

Guano-Induced Germination and Responses of Wheat Seedlings to Guano Under Water Stress Treatments

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Abstract: The present study was conducted to investigate the effects of guano (organic fertilizer) on germination and growth parameters of wheat (*Triticum durum* L. Karakılıç). Guano was applied into germination medium at a rate of 6g /100 ml - for 3 days. Following the germination of seeds, one group was water stressed by applying a water deficit for 7 days and the other group was irrigated ordinarily. The germination rate and the dry weights of germinated seeds were higher in guano-applied group. In vegetative stage, guano treatments improved relative water content (RWC) and relative growth rate (RGR) under water stress. Water stress increased malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) content but both parameters did not significantly change in combined water stress and guano treatments. At the seedling stage, ABA (abscisic acid) content decreased in guano group and under water stress but guano treatment increased ABA contents under water stress. It was concluded herein that guano treatments improved germination and growth parameters of wheat seeds and protected the wheat seedlings from oxidative damages under water stress.

Key words: Guano, water stress, wheat, abscisic acid, hydrogen peroxide

Guanonun Çimlenmeyi Teşviki ve Guano ve Su Stresi Uygulamalarına Karşı Buğday Fidelerinin Verdiği Yanıtlar

Özet: Bu çalışma organik bir gübre olan guanonun buğday (*Triticum durum* L. Karakılıç) bitkisinin çimlenme ve büyüme parametreleri üzerine olan etkisini tespit etmek amacı ile yapılmıştır. Guano çimlenme ortamına üç gün boyunca 6g /100 ml oranında uygulanmıştır. Çimlenmenin ardından bir grup yedi gün boyunca su kıtlığına maruz bırakılıp diğer bir grup düzenli olarak sulanmıştır. Çimlenme oranı ve çimlenen tohumlardaki kuru ağırlık guano uygulanan grupta daha yüksek bulunmuştur. Vejetatif aşamada guano uygulamaları su stresi altında bağıl su içeriği ve bağıl büyüme oranını geliştirmiştir. Su stresi malondialdehid ve hidrojen peroksit (H₂O₂) içeriğini arttırmış fakat bu parametreler su stresi ve guano uygulamalarının birlikte yapıldığı grupta belirgin bir değişikliğe uğramamışlardır. Fide aşamasında aba miktarı guano ve su stresi grubunda azalmış fakat guano uygulaması su stresi altında ABA (absisik asit) miktarını arttırmıştır. Bu sonuçlara göre guano uygulamasının buğday tohumlarında çimlenme ve büyüme parametrelerini iyileştirdiği ayrıca buğday fidelerinin su stresi altında oluşan oksidatif hasardan koruduğu ortaya konulmuştur.

Anahtar kelimeler: Guano, su stresi, buğday, absisik asit, hidrojen peroksit

Introduction

The higher plants usually store materials as carbohydrates and proteins in their cotyledons or endosperms (Bewley, 1997). Such plants obtain the energy by decomposition of these materials while they were germinating (Subbarao et al., 1988).

For a continuous germination process, carbohydrates and glucose must be provided to meet the energy demand of embryo. However, carbohydrates are generally stored in starch forms and therefore they are not available to be used in embryo (Ricard et al.,

1998). Thus, syntheses of the hydrolytic enzymes converting such materials into available forms are induced at the beginning of germination process (Uriyo, 2001). The α -amylase is an enzyme with a significant role in starch breakdown into glucose (Perata et al., 1997). Gibberellins hormone promotes amylase secretion and consequently promotes the germination by breaking dormancy of the seeds (Akgul, 2008). The seeds must take water from the soil as much as 50 % of their weights to initiate the germination process.

The water stress or excess moisture, both have adverse effects on plant growth. Plant water requirement increases with the progress of growth and usually reaches to maximum levels during the flowering period (Simsek et al., 2001). Water deficit creates a water stress and subsequently inhibits plant growth and development. Water absorption capacity of the roots usually decreases under water stress (Pessarakli, 1999). Nutrient uptakes also decrease with water stress-induced decrease in transpiration rates. During the water stress, the motion of roots and permeability characteristics may be altered in various ways. In this case, ABA synthesis may be increased in chloroplasts and moved to the other parts of the plant (Ozen and Onay, 1999). ABA prevents transpiration by promoting the stomatal conductance (Aherin and Christianson, 2003).

"Guano" is the term usually used for gull or bat scat (Keleher, 2004). It is rich in nitrogen (N), phosphorus (P) and potassium (K) and commonly used as a natural fertilizer for plants (Bozcuk, 1978). Organic acids produced by decomposition of organic matter provides the water and nutrient for the plants through increasing the solubilization of phosphorus and micro-elements (Gunes et al., 2000). Thus, guano is considered as the most significant organic fertilizer for crops to induce the growth and development. Wheat (*Triticum* spp) is the most important cereal crop worldwide in terms of both cultivated land area (232 million ha) and amount of production (595 million t) (FAO, 2002).

In literature, there are several studies reporting the germination and vegetative

growth of wheat under stress conditions. But there isn't any study about the effects of organic fertilizer "guano" on seed germination and plant growth under environmental stress factors. Therefore, present study was designed to investigate the effects of guano treatments on seed germination, physiological and biochemical responses of wheat under water stress.

Material and Methods

Experimental Design

Germination stage

Initially, the seeds were exposed to 5 % sodium hypochlorite solution for 15 minutes and washed with dH_2O for three times. Then, the seeds were germinated in petri dishes at $25\pm 1^\circ\text{C}$ in the dark medium for four days.

Seedling stage

The germinated seeds in petri dishes were inoculated into pots in soil medium with equal intervals and depths. Some properties of soil used in the experiment (soil, clay and clay-loam (2:1:1), pH:6-7) and guano was dissolved in a Hoagland solution at a rate of 6g/100ml. This concentration was recommended as the efficient concentration by previous experiments (Turkuvaz Bat Guano Agriculture Products Industry and Mining Company Limited, Eskischir, Turkey). Following the germination, plants were grown at 16h light / 8h dark intervals at the 25°C for 5 days.

The assessments were done in two stages. In the first group, the effect of guano on germination in wheat seeds and in the second group, guano on wheat seedlings under water stress were determined. The treatment groups were designed as follows:

Guano treatments were done with dH_2O whereas control groups were watered with dH_2O in the germination stage.

Control: (Watered with dH_2O from germination to seedlings and watered with Hoagland for 5 days and watered for 7 days)

G: Guano: (Watered with guano in dH_2O from germination to seedlings and watered

with guano in Hoagland for 5 days and watered for 7 days).

WS: Water Stress: (Watered with dH₂O from germination to seedlings and watered with Hoagland for 5 days and not - watered for 7 days).

WS+G: Water Stress + Guano: (Watered with guano in dH₂O from germination to seedlings and watered with guano in Hoagland for 5 days and not watered for 7 days).

Analysis

Germination rate (%): (number of seeds germinated / number of seeds in total) x 100 (Killi, 2004).

On the 4th day of germination stage, the length of radicle and hypocotyl and fresh and dry weights were measured. For dry weight, the samples were dried in at 70 °C for 36 h and then dry weights (DW) were determined. The relative growth rate (RGR) of seedling was calculated from the dry mass data taken at initial and final harvests, using the formula given by (Venus and Causton, 1979).

The relative water content (RWC) was calculated in accordance with Smart and Bingham (1974). The seedlings were floated on de-ionized water for 4h under low irradiance and then the turgid tissue was quickly blotted to remove excess water and their turgid weights (TW) were determined. The samples were dried in at 70 °C for 36 h and then dry weights (DW) were determined.

ABA content was determined in accordance with Flores et al., (2011) with an UHPLC-MS/MS device in Pure Analysis Laboratory.

The level of lipid peroxidation in leaf samples was determined in terms of MDA content according to the method specified by (Madhava and Sresty; (2000). MDA is an end product of lipid peroxidation and MDA content was determined by using the thiobarbituric acid reaction. MDA concentration was calculated from the absorbance at 532 nm and measurements were corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The

MDA concentration was then calculated by using an extinction coefficient of 155 mM⁻¹ cm⁻¹.

The H₂O₂ content was determined in accordance with (Velikova et al.; (2000) and Wahid et al.; (2007). Fresh leaves (0.1 g) was homogenized in 5ml of 0.1% Trichloroacetic acid (TCA) and centrifuged at 12,000 rpm for 15 minutes. A total of 0.5 ml supernatant is then mixed with 0.5 ml buffer (Potassium phosphate 10 mM pH 7) and 1ml 1M KI. The absorbance reading was taken at 390 nm.

Statistical Analysis: Resultant data were subjected to analyses of variance (ANOVA). Each data point was the mean of six replicates and means were compared at p<0.05 significance level.

Results and Discussion

Germination occurs with the uptake of water by the seeds and ends with elongation of the embryonic axis (Bewley, 1997). The seed consists of nutrient reserve storage tissues, an embryo and encapsulating structures protecting and regulating the germination. Many studies reported that factors such as light, temperature, water and chemicals affected the germination rate of crops (Bau et al., 1997). In the present study, organic fertilizer “guano” was applied to investigate the effects of guano on the germination capacity of wheat seeds. Guano contains nitrogen (N), phosphorus (P) and potassium (K) and provides nutritional support for the crops (Bozcuk, 1978). While the germination rate was (81 %) in guano group, the value was observed as 66 % in the control group. The reason for higher germination rate in guano group can be related to the higher water uptake capacity of seeds (Table 1). Parallel to these findings, higher dry weight percentages (67 %) were observed in guano groups than the control group (39 %) (Table 1). Such findings indicate that guano application might have led to increase in mineral nutrition in germinated seeds.

Table 1. Changes in germination rate, seed dry weight, radicle and hypocotly length, radicle and hypocotly dry weight in seedlings of *T.aestivum*.Tablo 1. *T. aestivum* bitkisinde çimlenme oranı, tohum kuru ağırlığı, kök ve hipokotil uzunluğu ile hipokotil kuru ağırlığındaki değişiklikler.

Groups Gruplar	Germination Rate (%) Çimlenme oranı (%)	Seed Dry Weight (%) Tohum kuru ağırlığı (%)	Hypocotyls Length (cm) Hipokotil uzunluğu (cm)	Radicle Length (cm) Radikula uzunluğu (cm)	Hypocotyls Dry Weight (%) Hipokotil kuru ağırlığı (%)	Radicle Dry Weight (%) Radikula kuru ağırlığı (%)
C K	66±0.12 ^a	39 ^a	2,6±0.11 ^a	5,9±0.21 ^a	13 ^a	63 ^a
G G	81±0.18 ^b	67 ^b	4,6±0.45 ^b	5,7±0.27 ^a	50 ^b	65 ^a

The values shown with different letters are significant ($p < 0.05$). C: control; G: guano
Farklı harfler birbirinden belirgin biçimde ($p < 0.05$) farklı olan değerleri göstermektedir.
C: kontrol; G: guano

Compared to control group, while the length of radicle did not change in guano group, hypocotly length increased by 77 % in guano group. Similarly, the dry weight of hypocotly increased about 3,85 fold in guano group but radical dry weight did not change (Table 1). It's clear that "guano" may have induced mineral uptake from radicle to hypocotly by unchanged length and dry weight of radicle in current experiments. Parallel to findings of the present study, Toledo et al., (2011) also observed an increase in seedling length with phosphorus fertilization in white oat plants. In contrast, Hartwigsen and Evans (2000) reported that humic acid (organic fertilizer) application improved root development in cucumber plants.

In current study, increasing RWC values were observed in guano group compared to control group but such an increase was not found to be significant. The value decreased by 5,3 % under water stress treatment. However, compared to the control group, RWC did not change in combined water stress and guano treatment. The RWC of combined treatment was 4,5 % higher than single water stress treatment (Table 2). In literature, it has been well documented that the RWC decreases in leaves under water stress in plants by increasing water loss (Sairam and Srivastava, 2001; Flexas et al., 2006). The improvement in guano-induced RWC can be related to more efficient water uptake and decrease water losses. The current findings are in good agreement with

the results of (Anjum et al.; (2011) who reported that exogenous fulvic acid (organic fertilizer) increased RWC in maize under drought stress.

It's well known that water stress reduced the relative growth rate in plants (Schubert et al., 2004; Franca et al., 2005). In parallel to previous studies, water stress reduced the relative growth rate of wheat seedlings by 9,5 % in current experiment (Table 2). Nevertheless, it did not change significantly under water stress and guano treatment compared to control group. Guano application led to increase in RGR under water stress by 7,2 %. Guano treatment also increased the RGR by 11 % compared to control group. These results indicate that the growth of seedlings was induced by application of guano and guano alleviated the water deficit- induced inhibition in growth.

Production of reactive oxygen species by mitochondria and chloroplast can cause damages membranes, proteins and lipids. MDA is a lipid peroxidation product and accepted as a good indicator of the prevalence of free radical reactions in tissues (Halliwell and Gutteridge, 1989). Compared to control treatment, MDA content did not change in a guano group of the present study. However, water stress treatment increased MDA content of wheat seedlings by 70 %. Similar results were also reported by Sudhakar et al., (2001) and Vendruscolo et al., (2007). Water stress induces numerous biochemical and physiological responses in

Table 2. Changes in relative water content, relative growth rate, hydrogen peroxide, malondialdehyde, abscisic acid content in seedlings of *T.aestivum* under water stress.
 Tablo 2. *T.aestivum* bitkisinde bağıl su içeriği, bağıl büyüme oranı ile hidrojen peroksit, malondialdehit ve absisik asit miktarındaki değişiklikler.

Groups Gruplar	RWC (%) Bağıl su içeriği (%)	RGR (mg mg ⁻¹ d ⁻¹) Bağıl büyüme oranı (mg mg ⁻¹ d ⁻¹)	H ₂ O ₂ (μM/g) Hidrojen peroksit (μM/g)	MDA (nmol g FW ⁻¹) Malondialdehid (nmol g YA ⁻¹)	ABA (μg/kg) Absisik asit (μg/kg)
C	93±4.25 ^b	0,063±0.24 ^b	33.43±0.14 ^a	14.19± 4.35 ^a	0,33±2.15 ^b
G	96±3.89 ^b	0,07±0.57 ^c	35.47±0.58 ^a	16.03±3.97 ^a	0,19±3.09 ^a
WS	88±2.60 ^a	0,057±0.29 ^a	41.16±0.69 ^b	24.19±2.67 ^b	0,15±3.14 ^a
WS+G	92±2.27 ^b	0,061±0.48 ^b	34.78±0.37 ^a	15.49±4.57 ^a	0,51±4.22 ^c

The values shown with different letters are significant (p < 0.05). C: control; G: guano WS: water stress WS+G: water stress + guano.

Farklı harfler birbirinden belirgin biçimde (p < 0.05) farklı olan değerleri göstermektedir. C: kontrol; G: guano WS: su stresi WS+G: su stresi + guano.

plants. The generation of reactive oxygen species (hydrogen peroxide, superoxide radical, hydroxyl radical) and subsequent oxidative damage during the stress conditions are well documented for various crops (Jiang and Huang, 2001). The production of ROS increases under water stress conditions and such an increase can cause oxidative damages and impairment of normal metabolism. However, in the present study, significant differences were not observed between control group and treatment groups. A 25 % decrease was observed in the MDA content of water stress guano combined treatment compared to water stress alone (Table 2). The current results indicate that guano treatment prevented oxidative damage in wheat seedlings by decreasing MDA content.

ABA also plays a major role in plant responses to biotic and abiotic stresses. Increases in ABA levels have been reported during salt, cold, drought exposures and wounds (Zeevaart and Creelman, 1988; Pen a-Cortes et al., 1989; Shinozaki and Yamaguchi-Shinozaki, 2000). Compared to control group ABA content decreased by 42,4 % in the guano group at seedling stage (Table 2). This decrease shows that wheat seedlings did not need to increase ABA for regulating the water content as it was RWC values. Under water stress, ABA content decreased by 54.5 % as compared to control

group. In literature, it was reported that the accumulation of ABA was induced by water stress in plants (Davies and Zhang, 1991; Yang et al., 2001; Pustovoitova et al., 2004; Domash et al., 2006). Current findings suggest that water stress decreases the ABA content by increasing MDA content in wheat seedlings. This decrease can lead to loss in water content. However, ABA content increased 3,4 fold in guano and water stress combined treatment compared to water stress alone. These results clearly suggest that guano protected the wheat leaves from water stress by increasing the ABA content. Such findings are also in good agreement with the results of Yuling et al., (2000), who reported that the fulvic acid treatment increased ABA content in wheat plant under drought conditions. In parallel to these results it was reported that nitrogenous fertilizers played a significant role in the regulation of stomatal responses and density of ABA symptoms under stress conditions (Zhang and Shan, 2003). Beside this, guano may increase calcium uptake from the soil and may play a role as a signal hormone for increasing the ABA biosynthesis (Zhang et al., 2006).

Water stress induced the hydrogen peroxide content as an oxidative stress agent or signal for antioxidant enzymes. Hydrogen peroxide content increased by 23.12 % under water stress (Table 2). This is also in

agreement with the results of (Sairam et al., 1998) indicating increased hydrogen peroxide and MDA contents in wheat plants under water stress. It can be suggested that the lower ABA content did not protect the plants from oxidative damage by increasing the MDA content. This increase shows that it was not a signal for activation of antioxidant enzymes and was not induced by ABA. In a previous study, it was reported that the ABA can induce hydrogen peroxide in plants under stress conditions (Hu et al., 2008). In contrast with this result, while ABA content increased in the combined water stress guano treatment, hydrogen peroxide was still around the same levels with the control group.

Conclusions

It is known that guano is an important natural fertilizer for plant growth. In the present study, guano-induced germination and responses of wheat seedlings to guano under water stress treatments were investigated. Results revealed that guano treatments protected the wheat seedlings from oxidative damages by unchanged MDA and H₂O₂ under water stress. The present study suggested that guano application to crops under stress conditions will be useful for increasing the wheats tolerance against water stress and to increase the yields. However, the investigation of the effects of different levels of guano treatments on wheat seedlings is needed.

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