Agrotechniques in Industrial Crops

Journal Homepage: https://atic.razi.ac.ir

Razi University

Evaluation of Drought Indices in Rapeseed (*Brassica napus* L.)

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ARTICLE INFO

Original paper

Article history:
Received: 25 May 2021
Accepted: 29 Aug 2021
Published: 31 Aug 2021

Keyword:
Rapeseed *(Brassica napus L.)*
Water stress
Cell membrane stability
PEG

ABSTRACT

Rapeseed is one of the most important oil plants in the world, which due to the increase in world population and improving living standards, should increase the production and consumption of oil of this plant. It is a plant that is cultivated both in winter and spring. The use of drought tolerance indices can help us identify drought tolerant genotypes. To determine drought tolerance indices, a study was carried out with 16 autumn rapeseed genotypes. The experiment was performed on a randomized complete block design with three replications under rainfed and irrigation condition. Drought tolerance indices including MP, GMP TOL, STI and SSI were calculated using grain yield data. Measurement of cell membrane stability (CMS) using polyethylene glycol (PEG) was used as a drought tolerance test. The results of analysis of variance showed a significant difference for all indices. Stress tolerance index (STI) was the best index to identify tolerant genotypes in both stress and non-stress conditions. Estimation of STI from the average of genotypes showed that Dante (1.22) genotype has the highest value. The results of analysis of variance for CMS showed a significant difference between genotypes at the 1% level of probability and the highest value (65.52) was for ARC5 genotype and the lowest (32.08) was for SLM046 genotype. There were a significant and strong correlation between STI, MP and GMP with CMS, as a result, cell membrane stability can be introduced as a fast and inexpensive method to identify drought tolerant genotypes. Based on STI, MP, GMP, CMS and grain yield indices in both stress and non-stress conditions, cluster analysis was performed, and genotypes were divided into 4 groups.

DOI: 10.22126/ATIC.2021.6515.1014

1. Introduction

Rapeseed (*Brassica napus* L.) is one of the most important agricultural products, the oil of which is the best edible oil with the lowest amount of erucic acid and glucosinolate. After extraction, the remaining oil is used as a valuable source of protein for the livestock feed industry. Iran is one of the arid and semi-arid regions of the world and rapeseed production is mainly limited by drought and soil salinity. Therefore, to have successful agriculture in arid regions, it is important to choose drought tolerant genotypes (Robertson and Holland, 2004). Drought tolerance is different in native and agricultural species. In native species it is defined as survival, while in agricultural species it means yield and productivity (Passioura, 1983). The difference between grain yield under stress and non-stress conditions is called drought tolerance index (TOL) (Rosielle and Hamblin, 1981).

Polyethylen glycols (PEGs) are a group of neutral osmotically active polymeras with a certain molecular weight, which cannot cross the cell wall due to its high molecular weight. It is widely used to induce drought stress (Meher *et al*., 2018). One of the methods used to identify drought tolerant cultivars is to measure the stability of cell membranes (Sullivan, 1972) that have been used in various products such as *Sorghum bicolar* (Sullivan and Ross, 1979), wheat (Blum and Ebrecon, 1981), maize (Premachandra *et al*., 1989), Populus deltoids (Michael *et al*., 1994), rice (Tripathy *et al*.,

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2000),wheat and wild relatives of wheat (Faroog and Azam, 2002).

Breeders are introduced to cultivars that have high yields in a variety of conditions, so SSI is not used for intense stress, while the MP average yield index and STI stress tolerance index are used for intense conditions (Naeemi *et al.*, 2007). Selection of drought tolerant genotypes with high grain yield has not been useful in non-stress conditions (Blum, 1979; Ceccarelli and Grando, 1991; Rathjen, 1994). Researchers have suggested that the selection of drought tolerant genotypes for high grain yield should be done under both stress and non-stress conditions (Sinmena *et al.*, 1993; Rajaram and Van Ginkle, 2001; Betran *et al*., 2003). The difference between grain yield under stress and non-stress conditions is called tolerance (TOL), which is considered as one of the indicators of drought tolerance (Rosielle and Hamblin, 1981). Based on the tolerance index, the selected genotypes have relatively high grain yield under stress conditions and low productivity under non-stress conditions. Mean performance under both stress and non-stress conditions is known as mean productivity (MP) (Rosielle and Hamblin, 1981). Stress tolerance index (STI) was introduced as a criterion for selection of drought-united genotypes (Fernandez, 1992). Genotypes with high STI in both stress and non-stress conditions are known as superior genotypes with high yield in both conditions. Fernandez, 1992 also suggested the geometric mean productivity (GMP) as another useful indicator of drought tolerance. Fisher and Maurer (1978) introduced the stress sensitivity index (SSI) as an index of drought tolerance, Low index indicates low grain yield difference under stress and non-stress conditions. Ilyas Khokhar et al. (2012) and, Aliakbari et al. (2014) Showed that based on the principal component analysis of the geometric mean of performance (GMP), Stress tolerance index (STI) and average yield (MP) for selection of drought tolerant genotypes are the best parameters. Genotypes are divided into 4 groups based on their performance under stress and non-stress conditions: Genotypes that perform the same function under both stress and non-stress conditions (Group A); Genotypes that perform well under stress-free conditions (Group B); Genotypes that perform relatively well only under stress conditions (Group C) and Genotypes that perform poorly under both stress and non-stress conditions (Group D). Appropriate selection criteria and indicators should be able to distinguish group A from the other three groups (Fernandez, 1992). This study was conducted to evaluate drought tolerance indices in autumn rapeseed genotypes to introduce drought tolerant genotypes.

2. Materials and methods

In this study 16 rapeseed (*Brassica napus* L.) were planted under moisture-stress and non-stressed conditions. The experiment was performed in a randomized complete block design (RCBD) with three replications. Sowing was done by hand in plot with four rows 4 m in length, 30 cm apart and between each plot 60cm. The yield (kg ha $^{-1}$) was obtained by converting the seed yield per plot to hectares. Non-stress plots were irrigated three times, at the bud formation, flowering, and grain filling stages, while stressed plot received no water other than rainfall. Origin and characters of genotype are given in table 1. Five drought tolerance indices were calculated as below: (Fischer and Maurer, 1978; Rosielle and Hamblin, 1981; Fernandez, 1992)

1. Stress Susceptibility Index

$$SSI = \frac{1-(Y_S/Y_P)}{SI} \quad , \quad SI = 1 - \left[\frac{\bar{Y}_S}{\bar{Y}_P}\right] \qquad (1)$$

2. Tolerance

$$TOL = Y_P - Y_S \qquad (2)$$

3. Mean of Productivity

$$MP = \frac{Y_s + Y_p}{2} \qquad (3)$$

4.Geometric Mean of Productivity

$$GMP = \sqrt{(y_s)(Y_p)} \qquad (4)$$

5. Stress Tolerance Index

$$STI = \left(\frac{Y_p}{\bar{Y}_p}\right)\left(\frac{Y_s}{\bar{Y}_s}\right)\left(\frac{\bar{Y}_s}{\bar{Y}_p}\right) = \frac{(Y_p)(Y_s)}{(\bar{Y}_p)^2} \qquad (5)$$

Where: Y_S: yield of a genotype under stress conditions; Y_P: yield of a genotype under non-stress conditions. $\bar{Y}_s$: mean yield under stress conditions; $\bar{Y}_P$: mean yield under non-stress conditions.

2.1. Cell membrane stability (CMS)

First, the developed leaves were separated. The middle part of the leaves was cut into one-centimeter pieces and washed three times with distilled water. The leaf pieces were placed in containers containing 25 ml of distilled water (control) or 24 ml of 40% solution of PEG6000. The samples were then incubated at 10 °C for 24 h. The dishes were taken out of the incubator and the liquid inside the container was emptied and the leaves were washed. Controlled and PEG-treated samples were again immersed in distilled water at 10 °C for 24 h. Electrical conductivity was measured. Containers containing the sample and distilled water were then autoclaved for 15 minutes and their final electrical conductivity was recorded. Then the percentage of cell membrane damage was calculated based on the following formula (Sullivan, 1972).

Injury (%) =1- {[1-T1/T2] / [1-C1/C2]} ×100 CMS (%) = 1 – I (also in %) (6)

T1 and T2 = first and second conductivity measurement of desiccation treatment, respectively.
C1 and C2 = first and second conductivity measurement of control, respectively.
Statistical analyses were performed using SPSS and MSTAT-C software.

Table 1. Origin and characters of genotypes

No.	Genotypes	Origin	Appearance
1	Geronimo	Rosticafrance (European=Winter) - (Mexican-China-Canadian = Spring)	Winter
2	Celecious	Sralof	Winter
3	Milena	Germany	Winter
4	Sahra	Danisco	Winter
5	Sunday	Danisco	Winter
6	Zarfam	Iran	Winter
7	Dante	Germany	Winter
8	SLM-046	Germany	Winter
9	Talaye	Iran	Winter
10	Talent	Germany	Winter
11	ARC2	U.S.A	Winter
12	Opera	SW-sweden	Winter
13	ARC5	U.S.A	Winter
14	Licord	Germany	Winter-Spring
15	Elite	Rosticafrance (European = Winter) - (Mexican-China-Canadian = Spring)	Winter
16	Ebonite	Rosticafrance (European = Winter) - (Mexican-China-Canadian = Spring)	Winter

3. Results and discussion

Drought tolerance indices were calculated for all the genotypes at each in replications. The results of analysis of variance (Table 2) showed that there is a significant difference between genotypes for drought tolerance indices at the level of 1% probability. The mean of the yield based indices and mean yield under both conditions (Table 3) showed that the Dante had the highest STI, MP and GMP, giving a high yield under both stressed (2968.6 kg/ha) and a low yield under non-stressed (4042.07kg/ha) conditions. The results of mean grain yield for all genotypes showed that grain yield under stress conditions was lower than grain yield under non-stress conditions. The results showed that the STI index was well able to identify high-yield genotypes in both stress and non-stress conditions. It is also able to detect drought tolerant genotypes. Suitable STI and TOL indices for selection of drought tolerant genotypes were introduced (Liravi, 2005; Yousefi, 2017).

Table 2. Analysis of variance for drought tolerance indices in rapeseed genotypes

		Mean of squares				
S.O.V	DF	STI	TOL	SSI	GMP	MP
Replication	2	0.003^{**}	1530.573^{ns}	0.010^{**}	15278.258^{ns}	6370.287^{ns}
Genotype	15	0.132^{**}	516916.674^{**}	0.160^{**}	1000801.633^{**}	1029228.320^{**}
Error	30	0.009	915.384	0.001	2956.116	4344.612

STI; Stress Tolerance Index; TOL: Tolerance; SSI: Stressed Susceptibility Index; GMP: Geometric Mean of Productivity; MP: Mean of Productivity; ** are Significant at 1%; NS: Non-significant.

Table 3. Mean of drought tolerance indices and mean yield under stress and non-stress conditions in rapeseed genotypes

Genotype	TOL (gm^{-2})	SSI	STI	GMP (gm^{-2})	MP (gm^{-2})	Y_S (gm^{-2})	Y_P (gm^{-2})
Geronimo	840.07	0.24	0.93	3025.20	3054.30	2634.27	3474.33
Celecious	715.40	0.19	1.06	3231.63	3251.77	2894.07	3609.47
Milena	93.47	0.04	0.48	2576.97	2688.67	2141.93	2235.40
Sahra	421.40	0.16	0.60	2428.87	2438.37	2227.67	2649.07
Sunday	1092.63	0.34	0.66	2556.00	2614.27	2067.93	3160.57
Zarfam	16040	0.55	0.37	3509.10	3578.10	1276.10	2880.10
Dante	1073.47	0.26	1.22	3463.93	3505.33	2968.60	4042.07
SLM-046	676.07	0.18	1.11	3309.80	3327.17	2989.13	3665.20
Talaye	481.77	0.15	0.89	2962.30	2972.20	2731.30	3213.07
Talent	411.80	0.17	0.46	2141.40	2151.30	1945.40	2357.20
ARC2	935.10	0.25	1.10	3193.20	3227.37	2759.83	3694.93
Opera	1180.34	0.42	0.46	2123.13	2204.10	1613.93	2794.27
ARC5	386.93	0.17	0.43	2063.57	2070.40	1876.93	2263.87
Licord	1444.67	0.34	1.16	3379.30	3456.60	2734.27	4178.93
Elite	1035.33	0.46	0.28	1663.90	1743.07	1225.40	2260.73
Ebonite	1059.93	0.29	0.96	3079.13	3125.10	2595.13	3655.07

TOL: Tolerance; SSI: Stress Susceptibility Index; STI; Stress Tolerance Index; GMP: Geometric Mean of Productivity; MP: Mean of Productivity; Y_S: Yield under stress conditions, Y_P: Yield under non- stress conditions.

A three-dimensional plot between Y_P, Y_S and STI (Fig.1) was used to distinguish the group A genotypes from the other three groups (B, C and D) (Fernandez, 1992; Farshadfar *et al.*, 2001; Yarahmadi *et al.*, 2020). In this case, Zarfam, Dante, SLM046 and Licord genotypes were introduced as suitable genotypes in both stress and non-stress conditions.

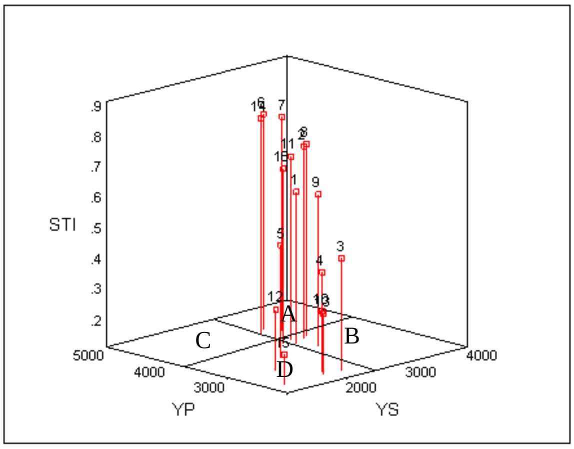

Figure.1 Mean of yield under stress and non-stress conditions and STI in rapeseed genotypes.

The results of analysis of variance of cell membrane stability (CMS) showed that there is a significant difference between genotypes at the level of one percent probability (Table 4). The highest and lowest values of CMS were recorded for ARC5 and SLM046, respectively (Table 5).

Table 4. Analysis of variance for cell membrane stability in rapeseed genotypes

S.O.V	DF	CMS
Replication	2	8.181
Genotype	15	253.265**
Error	30	15.778

** Significant at 1%

Table 5. Mean comparison of cell membrane stability in rapeseed genotypes

No.	Genotype	Means
1	Geronimo	47.80^{cde}
2	Celecious	64.94^{cde}
3	Milena	15.02^{bcd}
4	Sahra	37.90^{ef}
5	Sunday	35.35^{f}
6	Zarfam	52.18^{bc}
7	Dante	55.32^{bc}
8	SLM-046	32.08^{f}
9	Talaye	38.44^{ef}
10	Talent	41.20^{def}
11	ARC2	53.54^{bc}
12	Opera	45.76^{cde}
13	ARC5	65.52^{a}
14	Licord	45.34^{cde}
15	Elite	38.89^{ef}
16	Ebonite	59.31^{ab}

Different letters represent significant differences at the 1% probability level.

Table 6. Cluster analysis based on the indices MP, GMP, STI, CMS and yield under stress and non-stress condition

Genotype	Cluster	Mean of Cluster					
		CMS	STI	GMP	MP	Y_S	Y_P
Geronimo,Celecious,Dante,SLM046,Talaye,ARC2, Licord, Ebonit	1	49.6	1.05	3205.56	3239.98	2788.32	3691.63
Milena,Sahra,Sunday,Talent,Opera,ARC5, Elite	2	39.95	0.48	2221.97	2272.88	1871.31	2531.59
Zarfam	3	38.69	0.28	1663.90	1743.07	1225.40	2660.73

Cluster analysis based on grain yield under both stress and non-stress conditions and tolerance indices including STI, MP, GMP and CMS divided the genotypes into three groups (Table 6). The first group consisted of eight high-yield genotypes under both stress and non-stress conditions, high drought tolerance indices and CMS. The second and third groups consisted of seven and one genotype with medium and low parameters, respectively.

As a result, STI was introduced as the most suitable index for selection of drought tolerant genotypes in both stress and non-stress conditions. Cell membrane stability can be used as a rapid and inexpensive method for screening drought tolerant genotypes in rapeseed breeding programs. Cluster analysis based on suitable drought tolerance indices and CMS can be a useful method for grouping plant materials into different drought tolerance clusters.

Acknowledgement

We would like to thank Razi University for helping this research.

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HOW TO CITE THIS ARTICLE

Chaghakaboodi Z., Dogan H. 2021. Evaluation of Drought Indices in Rapeseed (*Brassica napus* L.). *Agrotechniques in Industrial Crops* 1(2): 97-102. 10.22126/ATIC.2021.6515.1014