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FRESH AND DRY WEIGHT CHANGES AND GERMINATION CAPACITY OF NATURAL OR PREMATURE DESICCATED DEVELOPING WHEAT SEEDS

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Summary. Throughout the course of development wheat seeds undergo changes in both fresh and dry weight and in moisture content. Between 10 and 20 DAF, both fresh and dry weights increase very rapidly. Accumulation of dry matter ceases at the same time as the seed dried out at 40 DAF. The natural desiccation period for wheat seeds is established between 30 and 40 DAF. Freshly harvested wheat seeds are capable of germinating during the period between 20 and 30 DAF but germinating capacity is very low. Increased frequency of germination along with the increase in seed age was observed in case of artificially desiccated seeds. It is noteworthy that the capacity of desiccated seeds to germinate does not depend on the degree of desiccation but only on their age at harvest time. The amount of water absorbed by desiccated seeds during germination depends on developmental stage of seeds at harvest time. It was established that to the lower initial moisture content of desiccated seeds correspond higher amounts of water absorbed in the course of germination. Moreover for every investigated phase of seed development there is a determined degree of moisture and the water uptake of desiccated seeds can not overcome this value. Full maturity of field grown wheat seeds was reached at 50 DAF.

Key words: desiccation, germination, seed development, seed moisture

Abbreviations: ABA – abscisic acid, DAF – days after flowering

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Introduction

Seeds pass through various phases in the course of their development – histodifferentiation (initial morphogenesis), maturation (seed expansion) and maturation drying (desiccation) (Muntz, 1982; Kermode, 1990, 1995). These phases are outlined by distinctive changes in fresh and dry weight and water content (Grilli et al., 1989; Kermode, 1990). Thomas (1993) claims that morphologically and biochemically, the most dramatic events of seed development occur during maturation. During maturation, the developing seed increases considerably in volume and mass due to significant cell expansion and the accumulation and storage of proteins, lipids and carbohydrates (Long et al., 1981; Bechtel et al., 1982; Rosenberg and Rinne, 1986). The third final phase of seed development – maturation drying (desiccation) is characterized by a general reduction in metabolism as water is lost from the seed (Kermode and Bewley, 1986; Bartels et al., 1996). During this phase orthodox seeds acquire desiccation tolerance and become germinabile (Vertucci and Farrant, 1995; Ingram and Bartels, 1996). They undergo desiccation in a programmed manner at the termination of their development (Black, 1991; McCarty, 1995; Kermode, 1995; Vertucci and Farrant, 1995). Desiccation serves as a boundary between seed maturation and germination (Thomas, 1993). Little is known about the physiological regulation of the induction of desiccation tolerance. According to Black et al. (1999) the amount of water loss required to initiate tolerance has never been defined. The ability of seeds to germinate is related to developmental stage (time from anthesis), degree of desiccation and rate of the imposed drying treatment (Gosling et al., 1981). It has been shown that seeds removed in the fresh state from mother plant will germinate either after achievement of maximum dry weight, followed by the beginning of water loss or after imposed artificial drying (King, 1976; Long et al., 1981; Rosenberg and Rinne, 1986; Black et al., 1999). In the first case full germination is not achieved until the orthodox seeds have nearly completed the natural desiccation phase of their development (Dasgupta et al., 1982; Greenwood and Bewley, 1982; Kermode and Bewley, 1985; Kermode et al., 1986). But in the case of imposed drying the capacity of seeds to germinate does not require the completion of major developmental events related to storage deposition (Kermode and Bewley, 1986; Kermode, 1990). Nevertheless premature drying treatment during seed development is effective only after a specific stage of embryo development has been reached (Rosenberg and Rinne, 1986).

The aim of the present investigation was to determine at which stage of development wheat seeds acquire desiccation tolerance and become capable to realize the transition from developmental to germination programme and growth of seedling. Data are presented about the changes in fresh and dry weights and water content in the course of wheat seed development and their germination capacity after natural or premature desiccation.

Materials and Methods

Plant material

Wheat (*Triticum aestivum* L. cv. Sadovo 1) seeds were used in this study and plants were grown to maturity in a field environment. During plant development, the age of seeds was monitored and expressed as days after flowering (DAF). Seeds at 10, 20, 30, 40 and 50 DAF were analyzed.

Determination of seed weights and moisture

Seed development was followed by assessment of seed weight and moisture content. Calculations of seed fresh weight, dry weight and moisture content were based on weights determined before and after oven drying seed samples at 80 °C for 24 h.

Desiccation procedure

In some cases samples of seeds were dried artificially to a water content of 64.6–8.6% by keeping the grains in open petri dishes at 4 °C temperature in the refrigerator for 1–7 days.

Germination and seedling growth testing

Fresh harvested seeds at various DAF and desiccated seed lots were surface sterilized in a solution containing 1% w/v $Ca(OCl)_2$ for 10 min and washed 5 times with sterile water. Germination (48 h) and seedling growth testing (7 days) was carried out in a constant environmental chamber at 24 °C in the dark. Triplicate samples of 100 seeds were imbibed in filter paper roles. Germination (radicle protrusion) and seedling growth were evaluated daily for each sample at the end of 7 days. Seed germination was defined as the appearance of a radicle 0.5 cm in length. Seedling growth was defined at the same time as the appearance of a radicle greater than 0.5 cm in length.

Percent of germinated seeds, growth rate of seedlings, fresh and dry weights and moisture content of freshly harvested seeds, germinating freshly harvested seeds, artificially dried seeds and germinating artificially dried seeds were determined.

Results and Discussion

Determination of grain age by days past anthesis is considered as an inaccurate marker of seed development as the environment in which the plants are grown affects the rate of grain development and maturation. King (1976) and Symons et al. (1983) proposed percentage grain moisture to be used as an indication which correlates better with physiological development. Such approach enables direct comparisons to be made between results reported by different investigators. Based on these considerations in the present investigation stage of wheat seed development was determined by measuring seed moisture content.

Maturation in cereal seeds occurs midway or late in seed development as ABA levels rise (Grilli et al., 1989; Lopes and Larkins, 1993). Our results show that full maturity of field grown wheat seeds was reached at 50 DAF. This period is shorter compared with that of harvest ripeness (60 days) established by King (1976) and longer in comparison with the period (34 DAF) determined by Bechtel et al. (1982) for wheat seeds. The differences might be explained with the results of Symons et al. (1983) showing that the environment in which the plants are grown affects rate of grain development and maturation.

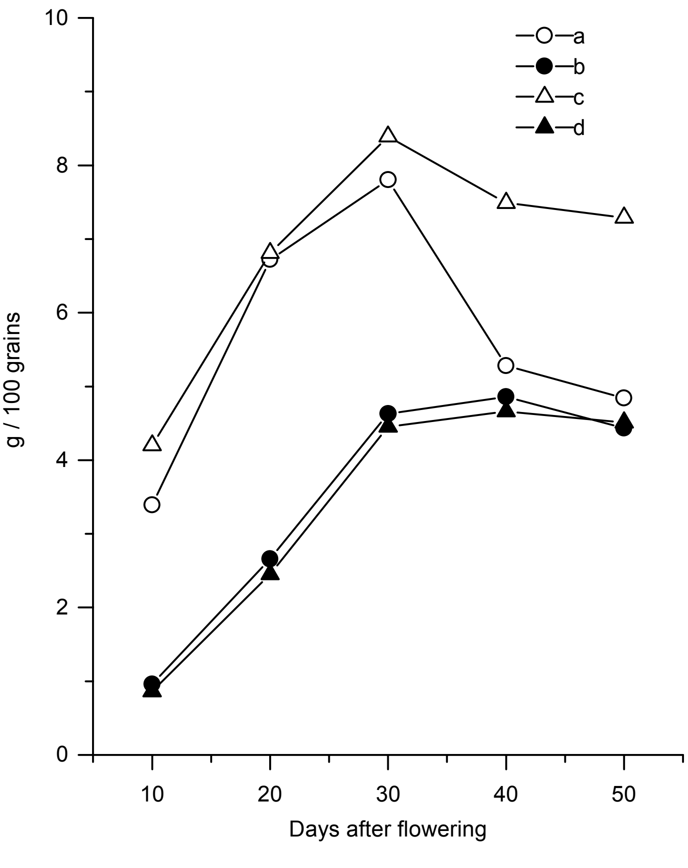

Fig. 1. Fresh (a, c) and dry (b, d) weight of freshly harvested (a, b) and germinating freshly harvested (c, d) seeds of wheat in the course of development

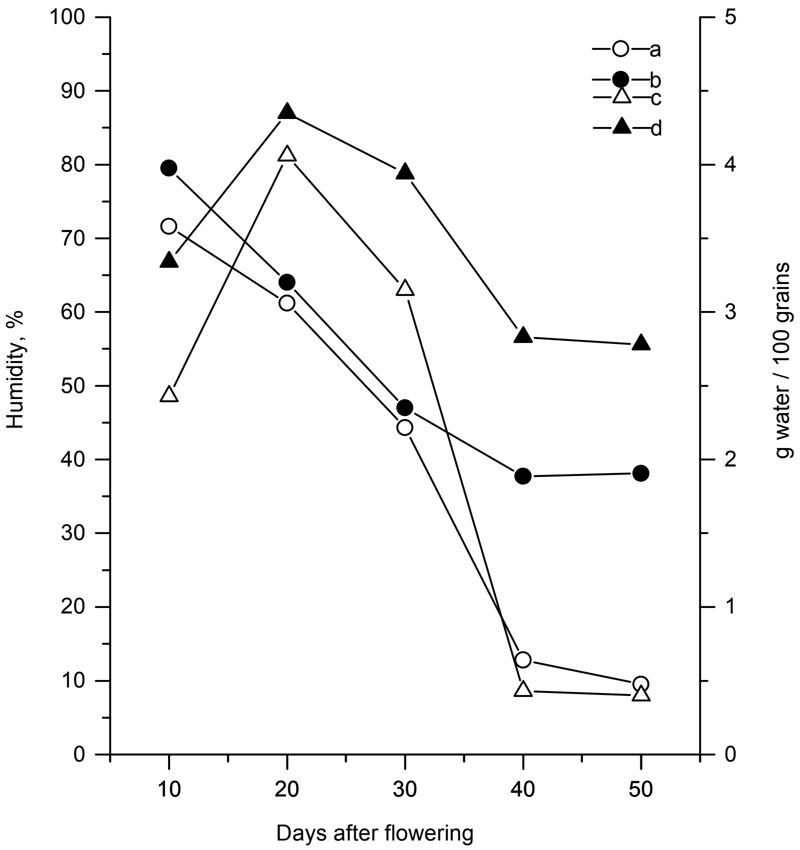

Fig. 2. Percentage (a, b) of moisture content and water content (c, d) of freshly harvested (a, c) and germinating freshly harvested (b, d) developing wheat seeds

Throughout the course of development wheat seeds undergo changes in both fresh and dry weight (Fig. 1). Between 10 and 20 DAF, both fresh and dry weights increase very rapidly. Fresh weight increases two fold due to simultaneous accumulation of dry mass and water uptake (Fig. 2). In developing seeds fresh weight continues to increase slowly between 20 and 30 DAF. This enhancement is a result of dry mass accumulation. During this period reserve material accumulation occurs with such intensity that fresh weight goes on increasing in spite of the decrease in water content which has already begun. Concerning dry weight of the seeds five times enhancement was observed between 10 and 30 DAF due to cell expansion and the accumulation of reserve materials mainly by the endosperm. At about 40 DAF, dry weight reaches a constant value while fresh weight rapidly declines from 30 DAF. At the last developmental phase (50 DAF) the values of all investigated parameters resemble those for 40 DAF. Accumulation of dry matter ceases at the same time as the seed dried out at 40 DAF. However in some cases reported by King (1976) dry matter accumulation ceases 5–10 days before natural drying of the grain. Our results about fresh (30–48 mg) and dry weights (9.6–44 mg) per seed for each investigated stage are very similar to those established by this author.

The results presented in this study are in contrast to those reported by Kermode and Bewley (1986) for castor been seeds. According to their general scheme there is a rapid increase in whole seed fresh weight and water content during histodifferentiation and early cell expansion. They conclude that generally a period of rapid dry weight gain occurs during the latter part of seed expansion (or maturation) phase of development when whole seed fresh weight is relatively stable. Most seeds lose water during this phase as reserves are deposited primarily within storage tissues, displacing water from the cells (Kermode, 1990).

The main differences between our results and those for castor been seeds are that fresh and dry weight and moisture content changes occur earlier in the case of wheat seed development and they pass in a different manner. There is no lag-phase in dry weight accumulation at the early stages and dry weight changes closely paralleled the fresh weight data from the very beginning of seed formation and cease at about 30 DAF.

There was an overall loss in seed moisture percentage from 10 to 40 DAF as reserve materials accumulated. Seed moisture dropped from 72.3% at 10 DAF to 9.5% at 50 DAF (Fig. 2, Table 1). Developing seeds at early stages of formation have a relatively high water content compared with the mature, dehydrated seeds. These results are in agreement with those of Bechtel et al. (1982) who reported that moisture

Table 1. Percentage of moisture content and germination of fresh and desiccated wheat seeds harvested at various stages of seed development

Days after	Fresh seeds		Desiccated seeds	
flowering	moisture	germination	moisture	germination
10	71.6	0.0	64.6	0.0
			55.5	0.0
			40.6	0.0
			37.8	0.0
			8.4	0.0
20	61.2	0.8	56.9	3.3
			53.3	0.0
			27.9	3.3
			8.6	8.3
30	44.4	5.0	28.8	50.8
			17.6	69.2
			9.3	47.5
40	12.8	7.5	10.4	18.3
50	9.5	17.5	-	-

content of wheat seeds drops rapidly past 12 DAF. But if the water content is expressed on a hundred seeds then an increase between 10 and 20 DAF is observed. After 20 DAF the water content decreases rapidly to 40 DAF.

The results about water content described in this investigation are quite similar to those presented by King (1976) for wheat seeds. He established that the moisture content of seeds at 14 DAF is 72% and at 20 DAF – 57%. Based on this comparison and on data about fresh and dry weight per seed it can be assumed that the phase of seed development could be successfuly defined by determining seed moisture content.

A drastic loss of water (about 70%) is observed between 30 and 40 DAF. This is exactly the natural desiccation period for wheat seeds. But the rapid water loss starts from 20 DAF (1.53 g $H_2O.g^{-1}$ DW) and at the time of 30 DAF it reaches to 0.68 g $H_2O.g^{-1}$ DW. Black et al. (1999) reported that desiccation tolerance in wheat embryos was fully induced by a decrease in water content from about 73% FW (2.7 g $H_2O.g^{-1}$ DW) to approximately 69% FW (2.2 g $H_2O.g^{-1}$ DW) for only 1 day (between 22–24 DAF). Comparison of the results presented by Black et al. (1999) with our data about natural desiccation period in wheat seeds is in support of the conclusion of Kermode (1995), that acquisition of desiccation tolerance is usually substantially earlier than the onset of the natural drying event itself. Dry mature wheat seeds (50 DAF) show a moisture content of 0.083 g $H_2O.g^{-1}$ FW. Such is the typical value for orthodox seeds that could be dried to a low moisture content of 0.05 g $H_2O.g^{-1}$ FW (Chin et al., 1989).

In seeds that dehydrate at maturity, the germination process is initiated by imbibition of water by the dry seed (Bradford, 1995). It was of interest to determine at which stage developing wheat seeds would become competent to undergo the transition from seed development to seed germination. The freshly harvested wheat grains are capable of germinating during the period between 20 and 30 DAF (Table 1), but germinating capacity is very low (0.8 and 5.0% respectively). These data are in contrast with those reported by King (1976) for wheat seeds that show significantly higher percent of germination. Grains of less than 10 DAF cannot be provoked to germinate by either treatment. Comparable results have been observed for wheat (Symmons et al., 1983), castor bean (Kermode et al., 1986; Kermode, 1990) and other seeds (Dasgupta et al., 1982). These results could be explained with the fact established by Symmons et al. (1983) that the embryo appears to be morphologically mature from as early as 12 DAF. As could be seen in Table 1 the frequency of germination upon rehydration increases when seed moisture declines naturally as seeds rise in age. An increased germinability with the progress of seed development is also observed in the case of premature desiccated seeds. Wheat seeds in fresh state as small as 60% of normal mature seed weight (less than 30 DAF) are non-germinabile. They germinate only after a drying treatment and rehydration. Dried wheat seeds at 30 DAF show germination frequency in excess of 47.5% in contrast to non-dried seeds of the same developmental stage only a small percent (5%) of which germinate. When the moisture of the seed is less than 12.8% (40 DAF), seeds could be considered to be "physiologically mature". Developing seed harvested midway or late in development (30 DAF) are already somewhat competent to germinate (5%) without imposed drying treatment. It is noteworthy that the capacity of desiccated immature seeds to germinate does not depend on the degree of desiccation reached by artificial drying but only on their age at harvest time (Table 1). These data are in contrast with the statement of Gosling et al. (1981) that the ability of seeds to germinate is related to the degree of desiccation. They are also in disagreement with the conclusion of Symmons et al. (1983) that phases of germination capacity are related to total grain moisture and not to the chronological age of the grain, i.e. days post-anthesis. Premature drying treatment applied is effective only after 30 DAF as can be seen from Table 1, i. e. after a specific stage of embryo development has been reached (Rosenberg and Rinne, 1986).

The course of dry weight changes for germinating seeds from different stages of development resembles that for freshly harvested seeds (Fig. 1). These changes are connected only with developmental stage and not with the germination process. Significant differences are observed in respect of fresh weight of freshly harvested and germinating freshly harvested seeds after 30 DAF. The amount of water taken up by wheat seeds for 48 h of germination is quite small and does not exceed 2–3 times the dry weight of the seed as was reported for imbibing seeds of different plant species (Bewley and Black, 1978). Freshly harvested seeds from different stages of development reach different moisture content in the course of germination which is typical for the particular stage. The seeds at 10 DAF have highest seed moisture – 80%. After this stage it declines parallel to the progress of seed development. Germinating mature seeds reach moisture content about 40%. But really in germinating mature seeds moisture content increases more intensively (about 30%) than in immature seeds (10 DAF) where this enhancement is only 8%. These results could be explained by the differences in the composition and the state of macromolecules from seeds at the various stages of their development (Grilli et al., 1989) and their ability for hydration. It is known that imbibition is affected by seed properties (morphology, structure, composition, moisture) as well as by environment in which they germinate (Vertucci, 1989). Besides that it shoud be considered that the seeds at 40 and 50 DAF are capable to germinate and to develop a normal seedling which also takes up water due to cell elongation and expansion and vacuole formation (Kermode, 1990).

Lots of desiccated seeds differ in fresh weight as a result of imposed loss of water. The dry weight of desiccated seeds is not changed after germination for 48 hours as was established for freshly harvested germinating seeds (Fig. 3). A possible explanation is that the period of germination is very short. Fresh weight of germinating desiccated seeds is always lower than that of the freshly harvested germinating seeds from the corresponding developmental stage. As development proceeds the fresh weight of germinating desiccated seeds approaches that of freshly harvested germinating seeds.

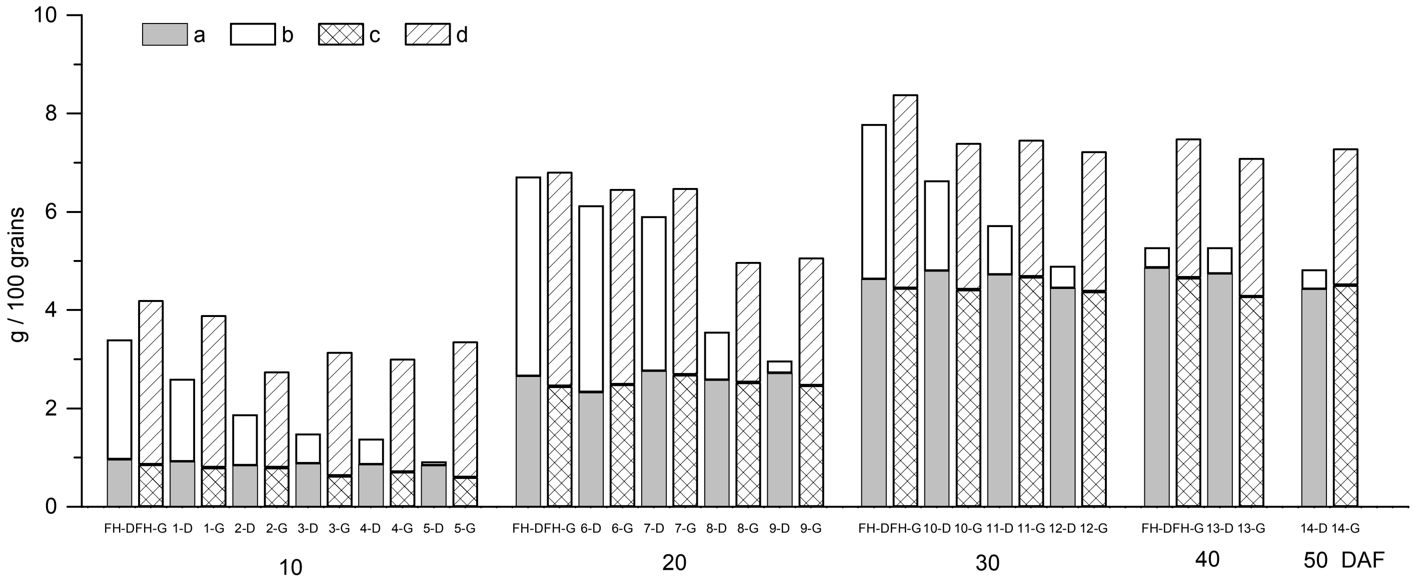

Fig. 3. Fresh (b, d) and dry (a, c) weight of freshly harvested (FH), desiccated (1–14) and germinating (G) seeds of wheat in the course of development. Moisture content of lots of desiccated seeds: 1 – 64.6%, 2 – 55.5%, 3 – 40.6%, 4 – 37.8%, 5 – 8.4%, 6 – 57.0%, 7 – 53.3%, 8 – 27.9%, 9 – 8.6%, 10 – 28.8%, 11 – 17.6%, 12 – 9.3%, 13 – 10.4%, 14 – 9.4%. D – freshly harvested or desiccated seeds at the corresponding phase before germination

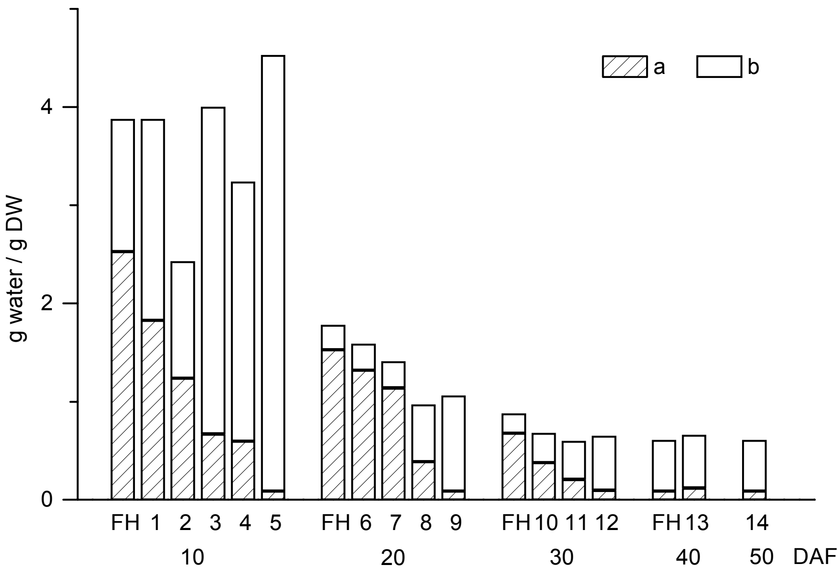

Fig. 4. Water content (g $H_2O.g^{-1}$ DW) of dry desiccated (a) and germinating desiccated (b) seeds of wheat in the course of development. FH and 1–14 – as in the Fig. 3.

Data established about the amount of water absorbed by germinating freshly harvested seeds are also valid for germinating desiccated seeds. Comparison between desiccated seed lots with similar moisture content but differing in developmental stage origin shows that they absorb different amount of water in the course of germination depending on the stage of development at harvest time (Fig. 4). Moreover, as a rule, the seed moisture of germinating desiccated seeds does not exceed that for germinating freshly harvested seeds.

It is noteworthy that the amount of water absorbed by desiccated seeds during germination depends on the developmental stage of seeds at harvest time and on the degree of desiccation. For all lots of desiccated seeds this amount, expressed as g $H_2O.g^{-1}$ DW is always higher than that for freshly harvested seeds from the corresponding phase (Fig. 4). Moisture content of germinating desiccated seeds approaches that of freshly harvested seeds from the corresponding phase as seed development proceeds. Lots of desiccated seeds differing in moisture content, obtained from the same developmental phase, show that to lower initial moisture content of dry seeds corresponds higher degree of water uptake in the course of germination. Moreover, for every investigated phase of seed development there is a determined degree of moisture and the water uptake of desiccated seeds can not overcome this value (Fig. 4). It is very interesting that at different stages of development one unit of dry weight can retain various amounts of water. The values for this specific moisture decrease as seed development proceeds. For desiccated seeds from 10 DAF this value is about 4.0 g $H_2O.g^{-1}$ DW and for mature dry seeds – 0.61. From this point of view the water uptake during 48 h of germination of developing seeds (at about 20 DAF) can be considered only as a physicochemical process and at the later stages it probably is transformed to a complex physiological-biochemical process.

Conclusions

Natural desiccation of wheat seeds occurs between 30 and 40 DAF.

– Developing wheat seeds harvested midway through development (30 DAF) are already competent to germinate (5%) without imposed drying treatment.
– The capacity of desiccated immature seeds to germinate does not depend on the degree of desiccation reached by artificial drying but only on their age at harvest time.
– Seed moisture of germinating desiccated seeds does not exceed as a rule that for germinating freshly harvested seeds.
– For every investigated phase of development there is a determined degree of moisture content for germinating freshly harvested seeds. The germinating desiccated seeds from corresponding phases can not overcome this value.
– One unit of dry seed weight can retain a various amount of water depending on the seed development stage. Values for this specific moisture content decrease as seed development proceeds.

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