Naser Sabaghnia* and Mohsen Janmohammadi

Graphic analysis of compatible organic solutes treatments × trait interaction on sunflower

https://doi.org/10.1515/helia-2023-0001
Received January 11, 2023; accepted March 17, 2023; published online March 31, 2023

Abstract: Compatible solutes control cell osmotic balance and compatible the cells' metabolism whereas nano-particles have been introduced to increase crop production with inadequate knowledge. This investigation is done to evaluate the effects of application of salicylic acid, glycine betaine and nano titanium dioxide on sunflower. Measured traits were plant height (PH), leaf length (LL), chlorophyll content (CHL), number of seeds per head (NSH), 1000-seed weight (TSW), day to maturity (DM), husk percentage (HP), kernel weight (KW), percentage of empty achenes (PEA), head number per plant (HNP), grain yield (GY) and oil percent (OIL). Results indicated that the first two principal components accounted 92% of total variation of the treatment by trait (T × T) interaction and were used to generate a T × T biplot. All treatments were identified as the vertex treatments in polygon of TT biplot and the nano titanium dioxide treatment had high values for all traits expect PEA, HP and TSW. The nano titanium dioxide was identified as ideal treatment and GY and HNP were identified as ideal traits. The positive correlations between HNP and GY, and between OIL and KW; and near zero correlations between TSW and HP, and between TSW with DM as showed. Application of compatible organic solutes (glycine betaine and salicylic acid) had not any considerable improvement on traits while application of nano-titanium dioxide indicated considerably enhanced the yield and most traits of sunflower.

Keywords: glycine betaine; nano-titanium dioxide; oil content; salicylic acid.

1 Introduction

Sunflower (*Helianthus annuus* L.) is one of the oil crops in the world which has important role in diet of some nations due to its high quality. Approximately, 58

***Corresponding author: Naser Sabaghnia**, Department of Plant Production and Genetics, Faculty of Agriculture, University of Maragheh, Maragheh, Iran, E-mail: sabaghnia@maragheh.ac.ir
Mohsen Janmohammadi, Department of Plant Production and Genetics, Faculty of Agriculture, University of Maragheh, Maragheh, Iran

million tons sunflower is harvested from about 30 million ha indicating 2.1% of the total harvested area of world, while the yearly production of sunflower in Iran is close to 60,000 tones achieved from 60,000 ha (FAOSTAT 2021). It was produced with total mean yield of 1.0 ton per hectare during 2021 while global mean yield of wheat was 1.9 tons per hectare, which showing 47% low yield performance in Iran regarding world mean yield record (FAOSTAT 2021). Semi-arid Mediterranean regions of Iran are characterized by low erratic rainfall and due to the unpredictable rainy season, low soil fertility, environmental stresses, the yield performance of sunflower in such regions is less than the expected amount. The rain-fed regions have an important role in food security because they form 80% cultivated areas of world and two thirds of world food production quantity (Janmohammadi et al. 2017). To improve the yield performance of this crop, some nanoparticles and compatible organic solutes have been used for better growth and development induction, and some treatments have been obtained with beneficial traits.

Utilization of compatible solutes in semi-arid regions seems to be an effective tool for improving crop performance which is used to mitigate the adverse effects of environmental stress. They are compounds that protect plant cells from desiccation by maintaining a high intracellular osmolality (Paliwal et al. 2021). Glycine betaine (G-B), as one of the compatible solutes, has an important role in protection of crops under different stressful environmental circumstances. It is also promoting expression of some proteins, such as heat-shock protein (HSP) and photosystems II (PSII), as a pigment–protein compound (Huang and ZuoNi 2020). Some investigations have showed that using of G-B, increase PSII's tolerance to environmental stresses through the stabilizing in the structure and function of PSII through indirect stabilize of the subunits and cofactors of PSII (Wei et al. 2017). Salicylic acid (S-A), as a phenolic compound, affects some physiological functions in plant cells and play an important role as a regulatory signal causing the proper response of crops to various stresses. It can regulate different physiological processes such as photosynthesis, and its established effects on stomatal function and chlorophyll content cause to possess other physiological possess, such as photosynthetic reactions (Arif et al. 2020).

Nanotechnology has indicated promising potential to enhance agriculture and numerous nanoparticle have been investigated for application in agriculture (Usman et al. 2020). Nanoparticles have special properties compared with bulk forms such as small size and ability to cross biological barriers, and their large specific surface can result in of interaction with cellular organelles. Nano-titanium dioxide (Nano-Ti) possesses biological properties and has caught the attention of researchers. Nanoparticles of TiO_2 can be used to improve biomass production and increase the activity of antioxidants (Shariatzadeh-Bami et al. 2021). Priya et al. (2020) found that application of TiO_2 nanoparticles on mung bean (*Vigna radiata* L.) can significantly improve shoot and root characteristics in germination and seedling growth stages.

Application of nanoparticles can regulate plant growth and improve the tolerance of plants to environmental stresses in semi-arid Mediterranean regions while they are not categorized as plant growth regulators.

Thus, investigation such interrelationships would be useful in yield selection but simple correlation coefficients may be insufficient in the detecting of the true value of each target trait (Fischer and Rebetzke 2018). The significant influence of the treatment by trait (T × T) biplot model has been indicated (Yan and Frégeau-Reid 2018). This model which is based on the principal component (PC) analysis, enables the clear point view of any interaction structure. The T × T biplot model provides better understanding of genotypes performances regarding several measured traits, and facilitates the selection process of the high performance genotypes (Singamsetti et al. 2021). Also, this method is suitable for evaluating newly improved genotypes under different environmental conditions and it is very useful for plant breeders for strong supporting from their decisions in genetic improvement projects. Sabaghnia and Janmohammadi (2014) used this model for analysis of interaction between nano-silicon and salinity stress on germination properties of lentil and showed that germination delayed by salt stress while application of nano-silicon dioxide could considerably alleviate the adverse effect of salt stress on germination and other traits such as root and shoot length. In this investigation, the T × T biplot model used in order to: (i) indicate the interrelationships among measured traits, (ii) compare treatments based on the multiple traits, (iii) detection the best treatments for semi-arid regions, and (iv) evaluation of different tools of T × T biplot model for graphical analysis of T × T pattern.

2 Materials and methods

A trial was performed in the randomized complete block design with four replications. Seeds were hand sown on the 28th of March in plots (8 rows, 4.5 m length and 0.6 m width) under rain-fed conditions but three supplemental irrigations during reproductive stage. The chemical fertilizers (150 kg nitrogen and 100 kg phosphate ha^{-1}) were applied in the form of urea and triple super-phosphate at the field preparation. Weeds were hand controlled. Experimental treatments included Control (spraying with water), G-B (100 mM foliar spraying with glycine betaine), S-A (1 mM foliar spraying with salicylic acid), and Nano-Ti (2 mM spraying with nano-titanium dioxide). Foliar spraying was done at the stem elongation and flowering stages. Chlorophyll content (CHL) was recorded with an SPAD-502 m using fully expanded upper leaves at the flowering stage. Leaf length (LL) and width were measured at the end of the flowering stage using 10 randomly selected plants. Plots were monitored for recording days to maturity (DM) and plants were harvested (6 center rows, 3.5 m length) at the stage of physiological maturity and grain yield (GY) was measured. The seed oil percentage (OIL) was measured using a portable near-infrared seed analyzer (Zeltex). At harvest, 10 randomly selected plants from each experimental plot were sampled and used for measuring plant height (PH), number of seeds per head (NSH); 1000 seed weight (TSW), kernel weight (KW), husk percentage (HP), percentage of empty achenes (PEA) and head number per plant (HNP).

A linear pairwise Pearson correlation analysis was performed to all the measured traits and then the treatment by trait (T × T) biplot model (Yan and Frégeau-Reid 2018) was used to indicate the pattern of T × T two-way interaction data in a graph. The T × T biplot graph is drawn by plotting the symmetric scaled scores of the treatments and traits, so that each treatment or trait is shown by a special marker in the graph using GGEbiplot software. For more details about T × T biplot model and other types of biplots for two-way structures, see Yan and Frégeau-Reid (2018).

3 Results and discussion

The first two PCs of T × T biplot model described 92% of the total variation (65% and 27% for PC1 and PC2, respectively). The T × T biplot model described relatively the all of the observed variation and almost all information of the routine statistical methods can be graphically showed in a T × T biplot. Yousefzadeh and Sabaghnia (2016), and Buenrostro-Rodríguez et al. (2019), also reported similar results while studying genotype by traits two-way structures in different crops, using biplot methodology. The vector view of T × T biplot (Figure 1), indicates the most prominent

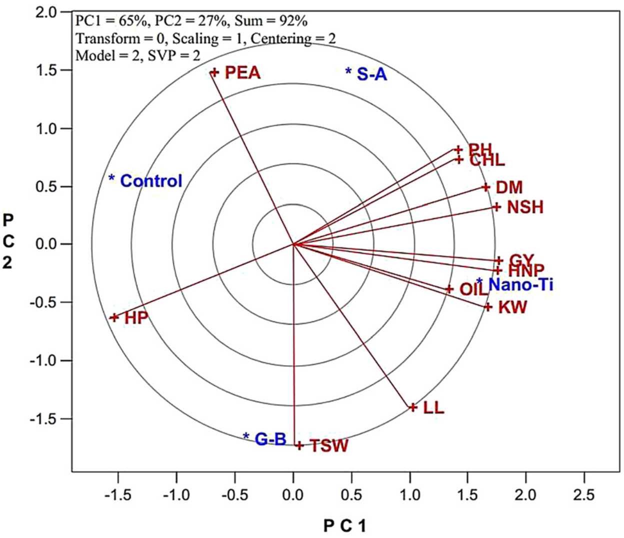

Figure 1: Vector view of treatment by trait biplot showing the interrelationship among traits. Traits are: plant height (PH), leaf length (LL), chlorophyll content (CHL), number of seeds per head (NSH), 1000-seed weight (TSW), day to maturity (DM), husk percentage (HP), kernel weight (KW), percentage of empty achenes (PEA), head number per plant (HNP), grain yield (GY) and oil per cent (OIL).

Table 1: Pearson correlation coefficients among sunflower traits.

	PH	LL	CHL	NSH	TSW	DM	HP	KW	PEA	HNP	GY
LL	0.13										
CHL	1.00	0.18									
NSH	0.88	0.42	0.88								
TSW	−0.46	0.81	−0.41	−0.18							
DM	0.78	0.26	0.76	0.96	−0.28						
HP	−0.83	−0.19	−0.81	−0.96	0.37	−0.99					
KW	0.34	0.98	0.39	0.58	0.67	0.41	−0.36				
PEA	−0.05	−0.97	−0.11	−0.26	−0.86	−0.07	0.01	−0.94			
HNP	0.74	0.67	0.76	0.95	0.13	0.88	−0.85	0.79	−0.53		
GY	0.75	0.63	0.77	0.96	0.09	0.90	−0.88	0.76	−0.48	0.99	
OIL	0.24	0.51	0.24	0.67	0.22	0.78	−0.71	0.53	−0.30	0.74	0.76

Critical values of correlation $p < 0.05$ and $p < 0.01$ are 0.81 and 0.92, respectively. Traits are plant height (PH), leaf length (LL), chlorophyll content (CHL), number of seeds per head (NSH), 1000-seed weight (TSW), day to maturity (DM), husk percentage (HP), kernel weight (KW), percentage of empty achenes (PEA), head number per plant (HNP), grain yield (GY) and oil per cent (OIL).

correlations as: positive correlations between CHL and PH, between HNP and GY, between DM and NSH, and between OIL and KW as showed by the low angles between projections (r = cosine 0 = +1). The positive correlation of yield and number of head per plant (Riaz et al. 2019), and the positive association of number of seeds per head and days to maturity (Siahbidi et al. 2022) has been reported previously. Also, there was relatively strong negative correlation between PEA and LL, and between HP with CHL and PH, as showed by the obtuse angle between their projections (r = cosine 180 = −1) (Figure 1). Finally, based on Figure 1, there were near zero correlations between TSW and HP, between PEA and HP, between PEA with PH and CHL, between TSW with DM and NSH and between LL with CHL and HP, as showed by the near perpendicular projections (r = cosine 90 = 0). The correlations between traits are presented in Table 1, and about all of the information of Figure 1, can be checked from the original table with very low disagreement because the T × T biplot model could not describ 8% of the observed variability.

In the T × T biplot model, an ideal trait should be distinguishing of the traits and at the same time representative of the considered trait. In Figure 2, the traits are ranked according to both discriminating property and representativeness, simultaneously. The marker of ideal trait on the average trait coordinate (ATC) x-axis was located to be equal to the longest vector of all traits while its marker on the ATC y-axis was zero, showing that it is representative of the average trait. Thus, the closer a trait is to the ideal trait, the better it is as a test trait. Thus, GY and HNP following to OIL, KW and NSH were relatively the most favorable traits (Figure 2), Thus, regarding the ideal trait position, grain yield had the high discriminating

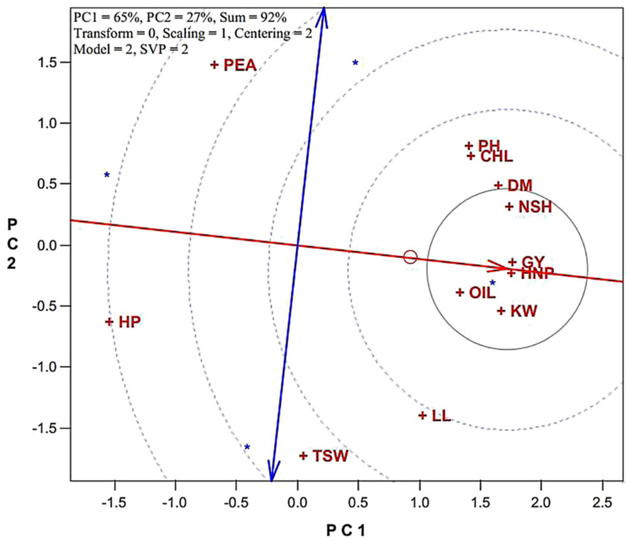

Figure 2: Ideal tester view of treatment by trait biplot showing the position of the ideal trait. Traits are: plant height (PH), leaf length (LL), chlorophyll content (CHL), number of seeds per head (NSH), 1000-seed weight (TSW), day to maturity (DM), husk percentage (HP), kernel weight (KW), percentage of empty achenes (PEA), head number per plant (HNP), grain yield (GY) and oil per cent (OIL).

ability and representativeness. Also, HP was a relatively the most unfavorable trait, followed by PEA and TSW, thus these traits cannot be used for discriminating of genotypes and they are not good for representativeness (Figure 2). The high discriminating ability and representativeness potential of yield performance and kernel weight is verified by Shojaei et al. (2022), thus using these traits for screening of the best treatments or even the best genotypes can be advised. Graphical visualization of biplot demonstrated that analyzing representativeness and discriminating ability of the testers this study can identify the candidate traits as the most "ideal" ones.

In the T × T biplot model, an ideal treatment, is one that has both high performances for all traits and low variability whereas its vector on the ATC *x*-axis is planned to be equal to the longest projection of all the treatments (Figure 3). A treatment is more favorable if it is closer to the ideal treatment position, thus, Nano-Ti followed by S-A were more desirable than the other treatments. These favorable treatments were identified as the most superior treatments for almost all of measured traits, based on mean comparisons after ANOVA test (Results are not shown). The most unfavorable treatments based on the ideal treatment, was Control followed by G-B treatments (Figure 3). Thus, all treatments could improve the means

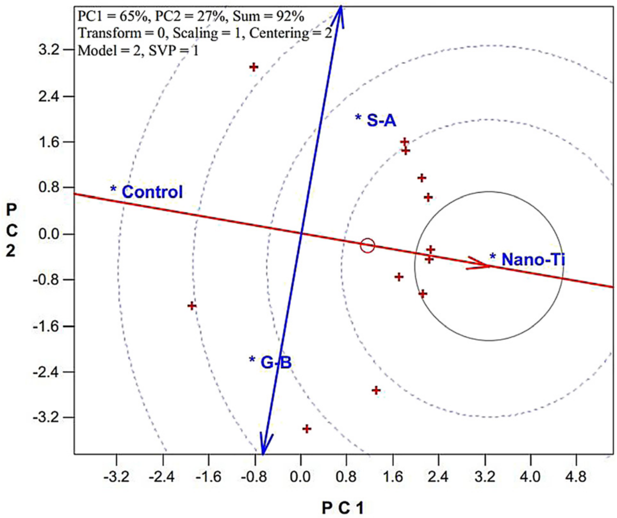

Figure 3: Ideal entry view of treatment by trait biplot showing the position of ideal treatment. Traits are: plant height (PH), leaf length (LL), chlorophyll content (CHL), number of seeds per head (NSH), 1000-seed weight (TSW), day to maturity (DM), husk percentage (HP), kernel weight (KW), percentage of empty achenes (PEA), head number per plant (HNP), grain yield (GY) and oil per cent (OIL).

of the most traits and were better than Control treatment. However, using nanoparticles like nano-titanium dioxide or application compatible organic solutes like salicylic acid or glycine betaine had positive effects on all measured traits of sunflower and were better than control treatment. Sabaghnia et al. (2018) reported relatively similar results in evaluation of nano-TiO_2 and nano-silica particles on yield performance and some morphological traits of sunflower and reported the nano-TiO_2 as the ideal treatment.

Another tool of T × T biplot model is examination of discrimination versus representativeness of traits (Figure 4), and the position of the average trait is shown by a small circle on ATC *x*-axis. The projection length of a trait indicates its discriminating potential and the magnitude of the angle between a trait and the ATC *x*-axis shows its representativeness. Thus, the discriminating potential of almost all traits were high (Figure 4). The small angle between a trait and the ATC *x*-axis, improves its high representative ability, thus GY and HNP showed high representative ability (Figure 4). Also, the large angle between a trait and the ATC *x*-axis, improves its low representative ability, thus HP and PEA indicated low representative ability (Figure 4). It can be concluded that, the high discriminating potential as well as the high representative ability of GY and HNP is verified and these traits can determine the differences among treatments and it is a good representativeness of

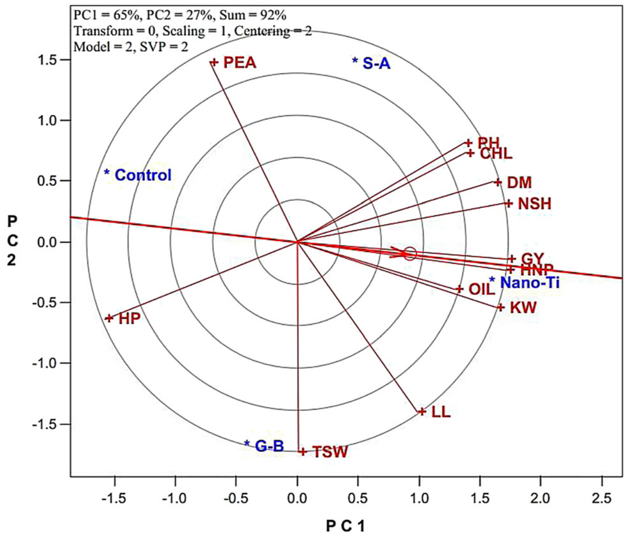

Figure 4: Discrimination versus representativeness of treatment by trait biplot showing the potential of traits. Traits are: plant height (PH), leaf length (LL), chlorophyll content (CHL), number of seeds per head (NSH), 1000-seed weight (TSW), day to maturity (DM), husk percentage (HP), kernel weight (KW), percentage of empty achenes (PEA), head number per plant (HNP), grain yield (GY) and oil per cent (OIL).

measured traits. In other word, head number per plant (HNP) as one of the most important components of yield and yield performance are adequate for detection of differences among treatments as well as genotypes. Similarly, Sabaghnia et al. (2018) reported sunflower yield as discriminative and representativeness trait.

The polygon tool of the T × T biplot provides an option to identification of the most superior treatments in the one or more traits and permits to visualization of the T × T interaction structures by connecting the furthest treatments to form a polygon. According to Figure 5, the four vertex treatments were Nano-Ti, Control, G-B, and S-A which were the best or the poorest treatments in some or all of the traits due to their longest distance from the origin. Treatment Nano-Ti, had the highest values for most traits expect PEA, HP and TSW, treatment G-B had the highest values for TSW, treatment Control had the highest values for PEA and HP, and treatment S-A had the highest values for HP and PEA (Figure 5). The other vertex treatment (S-A) and related sectors were not high for none the measured traits (Figure 5). Thus, it seems that for obtaining the high magnitudes of most traits especially economic traits like GY and OIL, application of treatment Nano-Ti is sufficient and effective. These four treatments showed very different characteristics and were completely different due to various position in vertex treatments.

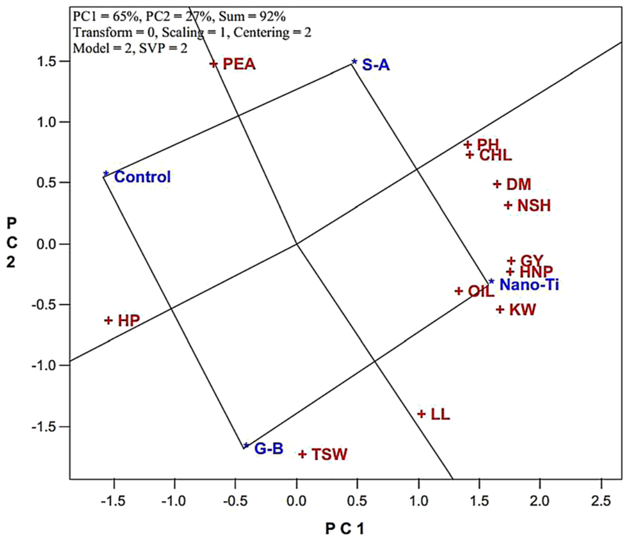

Figure 5: Polygon-view of treatment by trait biplot showing which treatment had the highest values for which traits. Traits are: plant height (PH), leaf length (LL), chlorophyll content (CHL), number of seeds per head (NSH), 1000-seed weight (TSW), day to maturity (DM), husk percentage (HP), kernel weight (KW), percentage of empty achenes (PEA), head number per plant (HNP), grain yield (GY) and oil per cent (OIL).

We found that, grain yield had the high discriminating potential as well as the high representative ability based on ideal trait biplot and discriminating versus representative biplot which had a more PC1 values for showing more discriminating of the treatment in terms of the genotypic effects and low PC2 values for showing more representative of the overall trait. The grain yield indicated both properties and can be advised for screening of genotypes reliably in a large scale, because it was good representative of all measured traits and cold discriminated studied genotypes. Similarly, Sabaghnia et al. (2016), reported leaf yield as the high discriminating and representative ability in spinach and verified that having ideal trait characteristics in an economic trait like yield performance, can help researchers to manipulate treatments in a simple way. However, finding grain yield as the discriminating and representative trait is a good news for promote plant optimizing projects.

4 Conclusions

Application of glycine betaine (100 mM), salicylic acid (1 mM), nano-titanium dioxide (2 mM). Control (spraying with water) indicated that only nano-titanium dioxide nanoparticles considerably enhanced the yield and most traits of sunflower. In contrast application of compatible organic solutes (glycine betaine and salicylic acid) had not any considerable improvement on sunflower.

Acknowledgements: We like to thank Professor Weikai Yan (Eastern Cereal Oilseed Research Center of Agriculture and Agri-Food Canada) for making available a time-limited version of GGEbiplot software as “Test Biplotxlsx.”
Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

References

Arif, Y., Sami, F., Siddiqui, H., Bajguz, A., and Hayat, S. (2020). Salicylic acid in relation to other phytohormones in plant: a study towards physiology and signal transduction under challenging environment. Environ. Exp. Bot. 175: 104040.

Buenrostro-Rodríguez, J.F., Solís-Moya, E., Gámez-Vázquez, A.J., Raya-Pérez, J.C., Mandujano-Bueno, A., Cervantes-Ortiz, F., and Covarrubias-Prieto, J. (2019). Yield performance and GGE biplot analysis of wheat genotypes under two irrigation treatments at El Bajío, Mexico. Chil. J. Agric. Res. 79: 234–242.

FAOSTAT (2021). *Food and agricultural organisation of the united nations*, Available at: http://faostat.fao.org.

Fischer, R.A. and Rebetzke, G.J. (2018). Indirect selection for potential yield in early-generation, spaced plantings of wheat and other small-grain cereals: a review. Crop Pasture Sci. 69: 439–459.

Huang, S., Zuo, T., and Ni, W. (2020). Important roles of glycinebetaine in stabilizing the structure and function of the photosystem II complex under abiotic stresses. Planta 251: 36.

Janmohammadi, M., Yousefzadeh, S., Dashti, S., and Sabaghnia, N. (2017). Effects of exogenous application of nano particles and compatible organic solutes on sunflower (*Helianthus annuus* L.). Bot. Serbica 41: 37–46.

Paliwal, A., Verma, A., Tiwari, H., Singh, M.K., Gour, J.K., Nigam, A.K., Kumar, R., and Sinha, V.B. (2021). Effect and importance of compatible solutes in plant growth promotion under different stress conditions. In: Wani, S.H., Gangola, M.P., and Ramadoss, B.R. (Eds.), *Compatible solutes engineering for crop plants facing climate change*. Cham: Springer.

Priya, B., Mukherjee, S., and Srinivasarao, M. (2020). TiO_2 nanoparticles can enhance germination and seedling growth of mung bean (*Vigna radiata* L.). Pharma Innov. J 9: 107–112.

Riaz, A., Tahir, M.H.N., Rizwan, M., Fiaz, S., Chachar, S., Razzaq, K., and Sadia, H. (2019). Developing a selection criterion using correlation and path coefficient analysis in sunflower (*Helianthus annuus* L.). Helia 42: 85–99.

Sabaghnia, N., Javanmard, A., Janmohammadi, M., and Nouraein, M. (2018). The influence of nano-TiO_2 and nano-silica particles effects on yield and morphological traits of sunflower. Helia 41: 213–225.

Sabaghnia, N. and Janmohammadi, M. (2014). Graphic analysis of nano-silicon by salinity stress interaction on germination properties of lentil using the biplot method. Agric. For. 60: 29–40.

Sabaghnia, N., Mohebodini, M., and Janmohammadi, M. (2016). Biplot analysis of trait relations of spinach (*Spinacia oleracea* L.). Genetika 48: 675–690.

Shariatzadeh-Bami, S., Khavari-Nejad, R.A., Ahadi, A.M., and Rezayatmand, Z. (2021). TiO_2 nanoparticles effects on morphology and physiology of *Artemisia absinthium* L. under salinity stress. Iran J. Sci. Technol. Trans. A Sci. 45: 27–40.

Shojaei, S.H., Ansarifard, I., Mostafavi, K., Bihamta, M.R., and Zabet, M. (2022). GT biplot analysis for yield and related traits in some sunflower (*Helianthus annuus* L.) genotypes. J. Agric. Food Res. 10: 100370.

Siahbidi, A.Z., Rezaeizad, A., and Ghaffari, M. (2022). Combining ability of some sunflower parental lines in both normal and drought stress conditions. Helia 45: 135–150.

Singamsetti, A., Shahi, J.P., Zaidi, P.H., Seetharam, K., Vinayan, M.T., Kumar, M., and Madankar, K. (2021). Genotype × environment interaction and selection of maize (*Zea mays* L.) hybrids across moisture regimes. Field Crops Res. 270: 108224.

Usman, M., Farooq, M., Wakeel, A., Nawaz, A., Cheema, S.A., Rehman, H., and Sanaullah, M. (2020). Nanotechnology in agriculture: current status, challenges and future opportunities. Sci. Total Environ. 721: 137778.

Wei, D., Zhang, W., Wang, C., Meng, Q., Li, G., Chen, T.H.H., and Yang, X. (2017). Genetic engineering of the biosynthesis of glycinebetaine leads to alleviate salt-induced potassium efux and enhances salt tolerance in tomato plants. Plant Sci. 257: 74–83.

Yan, W. and Frégeau-Reid, J. (2018). Genotype by yield*trait (GYT) biplot: a novel approach for genotype selection based on multiple traits. Sci. Rep. 8: 1–10.

Yousefzadeh, S. and Sabaghnia, N. (2016). Nano-iron fertilizer effects on some plant traits of dragonhead (*Dracocephalum moldavica* L.) under different sowing densities. Acta Agric. Slov. 107: 429–437.