10.17951/c.2017.72.2.15-27

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. LXXII, 2 SECTIO C 2017

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Genotypic response of barley to exogenous application of nanoparticles under water stress condition

ABSTRACT

Beneficial nanoparticles (SiO_2 and TiO_2) can have various profound effects on the crop physiological, biochemical and morphological characteristics. Here, we evaluated the mitigation of drought stress in barley genotypes by foliar application of SiO_2 and TiO_2 nanoparticles under filed condition in North West of Iran. Nanoparticles were foliar applied in late vegetative phase and during reproductive stages. Drought was imposed at by irrigation withdrawals during the dry months in the end of the growing season. We measured parameters related morphological growth, yield, and yield component. The genetic diversity between the genotypes was quite evident and the highest seed yield and yield component were recorded for G1, G2, G4, G11, G12 and G13. Foliar application of nanoparticles considerably affected the plant height, thousand seed weight, biological and seed yield. The best performance was observed for plant treated with SiO_2 nanoparticles. Spike length of G2, G6, G13 and G20 considerably responded to nano silicone foliar application. However, the best results for G8, G11 and G20 were obtained by foliar application TiO_2 nanoparticles while this treatment decreased the seed yield components in G1, G5, G9, G10, G15 and G20. This could be due to genetic variation between the evaluated genotypes and high sensitivity of some genotypes to the applied concentration. The results of current study showed that application of SiO_2 nanoparticles under water stress condition could have more beneficial effects on yield component of barley genotypes.

Keywords: alleviating drought stress, correlation, nano-silicon dioxide, TiO_2 nanoparticles, yield component

INTRODUCTION

Barley (*Hordeum vulgar* L.) is one of the most important crops in irrigated and rainfed areas and it is one of the four important cereals of the world. Despite the high importance of wheat, barley in semi-arid regions is strategic crop and this is partly due to higher drought resistance in the barley plant (27).

According to FAO, cultivated area of barley in Iran during 2016 growing season was about 1.61 million hectares and harvested production was estimated at 2.90 million tons, which is equivalent to 65 percent of domestic demand (3). Barley is a dual-purpose plant and it is very important for livestock industry. These statistics indicate that there are still steps to self-sufficiency in the production of barley. The importance of this issue becomes even greater when it has been recognized that its forage shortages are very evident in North West semi-arid areas of Iran. The major part of the barley cultivated area (60%) is rainfed (1). Water is a major restraining aspect for the world's economy because of its reducing quality and quantity and changes in distribution (22).

Food security for the hundreds of millions of rural poor necessitates improved crop productivity through breeding for enhanced drought tolerance (21). Furthermore, the importance of agronomic management in improving production in extreme conditions should not be ignored, so that drought stress is limiting factor in semi-arid region. Droughts stress can be categorized into meteorological drought, hydrological drought and agricultural drought. Time-series of annual rainfall, number of rainy days per year and monthly rainfall in semi-arid region are very variable (20). The occurrence of all three stresses is possible in terms of dry periods. However, in most of the years, due to reduced rainfall during reproductive growth, rising temperatures, and increasing amount of evapotranspiration of the barley plants experience terminal drought and heat stress. Terminal drought of varied intensities is, therefore, a primary constraint to barley productivity.

Nanoparticles are proposed to be the materials for the new millennium. Nanoagriculture involves the employment of nanoparticles in agriculture. These particles will impart some beneficial effects to crops. The emergence of nanotechnology and the development of new nanodevices and nanomaterials open up potential novel applications in agriculture and biotechnology (6, 8, 10, 23). However, with the advancement of science the ability to construct and manipulate materials at the nanoscale has increased dramatically in the last decade.

Although it seems that there is a widespread potential of nanoparticles applications in agricultural sector, nanoparticles are still unexplored, especially their mechanism and role on plant growth and development (24). Despite the prominence of Si as a mineral constituent of plants, Si is not considered as "essential" nutrient, for any terrestrial higher plants (2, 18). The beneficial elements are not deemed essential for all crops but may be vital for particular plant taxa. Silicon (Si) has not been proven to be an essential element for higher plants, but its beneficial effects on growth have been reported in a wide variety of crops, including rice, wheat, barley (11, 16). In plants, silicon is deposited in cell walls in the form of amorphous silica (SiO_2-nH_2O) and enhances cell wall rigidity and strength, interacting with pectins and polyphenols. Marschner (18) demonstrated that Si4+ is deposited in epidermal cells of leaves, hence improving leaf exposure to light by keeping leaves more erect; in roots, it increases cell elongation thus enhancing cell wall elasticity. As the beneficial effect of silicon has been proved as shown above, the application of nanosilicon can be more effective than the large applied particles, which means a more efficient input use (26). Si as a physical-mechanical barrier can prevent penetration of pesticides or pathogens into the plant cell. Silicon can also deposit on the walls of epidermis and vascular tissues of the stem and leaf surface in most plants, especially monocots, and also controls physiological properties of plants (9). Considering the increasing importance of nanoscience in agriculture and the remarkable effects of beneficial nanoparticles such as nano-SiO_2, evaluating their effect on plants is very important.

In this regard, there is not much information in the literature indicating whether nano-SiO_2 application may have similar beneficial effects on barley genotypes under drought stress. This information will help scientists choose genotypes that are more tolerant to hydric deficiency for breeding.

The present study was carried out to evaluate exogenous application of nano-SiO_2 and nano-TiO_2 on the yield and yield components of barley genotypes under water stress condition in northwest of Iran.

MATERIALS AND METHODS

Field experiments were carried out at the Research Farm of the Moghan College of Agriculture and Natural Resources, Parsabad, Ardabil, Iran, during the growing season of 2016.The field was located at 46°46' east longitude and 39°36' north latitude, at an altitude of 32 meters above the sea level. Based on Koppen's classification, this region has a semi-arid temperate climate. Moghan has warm and humid summers and temperate winters with dry winds and a short freezing period. Top 0–30 cm soil samples were randomly collected from field and analyzed for physicochemical properties. The soil type was clay loam, pH 7.22 and EC 2.35 $dS.m^{-1}$, organic matter 0.85%, potassium 306.4 mg kg^{-1}, phosphorous 15.8 mg kg^{-1}. Moghan is located in the plain of the Aras River and its level areas with deep, well-drained soils, and cropping in this region is most reliable. The mean annual temperature was 15 °C while the mean maximum and minimum temperatures were 31.4 and 1.4 °C, respectively. The mean temperature during the growth season was 21°C and average annual rainfall was about 335 mm.

The germplasm of 20 genotypes was studied in the current experiment. Genotypes were obtained from Seed and Plant Improvement Institute (SPII), Iran and they were differing in growth and morphological characters. The experiment was laid out as factorial (3×20) based on Randomized Block Design with three replications. The first factor was foliar treatment included control (Check; no treatment), nano-Ti (20 ppm) and nano-Si (20 ppm). The second factor was twenty genotypes of barley. Each genotype was sown in a double row with spaces apart at 30 v 10 cm in a plot of 5×3 m^2 size. The middle two rows were used for data collection. The field was mouldboard-ploughed and twice disked before seed sowing. After primary and secondary tillage, planting was done by hand drilling using a seed rate of 80 kg/ha for each variety at the second week of November. Nitrogen and phosphorous fertilizers were applied at the rate of 100 kg/ha urea and100 kg/ ha triple superphosphate at planting. All other management practices were uniformly applied to all plots at planting. There was no incidence of pest or disease on plants during the experiment. Weeds were controlled by systemic selective chlorophenoxy herbicides including 2, 4-D and MCPA. Plants were grown under rainfed condition that received natural rainfall.

The nano-SiO_2 and TiO_2 were procured from Nano-Pishgaman (Iran). The shape of SiO_2 and TiO_2 nanoparticles was spherical. Their average size and purity were 100 nm and 99.5%, respectively. Characterization of SiO_2 and TiO_2 nanoparticles by Scanning electron microscopy (TSCAN, Czech Republic) image (SEM) are shown in Figure 1. NPs of TiO_2 and SiO_2 solutions were prepared at concentrations of 20 ppm with filtered, double-distilled water. Working solutions were made by vigorous vortexing (using ultrasonic) before the applications. Foliar application was carried out during leaf development (BBCH-scale=17; seven leaves unfolded), tillering (BBCH-scale=21; beginning of tillering: first tiller detectable), stem elongation (BBCH-scale=34; node 4 at least 2 cm above node 3) and heading (BBCH-scale=55; middle of heading: half of inflorescence emerged).

Genotypic and phenotypic correlation coefficients of yield with the contributing characters and among themselves were calculated by using the genotypic and phenotypic variances and covariances as described by Singh et al. (25).

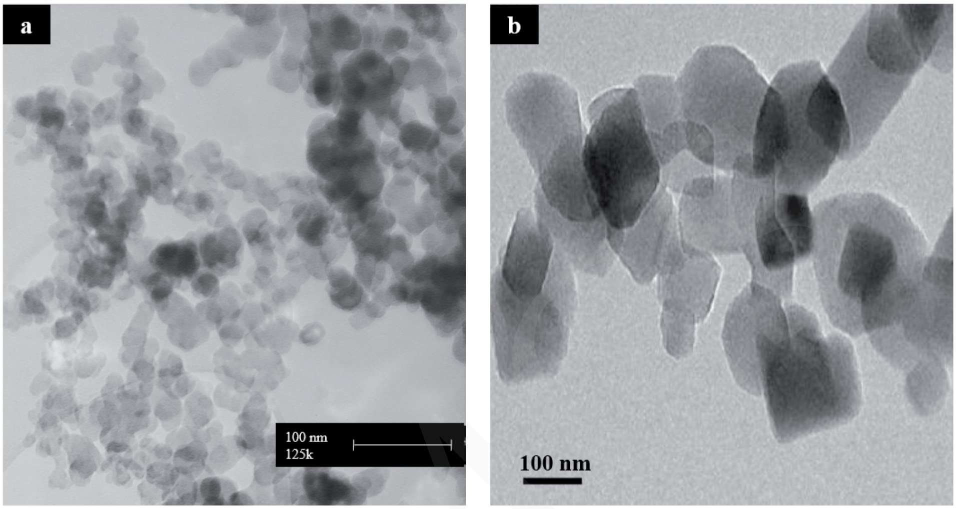

Fig. 1. Images of scanning electron microscopy (SEM) from silicon dioxide (a) and titanium dioxide (b) nanoparticles.

At fully ripe stage (BBCH-scale=89; grain hard, difficult to divide with thumbnail (19), yield components and morphological traits, such as plant height, number of fertile tillers, straw mass, spike length, number of grains/spike, 1000-seed weight and seed yield, were evaluated. The data were analyzed using the SAS statistical software. Statistical significance was accepted when the probability of the result assuming the null hypothesis, p is less than 0.05 (level of probability). Correlation analysis and principal component analysis (PCA), based on the rank correlation matrix and biplot analysis were performed by SPSS ver. 16, STATISTICA ver. 8 and Minitab ver. 16.

RESULTS AND DISCUSSION

The results of variance analysis revealed that there are significant effects of nanoparticles spray and genotypes on plant height (PH). Mean comparison of PH between foliar treatments showed that nanoparticles spray increased this trait by 14% over control (Table 1). The highest PH was recorded for G11, G15, G17, G18, G19 and G7 (Table 2). The interaction effect of nanoparticles spray × genotypes was significant for spike length ($P < 0.01$). Although the application of nanoparticles, especially SiO_2, caused significant increase in spike length in most genotypes, spray of nano-TiO_2 significantly reduced spike length in G3, G4, G5, G18 and G12 (Fig. 2). Number of tillers and number of fertile tillers significantly was different between the evaluated genotypes and the highest tiller number was recorded for G11, G2, and G17. Furthermore, the comparison between total tiller and fertile tiller number revealed that G6 and G9 had the highest survival rate for produced tillers. The main effect of the nanoparticles spray and genotypes as well

Table 1. The effect of foliar spray of nano-SiO_2 and nano-TiO_2 on some yield components of barley (*Hordeum vulgare* L.) genotypes under water stress condition

	PH	SL	TN	FTN	TSW	SNP	SWP	STY	BY	SY
Control	53.00a	5.87a	2.89a	2.23a	32.45b	55.20a	1.58ab	5448.9a	7567.2ab	2118.3b
Nano-Si	60.00a	5.87a	2.85a	2.19a	33.50a	55.46a	1.75a	5559.3a	7767.2a	2208.5a
Nano-Ti	61.00a	5.89a	2.83a	2.16a	31.34c	56.30a	1.25b	5237.0a	7320.8b	2083.8b
Treatment	*	NS	NS	NS	**	NS	*	NS	*	*
Genotype	**	**	**	**	**	**	**	*	**	**
G×T	NS	**	NS	NS	**	NS	*	NS	NS	*

PH: plant height (cm), SL: spike length (cm), TN: tiller number, FTN: fertile tiller number, TSW: 1000-seed weight (g), SNP: seed number per plant, SWP: seed weight per plant, STY: straw yield (kg ha^{-1}), BY: biological yield (kg ha^{-1}), SY: seed yield (kg ha^{-1}). G: genotype, T: foliar treatment. NS = Not significant, *= Significant at 5% level of probability, ** = Significant at 1% level of probability. Means followed by the same lower case letters in a column and capital letters on the lines do not differ significantly to the level of 5% probability.

as their interaction effects was significant on 1000-seed weight at the 0.01 level (Table 1). Although SiO_2 spray increased the grain weight by about 4%, foliar application of TiO_2 reduced this component by 3%. Foliar spray, especially SiO_2, significantly increased the seed weight in G4, G10, G14, G16, G17, and G20. However, the foliar application of nanoparticles reduced the seed weight in G2, G3, G5, G7, and G8 (Fig. 3).

ANOVA showed that seed number per plant (SNP) was not affected by foliar treatments. However, there were significant differences between SNP of genotypes and the highest SNP was recorded for G6, G7, G8, G14, G16, G17 and G19. Evaluation of seed yields revealed that the main effect of the nanoparticles spray and genotypes as well as their interaction effects were on this trait (Table 1). Mean comparison of seed yield between the foliar treatment showed that the best performance was obtained by nano-SiO_2. However, the response of genotypes seed yield was significantly different against the foliar treatments. The highest seed yield was recorded for G8 under nano-TiO_2 foliar application. However, other genotypes like G1, G4, G2, and G12 produced the highest seed yield under nano-SiO_2 foliar application (Fig. 4).

Cluster analysis divided the genotypes into four clusters (Fig. 5). The first group included G1, G2, G4, G6, G7, G8, G9, and G10, where the stimulating effect of nanoparticulate treatments, especially nano-SiO_2, was somewhat evident. The second cluster included G5, where the foliar spray of nano-TiO_2 significantly

Table 2. Mean comparison of seed yield components between the barley (Hordeum vulgare L.) genotypes under rainfed condition in north-west of Iran

	PH	SL	TN	FTN	TSW	SNP	SWP	STY	BY	SY
G1	76.02ab	5.32efgh	3.25bcd	2.31bcd	35.47ab	50.22bc	1.78abcde	4161hij	6931fgh	2770a
G2	7398b	5.02h	3.35abc	2.45ab	34.93ab	51.66bc	1.80abcd	3832ij	6472gh	2640ab
G3	77.33ab	5.25gh	2.88cde	2.10bcde	33.48bcde	49.33c	1.64bcde	5105fg	7297efg	2192def
G4	73.65b	5.63defg	2.84de	2.17bcde	34.15abc	54.11bc	1.85abcd	4612hij	7359efg	2747a
G5	76.67ab	6.11bcd	2.54e	2.06cde	30.32fgh	56.66bc	1.71abcd	2267k	3637j	1370j
G6	77.66ab	6.22bc	2.40e	2.12bcde	30.94efgh	59.11abc	1.82abcd	3323j	5340i	2016efg
G7	82.08ab	6.33b	2.92cde	2.36abcd	29.98fgh	68.00a	2.03ab	3954hij	5934hi	1980fg
G8	73.26b	5.98bcde	2.75e	2.14bcde	31.74cdefgh	59.12abc	1.88abc	4157hij	6343gh	2185def
G9	74.77ab	5.84bcdef	2.48e	2.11bcde	33.00bcdef	52.44bc	1.73abcde	4776fghi	6746fgh	2065efg
G10	81.83ab	5.55defgh	2.82de	2.32bcd	29.05h	48.66c	1.42e	5419fg	7124efg	1705i
G11	87.31a	5.77cdefg	3.80a	2.66a	33.73bcd	57.77abc	1.95abc	7987a	10573a	2585ab
G12	79.82ab	5.50efgh	3.35abc	2.37abc	34.99ab	54.44bc	1.90abc	4814fgh	6579gh	1765hi
G13	78.16ab	6.00bcde	2.64e	2.06cde	36.89a	56.88bc	2.10a	7610abc	9777ab	2165def
G14	77.38ab	5.70cdefg	2.68e	2.15bcde	34.89ab	57.22abc	2.00abc	7093bc	9589bc	2495bc
G15	83.82ab	5.93bcde	2.55e	1.92e	30.00fgh	48.66c	1.45de	4809fgh	6442gh	1632i
G16	81.40ab	6.98a	2.47e	1.94e	30.52efgh	59.66abc	1.83abcd	7649abc	9887ab	2238de
G17	81.55ab	6.14bcd	3.51ab	2.38abc	29.18gh	61.33ab	1.73abcde	6841cd	8784cd	1942gh
G18	84.55ab	6.00bcde	2.68e	2.21bcde	32.31bcdef	56.11bc	1.79abcde	5519ef	7677ef	2157defg
G19	80.66ab	6.22bc	2.46e	2.01de	32.95bcdef	57.33abc	1.89abc	7836ab	10167ab	2331cd
G20	74.32b	6.12bcd	2.73e	2.03cde	30.13fgh	53.77bc	1.60cde	6236de	7984de	1748ih

PH: plant height (cm), SL: spike length (cm), TN: tiller number, FTN: fertile tiller number, TSW: 1000-seed weight (g), SNP: seed number per plant, SWP: seed weight per plant, STY: straw yield (kg ha^{-1}), BY: biological yield (kg ha^{-1}), SY: seed yield (kg ha^{-1}). Means followed by the same lower case letters in a column and capital letters on the lines do not differ significantly to the level of 5% probability.

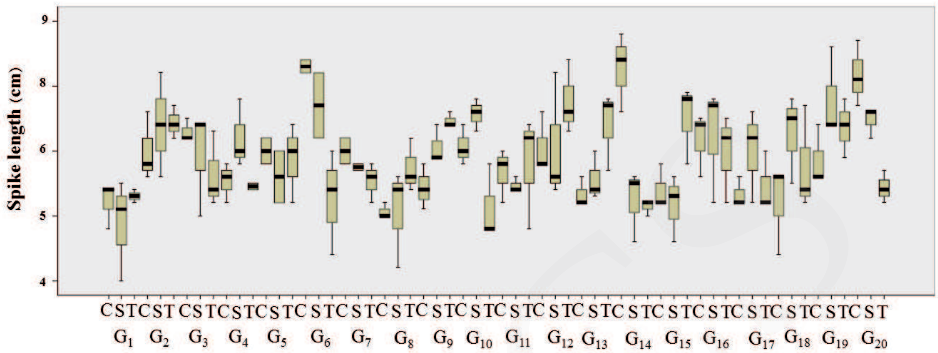

Fig. 2. The effect of beneficial nanoparticles (C: control, S: nanosilicon dioxide and T: nanotitanium dioxide) on spike length of barley genotypes under rainfed condition in northwest of Iran. Vertical bar is SE and the dark line present the mean value of replications.

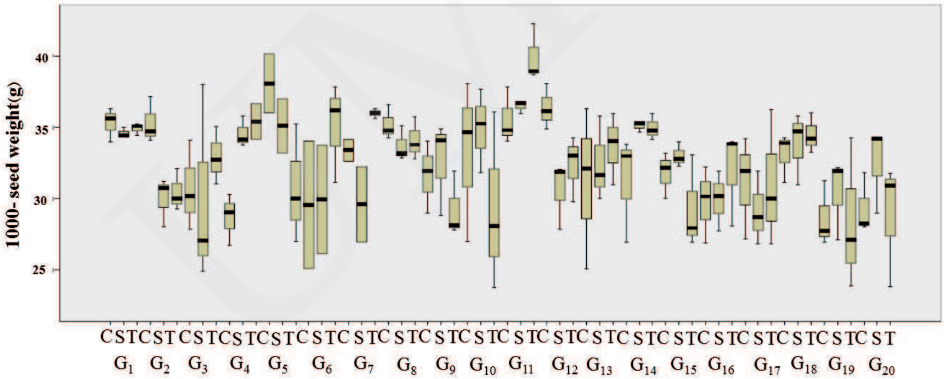

Fig. 3. The effect of beneficial nanoparticles (C: control, S: nanosilicon dioxide and T: nanotitanium dioxide) on 1000-seed weight of barley genotypes under rainfed condition in northwest of Iran.

decreased most of the traits. The third group includes G3, G12 and G15, where the effect of nano-SiO_2 on the growth characteristics and seed yield was very poor and in some cases foliar treatments were ineffective. The fourth group includes G11, G13, G14, G16, G17, G18, and G19, where the effect of silicon was greatly positive and the titanium effect was negligible (Fig. 5).

A number of central physiological processes contribute to the formation of grain in crops. Major ones are photosynthesis and the translocation of photosynthate to the grain, cell division and enlargement, and the accumulation and transport of nutrient elements for storage in the grain and for the general functioning of cell metabolism. These processes must occur during the appropriate stages of development, and consequently the timing of each contribution is important.

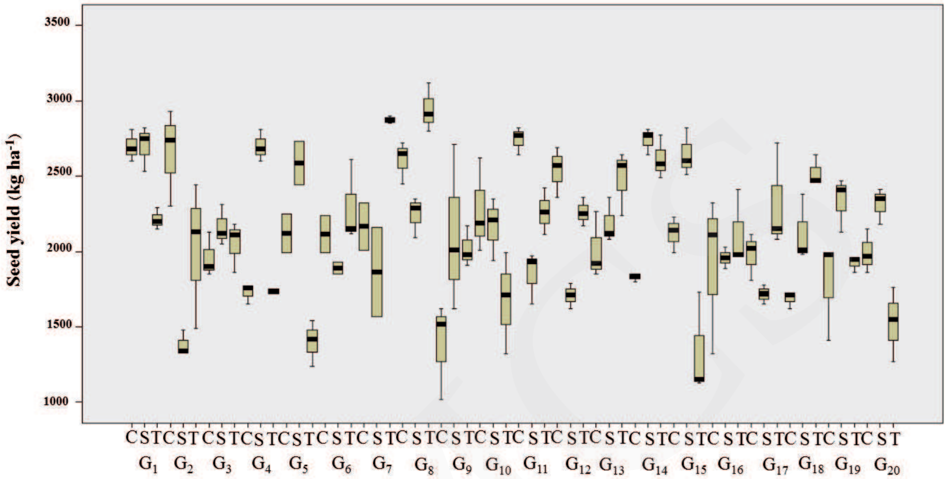

Fig. 4. The effect of beneficial nanoparticles (C: control, S: nanosilicon dioxide and T: nanotitanium dioxide) on seed yield of barley genotypes under rainfed condition in northwest of Iran.

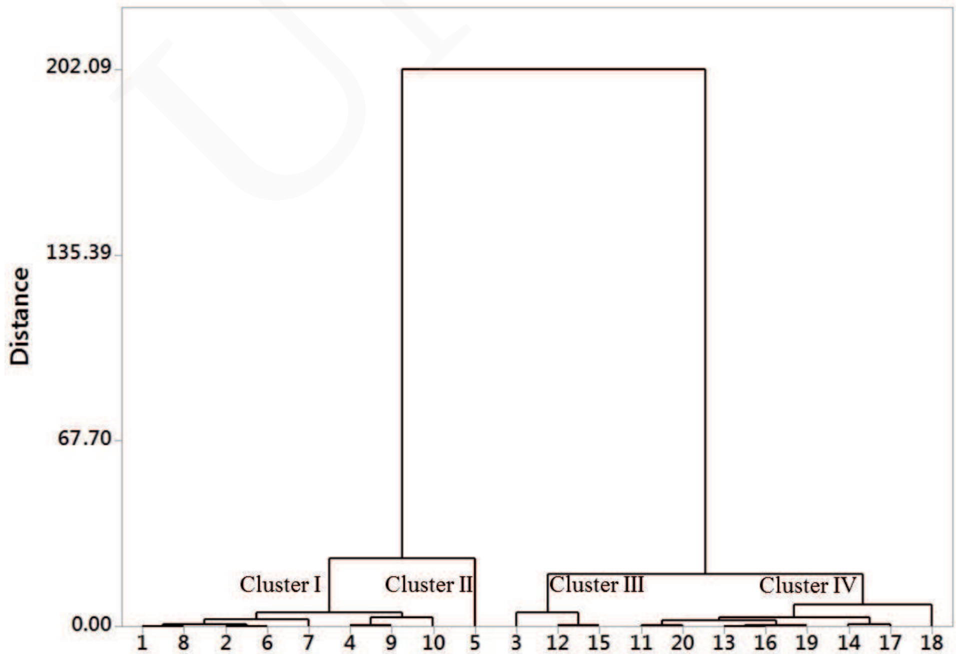

Fig. 5. Cluster analysis of agronomic traits of barley (*Hordeum vulgare* L.) genotypes according to similarity in response to foliar application of silicon and titanium nanoparticles under rainfed condition in northwest of Iran.

Superimposed on this set of circumstances is the suitability of the environment for supplying light, water, and nutrients for the completion of each stage of growth (28). Some of the beneficial effects of nanoparticles, especially silicon, on plant regeneration can be attributed to the increase and excitation of defensive systems under abiotic stress conditions. Current experiment has been carried out under rainfed condition in semi-arid region, where plants are faced with terminal drought stress. Water content is an important property of soils, influencing soil solution chemistry and nutrient uptake by plants. Morphology and other specific properties of the root, nutrient concentration in the soil solution, the mobility of nutrients in the soil, and supply from solid phases, affect nutrient uptake. Therefore, when the terminal dry period and water stress emerged, vitality of plants was weakened, their growth reduced, and mortality increased. Drought stress, as a multidimensional abiotic stress, strongly effects growth, development and yield of plants. Under drought condition, the plants initiate two strategies for survival – avoidance or tolerance; the strategies include morphological and/or physiological adjustments. Finding resistance genotypes to biotic and abiotic stress is very important for plant research. The role of silica in plants under stress conditions is more pronounced, but there is no report for using of silica as nanoparticles in plant drought stress. Silicon is also known as an anti-stress agent and can reduce cuticle transpiration (17) or increase water efficiency (4).

Likewise, foliar application of nanoparticles can also increase the plant growth and seed yield through improving the function of photosynthetic apparatus (source activities) and photo-assimilate translocation. These results are consistent with those of other studies and suggest that the application of Si may represent an approach to improve the growth of this crop and increase its production in arid or semi-arid areas where water is at a premium; this technique, however, would not fully substitute for an adequate water supply (7, 12).

Improvement of plant growth by foliar application of nano-SiO_2 and nano-TiO_2 can be due to modification of source and sink relationships. **Sources** are plant organs such as leaves that produce sugars. **Sinks** are plant organs such as roots, bulbs (swollen leaves) and filling seeds that consume or store sugars. It has been revealed that SiO_2 and TiO_2 play a role in enhancement of source size by increasing the photosynthesis rate, increasing the strength of leaves, chlorophyll concentration per leaf area and leaf area duration (5, 14). On the other hand, the activity and size of the reservoir are largely influenced by the proportion of phytohormones. In this case it has been showed that Si reduced endogenous concentration of jamonic acid (JA) and salicylic acid (SA), while abscisic acid (ABA) first increased and then decreased two weeks after exogenous application (13). However, it was recently demonstrated that Si increases cytokinin biosynthesis in *Sorghum* and *Arabidopsis* and that such an increase may strongly contribute to delay of senescence (15). Phytohormones communications are responsible for a complex biochemical and physiological network and a deep understanding of nanopartices influence on hormonal properties can facilitate the breeding process.

This also accords with our earlier observations, which showed that foliar application nano-SiO_2 improved canopy spread, ground cover, number of capitula in main branch and accelerated canopy closure; however, it did not significantly affect the achene yield under different fertilizer system (6, 8).

In addition, the principle component analysis (PCA) described a suitable amount of the total variation; the correlation coefficient between any two traits is approximated by the cosine of the angle between their vectors. In Figure 6, the most prominent relations are: a strong positive association among seed, tiller number, fertile tiller number and 1000-seed weight as indicated by the small obtuse angles between their vectors (r =cos 0=+1). There was no correlation between plant height, spike length and seed as indicated by the near perpendicular vectors (r =cos 90=0). This state also was confirmed by correlation curve for phenotypic and genotypic characters (Fig. 7).

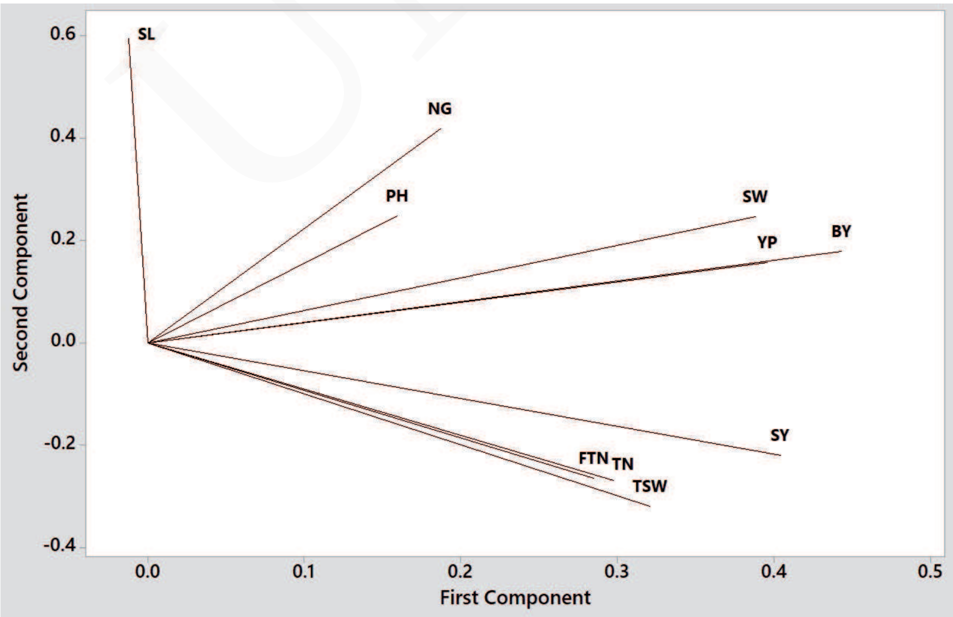

Fig. 6. The principle component analysis (PCA) for morphophysiological traits of barley (*Hordeum vulgare* L.) genotypes under rainfed condition in northwest of Iran. PH: plant height, SL: spike length, TN: tiller number, FTN: fertile tiller number, TSW: 1000-seed weight, SNP: seed number per plant, SWP: seed weight per plant, BY: biological yield, SY: seed yield.

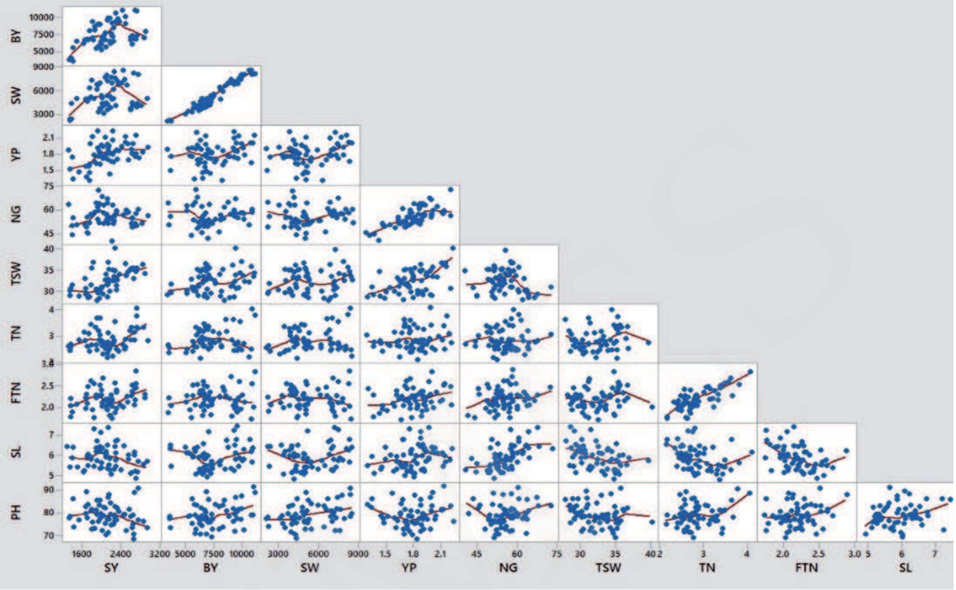

Fig. 7. Phenotypic and genotypic correlation between different characters in barley through the curve. PH: plant height, SL: spike length, TN: tiller number, FTN: fertile tiller number, TSW: 1000-seed weight, SNP: seed number per plant, SWP: seed weight per plant, biological yield, SY: seed yield

CONCLUSION

Crop plants in semi-arid region are faced with different challenge such as drought and heat stress. However, responding to these and other challenging issues in these regions will require new, impactful technologies. In current study we evaluated that possibility to exogenous use of nano-scaled beneficial elements on barley genotypes in semi-arid region. Nanotechnology is one of the exciting new fields of research that holds great promise in addressing many of the pressing needs in the food and agriculture sectors. Nanomaterials typically have at least one dimension that is in the size range of 1–100 nm. Our results showed that application of nanomaterial significantly affected plant height, seed weight, biological yields and economic seed yield. However, the response of the genotypes against the applied nanoparticles was somewhat different. In general, the stimulating effects of nano-SiO_2 on growth and yield components were much more pronounced than that of nano-TiO_2. Application of nano-TiO_2 in some genotypes caused a significant reduction in the evaluated traits. The most obvious reactions to the use of nano-SiO_2 were recorded for G1, G8, G2, G6, G7, G4, G9, and G10. Findings revealed that application of nano-SiO_2 solutions in semi-arid region can improve barley seed yield and can be introduced as beneficial fertilizer for foliar application.

ACKNOWLEDGMENTS

The authors are grateful for a financial support from the University of Maragheh. We thank and appreciate M. Amini's participation in constructive discussions about the properties of nanoparticles. The authors wish to thank all of those who contributed, collaborated or technically supported field experiments.

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