls unfolded PO:0007103 1.10 ten leaves, leaf pairs or whorls unfolded PO:0007116 1.11 eleven leaves, leaf pairs or whorls unfolded PO:0007092 3.04 fourth node/internode visible PO:0007086 3.05 fifth node/internode visible PO:0007062 3.06 sixth node/internode visible

This is so because when the stage 1.09 is visible in maize, stage 3.04 is also occurring. Similarly when 1.10 is visible 3.05 is visible too. Also by definition from MaizeGDB termid=67357 it says that late whorl stage in maize: "In adapted materials in the US Corn Belt, the collars of leaves 9-11 are visible (some of the lowest leaves may already have degenerated by this stage). By 10-leaf stage, new leaves appear every 2-3 days. Tassel development accelerates, and rapid stem elongation continues."

#### Synonym based approach:

Since the actual growth stage term may not represent the exact terminology used by the species specific growth staging system, every effort has been made to include them as synonyms. Thus allowing the users to retrieve the growth stages specific to their interest and species and still be able to use the common platform for annotation and representation of growth stages. This is important because, the whole ontology is built around the use of synonyms to enhance comfort and help is accurate query searches that will be used in searching plant databases. It will also address the comparisons across growth stages as well because there are known overlaps as mentioned in the maize example given in methodology section.

# The top eight nodes of the growth stage ontology are those shown in the image

The comparison of BBCH scales Vs the growth stage ontology developed by the POC.

Principal	growth stages
Stage	Description
0	Germination / sprouting / bud development
1	Leaf development (main shoot)
2	Formation of side shoots / tillering
3	Stem elongation or rosette growth / shoot development (main shoot)
4	Development of harvestable vegetative plant parts or vegetatively propagated organs / booting (main shoot)
5	Inflorescence emergence (main shoot) / heading
6	Flowering (main shoot)
7	Development of fruit
8	Ripening or maturity of fruit and seed
9	Senescence, beginning of dormancy

**BBCH SCALES** 

**POC SCALES** 

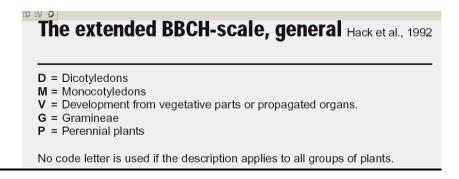
You may like to browse and look at these terms by visit the ontology browser at <a href="http://dev.plantontology.org/amigo-temporal/go.cgi?action=minus\_node&search\_constraint=terms&query=PO:0007004&session\_id=4948b1110323684">http://dev.plantontology.org/amigo-temporal/go.cgi?action=minus\_node&search\_constraint=terms&query=PO:0007004&session\_id=4948b1110323684</a>

#### Suggestions to revierws:

- To view the growth stages click on the + sign before the term name
- To view the details (definitions, synonyms and comments) of a term, click on the term name
- For more help on ontologies and how to use the browser please visit the following links

Tutorials (<a href="http://dev.plantontology.org/docs/otherdocs/tutorials.html">http://dev.plantontology.org/docs/otherdocs/tutorials.html</a>)
Browser help (<a href="http://dev.plantontology.org/amigo/docs/user\_guide/index.html">http://dev.plantontology.org/amigo/docs/user\_guide/index.html</a>)

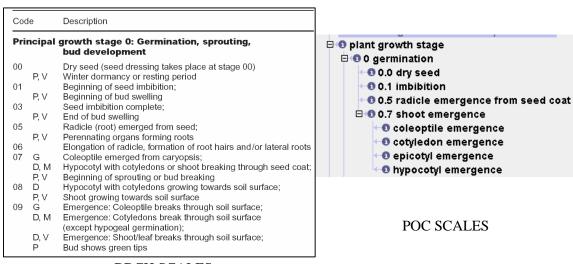
In this document on the BBCH scale images you may find the letter codes as described below. This will help in finding the appropriate explanation of the growth stage term being used and its equivalences.



# **Growth stage 0: Germination**

The list of growth stages starts with the germination of plants. This stage is numbered '0' and sub stages or instances are numbered 00, 0.1, 0.2...07.

- The first landmark temporal event in germination is denoted 'dry seed stage'
- Followed by 'imbibition'.
- The next common event to most plants during germination is the stage at which the 'radicle emerges from the seed coat' this stage is numbered 05.
- Stage 0.7 shoot emergence will be used to accommodate data where distinct events occurring prior to radicle emergence. This would apply to all growth stages wherever there is a lack of information. Instances below these nodes are not numbered as they are plant species/family specific singular events of coleoptile emergence, cotyledon emergence, epicotyl and hypocotyl emergence. These modifications to the BBCH scale were done as non-overlapping biological events were reexamined and correlated with the actual sequence of germination events of monocots and dicots. The significant differences related to germination in these two groups justifies that these events be treated as separate instances below 'shoot emergence'.



**BBCH SCALES** 

#### **Growth stage 1: Leaf production.**

Following germination is complete the plants growth, is marked by a series of macroscopic landmarks, the first of which is leaf production. Leaves are produced in a successive manner, and the progression through this stage is measured by counting the actual number of visible leaves. The emergence of leaves are indicators of robustness, disease susceptibility etc. This stage is defined as the stage when the 'shoot apical meristem is producing leaves'.

- The sub stages or instances of leaf production include the number of leaves a plant possibly unfolds during its growth.
- The stage at which the plant has one leaf or one pair of leaves (or whorl) is numbered 1.01. This includes the stages(s) that has cotyledons plus one leaf or a pair or whorl of leaves visible above the ground, typical of species.
- In taxa with a hypogeal type of germination, the first leaf on the epicotyl, is considered to be 'leaf one' and in grasses the coleoptile, is leaf one.
- The instances under this node continue up to numbers "1.20 twenty leaves/pairs/whorls of leaves unfolded". Unlike the BBCH scale, where secondary growth stages are given, only nine stages, this ontology was modified from BBCH to accommodate 99 instances. One such example of maize lead us to increase the numbered stages to 20.

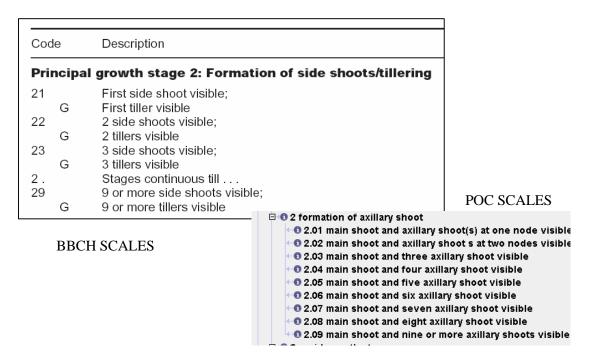


**BBCH SCALES** 

**POC SCALES** 

# **Growth stage 2: Formation of axillary shoot.**

The instances under this growth stage proceed from 2.00 to 2.09, each stage representing the number or pair of axillary shoots or tillers that are formed at one node.

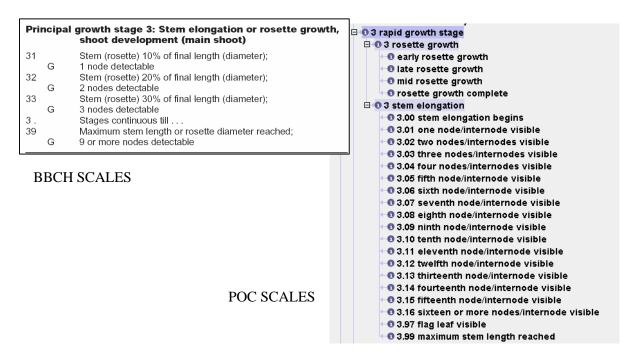


This stage may not be applicable to Arabidopsis (Boyes et al 2001)

# **Growth stage 3: Rapid growth stage**

01/serveFile?name=publication.

This is the stage at which the stem elongation or the rosette growth becomes apparent. In most cases of plants, elongating nodes are visible at this time. In grasses, the flag leaf is visible at stage 3.97, and by stage 3.99 'maximum stem length has been reached. Since this stage reflects the changes in the biomass acquisition, the stem elongation and the rosette growth stages are grouped together at the higher level. However the two instances (3.0 stem elongation and 3.0 rosette growth are kept separate with no known knowledge of the equivalences in their children terms). Which means unless there is information available to suggest that when rosette has reached 20% of the growth how many elongating nodes are visible, there is no mechanism to make equivalences with the children of 3.0 stem elongation..



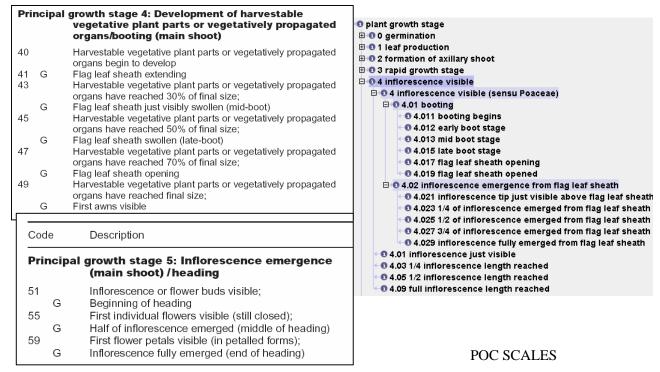
In the Zadoks/BBCH scale the stem elongation stages start when the first (elongating) node becomes detectable. This is usually equivalent to the actual node number 6-7, in the grasses, since earlier nodes are not visible out of the leaf sheath of the previous leaf.

The maize using Ritchie's scale which is popular as V and R staging system the 'first node detectable', is the seventh node carrying seven leaves in maize or 'V7 stage' with synonyms-rapid stem elongation/ seven leaves/ mid-whorl stage mentioned for both the 3.01 one node/internode visible and 1.06 six leaves, leaf pairs or whorls unfolded.

As mentioned before, the BBCH system equates the stem elongation stage to rosette growth in Arabidopsis, the POC biologists are of the opinion that these stages are non-comparable and treat rosette growth under a separate node. Ref: http://www.icoria.com/pubs\_patents/scientific\_publications/publication\_2003-09-

# Growth stage 4: Inflorescence visible

This stage is defined as the stage at which the inflorescence is visible on the plant. This stage does not mention about when the inflorescence development (initiation) occurs, instead when the inflorescence development has passed its initial phases of development and has acquired a substantial form (to be defined later), that it can be called as one. Stage 4.01 occurs when 'inflorescence is just visible", followed by the growth of the inflorescence (length wise) measured at quarter (1/4) and half (1/2) the length of final size. Stage 4.09 coincides with completion of inflorescence formation and attaining the final length of the inflorescence. Often it is difficult to assess this length because of the lack of information on the final size or the indeterminacy of inflorescence development.



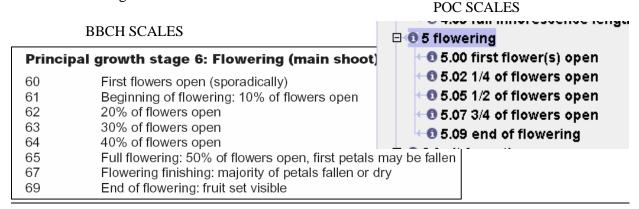
**BBCH SCALES** 

When the inflorescence is visible the plants of Poaceae, is markedly different from that of the other plant families, considering that it is not visible to the naked eye until it approaches heading, The earliest stages can be visually observed by the presence of the boot or the swelling, (4 inflorescence visible (sensu Poaceae). Stage 4.01 booting stage, has 6 instances below it (see above image). In the BBCH scale this stage is common to the plants having harvestable body parts, and the authors note that "in non-cereals the vegetative plant or vegetatively propagated organs begin to develop" as we have restricted at present, the growth stages to Arabidopsis, rice and maize plants only.

Stage 4.02 inflorescence emergence from flag leaf sheath under 4 inflorescence visible (sensu Poaceae), relates to the emergence of the inflorescence from the flag leaf sheath in the Poaceae (a heading event). Instances of this stage proceed from the inflorescence being just visible above the flag leaf sheath to when it is fully emerged from it.

# **Growth stage 5: Flowering**

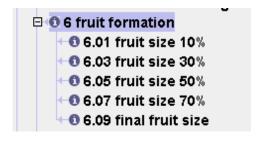
This landmark stage in the life of a plant begins with the anthesis. Anthesis includes bursting of anthers, shedding of pollen and the stigma becoming receptive to pollen. This stage is partitioned into sub-stages by the number of flowers in an inflorescence undergoing anthesis. As shown in image below.



#### **Growth stage 6: Fruit formation**

Fruit formation deals with the developmental changes that occur in the whole plant after fertilization and during fruit development. The development of the fruit in conceptualized in this stage by measuring the size it has acquired compared to the final size. The fruit size is a marker of a variety of physiological processes and provides a visible estimate corresponding to a whole plant growth stage. In cereals it refers to the earliest phases of starch accumulation (milk stage) in the endosperm of kernel/seed (caryopsis) and acquisition of the full grain size.

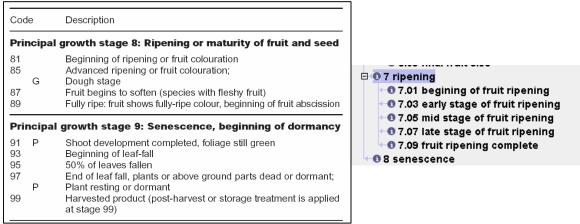
Dei	nainal	growth stage 7. Development of fruit
PI	псіраі	growth stage 7: Development of fruit
71		10% of fruits have reached final size or fruit has reached 10% of final size¹
	G	Caryopsis watery ripe
72		20% of fruits have reached final size or fruit has reached 20% of final size1
73		30% of fruits have reached final size or fruit has reached 30% of final size¹
	G	Early milk
74		40% of fruits have reached final size or fruit has reached 40% of final size <sup>1</sup>
75		50% of fruits have reached final size or fruit has reached 50% of final size <sup>1</sup>
	G	Milky ripe, medium milk
76		60% of fruits have reached final size or fruit has reached 60% of final size
77		70% of fruits have reached final size or fruit has reached 70% of final size¹
	G	Late milk
78	Ū	80% of fruits have reached final size or fruit has reached
79		Nearly all fruits have reached final size <sup>1</sup>



**POC SCALES** 

# **Growth stage 7: Ripening**

This stage relates to the maturation of fruit after they have attained full size. Instances of ripening include the beginning, early, mid and late ripening stages. Stage 7.09 occurs when fruit ripening is complete. Particular ripening stages properly refer to the development of an individual fruit and are commonly used to extrapolate to the growth stage of whole plant or a group of plants. In cereals it refers to the accumulation of starch in the endosperm of kernel/seed (caryopsis). Thus including the dough and dent stages of kernel development.



**BBCH SCALES** 

**POC SCALES** 

# **Growth stage 8: Senescence**

The last growth stage of a plant is senescence, which is defined as the beginning of death processes in a plant or plant parts (death-related). All the stages related to seed dormancy must be mapped to this stage. At this time no effort has been made to extend this stage into sub stages, however the species specific stages falling under this growth stage were included as synonym.