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Relationships Among Traits Using Correlation, Principal Components and Path Analysis in Safflower Mutants Sown in Irrigated and Drought Stress Condition

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Abstract: Safflower *Carthamus tinctorius* comes from Asteraceae family. Safflower is annual plant and used as oil and forage crop. In this research the seeds of Zarghan 279 as a winter variety was irradiated by Gamma ray in 80, 100, 150 and 200 Gy and sown in farm. The mutants after M5 generation grown in irrigated and drought stress condition in farm. The results indicated that differences among mutants for the height, capitulum diameter, number of seeds in capitulum, capitulum weight, days to 50% flowering, days to maturity and yield were significantly deferent in irrigated and drought stress condition. There were not significant differences between oil content and number of capitulum in two condition. On the basis of correlations between principal components and original traits, a classification was made to observe the relation between different traits. It was observed that for the first principal component, in irrigated condition height, stem diameter, capitulum diameter, number of seeds in capitulum, capitulum weight, oil content and days to maturity and in drought stress condition capitulum diameter, number of seeds in capitulum, 100 seeds weight, oil content and days to maturity had positive correlation between themselves i.e., varies in the same aspect. Path analysis suggested that in irrigated condition number of seeds in capitulum, 100 seeds weight and stem diameter and in drought stress condition number of seeds in capitulum, 100 seeds weight, days to 50% flowering and capitulum diameter had greatest positive direct effects, capitulum weight had greatest negative direct effect on seeds yield.

Key words: Safflower, mutation, path analysis, gamma rays

INTRODUCTION

Development of oilseeds cultivation has an important role in provision of requisite edible oils for country people (Pasban, 2004). Safflower (*Carthamus tinctorius*) is an annual, brood leaf, oilseed crop adapted chiefly to the small-grain production areas (Oelke *et al*., 1990).

Safflower is an important crop in the traditional farming of the semi arid areas of India, Iran, Egypt, Pakistan and other Mediterranean counters. It belong to the family composite (Oad *et al*., 2002; Tuncturk and Ciftici, 2004). This crop is adapted to dryland or irrigated cropping systems and was originally grown for the flowers that were used in making red and yellow days for clothing and cooking (Oelke *et al*., 1990).

Today this crop supplies for oil, meal birdseed and foots for the food and industrial products markets, although this crop is now primarily grown for the oil (Oelke *et al*., 1990; Esendal, 2001). Safflower has been cultivated in Iran for centuries small amounts for the extraction of dye from it's florets. Iran is one of the richest germplasm source of safflower (Omidi, 2000; Pasban, 2004). For instance of the 2024 safflower genotypes deposited at the Western Regional Plant Introduction Station, Pullman, WA, USA, 199 of them are of Iranian origin (Omidi, 2000). With comparison of the sunflower and safflower, although both oilseed crops show great promise and good economic returns with a minimal irrigation, safflower gives more response and yield and a high economic result because of it's greater tolerance to drought, oil content and composition and other oil characteristics (Kumar, 2000). Combination of number of capitulum per plant, capitulum diameter, number of seeds per capitulum and test weight constitute the most desirable plant type in safflower. However the strong negative associations between these desirable traits are major problems for the improvement of safflower (Bagawan and Ravikumar, 2001). Conventional breeding has not been successful in boosting the yields of either oil or seed. Genetically upgrading the ultimate product in safflower is complicated, as it would require simultaneous improvement of seed yield and oil content. Such problems may be facilitated by mutagenesis through which genetic variability can be created (Khadeer *et al*., 1990). Khadeer *et al*. (1990) reported the complex traits such as oil quantity, quality, as well as polygenic traits, variability

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can be created through mutagenesis and that induced variability can be exploited in hybridization programs. They indicated a wide range of mutants with changes in branching pattern, flower morphology and seed characters in the safflower M_2 generation. Maluszynski *et al.* (2002) stated that induced mutation has been extensively used for creating new genetic variation in crop plant. More than 2200 mutant varieties of different crops with improved agronomic traits have been developed and released to the farmers for general cultivation in world. Patil *et al.* (2001) reported that the variation from the mutation of heterozygous genotypes was found to be more than that in untreated F_2 population for important characters such as seed weight, number of seeds and yield per plant vice versa for number of branches, number of capitulum and head diameter. Bagawan and Ravikumar (2001) stated that there were some desirable changes in character associations due to mutations depending on genetic architecture of the traits, mutagen and genotypes. Parameshwarpa and Meghannavar (2001) reported that the number of Capitulum, seed weight and oil content had a higher range and variance in F_3 M_3 compared to the F_3 population. This is attributed that hidden variability was released by irradiation at the early stage. As a result of irradiation, the strong negative relationships existing between hull and oil content and between seed yield and oil content were decreased. Thus additional variability created for some of the important yield traits coupled with shifts in correlations from negative to positive would offer greater scope for selection in the irradiated populations. The objectives of this study were to estimate the heritable variation, relationships between yield and yield components and the best selection criteria for yield improvement in safflower.

MATERIALS AND METHODS

This research was conducted at Nuclear Agriculture Medicine and Industrial institute of Karaj, Iran during 2000-2005. Seeds of safflower, *Carthamus tinctorius*, var. Zarghan 279, having (11-13) per-cent moisture contents with attention average of 100 seeds weight (30-35 g), were exposed to 80, 100, 150 and 200 Gy doses of Co ^{60} gamma rays for Induction of genetic variability for selection of desirable genotypes. Irradiated seeds were sown in farm and were screened at M_3 generation. Seeds of selected plants were divided into two parts. One part of seeds was sown in irrigated condition and next part was sown in drought stress condition. Since M_2, generation selection for the desirable traits such as earliness and drought tolerance were carried out. Selection of single plant to be continued to M_5 generation according to desirable traits in two mentioned conditions. In M_5 generation 14 mutants are selected based on promising performance for high yield earliness and other characters. Selected mutants for four generation have been shown in Table 1. Thirteen promising mutants, parent variety and Mexican variety were evaluated in yield trail in randomized complete block design with four replications in two condition. Experimental plots were consisted of rows 3 m length and 615 m apart. Yield components and other agronomic traits were obtained by calculating the mean of five representative plants.

Principal component analysis was used to explain the correlation structure of yield related characters of safflower mutants. Path analysis was used to measure both direct and indirect effects that morphological characters may have upon the grain yield of safflower mutants. Statistical analysis of the results were done by using MSTAT-C and SAS programs.

RESULTS AND DISCUSSION

The results of analysis of variance are presented in Table 2. Differences among mutants for the height, capitulum diameter, Number of seeds in capitulum, capitulum weight, days to 50% flowering, days to maturity and yield were significant in irrigated and drought stress condition. There were not significant differences between oil content and number of capitulum in two condition. 100 seeds weight was significantly different in irrigated condition but wasn't significant under drought stress condition whereas stem diameter was significant difference in drought stress condition but wasn't significant in irrigated condition. Comparison of mutants by Duncan test in 5% levels indicated that in irrigated and drought stress condition mutants number 12 and 5 had the greatest averages in most of traits, respectively, in comparison with other mutants and parental variety. Investigation of correlation coefficients matrix (Table 3) between variables under two mentioned condition indicated that number of capitulum had significant and positive correlation with seed yield in irrigated conditions whereas in drought stress condition this relation was weak. Because in drought stress number of seedless

Table 1: Dose rates irradiation and achived mutants

Condition	Dose	Zarghan 279 (prarental variety)	80 Gy	100 Gy	150 Gy	200 Gy	Mexican (control)
Irrigated	Line number	1	2 3 4 5 6	7 8 9	10 11 12	13 14	15
Drought stress	Line number	1	2 3 4 5 6 7 8	9 10	11 12	13 14	15

Table 2: Analysis of variance for 11 characters in irrigated and drought stress condition

		Mean of squares										
Source	Condition	H	SD	NC	CD	NSC	CW	100 SW	OC	DM	D 50%F	Y
Mutant	Irrigated	924.46**	11.36^{ns}	0.992^{ns}	81.01**	757.51**	7.18**	0.808**	6.2^{ns}	19.36**	60.114**	9.914*
CV%	Irrigated	8.84	12.84	40.67	8.32	30.44	22.93	16.89	19.19	0.47	0.796	19.98
Mutant	Drought	71546**	0.865*	7.22^{ns}	81.99**	1198.16**	8.089**	0.147^{ns}	8.406^{ns}	25.64**	28.63**	10.91**
CV%		8.59	15.29	19.07	9.18	27.6	20.77	14.16	9.042	0.686	0.684	21.35

**, * and ns: significant at 1 and 5% and non significant, respectively, H: Height SD: Stem Diameter, NC: No. of Capitulum CD: Capitulum Diameter NSC: No. of Seeds in Capitulum, CW: Capitulum Weight 100 SW: 100 Seeds Weight OC: Oil Content, DM: Days to Maturity D 50% F: Days to 50% Flowering Y: Yield

Table 3: Correlation coefficient in two conditions

Parameters	Condition	H	SD	NC	CD	NSC	CW	100 SW	OC	DM	D 50% F
H	1										
	2										
SD	1	0.103									
	2	0.306									
NC	1	-0.536*	0.217								
	2	0.045	0.476								
CD	1	0.719**	0.409	-0.396							
	2	0.187	0.162	-0.398							
NSC	1	0.527*	0.498	-0.155	0.867**						
	2	0.149	0.135	-0.463	0.932**						
CW	1	0.69**	0.487	-0.51	0.945**	0.812**					
	2	0.175	0.121	-0.481	0.924**	0.96**					
100 SW	1	0.106	0.182	-0.41	0.329	0.005	0.439				
	2	-0.103	0.05	-0.033	0.411	0.517*	0.64				
OC	1	0.128	-0.082	-0.236	0.165	0.372	0.103	-0.391			
	2	0.504	0.228	0.121	-0.109	-0.177	-0.241	-0.413			
DM	1	0.099	0.28	0.128	0.311	0.293	0.383	0.472	-0.119		
	2	-0.163	-0.072	-0.332	0.619*	0.491	0.488	0.193	0.001		
D %50	1	0.24	0.398	-0.064	0.626*	0.479	0.634*	0.599*	0.057	0.842**	
	2	-0.297	0.01	-0.07	0.405	0.177	0.259	0.191	-0.044	0.85**	
Y	1	0.2	0.692**	0.537*	0.439	0.571*	0.37	0.28	-0.021	0.346	0434
	2	0.283	0.621*	0.328	0.58*	0.598*	0.59*	0.604	-0.058	0.084	0.066

1 = Irrigated, 2 = Drought, ** and *: Significant at 1 and 5%, respectively, H: Height SD: Stem Diameter, NC: No. of Capitulum CD: Capitulum Diameter NSC: No. of Seeds in Capitulum, CW: Capitulum Weight 100 SW: 100 Seeds Weight OC: Oil Content, DM: Days to Maturity D %50 F: Days to %50 Flowering, Y: Yield

capitulum increased. Stem diameter in two condition had high correlation with yield. This matter indicated that stem is important reserve members in safflower and it can be morphological index for selection. Number of seeds in capitulum had positive and high correlation with seed yield in two conditions. Capitulum weight in drought condition had higher correlation with yield than irrigated conditions. In drought condition, because of unfavorable conditions, the pollination and fertilization rates decreased and although number of seedless capitulum increased. Correlation of 100 seeds weight with yield under drought stress condition is high, but under irrigated condition is weak. Correlation of capitulum number with height in irrigated condition was high and negative but in drought stress was low and positive. In irrigated condition whatever height increased, number of capitulum decreased. This mater in farm conditions was distinected. Coefficient correlation matrix indicated that in drought stress conditions correlation between morphological traits related to growth phase as compared with irrigated conditions was changed and relations between traits in growth phase was weaked. Oil content with other traits, had not significant correlation under two condition. Days to maturity in irrigated condition had not significant correlation with other traits, but in drought stress had positive and significant correlation with capitulum diameter. It was indicated that whatever capitulum diameter increased days to maturity increased also.

Under irrigated condition, there were positive and significant correlation between days to 50% flowering with capitulum weight, capitulum diameter and 100 seeds weight. As the 50% flowering increased infect growth phase increased and plant had most vigor for intering the production phase and also in production phase capitulum weight and 100 seeds weight increased. Omidi Tabrizi (2000) reported significant differences between new spiny safflower genotypes in yield, 100 seeds weight, number of capitulum in plant and oil yield. Rudra Naik *et al.* (2005) reported that seed yield contributing characters such as plant height were significantly correlated with number of branches and main head diameter. Khan *et al.* (2003) indicated that large variation in agro morphological characteristics and fatty composition exists in germplasm evaluated. Positive and significant relationships were found between days to flowering and plant height.

Table 4: Path analysis showing direct and indirect effect in irrigated and drought stress condition

		Indirect effects										
Parameters	Condition	H	SD	NC	CD	NSC	CW	100 SW	OC	DM	D 50% F	Ray
H	1	(0.141)	-0.303	-0.032	0.0326	0.139	-1.270	0.094	-0.007	-0.004	-0.027	0.200
	2	(0.364)	0.096	0.016	0.0128	0.274	-0.387	-0.081	-0.049	0.120	-0.184	0.283
SD	1	0.015	(0.566)	0.013	0.186	0.699	-0.897	0.160	0.004	-0.012	-0.044	0.692
	2	0.111	(0.314)	0.027	0.111	0.248	-0.269	0.039	-0.022	0.053	0.006	0.621
NC	1	-0.076	0.122	(0.059)	-0.163	-0.217	0.939	-0.124	0.013	-0.006	0.007	0.537
	2	0.016	0.149	(0.059)	-0.273	-0.851	1.065	-0.026	-0.018	0.240	-0.043	0.328
CD	1	0.102	0.231	-0.023	(0.456)	1.216	-1.740	0.291	-0.008	-0.016	-0.070	0.436
	2	0.068	0.050	0.022	(0.686)	1.630	-2.045	0.325	0.010	-0.450	0.250	0.580
NSC	1	0.074	0.282	-0.009	0.394	(1.403)	-1.495	0.004	-0.020	-0.013	-0.054	0.571
	2	0.054	0.042	-0.026	0.639	(1.84)	-2.125	0.408	0.017	-0.360	0.110	0.600
CW	1	0.097	0.276	-0.030	0.429	1.139	(-1.842)	0.3896	-0.005	-0.016	-0.071	0.370
	2	0.064	0.038	-0.027	0.633	1.740	(-2.214)	0.505	0.023	-0.361	0.161	0.590
100SW	1	0.015	0.103	-0.008	0.149	0.007	-0.808	(0.881)	0.021	-0.021	-0.067	0.280
	2	-0.037	0.016	-0.002	0.282	0.951	-1.417	(0.79)	0.040	-0.143	0.119	0.604
OC	1	0.018	-0.046	-0.014	0.740	0.520	-0.189	-0.347	(0.053)	0.005	-0.006	-0.210
	2	0.183	0.0715	0.007	-0.074	-0.325	0.530	-0.326	(-0.097)	-0.001	-0.027	-0.058
DM	1	0.014	0.158	0.007	0.171	0.411	-0.705	0.419	0.006	(-0.043)	-0.094	0.346
	2	-0.060	-0.023	-0.019	0.424	0.903	-1.080	0.159	0.000	(-0.74)	0.528	0.084
D 50% F	1	0.003	0.225	0.004	0.284	0.672	-1.160	0.532	-0.003	-0.037	(-0.112)	0.434
2		-0.110	0.003	-0.004	0.278	0.329	-0.573	0.151	0.004	-0.629	(621)	0.066

1 = Irrigated, 2 = Drought stress, Irrigated : Adjusted R^2 0/77, Drought stress: Adjusted R^2 0/907, (Figures in parentheses are direct effects), H: Height SD: Stem Diameter, NC: No. of Capitulum CD: Capitulum Diameter NSC: No. of Seeds in Capitulum, CW: Capitulum Weight 100 SW: 100 Seeds Weight OC: Oil Content DM: Days to Maturity D 50% F: Days to 50% Flowering, Y: Yield

Path analysis: The direct and indirect effects of ten examined traits on seed yield in two condition were estimated by path coefficient (Table 4). Under irrigated condition the number of seeds in capitulum, 100 seeds weight and stem diameter had the greatest positive direct effects respectively and capitulum weight had the negative direct effect on seeds yield. In drought stress the number of seeds in capitulum, 100 seeds weight, days to 50% flowering and capitulum diameter had greatest positive direct effects respectively and capitulum weight had greatest negative direct effect on seed yield. Although 100 seeds weight in irrigated condition and days to 50% flowering in drought stress condition had high positive direct effects on yield but it's indirect effects through capitulum weight that were negative which consequently reduced correlation coefficient of 100 seeds weight and days to 50% flowering wit yield. capitulum weight in two condition had high negative direct effect on grain yield but indirect effects through number of seeds in capitulum, capitulum diameter and capitulum weight increased correlation coefficient of capitulum weight and yield. In irrigated conditions Capitulum number had low direct effect but through Capitulum weight had high positive indirect effect on seed yield.

The result of path analysis indicated that even though the relationships among some characters were statistically significant, the path coefficient values were found non significant. According to these results relations among examined characters are insufficient for plant breeding program. Corlto *et al*. (1997) reported that the number of capitulum per plant is the most important character for yield of safflower. Abel and Driscol (1993) reported that number of capitulum per plant and number of seeds per capitulum were important traits for high yielding in safflower lines. Tucturk and Ciftici (2004) reported negative and significant relationship between seed yield and percent of protein but negative and non significant relationship was found between seed yield and 100 seeds weight. Digming *et al*. (1993) in study of 30 safflower cultivars, reported that the number of effective branches, main stem diameter, diameter of top fruit, 100 seeds weight, oil content and angle of the first branch were the six principal components. Uslu *et al*. (1998) reported that the plant height, number of branches per plant number of capitulum per plant and capitulum diameter always more closely associated with high yields of each cultivar than seed weight. Rudra Naik *et al*. (2005) revealed that number of branches per plant and number of heads per plant were the most important characters and had the highest positive indirect effects on seed yield. Zheng *et al*. (1993) stated that the high yielding safflower varieties have taller individuals, lower branches, more effective capitulums, Fewer ineffective capitulum, lower weight seeds, higher average number of capitulum and longer flowering period. Bagawan and Ravikumar, (2001) reported that number of capitulum per plant and test weight are the most important characters for seed yield. These two traits were negatively associated with capitulum diameter and seed number. Ben Salah *et al*. (2001) indicated that there was strong positive association between the number of primary branches and yield. Therefore, significant positive correlation between seed yield and number of seeds in capitulum. Rang Rao *et al.* (1997) stated that correlation between number of capitulum per plant and capitulum weight with yield per plant was pronounced and they showed large direct effects on yield. All other components influenced seed yield mainly through these two components. Seed size had little effect on yield while seed number exerted appositive influenced. Patil *et al.* (1994) indicated that in the F_2 generation, number of capitulum per plant, number of seeds per capitulum, test weight and seed yield were the criteria used for single plant selection. In this study indicated that in irrigated condition selection based on number of seeds in capitulum, 100 seeds weight stem diameter and capitulum weight and under drought stress condition selection based on number of seeds in capitulum, 100 seeds weight, capitulum diameter, capitulum weight and days to 50% flowering would be effective.

Correlation between original variables and principal components: Correlation of the variables with principal components (PC's) often helps to interpret the components (Arshad *et al.*, 2005). On the basis of correlation coefficient give equation, original variables are classified in to the mutually exclusive sets A, B and C. Original variables having positive correlation with PC's are placed inset A whereas set B and set C contain all variables having negative correlation and no correlation with PC's respectively. This subdivision is useful for explaining correlation structures. By applying principal component analysis technique on safflower data, the eigen values and cumulative variances of correlation matrix of safflower plant traits are given in Table 5. It is clear that in irrigated condition the first principal component of safflower data accounts 46.1% of total variability percent in the data. While the second principal component accounts 19.88% of the total variability. So the first four principal components which are orthogonal with each other and extract maximum of total variability (about 86.9%). in drought stress condition the first principal component accounts 39.87% while, the second principal component accounts 21.08% of the total variability. The first four principal component extract maximum of total variability (about 88.39). On the basis of correlation between original variables and principal component give in Table 6, the original variables were divided in to tree sets A, B and C for positive, negative and no correlation, respectively. Testing of significance

Table 5: Eigen- analysis of correlation matrix

Condition	Prin comp	Eigen value	Proportion	Cumulation
Irrigated	PC1	5.072	0.4610	0.4611
	PC2	2.1873	0.1988	0.6599
	PC3	1.4065	0.1278	0.7878
	PC4	0.8957	0.0814	0.8692
Drought Stress	PC1	4.3863	0.3987	0.3987
	PC2	2.3189	0.2108	0.6095
	PC3	1.5579	0.1416	0.7512
	PC4	1.4599	0.1327	0.8839

Table 6: Correlation between original traits and principal component

Traits	Condition	PC1	PC2	PC3	PC4
H	1	0.624*	-0.438	-0.107	-0.135
	2	0.082	0.64*	0.497	-0.267
SD	1	0.561*	0.418	0.586*	-0.330
	2	0.171	0.78**	0.000	0.385
NC	1	-0.648**	0.367	0.224	0.005
	2	-0.376	0.596*	-0.337	0.537*
CD	1	0.950**	-0.072	0.148	-0.081
	2	0.946**	0.027	0.205	-0.068
NSC	1	0.801**	-0.413	0.31	0.088
	2	0.937**	0.052	0.083	-0.278
CW	1	0.958**	-0.093	0.028	-0.083
	2	0.965**	0.027	0.008	-0.284
100 SW	1	0.457	0.572*	-0.494	-0.153
	2	0.649**	0.075	-0.566*	-0.153
OC	1	0.021	-0.676**	-0.245	0.449
	2	-0.234	0.379	0.766**	0.228
DM	1	0.559*	0.541*	-0.201	0.45
	2	0.643**	-0.43	0.363	0.464
D 50%	1	0.768**	0.434	-0.196	0.283
	2	0.45	-0.395	0.164	0.694**
Y	1	0.552*	0.453	0.575*	-0.239
	2	0.642**	0.672**	-0.26	0.053

1 = Irrigated, 2 = Drought stress, ** and *: significant at 1, 5%, respectively, H: Height SD: Stem Diameter, NC: No. of capitulum CD: Capitulum Diameter NSC: No. of Seeds in Capitulum, CW: Capitulum Weight 100 SW: 100 Seeds Weight OC: Oil Content, DM: Days to Maturity D %50 F: Days to %50 Flowering, Y: Yield

Table 7: Subdivision of the original variables on the basis of correlation

Pc	Condition	SET A	SET B	SET C
1	Irrigated	H, SD, CD, NSC, CW, DM, D50%F, Y	NC	100SW, OC
	Drought stress	CD, NCS, CW, 100SW, DM, Y	--------	H, SD, NC, OC, D50%F
2	Irrigated	100SW, DM	OC	H, SD, NC, CD, NSC, CW, D50%F, Y
	Drought stress	H, SD, NC, Y	--------	CD, NCS, CW, 100SW, OC, DM, D50%F
3	Irrigated	SD, Y	--------	H, NC, CD, NSC, CW, 100SW, OC, DM, D50%F
	Drought stress	OC	100SW	H, SD, NC, CD, NSC, CW, DM, D50%F, Y
4	Irrigated	--------	--------	H, SD, NC, CD, NSC, CW, 100SW, OC DM, D50%F, Y
	Drought stress	NC, D50%F	--------	H, SD, CD, NSC, CW, 100SW, OC DM,, Y

H: Height SD: Stem Diameter, NC: No. of Capitulum CD: Capitulum Diameter NSC: No. of Seeds in Capitulum, CW: Capitulum Weight 100 SW: 100 Seeds Weight OC: Oil Content, DM: Days to Maturity D %50 F: Days to %50 Flowering, Y: Yield

procedure at α = 5% was made for each correlation between original variables and principal components so as to test for positive, negative or zero correlation and classified in Table 7 for the first principal component there exists a positive relationship within set A and within set B. while the correlation between each possible pair of members of set A and set B is non significant uncorrelated. In irrigated condition in first principal component, height, stem diameter, capitulum diameter, number of seeds in capitulum, capitulum weight, days to maturity, days to 50% flowering and yield have positive correlation between themselves i.e., varies in the same direction. In drought stress condition capitulum diameter, number of seeds in capitulum, capitulum weight, 100 seeds weight, days to maturity and yield have positive correlation between themselves and varies in the same direction. Set B is empty. For the second principal component in irrigated condition H height and oil content had positive correlation between themselves and when height and days to maturity increased oil content will decreased. In drought stress condition height, stem diameter, number of capitulum and yield had positive correlation between themselves and set B was empty. For third condition in irrigated stem diameter and yield had positive correlation and set B as empty. In drought stress condition represent a contrast between set A and set B. thus as oil content increases height decreased. For four principal component in irrigated condition set A and set B is empty but in drought stress condition number of capitulum and days to 50% flowering had positive relationship and set B is empty.

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