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Path analysis of the relationships between yield and some related traits in diallel population of sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions

R. Darvishzadeh[1,2], H. Hatami Maleki[3] and A. Sarrafi[4]

[1]Department of Agronomy and Plant Breeding, Urmia University, Urmia, Iran;
[2]Institue of Biotechnology, Urmia University, Urmia, Iran
[3]Department of Agronomy and Plant Breeding, University of Maragheh , Maragheh, Iran
[4]Laboratoire Symbiose et Pathologie des Plantes, INP-ENSAT, 18 Chemin de Borde Rouge, BP 32607, 31326 Castanet-Tolosan, France

***Corresponding author: r.darvishzadeh@mail.urmia.ac.ir**

Abstract

In order to study the association among yield components and their direct and indirect effects on the seed yield of sunflower under well-watered and water-stressed conditions, six sunflower inbred lines and their 15 F_1 hybrids were investigated using a randomized complete block design with three replications in each water treatment conditions. Different traits such as: chlorophyll content (CC), head diameter (HD), head weight (HW), leaf number (LN), aerial part dry weight (APDW), number of achene (NA), plant height (PH) and seed yield per plant (SY) were measured. Morphological traits were measured in full flowering stage and traits related to seed were recorded after seed harvesting. Genotypic correlations manifest that seed yield per plant was positively and significantly associated with head diameter, plant height and number of leaf and achene traits at well-watered condition. While, in the water-stressed state, head weight, head diameter, number of achene and chlorophyll content showed positive and significant correlation with seed yield per plant. Path coefficient analysis revealed that traits including head diameter and number of achene in both conditions and chlorophyll content in water-stressed condition have positive direct effect on seed yield per plant. Therefore, selection based on these traits would be more effective to improving seed yield of sunflower in well-watered and water-stress conditions.

Keywords: *Helianthus annuus* L., direct effect, F_1 hybrids, genotypic correlation, indirect selection, water treatment.
Abbreviations: chlorophyll content (CC), head diameter (HD), head weight (HW), leaf number (LN), aerial part dry weight (APDW), number of achene (NA), plant height (PH), seed yield per plant (SY).

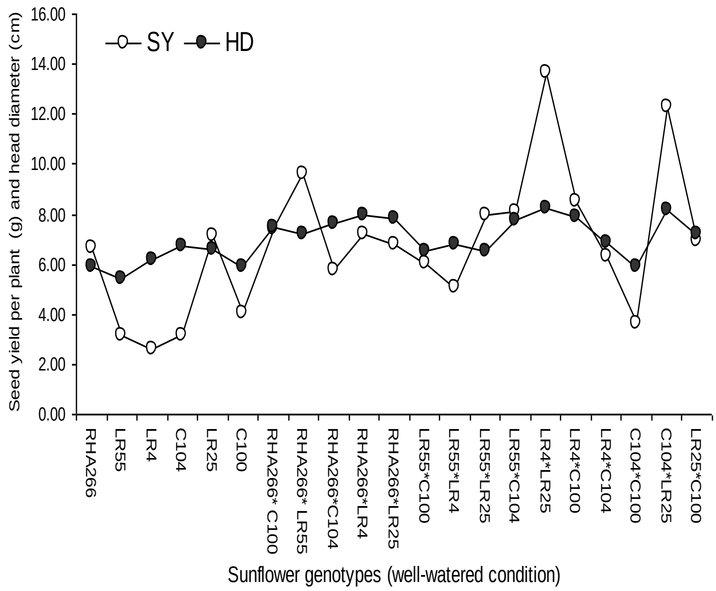
Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops that widely grown on many parts of the world (Hu et al., 2010). Sunflower seed contains high oil content ranging from 35-48% with some types yielding upto 50% (Skoric and Marinkovic, 1986), 20-27% protein (Nazir et al., 1994) and high percentage of poly-unsaturated fatty acids (60%) including oleic acid (16.0%) and linoleic acid (72.5%), which control cholesterol in blood (Satyabrata et al., 1988). Sunflower oil is considered as premium oil due to its light color, mild flavor and ability to withstand at high cooking temperatures. Furthermore, sunflower oil contains fat-soluble vitamins A, B, E and K, well for heart proteins (Evertt et al., 1987; Gossal et al., 1988). The majority of country edible oil requirements are met through imports. It has great ability to meet domestic needs of the country. In northwest region, sunflower has potential to plant as a second crop after cereal harvest. Iranian farmers evaluate sunflower varieties only in terms of yield potential, earliness and yield fluctuations by location and year. For that purpose, superior varieties must be developed by selection among and within populations that have very rich variations in important agronomic traits. The success of selection depends on the choice of selection criteria for improving seed yield (Samonte et al. 1998). Seed yield is combination of many traits, which are polygenic in nature and it is difficult to make direct selection for these traits. Therefore, indirect selection through associated component traits is possible to improve the seed yield. To separate correlation coefficients into components of direct and indirect effects, the path-coefficient analysis provides an excellent tool as it can measure the direct and indirect effects of interrelated components of a complex trait like yield (Alba et al., 1979; Chaudhary, 1993; Punia and Gill, 1994; Yasin and Singh, 2010). Sunflower breeders reported different types of character-associations (Marinkovic, 1992; Punia and Gill, 1994; Patil et al., 1996; Lal et al., 1997; Teklewold et al., 2000; Ashok et al., 2000; Khan, 2001; Kaya and Atakisi, 2003; Yasin and Singh, 2010). Their results differ widely for trait to trait which could be attributed due to differences in genetic material used for their studies. Iran, with about 220 mm of average annual rainfall is located in dry part of the world and except some northern provinces that are located in the vicinity of the Caspian Sea; in most areas the crop production encounters serious drought stress. Sunflower has good potential for drought tolerance because of well developed root system (Tahir et al., 2002; Rauf, 2008; Fulda et al., 2011). Although sunflower is moderately tolerant to water stress but its distribution and production is greatly influenced by drought (Fereres et al., 1986; Andrich et al., 1996; Krizmanic et al. 2003; Reddy et al., 2003; Grieu et al., 2008; Rauf, 2008;

Table 1. Genotypic correlation coefficients among different characters in well-watered condition

Traits	SY	HW	HD	APDW	PH	LN
NA	0.67**	-0.17ns	0.67**	-0.52ns	0.68**	0.67**
SY		0.26ns	0.86**	-0.35ns	0.78**	0.63**
HW			0.67**	0.29ns	0.87**	0.22ns
HD				-0.45*	0.96**	0.72**
APDW					-0.61**	-0.15ns
PH						0.59**

Leaf number (LN), plant height (PH), aerial part dry weight (APDW), head diameter (HD), head weight (HW), number of achene (NA) and seed yield per plant (SY).



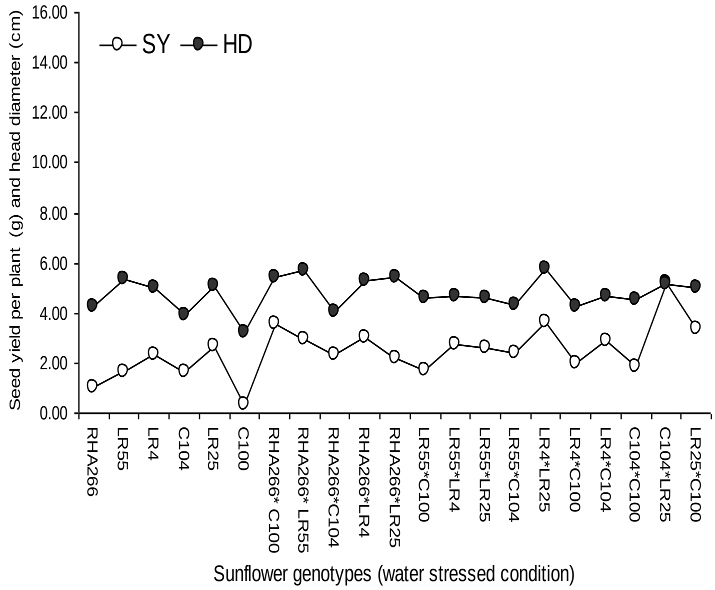


Fig 1. Genetic potential of sunflower genotypes for seed yield per plant (g) and head diameter (cm)

Iqbal et al., 2009). The interrelationships among plant yield and other traits may be varied in different water treatment conditions (Razi and Assad, 1999; Cicek et al., 2006; Cobos et al., 2007). Cobos et al. (2007) reported that correlation between seed yield and seed size in chickpea was negative and significant at one condition but positive and significant at another condition. Cicek et al. (2006) reported significant correlation at one condition but non-significant correlation at another condition between several agronomic and seed quality traits in soybean. There are enormous reports about correlation coefficient and path analyses of sunflower in determining interrelationships between traits under well-watered conditions (Marinkovic, 1992; Kaya and Atakifli, 2003; Vidhyavathi et al., 2005; Kaya et al., 2007; Arshad et al., 2007; Göksoy et al., 2007; Yasin and Singh, 2010). Review of literature showed very limited work on the use of correlation and path analysis under water stress conditions for sunflower (Razi and Assad, 1999; Iqbal et al., 2009), however, it was utilized to assess correlated effects of various traits on yield under water stress conditions for wheat (Subhani and Chowdary, 2000; Khan et al., 2003) maize (Saleem et al., 2007), chickpea (Talebi et al., 2007), rice (Yogameenakshi et al., 2004), barley (Campbell et al., 1980), and safflower (Mozaffari and Asadi, 2006). The main objectives of present study were: 1) estimation of genotypic correlation coefficient of several traits with seed yield of sunflower 2) determination of direct and indirect effects of studied traits by path analysis in well-watered and stressed conditions. Study on the relationships between yield and yield related traits will improve the efficiency of breeding program by determining appropriate selection criteria.

Material and methods

Plant material

According to our previous experiments on genetic analysis of sunflower tolerance to drought stress (Poormohammad Kiani et al., 2007a, 2007b, 2008, 2009) five sunflower recombinant inbred lines (RILs) out of 126 including "C104", "LR25", "LR4", "C100", "LR55" and their paternal line "RHA266" were selected. These 6 genotypes were grown in greenhouse and then crossed in a diallel mating system without reciprocals to produce 15 F_1 hybrid combinations.

The RILs used in this experiment were F_9 pure lines which were developed through single seed descent from F_2 plants derived from a cross between 'PAC2' and 'RHA266'. 'RHA266' was obtained from a cross between wild *H. annuus* and *peredovik* by USDA and 'PAC2' is an INRA-France inbred line from a cross between *H. petiolaris* and 'HA61' (Gentzbittel et al., 1995). This public RILs population is widely used for genetic analysis of complex traits in sunflower (Poormohammad Kiani et al., 2007a, b, 2008, 2009; Darvishzadeh et al., 2007). C104 has good water status and osmotic adjustment as well as biomass and yield under water stress condition. LR25 has good water status and osmotic adjustment as well as biomass under water stress condition. LR4 has average water status and osmotic adjustment as well as biomass and yield under water-stressed condition. C100 has good water status and osmotic adjustment but very low in yield under both well-watered and water-stressed conditions. LR55 has the lowest water status traits and osmotic adjustment as well as biomass and yield under water-stressed condition.

Methodology of experiment

Seed of 15 F_1 hybrid and their 6 parental lines were sown in triplicate randomized complete block design (RCBD) in both stress and non stress conditions. The trial was conducted in the greenhouse condition. In order to simulate natural water deficit conditions (similar to field) a progressive water stress from mild stress to severe stress was imposed on 45-day-old plants at stage near flower bud formation (R_1) (Schneiter and Miller, 1981) for a period of 12 days (Poormohammad Kiani et al., 2007a, b). Well-watered plants (control) received sufficient water to maintain soil water content close to field capacity. Water-stressed plants were subjected to a progressive water stress and irrigated with a water volume of 60%, 50% and 40% of field capacity (each for 4 days) during 12 days and continued up to harvest.

Measurement of traits

All the recommended agronomic and protection practices were followed from sowing until harvesting. Different traits such as: head diameter (HD), head weight (HW), leaf number (LN), aerial part dry weight (APDW), number of achene (NA), plant height (PH) and seed yield per plant (SY) were measured. Morphological traits were measured in full flowering stage and traits related to seed were recorded after seed harvesting. The greenness of the upper most fully expanded leaves as an indicator of total chlorophyll content (CC) was determined using a portable chlorophyll meter, SPAD-502, Soil-Plant Analysis Development Section, Minolta Camera, Osaka, Japan, in SPAD values according to Castelli et al. (1996).

Statistical analysis

The estimation of genotypic variances of each trait was done according to expected values of mean squares by using this formula:

$$\sigma^2 g = \frac{MSG - MSE}{r};$$

where MSG is the genotype mean square, MSE is the error mean square and r is the number of replication. Genotypic covariance calculated based on this formulas:

$$Cov_{g\,xy} = \frac{MP_g - MP_e}{r};$$

where MP is considered as mean of product. Genotypic correlations were determined using following formula (Kearsey and Pooni, 1996; Falconer and Mackay, 1997):

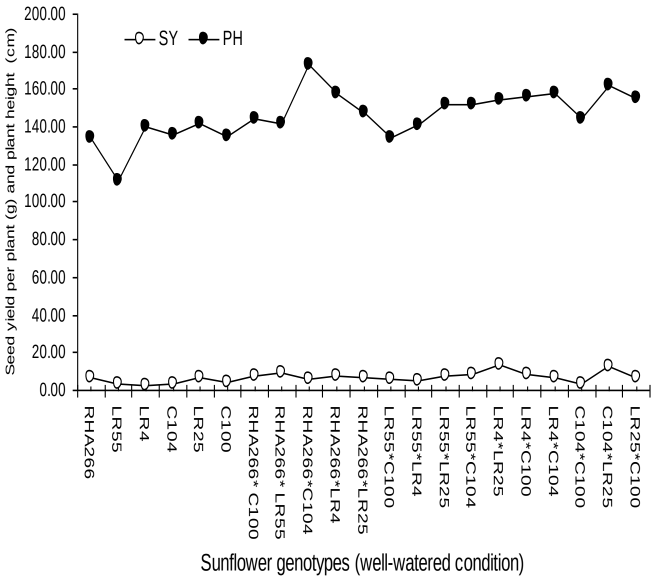
$$r_g = \frac{Cov_{g\,xy}}{\sqrt{\sigma^2_{g\,x}\sigma^2_{g\,y}}};$$

where x represent the first trait, y represent the second trait. The path coefficient analysis using significant genotypic correlation coefficients (Razi and Assad, 1999) was carried out according to Dewy and Lu (1959). Statistical analyses were conducted using SPSS version 16.0 computer programme.

Table2. Genotypic correlation coefficients among different characters in water-stressed condition

Traits	YS	HW	HD	APDW	CC	PH	LN
NA	0.85^{**}	0.24^{ns}	0.77^{**}	-0.11^{ns}	0.25^{ns}	-0.35^{ns}	0.41^{ns}
SY		0.46^{*}	0.84^{**}	-0.05^{ns}	0.48^{*}	-0.22^{ns}	0.34^{ns}
HW			0.41^{ns}	0.22^{ns}	0.37^{ns}	0.43^{ns}	0.87^{**}
HD				0.18^{ns}	0.04^{ns}	-0.19^{ns}	-0.17^{ns}
APDW					0.43^{ns}	-0.13^{ns}	-0.21^{ns}
CC						-0.19^{ns}	0.19^{ns}
PH							0.43^{ns}

Leaf number (LN), plant height (PH), aerial part dry weight (APDW), chlorophyll content (CC), head diameter (HD), head weight (HW), number of achene (NA) and seed yield per plant (SY).



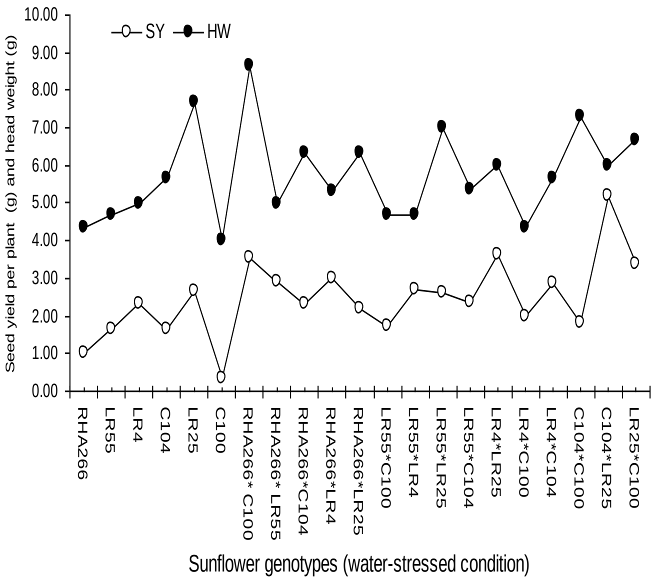


Fig 2. Genetic potential of sunflower genotypes for seed yield per plant (g) and plant height (cm) as well as for head weight (g)

Table 3. Direct and indirect effects of agronomic traits on seed yield per plant in sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions

Well-watered condition						
Traits	Genotypic correlation	Direct effects	Indirect effects			
			NA	HD	PH	LN
NA	0.67	0.39		1.42	-0.87	-0.27
HD	0.86	2.11	0.26		-1.23	-0.29
PH	0.78	-1.28	0.27	2.03		-0.24
LN	0.63	-0.40	0.26	1.52	-0.76	
water-stressed condition						
Traits	Genotypic correlation	Direct effects	Indirect effects			
			NA	HW	HD	CC
NA	0.85	0.30		0.00	0.46	0.10
HW	0.46	0.00	0.07		0.24	0.14
HD	0.84	0.60	0.23	0.00		0.02
CC	0.48	0.38	0.07	0.00	0.02	

Leaf number (LN), plant height (PH), chlorophyll content (CC), head diameter (HD), head weight (HW), number of achene (NA) and seed yield per plant (SY).

Results and discussion

Well-watered

In the well-watered conditions, the strongest genotypic correlation of seed yield per plant was observed with head diameter (r = 0.86) (Table 1). This is in agreement with the findings of Vanishree et al. (1988), Razi and Assad (1999), Teklewold et al. (2000) and Göksoy and Turan (2007). Razi and Assad (1999) reported that head diameter is the most important yield component in sunflower under well-watered conditions. Significant and positive genotypic correlations between seed yield per plant and head diameter imply that either gene(s) involve in controlling traits are closely related or it might be explained by the pleiotropical effect of given genes. Pleiotropic effects are the main causes for correlations among traits (Aastveit and Aastveit, 1993; Falconer and Mackay, 1997). In the next step, some other studied traits including plant height, number of achene per head and number of leaf showed significant genetic relationships with seed yield per plant (Table 1). Plant height also showed positive and significant correlations with all other studied traits (Table 1) which was in accordance with previous reports in sunflower (Vanishree et al., 1988; Tahir et al., 2002; Göksoy and Turan, 2007). Plant height is a function of both genetic constitution and the environmental conditions under which it is grown. The greater plant height indicated maximum chances for radiation interception, its utilization efficiency and consequently higher photosynthetic rate (Al-Thabet, 2006). Leaves are the food manufacturing factories of the plant and the greater number of leaves ensured the better crop yield due to higher photosynthetic capacity by increasing LAI and resultantly higher Fi (fraction of intercepted radiation), intercepted PAR and its utilization efficiency. Based on simple correlation coefficient analysis under well-watered condition, screening for high head diameter, plant height, number of achene and number of leaf may be useful to achieving high seed yield (Figure 1, 2). Since we could not establish the cause and effect relationships between the examined yield components and seed yield per plant on the basis of simple correlation coefficients, we processed the data by the path-coefficient analysis which enabled the partitioning of the direct and indirect effects of individual yield components and identification of yield components applicable as selection criteria in sunflower breeding. Path coefficient analysis was conducted by considering yield-related traits as pridictor variables and seed yield as the response variable. This method allowed separation of the correlation coefficients with components of direct and indirect effects (Table 3). Significant genotypic correlations were used to path analysis and results revealed that traits including plant height and leaf number had negative direct effects on seed yield per plant and the head diameter and number of achene had positive direct effect on seed yield per plant (Table 3). The negative direct effects of plant height and leaf number traits were compensated by the highly positive indirect effects of head diameter and number of achene, respectively (Table 3). Therefore, they cannot be generalized as traits for indirect selection for higher seed yield. In order to identify a trait as an indirect selection criterion for seed yield through path coefficient, the trait should have positive direct effect on seed yield as well as significant positive correlation with seed yield (Das and Taliaferro 2009). Identifying a trait as an indirect selection criterion based solely on positive direct effect and disregarding the nature and magnitude of correlation of that trait with seed yield would be misleading (Das and Taliaferro 2009).

Water-stress

In the water-stressed condition, to some extent, correlations values were similar to well-watered conditions (Table 2). In this state, seed yield per plant was positively correlated with head weight, head diameter, number of achene and chlorophyll content (Table 2). Interestingly, head diameter is still best criterion for improving achene weight in sunflower. In this way, screening for high head weight and diameter may bring increase in sunflower seed yield under water-stressed condition (Figure 1, 2). According to path analysis, based on significant genotypic correlations in this condition, head diameter had highest positive direct effects on seed yield per plant (Table 3). Moderately high positive indirect effect of head diameter was by the number of achene (Table 3). Also, number of achene had positive direct effects on seed yield per plant under water-stressed condition as same as well-watered conditions (Table 3). In addition to direct load of number of achene on seed yield, it also contributes indirectly and positively to seed yield per plant through chlorophyll content (Table 3). Weight of head didn't have any direct effect while had indirect effects via chlorophyll content and head diameter traits (Table 3). Chlorophyll content presents direct effect

(0.38) on response variable while it hadn't noticeable indirect effects via predictor variables (Table 3). Different studies reported that chlorophyll content is one of the most important traits in the water deficit states (Li et al., 2006; Poormohammad Kiani et al., 2008). Path analysis results of water-stressed condition were varied from normal condition and this is in consistent with Cicek et al. (2006) and Cobos et al. (2007) reports in soybean and chickpea respectively.

Conclusion

This investigation concluded that in well-watered conditions, genotypic association of seed yield with head diameter, plant height, number of leaf and number of achene is positive and significant. While, in the water-stressed conditions, seed yield per plant has positively and significantly correlated with head weight, head diameter, number of achene and chlorophyll content. Considering to our results, head diameter and number of achene can be good selection criteria because they could easily measure and have strong and highly significant positive correlations with seed yield in both water treatment conditions. This relationship was confirmed by the path analysis as they had a considerable positive direct effect on seed yield. Also, chlorophyll content in water-stressed state has positive direct effect on seed yield. Therefore, selection based on these traits would be more effective to improving sunflower seed yield under well-watered and water-stress conditions.

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