

Original article

Differential Response of Sunflower Maintainer and Restorer Inbred Lines to Salt Stress

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Abstract

In order to identify of salt tolerant sunflower inbred lines a set of 24 inbred lines were evaluated as a randomized block design with three replications in Isfahan and Zabol in 2017. Salt stress was imposed following seedlings establishment 20 days after planting as irrigation with two (fresh water) and 8 ds.m-2 electrical conductivity. According to the results, there were significant differences among locations and also salt treatment for all measured traits except for flowering time and oil content. There were also significant differences among inbred lines for all of the characteristics which could be used a source for improvement of salt tolerance in sunflower. Salt stress had a negative impact on agronomic futures of the inbred lines. Achene and oil yield had suffered more than other traits with 34 and 31% reduction respectively. Phenological traits were affected less than agronomic traits. The inbred lines BGK259 and RGK38 had the highest and lowest achene and oil yield respectively. Three lines BGK259, BGK369 and BGK375 with higher STI expressed as more salt tolerant lines. All 12 maintainer lines had a higher STI than all the 12 restorer lines and were more tolerant. Among the restorers RGK22, RGK15 and RGK2 were more salt tolerant than others. In accordance with STI, TOL and GM indices, principal component analysis differentiated BGK259, BGK369 and BGK375 as the most salt tolerant inbred lines.

Keywords: Genetic Variability, Inbred line, Principal Components, Tolerance index.

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INTRODUCTION

Following soybean and oilseed rape, sunflower with 26.6 M ha area of cultivation area is the third oil crop of the world (FAO.2018). Cultivation of oil type sunflower is started since 1967 and increased to 107,000 ha in 1993 however recently the area of cultivation is limited to about 15000 ha (Anonymous, 2018). Both oil and confectionary types of sunflower are cultivated in Iran, however due to the higher income, confectionary types are more interested. Cultivation of the oil types sunflower began since 1965 with Russian and Romanian cultivars as Record and Vnimik 8931 (Ghaffari et al., 2020). Salinity of irrigation water and soil is among the limiting factors for this crop in Iran. About 15% of agricultural lands suffer from salinity in Iran (Khan and Gulzar, 2003). Estimated that there is total of 27 M ha of saline and sodic soils in Iran (Kamkar et al. 2004). Salinity has a negative effect on crops in several ways, including drought stress, toxicity, oxidative stress, nutritional and metabolic disorders, reduced growth and cell division, and in general reduced growth, development and survival (Hasegawa et al. 2000; Munns, 2002). Salinity affects seed germination and growth by reducing the water potential and toxicity of certain ions such as sodium and chlorine, as well as the reduction of plant ions required by plants such as calcium and potassium (Khan and Gulzar, 2003). The negative effects of salinity on plants are manifested in the form of seedling and plant death or reduced growth and yield (Parida and Das, 2005). Some species are more flexible to saline conditions however, the general response of the plants depends on the intracellular response and the balance between salt uptake and division in plant tissues (Volkmar and Steppuhn, 1998).

Plants are classified into two groups in terms of salinity response: halophytes (able to grow at higher salt concentrations) and non-salinity tolerant and sensitive halophytes. Based on electrical conductivity (Ec), soils are divided into four groups: non-saline (4-0), relatively saline (4-7), very saline (7-15) and high salinity (more than 15) and crops based on yield curves in different salinity conditions are classified into four groups: sensitive, relatively sensitive, relatively tolerant and tolerant (Mass and Hoffman, 1977).

Sunflower is semi sensitive to salt stress however there is variability in response of cultivars to this stress (Levit, 1980; Katerji *et al.* 2000). The threshold for sunflower tolerance to salinity is 2.3 dS / m. Sunflower yield has decreased by 10, 25 and 50%, in electrical conductivity by 3.2, 4.7 and 6.3 dS / m respectively. But thresholds for irrigation water salinity for sunflower in sandy, loamy and clay soils have been reported to be 7.5, 3.4, and 2.5 dS / m, respectively (Mass and Hoffman, 1977). It is reported that sunflower achene yield is not reduced with salinity of 5 ds/m but 50% of yield is lost with salinity of 12ds/m (Khajepour, 2007). Flagella *et al.* (2004) indicated that for each unit of increase in salinity, sunflower yield is reduced about seven percent. Pirzad *et al.* (2013) and Dehghani *et al.* (2014) reported that sunflower is appropriately classified as moderately tolerant to salinity however Each unit increase in salinity above 48 dS/ m reduced yield by 5.0%. Kaya et al. (2019) reported that under saline condition

sunflower genotypes exhibited varying responses to salinity, mean germination time significantly prolonged and a dramatic reduction in seedling growth was observed. The aim of this study was to investigate the effects of irrigation water salinity on agronomic characteristics of sunflower inbred lines and to identify tolerant lines in Isfahan and Zabol conditions regions in Iran.

MATERIALS and METHODS

In order to identify of salt tolerance inbred lines 24 sunflower inbred lines were evaluated for salt tolerance as a randomized block design with three replications in Isfahan and Zabol in 2017. Each experimental plot consisted of four rows each three meter long with row spacing of 60 cm and plant spacing of 25 cm. Before planting, Triflouralin herbicide was used at the rate of two liters per hectare to control weeds. Super Galant herbicide at the rate of 2.5 liters per hectare was used to control of narrow-leaved weeds. Salt stress was imposed following seedlings establishment 20 days after planting as irrigation with 2 (fresh water) and 8 ds.m⁻² electrical conductivity. Salt stress was imposed following seedlings establishment 20 days after planting as irrigation with 2 (fresh water) and 8 ds.m⁻² electrical conductivity. Irrigation was performed after 60 mm evaporation from the pan and a total of 6 irrigations were performed in Zabol and 8 in Isfahan. About 800 cubic meters per hectare was irrigated each time. The results of soil test analysis at the two test sites at the end of the season for normal irrigation and salinity stress conditions are shown in Table 1. During the growing stages, phenological and agricultural characteristics were recorded. Following combined analysis of variance mean of inbred lines for evaluated traits were compared using least significant differences (LSD) test. Stress tolerance indices (Fernandez, 1992) and principal component analysis was used to identify of tolerant lines. Statistical analysis performed by SPSS (Ver.24).

				Isfahan				
Condition	EC (dS/m)	pН	% O.C.	P(Av) ppm	K(Av) ppm	Sand %	Silt %	Clay %
Normal	3.4	7.2	0.85	23.5	280	15	46	39
Salt stress	8.2	7.3	0.78	4.1	260	13	46	41
				Zabol				
Normal	2.4	7.9	0.37	18.6	131	18	48	34
Salt stress	8.5	8.2	0.34	2.4	105	19	50	31

Table 1. Soil test results for experimental field

Results and Discussion

There were significant differences between locations and salt treatments for all agronomic traits except days to flowering and oil content (Table 2). There was also considerable variability among inbred lines for agronomic characteristics that could be used as a genetic source for improvement of salt tolerance in sunflower. The three-way interaction effect of line \times location \times irrigation was significant

for days to the end of flowering, flowering duration, plant height, head and stem diameter, number of seeds per head, 1000-seeds weight and grain and oil yield which shows that the response of lines to salinity stress conditions were different in the two locations.

Salt stress had a negative impact on agronomic futures of the inbred lines. Achene and oil yield had suffered more than other traits with 30.7 and 34.5% reduction respectively. Phenological traits were affected less than agronomic traits, however days to maturity affected more than flowering time (0.2%) and reduced 3.3 %. Plant height, head diameter, stem girth reduced 15.4, 19.8 and 25.2 percent respectively. Among the yield components, seed number per head was significantly impaired (27.2 %) more than 1000-seeds weight (6.5 %) and oil percentage (5.2 %) due to salinity stress (Table 3). Francoise (1996) reported that oil concentration of sunflower was relatively unaffected by increased soil salinity up to 10.2 ds.m⁻¹. It seems that salinity stress cause to reduce the number of seeds per head by affecting seed setting. Ghaffari et al. (2019) reported seed number per head as the main determinant of seed and oil yield in sunflower. The results of this study are in accordance with who reported that yield reduction of sunflower under salinity was attributed primarily to a reduction in seeds per head (Francois, 1996).

The maintainer line BGK329 had the highest seed yield under normal irrigation (2412 and 973 kg ha⁻¹ respectively). The line BGK329 was characterized by late flowering (66 days), late maturing (108 days), higher plant height (122 cm) than other lines. The highest head diameter (19.1 cm) and stem girth (20.9 mm) were observed in BGK259. BGK 345 had the highest 1000-seed weight (58.7 g), while BGK375 had the highest seed number per head (1004 seeds) (Table 4). Under salt stress the inbred line BGK259 had the highest achene and oil yield (1865 and 756 kg ha⁻¹ respectively). Phenological stages was affected by salt stress in terms of reduction in days to flowering and physiological maturity. The line BGK329 was the latest flower (63 days) while RGK25 was the most late mature line in compared to the others. The highest plant height was observed in BGK329 (105 cm) while BGK369 had the highest 1000 seed weight was observed in BGK259 (55.7 g) and BGK369 had the highest number of seeds per head (648 seeds). The highest oil content was observed in R14 (42.2 %) however BGK343 had the lowest oil content (33.4%).

Drought sensitivity and tolerance indices of STI, MP and GM were calculated using the performance of lines in both optimal irrigation and stress conditions (Table 5). High values of these indices indicate more drought tolerance and can differentiate group A genotypes (with higher yield in both stress and non-stress environments) from sensitive genotypes (Fernandez, 1992). The tree lines BGK259, BGK369 and BGK375 had higher STI than the others and expressed as salt tolerant inbred lines. The two MP and GMP indices also differentiated tolerant lines similarly to the STI index. A noteworthy point in this study was that each of the 12 maintainer lines had higher STI compared to the 12 restorer lines and appeared more tolerant. This result shows the effect of grain yield on tolerance

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indices. Single-branch maintainer lines generally have a higher yield compared to multi-branch restorer lines, and this can be skewed in calculating the indices because the performance of sub-branches in restorer lines does not interfere with yield calculation. However, the results of previous studies have shown that these indices can differentiate tolerant and susceptible lines when single-genotype genotypes are evaluated (Darvishzadeh *et al.*, 2010; Ghaffari *et al.* 2012). Some reports also suggest that the SSI index cannot be used to identify drought tolerant lines (Clarke *et al.*, 1993). Among the restorers RGK22, RGK15 and RGK2 were more salt tolerant than the others, however, they were more sensitive than all maintainer lines.

Considering the one-dimensional role of grain yield in differentiating lines in terms of stress tolerance indices, in order to determine a comprehensive criterion that includes all the measured traits and also show the relationship between variables, principal components analysis which could cover all measured properties was used to identify the stress-tolerant lines. The results of this analysis in accordance with STI, TOL and GM indices, differentiated BGK259, BGK369 and BGK375 as the most salt tolerant inbred lines (Fig.1). This shows that these indicators have a high ability to identify salttolerant lines and by measuring grain yield under optimal and stressed conditions alone, the salt tolerant lines can be identified. As it shown in PCA biplot these lines were characterized by showing higher achene and oil yield, head diameter and seed weight under salt stressed condition. Considering the vectors there were high correlation between achene and oil yield with 1000 seed weight and head diameter, so these two traits which had higher weight in two first PCA components could be used as a simple indicator of salt tolerance of sunflower genotypes. Multivariate methods as cluster analysis were used by Kaya et al. (2019) for classification of sunflower genotypes for salinity tolerance. Principle component analysis have been used for determination relationships between parent inbred lines and related crosses (Haddadan et al. 2020) and also as a reflector of combining the abilities (Ghaffari et al., 2011) in sunflower.

According to this analysis, the line RGK38 had the highest sensitivity to salinity stress at the farthest opposite point of the grain and oil yield vectors. This line had the lowest STI index (0.22) and these results show the correspondence of specific results of principal component analysis and STI index in the identification of tolerant and sensitive lines. Figure 1 shows that the first component is able to discriminate almost all maintainer lines (except RGK22) from the restorer lines. The results suggests that theses indices had a higher potential for identify of salt tolerant genotypes and it is possible to identify salt tolerant genotypes by estimation of achene yield straightly.

Conclusions

Significant differences among sunflower inbred lines for agronomic characteristics indicated that this variability could be used for improvement of salt tolerance in sunflower. Salt stress had a negative impact on agronomic futures of the inbred lines. Achene and oil yield had suffered more than other traits with 34 and 31% reduction respectively. Phenological traits were affected less than agronomic traits. All maintainer lines in comparison with restorer lines expressed as more tolerant lines. Among the restorers RGK22, RGK15 and RGK2 were more salt tolerant than others. In accordance with STI, TOL and GM indices, principal component analysis differentiated BGK259, BGK369 and BGK375 as the most salt tolerant inbred lines. More tolerant inbred lines in this study can be used as parental inbred lines for development of salt tolerant sunflower hybrids.

Sources of variation	DF	Days to flowering	Days to maturity	Plant height	Head diameter	1000 seeds weight	Seed No. head ⁻¹	Oil content	Achene yield	Oil yield
Location	1	120.12	6912.92**	12983.32**	413.62**	15569.95**	5687861.81**	31.48	5799643.16**	952607.14**
Irrigation	1	0.89	986.42**	16569.58**	366.89**	507.16**	2747031.27**	312.31*	19006895.50**	3834982.04**
Location × Irrigation	1	138.89	477.92**	23.06	2.52	73.99	105367.28	11.21	485408.87	76451.58
Rep/Environ.	8	26.48	41.14	150.36	5.36	17.44	49255.70	7.10	161962.97	30450.91
Line	23	62.92**	98.96**	2086.80**	64.79**	1213.80**	129029.17**	97.99*	2185744.11**	300057.48**
Line × Location	23	39.99**	60.45**	896.03**	17.22**	210.19**	93155.86**	4.25	559510.43**	78601.29**
Line × Irrigation	23	8.66*	9.04	108.20*	8.45*	47.70**	65659.08**	3.14	158944.42**	23883.15**
Line × Location × Irrigation	23	5.06	7.32	105.18*	9.73**	54.54**	63358.59**	2.60	183043.59**	30078.47**
Error	184	4.91	7.25	57.19	4.53	24.38	27214.62	6.40	72380.08	11913.37
Coefficient of variation		3.76	2.68	8.29	22.55	12.55	27.20	6.43	18.99	19.70
*and ** denote to the significant at 5 and 1% probability										

Table 2. Combined analysis of variance for agronomic traits in Isfahan and Zabol conditions

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Treatment	Days to flowering	Days to maturity	Plant height (cm)	Head diameter (cm)	Stem girth (mm)
Normal Irrigation	58.92	102.26	98.75	10.36	18.67
Salt stress	58.81	98.89	83.58	8.31	13.97
Changes (%)	0.2	3.3	15.4	19.8	25.2
Treatment	1000 achene weight (g)	Achene No. head ⁻¹	Oil content (%)	Achene yield (kg.ha ⁻¹)	Oil yield (kg.ha ⁻¹)
Normal Irrigation	40.66	704.06	40.32	1673.47	669.37
Salt stress	38.00	508.73	38.24	1159.67	438.59
Changes (%)	6.5	27.7	5.2	30.7	34.5

Table 3. Effect of salt stress on agronomic traits of sunflower in Isfahan and Zabol

Treatme nt	Line	Days to flowerin g	Days to maturity	Plant heig ht (cm)	Head diamet er (cm)	Stem girth (mm)	1000 achen e weigh t (g)	Achen e No. head ⁻¹	Oil conten t (%)) kg.ha ⁻ ¹ (Achen e yield) kg.h a ⁻ 1 (Oil yield
	BGK35 5	57.7	98.7	90.4	14.1	17.6	48.2	593.4	36.5	1814.9	661.4
	BGK37 5	54.5	100.8	104. 9	14.1	21.6	45.3	1003.9	40.0	2390.3	955.6
	BGK36 9	56.0	100.0	88.6	13.7	19.5	49.6	761.8	37.6	2258.2	841.9
	BGK34 3	56.2	101.8	113. 2	10.1	17.0	51.7	509.6	33.8	1661.0	551.9
	BGK45	56.8	96.7	88.1	11.0	16.9	38.1	997.7	34.6	2388.8	828.8
	BGK25 9	58.7	104.7	105. 8	19.1	20.0	58.6	651.8	42.8	2315.5	990.6
	BGK10 9	56.3	98.5	78.6	11.9	18.6	50.7	618.8	44.0	1940.9	861.1
	BGK32 9	65.7	108.2	121. 5	12.9	19.7	46.2	894.7	40.3	2411.8	973.4
	BGK22 1	60.7	103.5	114. 7	12.4	17.8	41.0	807.0	38.1	2073.0	787.0
_	BGK77	60.7	103.0	91.9	11.6	18.2	56.6	511.4	39.9	1744.5	696.3
igatior	BGK34 5	56.2	98.7	101. 3	11.2	18.3	58.7	520.9	40.4	1853.8	747.5
al Irr	BGK35	56.0	100.5	84.8	12.4	18.9	45.5	693.5	36.6	1861.2	682.9
Norma	RGK 22	61.0	104.2	115. 0	11.5	18.9	37.0	784.0	41.4	1829.5	757.9
-	RGK33	59.7	99.7	91.7	7.5	17.8	40.0	667.7	40.8	1524.8	632.1
	RGK15	59.8	103.7	98.7	7.4	19.1	34.5	749.4	39.7	1524.5	605.1
	RGK21	58.8	99.8	95.5	8.5	18.8	34.7	587.8	44.2	1179.7	524.0
	R14	56.8	100.5	95.8	7.9	17.7	38.9	431.9	43.0	923.0	396.2
	RGK38	61.0	105.7	91.7	7.4	18.6	22.6	879.9	37.9	1049.3	401.0
	RGK2	59.7	103.3	99.7	9.2	18.2	28.0	818.0	44.5	1430.2	638.9
	RGK29/ 1	55.3	101.7	65.4	10.0	16.9	32.2	466.7	44.3	889.6	394.0
	RGK47	60.5	105.5	116. 6	8.2	19.5	29.8	824.6	43.4	1414.6	614.5
	RGK25/ 2	61.0	104.2	118. 1	7.2	20.9	32.9	654.6	43.6	1334.3	581.4
	RGK24	65.0	107.2	90.7	7.2	18.4	25.6	733.5	41.2	1171.4	482.1
	RGK46	60.2	104.0	107. 5	7.2	19.1	29.1	734.8	38.8	1178.7	459.4
LSD 5%		1.53	1.87	5.24	1.48	1.19	3.42	114.32	1.75	186.43	75.64

Table 4. Mean of agronomic traits in sunflower inbred lines in normal and salt stressed condition (Isfahan and Zabol)

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Table 4. Continue

Treatment	Line	Days to flowering	Days to maturity	Plant height (cm)	Head diameter (cm)	Stem girth (mm)	1000 achene weight (g)	Achene No. head -1	Oil content (%)) kg.ha ⁻¹ (Achene yield) kg.ha ⁻¹ (Oil yield
	BGK35	59.2	94. 8	72.5	8.9	14.1	36.0	584.3	33.9	1361.	458.0
	BGK37	59.3	96. 5	86.2	10.4	14.0	48.3	564.1	38.2	1704.	656.7
	BGK36	57.8	95. 7	64.6	10.9	15.3	48.2	648.2	35.2	1848. 6	647.2
	BGK34	58.0	, 94. 7	102. 3	8.9	12.5	44.8	462.4	33.4	1317. 0	443.8
	BGK45	57.8	, 95. 8	68.8	8.2	12.1	33.8	618.0	34.4	1282. 0	440.7
	BGK25	59.0	10 1 2	92.4	11.5	14.8	55.7	566.3	40.5	1864. 5	755.7
	BGK10	56.0	96. 2	74.5	8.7	15.6	53.8	520.0	40.1	1635. 6	661.5
	BGK32	62.7	10 2.3	105. 3	10.4	14.3	49.2	464.8	37.7	1387. 3	518.0
	BGK22	59.0	99. 3	98.5	10.4	14.4	41.6	594.2	34.5	1521. 3	523.5
	BGK77	59.8	10 1.3	75.9	9.8	14.1	50.5	548.1	37.2	1574. 6	583.7
	BGK34 5	56.2	97. 0	73.2	8.8	13.7	49.9	438.5	38.9	1347. 6	522.7
tress	BGK35	57.3	99. 0	72.8	9.8	13.5	42.8	501.4	35.3	1242. 0	436.8
Salt S	RGK 22	59.0	10 0.2	102. 9	10.1	15.7	35.5	531.1	40.0	1172. 6	468.5
	RGK33	57.5	94. 3	75.2	6.4	13.3	35.9	340.7	39.0	741.6	285.9
	RGK15	59.5	10 0.3	87.1	7.0	14.5	32.9	591.3	37.4	1065. 7	398.5
	RGK21	59.0	95. 3	73.1	7.3	13.0	32.3	392.7	40.6	725.6	291.3
	R14	58.2	93. 8	89.2	5.6	13.4	29.6	400.4	42.2	675.2	286.1
	RGK38	59.8	10 1.2	83.4	6.8	13.5	25.4	386.9	36.6	571.5	209.1
	RGK2	58.0	10 1.5	78.9	7.4	13.7	27.3	508.2	41.2	861.7	356.5
	RGK29/ 1	54.5	96. 5	59.5	6.6	13.1	28.3	439.7	41.4	748.2	310.2
	RGK47	59.8	10 2.2	95.1	5.7	14.5	27.2	523.4	40.8	843.9	343.7
	RGK25/ 2	60.5	10 2.5	100. 5	7.8	14.9	33.1	393.6	41.4	770.8	319.7
	RGK24	63.3	10 1.8	76.3	5.6	12.8	22.3	563.5	39.8	729.1	290.0
	RGK46	60.2	10 2.0	98.0	6.7	14.6	27.8	628.2	38.1	839.9	318.4
L	SD 5%	1.53	1.8 7	5.24	1.48	1.19	3.42	114.3 2	1.75	186.4 3	75.64

		Achene yie						
No.	Line	Normal	Stressed	STI	SSI	TOL	MP	GM
1	BGK355	1814.86	1361.63	0.88	0.81	453.23	1588.25	1572
2	BGK375	2390.33	1704.27	1.45	0.93	686.06	2047.3	2018.36
3	BGK369	2258.17	1848.62	1.49	0.59	409.55	2053.39	2043.15
4	BGK343	1661.00	1317.00	0.78	0.67	344.00	1489	1479.03
5	BGK45	2388.80	1281.97	1.09	1.51	1106.83	1835.39	1749.96
6	BGK259	2315.54	1864.54	1.54	0.63	451.00	2090.04	2077.84
7	BGK109	1940.90	1635.56	1.13	0.51	305.35	1788.23	1781.7
8	BGK329	2411.75	1387.25	1.19	1.38	1024.50	1899.5	1829.13
9	BGK221	2072.96	1521.31	1.13	0.87	551.65	1797.13	1775.84
10	BGK77	1744.46	1574.58	0.98	0.32	169.87	1659.52	1657.34
11	BGK345	1853.75	1347.56	0.89	0.89	506.20	1600.65	1580.52
12	BGK35	1861.22	1242.00	0.83	1.08	619.22	1551.61	1520.41
13	RGK 22	1829.53	1172.60	0.77	1.17	656.93	1501.07	1464.69
14	RGK33	1524.75	741.64	0.40	1.67	783.11	1133.19	1063.4
15	RGK15	1524.50	1065.70	0.58	0.98	458.80	1295.1	1274.62
16	RGK21	1179.72	725.63	0.31	1.25	454.10	952.673	925.222
17	R14	922.96	675.20	0.22	0.87	247.76	799.078	789.416
18	RGK38	1049.33	571.50	0.21	1.48	477.83	810.417	774.399
19	RGK2	1430.17	861.73	0.44	1.29	568.43	1145.95	1110.15
20	RGK29/1	889.61	748.15	0.24	0.52	141.46	818.878	815.817
21	RGK47	1414.58	843.92	0.43	1.31	570.67	1129.25	1092.61
22	RGK25/2	1334.30	770.83	0.37	1.38	563.46	1052.57	1014.16
23	RGK24	1171.35	729.08	0.30	1.23	442.27	950.217	924.128
24	RGK46	1178.71	839.95	0.35	0.94	338.77	1009.33	995.016

Table 5. Tolerance and susceptibility indexes of salt stress for sunflower inbred lines



Figure 1. Principal component analysis using agronomic traits for classification of sunflower inbred lines. The vectors and rectangular represent traits and inbred lines respectively. The abbreviations areas: FI; Flower initiation, PM; Physiological maturity, PH; Plant height, SD; Stem diameter, HD; Head diameter, OC; Oil content, AY; Achene yield and OY; Oil yield.

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