

CHARACTERIZATION OF BARLEY

(*Hordeum vulgare* L.)

VARIETIES FOR DISTINCTIVENESS, UNIFORMITY AND STABILITY



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S.R. Vishwakarma and Indu Sharma



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Correct Citation: R.P.S. Verma, Vishnu Kumar, A. S. Kharub, Dinesh Kumar, S. R. Vishwakarma and Indu Sharma. 2013. “Characterization of Barley (*Hordeum vulgare* L.) Varieties for Distinctiveness, Uniformity and Stability”. Directorate of Wheat Research, Karnal-132001 (Haryana). *Research Bulletin No. 30*: P 26.

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Published by : **Project Director,**
Directorate of Wheat Research,
Karnal - 132001, Haryana, India
Tel.: 0184-2267490, Fax: 0184-2267390
E-mail: dwr@vsnl.com, wheatpd@gmail.com
Web site: www.dwr.in

First Print: August, 2013

Copies: 500

Printing : Intech Printers & Publishers
SCO 343, 1st Floor, Mughal Canal
Karnal – 132 001 (Haryana)
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Email: jobs.ipp@gmail.com

FOREWORD

India being a signatory to Trade Related Aspect of Intellectual Property Rights (TRIPs) is required to protect plant varieties either by patent or by an effective *sui generis* system of protection or by combination of both. The Government of India opted for *sui generis* system (of its own kind) of Protection of Plant Varieties and enacted “Protection of Plant Varieties and Farmers Rights Act” (PPV&FR) in 2001 to encourage research, variety development, protection to breeders varieties, ensures farmers rights and growth of seed industry. To claim this right the variety has to be distinct, uniform, stable (DUS) and novel. To validate these stipulations DUS tests need to be conducted for which national guidelines for testing of distinctiveness, uniformity and stability are being developed for different crops on India. The Directorate of Wheat Research in consultation with National Core Group Experts has characterized barley varieties, which is being published as a bulletin entitled, “Characterization of Barley (*Hordeum vulgare* L.) Varieties for Distinctiveness, Uniformity and Stability”. The bulletin will be quite useful to researchers, students, administrators and policy makers dealing with the DUS related activities and malt, feed and dual purpose barley improvement.



(Indu Sharma)
Project Director

ACKNOWLEDGEMENT

The authors are highly grateful to Dr. S. Ayyappan, Secretary, DARE & Director General, ICAR for his overwhelming guidance, encouragement and his keen efforts in the protection of Plant Varieties and Farmer's Rights in the country. The team also expresses their sincere gratitude to Dr. Swapan Kumar Datta, Deputy Director General (CS) for his constant support and vital suggestions. Our sincere thanks are due to Dr. R.P. Dua, Assistant Director General (FFC) for facilitating the publication of this important bulletin. Authors are highly grateful to Dr. P L Gautam, Ex-chair person, PPV&FRA and incumbent Chair person, PPV&FRA Dr. R R Hanchinal for their constant support, encouragement and guidance. The members of Core Committee viz., Drs. S.C. Gulati, Sushila Kundu, Mahesh Shrimali, Tejbir Singh deserves special appreciation for their help and critical suggestions during the course of finalization of barley guidelines. The authors are grateful to Drs. R. C. Agrawal, Manoj Srivastava, Ravi Prakash, D. R. Choudhury, A. C. Sarma, D. S. Mishra and D. S. Paliania for their immense help and valuable technical inputs and suggestions. Mr. Amit Kumar deserves special appreciation for his constant support and arduous work in data recording under the project. The authors are highly thankful for the generous support extended by DAC, Ministry of Agriculture, Government of India for funding DUS programme in barley under the Central Scheme for implementation of PPV&FR Act in the country. Authors are highly thankful to all the persons, who directly or indirectly helped to bring out and publish this document.

(Authors)

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INTRODUCTION

India is a signatory to World Trade Organization (WTO), which was established in January 1995. General Agreement on Tariffs and Trade (GATT) recognized agriculture as a rule-bound enterprise of investment and profit making and included it in the negotiations for the first time in Uruguay Round (1986-1994). India became signatory to the Trade Related Aspects of Intellectual Property Rights Agreement (TRIPs) in 1994, such a legislation was necessitated. Article 27.3 (b) of this agreement requires the member countries to provide for protection of plant varieties either by a patent or by an effective *sui generis* system or by any combination thereof. The existing Indian Patent Act, 1970 excluded agriculture and horticultural methods of production from patentability. During 2001, significant developments have taken place with respect to the realization of the rights of breeders, farmers and local communities. The Protection of Plant Varieties and Farmers' Rights Act (PPV&FR) was passed by the Indian Government. The *sui generis* system for protection of plant varieties was developed integrating the rights of breeders, farmers and village communities, and taking care of the concerns for equitable sharing of benefits. It offers flexibility with regard to protected genera/species, level and period of protection, when compared to other similar legislations existing or being formulated in different countries. The Act covers all categories of plants, except microorganisms. The objective is to provide an effective system of protection of plant varieties, by an effective *sui generis* system. The objectives of the Act are:

- i) To provide for the effective system for protection of plant varieties.
- ii) To provide for rights of farmers and plant breeders.
- iii) To stimulate investment for research and development and to facilitate growth of the seed industry.
- iv) To ensure availability of high quality seeds and planting material to the farmers.

The genera and species of the varieties for protection shall be notified through a Gazette, after the appropriate rules and by-laws are framed for the enforcement of the Act. DUS testing is compulsory and an essential part in order to implement the *sui generis* system for plant varieties protection and for granting rights to breeders or farmers or institutions. A new variety shall be registered if it conforms to the criteria of novelty, distinctness, uniformity and stability. An extant variety, a variety about which there is a common knowledge shall also be registered within a specified period if it conforms such criteria as distinctness, uniformity and stability as shall be specified. A new variety shall be deemed to be:

- a) **Novel**, if at the date of filling of the application for registration for protection, the propagating or harvested material of such variety has not been sold in India, earlier than one year, or outside India, earlier than four years, before the date of filling such application.
- b) **Distinct**, if it is clearly distinguishable by at least one essential characteristic from any other variety whose existence is a matter of common knowledge in any other country at the time of filling of the application.

- c) **Uniform**, if subject to the variation that may be expected from the particular features of its propagation, it is sufficiently uniform in its essential characteristics.
- d) **Stable**, if its characteristics remain unchanged after repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle.

Once a valid application for Plant Breeder Right is accepted, DUS tests need to be conducted by crop based institutions on behalf of the authority *i.e.* PPVFRA by following universally acceptable test procedures. The recommended seed/planting material of the variety will be requested from the respective breeder for official DUS test. This necessitated in the development of the test guidelines, descriptors and procedures to, be followed in each crop. National Test Guidelines have been developed for 57 crops including barley representing coordinated approach for the testing of new varieties for novelty, uniformity and stability, which will form the basis for DUS examination. These contain details on, (i) subject of the guidelines (ii) material required (iii) conduct of tests (iv) methods and observation (v) grouping of varieties (vi) characteristic and symbols (vii) table of characteristics (viii) explanation on table of characteristics (ix) biochemical character (x) system for growth stages in barley. The characteristics in the table follow the botanical and chronological order of recording from seed (submitted), seedling, plant, stem, leaf, inflorescence, seed etc.

The table of characteristics indicates the various characters viz. culm, spike, flag leaf, awn and grain etc. of barley species, which should be examined critically and included in the description of varieties. Essential characters have been marked with an asterisk (*). It also contains additional characteristics, which are considered to be helpful in taking the final decision on the variety. In this table of characteristics, a scale of possible states of expression (so-called “states”) is indicated for each characteristic. The states are accompanied by “Note” containing code numbers, which permit the computerization of variety description. As far as possible, “Example varieties” are also cited for each state. Some characteristics are marked with the sign (+), which indicates that the characteristics are illustrated by explanations and drawing or that testing method is indicated in the chapter entitled “Explanations on the table of characteristics”.

In consultation with the National Core Group Experts, Directorate of Wheat Research has played a key role in developing national test guidelines for DUS test in barley. The NDUA&T, Faizabad (co-nodal centre) has also played an important role in the data generation. Due care has been taken to include observations of special task force in drafting the guidelines. This technical bulletin includes test guidelines, descriptors to be observed for establishing the distinctness, explanations to the descriptors and procedures to be followed.

I Subject

These morphological and biochemical descriptors are for characterization of all varieties, hybrids and parental lines of Barley (*Hordeum vulgare* L.)

II Material required

1. The minimum quantity of seed to be provided by the applicant shall be 1500 grams in the case of the candidate variety or hybrid and 1000 grams for each of the parental lines of the hybrid. Each of these seed lots shall be packed and sealed in ten equal weighing packets and submitted in one lot. Applicants submitting such seed material from a country other than India shall make sure that all customs and quarantine requirements stipulated under relevant national legislation are complied with.
2. At least 100 spikes, each representing the normal spike size and drawn from the main tiller of the candidate variety shall be submitted. The spikes shall be individually packed and submitted along with the said seed lot.
3. The seed and spikes submitted shall have at least 95% germination, 98% physical purity, highest genetic purity, uniformity, sanitary and phytosanitary standard. In addition the moisture content of the seed shall not exceed 8% to meet the safe storage requirement. The applicant shall submit along with the seed a certified data on germination test made not more than one month prior to the date of submission.
4. The seed material shall not be subjected to chemical and biophysical treatment.

III Conduct of tests

1. The minimum duration of the DUS tests for the new varieties shall normally be at least two independent similar growing seasons.
2. The test shall normally be conducted at least at two locations. If any essential characteristic of the candidate variety is not expressed for visual observation at these locations, the variety shall be considered for further examination at another appropriate test site or under special test protocol on expressed request of the applicant.
3. The field tests shall be carried out under conditions favoring normal growth and expression of all test characteristics. The size of the plots shall be such that plants or parts of plants could be removed for measurement and observation without prejudicing the other to the observations on the standing plants until the end of the growing period. Each test shall include about 500 plants, in the plot size and planting space specified below across three replications. Separate plots for observation and for measuring can only be used if they have been subjected to similar environmental conditions. All the replications shall be sharing similar environmental conditions of the test location.

4. Test plot design

Number of rows	:	4
Row length	:	4 m
Row to row distance	:	30 cm
Plant to plant distance	;	10 cm
Expected plants/replication	:	160
Number of replications	:	3

5. Observations should not be recorded on plants in border rows

IV Methods and observations

1. The characteristics described in the table of characteristics shall be used for the testing of varieties, inbred lines and hybrids for their DUS
2. For the assessment of distinctiveness and stability observation shall be made on 30 plants or parts of 30 plants, which shall be equally divided among 3 replications (10 plants per replication).
3. For the assessment of uniformity of characteristics on the plot as a whole, this shall be done on simple visual observation of a group of plants or parts of plant. During such observation the entry shall be deemed uniform when the number of aberrant or odd plants or parts of plant shall not be exceeding 2 in 500.
4. For the assessment of uniformity of characteristics on single spike-rows, plants or parts of plant shall be visually observed on all individual spike-rows, plants or parts of plants. A spike-row having at least one aberrant or odd plant or parts of plant is dealt as an aberrant row. A variety shall be deemed uniform when the number of such aberrant spike-rows shall not exceed 2 in 100.
5. For the assessment of color characteristics, the latest Royal Horticultural Society (RHS) color chart shall be used.

V. Grouping of varieties

1. The candidate varieties for DUS testing shall be divided into groups to facilitate the assessment of Distinctiveness. Characteristics, which are known from experience not to vary or to vary only slightly, within a variety and which in their various states are fairly evenly distributed across all varieties in the collection, are suitable for grouping purposes.
2. The following characteristics are proposed to be used for grouping barley varieties:
 - a) Stem: Basal pigmentation (Characteristic 2)
 - b) Auricle: Anthocyanin pigmentation (Characteristic 3)
 - c) Spike emergence (Characteristic 7)
 - d) Spike type (row number) (Characteristic 8)
 - e) Plant height (Characteristic 20)
 - f) Spike density (Characteristic 25)
 - g) Grain hullness (Characteristic 26)
 - h) Grain: colour (Characteristic 27)

VI. Characteristics and symbols

1. To assess Distinctiveness, Uniformity and Stability, the characteristics and their states as given in the table of characteristics (Section VII) shall be used.
2. Scale 1 to 9 is used to describe the state of each character for the purpose of digital data processing.
3. The optimum stage for taking the observation of each characteristic during the plant growth and development is indicated by a decimal code. The Zadoks system being the most universally accepted is described here. It is applicable to any small grain, and its stages are easy to identify in the field. The Zadoks system is a two-digit code where the first digit refers to the principal stage of development beginning with germination and ending with kernel ripening. The second digit (between 0 and 9) subdivides each principal growth stage. The relevant growth corresponding to the decimal code number are described in section VII, column 5.
4. Legend :
 - (*) Characteristics that should be observed during every growing period on all varieties and should always be included in the description of the variety, except when the state of expression of any of these characters is rendered impossible by a preceding phenological characteristic or by the environmental conditions of the testing region. Under such exceptional situation, adequate explanation should be provided.
 - (+) See Explanations on the table of characteristics in Chapter VIII.
5. Type of assessment of characteristics indicated in the table of characteristics is as follows:
 - MG: Measurement by a single observation of a group of plants or parts of plants
 - MS: Measurement of a number of individual plants or parts of plants
 - VG: Visual assessment by a single observation on a group of plants or parts of plants
 - VS: Visual assessment by observations of individual plants or parts of plants

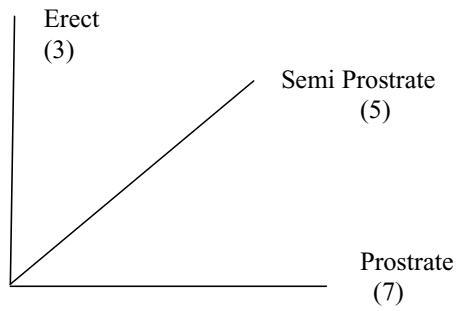
VII Table of characteristics

S.NO (1)	Characteristics (2)	State (3)	Note (4)	Stage of observation (5)	Example variety (6)	Type of assessment (7)
1. * (+)	Growth habit	Erect Semi-prostrate Prostrate	3 5 7	23-25	Amber Alfa93 ---	VG
2. *	Stem: Basal pigmentation	Absent Present	1 9	25-33	Amber Alfa93	VG
3. *	Auricle (Flag leaf): Anthocyanin Pigmentation	Absent Present	1 9	49-59	Amber Alfa93	VG
4. *	Upper node Pigmentation	Absent Present	1 9	49-59	Amber Alfa93	VG
5. * (+)	Flag leaf attitude	Erect Semi-erect Drooping	1 5 9	51-59	Amber BCU73 Alfa93	VG
6. *	Flag Leaf: Waxiness of sheath	Absent Present	1 9	51-59	Ratna Alfa93	VG
7. *	Spike emergence	Very early (<65 days) Early (65-75 days) Medium (76-86days) Late (87-96 days) Very late (> 96days)	1 3 5 7 9	51-59	----- BCU73 Amber Dolma Alfa93	MG
8. * (+)	Spike type	Two-row Six-row	3 7	59-69	Alfa93 Amber	VG
9. (+)	Lateral florets (Two-row barley)	Rudimentary Developed	1 9	59-69	DWRB73 Alfa93	VG
10. * (+)	Spike: Waxiness	Absent Present	1 9	59-85	BCU73 Alfa93	VG
11.	Spike: colour	Pale green Green Dark green	1 2 3	69-77	DWR28 Alfa93 DWRUB64	VG
12. * (+)	Spike: attitude	Erect Semi-erect Drooping	3 5 7	69-77	Amber BHS169 Alfa93	VG
13.	Awn: roughness	Smooth Rough	3 7	69-77	BH393 Alfa93	VG
14.	Flag leaf length	Short (<10cm) Medium (10-14 cm) Long (> 14 cm)	3 5 7	71-85	Alfa93 Amber Ratna	MS
15.	Flag leaf breadth	Narrow (<1.0 cm) Medium (1.0-1.5 cm) Wide (>1.5 cm)	3 5 7	71-85	Alfa93 Amber BH75	MS
16.	Awn: Tip pigmentation	Absent Present	1 9	73-87	Amber BH393	VG
17.	Spike: basal sterility	Absent Present	1 9	73-89	Alfa93 RD2715	VS
18. (+)	Lemma: pigmentation	Absent Nerve pigmented Present	3 5 7	75-87	Dolma Alfa93 DWRB91	VG
19.	Spike: length	Small (< 7cm) Medium (7.1-10cm) Long (>10cm)	3 5 7	75-89	Ratna BH75 Alfa93	MS

20. *	Plant: height	Very short (< 75.0 cm) Short (75.1-85.0 cm) Medium (85.1-95.0 cm) Tall (95.1-105.0 cm) Very tall (> 105.0 cm)	1 3 5 7 9	75-89	---- BCU73 Alfa93 BHS169 Amber, Jyoti	MG
21.	Peduncle: length	Short (<22.0 cm) Medium (22.0 -27.0 cm) Long (> 27.0 cm)	3 5 7	75-89	Alfa93 Amber Dolma	MS
22. (+)	Awns	Absent Present	1 9	83-87	---- Alfa93	VG
23. * (+)	Awns: type	Hooded Awnletted Normal	1 3 5	83-87	--- --- Alfa93	VG
24.	Awn: length	Short (< 8.0 cm) Medium (8.0-11.0 cm) Long (>11.0 cm)	3 5 7	83-87	BH75 BHS169 Alfa93	MS
25. * (+)	Spike: density	Lax Intermediate Dense	3 5 7	83-89	Azad, Jyoti Alfa93 Ratna	VG
26. (+)	Grain: hullness	Naked (hulless) Covered (Hulled)	1 9	87-92	Dolma Alfa93	VS
27. *	Grain: color	White Yellow Purple Black	1 2 3 4	92	Dolma Alfa93 Bilara2, Jyoti ----	VG
28. * (+)	Grain: shape	Oval Oblong Elongated	1 5 7	92	Alfa93 BHS169 Amber	VG
29. * *	Grain: size (1000 grain weight)	Small (<30g) Medium (30-40g) Large (41-50g) Very large (>50g)	1 3 5 7	92	Dolma Alfa93 Amber BCU73	MS
30. (+)	Grain: surface	Smooth Wrinkled	1 9	92	Amber JB58	VG
31. (+)	Rachilla hairs	Rudimentary Prominent	1 9	92	Alfa93 BHS46	VS
32. * (+)	Grain: Crease width	Narrow Intermediate Wide	3 5 7	92	BHS169 Alfa93 -----	VS

VIII. Explanations on the Table of characteristics-

Characteristic 1. Growth habit

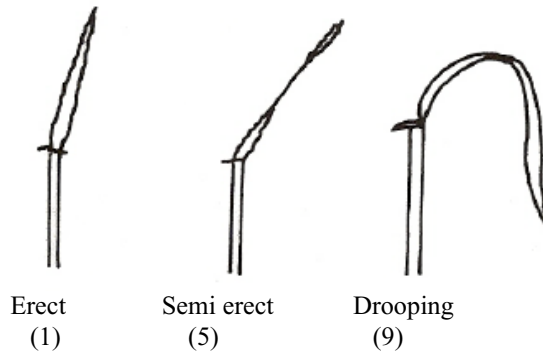


Erect



Semi-prostrate

Characteristics 5: Flag leaf attitude

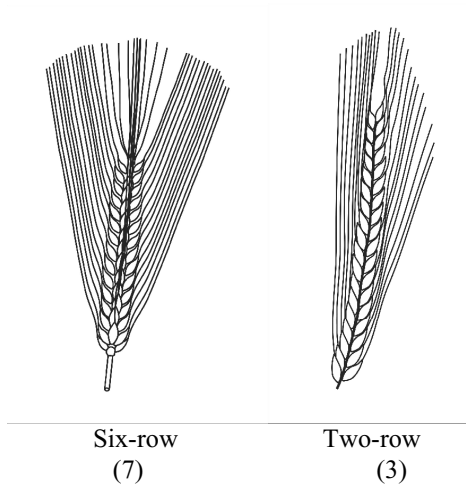


Erect



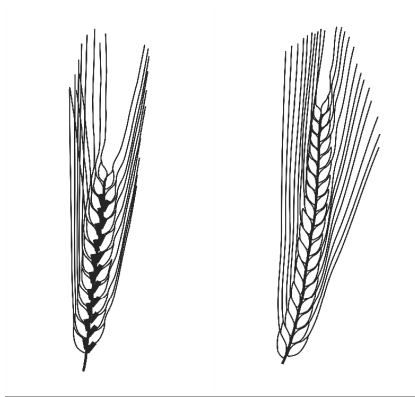
Drooping

Characteristics 8: Spike type



Six-row Two row

Characteristics 9: Lateral florets (Two-row barley)



Developed
(9)

Rudimentary
(1)



Developed

Rudimentary

Characteristics 10: Spike waxiness



Waxy



Non-waxy

Characteristics 12: Spike attitude



Erect



Drooping

Characteristics 18: Lemma pigmentation



Pigmented



Nerve pigmentation



Absent

Characteristics 22: Awns



Absent
(1)



Present
(9)

Characteristics 23: Awns: Type



Hooded
(1)



Awnleted
(3)



Normal
(5)



Hooded

Awnleted

Normal

Characteristics 25: Spike: density



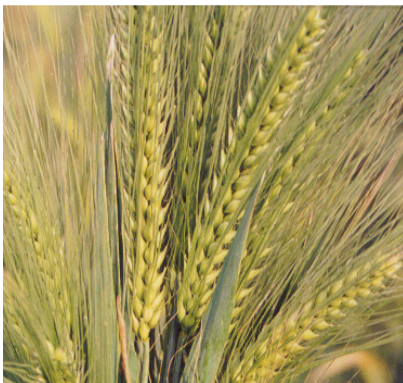
Lax
(3)



Intermediate
(5)



Dense
(7)



Lax



Dense

Characteristics 26: Grain hullness



Naked



Hulled

Characteristics 28: Grain: Shape



Oval
(1)



Oblong
(5)



Elongated
(7)



Oval



Oblong



Elongated

Characteristics 30: Grain surface

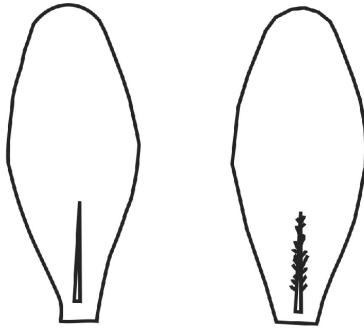


Smooth



Wrinkled

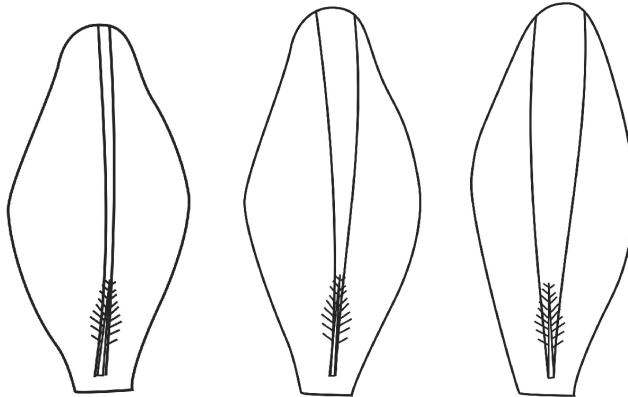
Characteristics 31: Rachilla hairs



Rudimentary
(1)

Prominent
(9)

Characteristics 32: Grain crease width



Narrow
(3)

Intermediate
(5)

Wide
(7)

IX. Biochemical characters (Additional character)

Procedure for estimation of hordein profile:

Hordeins are the major storage proteins of barley endosperm. They are extremely heterogeneous in composition in the different barley cultivars, allowing the differentiation of genotypes by their protein electrophoretic patterns. In general, barley hordeins are divided into A, B, C and D groups on the basis of the molecular weight differences due to their amino acid compositions. Since hordein composition is normally not affected by environmental factors (e.g. growing location, soil types, fertilization level, etc.), hordein electrophoretic composition could be used as genetic character for cultivar identification. By far, poly-acrylamide gel electrophoresis (PAGE) is the most common methods for the protein separation, characterization and varietal identification in barley (Heisel *et al.* 1986).

Heisel S E, Peterson D M, and Jones B L. 1986. Identification of United States barley cultivars by sodium dodecyl sulfate poly-acrylamide gel electrophoresis of hordeins. *Cereal Chem*, **63**:500-505.

Annexure-I System for growth stages in barley

Zadok's code		Description
Principal stage	Secondary stage	
0		Germination
	0	Dry kernel
	1	Start of imbibition (water absorption)
	5	Radical emerged
	7	Coleoptile emerged
	9	Leaf just at coleoptile tip
1		Seeding development
	0	First leaf through coleoptile
	1	First leaf at least 50% emerged
	2	Second leaf at least 50% emerged
	3	Third leaf at least 50% emerged
	4	Fourth leaf at least 50% emerged
	5	Fifth leaf at least 50% emerged
2		Tillering
	0	Main shoot only
	1	Main shoot plus 1 tiller visible
	2	Main shoot plus 2 tillers
	3	Main shoot plus 3 tillers
	4	Main shoot plus 4 tillers
	5	Main shoot plus 5 tillers
3		Stem elongation
	1	First node detectable
	2	Second node detectable
	3	Third node detectable
	7	Flag leaf just visible
	9	Flag leaf collar just visible
4		Boot
	1	Flag leaf sheath extending
	3	Boot just beginning to swell
	5	Boot swollen

	7	Flag leaf sheath opening
	9	First awns visible
5		Head emergence
	1	First spikelet of head just visible
	3	One-fourth of head emerged
	5	One-half of head emerged
	7	Three-fourths of head emerged
	9	Head emergence complete
6		Flowering (not readily visible in barley)
	1	Beginning of flowering
	5	Half of florets have flowered
	9	Flowering complete
7		Milk development in kernel
	1	Kernel watery ripe
	3	Early milk
	5	Medium milk
	7	Late milk
8		Dough development in kernel
	3	Early dough
	5	Soft dough
	7	Hard dough, head losing green color
	9	Approximate physiological maturity
9		Ripening
	1	Kernel hard (difficult to divide with thumbnail)
	2	Kernel cannot be dented by thumbnail, harvest ripe



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