Acta Physiol Plant (2017) 39:252
DOI 10.1007/s11738-017-2552-0

ORIGINAL ARTICLE

Application of hydrotime model to predict early vigour of rapeseed (*Brassica napus* L.) under abiotic stresses

Elias Soltani[1] · Roqia Adeli[1] · Gholam Abbas Akbari[1] · Hossein Ramshini[1]

Received: 18 March 2017 / Revised: 19 June 2017 / Accepted: 9 October 2017 / Published online: 19 October 2017

Abstract Rapeseed (*Brassica napus* L.) is important for edible oil production in semi-arid areas. Abiotic stresses are threatening rapeseed production in such areas. This study was conducted to find tolerant genotypes of rapeseed and to determine which traits of crop establishment is related to abiotic stress tolerance. Hydrotime model parameters were determined in a laboratory germination test, and seedling emergence and growth were evaluated in pot experiments under control, drought, salinity, deep sowing, low and high temperatures for 19 rapeseed genotypes. Results indicated that the predicted germination time courses at the various water potentials generally fitted well with the observed germination data. The estimated values of θ_H, $\psi_b(50)$, and $\sigma\psi_b$ differed significantly across genotypes. Seedling emergence and growth differed significantly under each environmental condition. PCA showed that genotypes of Hayola 401 and line 285 were the most tolerant to abiotic stresses during crop establishment and seedling growth. The first PC explained 40% of variations, and a correlation was observed between PC1 and $\psi_b(50)$. Correlations among hydrotime model parameters and early seed vigour variables indicated that $\psi_b(50)$ negatively correlated with seedling emergence percentage and rate (day^{-1}) under all abiotic stresses. It shows that genotypes with more negative values of $\psi_b(50)$ have more seedling emergence percentage and a larger seedling emergence rate (days^{-1}) under a wide range of environmental conditions. Thus, it can be concluded that, to identify tolerant genotypes of rapesee to abiotic stresses, $\psi_b(50)$ is a good trait and that breeders can focus on reducing $\psi_b(50)$ to increase tolerance of abiotic stresses.

Keywords Crop establishment · Drought · PCA · Salinity · Sowing depth · Temperature

Communicated by W. Zhou.

✉ Elias Soltani
elias.soltani@ut.ac.ir

[1] Department of Agronomy and Plant Breeding Sciences, College of Aburaihan, University of Tehran, P.O. Box 11365/7117, Pakdasht, Tehran, Iran

Introduction

Rapeseed (*Brassica napus* L.) is one of the most important oilseed crops in the world. Grains of rapeseed are commonly used as edible oil (Flakelar et al. 2015) and biodiesel (Aoun et al. 2016). Rapeseed contains both spring and winter types that are distinguished by vernalization requirement. Spring types also can be cultivated in winter when temperature is not very low and does not limit the growth. Winter cultivation of rapeseed has advantages compared to spring cultivation under rainfed conditions in semi-arid areas. In these conditions, water stress can restrict the production of crop, but a winter crop can use water from winter rainfalls. Drought, salinity, temperature, and deep sowing (physical stress) are the most common abiotic stresses limiting crop establishment and early growth of rapeseed in semi-arid area. Drought and salinity stresses decrease germination and early growth in many crops (Katembe et al. 1998; Jabbari et al. 2013; Fernández-Torquemada and Sánchez-Lizaso 2013). Rainfalls are low and irregularly distributed in semi-arid areas, and it may delay seedling emergence of rapeseed by inducing secondary dormancy (Momoh et al. 2002; Gulden et al. 2003) or inhibiting seed germination. Salinity results in lower osmotic potential (negative values), thereby inhibiting water uptake by seeds (Zhang et al. 2010). Salinity stress also affects seed germination through ion toxicity (Fernández-Torquemada and Sánchez-Lizaso 2013). Low and high temperatures can lead progressively poorer germination and emergence (Farzaneh et al. 2014), and effects on rapeseed growth (Tian et al. 2017). It is possible to experience low or high temperatures during seedling emergence and early growth of rapeseed under winter cropping system. It is not surprising to have late summer heat or early winter cold during autumn in semi-arid areas. Soil compaction, inappropriate seed bed, and deep sowing depth can cause physical stresses and decrease seedling emergence percentage and uniformity (Soltani et al. 2009; Zuo et al. 2017).

Higher seedling emergence and early vigour are important for crop establishment and final yield, especially under abiotic stresses. Genotypes vary in their ability to respond to adverse conditions, and genotypes with higher tolerance can successfully pass this stage. However, it is difficult to find a genotype with all favourable characteristics to overcome stress conditions. For example, Farzaneh et al. (2014) indicated that cold and heat tolerances were inversely related and made it difficult to identify a rapeseed genotype that possesses both heat and cold tolerance characteristics. If there was a characteristic(s) with high correlation on plant tolerance to abiotic stresses, it was possible to change this characteristic(s) to increase stress tolerance. It is also possible to focus on breeding programmes to get this characteristic(s) in new cultivars.

Gummerson (1986) found that time to germination is related to the magnitude of the difference between the water potential (ψ) of environment and the physiological ψ threshold for radicle emergence of seed (base ψ; ψ_b). Multiplication of the difference between ψ and ψ_b by time (hours or days) is equal to hydrotime (θ_H; MPa-hours or MPa-days) (Bradford 1990; Dahal and Bradford 1990; Bradford and Still 2004). It has been indicated that the values of ψ_b for different germination fractions ($\psi_b(g)$) vary among seeds in a population with a normal distribution at sub-optimal temperatures (Dahal and Bradford 1990; Soltani et al. 2013; Patané et al. 2016). This distribution can be defined by its mean ($\psi_b(50)$) and standard deviations ($\sigma\psi_b$). Therefore, hydrotime model has three parameters: (1) the hydrotime constant, θ_H, (2) base water potential, $\psi_b(50)$, and (3) germination uniformity ($\sigma\psi_b$). Bradford and Still (2004), who explained that hydrotime analysis can be applied to investigate the physiological status of seed lots, showed that there was an association between hydrotime parameters and stand establishment in broccoli. It has been indicated that seed priming can increase seed germination rate by reducing θ_H (Dahal and Bradford 1990; Bradford and Somasco 1994) or by reducing $\psi_b(50)$ (Patané et al. 2016). Reports showed that $\psi_b(50)$ was correlated with early vigour in sugar beet (Farzane and Soltani 2011) and cotton (Soltani and Farzaneh 2014). However, it is unknown if there is a relationship between hydrotime parameters and crop establishment in abiotic stresses for rapeseed. Based on the explained details, the aims of this study were as follows: (1) to model seed germination by hydrotime model for different genotypes of rapeseed, (2) to evaluate different rapeseed genotypes tolerance to abiotic stresses (drought, salinity, deep sowing, low, and high temperatures), (3) to find the most tolerant genotypes of rapeseed under a wide range of abiotic stresses, and (4) to test whether hydrotime parameters can be used as a measure of abiotic stresses tolerance.

Materials and methods

Developing of hydrotime model

Seeds of 19 rapeseed genotypes (Table 1) were provided by Seed and Plant Improvement Institute, Karaj, Iran. Four replicates of 50 seeds for each genotype were germinated in 90 mm-diameter Petri dishes on filter paper at 20 °C at each of the five water potentials: 0, − 0.15, − 0.3, − 0.5, and − 0.8 MPa. Osmotic potentials were maintained with solutions of polyethylene glycol 6000 (PEG) according to the desired water potentials at 20 °C (Michel and

Table 1 Oilseed rape genotypes and their characteristics used for experiments with dormancy induction, produced in Karaj in the growing season 2014–2015

Genotype	Origin	Type of cultivar	Growing type
Line 205	Iran	Open pollinated	–
Line 280	Iran	Open pollinated	–
Line 285	Iran	Open pollinated	–
Line 389	Iran	Open pollinated	–
H50	Canada	Hybrid	Spring
Hayola 401	Canada	Hybrid	Spring
Modena	Denmark	Open pollinated	Facultative
RGS	Germany	Open pollinated	Spring
SLM046	Germany	Open pollinated	Winter
Sarigol	Iran	Open pollinated	Facultative
Talayeh	Germany	Open pollinated	Facultative
Karaj1	Iran	Open pollinated	Winter
Karaj2	Iran	Open pollinated	Winter
Karaj3	Iran	Open pollinated	Winter
Licord	Germany	Open pollinated	Facultative
Okapi	France	Open pollinated	Facultative
Opera	Sweden	Open pollinated	Facultative
RGS003	Germany	Open pollinated	Spring
Zarfam	Iran	Open pollinated	Facultative

Kaufmann 1973; Momoh et al. 2002). Before seed placement, the filter papers were soaked for 24 h in Petri dishes containing the mentioned water potentials. Seeds were monitored for germination twice a day or daily until no further germination was observed for 3 days, and they were considered germinated when radicle protrusions were approximately 2 mm.

The hydrotime model (Gummerson 1986; Bradford 1990; Bradford and Still 2004) is described by the following equation:

$$\theta_H = \left(\psi - \psi_b(g)\right) \text{tg}, \quad (1)$$

where ψ is the actual water potential (MPa), θ_H is the hydrotime constant (MPa h), $\psi_b(g)$ is the base water potential (MPa) defined for a specific germination fraction (g), and tg is the time (h) to radicle protrusion of fraction g (%) of the seed population.

The normal distribution of $\psi_b(g)$ values among seeds in a population is characterized by its median $\psi_b(50)$ and standard deviation ($\sigma\psi_b$) that can be estimated using repeated probit analyses, varying θ_H until the best fit is reached (Dahal and Bradford 1990; Huarte 2006; Soltani and Farzaneh 2014) as follows:

$$\text{probit}\ (g) = \left[\psi - (\theta_H/\ \text{tg}) - \psi_b(50)\right] / \sigma\psi_b \quad (2)$$

which separately model the germination time course at different water potentials for each genotype.

Pot experiments

Seeds of the 19 mentioned rapeseed genotypes were sown at control ($EC_e = 0.8$ dS m^{-1}, field capacity, 2 cm depth) and different abiotic stresses of drought (− 0.7 MPa), salinity (7 dS m^{-1}), deep sowing (5 cm), and low (10 °C) and high (30 °C) temperatures. Abiotic treatments of control, drought, salinity, and deep sowing were sown in greenhouse (at 20 °C), and low and high temperatures in growth chambers. At each stress treatment, we only had one stress and other conditions were as control. There were control conditions for greenhouse and growth chambers. Pots (15 cm diameter and 20 cm deep) were filled with a clay loam soil (34% clay, 26% silt, 40% sand). The experiments began on 30 January 2016. Soil water was determined based on soil moisture release curve, which indicates the relationship between soil water potential and soil moisture content (Saxton et al. 1986). Prior to the pot experiments, three samples of wet soil (wet soil = dry soil + soil moisture content) were dried and soil moisture content at the beginning of experiment was determined. Then pots were equally filled with wet soil. Two pots were considered as references and were weighted each day: one for drought stress and one for other abiotic stresses. The soil moisture content could be obtainable after weighing and it was possible to calculate soil water potential from soil moisture release curve. Soil water potentials were kept around field capacity and − 0.7 MPa for different treatments during the experiment.

Salinity stress was created according to methods mentioned by Soltani et al. (2004) and Soltani et al. (2009). The amount of salt required for the salinity level of 7 dS m^{-1} was calculated using the method of staff (US Salinity Laboratory 1954; Richter et al. 1995). NaCl and $CaCl_2$ salts with a weight ratio of 1:1 were used for soil salinization.

Pots were observed daily for seedling emergence until complete seedling emergence. Final emergence percentage and emergence rate (day^{-1}) were determined as follows:

$$\text{Emergence rate} = 1/t10, \quad (3)$$

where $t10$ (day) is time to 10% of seedling emergence. Estimates of the time taken for cumulative emergence to reach 10% in each replicate and treatment were interpolated from the emergence progress curve versus time. Time to 10% of emergence was used because most treatments had a $t10$ and not reached 50% of emergence (Bewley et al. 2013; Soltani et al. 2015). Leaf number, leaf area, and shoot dry weight were measured at 45 days after sowing. Due to the limitation of using the growth chambers, data from seedling growth were not available for low and high temperatures, and these experiments were finished after seedling emergence completed.

Data analysis

Analysis of variation was conducted as factorial experiment based on completely randomized design (CRD) with four replications for pot experiments. Hydrotime model was fitted to the data as indicated in Eq. 2. Analysis of variation was conducted as CRD with four replications for hydrotime model parameters. Significant differences for all data among genotypes were determined using Proc GLM (SAS Institute Inc., Cary, NC, USA, 2011). Mean comparisons among genotypes were considered by least significant difference ($P < 0.05$). Simple correlation coefficients and principal component analysis (PCA) were carried out using SAS (SAS Institute Inc., Cary, NC, USA, 2011).

Results

Hydrotime model for germination

The time courses of cumulative germination at different water potentials for each genotype are shown in Fig. 1. A single distribution of ψ_b(g) and a constant value of θ_H was

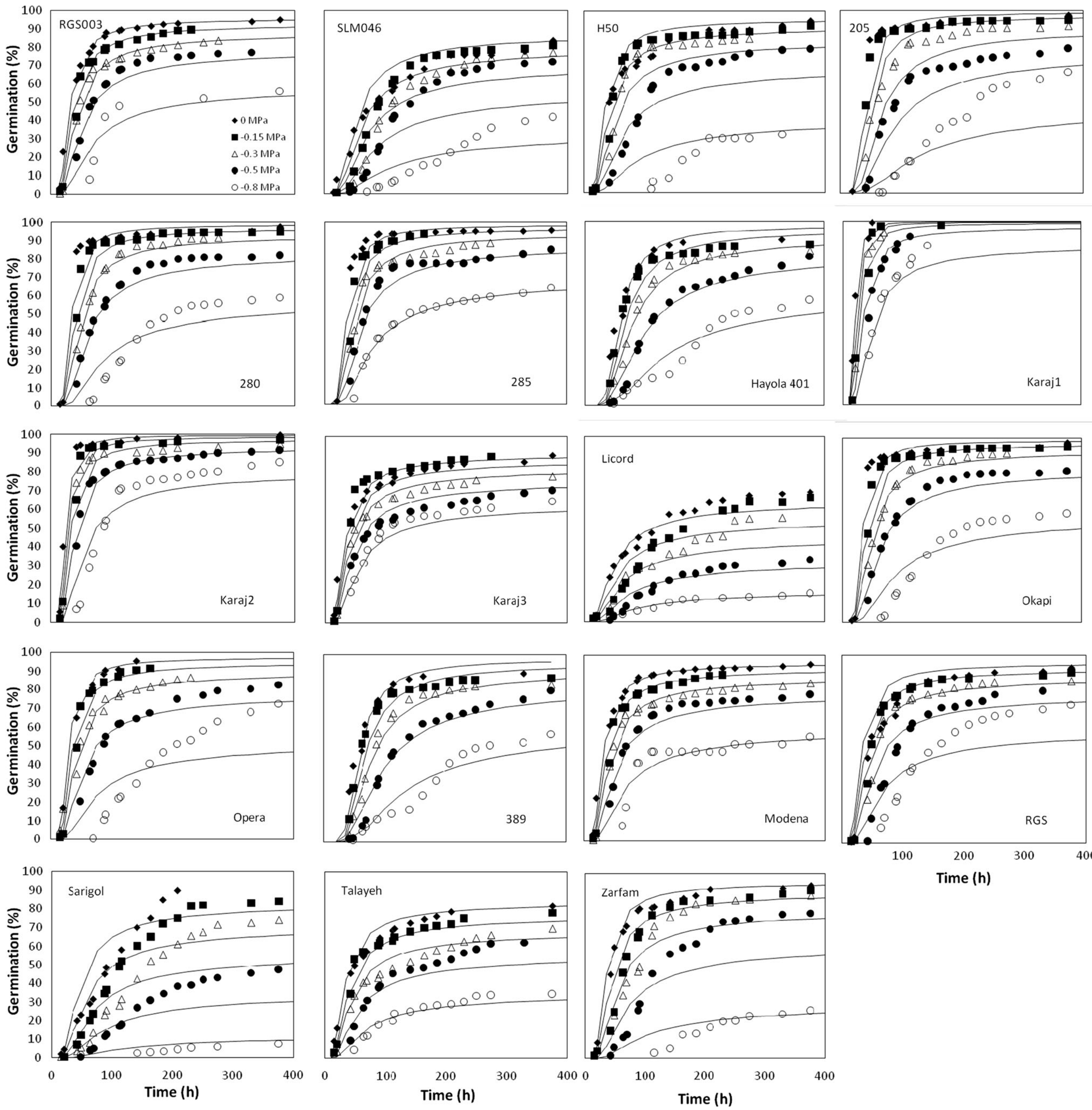

Fig. 1 Germination time-courses of 19 genotypes of oil seed rape. The symbols are the actual data and the curves are the results of fitting the hydrotime model. The name of each genotype is indicated

obtained for each genotype. Coefficients of determination (R^2) values indicate a good fit of the hydrotime model at 20 °C. The predicted germination time courses at the various water potentials generally fitted well with the observed germination data, with R^2 values of 0.61–0.88 (Fig. 1; Table 2). The estimated values of θ_H, $\psi_b(50)$, and $\sigma\psi_b$ differed significantly across genotypes (Table 2). The estimated θ_H was lowest for ''Zarfam'' (22.8 MPa-hours) and highest for ''line 389'' (50.9 MPa-hours). The lowest $\psi_b(50)$ was observed in ''Karaj1'' (− 1.23 MPa), and it did not significantly differ from ''Karaj2'', ''Karaj3'', ''Opera, line 285'', and ''Hayola 401''. The estimated $\sigma\psi_b$ ranged between 0.326 and 0.750, and significantly changed among genotypes.

Table 2 Estimated hydrotime model parameters for different genotypes of oilseed rape

Genotype	θ_H (MPa h)	$\psi_b(50)$ (MPa)	$\sigma\psi_b$	R^2
Line 205	28.21	− 0.853	0.326	0.85
Line 280	24.56	− 0.798	0.352	0.87
Line 285	36.87	− 1.057	0.436	0.84
Line 389	50.93	− 0.939	0.419	0.87
H50	24.43	− 0.681	0.372	0.85
Hayola 401	33.63	− 0.988	0.490	0.85
Modena	29.34	− 0.360	0.484	0.61
RGS	40.31	− 0.892	0.589	0.80
SLM046	40.16	− 0.603	0.612	0.68
Sarigol	28.52	− 0.377	0.524	0.70
Talayeh	23.73	− 0.665	0.488	0.86
Karaj1	25.03	− 1.234	0.366	0.76
Karaj2	27.38	− 1.153	0.393	0.87
Karaj3	40.25	− 1.023	0.750	0.87
Licord	34.03	− 0.230	0.801	0.76
Okapi	30.72	− 0.867	0.443	0.80
Opera	48.07	− 1.202	0.726	0.79
RGS003	30.81	− 0.950	0.523	0.88
Zarfam	22.76	− 0.611	0.366	0.84
F value	3.05**	18.43**	3.58**	–
$LSD_{0.05}$	13.27	0.254	0.244	–

Coefficients determination (R^2) is showing the goodness of fitness of the model fitting. *F* values and LSD are indicated to compare the parameters among genotypes

The ** indicate significance at $P < 0.01$

Pot experiments

Seedling emergence percentage (%) and rate (days^{-1}) differed significantly across genotypes under each experimental condition (Table 3). Leaf number, leaf area, and shoot dry matter also changed significantly cross genotypes in control, deep sowing, water stress, and salinity stress (Table 4). Abiotic stresses decreased emergence percentage and rate of emergence (Table 3) and all seedling variables compared to control conditions (Table 4). However, there were no significantly changes for the emergence and seedling growth in some genotypes under stress conditions compared with control. Top genotypes differed for each variable and environmental condition, and it was not easy to select a top genotype in a wide range of environmental conditions from means comparison. For example, ''Karaj1'' was included in top genotypes for emergence (%) in control, deep sowing, low temperature, and high temperature, but it was not included in water and salinity stresses.

Principal component analysis (PCA)

PCA showed that 17 PCs (1–17) explained all early vigour variations across genotypes, and six PCs (1–6) explained 80% of variations (Table 5). The first PC explained 40% of variations, and a correlation was observed between PC1 and $\psi_b(50)$, germination percentage and rate (hour^{-1}) in different water potentials, and seedling emergence percentage and rate (day^{-1}) in different environmental conditions. The correlation between PC1 and $\psi_b(50)$ was negative and positive between PC1 and germination and seedling emergence [both rate (time^{-1}) and percentage]. The second and third PCs explained about 22% of variations. PC2 had negative correlations with hydrotime constant (θ_H), shoot dry matter, and leaf area (in deep sowing), but had positive correlations with leaf number (in control and water stress), shoot dry matter (in control, and water stress), and leaf area (in control and water stress). PC3 had positive correlations with hydrotime (θ_H), leaf area (in control and salinity stress), shoot dry matter (in control and salinity stress), and leaf number (in deep sowing and salinity stress).

The score plots of PCA are indicated to study overall distribution of different variables of early seedling growth in different environmental conditions (Fig. 2). It can be observed that ''Hayola 401'', ''line 205'', ''line 280'', ''RGS003'', ''line 285'', and ''Karaj1'' have more negative values of $\psi_b(50)$, high germination and seedling emergence percentage rate (PC1) and lower hydrotime, and high seedling growth under control and water stress (PC2) (Fig. 2a). Genotypes of ''Karaj3'', ''Opera'', ''RGS'', ''line 285'', ''Hayola 401'', ''Karaj2'' also have more negative values of $\psi_b(50)$, high germination and seedling emergence percentage and rate (PC1) and high seedling growth under control, salinity and deep sowing (Fig. 2b). Overall, genotypes of ''Hayola 401'' and ''line 285'' were the most tolerant to abiotic stresses during crop establishment and seedling growth.

Correlation analysis

Correlations among hydrotime model parameters and early seed vigour variables indicated that $\psi_b(50)$ negatively correlated with seedling emergence percentage and rate (day^{-1}) under control, deep sowing, water stress, salinity stress, low and high temperatures (Table 6). It showed that genotypes with more negative values of $\psi_b(50)$ have more seedling emergence percentage and more seedling emergence rate under a wide range of environmental conditions. Hydrotime constant (θ_H) had only negative correlations with seedling growth variables under water stress. The correlations were observed between $\sigma\psi_b$ and rate of

Table 3 Effect of environmental conditions of control, deep sowing, water stress, salinity stress, low and high temperatures on seedling emergence (E) and rate of emergence (RE) in relation to the genotypes

Genotyzpe	Control Condition		Deep sowing		Water stress		Salinity stress		Low temperature		High temperature	
	E (%)	RE (days^{-1})	E (%)	RE (days^{-1})	E (%)	RE (days^{-1})	E (%)	RE (days^{-1})	E (%)	RE (days^{-1})	E (%)	RE (days^{-1})
Line 205	57.3	0.0064	61.3	0.0043	44.0	0.0048	57.3	0.0046	34.2	0.0020	48.1	0.0127
Line 280	61.3	0.0052	24.0	0.0039	37.3	0.0039	58.7	0.0056	44.9	0.0028	58.1	0.0343
Line 285	57.8	0.0065	66.7	0.0072	52.0	0.0058	25.3	0.0044	36.6	0.0032	77	0.0126
Line 389	58.7	0.0069	20.0	0.0036	34.7	0.0045	29.3	0.0039	58.3	0.0030	50	0.0177
H50	52.3	0.0055	57.3	0.0053	22.7	0.0031	20.0	0.0024	26.6	0.0023	74	0.0404
Hayola 401	61.3	0.0070	37.3	0.0051	57.3	0.0045	57.3	0.0049	31.6	0.0023	49	0.0131
Modena	42.6	0.0038	10.0	0.0013	30.0	0.0033	6.0	0.0000	51.1	0.0033	26.1	0.0112
RGS	57.3	0.0078	13.3	0.0021	40.0	0.0044	45.3	0.0044	28.9	0.0027	31	0.0170
SLM046	30.6	0.0035	5.3	0.0008	6.7	0.0000	18.7	0.0021	35.0	0.0025	20	0.0108
Sarigol	50.6	0.0038	6.7	0.0000	36.0	0.0033	20.0	0.0025	22.2	0.0023	22	0.0061
Talayeh	42.6	0.0051	30.7	0.0046	36.0	0.0050	36.0	0.0064	33.3	0.0033	15	0.0097
Karaj1	84.0	0.0092	58.7	0.0068	45.3	0.0041	29.3	0.0031	60.0	0.0044	65	0.1208
Karaj2	72.0	0.0080	20.0	0.0040	65.3	0.0047	45.3	0.0059	69.9	0.0042	66	0.0523
Karaj3	57.3	0.0071	17.3	0.0027	29.3	0.0038	37.3	0.0059	25.0	0.0024	29	0.0226
Licord	33.3	0.0042	14.7	0.0014	8.0	0.0000	13.3	0.0016	11.7	0.0013	23	0.0079
Okapi	48.0	0.0058	16.0	0.0025	28.0	0.0031	14.7	0.0017	28.3	0.0026	48	0.0100
Opera	62.7	0.0062	10.7	0.0009	30.7	0.0034	25.3	0.0039	22.2	0.0021	53	0.0203
RGS003	54.7	0.0064	13.3	0.0019	30.7	0.0042	50.7	0.0046	30.0	0.0030	41	0.0203
Zarfam	57.3	0.0051	8.0	0.0010	37.3	0.0036	6.7	0.0008	13.3	0.0018	39	0.0093
$LSD_{0.05}$	25.97	0.0022	17.82	0.0031	15.51	0.0014	20.60	0.0026	15.58	0.0011	18.75	0.0348

Source of variation	DF	E		RE	
		F value	*P*	*F* value	*P*
G	18	18.35	< 0.0001	6.51	< 0.0001
EC	5	36.93	< 0.0001	58.73	< 0.0001
G × EC	90	3.66	< 0.0001	5.08	< 0.0001

Analysis of variance are indicated for seedling emergence and rate of emergence as affected by genotype (G), environmental condition (EC) and their interactions (G × EC). The values of $LSD_{0.05}$ are indicated for each variable and environmental condition

emergence (under control, water stress, and salinity stress), leaf number (under water stress), and seedling emergence percentage (under salinity stress).

Discussion

Our results showed wide variations among genotypes for germination characteristics, hydrotime parameters, and early vigour of seedlings under different environmental conditions. Considerable variations were observed for seedling emergence and rate under abiotic stresses. Abiotic stresses decreased seedling emergence compared to control by 52, 36, 42, 36, and 20% for deep sowing, water stress, salinity stress, low and high temperatures, respectively. However, abiotic stresses decreased shoot dry weight (mg) vis-à-vis control by 16, 77, and 5% for deep sowing, water stress, and salinity stress, respectively. It showed that seedling emergence of rapeseed is most sensitive to deep sowing, but seedling growth is most sensitive to water stress.

Sowing depth is one of the major factors known to influence seedling emergence and crop establishment of rapeseed (Zuo et al. 2017). Gurber et al. (2010) showed that increasing soil depth from 1 to 5 cm had no effect on seedling emergence of rapeseed, but it was clearly decreased at depth of 7 cm and completely inhibited at 12 cm. Soltani et al. (2013) indicated that no seedling of rapeseed emerged from soil depth of 10 cm and seedling emergence was the highest at the soil depth of 1–3 cm. Thomas et al. (1994) also observed that seedling emergence of rapeseed decreased at sowing depths deeper than

Table 4 Effect of environmental conditions of control, deep sowing, water stress and salinity stress on leaf number (LN), leaf area (LA) and shoot dry weight (SDW) in relation to the genotypes

Genotype	Control Condition			Deep sowing			Water stress			Salinity stress		
	LN	LA (mm^2)	SDW (mg)	LN	LA (mm^2)	SDW (mg)	LN	LA (mm^2)	SDW (mg)	LN	LA (mm^2)	SDW (mg)
Line 205	3.75	1828.59	88.11	3.55	1775.01	87.50	1.98	593.44	33.67	2.26	969.15	53.89
Line 280	3.34	2414.15	118.17	2.72	1732.75	80.15	1.69	672.66	32.29	2.91	2592.81	102.27
Line 285	3.72	2122.26	97.02	3.29	1432.11	63.25	2.24	468.90	29.62	4.03	2915.38	126.17
Line 389	3.28	1977.03	106.20	3.14	2300.52	100.60	0.75	93.00	13.62	2.89	1894.31	93.87
H50	3.38	2133.18	92.66	3.49	2867.57	127.16	1.64	233.82	19.43	2.87	1441.46	75.63
Hayola 401	3.31	2360.21	117.54	2.80	1750.62	66.22	2.33	578.22	36.42	3.21	3890.83	187.98
Modena	2.50	1872.34	92.01	2.65	1672.81	81.37	0.92	108.24	14.21	2.17	1228.33	65.29
RGS	3.44	1643.17	78.64	3.00	2352.53	108.37	1.70	300.85	17.24	3.90	3373.76	144.31
SLM046	2.62	1315.13	62.99	2.89	2502.07	137.89	2.33	484.38	23.33	2.33	1809.07	83.98
Sarigol	3.01	1350.10	83.18	3.33	1216.94	86.67	1.81	363.57	28.54	1.89	780.12	47.17
Talayeh	3.96	4383.02	185.83	3.11	2241.05	97.43	2.11	784.57	39.83	2.83	1627.85	86.47
Karaj1	3.11	2144.27	110.71	2.65	1695.02	84.35	2.59	591.27	53.45	2.73	1685.88	94.92
Karaj2	2.84	2238.47	104.24	2.63	1300.31	44.00	1.63	488.32	25.50	3.10	2882.03	129.37
Karaj3	3.27	3155.68	147.93	3.08	1993.00	97.56	1.67	222.46	22.22	3.06	2451.32	124.48
Licord	3.32	3294.22	194.50	2.42	581.72	37.58	1.33	141.15	12.33	2.78	2910.91	123.17
Okapi	3.92	2754.91	122.99	3.54	1715.28	91.13	2.00	632.19	37.17	2.87	1143.04	56.11
Opera	3.39	2785.45	117.78	3.67	3917.11	172.83	1.36	238.88	16.34	3.32	3407.63	194.48
RGS003	3.63	2470.14	100.38	3.73	2073.32	86.31	2.10	412.60	26.68	3.37	1828.55	98.21
Zarfam	2.53	2212.51	87.69	3.42	2927.22	104.50	1.40	201.60	15.75	2.89	1296.33	83.33
$LSD_{0.05}$	0.68	1447.2	53.41	0.99	1931.00	82.98	0.82	522.65	21.46	0.98	1768.10	74.73

Source of variation	DF	LN		LA		SDW	
		F value	*P*	*F* value	*P*	*F* value	*P*
Genotype (G)	18	2.85	0.0004	1.88	0.0246	1.91	0.0211
Environment (E)	3	89.04	<0.0001	49.41	<0.0001	57.66	<0.0001
G × E	54	1.86	0.0023	1.71	0.0072	2.16	0.0002

Analysis of variance are indicated for leaf number, leaf area and shoot dry weight as affected by genotype (G), environmental condition (EC) and their interactions (G × EC). The values of $LSD_{0.05}$ are indicated for each variable and environmental condition

Table 5 Eigenvalues of first six principal components and the percentage of variance explained by them for hydrotime model parameters, germination, emergence and seedling growth properties of oil seed rape under different environmental conditions

PCs	Eigenvalue	Percentage of variance	Cumulative (%)
1	16.51	40.26	40.26
2	4.91	11.97	52.23
3	4.14	10.09	62.32
4	3.52	08.59	70.91
5	2.17	05.28	76.19
6	1.93	04.70	80.89

3 cm in field. In the current study, soil depth of 5 cm reduced seedling emergence compared with soil depth of 2 cm (control). In general, failure of rapeseed seedlings emergence in deep sowing could result from (1) unfavourable conditions for germination, (2) fatal germination, and (3) dormancy induction (Soltani et al. 2013, 2016). Our results showed that deep sowing also increases the time between seed germination and seedling emergence. This can result in an increase in hypocotyl or epicotyl length, and reduce the ability to overcome soil strength and increase the risk of exposure to pathogens (Zuo et al. 2017).

Insufficient rainfall affects rapeseed establishment, seedling growth, and foliar expansion in semi-arid areas. The present study showed soil water potential of − 0.7 MPa decreased seedling emergence and growth of rapeseed crops. Sangtarash et al. (2009) showed that water stress decreased seedling growth of rapeseed crops. Jabbari et al. (2013) found that reduction in soil moisture content from 50 to 20% field capacity decreased seedling emergence of rapeseed from 94.3 to 82.7%. The effects of water

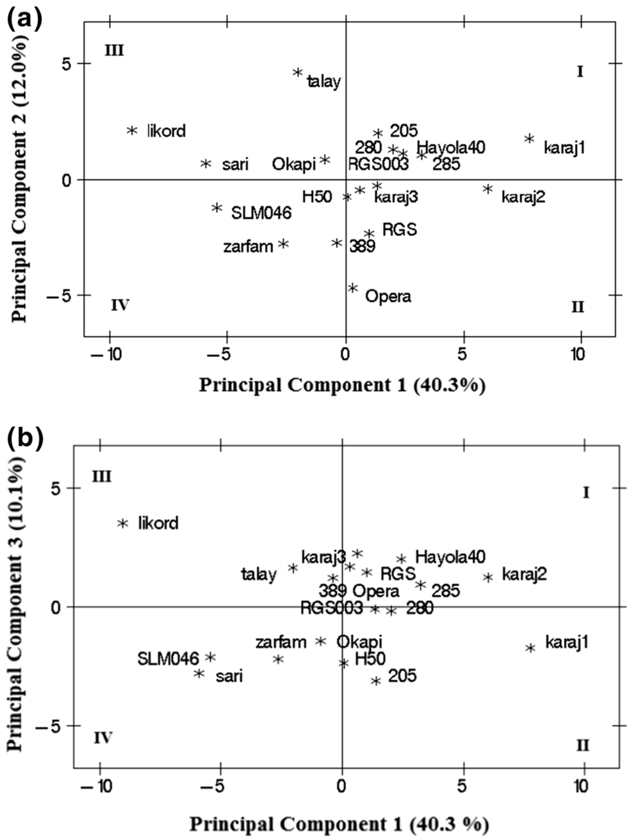

Fig. 2 Biplot for diversity of 19 oil seed rape genotypes at five water potentials in germination stage and at six environmental conditions in seedling emergence stage, showing which tolerant genotypes to abiotic stresses and had higher values PC1, PC2 and PC3. PC1, PC2 and PC3 are first, second, and third principal components. Genotypes name are defined in Table 1

stress on crop establishment are well understood. A reduction in soil water potential leads to decrease difference between ψ and ψ_b and increasing seedling emergence time. Water stress also affects photosynthesis, translocation of assimilates, shoot dry weight and chlorophyll content (Mundree et al. 2002; Liu et al. 2013), and severe drought stress can lead to complete death (Kivuva et al. 2015).

Salinity is a major problem affecting semi-arid areas. Grewal (2010) indicated that water uptake and shoot dry weight of rapeseed declined by 26 and 34% under highest subsoil salinity (1500 and 2000 mg/kg soil). The reduced water uptake is probably due to more osmotic potential under salinity conditions. Naeem et al. (2011) found a positive correlation between osmotic potential and relative water content of first leaf and third leaf in rapeseed. They also showed that increasing salinity imposed negative impact on relative growth rate of rapeseed. Our study revealed that salinity significantly decreased seedling emergence, but shoot dry weight of emerged seedling has little difference from control.

Table 6 Correlation coefficients (*r*) between seedling growth variables and hydrotime parameters at different environmental conditions of control, deep sowing, water stress, salinity stress, low and high temperatures for 19 oil seed rape

Variable	θ_H	$\psi_b(50)$	$\sigma\psi_b$
Control condition			
Emergence (%)	− 0.09	− 0.45**	0.21
Rate of emergence (days^{-1})	0.03	− 0.58**	0.27*
Leaf number	0.08	− 0.10	0.02
Leaf area	− 0.09	0.07	0.17
Shoot dry matter	− 0.07	0.17	0.05
Deep sowing			
Emergence (%)	− 0.18	− 0.29*	0.18
Rate of emergence (days^{-1})	− 0.07	− 0.42**	0.11
Leaf number	0.01	0.01	0.07
Leaf area	0.06	− 0.01	0.14
Shoot dry matter	0.10	0.05	0.05
Water stress			
Emergence (%)	− 0.14	− 0.42**	0.18
Rate of emergence (days^{-1})	− 0.05	− 0.43**	0.27*
Leaf number	− 0.23*	− 0.11	− 0.22*
Leaf area	− 0.28*	− 0.06	− 0.12
Shoot dry matter	− 0.26*	− 0.17	− 0.20
Salinity stress			
Emergence (%)	− 0.04	− 0.41**	0.26*
Rate of emergence (days^{-1})	0.03	− 0.46**	0.28*
Leaf number	0.15	− 0.28*	0.18
Leaf area	0.14	− 0.15	0.05
Shoot dry matter	0.15	− 0.23*	0.10
Low temperature (10 °C)			
Emergence (%)	− 0.02	− 0.25*	− 0.03
Rate of emergence (days^{-1})	− 0.10	− 0.24*	− 0.11
High temperature (30 °C)			
Emergence (%)	− 0.12	− 0.43**	0.20
Rate of emergence	− 0.22	− 0.32**	− 0.04

The * and ** indicate significance at $P < 0.05$ and $P < 0.01$, respectively

Temperature is the single-most important factor for germination and seedling growth when light, oxygen, nutrients, and moisture are not the limiting factors (Steinmaus et al. 2000). Cardinal temperatures determine the range for seed germination and seedling growth (Seefeldt et al. 2002). Maximum rate of growth is obtainable at optimum temperature(s), while decreasing and increasing temperatures lead to declined seedling growth. Farzaneh et al. (2014) showed wide variations of cardinal temperatures and cold or heat tolerance among rapeseed genotypes. The base, optimum, and ceiling temperatures ranged from 0 to 5, 21–35, and 41–46 °C for germination rate (hour^{-1}) of rapeseed (Farzaneh et al. 2014). These values were similar to the range of values reported in other studies (Marshall and Squire 1996; Squire 1999; Soltani et al. 2013). Low or high temperatures reduce the seedling emergence and increase time from sowing to seedling emergence. It is important to develop cultivars with chilling or heat tolerance, as these allow cultivars to tolerate the cold or heat stresses during early rapeseed growth, allowing for better crop establishment (Farzaneh et al. 2014).

In the current study, analysis of PCA showed that PC1 is related to crop establishment under control and abiotic stresses, and PC2 and PC3 are related to seedling growth. PC2 had correlations with seedling growth variables under control and water stress. However, PC3 was related to seedling growth under control, salinity, and deep sowing stresses. Based on PCA biplot analysis, rapeseed genotypes were categorized into four groups based on tolerant to water stress, low and high temperatures (Fig. 2a), or tolerant to salinity, deep sowing, and low and high temperatures (Fig. 2b): tolerant (I), intermediate (II and III), and sensitive (IV). The genotypes in Groups I and II had good establishment in a wide range of environmental factors (control, water stress, salinity, deep sowing, low and high temperatures; Fig. 2a, b). Genotypes in Group III had good seedling growth under control and water stress (Fig. 2a), or under control, salinity and deep sowing (Fig. 2b). Genotypes in Group IV did not well crop establishment (in all environmental conditions) and did not well seedling growth under control, water stress (Fig. 2a), or under salinity and deep sowing (Fig. 2b) conditions. Genotypes of ''Hayola 401'' and ''line 285'' were included in Group I in both PCA graphs, showing these genotypes had well crop establishment and seedling growth under control and abiotic stresses (Fig. 2). ''Hayola 401'' and ''line 285'' were the most tolerant genotypes to water stress, salinity, deep sowing, and low and high temperatures.

PC1 negatively correlated with $\psi_b(50)$, thus showing that genotypes with more negative values of $\psi_b(50)$ have better crop establishment. Our results indicated that among hydrotime parameters, $\psi_b(50)$ was correlated with seedling emergence percentage and rate (day^{-1}) under control and abiotic stresses (Table 6). There were close relationships between the overall mean (under control, water stress, deep sowing, salinity, and low and high temperatures) of seedling emergence (%) or rate (days^{-1}) and $\psi_b(50)$ (Fig. 3). It shows that $\psi_b(50)$ can be applied to predict crop establishment in rapeseed under a wide range of environmental conditions. Some reports showed that hydrotime parameters could be used to predict early seed vigour in other crops (Dahal and Bradford 1990; Bradford and Somasco 1994; Bradford and Still 2004; Farzane and Soltani 2011; Soltani and Farzaneh 2014). Farzane and Soltani (2011) indicated that $\psi_b(50)$ was related to seedling vigour of

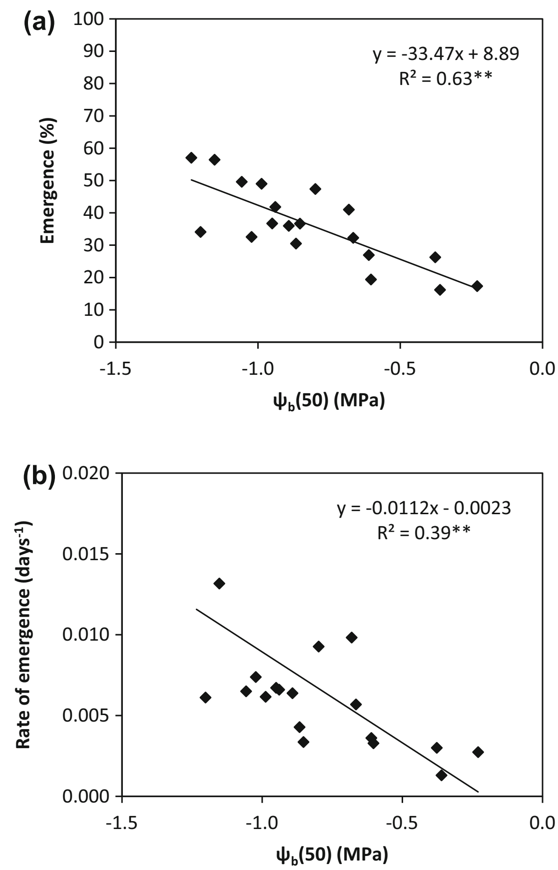

Fig. 3 Relationships (regressions) between overall mean (mean of six levels of environmental conditions) of seedling emergence percentage (**a**) and rate of emergence (**b**) with base water potential ($\psi_b(50)$) for 19 oil seed rape genotypes. Significant ($P < 0.01$) R^2 values are indicated by double asterisk

sugar beet. Soltani and Farzaneh (2014) also revealed a relationship between $\psi_b(50)$ and seedling emergence percentage, suggesting that $\psi_b(50)$ can be used for predicting early seed vigour in cotton.

Successful breeding programmes to improve abiotic stress tolerant of crops depend on the identification of tolerant germplasms and their traits. There were studies on identification indices of drought tolerance by the PCA method during rapeseed germination (Xia and Xiaoyu 2012) and seedling establishment (Jabbari et al. 2013). Xia and Xiaoyu (2012) reported that seed germination and germination rate are the most important traits for screening of rapeseed genotypes under drought conditions. Jabbari et al. (2013) showed that tolerant rapeseed genotypes were characterized by their higher germination rate, final emergence, mean daily germination, and seedling vigour index. Farzaneh et al. (2014), who screened rapeseed genotypes for thermotolerance, stated that base temperature (T_b) had positive relationship with heat tolerance and negative relationship with cold tolerance, and that T_b is the most important trait for screening rapeseed genotypes under heat or cold stresses. There was no study to identify indices of salinity or deep sowing tolerance during rapeseed establishment. Our study showed that tolerance to rapeseed genotypes to water stress, salinity, deep sowing, and low and high temperatures can be identified by lower $\psi_b(50)$.

On the whole, this study estimated hydrotime parameters for rapeseed germination and related them to abiotic stress tolerance for the first time. Abiotic stresses decreased seedling emergence and seedling growth. Seedling emergence of rapeseed was most sensitive to deep sowing, but seedling growth was most sensitive to water stress. PCA analysis showed that ''Hayola 401'' and ''line 285'' were the most tolerant genotypes to abiotic stresses. Among the hydrotime parameters, $\psi_b(50)$ was the most correlated one with seedling emergence under all abiotic stresses. Based on our results, the $\psi_b(50)$ ranged between − 1.234 and − 0.230 MPa, and the most tolerant genotypes had more negative values of $\psi_b(50)$. Therefore, it can be concluded that identifying tolerant genotypes of rapeseed to abiotic stresses, $\psi_b(50)$ is a good trait and that breeders can focus on reducing $\psi_b(50)$ to increase tolerance to abiotic stresses. It may be difficult to identify a cultivar that possesses all stresses tolerance characteristics, but selection and breeding based on a single trait ($\psi_b(50)$) would be more applicable. Evaluation of the relationships between stress tolerance characteristics and hydrotime model parameters, in particular $\psi_b(50)$, might reveal similar significant relationships in other crops.

Author contribution statement This paper is based on the MS thesis for Roqia Adeli. Elias Soltani was her supervisor and he has written the paper. Gholam Abbas Akbari and Hossein Ramshini worked as advisors.

References

Aoun WB, Akkari ME, Flénet F, Jacquet F, Gabrielle B (2016) Recommended fertilization practices improve the environmental performance of biodiesel from winter oilseed rape in France. J Clean Prod 139:242–249

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Seeds: physiology of development, germination and dormancy, 3rd edn. Springer, New York

Bradford KJ (1990) A water relations analysis of seed germination rates. Plant Physiol 94:840–849

Bradford KJ, Somasco OA (1994) Water relations of lettuce seed thermoinhibition. I. Priming and endosperm effects on base water potential. Seed Sci Res 4:1–10

Bradford KJ, Still DW (2004) Applications of hydrotime analysis in seed testing. Seed Technol 26:74–85

Dahal P, Bradford KJ (1990) Effects of priming and endosperm integrity on seed germination rates of tomato genotypes. II. Germination at reduced water potential. J Exp Bot 41:1441–1453

Farzane S, Soltani E (2011) Relationships between Hydrotime parameters and seed vigor in sugar beet. Seed Sci Biotech 5:7–10

Farzaneh S, Soltani E, Zeinali E, Ghaderi-Far F (2014) Screening oil seed rape germination for thermotolerance using a laboratory-based method. Seed Tech 36:15–27

Fernández-Torquemada Y, Sánchez-Lizaso JL (2013) Effects of salinity on seed germination and early seedling growth of the Mediterranean seagrass *Posidonia oceanica* (L.) Delile. Estuar Coas Shelf Sci 119:64–70

Flakelar CL, Luckett DJ, Howitt JA, Doran G, Prenzler PD (2015) Canola (*Brassica napus*) oil from Australian cultivars shows promising levels of tocopherols and carotenoids, along with good oxidative stability. J Food Comp Ana 42:179–186

Grewal HS (2010) Water uptake, water use efficiency, plant growth and ionic balance of wheat, barley, canola and chickpea plants on a sodic vertosol with variable subsoil NaCl salinity. Agric Water Manag 97:148–156

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

Gummerson RJ (1986) The effect of constant temperatures and osmotic potentials on the germination of sugar beet. J Exp Bot 37:729–741

Gurber S, Bühler A, Möhring J, Claupein W (2010) Sleepers in the soil—vertical distribution by tillage and long-term survival of oilseed rape seeds compared with plastic pellets. Eur J Agron 33:81–88

Huarte R (2006) Hydrotime analysis of the effect of fluctuating temperatures on seed germination in several non-cultivated species. Seed Sci Tech 34:533–547

Jabbari H, Akbari GA, Khosh Kholgh Sima NA, Shirani Rad AH, Alahdadi I, Hamed A, Shariatpanahi ME (2013) Relationships between seedling establishment and soil moisture content for winter and spring rapeseed genotypes. Ind Crop Prod 49:177–187

Katembe WJ, Ungar IA, Mitchell JP (1998) Effect of salinity on germination and seedling growth of two *Artiplex* species (Chenopodiaceae). Ann Bot 82:167–175

Kivuva BM, Githiri SM, Yencho GC, Sibiya J (2015) Screening sweetpotato genotypes for tolerance to drought stress. Field Crop Res 171:11–22

Liu D, Wu L, Naeem MS, Liu H, Deng X, Xu L, Zhang F, Zhou WJ (2013) 5-Aminolevulinic acid enhances photosynthetic gas exchange, chlorophyll fluorescence and antioxidant system in oilseed rape under drought stress. Acta Physiol Plant 35:2747–2759

Marshall B, Squire GR (1996) Non-linearity in rate-temperature relations of germination in oilseed rape. J Exp Bot 47:1369–1375

Michel BE, Kaufmann MR (1973) The osmotic potential of polyethylene glycol 6000. Plant Physiol 51:914–916

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

Mundree SG, Baker B, Mowla S, Peters S, Marais S, Willigen CV, Govender K, Maredza A, Muyanga S, Farrant JM, Thomson JA (2002) Physiological and molecular insights into drought tolerance. Afr J Biotechnol 1:28–38

Naeem MS, Rasheed M, Liu D, Jin ZL, Ming DF, Yoneyama K, Takeuchi Y, Zhou WJ (2011) 5-Aminolevulinic acid ameliorates salinity-induced metabolic, water-related and biochemical changes in *Brassica napus* L. Acta Physiol Plant 33:517–528

Patané C, Saita A, Tubeileh A, Cosentino SL, Cavallaro L (2016) Modeling seed germination of unprimed and primed seeds of sweet sorghum under PEG-induced water stress through the hydrotime analysis. Acta Physiol Plant 38:115–127

Richter C, Heiligtag B, Gerting M, Abdullahzadeh A (1995) Salt tolerance of different varieties of *Sorghum bicolor* and *Vicia faba*. Der Tropen 96:141–152

Sangtarash MH, Qaderi MM, Chinnappa CC, Reid DM (2009) Differential sensitivity of canola (*Brassica napus*) seedlings to ultraviolet-B radiation, water stress and abscisic acid. Environ Exp Bot 66:212–219
SAS Institute INC (2011) SAS/STAT 9.3 user's guide, the PLSprocedure, Cary
Saxton KE, Rawls WJ, Romberger JS, Papendick RI (1986) Estimation generalized soil water characteristics from texture. Soil Sci Soc Am J 50:1031–1036
Seefeldt SS, Kidwell KK, Waller JE (2002) Base growth temperatures, germination rates and growth response of contemporary spring wheat (*Triticum aestivum* L.) cultivars from the US Pacific Northwest. Field Crop Res 75:47–52
Soltani E, Farzaneh S (2014) Hydrotime analysis for determination of seed vigour in cotton. Seed Sci Tech 42:260–273
Soltani A, Ghorbani MH, Galeshi S, Zeinali E (2004) Salinity effects on germinability and vigor of harvested seeds in wheat. Seed Sci Tech 32:583–592
Soltani E, Galeshi S, Kamkar B, Akramghaderi F (2009) The effect of seed aging on seedling growth as affected by environmental factors in wheat. Res J Environ Sci 3:184–192
Soltani E, Soltani A, Galeshi S, Ghaderi-Far F, Zeinali E (2013) Seed bank modelling of volunteer oil seed rape: from seeds fate in the soil to seedling emergence. Plant Danin 31:267–279
Soltani E, Ghaderi-Far F, Baskin CC, Baskin JM (2015) Problems with using mean germination time to calculate rate of seed germination. Aust J Bot 63:631–635
Soltani E, Baskin CC, Baskin JM, Soltani A, Galeshi S, Ghaderi-far F, Zeinali E (2016) A quantitative analysis of seed dormancy and germination in the winter annual weed *Sinapis arvensis* (Brassicaceae). Bot 94:289–300
Squire GR (1999) Temperature and heterogeneity of emergence time in oilseed rape. Ann Appl Biol 135:439–447
Steinmaus SJ, Prather TS, Holt JS (2000) Estimation of base temperatures for nine weed species. J Exp Bot 5:275–286
Thomas DL, Raymer PL, Breve MA (1994) Seeding depth and packing wheel pressure effects on oilseed rape emergence. J Prod Agric 7:94–97
Tian T, Wu L, Henke M, Ali B, Zhou WJ, Buck-Sorlin G (2017) Modeling allometric relationships in leaves of young rapeseed (*Brassica napus* L.) grown at different temperature treatments. Front. Plant Sci 8:313. doi:10.3389/fpls.2017.00313
U. S. Salinity Laboratory Staff (1954) Diagnosis and improvement of saline and alkali soils. USDA Handbook 60, US Gov. Print. Office, Washington, D.C.
Xia Z, Xiaoyu X (2012) Studies on identification indexes of drought resistance by peg during seed germination of rapeseed (*Brassica napus* L.). Acta Agric Boreali-Occidentalis Sinica 2:1–17
Zhang H, Irving LJ, McGill C, Matthew C, Zhou D, Kemp P (2010) The effects of salinity and osmotic stress on barley germination rate: sodium as an osmotic regulator. Ann Bot 106:1027–1035
Zuo Q, Kuai J, Zhao L, Hu Z, Wu J, Zhou G (2017) The effect of sowing depth and soil compaction on the growth and yield of rapeseed in rice straw returning field. Field Crop Res 203:47–54