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Graphical biplot assessment of pre-sowing procedures on the seeds of *Dracocephalum moldavica* L. employing diverse nanoparticles

Naser Sabaghnia[1*],

Mohsen Janmohammadi[1],

Saeed Yousefzadeh[2]

[1] *Department of Plant Production and Genetics, University of Maragheh, Maragheh, Iran*

[2] *Department of Agriculture, Payame Noor University, Tehran, Iran*

Dracocephalum moldavica L., the Moldavian dragonhead, has found its place in folk medicine and has been used to address kidney complaints. Pre-hydration methodologies were administered to dragonhead seeds utilising nano-silicon dioxide at three concentrations: Si1 0 mM as control, Si2 and Si3, 1 and 2 mM, respectively. Concurrently, treatments with nanoparticles of Fe in oxide form encompassed: Fe1 0 mM as control, Fe2 and Fe3, 1 and 2 mM, respectively. A range of parameters, including the germination percentage, weight (fresh and dry) and length of roots and shoots, seed residue dry weight, and root and shoot dry weights were meticulously gauged. The utilisation of the treatment-by-trait biplot facilitated the visualisation of interrelationships of traits and treatments, with the initial two principal components elucidating 80% of the observed variation. The majority of the traits are located in a specific sector of graph with Si2-Fe3 as the optimal pre-hydration treatment. The ideal treatment for eliciting elevated seed germination properties was detected as Si2-Fe3 (1 mM nano-silicon dioxide and 2 mM of Fe oxide nanoparticles) through the ideal entry biplot. Our findings strongly suggest that priming with silicon and Fe in form of nanoparticles holds the potential to expedite both germination and seedling growth in *D. moldavica* L. In summary, the text emphasises the potential benefits of nanoparticle technology in agriculture, underscores the importance of precise experimental optimisation, highlights the objectivity of the biplot, and suggests promising strategies for seed priming in the cultivation of the Moldavian dragonhead.

Keywords: seed germination properties, nano-Fe oxide, nano-silicon dioxide

* Corresponding author: Email: sabaghnia@yahoo.com

INTRODUCTION

The Moldavian balm or the Moldavian dragonhead (*Dracocephalum moldavica* L.), a plant that falls within the Lamiaceae family, has found its place in folk medicine due to its perceived pain-relieving and anti-rheumatic properties (Amirnia et al., 2017). Dragonhead is an aromatic herb popular in culinary applications: it can be used to flavour various dishes, teas, or infusions. The plant has potential for the extraction of essential oils, which can have applications in perfumery, aromatherapy, and various cosmetic products. It produces attractive flowers that are known to attract pollinators, particularly bees (Konarska et al., 2022). This makes it a beneficial plant for supporting pollinator populations and promoting biodiversity. This botanical gem is typically distributed in high-altitude regions in central and northern areas of Iran. Dragonhead is known for its adaptability to different climates, which makes it a versatile plant for cultivation in various geographical locations (Borghei et al., 2024).

In the context of agricultural practices, germination of the seeds of *D. moldavica* L. is a critical process, especially when plants face envFement stresses. Planting seeds early in stressful conditions can accelerate plant growth, potentially helping plants to navigate critical developmental stages more resiliently and avoid the adverse impacts of terminal stresses (Waqas et al., 2021). The challenge of weak plant establishment is a significant factor for overall production. Seed priming, a technique involving the treatment of seeds before sowing, has shown promise in enhancing seed germination. Various seed pre-treating are used via different procedures to improve both pre and after germination reactions. In the case of *D. moldavica* L., these treatments play a crucial role in optimising its growth and development. Seed priming often involves carefully hydrating seeds with controlled amounts of water. This controlled hydration aims to provide the seeds with the necessary moisture for germination while preventing excessive water absorption that might lead to premature germination or other undesirable effects. The goal is to kickstart physiological processes effectively, ensuring a robust start to the plant's life cycle.

The physiological barrier plays a crucial role in plant protection, and, owing to its physiological attributes, the Moldavian dragonhead contributes significantly to this defence mechanism. Fe oxide nanoparticles have been identified for their positive impact on plants, facilitating photosynthesis and enhancing Fe transfer to peanut leaves (Najafi-Disfani et al. 2016). This, in turn, results in increased total Fe concentration and the overall Fe absorption in soybeans (Delkhosh et al. 2011). Another instance of nanoparticle influence involves the treating of sunflower achenes with silicon nanoparticles and their next dehydration process, which has been found to significantly boost seed germination (Gomes-Junior et al., 2022). The advantageous effects observed in primed seeds treated with nano silicon suggest potential benefits for improved sunflower properties. The utilisation of nanosized components has garnered interest among researchers, particularly seed scientists. In the case of the dragonhead, investigating the use of silicon dioxide and Fe oxide in form of nanoparticles becomes essential to enhance germination ability. The current research aims to explore the impact of seed priming with above particle treatments on *D. moldavica* L. The objective is to identify the most promising concentration through the application of the biplot method, shedding light on the intricate relationships between treatments and seed germination traits.

MATERIALS AND METHODS

The seeds underwent priming involving drenching in distinct concentrations of silicon dioxide as nanoform: Si1, 0 mM as control; Si2 and Si3, 1 and 2 mM, respectively. Simultaneously, three concentrations of Fe oxide as nanoform were applied: Fe1, 0 mM as control; Fe2 and Fe3, 1 and 2 mM, respectively. Each treatment involved 50 g of seeds received from local farmers, which were positioned on special paper (GE Healthcare, Chicago, IL, USA). To conduct the experiment, the paper was placed into 10 × 15 cm Petri dishes, and 10 mL of treating solution (own production from nano-ingredients by Pishgaman Nano Company, Iran) was subjoined. About 45 seeds were chosen and arranged, covered and packed with special tape, and then transferred to an experimental incubator with optimal regime (20/30°C – night/day) and photoperiod (12/12 h, light/dark). The germinated seeds were monitored daily and the germination percentage (GP) was computed based on radical emergence. Various measurements, including root fresh weight (RFW), shoot fresh weight (SFW), root length (RL), shoot length (SL), dry weight of the seed residue (DWS), root dry weight (RDW), and shoot dry weight (SDW), were recorded. The Seedling Evaluation Handbook (Bekendam, Grob, 1979) guided the execution of these measurement based on five replications.

The obtained data were subjected to a treatment by trait (TT) interaction biplot analysis using the GGEbiplot software version Biplotxlsx (Yan, 2001). This type of biplot graphically represents the pattern of TT interaction in two-way interaction data using the following formula:

$$X_{ij} - \mu_j = \sum_{n=1}^{2} \alpha_n \beta_{in} \gamma_{jn} + E_{ij}$$

where X_{ij} represents the mean of treatment i for trait j, μ_j is the mean of all treatments for trait j, S_j is the standard deviation of trait j among treatments, α_n is the singular value for PC n, β_{in} and η_{jn} are scores for treatment i and trait j on PC n, respectively, E_{ij} is the residual magnitude of the model related to treatment i for trait j.

To achieve symmetric scaling in the values of both treatments and traits, the singular value αn needs to be adjusted via absorption of their vectors (βin and γjn). This adjustment helps in obtaining a balanced representation of treatments and traits in the analysis; $\beta^*_{in} = \sqrt{\lambda_n}\xi_{in}$ and $\gamma^*_{jn} = \sqrt{\alpha_n}\gamma$. The TT interaction biplot graphs are created by plotting the symmetrically scaled scores of the genotypes and traits. In these graphs, each treatment (entry) or trait (tester) is represented by a unique marker, allowing for a graphical indication of the associations between traits and treatments. This comprehensive approach allowed for a detailed exploration of the relationships between treatments and traits.

RESULTS AND DISCUSSION

The first and second principal components, derived from the model, collectively accounted for 80% of the total variability in the dataset (Fig. 1). Specifically, PC1 contributed 66%, while PC2 contributed 14% to the overall explained variation. The noteworthy amount of the T × T interaction observed implies both additive and crossover interactions in our dataset, indicating differential rankings of measured traits across treatments. This finding aligns with similar observations in other plant studies (Woyann et al., 2019; Ene et al., 2022; Fantahun et al., 2023), highlighting the challenge of achieving an indirect response to selection overall treatments without considering the T × T interaction.

Recognizing the importance of the T × T interaction data, the study employs site regression (SREG) models as an appropriate analytical tool, as recommended by Yan and Frégeau-Reid (2018). Biplots are employed to effectively identify the T × T interaction and provide valuable information (Yan et al., 2022), because it allows for the evaluation of seed priming treatments. Figure 2 visually represents the treatments that excelled in specific aspects of seed germination. Most characteristics, including GP, RFW, SFW, RL, SL, and RDW, were located in this section. Notably, the Si2-Fe3 emerged as the most effective treating method (Fig. 1). This comprehensive approach provides insights into the performance of seed priming treatments, highlighting their effectiveness across multiple traits, and facilitating the identification of optimal treatments.

The DWS was situated in a distinct section, with the Si1-Fe2 method identified as the most effective priming method. Conversely, the sector of shoot dry weight (SDW) featured the Si1-Fe3 as the top-performing priming procedure

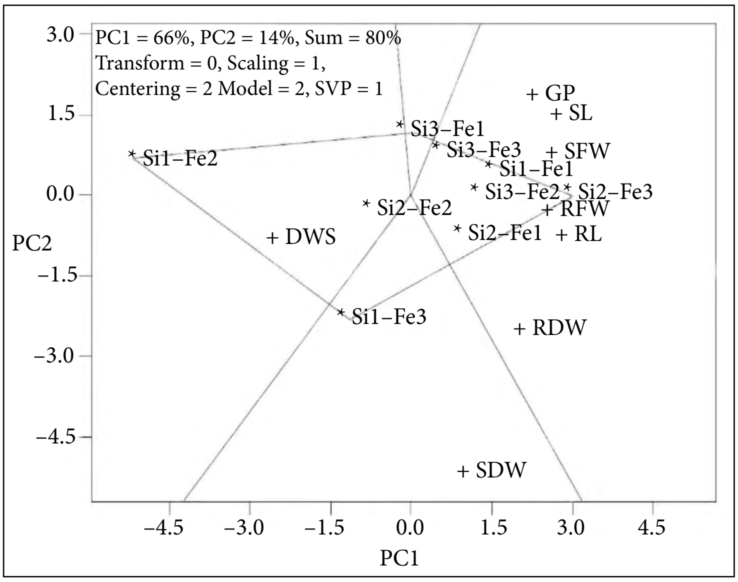

Fig. 1. Polygon of the biplot indicating the winning treatments for specific characteristics of the dragonhead.

Trait abbreviations: GP – germination percentage; RFW – root fresh weight; SFW – shoot fresh weight; RL – root length; SL – shoot length; DWS – dry weight of the seed residue; RDW – root dry weight; SDW – shoot dry weight.
Treatment abbreviations: Si1-Fe1 – 0 mM of silicon dioxide and 0 mM Fe oxide; Si1-Fe2 – 0 mM of silicon dioxide and 1 mM Fe oxide; Si1-Fe3 – 0 mM of silicon dioxide and 2 mM Fe oxide; Si2-Fe1 – 1 mM of silicon dioxide and 0 mM Fe oxide; Si2-Fe2 – 1 mM of silicon dioxide and 1 mM Fe oxide; Si2-Fe3 – 1 mM of silicon dioxide and 2 mM Fe oxide; Si3-Fe1 – 2 mM of silicon dioxide and 0 mM Fe oxide; Si3-Fe2 – 2 mM of silicon dioxide and 1 mM Fe oxide; Si3-Fe3 – 2 mM of silicon dioxide and 2 mM Fe oxide.

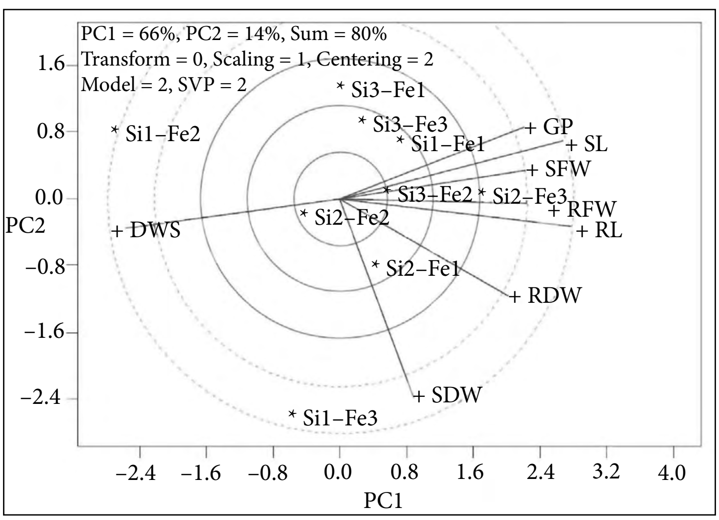

Fig. 2. Vectors of the biplot indicating the associates among the characteristics of the dragonhead across priming treatments.

Trait abbreviations: GP – germination percentage; RFW – root fresh weight; SFW – shoot fresh weight; RL – root length; SL – shoot length; DWS – dry weight of the seed residue; RDW – root dry weight; SDW – shoot dry weight.
Treatment abbreviations: Si1-Fe1 – 0 mM of silicon dioxide and 0 mM Fe oxide; Si1-Fe2 – 0 mM of silicon dioxide and 1 mM Fe oxide; Si1-Fe3 – 0 mM of silicon dioxide and 2 mM Fe oxide; Si2-Fe1 – 1 mM of silicon dioxide and 0 mM Fe oxide; Si2-Fe2 – 1 mM of silicon dioxide and 1 mM Fe oxide; Si2-Fe3 – 1 mM of silicon dioxide and 2 mM Fe oxide; Si3-Fe1 – 2 mM of silicon dioxide and 0 mM Fe oxide; Si3-Fe2 – 2 mM of silicon dioxide and 1 mM Fe oxide; Si3-Fe3 – 2 mM of silicon dioxide and 2 mM Fe oxide.

(Fig. 1). Thus, the Si3-Fe1 method did not emerge as the best for any of the characteristics (Fig. 1). Thus, to achieve optimal performance across most of the measured traits, it appears that Si2-Fe3 method may be beneficial for promoting high germination, followed by Si1-Fe1, Si2-Fe1, Si3-Fe2, and Si3-Fe3 priming methods.

These results align with other research demonstrating important effects of nano-silicon on the germination characteristics of various crops, including lentils (Sarkar et al., 2022), rice (Badawy et al., 2021), and sunflower (Yurekli, Kilicaslan, 2019). Similar positive impacts of silicon on seed germination have been reported in maize (Sun et al., 2021) and melon (Zhang et al., 2020). However, it is worth noting that Shi et al. (2014) did not find any positive impact of silicon on the germination of tomato seeds under non-stress conditions. Therefore, it can be concluded that under normal circumstances, the beneficial impact of silicon on plant germination may be dependent on the plant species in question.

Figure 2 illustrates the interrelationships of seed characteristics via lines that connect each characteristic to the plot origin. In this representation, the stature of the vectors estimates the mean squares of error of the corresponding characteristic, while the cosine of the vectors estimates the magnitude of association. As seen in Fig. 2, traits such as GP, SL, SFW, RFW, and RL exhibit a high positive correlation, indicating that they provide the same witting about the variation of the priming methods, and such findings align with those reported by Nejatzadeh (2021).

On the other hand, dry weight of the DWS showed a relatively a negative association with GP, SL, SFW, RFW, and RL. Moreover, there appears to be a near-zero correlation between SDW and DWS, as well as between SDW and GP, SL, SFW, RFW, and RL, as evidenced by the relatively staple lines in Fig. 2. Some of these observations can be cross-verified using the original values of correlation coefficients in Table. It is important to note that certain discrepancies between the biplot model predictions and the original data were expected. This is because the T × T biplot model only accounts for less than 100% of the total observed variation (in this case, approximately 80%) and not exactly the total variation. The seed priming with nano-silicon has demonstrated its potential to break seed dormancy in tall wheatgrass, suggesting that such materials could serve as a next approach for overcoming the problem of dormant seed issues (Gomes-Junior et al., 2022). However, it is essential to note that our study hypothesises that large magnitudes of nano-silicon dioxide (2 mM) might have a harmful effect on seeds, adversely impacting their performance. This implies that while nanoparticles can be beneficial, using excessively high concentrations may have negative consequences on seed viability and germination.

Table. **Pearson's correlation coefficients for the traits of *D. moldavica* L.**

	GP	RFW	SFW	RL	SL	DWS	RDW
RFW	0.517ns						
SFW	0.557ns	0.482ns					
RL	0.718*	0.788*	0.751*				
SL	0.774*	0.783*	0.776*	0.912**			
DWS	–0.829**	–0.690*	–0.794**	–0.944**	–0.923**		
RDW	0.319ns	0.801**	0.363ns	0.732*	0.600ns	–0.522ns	
SDW	0.135ns	0.090ns	0.245ns	0.414ns	0.071ns	–0.267ns	0.342ns

*, **, and ns = significant at 5%, and 1% probability level, and non-significant.

Trait abbreviations: GP – germination percentage; RFW – root fresh weight; SFW – shoot fresh weight; RL – root length; SL – shoot length; DWS – dry weight of the seed residue; RDW – root dry weight; SDW – shoot dry weight.

Our results also indicate that the reaction of seed germination to priming at different levels of silicon dioxide and Fe dioxide nanoform solutions was highly variable. This variability suggests that the effects of nanomaterials on the plant growth are somewhat not predictable, as reported by Kolenčík et al. (2020). Contrary to our findings, the use of bulk silicon dioxide at large amounts significantly reclaimed positively the seed germination of *Astragalus fridae* (Moghanloo et al., 2019). However, our study underscores the importance of considering optimal concentrations, suggesting that the best results for *D. moldavica* L. seed germination may be achieved at lower concentrations of nano-silicon. This highlights the need for careful consideration and optimisation of nanoparticle concentrations in seed priming treatments to avoid potential adverse effects on seed performance.

Earlier research proposed that the use of our studied nanomaterials could serve as a promising strategy to mitigate the detrimental impacts of salt on germination (Tiwari et al., 2021) and improve water-use efficiency in plants (Esmaili et al., 2022). In our current research, we observed that priming with a combination of both nanoparticles positively influenced the seed germination. However, the physiologic physiological impacts of nanomaterials in seed germination are not clear which emphasises, warranting further investigation to unravel the intricate mechanisms at play. The improving effect of silicon dioxide on germination-related parameters, such as the germination percentage, root and shoot characteristics have been documented in previous studies, particularly in plants such as *Jasminum elongatum* (Azimi et al., 2014). Similarly, beneficial impacts of nano-Fe particles on the mean germination time of barley have been reported (Najafi-Disfani et al., 2016). These findings collectively suggest the potential of nano-silicon and nano-Fe to positively influence various aspects of the germination process and related traits, indicating their promising applications in agriculture. Nonetheless, a more in-depth exploration of the specific physiological roles and underlying mechanisms of nanoparticles in germination is crucial for a comprehensive understanding and optimised utilisation of these nanomaterials in agricultural practices.

The vitality of seeds and their robust germination properties are crucial for the cultivation of crops, ultimately leading to high economic production. Various materials are employed to enhance germination and foster plant growth. Recent investigations into seed treatments using nanoparticles have shown promising results, positioning them as potentially more effective tools compared to traditional materials for promoting seed germination and seedling growth. In our study, we observed that nano-silicon particles contribute to improved seed germination, aligning with findings discussed in the review by Abbasi-Khalaki et al. (2021). The review concludes that the application of silicon nanoparticles can enhance physiological properties and overall plant growth and reduce evapotranspiration. Similarly, our findings regarding Fe nanoparticles align with the positive effects reported by Najafi-Disfani et al. (2016), who highlighted the benefits of Fe nanoparticles in enhancing germination of barley seeds. Additionally, Fe nanoparticles are utilised as fertilisers to enhance the availability of Fe to plants and regulate antioxidant enzymes, further showcasing their potential in promoting plant health and growth.

These observations collectively emphasise the promising role of nanoparticles, particularly nanoparticles in improving germination and fostering robust seedling growth, which has significant implications for agricultural practices aiming at high crop yields and economic productivity. Indeed, reports highlight potential negative impacts of nanomaterials on germination and next plant growth (Goswami, Mathur, 2019). The extent of phytotoxic impacts related with nanomaterials depends on various factors, including the type and concentration of nanoparticles as well as plant properties such as seed size and plant species. These effects are often studied under controlled conditions, but to obtain more reliable information about the impact of nanoparticles on crops, ecosystems, and human health, investigations under natural field conditions are crucial.

Achieving high economic yield performance requires the optimisation of nanoparticle experiments. This involves careful consideration of factors such as the type of nanoparticles, their concentration, and the duration of exposure, all of which can interact differently with target species under specific envFemental conditions. The application of nanoparticle technology has the potential to enhance target production, provided that these experiments are precisely tailored to the needs of the specific crop and envFemental context. Furthermore, ongoing research should focus on identifying new nanoparticles that can induce more desirable changes while minimizing toxicity concerns for both human and animal immunity properties and envFemental ecosystems. This nuanced approach is essential to harness the benefits of nanoparticles in agriculture while minimizing potential risks and ensuring sustainable practices.

The T × T (treatment by trait) biplot model emerges as a robust and insightful tool for extracting valuable information from experimental data. Its strength lies in its ability to visualize the complex interplay between treatments and traits in a two-way data table, making it an indispensable asset for experimental studies. In the specific context of this investigation, where multiple traits and treatments are considered, the T × T biplot proves to be an excellent means of representation. While existing research has extensively explored genotype by envFement (GE) biplots on yield stability analysis, the application of T × T biplots for multiple traits has been comparatively limited. What sets the T × T biplot apart is its capacity to comprehensively evaluate treatments based on their effects across a range of traits. This dynamic model not only graphically ranks treatments, indicating their importance in influencing multiple traits, but also provides insights into their potential impact. One notable advantage of the biplot model is its objectivity. Unlike some other analytical methods, it does not rely on subjective weighting, ensuring that the outcomes are solely dependent on the specific traits included in the analysis. This feature enhances the reliability and unbiased nature of the model, making it a powerful tool for visually assessing the effects of treatments across a diverse array of traits. The biplot thus stands as a good and objective method for researchers seeking a comprehensive understanding of the associations among various treatments and multiple traits in their experimental data.

CONCLUSIONS

This research underscores the effectiveness of the biplot as an outstanding visual tool for interpreting treatment by trait data. The application of seed priming with nanomaterials emerges as a promising strategy to promote germination and enhance Moldavian dragonhead cultivation. Specifically, our findings suggest that the application of nanoparticles, particularly in the treatment Si2-F3 (involving 1 mM silicon dioxide as nanoform and 2 mM Fe dioxide as nanoform), holds significant potential for improving seed germination properties in Moldavian dragonhead.

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Naser Sabaghnia, Mohsen Janmohammadi, Saeed Yousefzadeh

DARŽELINĖS ŽIOMENĖS SĖKLŲ PRIEŠSĖJINIŲ PROCEDŪRŲ, PANAUDOJANT ĮVAIRIAS NANODALELES, DVIMATĖ GRAFINĖ ANALIZĖ

Santrauka

Daržėlinė žiomenė (*Dracocephalum moldavica* L.) liaudies medicinoje naudojama sutrikus inkstų veiklai. Daržėlinės žiomenės sėkloms buvo taikoma išankstinė hidratacija trijų koncentracijų nanosilicio dioksidu: kontrolinė Si1 (0 mM), Si2 ir Si3 (atitinkamai 1 ir 2 mM). Kartu naudotos Fe nanodalelės oksido pavidalu: kontrolinė Fe1 (0 mM), Fe2 ir Fe3 (atitinkamai 1 ir 2 mM). Kruopščiai išmatuoti įvairūs parametrai – daigumas, svoris (šviežios ir sausos medžiagos), šaknų ir ūglių ilgis, sausų sėklų, sausų šaknų ir ūglių svoris. Dvimatis grafikas palengvino požymių ir apdorojimo būdų sąsajos vizualizaciją, o du pagrindiniai komponentai padėjo išaiškinti 80 % stebėtų pokyčių. Nustatyta, kad Si2-Fe3 yra optimali išankstinė hidratacija, pagerinanti sėklų daigumo savybes. Mūsų tyrimas rodo, kad Si ir Fe nanodalelės gali pagreitinti daržėlinės žiomenės dygimą ir sėjinukų augimą. Straipsnio autoriai pabrėžia nanodalelių technologijos pranašumą žemės ūkyje, eksperimentinio optimizavimo svarbą, dvimačio grafiko objektyvumą ir perspektyvią daržėlinės žiomenės sėklų apdorojimo strategiją.

Raktažodžiai: sėklų daigumas, nanogeležies oksidas, nanosilicio dioksidas