DOI: 10.1515/plass-2016-0008

Naser Sabaghnia*, Mohsen Janmohammadi

Department of Agronomy and Plant Breeding, Agriculture College,
University of Maragheh, Iran.*Corresponding Author's e-mail sabaghnia@maragheh.ac.ir

BIPLOT ANALYSIS OF SILICON DIOXIDE ON EARLY GROWTH OF SUNFLOWER

ABSTRACT

Research into nanotechnology has advanced in almost all fields of technology and the aim of this study was to evaluate the role of nano-silicon dioxide (nano-SiO₂) in germination performance sunflower. Germination and seedling growth are the most important stage of plant development and are critical factors to crop production and are essential to achieve optimum performance. The effects of pre-germination hydration in solutions of nano-SiO₂ (0, 0.2, 0.4, 0.6, 0.8, 1 and 1.2 mM for 8 h) on germination characteristics of sunflower were investigated. The trait by treatment (TT) biplot explained 93% of the total variation of the standardized data (77% and 16% for the first and second principal components, respectively). According to polygon-view of TT biplot, T2 (0.2 mM) had the highest values for all of the measured traits except mean germination time and the time to 50% germination. The germination percentage was determined as the best trait and showed the high association with promptness index, energy of germination and germination rate traits. The results of the present study indicated that pre-sowing seed treatments with low concentration of nano-SiO₂ had favorable effect sunflower seed germination and seedling early growth. Such a similar outcome could be applied in the future to outline other crops in response to nano-particles as well as to help define tolerance tools for recommendations in stressful conditions in the world.

Key words: germination rate, nano-sized materials, seed priming, TT biplot

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is important oilseed crops in the world and it is arousing the interest of producers, due to the possibility of using its oil as human food or as raw material for industrial proposes. Its growing area has been very variable and ranged from 110000 to 30000 hectares in Iran in last two decades (1990-

2010), but at recent years its cultivation areas is increased to 68000 hectares (FAOSTAT 2012). It has been estimated that world production of sunflower is exceeded 37 million tones from 25 million hectares cultivated areas (FAOSTAT 2012). Sunflower is primarily grown for edible oil, but also as a raw material for the processing industry, livestock feed and beekeeping proposes. In the recent few years, sunflower oil is used for industrial biodiesel production. It is harvested early in the fall for providing enough time for planting winter cereal crops and plays an important role in crop rotation systems (Mijic *et al.* 2009).

Poor crop establishment was identified as one of the major problems in crop production under arid and semi-arid regions (Harris *et al.* 1999). It seems that seed priming would improve seed germination and plant growth with adjusting the hydration level within seeds which permits seedlings to emerge more rapidly and to facilitate the uniform seedling stand (BASRA *et al.* 2006). Most priming treatments involve imbibing seed with constrained amounts of water to allow adequate hydration and progress of biochemical processes but hindering the protuberance of the radicle (Ashraf and Foolad 2005). In recent years, the progress of nanotechnology has appreciably increased the utilization domain of nano-particles in agriculture and some studies indicated the potential of nano-particles in improving seed germination and seedling early growth (Siddiqui & Al-waibi 2014; Sabaghnia and Janmohammadi 2014). Nano-particles can easily enter into plant by overcoming the cell wall barrier in comparison with bulk materials, but their behavior inside the plant system is unpredictable (Nair *et al.* 2011).

The correlation coefficients are important issues in determining the degree of association of different germination properties. This analysis is a reliable statistical method, which provides tool to quantify the associations among different traits. Effective interpretation of the data is important at all stages of plant evaluation and several methods have been used in exploration for an understanding of the data structure. These methods may differ in overall appropriateness; different methods usually lead to the similar conclusions for a given dataset. Yan *et al.* (2000) have developed a genotype main effect plus genotype by environment (GGE) biplot methodology for the graphical analysis of multi-environment trial data. This method can be used for all types of two-way dataset such as treatments with multiple traits. Dehghani *et al.* (2008) have used a treatment by trait (TT) biplot, which is an application of the GGE biplot to study the treatment by trait data.

Silicon as the second most abundant mineral element in the soil has been recognized as a beneficial nutrient for plant growth and development (Liang *et al.* 2007). It appears that silicon have an important function in plant protection because it deposit on the walls of epidermis and vascular tissues and play role as a physico-mechanical barrier, (Ma and Yamaji 2008), and also regulate some physiological process related to defense mechanisms in plants (Bao-Shan *et al.* 2004). It seems that, seed prehydration in solution of nano-silicon can be considered as a seed vigour enhancement treatment. The pervious studies revealed that some advantages are associated with priming treatments for seed vigor improvement of some crops, but

there is dearth of information about the germination performance of nano-particles primed achenes of sunflower. Therefore it was essential to evaluate the application of nano-silicon in order to improve sunflower germination capability. The purpose of this research was to evaluate the effect seed priming in nano-silicon on seed germination performance of sunflower to find out the most promising concentration using biplot statistical tool.

MATERIALS AND METHODS

Fig. 1. X-Ray diffraction pattern of SiO₂ nano-particles.

Seeds of sunflower (cultivar Azargol) were immersed in a 5% sodium hypochlorite solution for 10 min to ensure surface sterility then washed with distilled water for three times. Pre-hydration treatments were including control (non-primed seeds) and seeds soaking in solutions containing different concentrations of nano-silica (0.2, 0.4, 0.6, 0.8, 1 and 1.2 mM for 8 h). Nano-particles of SiO₂ were prepared from the Pishgaman-Nano, Iran. The particle sizes of SiO₂ ranged from 20 to 30 nm and specific surface area of nano-sized silicon was 180-600 m² × g⁻¹ and purity was 99.7%. The result of X-ray analysis of SiO₂ (Fig. 1) and the high-resolution transmission electron microscopy (HRTEM) image of the nano-particle sample (Fig. 2)

are shown. After presoaking, all seeds were washed with distilled water then allowed to air dry for 48 h at 25°C (Iqbal & Ashraf 2007). The experiment was carried out based on completely randomized design with three replications. Of 30 primed seeds were placed on 9 cm petri dishes among papers and wetted with 10 ml sterilized distilled water.

Fig. 2. Large area TEM image of SiO₂ nano-particles

The experiment is performed in a dark incubator at $20 \pm 1^\circ\text{C}$ and germinated seed were recorded daily for eight days. Germination was considered to have occurred when the seed developed at least 2 mm long radical. The final germination percentage was calculated based on total number of germinated seeds at the end of tenth day. The all evaluations were executed as described in Seedling Evaluation Handbook (AOSA, 1991). Mean germination time (MGT) was computed according to ELLIS & ROBERTS (1981) and germination rate (GR) considered as the reciprocal of the mean germination time (Ranal & Santana 2006). The time to 50% germination (T50) was calculated according to the following formula of Farooq *et al.* (2005) and energy of germination (EG) was calculated four day after start of the experiment. It is the percentage of germinating seeds 4 days after planting relative to the total number of seeds tested (RUAN *et al.* 2002). Promptness index (PI) according to Ashraf *et al.* (2006). The fresh weight of root and shoot of seedlings was determined by weight of the root and shoot separately on electric balance. After the fresh weight taken then the seedlings was kept in a hot air oven at 60°C for 48 hrs then the weight of dry matter was recorded.

Because the traits were measured in different units, the biplots were generated using the standardized values of the traits means using GGEbiplot software (YAN 2001). Up-to-date information on GGE biplot and new version of GGEbiplot package are available at <http://www.ggebiplot.com>. The biplot analysis was according to

Model 2 (Transform=0) within-trait standard deviation standardized (Scale=1), and trait-centred (Centering=2). The polygon views were based on cultivar-focused singular value partitioning (SVP=2), while the vector views were based on the trait-focused singular value partitioning and is, therefore, appropriate for visualizing the relationships among traits and cultivars. The cultivar by trait (GT) biplot model for cultivar by trait interaction biplot analysis was used according to Yan & Rajcan (2002). The treatment by trait (GT) biplot model equation (Yan & Rajcan 2002) is presented as follows:

$$\frac{\alpha_{ij} - \beta_j}{\sigma_j} = \sum_{n=1}^2 \lambda_n \xi_{in} \eta_{jn} + \varepsilon_{ij} = \sum_{n=1}^2 \xi_{in}^* \eta_{jn}^* + \varepsilon_{ij}$$

where α_{ij} is the mean value of treatment i for trait j , β_j is the mean value of all treatments in trait j , σ_j is the standard deviation of trait j among the treatment means, λ_n is the singular value for principal component n (PCn), ξ_{in} and η_{jn} are scores for treatment i and trait j on PCn, respectively, and ε_{ij} is the residual associated with treatment i in trait j . To achieve symmetric scaling between the treatment scores and the trait scores the singular value λ_n has to be absorbed by the singular vector for treatments ξ_{in} and that for traits η_{jn} and $\eta_{jn}^* = \eta_{jn} \times \lambda_n^{0.5}$. Because of $n=2$, only PC1 and PC2, are retained in the model and such a model tends to be the best for extracting pattern and rejecting noise from the data.

RESULTS

The TT biplot for sunflower dataset based on the method described by Yan & Rajcan (2002), explained 93% of the total variation of the standardized data. This high percentage reflects the relatively moderate complexity of the relationships among the measured traits. The first two principal components (PC1 and PC2) explained 77% and 16%, respectively. In the TT biplot, a vector is drawn from the biplot origin to each marker of the tester (treatment) to facilitate visualization of the relationships between and among the measured traits as well as treatments. According to Rubio *et al.* (2004), Dehghani *et al.* (2008) and Sabaghnia *et al.* (2011), the basic structure among the traits should be captured by the biplots. The polygon view of the TT biplot aids identifies entries (treatments) with the highest values for one or more traits. For this target, the genotypes that are connected with straight lines so that a polygon is formed with all other traits contained within the polygon. The biplot in Fig. 3A presents the data of seven treatments in nine different traits and the following information can be obtained: the vertex treatments in this investigation are T2, T3, T6 and T7. These treatments are the best or the unsuitable treatments in some or all of the traits since they had the longest distance from the origin of biplot. Therefore, T2 had the highest values for all of the measured traits except

MGT and T50 (i.e. RDW, SDW, SL, PI, GP, EG and GR). The T2 and the other treatments (in this case: T4) of this sector had suitable amounts for the above mentioned traits (Fig. 3A). The vertex treatment T6 was suitable for MGT while the vertex treatment T7 and its related treatments (T1 and T5), which fall in its sector, were suitable for T50 (Fig. 3A). The other vertex treatment (T3) and related sector was not suitable performance for the measured traits (Fig. 3A).

Fig. 3. (A) Polygon view of TT biplot showing which treatment had the highest values for which traits and (B) vector view of TT biplot, showing the interrelationship among all measured traits of sunflower

Fig. 4. (A) vector view of TT biplot, showing the interrelationship among all treatments and (B) ideal entry view of TT biplot, showing the relationships of treatments with ideal entry in sunflower

Provided that the biplot model described a sufficient amount of the total variation (in this case, 93%), the correlation coefficient between any two traits is approximated by the cosine of the angle between their vectors (Yan & Rajcan 2002). The correlation coefficients among the traits indicate that the biplot currently shows relationship among the traits that had relatively large loading on both PC1 and PC2. Therefore, the most prominent relations by Fig. 3B are: a strong positive correlation between RDW and SDW; between SL and PI; and between GP and EG; as indicated by the small obtuse angles between their vectors ($r = \cos 0 = +1$). The correlations between RDW and GR; RDW and MGT; and T50 and GR were near zero (Fig. 3B) as indicated by the near perpendicular vectors ($r = \cos 90 = 0$). There was a negative correlation between GR and MGT, and between RDW and T50 (Fig. 3B) as indicated by the near angle of approximately 180 degrees ($r = \cos 180 = -1$). Most of the mentioned results can be verified using original correlation coefficients, but less of them can not be verified using original correlation coefficients, because the biplot accounted for <100% of the total variation (93%). The statistical properties of this method have been described in detail by Yan *et al.* (2000) and Yan & Rajcan (2002).

Similar to tester vector-view biplot, the most prominent relations by entry vector-view biplot (Fig. 4A) are: a strong positive correlation between T2 and T3 as indicated by the small obtuse angles between their vectors ($r = \cos 0 = +1$). The correlation between T2 and T7 was near zero (Fig. 4A) as indicated by the near perpendicular vectors ($r = \cos 90 = 0$). There was a negative correlation between T2 and T6, and between T3 and T7 (Fig. 4A) as indicated by the near angle of approximately 180 degrees ($r = \cos 180 = -1$). An ideal entry (treatment) has been defined as the entry that combines several suitable traits in its characteristics. Based on Fig. 4B, the ideal treatment is strongly related to as T2 and T3 treatments while treatment T6 following to treatments T7 and T5 were not suitable. Therefore, it seems that application of SiO₂ nano-particles in low concentration (0.2 and 0.4 mM) would be more effective than moderate or high concentrations. Maybe, in high concentrations of SiO₂ nano-particles, the toxicity of nano-particles is more effective than its improving property.

Due to great importance of germination percentage (GP) in crop establishment of sunflower, this trait was compared with the other traits and visually displayed in Fig. 5. In the context of TT biplot, the GP was determined as the best trait, which showed the above average performance, was EG following to PI and GR. The other remaining traits including SL > SDW > RDW > T50 > MGT indicated below performance (Fig. 5). Therefore it seems that, in germination percentage characteristic, energy of germination, promptness index and germination rate are important. In other word, the rate and power of seed germination are greatly related and influenced the germination percentage in sunflower.

Fig. 5. Tester comparison view of TT biplot showing the relationships of traits with germination percentage (GP) in sunflower

DISCUSSION

Nanotechnology has emerged as a new field, and nano-particles have become a centre of attraction due to their unique properties compared to bulk materials. The SiO_2 nano-particles could be acts as a delivering agent into plants but the mechanism of their action on plants is still scarce. Seed germination prepares a proper foundation for plant growth and yield performance. In the present study application of SiO_2 nano-particles enhanced seed potential by increasing the related traits or parameters of seed germination in sunflower at low concentrations. Among the treatments, application of 0.2 and 0.4 mM of SiO_2 nano-particles proved best by giving the highest values for most of the seed germination parameters. Also, our findings revealed that seed soaking in SiO_2 nano-particles solution resulted in vigour enhancement. These results confirmed the pervious study on tomato germination by utilization of nano-silicon dioxide (Siddiqui & Al-Whaibi 2014).

This research demonstrated that TT biplot is a useful tool for the analysis of sunflower dataset. Based on TT biplots, the similarities and differences among traits and treatments and the similarities among the treatments from traits aspects can be visualized. The results of analysis by the TT biplot showed that

treatments T2 and T3 showed better performance than other treatments tested in this experiment. These two treatments, in the present investigation, identified as the most consistent-performing treatments. The use of these treatments would be ensuring high performance in germination of sunflower with application of SiO₂ nano-particles. Similar conclusions we can address about correlations among traits from TT biplot analysis. In germination percentage, some traits including energy of germination, promptness index and germination rate were important and influenced the germination percentage in sunflower from the rate and power of seed germination aspects. The GT biplot analysis was shown to be more informative than conventional methods for distinguishing special features in treatments and this statistical method was found to be proper for test of treatments.

It appears that silicon has an important role in plant protection against biotic and abiotic stress (Ma 2004) and it has been reported that silicon application could alleviate the adverse effects of salinity stress on seed germination (Haghighi *et al.* 2012). The faster and synchronized seed germination might be attributed to enhanced metabolic activities in the primed achenes (Wahid *et al.* 2008). In the arid and semi-arid regions which are adverse with abiotic stresses, the rate or entirely hinder seed germination and seedling emergence in late spring and early summer are decreased. In this respect, the application beneficial nano-particles for pre-sowing treatments and utilization of seeds with enhanced vigor can be a practicable strategy to obtain healthy seedlings and a better crop stand under unfavorable environmental conditions.

It is clear that the TT biplot is an excellent statistical tool for visual data analysis. Compared with conventional statistical methods of data analysis, the TT biplot approach has some advantages including: (i) the graphical presentation of the dataset which greatly enhances researcher ability to understand the pattern of the data; (ii) the more interpretability which is facilitates pair-wise genotype comparisons; and (iii) facilitating the identification of possible sectors or groups of testers or entries (Yan & Hunt 2001; Yan *et al.* 2007). A potential constraint of the biplot method is that it may fail to explain most of the variation and therefore fail to display all patterns of the data. This is most likely to occur with large datasets, but in our dataset 93% of total variation is explained by the two first PCs and this constraint was not observed. However, even when this is the case, it can be ensured that the biplot of PC1 versus PC2 still displays the most important linear patterns of the data (Yan and Hunt 2001). Also, other types of biplots, such as one consisting of PC3 versus PC4, may be required to fully understand the dataset's pattern.

Result emphasized that controlled imbibition of sunflower seeds in SiO₂ nano-particles solution followed by dehydration could significantly enhance germination potential. It appears that treated seeds usually would exhibit rapid germination when absorb water under field conditions where the time from sowing to seedling establishment is of considerable importance. According to the results,

it seems that the SiO₂ nano-particles with the increased penetration power of the seed facilitates the entry of water and oxygen into the cell and that with increasing absorption of nutrients in the seed, thus invigorated seeds can germinate very quickly and produce stronger seedlings. However, further research should be done to investigate the interaction mechanism between SiO₂ nano-particles and seed in cellular and molecular levels.

CONCLUSION

In conclusion, obtained results revealed that the application of SiO₂ nano-particles enhanced seed germination potential in sunflower. An increase in germination parameters by the application of low concentrations of SiO₂ nano-particles may be effective for the growth and yield of crops. Also, the most important traits were germination percentage, energy of germination, promptness index and germination rate which are reflect the rate and power of seed germination and seedling growth in sunflower.

ACKNOWLEDGMENTS

We wish to thank Dr. Wei-Kai Yan (Eastern Cereal Oilseed Research Center of Agriculture and Agri-Food, Canada) for making available a time-limited version of GGEbiplot as “testBiplotxlsx”.

REFERENCES

- AOSA (Association of Official seed Analysis). 1991. Rules for testing seeds. *Seed Science and Technology* 12: 18–19.
- Ashraf M., Foolad M. R. 2005. Pre-sowing seed treatment—a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Advances in Agronomy* 88: 223–271.
- Ashraf M.Y., Akhtar K., Hussain F., Iqbal J. 2006. Screening of different accessions of three potential grass species from Cholistan desert for salt tolerance. *Pakistan Journal of Botany* 38: 1589–1597.
- Bao-Shan L., Shao-Qi D., Chun-Hui L., Li-Jun F., Shu-Chun Q., Min Y. 2004. Effect of TMS (nanostructured silicon dioxide) on growth of Changbai Larch seedlings. *Journal of Forestry Research* 15: 138–140.
- Basra S.M.A., Farooq M., Wahid A., Khan M. B. 2006. Rice seed invigoration by hormonal and vitamin priming. *Seed Science and Technology* 34: 753–758.
- Dehghani D., Omid H., Sabaghnia N. 2008. Graphic analysis of trait relations of canola (*Brassica napus* L.) using biplot method. *Agronomy Journal* 100: 760–764.
- Ellis R. H., Roberts E. H. 1981. The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology* 9: 373–409.
- FAOSTAT. 2012. FAOSTAT. Food and Agricultural Organisation of the United Nations. Available at: <http://faostat.fao.org>.
- Farooq M., Basra S.M.A., Hafeez K., Ahmad N. 2005. Thermal hardening: a new seed vigour enhancement tool in rice. *Acta Botanica Sinica* 47: 187–193.
- Haghighi M., Afifpour Z., Mozafarian M. 2012. The effect of N–Si on tomato seed germination under salinity levels. *Journal of Biological and Environmental Science* 6: 87–90.
- Harris D., Joshi A., Khan P.A., Gothkar P., Sodhi P. S. 1999. On-farm seed priming in semi-arid agriculture: development and evaluation in maize, rice and chickpea in India using participatory methods. *Experimental Agriculture* 35: 15–29.

- Iqbal M., Ashraf M. 2007. Seed treatment with auxins modulates growth and ion partitioning in salt-stressed wheat plants. *Journal of Integrative Plant Biology* 49: 1003–1015.
- Liang Y., Sun W., Zhu Y.G., Christie P. 2007. Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. *Environmental Pollution* 147: 422–428.
- Ma J. F. 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science and Plant Nutrition* 50: 11–18.
- Ma J. F., Yamaji N. 2008. Functions and transport of silicon in plants. *Cellular and Molecular Life Sciences* 65: 3049–3057.
- Mijic A., Liovic I., Zdunic Z., Maric S., Jeromela A. M., Jankulovska M. 2009. Quantitative analysis of oil yield and its components in sunflower (*Helianthus annuus* L.). *Romanian Agricultural Research* 26: 41–46.
- Nair R., Poulouse A. C., Nagaoka Y., Yoshida Y., Maekawa T., Kumar D. S. 2011. Uptake of FITC labeled silica nanoparticles and quantum dots by rice seedlings: effects on seed germination and their potential as biolabels for plants. *Journal of fluorescence* 21: 2057–2068.
- Ranal M. A., Santana, D. G. D. 2006. How and why to measure the germination process?. *Brazilian Journal of Botany* 29: 1–11.
- Ruan S., Xue Q., Tylkowska K. 2002. The influence of priming on germination of rice (*Oryza sativa* L.) seeds and seedling emergence and performance in flooded soil. *Seed Science and Technology* 30: 61–67.
- Rubio J., Cubero J.I., Martín L.M., Suso M.J., Flores F. 2004. Biplot analysis of trait relations of white lupin in Spain. *Euphytica* 135: 217–224.
- Sabaghnia N., Dehghani H., Alizadeh B., Moghaddam M. 2011. Yield analysis of rapeseed (*Brassica napus* L.) under water-stress conditions using GGE biplot methodology. *Journal of Crop Improvement* 25: 26–45.
- Sabaghnia N., Janmohammadi M. 2014. Graphic analysis of nano-silicon by salinity stress interaction on germination properties of lentil using the biplot method. *Agriculture and Forestry* 60: 29–40.
- Siddiqui M. H., Al-Whaibi M. H. 2014. Role of nano-SiO₂ in germination of tomato (*Lycopersicum esculentum* seeds Mill.). *Saudi Journal of Biological Sciences* 21: 13–17.
- Wahid A., Noreen A., Basra S. M., Gelani S., Farooq M. 2008. Priming-induced metabolic changes in sunflower (*Helianthus annuus*) achenes improve germination and seedling growth. *Botanical Studies* 49: 343–350.
- Yan W. 2001. GGEbiplot—A Windows application for graphical analysis multi-environment trial data and other types of two-way data. *Agronomy Journal* 93: 1111–1118.
- Yan W., Hunt L.A., Sheng Q., Szlavnics Z. 2000. Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Science* 40: 597–605.
- Yan W., Kang M S., Ma B., Woods S., Cornelius P.L. 2007. GGE biplot vs. AMMI analysis of genotype-by-environment data. *Crop Science* 47: 643–655.
- Yan W., Rajcan I. 2002. Biplot evaluation of test sites and trait relations of soybean in Ontario. *Crop Science*, 42, 11–20.
- Yan, W., Hunt L. A. 2002. Biplot analysis of diallel data. *Crop Science* 42: 21–30.