# Seed development stages of kale (*Brassica oleracea* var. *acephala* L.) genotypes in Turkey

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**ABSTRACT**: This research determined the stages of kale (*Brassica oleracea* var. *acephala* L.) seed development in Samsun province, Turkey, between 2002 and 2003. Plants of inbred kale lines (55TE07, 55TK09, 52PE09, 61ÇY01 and 67DE01) were grown. On the basis of seed and embryo colour, seed morphology (seed length, seed width and embryo length), seed development can be divided into 7 discrete but contiguous stages. The germination rate of inbred lines was at its maximum, or near to its maximum, until the seed moisture declined to 50%. Germination rates reached a maximum level at 65 days after pollination (DAP). It was concluded that kale seeds should be harvested when pods became brown in stage 7 (65 DAP). This study will be used for comparisons with seed development in other *Brassica* species.

**Keywords**: *Brassica oleracea* var. *acephala*; kale; seed development; germination

Seed is a primary and essential starting point for a wide range of horticultural crops, including the majority of vegetables and many annual and biennial ornamentals. The seed phase of the plant life cycle commences with fertilization, followed by embryogenesis, maturation, dehydration and imbibition and terminates with germination (Chaudry et al. 2001). Seed development is affected by a variety of factors, including genetic, physiological and environmental elements, and the changes which occur cannot be correlated simply with time (days) after pollination (or anthesis). Due to the importance of these factors to harvestable yield in crops where the seed is the commodity, much is known about their influence on seed yield (Robani 1992; Bradford 2004). Each crop species undergoes characteristic changes leading to seed ripening which must be understood to establish the best time to harvest (Copeland, Mc Donald 1985). Successful seed production depends on thorough knowledge of the reproductive processes of a particular crop. Optimal timing of harvest is a prerequisite for the production of the maximum number of high quality seeds. Seed crops should be harvested when the physiological quality of seed is maximal. Research on seed development and maturation has revealed a great variability in seed quality. In Chinese cabbage (Ren, Bewley 1998), maximum seed quality occurred at the end of the seed-filling period, which has been described as physiological maturity for a long time (Harrington 1972). Elias and Copeland (2001) determined physiological and harvest maturity of canola (*Brassica napus* L.) cultivars using morphological and physiological markers. Canola attained physiological maturity when pods turned from green to greenish-yellow or light brown, and contained seeds ranging in colour from brownish-green to greenish-brown and light brown.

Vegetable brassicas are an important and highly diverse group of crops grown world-wide that belong mainly to the species *Brassica oleracea* and *Brassica campestris (*Monteiro, Lunn 1998*).* In Turkey, there is a group of grown local and imported vegetables which belong to *Brassica* spp., and in particular six *Brassica* species (kale, cauliflower, cabbage, Brussels sprouts, kohlrabi, broccoli). *Brassica oleracea* is a native of the Mediterranean region. Vegetable brassicas have been cultivated in Europe since very ancient times from where they have spread to other parts of the world (Nieuwhof 1969).

Kale is a biennial crop when grown for seed. There has been no detailed examination of the development of seed quality attributes during maturation in

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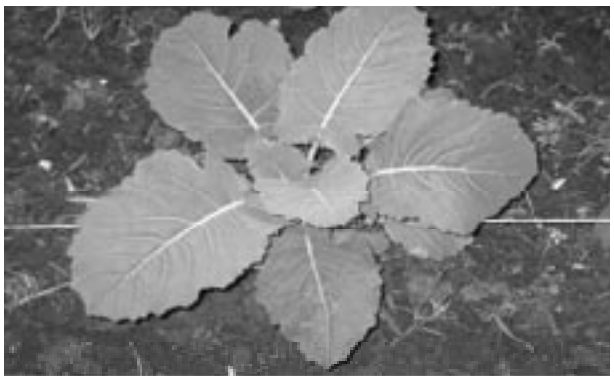

Fig. 1. The view of local kale (*Brassica oleracea* var. *acephala* L.) plant

kale. The identification of distinct morphological and physiological characteristics is an important initial step in the study of seed development in a species. Hence for researchers to be able to compare data on seed development, reference points other than time after pollination are essential (Ren, Bewley 1998). As a prelude to the study of kale seeds, it was necessary to identify the distinct morphological characteristics of each stage and to define the stages of seed development chronologically.

## MATERIAL AND METHODS

### Plant husbandry and seed harvest

This research was carried out in the field and in the laboratory of the Department of Horticulture, Faculty of Agriculture, Ondokuz Mayis University, from September 2002 to October 2003. There are no registered kale varieties in Turkey (Anonymous 2004). Therefore, local kale populations are commonly used in kale production (Fig. 1). For this reason, plants of inbred kale lines (55TE07, 55TK09, 52PE09, 61ÇY01 and 67DE01) developed by the breeding studies of Balkaya et al. (2001) were used in this research.

Seeds were sown in seedling trays on 5 September 2002 and seedlings were transplanted on October 10 when they reached the stage of 4–5 expanded leaves. Plant spacing was 40 cm between rows and 30 cm within rows. Standard fertilization and weed control practices were used.

Inbred lines (8 plants) were cultivated in an isolation cage. The plants began flowering in April–May. Each genotype was openpollinated under isolation. Although the selected inbred lines required the same length of time to complete the various stages of seed development, there were some variations among seeds on the same plant or even within a single silique. Siliques were harvested at intervals of 3 days after anthesis. Thus, seeds at different development stages were collected at intervals of 3 days, according to their DAP and then selected by their colour and size, according to staging standards.

### Measurement of the physical characteristics of seeds and embryos

Developing seeds at different stages were collected and the characteristics of representative seeds and embryos were described. The siliques used to determine the seed characteristics were immersed in an FAA solution prepared with 20 cc formaldehyde, 25 cc glacial acetic acid and 390 cc ethyl alcohol, made up to 1 litre with distilled water and stored in the refrigerator (Kavak, Yanmaz 1999).

Maximum diameter and length of seed and embryo length were measured with a micrometer for all seed development stages of the kale lines (Oliva et al. 1988). Embryos were dissected from freshly harvested seeds at different development stages using a dissecting microscope and their morphology was noted. Illustrative photos of the samples were taken with using a photo-optic microscope (Leica DMLS).

### Germination tests

Seed germination experiments were conducted in germination cabins. Germination tests were carried out on four replications of 50 seeds of all harvests at 20°C on Watman No. 1 paper in Petri dishes 9 cm in diameter. The seeds for the germination study were observed daily, with the seed having a radicle 1 cm long being the criterion for seed germination (Şehirali 1997).

## RESULTS

### Stages of seed development in kale

Using the criterion of the colour of seeds and embryos and morphology of the embryos, seed development was divided into seven discrete stages. Stages of developing seeds are shown in Fig. 2.

**Stage 1**: This period was identified as the preembryo period, from 1 to 10 DAP. It was observed by the binocular examination that the embryo was not formed at this stage. Seeds were soft, jelly-like and oval-shaped at this stage (Fig. 2a), and the seed colour was light yellow (Fig. 2b).

**Stage 2**: It was classified as the precotyledon-early cotyledon period, from 11 to 21 DAP. It was determined that the endosperm into the seed sac was growing rapidly and developed at this stage. Seeds were soft and jelly-like (Fig. 2c). The seed shape was nearly round. At this stage, embryos were too small

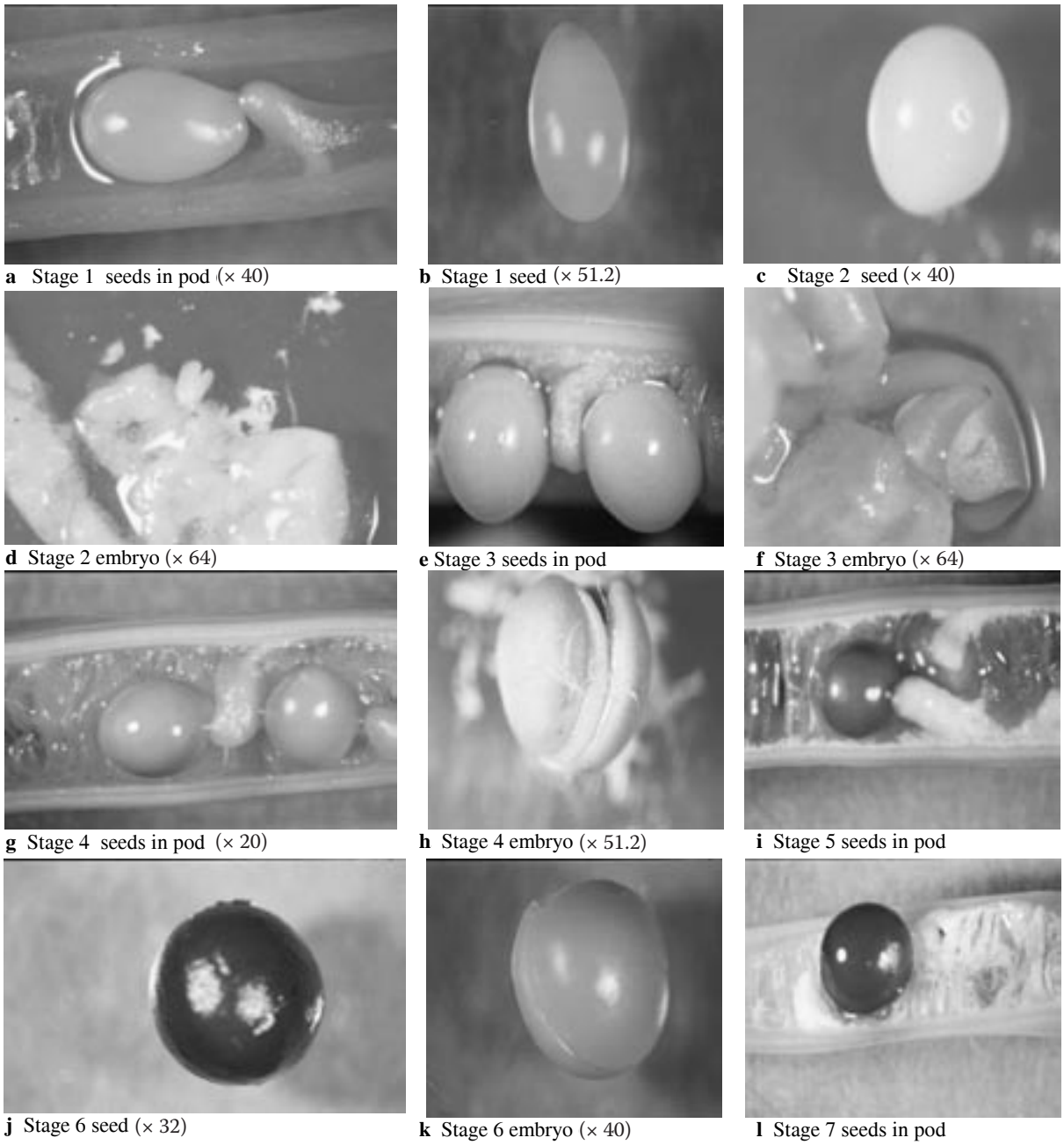


Fig. 2. Seed development stages

to be dissected and were globular or heart shaped (Fig. 2d). Seed colour bright light yellow as in stage 1.

**Stage 3**: During this period, the radicle and cotyledons began to develop and it was defined as the early cotyledon stage. This period was from 22 to 24 DAP. The seed was nearly round, jelly-like and yellow colour (Fig. 2e). The colour of the embryos was dull green tones (Fig. 2f).

**Stage 4**: This was the period of embryo enlargement, from 25 to 35 DAP. In this stage, it was determined that seeds were becoming less jelly-like (Fig. 2g). Seed shape was round and embryo colour was green tones. At this stage, the embryo was at its largest (Fig. 2h). This was the main stage for the accumulation of storage reserves in the embryo. Storage reserves were accumulated in the radicle and cotyledons (Demir 2004).

**Stage 5**: This period was identified as the period of the starting of seed maturation. This was the first stage of filling and maturation, from 36 to 43 DAP. The seed coat firmed in this stage and colour turned to brown from yellowish (Fig. 2i). Embryos lost their flexibility at this stage. The colour of embryos was beginning to turn yellowish from dull green.

**Stage 6**: This period was the second phase of filling and maturation and was from 44 to 49 DAP. The colour of the seed coat was turning to dark red or dark brown (Fig. 2j), and the embryo was dark yellow (Fig. 2k).

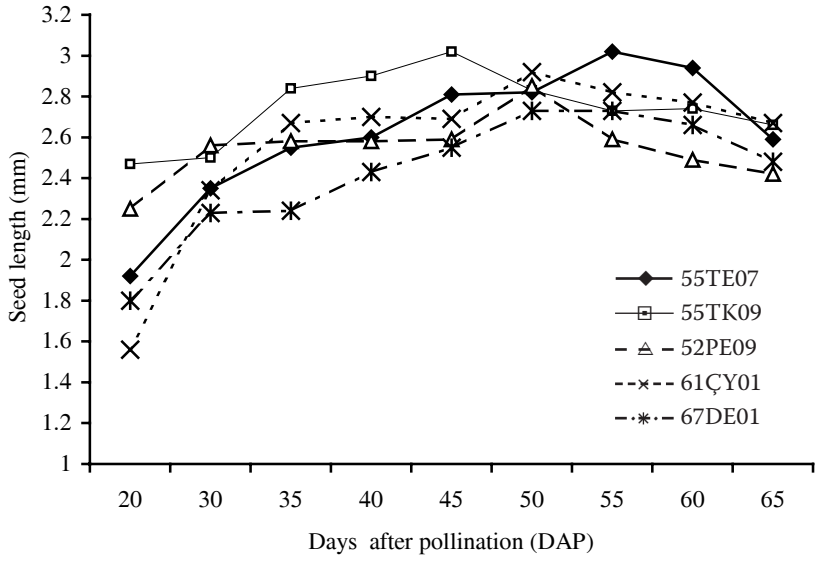


Fig. 3. Changes in seed length (mm) of kale seed during development

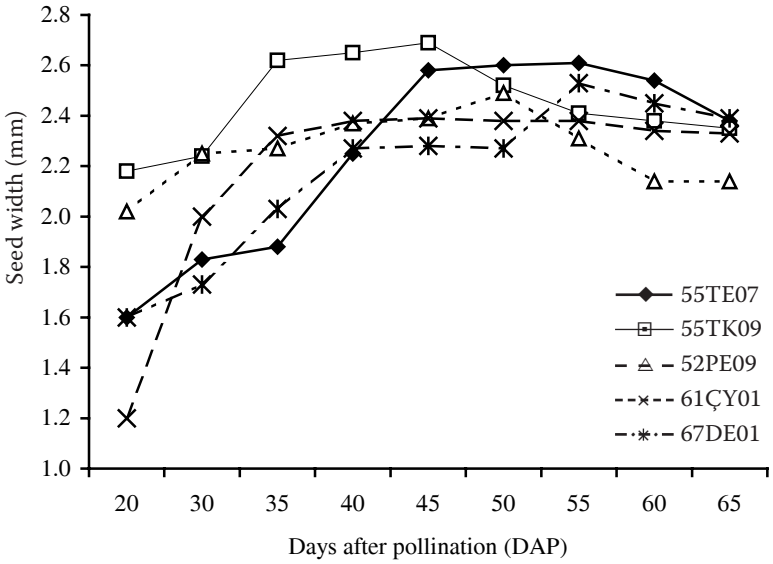


Fig. 4. Changes in seed width (mm) of kale seed during development

**Stage 7**: This period was the third phase of filling and maturation, namely desiccation, and was from 50 to 68 DAP. In this stage, the seed funicle into the pod was getting desiccated. The seed coat colour was brownish-red, and the hilum was brown and black tones (Fig. 2l), and the embryo colour was yellowish-orange.

### Seed and embryo measurement results

Maximum diameter and length of each seed for all seed development stages (Figs. 3 and 4) was measured with a micrometer. Seed length of inbred lines increased rapidly between 45 and 50 DAP. After the 50$^{th}$ day, there was a horizontal trend up to the field maturation stage, and afterwards a partial decrease in seed length. This may be attributed to a decrease in the moisture content of the seed. Seed width measurements were similar to seed length measurements. The highest seed width value for each type was obtained at a different duration. The highest seed width values were obtained for 55TE07, 67DE01, 52PE09, 55TK09 and 61ÇY01 types at the 40$^{th}$, 45$^{th}$, 50$^{th}$, 55$^{th}$ day, respectively (Fig. 4). Embryo measurements indicated that embryo length increased to a certain level and afterwards remained constant (Fig. 5). Embryo length reached its highest value at the 55$^{th}$ day for all types.

### Seed germination results at the various stages of seed development

Seed viability developed from 30 to 32 DAP in inbred kale lines. Germination rates increased rapidly from 40 DAP. Germination rates were above 90%, except for 55TK09 at 60 DAP, and reached the maximum level at 65 DAP (Fig. 6). At 70 DAP, seeds in which the moisture content had declined below

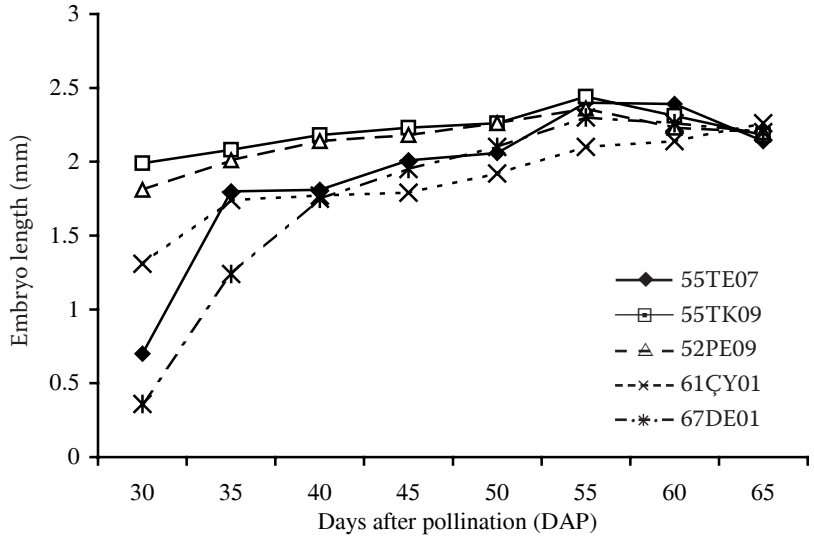


Fig. 5. Changes in embryo length (mm) of kale seed during development

10% were not tolerant of dehydration and germination rates decreased.

## DISCUSSION

Seed maturation in kale is the process during which characteristic changes occur from the time of fertilization until the matured ovules (seed) are ready for harvest. Especially, the changes appear in morphological (seed size and seed colour), physiological (dry weight, moisture content and germination), chemical (oil, protein and carbohydrate) and functional (vigour and viability) characteristics (Şeker 2002). During the maturation, the variety and ecological, geographical and climatic factors significantly affect the changes occurring in those characteristics, and therefore each locality and each type has a characteristic seed maturation pattern. Seed maturity effects on seed quality are particularly evident in indeterminate crops where flowering and seed production extend over a long period. For example in Brassicas, flowering progresses from the base to the apex and individual inflorescences are produced at different times (Bradford 2004). Seed development is not temporally uniform in any given kale type. The identification of distinct morphological and physiological characteristics is an important initial step in understanding seed development. It is so because the determination of the exact stages of physiological maturity and harvest maturity is very important for seed producers to guarantee the highest quality and yield of kale seeds.

There are many reports in the literature on seed embryogenesis and seed development of other Brassica species but there are no previous reports on the staging of kale (*Brassica oleracea* var. *acephala*) seed development. For this reason, the development stages of kale seed were determined in this

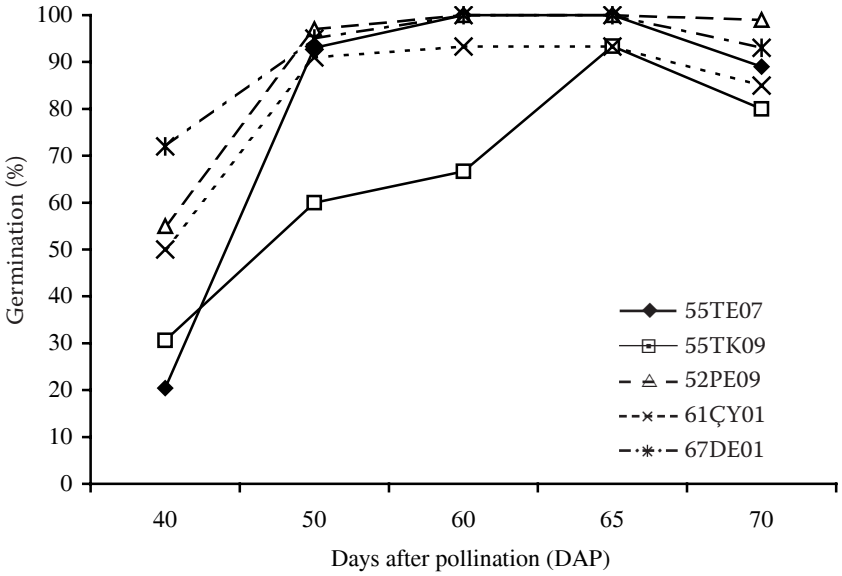


Fig. 6. Changes in germination rates (%) of kale seed during development

study. Some general staging of seed development has been reported for other closely related species or subspecies of Brassica. Lamba (1975) described the structure and development of seed in *Brassica nigra*. Dasgupta and Mandal (1993) divided seed development in oilseed mustard (*Brassica campestris*) into three stages; embryo cell division and differentiation, rapid accumulation of storage materials and dehydration. Similar stages were also reported for *Brassica napus* (Crouch, Sussox 1981; Elias, Copeland 2001; Beccacoui, Wilf 2003). Ren and Bewley (1998) divided seed development of Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) into 10 discrete but contiguous stages. In this study, we divided kale (*Brassica oleracea* var. *acephala*) seed development into 7 contiguous stages based on the morphology and colour of embryo or seed. These stages can be widely used as a uniform reference for the staging of kale seed development.

Ren and Bewley (1998) reported that seed maturation began 33–37 days after flowering in Chinese cabbage. In the current study, seed maturation began 25–35 days after flowering. The embryo fed on the endosperm and began to fill the major portion of the seed during the 4$^{th}$ stage. During the 7$^{th}$ stage, from 50 to 68 days after flowering, seeds became physiologically mature, the funicles by which they are attached to the silique began to dry and finally silique colour changed to yellowish-brown.

Ma and Zheng (1992) determined the stages of embryo development in Chinese cabbage seeds. They divided embryo development into transitional, heart shaped, torpedo-like, walking-stick-like and horseshoe stages. In other research, embryo morphology of Chinese cabbage seed during embryogenesis proceeds through the pre-embryo globular, heart shaped, torpedo and early cotyledon stages (Ren, Bewley 1998).

Embryo length increased due to development of seeds. However, there was no significant relationship between seed viability and embryo length. Increases in seed length and width values of inbred kale lines were similar to the results for onion and Chinese cabbage reported above (Ren, Bewley 1998; Yanmaz, Özçoban 2000). Seed size declined during the period of 60–65 DAP. Delay in harvest resulted not only in physiological aging but also in seed loss through shedding.

The germination rate of inbred lines was maximum or near to maximum until the seed moisture declined to 50%. The germination rate varied amongst inbred lines in this study. There are several reports that the testa structure affects seed germination behaviour (Meng 1985; Ren, Bewley 1998). The observations reported here are consistent with there being a relationship between germination and seed development stages in kale seeds.

Morphological characteristics of pods and colour of the seed are used to estimate harvest maturity in kale. It is concluded that kale seeds should be harvested when pods turn brown in colour in stage 7 (65 DAP) in Samsun ecological conditions. The result of this study will be used to make informative comparisons with development in other *Brassica* species. It is suggested that future studies work towards understanding biochemical changes during kale seed development.

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## Vývojové etapy semena u genotypů listové kapusty (*Brassica oleracea* var. *acephala* L.) v Turecku

**ABSTRAKT**: Výzkum byl zaměřen na stanovení jednotlivých etap vývoje semen listové kapusty (*Brassica oleracea* var. *acephala* L.) v provincii Samsun v Turecku v letech 2002 a 2003. V pokusech byly pěstovány inbrední linie kapusty (55TE07, 55TK09, 52PE09, 61ÇY01 a 67DE01). Na základě barvy semena a embrya a dále morfologie semena (délky semena, šířky semena a délky embrya) lze vývoj semena rozdělit do sedmi samostatných, ale navazujících etap. Klíčivost inbredních linií byla maximální nebo se blížila maximu, dokud vlhkost semen neklesla na 50 %. Procento klíčivosti dosáhlo nejvyšší hodnoty 65 dní po opylení (DPO). Došli jsme k závěru, že by se semena kapusty měla sklízet, když lusky v sedmé etapě zhnědnou (65 DPO). Studie bude využita pro kvalifikované srovnání s vývojem u jiných druhů rodu *Brassica*.

**Klíčová slova**: *Brassica oleracea* var. *acephala* L.; listová kapusta; vývoj semen; klíčivost

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