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A Modified Method to Assess Secondary Dormancy in the Seeds of Different Rapeseed Lines and Cultivars

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ABSTRACT

The exposure of seeds to undesirable soil conditions induces secondary dormancy and this causes many problems for seed producers. Assessing seed secondary dormancy potential is an important factor in developing rapeseed lines and cultivars. A relatively rapid, precise, and reproducible method derived from the Hohenheim standard dormancy test (HSDT) has been developed for secondary dormancy evaluation of 41 rapeseeds (*Brassica napus* L.) lines and 5 cultivars. The two previous methods, HSDT and rapid secondary dormancy (RDT) were compared with our suggested fast-reproducible dormancy test (FRDT) method. All three methods consider three stages for seeds *i.e.* dormancy induction, germination in darkness, and breaking secondary dormancy. In the FRDT method, time to germination in darkness and seed dormancy breakage duration decreased to 7 and 4 days, respectively, whereas the corresponding figures in the HSDT were 14 and 7 days. Meanwhile, the duration of the three stages in sum decreased from 35 to 25 days. The obtained ranges of seed dormancy in different lines and cultivars as assessed by HSDT, FRDT, and RDT varied from 6 to 98.75, 7.5 to 99, and 0 to 36%, respectively. Positive relationships were found between data of the seed secondary dormancy testing by HSDT and FRDT methods. Owe to the precise and reproducible estimates, both HSDT and FRDT methods can be used for testing seed secondary dormancy in different rapeseed lines and cultivars. This method helps seed breeders to improve the screening of new rapeseed lines and cultivars with lower potential for secondary dormancy and as a result reduce the risk of volunteer rapeseed emergence in the field, which compromises yield in the next growing season.

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Introduction

Seed shattering is one of the major concerns regarding rapeseed cultivation. This happens due to pod structure, seed arrangement within pods and pods dehiscence. The number of shattered seeds may range from 2000 to 10000 /m^2 (Gruber *et al*., 2018; Lutman *et al*., 2005), with an average of 4700 seeds/m^2 (Weber *et al*., 2010). Yield loss from 4000 to 7000 seed per m^2 has also been reported in other studies carried out in Europe and the United States (Gruber *et al*., 2007; Pekrun *et al*., 1998). The fates of shattered seeds would be different. Fast ploughing after harvest replaces the shattered seeds in depths of soil. In the absence of light

necessary for germination, seeds are induced for secondary dormancy (Weber *et al*., 2010; Pekrun *et al*., 1997a, 1998). Dormancy in seeds can be categorized as primary and secondary wherein the former is induced during seed development on the mother plant while in the latter it is induced in mature seeds after release from the mother plant and when exposed to undesirable conditions (Buijs, 2020). Several factors affect secondary dormancy in seeds. These include limited oxygen supply and excessive carbon dioxide levels, high soil osmotic pressure after harvest during summer (Momoh *et al*., 2002), light (Martel *et al*., 2018), seed longevity (Momoh *et al*., 2002), seed size (Gulden *et al*., 2004), harvest time (Haile and Shirtliffe, 2014; Zhu *et al*., 2012; Gulden *et al*., 2004), harvest method (Buijs, 2020), precipitation during harvest (Zhu *et al*., 2012), weather condition during seed development (Huang *et al*., 2016), ploughing method (Gruber *et al*., 2004) and seed burial (Postma *et al*., 2016). The volunteer rapeseed is originated from the secondary dormant seeds in the soil seed bank that subsequently germinate in the next growing season in the field (Soltani *et al*., 2019).

The suggested laboratory testing methods for inducing secondary dormancy consist of the application of osmotic stress to seeds and their incubation in darkness. Pekrun *et al*. (1997b) were the first who suggested a method for the induction of secondary dormancy in rapeseed seeds through their exposure to Polyethylene glycol (-15 bar) for 4 weeks. Seeds were then transferred to germination conditions for 14 days and their germination was recorded. The remaining non-germinated dormant ones were then transferred to low temperature for 3 days to be released from dormancy. Finally, seeds were returned to normal germination conditions. Gulden *et al*. (2003) categorized the Canadian canola genotypes based on seed secondary dormancy and seed persistence in the soil according to Pekrun *et al*. (1997b). Their study showed that genotype is an important factor for the expression of secondary dormancy in the spring rapeseed cultivars in the west of Canada. The importance of genotype in the expression of seed secondary dormancy has also been suggested in Chinese and European rapeseeds (Pekrun *et al*., 1999; Momoh *et al*., 2002).

The Hohenheim standard dormancy test (HSDT) takes a time of about 35 days and is another time-saving method for assessing the potential of rapeseed seeds for secondary dormancy induction (Gruber *et al*., 2004). So far, studies carried out using the HSDT method have shown that rapeseed seed in secondary dormancy is greatly affected by the genotype (Gruber *et al*., 2004; Momoh *et al*., 2002). This method has revealed that genotype may contribute from 44 to 82% to trait of rapeseed seed in secondary dormancy (Gulden *et al*., 2004).

The long duration (about 54 days) required for the first testing method of rapeseed seed secondary dormancy evaluation introduced by Pekrun *et al*. (1997b) is its major disadvantage. The HSDT method is more competent, however, as it takes shorter (35 days) time but it is still long (Gruber *et al*., 2004). Consequently, based on the HDST method, the rapid secondary dormancy (RDT) method has been suggested by other researchers (Weber *et al*., 2010). In this method, the duration of secondary dormancy induction and germination test in darkness decreased to 14 and 7 days, respectively. This method has been used in other studies to test rapeseed seed secondary dormancy (Weber *et al*., 2013, 2014). Establishing the competency of a method for evaluating a trait, demands demonstration of its applicability on other genotypes to make sure of its exactness, accuracy, and effectiveness. Accordingly, in this study, we have improved the former rapeseed secondary dormancy testing methods of Gruber *et al*. (2004) and Weber *et al*. (2010) to a more efficient, rapid, and accurate one. The suggested method would help seed breeders and producers in the selection of cultivars with the low ability for seed secondary dormancy and hence prevention of the volunteer rapeseed emergence in the next growing season.

Materials and Methods

Five rapeseed cultivars including Hyola50 (H50), Hyola40 (H40), Hyola308 (H308), RGS, and Zarfam along with 41 hybrids (H) and open pollination (OP) lines were procured from the Centre of Agricultural Research and Education of Golestan Province, Iran (Table 1). Two hundred seeds in four replicates of 50 were imbibed in distilled water and then incubated at

20°C in 15 cm Petri dishes under dark conditions. Final germination was recorded after 7 days. Seeds were considered germinated when radicle protrusion occurred to a length of about 2 mm (Schatzki *et al*., 2013). Means for the primary germination were greater than 94 percent in all investigated cultivars and lines. Table 1. The rapeseed lines and cultivars were used in this study. The 41 lines and five rapeseed cultivars were provided by the center of agricultural research and education of Golestan Province, Iran. The seed secondary dormancy-testing methods investigated in this study include:

Table 1. The rapeseed lines and cultivars were used in this study. The 41 lines and five rapeseed cultivars were provided by the center of agricultural research and Education of Golestan Province, Iran.

No.	Lines	Breeding system	No.	Lines	Breeding system
1	Gor[1]-O[2]-1	Pedigree Breeding	27	Gor-H[3]-1	Hybrid Breeding
2	Gor-O-2	Pedigree Breeding	28	Gor-H-2	Hybrid Breeding
3	Gor-O-3	Pedigree Breeding	29	Gor-H-3	Hybrid Breeding
4	Gor-O-4	Pedigree Breeding	30	Gor-H-5	Hybrid Breeding
5	Gor-O-5	Pedigree Breeding	31	Gor-H-6	Hybrid Breeding
6	Gor-O-6	Pedigree Breeding	32	Gor-H-7	Hybrid Breeding
7	Gor-O-7	Pedigree Breeding	33	Gor-H-8	Hybrid Breeding
8	Gor-O-8	Pedigree Breeding	34	Gor-H-9	Hybrid Breeding
9	Gor-O-9	Pedigree Breeding	35	Gor-H-10	Hybrid Breeding
10	Gor-O-10	Pedigree Breeding	36	Gor-H-11	Hybrid Breeding
11	Gor-O-11	Pedigree Breeding	37	Gor-H-12	Hybrid Breeding
12	Gor-O-12	Pedigree Breeding	38	Gor-H-13	Hybrid Breeding
13	Gor-O-13	Pedigree Breeding	39	Gor-H-14	Hybrid Breeding
14	Gor-O-14	Pedigree Breeding	40	Gor-H-15	Hybrid Breeding
15	Gor-O-15	Pedigree Breeding	41	Gor-H-16	Hybrid Breeding
16	Gor-O-16	Pedigree Breeding			
17	Gor-O-17	Pedigree Breeding	**No.**	**Cultivar**	
18	Gor-O-18	Pedigree Breeding	42	RGS	
19	Gor-O-19	Pedigree Breeding	43	Zarfam	
20	Gor-O-20	Pedigree Breeding	44	H308	
21	Gor-O-21	Pedigree Breeding	45	H401	
22	Gor-O-22	Pedigree Breeding	46	H50	
23	Gor-O-23	Pedigree Breeding			
24	Gor-O-24	Pedigree Breeding			
25	Gor-O-25	Pedigree Breeding			
26	Gor-O-26	Pedigree Breeding			

1=Gorgan; 2=Open pollinated Cultivar; 3=Hybrid Cultivar; GANRRTC = Golestan Agricultural and Natural Resources Research and Training Center Breeding company and distributor are GANRRTC.

Hohenheim standard dormancy test

A. Induction of seed secondary dormancy: Polyethylene glycol (PEG 6000) solution was prepared by dissolution of 354.37 g powder in one liter of distilled water which produces a water potential (ψ) of -15 bar at 20°C (Michael and Kaufman, 1973). PEG solution (8 ml) was added to each Petri dish (10 cm diameter) sterilized with ethanol (80%) and lined with two layers of filter papers (Whatman No.2) each containing 100 seeds. The procedure was carried out in darkness under green safe light (500 to 600 nm) conditions. Petri dishes were placed randomly in a large box covered by two layers of black nylon sheets to block the passage of light and incubated under controlled temperature (20°C) for 14 days.

B. Germination test in darkness: After 14 days of secondary dormancy induction in seeds, they were washed with distilled water and transferred into new Petri dishes as described before and irrigated with 6 ml distilled water. This stage is also done in darkness under green safe light (500 to 600 nm) conditions. Counting of germinated seeds was carried out for a second, fourth, seventh, 10th, and 14th days after transferring to germination condition. Seeds with radicle lengths of about 2 mm were considered as germinated (Schatzki *et al*., 2013, Gruber *et al*., 2004).

C. Breaking secondary dormancy: All non-germinated seeds from the previous step were incubated alternatively in temperatures of 30 and 3°C under light and dark (12/12 h) conditions for 7 days, respectively, for the release of secondary dormancy.

Rapid secondary dormancy

In this method, the durations for the induction of secondary dormancy and germination test in darkness were reduced from 14 to 7 days. For breaking the secondary dormancy of non-germinated seeds, they were incubated in alternative temperatures as described above for 7 days.

Fast-reproducible dormancy test

It is a modified form of the HSDT method. The duration for the induction of secondary dormancy (14 days) in seeds was not changed; however, the times required for germination test in darkness and breaking secondary dormancy in seeds were reduced to 7 and 4 days compared with 14 and 7 days in HSDT, respectively. This resulted in the shortening of the whole procedure in just only 25 days compared to 35 days in the HSDT method.

Statistical analyses

Statistical analyses were carried out using the R program (R core team, 2014), and the Office Excel 2013 program drew graphs. Datasets were tested for normality using *Shapiro. test* in the R program. Standard errors of the means were calculated. Euclidian distance and complete linkage method were used for the classification of different rapeseed lines and cultivars using cluster analysis. In addition, simple x - y ordinate lines in Fig.1, 3, and 4 were used to determine the relationships of different days after incubation in darkness for germination and also days after being in optimal condition for breaking secondary dormancy. Meanwhile, an FRDT method was used to investigate the extent of rapeseed seed secondary dormancy induction in parallel with RDT and HSDT methods. The clustering of seed secondary dormancy in different cultivars and lines was carried out by the Euclidean distance method using R statistical software. Moreover, division lines were drawn using as follows: *n* is equal to the number of cultivars and lines [Equation 1] (Farshadfar, 2011).

Number of clusters = $\sqrt{(n/2)}$ [Equation 1]

Results

The results showed significant differences between all lines and cultivars and the methods used and also their interactions for investigating secondary dormancy in rapeseed seeds (Table 2). All these refer to the usefulness of these methods to test secondary dormancy.

During the induction of secondary dormancy, germination in darkness and viability test were 14, 14, and 7, respectively in the HSDT method used in this study. Therefore, 35 days were needed for secondary dormancy testing (Table 3). Comparisons for the levels of seed secondary dormancy measured by either RDT or the suggested method (FRDT) with those of HSDT are shown in Fig. 1.

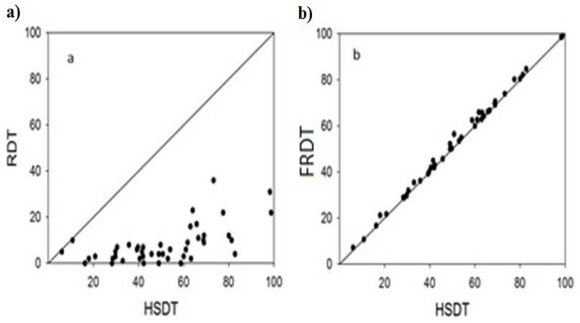

Fig. 1. The relationship for the average of secondary dormancy percentages is obtained from the comparison of the HSDT method to those obtained from RDT (a) and FRDT (b) methods.

Positive relationships between data from HSDT and FRDT methods were found in the secondary dormancy testing while the RDT method was much less efficient in the prediction of levels of secondary dormancy in seeds. Genetic diversity based on secondary dormancy was investigated for each line and cultivar to confirm the conformity of the new FRDT methods with either HSDT or RDT methods. The estimated levels of secondary dormancy in seeds were different for each line and cultivar and ranged from 6 to 98.75, 7.5 to 99, and 0 to 36% by HSDT, FRDT, and RDT methods, respectively (Fig. 2).

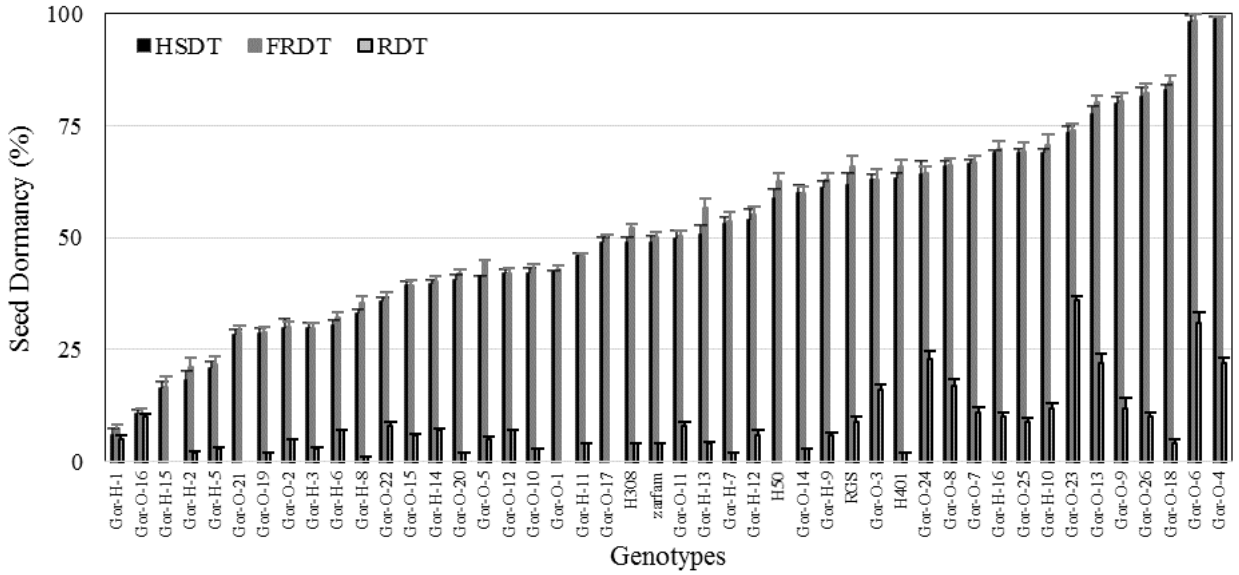

Fig. 2. Levels of secondary dormancy were determined in the seeds from 41 rapeseed lines and 5 cultivars according to HSDT, RDT, and FRDT methods. Significant differences in all lines and cultivars were shown by the standard error.

Estimates for the levels of secondary dormancy in seeds were nearly identical in FRDT and HSDT methods for all lines and cultivars; however, RDT led to the underestimation of dormancy levels. Moreover, failure in assessing seed secondary dormancy was detected using the RDT method.

Table 2. Analysis of variance for different rapeseed lines/cultivars and the methods used to test seed secondary dormancy.

Analysis of variances	df	MS
Cultivar and lines [CL]	45	2432***
Methods [M]	2	87988***
[CL] * [M]	90	338***
Error	276	0.0217
CV	0.398	

*p < 0.05; **p < 0.01; ***p < 0.001.

Table 3. Time comparison of the three stages used in the different methods for assessing the seed secondary dormancy in rapeseed.

	HSDT	RDT	FRDT
	Duration (day)		
Dormancy induction (DID)	14	7	14
Germination test in darkness (GTD)	14	7	7
Breaking seed secondary dormancy	7	7	4
Total	35	21	25

HSDT: Hohenheim standard dormancy test; RDT: Rapid secondary dormancy; FRDT: Fast-reproducible dormancy test.

Fig. 3 shows the relationships between germination percentages in darkness on the 2nd, 4th, 7th and 10th day against those on the 14th day. As it is evident, not only seed germination in all lines and cultivars in darkness was not terminated on the 2nd day but also it continued beyond. The mean germination percentage in darkness was equal to 42.52% on the 2nd day while it was 48.17% on the 4th day, which then reached 49.13% and 49.23% on the 7th and 10th days, respectively. This implies that the increase in seed germination percentage is not beyond 7 days and accordingly, test for secondary dormancy should not be prolonged. Therefore, the time to germination in darkness could be reduced from 14 to 7 days.

According to the HSDT method, the duration required for breaking secondary dormancy in rapeseed seeds corresponds to 7 days (Table 3). Fig. 4 shows the relationships between the percentage of seed germination on the 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} days with the 7th day after exposure to conditions necessary for breaking secondary dormancy in seeds (Fig 4). Means for germination percentage on the 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} days were 48.59, 48.97, 52, and 52.5%, respectively. It implies that the germination percentage did not increase after the 4th day (Fig. 3). Accordingly, the duration necessary for breaking secondary dormancy in rapeseed seeds

can be reduced from 7 days in the HSDT method to 4 days in the new FRDT method.

Entry of seeds into the secondary dormancy state is prevented due to having remained viable after exposure to alternative temperatures under light and dark conditions. Some physical features such as swelling and decay could identify seeds incapable of germination.

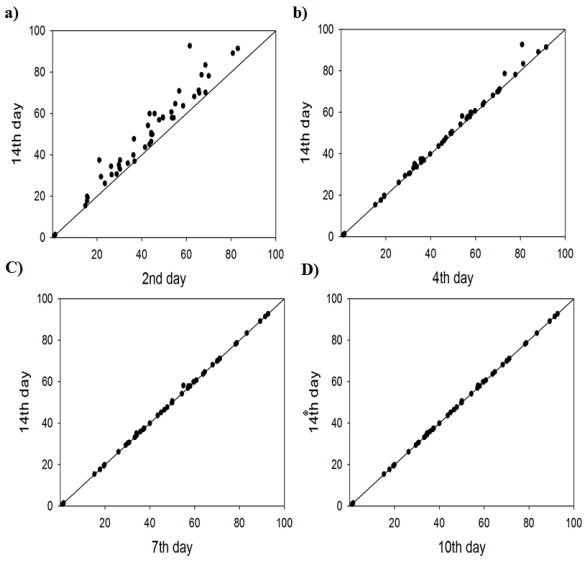

Fig. 3. Percent of germinated seeds are shown for different days (2nd (a), 4th (b), 7th (c), and 10th (d)) after exposure to the condition of germinating in darkness against 14th by line 1:1. The last day for counting was considered the 14th day according to the HSDT method.

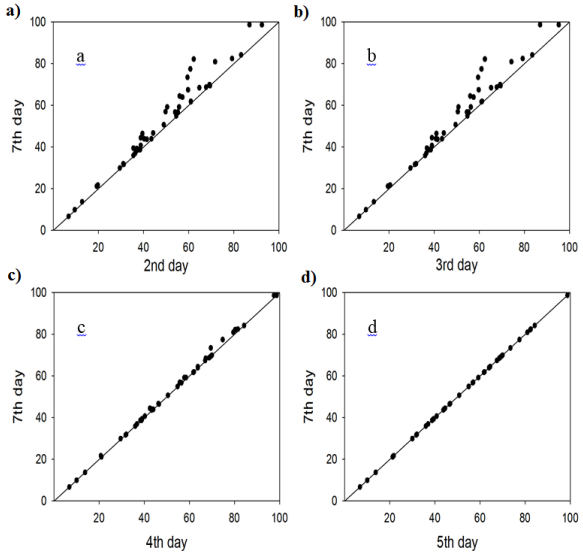

Fig. 4. Percent of seeds germinated in different days (2nd (a), 3rd (b), 4th (c), and 5th (d)) are shown after being placed in the condition of breaking secondary dormancy (alternative temperatures 3-30°C (light/darkness)) by line 1:1. The 7th day was considered the last day according to the HSDT method.

Cluster analysis categorized rapeseed lines and cultivars into 5 levels of seed secondary dormancy including very low, low, average, high, and very high. Clustering of seed secondary dormancy by the HSDT method displayed high conformity with that of the new FRDT method; however, weak conformity was found with the RDT method (Figs 5-7).

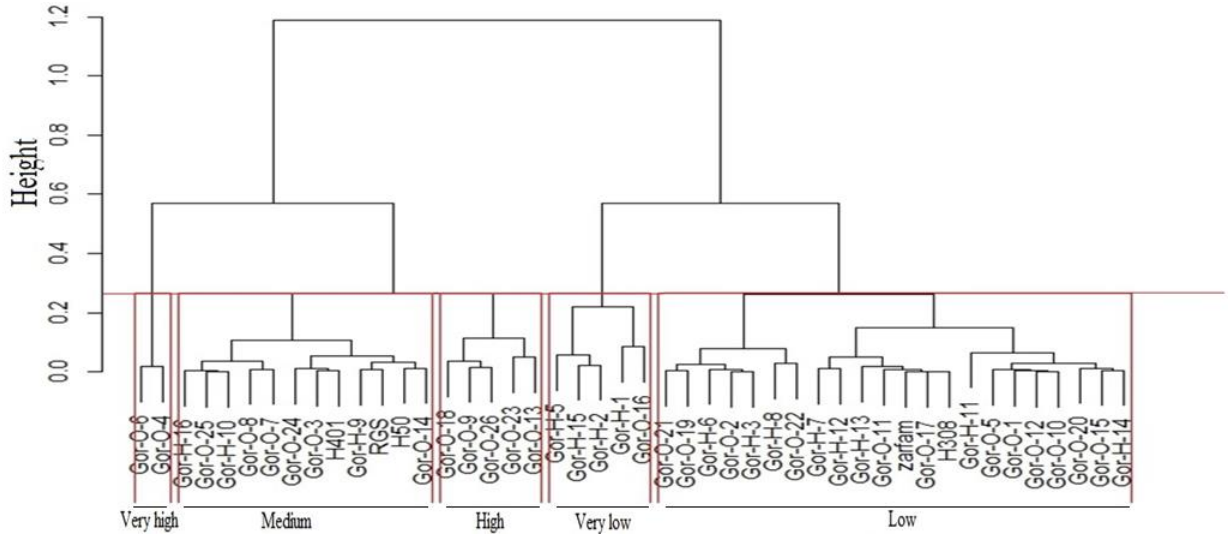

Fig. 5. Cluster analysis for the classification of 41 rapeseed lines and 5 cultivars based on assessing seed secondary dormancy by the HSDT method.

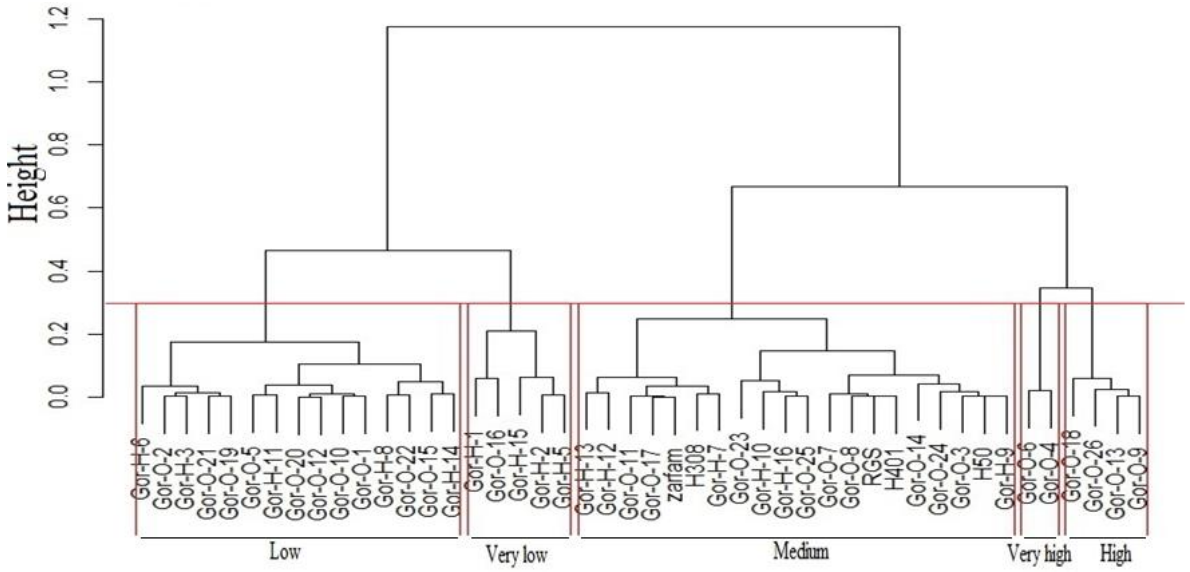

Fig. 6. Cluster analysis for the classification of 41 rapeseed lines and 5 cultivars based on assessing seed secondary dormancy by FRDT method.

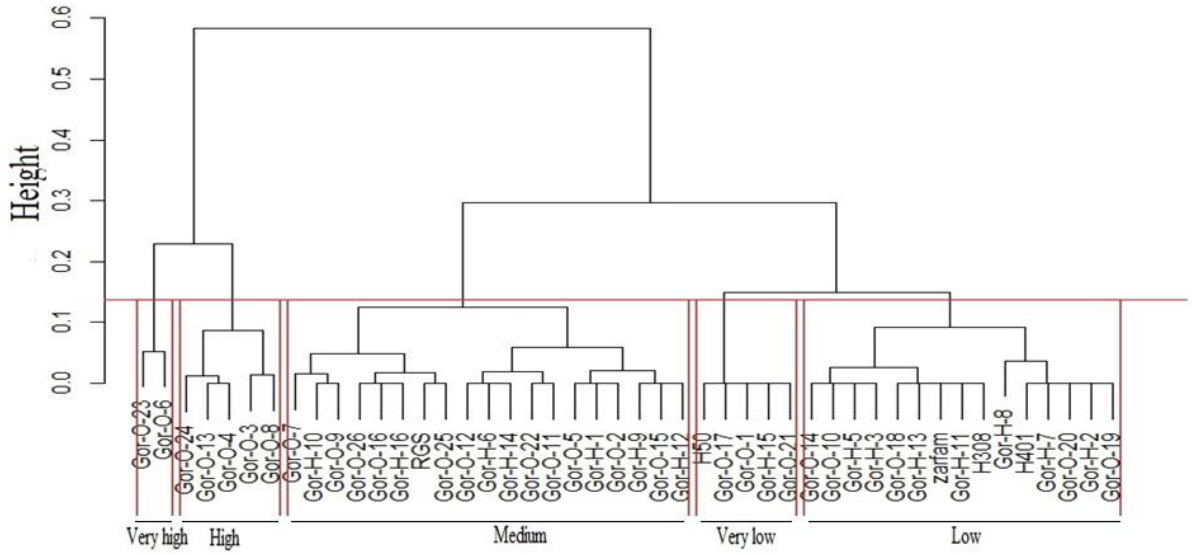

Fig. 7. Cluster analysis for the classification of 41 rapeseed lines and 5 cultivars based on assessing seed secondary dormancy by RDT method.

Discussion

The first suggested method for assessing secondary dormancy in rapeseed seeds is lengthy and takes 54 days (Pekrun *et al*., 1997b). The HSDT, which is another method for assessing secondary dormancy in rapeseed seeds, (Gruber *et al*., 2004) still has the same problem. Thus, based on HSDT, the rapid secondary dormancy (RDT) method was suggested in which the time for screening genotypes has been reduced to 21 days (Weber *et al*., 2010). As another alternative time-saving method, the FRDT is suggested in this study for the reliable assessment of secondary dormancy in different rapeseed seeds with high precision. The susceptibility for the induction of secondary dormancy in seeds of different genotypes was great and ranged from 0 to 99% in this study while Gulden *et al*. (2004) reported ranges between 44 to 82%. Cluster analysis of all rapeseed lines and cultivars based on seed secondary dormancy using HSDT and FRDT methods properly placed them in distinct clusters. However, this could not be achieved by the RDT method. The inefficiency of this test may be due to the short duration i.e. 7 days

considered for the induction of secondary dormancy. In fact, in the RDT method, the appropriate conditions for the induction of secondary dormancy in seed populations were not properly designed to be compatible with those prevailing under field conditions. Other researchers have also shown that reducing the duration required for secondary dormancy induction from 14 to 7-day results in the underestimation of rapeseed seeds' secondary dormancy by 50% (Pekrun *et al*., 1997b). Since the duration for the induction of secondary dormancy in the newly introduced FRDT method is the same as HSDT, problems associated with the proper induction of secondary dormancy in rapeseed seeds are eliminated.

Great relationships between germination percentages in darkness beyond 7th day with those at 14th day established that no more germination of seeds occurs after 7th day and accordingly, time to germination in darkness can be reduced from 14 to 7 days. Thus, the FRDT method is suggested to be used in future studies, as it is efficient time and more precise than former methods for assessing secondary dormancy in rapeseed seeds. Reducing the period for screening seed germination in darkness to 7th days helps breeders in assessing different rapeseed genotypes for the presence of seed secondary dormancy. Although this time reduction has also equally been considered in the RDT method (Weber *et al*., 2010); however, it suffers the required precision.

The steps necessary for breaking secondary dormancy in rapeseed seeds consisted of light/dark regimes and alternative temperatures (Momoh *et al*., 2002, Pekrun *et al*., 1997b). Secondary dormancy in various seeds is affected by light quality, which is related to wavelength (Martel *et al*., 2018). In addition, the temperature is another factor, which affects germination and secondary dormancy in the seeds of many species; however, some believe that light might not be considered as the main factor in the control of secondary dormancy (Fenner and Thompson, 2005; Shayanfar *et al*., 2020). Despite these claims, light is effective in promoting germination and affects secondary dormancy in some other studies (Shayanfar *et al*., 2018). As rape seeds are exposed to low osmotic potential under dark conditions, their sensitivity to light increases (Pekrun, 1994). This is beneficial as it has resulted in the prevention of seed germination in the depth of soil from 4 to 5 mm, which is not usually penetrated by light (Kołodziejek and Patykowski, 2015). In agricultural practices, two environmental changes can promote seed germination and contribute to better light signaling; First of all, soil ploughing during agricultural practices and next making gaps between dense canopies for light penetration (Benech-Arnold *et al*., 2000). Germination percentages in secondary dormant rapeseed seeds increased from 13.3 to 63% due to light of camera flashes of about 0.002 seconds and germination percentage reached 98.1% when the seeds were exposed to continuous light (Schlink, 1994). As a result, seed dormancy can be released by incubating rapeseed seeds under alternating temperatures and light/dark conditions for 4 days. Other studies have found that very low fluence response (VLFR) concerning seed germination of many species on the upper and lower soil surface layers (Benvenuti and Mazzoncini, 2021). Despite these, seeds from various plants responded differently to light and an even longer duration (10 days) of exposure to light might be needed to break secondary dormancy in some species such as *Setaria parvaliflora* while responding to VLFR were not reported (Mollard and Insausti, 2009). The phytochrome system has been considered responsible for sensitivity to light in the secondary dormant rapeseed and *Rosa canina* L. seeds (Pawłowski *et al*., 2020).

The germination competence of non-germinated seeds can be assessed after experiencing light/dark regimes at alternating temperatures based on their physical characteristics by pressure test with the thumb (Bonner, 1981). This method was also used in our study to distinguish between viable dormant and dead non-germinating rapeseed seeds. Furthermore, the spoiled and infected seeds can be removed in this way to save time and expenditure required for a tetrazolium viability test (Weber *et al*., 2010). The HSDT is known as a reliable method for investigating secondary dormancy in rapeseed seeds. The FRDT method introduced in this study, however, is as reliable as HSDT but with more advantages of rapidity and lower

costs. Thus, FRDT displays high conformity with the HSDT method in assessing secondary dormancy in rapeseed seeds and their categorization. These features warrant the utilization of this method by seed producers and researchers. On the other hand, the RDT method in contrast to the former claim (Weber *et al*., 2010) has very limited ability in the evaluation of seed secondary dormancy in different lines and cultivars, as it did not show high accuracy, exactness, and reproducibility in the testing of secondary dormancy. However, the modified HSDT is called here a reliable FRDT and in a shorter time compared to the HSDT, can assess secondary dormancy in different lines, cultivars, and genotypes. Genotypes with low potential for secondary dormancy might be selected in future breeding programs using these two methods (HSDT, FRDT) to minimize problems due to the cultivation of rapeseed genotypes with a high potential for seed secondary dormancy induction which inevitably leads to the presence of volunteer rapeseeds in the next growing season (Schatzki *et al*., 2013).

References

Benech-Arnold RL, Sanchez RA, Forcella F, Kruk BC, Ghersa CM. 2000. Environmental control of dormancy in weed seed banks in soil. *Field Crops Res* 67: 105-122.

Benvenuti S, Mazzoncini M. 2021. "Active" weed seed bank: soil texture and seed weight as key factors of burial-depth inhibition. *Agronomy* 11: 210.

Bonner FT. 1981. Measurement and management of tree seed moisture. New Orleans: USDA Forest Service, Southern Forest Experiment Station.

Buijs. G. 2020. A perspective on secondary seed dormancy in *Arabidopsis thaliana*. *Plants* 9: 749. doi: 10.3390/plants9060749

Farshadfar E. 2011. Principles of multivariate statistical methods. Kermanshah: Razi university press. pp. 734. [in Persian].

Fenner M, Thompson K. 2005. The ecology of seeds. Cambridge, UK: Cambridge University Press.

Gruber S, Claupein, W. 2007. Fecundity of volunteer oilseed rape and estimation of potential gene dispersal by a practice-related model. *Agric Ecol Environ* 119: 401-408.

Gruber S, Pekrun C, Claupein W. 2004. Population dynamics of volunteer oilseed rape (*Brassica napus* L.) affected by tillage. *Eur J Agron* 20: 351-361.

Gruber S, Weber EA, Thole H, Mohring J, Dietz-Pfeilstetter A, Claupein W. 2018. Impact of cultivar on survival of volunteer oilseed rape populations in fields is more important than field management. *Weed Res* 58(2): 89-98.

Gulden RH, Shirtliffe SJ, Thomas AG. 2003. Secondary seed dormancy prolongs persistence of volunteer canola in western Canada. *Weed Sci* 51: 904-913.

Gulden RH, Thomas AG, Shirtliffe SJ. 2004. Relative contribution of genotype, seed size and environment to secondary seed dormancy potential in Canadian spring oilseed rape (*Brassica napus*). *Weed Sci* 44: 97-106.

Haile TA, Shirtliffe SJ. 2014. Effect of harvest timing on dormancy induction in canola seeds. *Weed Sci* 62: 548-554.

Huang S, Gruber S, Stockmann F, Claupein W. 2016. Dynamics of dormancy during seed development of oilseed rape (*Brassica napus* L.). *Seed Sci Res* 26: 1-9.

Kołodziejek J, Patykowski J. 2015. Effect of environmental factors on germination and emergence of invasive *Rumex confertus* in central europe. *Scic World J* 170176: 1-10.

Lutman PJW, Berry K, Payne RW, Simpson E, Sweet JB, Champion GT, May MJ, Wightman P, Walker K, Lainsbury M. 2005. Persistence of seeds from crops of conventional and herbicide tolerant oilseed rape (*Brassica napus*). *Proc Royal Soc* 272: 1909-1915.

Martel C, Blair LK, Donohue K. 2018. PHYD prevents secondary dormancy establishment of seeds exposed to high temperature and is associated with lower PIL5 accumulation. *J Exp Bot* 69 (12): 3157-3169.

Michael BE, Kaufman MR. 1973. The osmotic potential of polyethylene glycol 6000. *Plant physiol* 51: 914-916.

Mollard FPO, Insausti P. 2009. Breaking *Setaria parviflora* seed dormancy by nitrates and light is part of a mechanism that detects a drawdown period after flooding. *Aquat Bot* 91: 57-60.

Momoh EJJ, Zhou WJ, Kristiansson B. 2002. Variation in the development of secondary dormancy in oilseed rape genotypes under conditions of stress. *Weed Res* 42: 446-455.

Pawłowski TA, Bujarska-Borkowska B, Suszka J, Tylkowski T, Chmielarz P, Klupczynska EA, Staszak AM. 2020. Temperature regulation of primary and secondary seed dormancy in *Rosa canina* L.: Findings from proteomic analysis. *Int J Mol Sci* 21: 7008. doi: https://doi.org/10.3390/ijms21197008.

Pekrun C, Hewitt JDJ, Lutman PJW, 1998. Cultural control of volunteer oilseed rape (*Brassica napus* L.). *J Agric Sci* 130: 155-163.

Pekrun C, Lane PW, Lutman PJW, 1999. Modelling the potential for gene escape in oilseed rape via the soil seedbank: its relevance for genetically modified cultivars. In: Proceedings No. 72 of the BCPC Symposium on Gene flow and Agriculture: Relevance for Transgenic Crops, Staffordshire, UK, 12-14 April 1999, pp. 101-106.

Pekrun C, Lutman PJW, Baeumer K. 1997a. Germination behaviour of dormant oilseed rape seeds in relation to temperature. *Weed Res* 37: 419-431.

Pekrun C, Lutman PJW, Baeumer K. 1997b. Induction of secondary dormancy in rape seeds (*Brassica napus* L.) by prolonged imbibition under conditions of water stress or oxygen deficiency in darkness. *Eur J Agron* 6: 245-255.

Pekrun C. 1994. Untersuchungen zur sekundaren Dormanz bei Raps (*Brassica napus* L.). Ph.D Thesis, Georg-August University, Gottingen, Germany [in German].

Postma FM, Lundemo S, Agren J. 2016. Seed dormancy cycling and mortality differ between two locally adapted populations of *Arabidopsis thaliana*. *Ann Bot* 117: 249-256.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Schatzki J, Allam M, Kloppel C, Nagel M, Borner A, Mollers C. 2013. Genetic variation for secondary seed dormancy and seed longevity in a set of black-seeded European winter oilseed rape cultivars. *Plant Breed* 132: 174-179.

Schlink S. 1994. Ökologie der Keimung und Dormanz von Körneraps (*Brassica napus* L.) und ihre Bedeutung für eine Überdauerung der Samen im Boden. Ph.D. thesis, University of Göttingen, Germany.

Shayanfar A, Ghaderifar F, Behmaram R, Soltani A, Sadeghipour H. 2018. Temperature and light effects on volunteer's rapeseed (*Brassica napus* L.) secondary dormancy and germination. *J Plant Prot* 32(2): 269-278.

Shayanfar A, Ghaderi-Far F, Behmaram R, Soltani A, Sadeghipour HA. 2020. Impacts of fire cues on germination of *Brassica napus* L. seeds with high and low secondary dormancy. *Plant Biol* 22: 647-654.

Soltani E, Baskin JM, Baskin CC. 2019. A review of the relationship between primary and secondary dormancy, with reference to the volunteer crop weed oilseed rape (*Brassica napus*). *Weed Res* 59: 5-14.

Weber EA, Frick K, Gruber S, Claupein W. 2010. Research and development towards a laboratory method for testing the genotype predisposition of oilseed rape (*Brassica napus* L.) to secondary dormancy. *Seed Sci Technol* 38: 298-310.

Weber EA, Gruber S, Claupein W. 2014. Emergence and performance of volunteer oilseed rape (*Brassica napus*) in different crops. *Eur J Agron* 60: 33-40.

Weber EA, Gruber S, Stockmann F, Claupein W. 2013. Can low-dormancy oilseed rape (*Brassica napus*) genotypes be used to minimize volunteer problems? *Field Crops Res* 147: 32-39.

Zhu YM, Li YD, Colbach N, Ma KP, Wei W, Mi XC. 2012. Seed losses at harvest and seed persistence of oilseed rape (*Brassica napus*) in different cultural conditions in Chinese farming systems. *Weed Res* 52: 317-326.