Re-classification of developmental stage of wheat grain

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Summary

Developmental stages of wheat seed that were identified easily have been classified by two different ways; one based on the endosperm development (six stages of watery, milky, soft dough, hard dough, ripe, and dead ripe) and the other based mainly on the embryo development (five stages of I to V). We examined these two types of the developmental stages by observing embryo and endosperm development, and appearance of seeds. Since we find characteristic developmental stages of wheat seed in respects of dry weight increase and morphological development of embryo and endosperm, we propose the following six development stages. At stage 1 (from fertilization to days post anthesis (DPA) 5), the embryo is globular and do not yet differentiate. The endosperm grows most rapidly. The seeds are white green and less than 4 mm in length. At stage 2 (from DPA 5 to 10), the embryo differentiates into embryo axis and scutellum. The endosperm still grows rapidly. At stage 3 (from DPA 10 to 15), the scutellum elongate. Dry weight of the seeds increases most rapidly. The endosperm becomes milky. At stage 4 (from DPA 15 to 20), the primary leaf, root and coleoptile differentiate in the embryo axis. The size of the endosperm and consequently the size of the seed reach their maximum. The seeds become fresh green. At stage 5 (from DPA 20 to 30), the embryo differentiates further and its dry weight increases most rapidly. The reserve accumulation in the endosperm shows the second peak. The endosperm has a soft dough consistency. The seeds show yellowish part. At stage 6 (from DPA 30 to 50), the seeds desiccate and turn brown. The stages of hard dough, ripe, and dead ripe are included in the stage 6.

Key Words : *Triticum aestivum* L., wheat, seed, developmental stage, morphology.

Introduction

Morphological and developmental studies of wheat seed have been carried out extensively due to its economic importance. Bradbury (1956a, b) reported the basic morphology of mature wheat seeds. Development of the endosperm has been also studied histologically and cytologically (Evers 1970, Mares *et al.* 1975, Morrison and O'Brien 1976, Morrison *et al.* 1978, Briarty *et al.* 1979, Campbell *et al.* 1981, Bechtel *et al.* 1982, Huber and Grabe 1987). There have been also many studies on the

development of wheat embryos (Percival 1921, Hoshikawa 1961a, Bennett *et al.* 1973 1975, Smart and O'Brien 1983). Hoshikawa (1961a) divided seed development into eleven embryonic stages. However, these stages were not characterized by the appearance of seed, such as color and size and not handy to use for collecting seeds at the same stage. To identify the development stage of the seed, agronomists tend to use the other classification based mainly on the starch accumulation in the endosperm; six stages such as watery, milky, soft dough, hard dough, ripe, and dead ripe (Peterson 1965, Zadoks *et al.* 1974).

However, recent research on the development of wheat seed in molecular level requires the classification that are based on the embryo and endosperm development, and can be used easily. Rogers and Quatrano (1983) studied the developmental process of wheat seed and proposed the five stages. Their stages reflects well to wheat seed development. However, as the development of the embryo and endosperm appear to be more characteristic than those of Rogers and Quatrano (1983), we re-examined the development of wheat seed and proposed six developmental stages.

Materials and Methods

Spikes of *Triticum aestivum* L. cv. Chihokukomugi (Chihoku) grown in a greenhouse were tagged at anthesis. Seeds were collected 5, 10, 15, 20, 30, 40, and 50 days post anthesis (DPA). Plants were raised in clay pots (diameter 6.5 inches) with sterilized soil including compost. Temperature of the greenhouse was maintained between 20 °C and 30 °C after anthesis. Thirty seeds of

Table 1. Plant growth condition and method to estimate developmental stages of wheat seeds in the following reports

Authors	Variety Used	Growth Condition	Stage Estimation
Hoshikawa (1961)	Norin 61	field	LM observation of paraffin section
Evers (1970)	Svenno, Caesar	?	LM observation of paraffin section
Bechtel <i>et al.</i> (1982)	Eagle	field	LM and EM observation
Rogers and Quatrano (1983)	Chinese spring	green house	LM observation of paraffin section measurement of seed and embryo size
Huber and Grabe (1987)	five cultivars	field	LM observation of paraffin section.

LM : light microscope, EM : electron microscope.

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the first and second florets at different developmental stages were used to measure the seed length, and fresh and dry weights of seed and embryo in three replications, but weights of embryo were measured once. The embryos were dissected out by a blade for vaccination or a disposable blade (Feather No. 11, Feather Co. LTD). The water content of seeds and embryos were estimated after the desiccation of 30 seeds and embryos in an oven at 130 °C for 1 h.

Developing seeds were freeze-sectioned into 10 μ m thick slices by a cryostat microtome, and the sections were double-stained with Feulgen and Fast-Green. Before sectioning, seeds were fixed with 2 % para-formaldehyde, 1 % glutaraldehyde at 4 °C for 7 to 20 h, depending on their water content. The lengths of embryo axis and the scutellum were measured in the longitudinal sections of three embryos by a micrometer in a microscope (10 μ m). To compare the present experiment condition to that of the other reports related to wheat seed development, the conditions of the five papers cited in this paper were listed in table 1.

Results and Discussion

Differentiation of embryo

The embryo was globular in shape, and did not show differentiation of tissues until DPA 5 (Fig. 1A). After DPA 5, the embryo axis and scutellum differentiated (Fig. 1B). Between DPA 10 and 15, the scutellum grew extensively (Table 2, Fig. 2 A). At DPA 15, the radicle and first leaf, and coleoptile were observed in the embryo axis (Fig. 1C). The epithelium of the scutellum did not differentiate yet. Between DPA 15 and 20, the length of embryo axis increased rapidly (Table 2, Fig. 2 A). At DPA 20, the epithelium and vascular system developed in the scutellum (Fig. 1D). After DPA 20, the embryo axis differentiated further. The dry weight of the embryo increased rapidly and its length reached their maximum at DPA 30 (Tables 2 and 3, Fig. 2B). The embryo appeared to have differentiated fully at DPA 30 (Fig. 3A). These observations agree well with the description of embryo development by Rogers and Quatrano (1983).

Table 2. The length (mm) of seed, scutellum, and embryo axis of *T. aestivum* L. cv. Chihoku during seed development

DPA	Seed Length	Scutellum Length	Embryo axis Length
5	4.15(0.39)	0.14(0.03)	0.14(0.03)
10	5.93(0.17)	0.80(0.13)	0.46(0.04)
15	6.97(0.25)	1.89(0.29)	1.18(0.34)
20	6.62(0.28)	2.40(0.10)	1.99(0.08)
25	6.66(0.23)	2.40(0.08)	2.01(0.10)
30	6.68(0.16)	2.54(0.12)	2.19(0.05)
40	6.27(0.30)	2.40(0.12)	1.98(0.10)
50	6.05(0.10)	2.42(0.07)	1.98(0.04)

(): standard deviation.

Development of endosperm

A small amount of starch granules was noted in the distal region of the seed at DPA 6-7 (Fig. 3 C). Hoshikawa (1961b) observed starch granules in the endosperm at DPA 5. Bechtel *et al.* (1982) reported the protein bodies in the endosperm at DPA 6-7. These observations show that starch and protein start to accumulate at a relatively early stage of the endosperm development.

Seed length increased rapidly between DPA 5 and 10 and reached its maximum at DPA 15 (Table 2, Fig. 2 A). Evers (1970) and Huber and Grabe (1987) reported that cell division of the endosperm tissue continued until about DPA 15. Differentiation of the aleurone cell was detected at DPA 12 (Fig. 3B) as reported by Huber and Grabe (1987).

Accumulation of the reserves in the endosperm and consequent rapid increase of dry weight of the seed were observed between DPA 10 and 15 (Table 3, Fig. 2 B). At this stage, the endosperm was milky. After DPA 15, the fresh weight increased gradually, and starch accumulation continued. The endosperm has a soft dough consistency. In the period DPA 25-DPA 30, the reserve accumulation showed the second peak (Fig. 2B). At DPA 30, the fresh and dry weight reached a maximum (Table 3), which corresponded to the physiological maturity as reported by Hanft and Wych (1982). After DPA 30, the endosperm become harder and reached the hard dough stage. Desiccation started and consequently the fresh weight of seed decreased (Table 2). Dry weight of seed also decreased slightly after DPA 30 as Hanft and Wych (1982) and Lingle and Chevalier (1985) observed during desiccation period, although Rogers and Quatrano (1983) noticed dry weight increase until DPA 50. It is not clear why Rogers and Quatrano (1983) observed the reserve accumulation in the desiccation period. Growth condition of plants might be one of the factors that make a period of assimilation transfer change.

Table 3. Fresh weight (mg), dry weight (mg) and water content (%) of *T. aestivum* L. cv. Chihoku seed and embryo

DPA	Seed			Embryo		
	Fresh Wt.	Dry Wt.	Water Cont.	Fresh Wt.	Dry Wt.	Water Cont.
5	12.9 (1.0)	3.0 (0.2)	76.7			
10	28.3 (2.7)	7.0 (0.8)	75.3			
15	52.6 (1.5)	16.4 (2.0)	68.8	0.60	0.20	66.6
20	53.7 (4.3)	18.7 (1.7)	65.2	1.70	0.57	66.5
25	58.4 (4.5)	23.4 (1.4)	59.9	2.90	1.20	58.6
30	64.0 (6.4)	29.7 (0.9)	53.6	3.60	1.55	56.9
40	44.7 (3.6)	29.4 (2.5)	34.2	2.70	1.66	40.7
50	29.7 (6.8)	26.6 (5.8)	10.4	1.60	1.50	6.3

(): standard deviation.

Fig. 1. Embryo development of wheat seed (*T. aestivum* L. cv. Chihoku) after anthesis.
A, DPA 5; B, DPA 10; C, DPA 15; D, DPA 20; E, DPA 30; F, DPA 50.
e : embryo, ea:embryo axis, sc:scutellum, r:root, co:coleoptile
— : 0.1 mm for A,B and C, 0.5mm for C,D and E.

Seed appearance

The ovary just before anthesis was white and about 2 mm in length (Fig. 4A). At DPA 5, the seed became white-green and its length increased to about 4 mm (Fig. 4B, Table 2). At DPA 10, the seed became about 6 mm long, and its color was similar to that at DPA 5 (Fig. 4C). Between DPA 15 and 20, the seeds showed a fresh green color. The embryo developed and formed a bump at the end of the seed (Fig. 4D). The length of the seed reached its maximum of about 7 mm. At DPA 25, the embryonic part was clearly visible from outside. The seeds show yellow part in the dark green background (Fig. 4E). After this stage, the seeds lost their green color and became yellow at DPA 30 (Fig. 4F). At DPA 40, the seeds were brown and became smaller (Fig. 4G). Around this stage, the seeds ripened. As desiccation proceeded, the seed became smaller and harder, and reach to the dead ripe stage (Fig. 4H). The present observation agrees with the seed appearance reported by Rogers and Quatrano (1983).

From these observations, an outline of wheat seed de-

velopment is generally accord to that of Rogers and Quatrano (1983). However, there is a characteristic pattern of the reserve accumulation in the endosperm and dry weight increase of the embryo between DPA 10 and 30 (Fig. 2), which were physiologically distinctive phase, and have not noticed before. It is necessary to include this phenomenon in classification of the seed development. So, we propose to classify the seed development into the following six stages (Table 4).

Proposal of classification of seed formation.

Stage 1 (from fertilization to DPA 5): The embryo shows globular shape and no differentiation. The seeds are white green and less than 4 mm in length.

Stage 2 (from DPA 5 to 10): The embryo axis and scutellum differentiate and grow. The endosperm develops most rapidly.

Stage 3 (from DPA 10 to 15): The scutellum elongate. The aleurone starts to differentiate. Accumulation of the reserve in the endosperm show a first peak. The endosperm becomes milky.

Stage 4 (from DPA 15 to 20): The embryo axis differentiates coleoptile, radicle and first leaf along with the

Fig. 2. Increase of lengths of seed, scutellum and embryo axis (A), and of dry weights of seed and embryo (B) during seed development.

Fig. 3. Embryo and endosperm of *T. aestivum* L. cv. Chihoku seed during their development.

A : Embryo at DPA 30,

B : Aleurone layer cells developing at DPA 12

C : Starch grains in the distal region of the endosperm at DPA 7.

rc : root cap, rs : root sheath or coleorhiza, r : root, e : epiblast,

1l : first leaf, 2l : second leaf, co : coleoptile, al : aleurone,

v : vascular bundle of scutellum, ep : epithelium of scutellum

— : 0.1 mm for A, 0.02 mm for B and C.

Table 4. Developmental stages of *T. aestivum* L. cv. Chihoku seed.

Stage	1	2	3	4	5	6
DPA	0 – 5	5 – 10	10 – 15	15 – 20	20 – 30	30 – 50
Embryo	Globular		Scutellum Development	Embryo Axis Development	Dry weight Increase	Desiccation
Endosperm		Development		Dry Weight Increase		Desiccation
			1st peak	2nd peak		
DPA	0 – 10		10 – 20		20 – 30	30 – 35 35 – 40 40 – 50
Endosperm stage	Pre-milk		Milk		Soft Dough	Hard Dough Ripe Dead Ripe
	Cell Division		Reserve Accumulation		Physiological Maturity	Desiccation

increase of its size. Epithelium and vascular system develop in the scutellum. The increase of the embryo size is observed as a bump at the end of the seed. The seed size reaches its maximum. The seeds become fresh green.

Stage 5 (from DPA 20 to 30): Embryo axis differentiates further and its dry weight increase very rapidly. The reserve accumulation in the endosperm show a second peak, and results in soft dough consistency. The

seeds become yellow.

Stage 6 (from DPA 30 to 50): No differentiation of the embryo and no accumulation of the reserve in the endosperm. The endosperm reaches the hard dough stage. The seeds desiccate and become brown and smaller. Its weight decreases.

In comparison with those of Rogers and Quatrano (1983), the present stages 2, 3, and 4 correspond to the stage II and III of Rogers and Quatrano (1983). Also,

Fig. 4. Appearance of wheat seed (*T. aestivum* L. cv. Chihoku) during seed development. A, Ovary just before anthesis. B-H, Seed at DPA 5, 10, 20, 25, 30, 40, and 50. — : 1 mm

compared with the classification of Peterson (1965), the stages 1 and 2 correspond to the pre-milk stage, and the stage 3 and 4 to the milk stage. The soft-dough, hard-dough, ripe, and dead-ripe stages are grouped into the stages 5 and 6 (Table 4).

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