Research Article
*THE INTERNATIONAL JOURNAL OF BIOLOGICAL RESEARCH (TIJOBR)*
*ISSN Online: 2618-1444*
*Vol. 2(3) July – Sep., 338-347; 2019*
*http://www.rndjournals.com*

# COMPARATIVE MORPHO-PHYSIOLOGICAL RESPONSES OF PEA GENOTYPES UNDER WATER STRESS WITH THE APPLICATIONS OF IRON OXIDE NANOPARTICLES

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**Abstract.** Nanoparticles are a group of environmental effluences. Drought hinders numerous physiological processes and decrease crop biomass and seed yields. Our principal focus is to check the interactive effects of nanoparticles and drought. However, no study has been performed on the effects of nanoparticles on Pea plant with the drought stress. In this study we exposed pea to 3 levels of nanoparticles concentration 0ppm, 20ppm and 50ppm under water deficit conditions during growing season 2017-2018. The experiment was performed using completely randomized design. Days to flowering were decreased 22% showing positive impacts of nanoparticles, there is 24% increase in Tendril Length, Relative water content, grain weight and Number of grains per pod due to drought stress and application of nanoparticles. Root length was increased 23%. Variety "Adventa selection" behaves much better than Climax with respect to certain agronomic traits because mean values for Tendril Length, Number of grains per pod and Days to flowering was better than Climax. Climax variety behave much better in some other agronomic traits like shoot length, root length and grain weight. Because mean values for above mentioned parameters were much better than Adventa selection. The application of iron oxide nanoparticles under water stress is recommended.
**Keywords:** Pea (*Pisum sativum L.*), Drought; Yield, nanoparticles

## Introduction

Pea (*Pisum sativum L.*) is the utmost significant cool season leguminous crop and used for multiple advantages on world level (Macas *et al*., 2007). This plant is predominantly easily damaged or distressed by slight changes in water availability (Okçu *et al*., 2005). Drought hinders numerous physiological processes and decrease crop biomass and seed yields (Hsiao, 1973). Nitrogen fixation is disturbed by numerous mechanisms of arid conditions by upsetting various steps in fixation like initiation of nodule formation, /enlargement of nodules or its function (Busse and Bottomley, 1989; Mnasri *et al*., 2007; Serraj *et al*., 1999; Zahran and Sprent, 1986). Peas are a valued and cheap protein source for humans and different animals. Peas contain 21·2–32·9% Protein, 36·9 to 49·0% Starch, 2·1 to 6·3% Resistant Starch, 20·7 to 33·7% Amylose, 14 to 26 % Dietary Fibers, 10 to 15% Insoluble Fibers, 2 to 9% Soluble fiber, 5·3 to 8·7% , Total lipid 1·2 to 2·4% Lipids (Hood-Niefer *et al*., 2012). Peas are rich in starch content (46%) and fiber (20%) of dry mass of seed, in respective manner, averagely (Tzitzikas *et al.,* 2006). Nanotechnology is an emergent field of science gifted with the far ranging uses in cancer treatment, battered drug distribution, biomedicines, waste water handling, cosmetic industries, electronics and biosensors (Nel *et al.,* 2006). Nanomaterials (NMs), was termed as Magic Bullets by a scientist named Paul Ehrlich, there are most widely studied by most researcher one of the most widely studied materials of this century that opens new doors to science and a new branch of science called "nanotechnology" is introduced (Khan *et al*., 2017). Commercial production of nanomaterials is increasing day by day and due to their greater use and production we must know their possible influences on the atmosphere (Dimkpa *et al*., 2012; Wang *et al*., 2012; Yin *et al*., 2012; Feng *et al*., 2013; Azimi *et al*., 2014; Soliman *et al*., 2015). Nutrients like Ag, Cu and Fe have been widely provided to different plants; they affect the crops both favorably and adversely mainly dependent on plants and quantity of Nps provided. To check the response of wheat towards NPs, extensive work was carried out because wheat is a crop used by most of population as staple food. For this study three different types of nanoparticles were selected and applied with three different types of treatment and results were documented. According to these results above mentioned types of nanoparticles influenced the following: Rate and Percentage of germination, Plant height, root elongation and seedling strength both harmfully and favorably. These results indicate that Fe NPs have favorable whereas Cu has adverse effects on *Triticum aestivum* (Yasmeen *et al*., 2015).

Zhu *et al*. (2008) completed his first investigation consuming $Fe_3O_4$ NPs in plants and described these nanoparticles are absorbed in a greater concentration by *Cucurbita maxima* and their following displacement and gathering in numerous cells and its larger aggregations. In *Glycine max*, it was stated that chlorophyll contents are influenced by iron oxide NPs and exerts same impact on enzymatic and biochemical efficacy in multiple steps of the photosynthetic products synthesis (Ghafariyan *et al*., 2013). Iron oxide magnetite nanoparticles are usually used in making images and chromatographic practices because of its magnetic possessions because they are considered to be biologically and chemically inactive (Ren *et al*., 2011). Additionally, these NPs can be used to acquire double functions: separation and detection if they are treated with catalysts or enzymes (Gao *et al*., 2007). Furthermore, $Fe_3O_4$ NPs are also used in the pharmaceutical industry (Kohler *et al*., 2005; Mahmoudi *et al*., 2011) and for getting rid of environmental problems (Liu *et al*., 2008; Yantasee *et al*., 2007). $Fe_2O_3$ NPs helps in reducing nutrients from the soil because a large amount of them stick to the soil particles griping the nutrients.

Very few researches have been carried out to check the consequence of Iron oxide nanoparticles on the morphology and physio-chemical activities *of Pisum sativum L*.under water stress. In this study *Pisum sativum L.* genotypes were exposed to Iron oxide nanoparticles to study their responses under these parameters. Key points of this investigation are to find out the Physiological responses of *Pisum sativum L.* genotypes under water stress. To observe the morphological changes compared to control genotypes of Pea and to find out the effect of Iron oxide nanoparticles and their fate in the plant systems.

## MATERIALS AND METHODS

Experiments to conclude the effect of iron oxide nanoparticles on Pea cultivar Adventa selection and Climax were conducted in the Crop Physiology Laboratory of University of Lahore Sargodha Campus**.**

## PRODUCTION OF IRON NANOPARTICLES

Fe Nps were produced by the chemical reduction of ferric chloride hexa-hydrate ($FeCl_3.6H_2O$) using onion extract. $FeCl_3.6H_2O$ in dissolved form was formed by mixing 968.37mg of ferric chloride in 1 liter of distill water. The solution was boiled for 4 min 10 sec. 30 ml Onion extract was added for the reduction stepwise. The solution was heated to boil uninterruptedly up until the color of solution altered to reddish brown. Solution as a result was the stock solution of Fe NPs having 200 ppm concentration (Fig 1). Stock solution was made Dilute to the required concentrations from stock solution for future use (Fig 1).

## CHARACTERIZATION OF IRON NPs

Size of Iron nanoparticles was calculated by Zeta Particle Analyzer from Nuclear Institute of Biotechnology and Genetic Engineering (NIBGE), Faisalabad. Size of the nanoparticles was determined in solution form. The size as tested by Zeta Particle Analyzer range from 8nm to 14nm (Fig 2).

Fig 1: Iron Nanoparticles (200ppm)

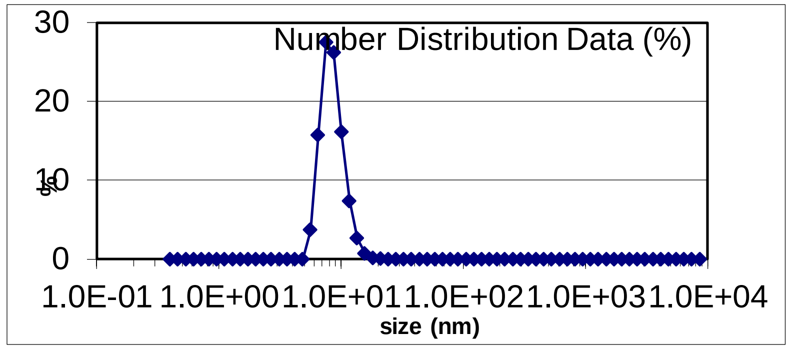


Fig 2: Zeta Analyzer Peak for Iron Nanoparticles

**EXPERIMENTAL PROCEDURE**

Earthen pots were filled with 6.25Kg of thoroughly mixed soil. Seven seedlings were transplanted from the petri dishes to each earthen pot. Solution equivalent to field capacity water with different concentrations of Fe NPs (20 ppm and 50 ppm) was applied to each pot. Water served as control. Water was used according to requirement of the crop. Different concentrations of Fe NPs were applied again at heading stage. For experimental layout, Completely Randomized Design (CRD) with 2 replications was used. When plants became mature and ready to be harvested, yield parameters were measured.

**Measurement of various morpho-physiological parameters**

***Days to Flowering***

Days to flowering was calculated by subtracting flowering date from days of sowing.

***Root length (cm) and plant height (cm)***

The distance between the tip of the root and the portion of the plant just touching the surface soil was measured as the root length. Plant height was measured starting from the base of the plant to the top of the main shoot. After taking average of five plants we calculated the mean height.

***Grain Weight***

Grain from each pod was taken out carefully and weigh.

***Tendril Length***

The length of Tendrils from each pot was calculated in centimeters.

***Relative water content (RWC)***

Leaf relative water content (RWC) was noted in fully extended leaves of four plants for each replicate. Five leaf discs of 10mm in diameter were carefully cut from the inter-veinal part of every plant. For each replicate, 20 discs were pooled and their Fresh Weight was calculated. The discs were floated on distilled water in Petri dishes for 4 h to attain turgidity, than defrosted and reweighed (TW). The samples were desiccated at 808C for 1 day to conclude the Dry Weight. Experiments indicated that complete hydration of the leaf discs occurred within 4 h. RWC was defined as follows:

RWC ð%Þ ¼ ðFW DW=TW DWÞ 100

***Pod Length***

The length of Pod from each pot was calculated in centimeters.

***Number of grains per Pod***

Number of grains per Pod was counted.

**Results**

Genotypic variation, Variation present due to treatment and the interaction between them measured under water stress conditions for the estimated yield parameters are presented in Table .1. Both genotypes along with treatments varied for all the evaluated parameters including days to flowering, tendril length, relative water contents (RWC), Number of grains per pod, Pod length, Shoot Length, Grain Weight and Root Length.

Interaction among genotypes and treatments also exhibit high variances for all the traits. Overall drought stress effect was inhibitory in case of tendril length, relative water contents (RWC), Number of grains per pod, Pod length, shoot length, Grain Weight and Root Length. Summary statistics of the studied traits are given in Table .2 All the traits not highly different (P≤ 0.001) but are significant between control and drought stress treatments. Further a mean decrease in Tendril length (1.25cm), RWC (71.74%), Number of grains per pod (4.083), Pod length (5.89 cm), shoot length (23.40cm), Grain weight (0.39g) and Root length (6.15cm) was evaluated in stress treatment as compare to control-group.

***Table1**: Summary statistics of the studied traits under control and drought condition*

| Treatment | Mean | Max | Min |
|---|---|---|---|
| **Days to flowering** | | | |
| Control | 57.8333 | 59 | 55.5 |
| Stress | 58.333 | 60 | 55.5 |
| **Tendril Length** | | | |
| Control | 1.475 | 1.6 | 1.25 |
| Stress | 1.25 | 1.45 | 1.05 |
| **Relative Water Content** | | | |
| Control | 74.7 | 76 | 70.57 |
| Stress | 71.74 | 73.1 | 70.07 |
| **Number of grains per pod** | | | |
| Control | 5.16667 | 6 | 4 |
| Stress | 4.0833 | 4 | 3.5 |
| **Pod Length** | | | |
| Control | 6.665 | 7.2 | 5.55 |
| Stress | 5.898 | 6.55 | 5.15 |
| **Shoot Length** | | | |
| Control | 25.925 | 26.65 | 24.85 |
| Stress | 23.4083 | 24.15 | 22.75 |
| **Grain weight** | | | |
| Control | 0.51733 | 0.61 | 0.375 |
| Stress | 0.39458 | 0.485 | 0.337 |
| **Root Length** | | | |
| Control | 7.7333 | 8.45 | 7.35 |
| Stress | 6.15 | 6.6 | 5.2 |

***Table 2**: Descriptive statistics results for 8 Studied Traits under water stressed and Control Conditions*

| **Days to Flowering** | | | | |
|---|---|---|---|---|
| SOV | DF | MS | F | P |
| Main effects | 4 | 4.8542 | 19.42 | 0.000 |
| Stress | 1 | 1.5000 | 6.00 | 0.004 |
| Varieties | 1 | 4.1667 | 16.67 | 0.000 |
| Treatments | 1 | 12.2500 | 49.00 | 0.000 |
| **Tendril Length** | | | | |
| SOV | DF | MS | F | P |



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| | | | | |
|---|---|---|---|---|
| Main effects | 4 | 0.131302 | 10.68 | 0.003 |
| Stress | 1 | 0.303750 | 24.71 | 0.001 |
| Varieties | 1 | 0.070417 | 5.73 | 0.044 |
| Treatments | 1 | 0.140625 | 11.44 | 0.010 |
| **Relative Water Content** | | | | |
| SOV | DF | MS | F | P |
| Main effects | 4 | 19.2777 | 9.44 | 0.004 |
| Stress | 1 | 51.2168 | 25.07 | 0.001 |
| Varieties | 1 | 11.5926 | 5.08 | 0.044 |
| Treatments | 1 | 13.8198 | 6.77 | 0.032 |
| **Number Of Grains Per Pod** | | | | |
| SOV | DF | MS | F | P |
| Main effects | 4 | 6.1146 | 9.78 | 0.004 |
| Stress | 1 | 12.0417 | 19.27 | 0.002 |
| Varieties | 1 | 3.3750 | 5.40 | 0.049 |
| Treatments | 1 | 9.0000 | 14.40 | 0.005 |
| **Pod Length** | | | | |
| SOV | DF | MS | F | P |
| Main effects | 4 | 1.86399 | 6.30 | 0.014 |
| Stress | 1 | 3.60375 | 12.18 | 0.008 |
| Varieties | 1 | 1.71735 | 5.81 | 0.043 |
| Treatments | 1 | 2.13160 | 7.21 | 0.028 |

| **Shoot Length** | | | | |
|---|---|---|---|---|
| SOV | DF | MS | F | P |
| Main effects | 4 | 10.4314 | 36.68 | 0.000 |
| Stress | 1 | 38.0017 | 133.63 | 0.000 |
| Varieties | 1 | 1.9267 | 6.78 | 0.031 |
| Treatments | 1 | 1.7556 | 6.17 | 0.038 |
| **Grain Weight** | | | | |
| SOV | DF | MS | F | P |
| Main effects | 4 | 0.4182 | 8.09 | 0.006 |
| Stress | 1 | 0.0904 | 17.49 | 0.003 |
| Varieties | 1 | 0.02891 | 5.59 | 0.046 |
| Treatments | 1 | 0.03841 | 7.43 | 0.026 |
| **Root Length** | | | | |
| SOV | DF | MS | F | P |
| Main effects | 4 | 4.7285 | 16.27 | 0.001 |
| Stress | 1 | 15.0417 | 51.76 | 0.000 |
| Varieties | 1 | 1.6017 | 5.51 | 0.047 |
| Treatments | 1 | 2.0306 | 6.99 | 0.030 |

***Table.3*** *Analysis of soil sample*

| Lab.No | 665 | 666 | 667 | 668 |
|---|---|---|---|---|
| Nature of sample | Lantana camara | Brousonatia | Parthurium | Eucalyptus |
| Dry matter % | 88.4 | 89.4 | 86.4 | 89.2 |
| Moisture % | 11.6 | 10.6 | 13.6 | 10.8 |

| | | | | |
|---|---|---|---|---|
| Crude Protein | 14.0 | 19.25 | 12.25 | 12.25 |
| Crude Fat | 3.1 | 2.5 | 1.6 | 4.1 |
| Crude Fiber | 15.5 | 14.0 | 23.0 | 14.0 |
| Total Ash | 11.0 | 12.5 | 11.0 | 5.5 |

## DISCUSSION

### Relative Performances of the Genotypes

***Days to Flowering***

Mean values for Days to Flowering (DF) was highly significant for the treatments stress and variety. A net decrease of 22% in DF was observed among mean of both treatments were averaged across all the genotypes. Lowest relative decrease in DF was noted for the genotypes 55.5 days both in drought and under control conditions in Climax genotype. Hence, Climax genotype is not affected by drought.

Highest Relative increase in DF was observed in Adventa Selection under drought conditions and were 60 days, showing it was highly affected by the drought and exhibit delayed flowering. Under 20ppm concentration of nanoparticles both varieties exhibit same number of days to flower. Genotypes ranged for DF from 55.5 days to 59 days under control conditions and 55.5 to 60 days under drought conditions.

***Tendril Length***

Mean values of Tendril length was significant for stress, variety and treatments. Tendril length overall decreased due to drought stress. 24% decrease in tendril length was observed in both varieties when compare to drought with control. Mean decrease in tendril length for both varieties was 1.475 cm under control and was 1.25cm under drought conditions. Tendril length was increased when compare to control due to application of nanoparticles. Tendril length was equally increased in for both varieties when 20ppm nanoparticles was applied that was treatment 2, and mean was 1.55 cm and same increase was observed for genotype Climax. When 50ppm nanoparticles were applied it was observed that mean effect of nanoparticles under 20ppm and 50ppm were same for Adventa selection.

***Relative Water Content***

Mean values for Pea genotypes for RWC were significant for stress, variety and treatments. (Table.1). A net decrease of 24% in relative water content (RWC) was observed under drought stress when compared to control set. Embiale *et al* (2016) had previously reported decrease in RWC in *Pisum sativum* in response to drought stress. High relative decrease was recorded in Adventa selection cultivar which is 70.07%. Mean values of the studied genotypes were from 70.57 to 76 under control conditions, whereas it ranges from 70.07 to 73 % under drought conditions.

***Number of Grains per Pod***

Mean values of Pea genotypes were significant for stress variety and treatments. A net decrease of 24% in the number of grains per pod was observed under drought stress when compared to control set. High relative decrease was recorded in both Adventa selection and Climax (3.5). Mean values of studied genotypes ranges from 4 to 6 under control conditions and it ranges from 3.5 to 4.5 under drought. Genotype Adventa selection and Climax behave similarly in 50ppm nanoparticles concentration. Whereas, Climax genotype have similar mean values for number of grains both in 20ppm and 50ppm nanoparticles concentration.

***Pod Length***

Mean values of Pea genotypes under control conditions ranges from 5.55cm to 7.2cm under control conditions and it ranges from 5.15cm to 6.55cm under drought conditions. Highest maximum decrease in mean pod length exhibited by 5.15cm by Climax genotype and highest pod length was observed in Climax genotype in 20ppm concentration of nanoparticles was 7.2cm. Decrease in pod length due to drought was previously studied by Desclaux *et al*, (2011) in Soybean plant.

***Shoot Length***

Mean values of shoot length were significant for stress variety and treatments ($P \leq 0.05$). Overall drought effect was inhibitory and a total decrease of 23% in shoot length was observed in comparison to drought treatment. Lowest relative decrease was 22.75 cm in Climax genotype at 50ppm nanoparticles concentration, while highest recorded shoot length was in Adventa selection at 20ppm concentration and is 26.65cm. The range of shoot length varied from 24.85cm to 26.65 cm under control conditions, whereas shoots length ranges from 22.75 cm to 24.15cm under drought conditions.

***Grain Weight***

Mean value for grain weight was significant for genotype and stress. Genotype Adventa Selection performed much better under stress conditions as they show smallest possible decrease in grain weight (0.485g) under drought and 20ppm nanoparticles concentration, Genotype Climax poorly performed by showing largest decrease in grain weight (0.337g) under control conditions and 0ppm nanoparticles concentration. Range of grain weight observed under control conditions were 0.375g to 0.61g, whereas under drought it ranges from 0.337 to 0.485g.

***Root Length***

Mean values of genotypes are significant for the stress and genotype (P≤0.05). A net decrease of 23% in root length was observed under drought conditions as compared to control. High relative decrease was recorded in the genotype Climax and was 5.25 cm, whereas Adventa Selection shows maximum increase in root length 8.45cm under 20ppm nanoparticles concentration. Range of root length was from 5.25 to 6.6 under drought conditions and from 6.85 to 8.45cm under control conditions.

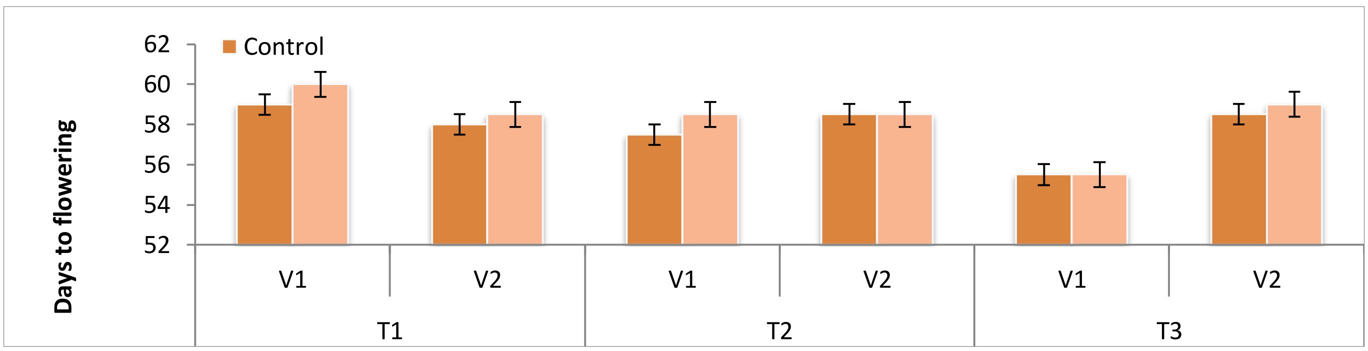


Figure4.1: Mean values of selected Pea genotype for days to flowering
V1=climax,V2=Adventa selection,T1=0ppm Ferric oxide nanoparticles,T2=20ppm Ferric oxide nanoparticles,T3=50ppm Ferric oxide nanoparticles

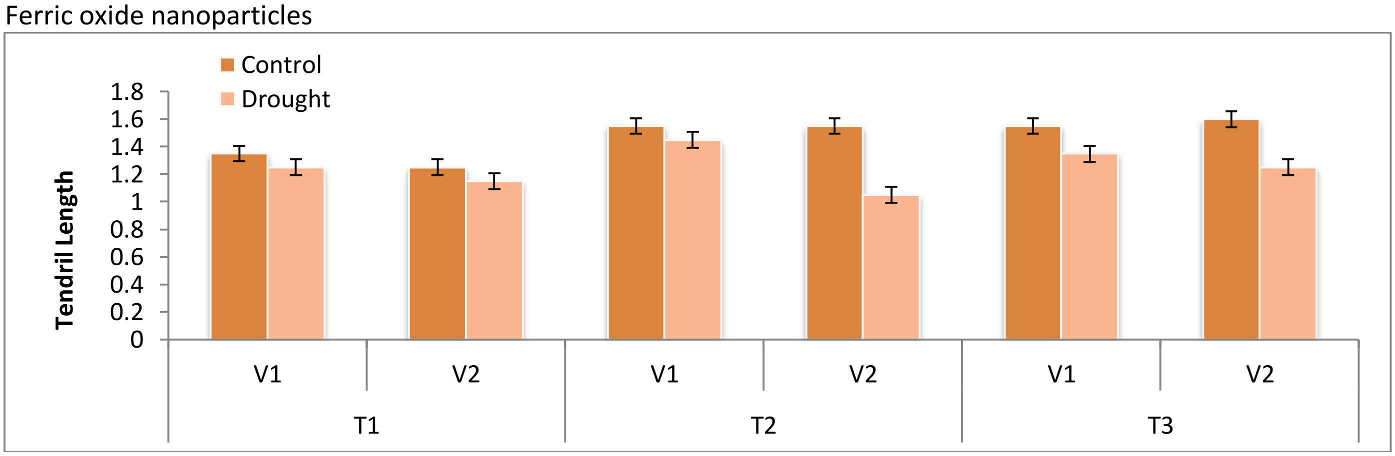


Figure 4.2: Mean values of selected Pea genotypes for Tendril Length V1=climax, V2=Adventa selection, T1=0ppm Ferric oxide nanoparticles, T2=20ppm Ferric oxide nanoparticles, T3=50ppm Ferric oxide nanoparticles



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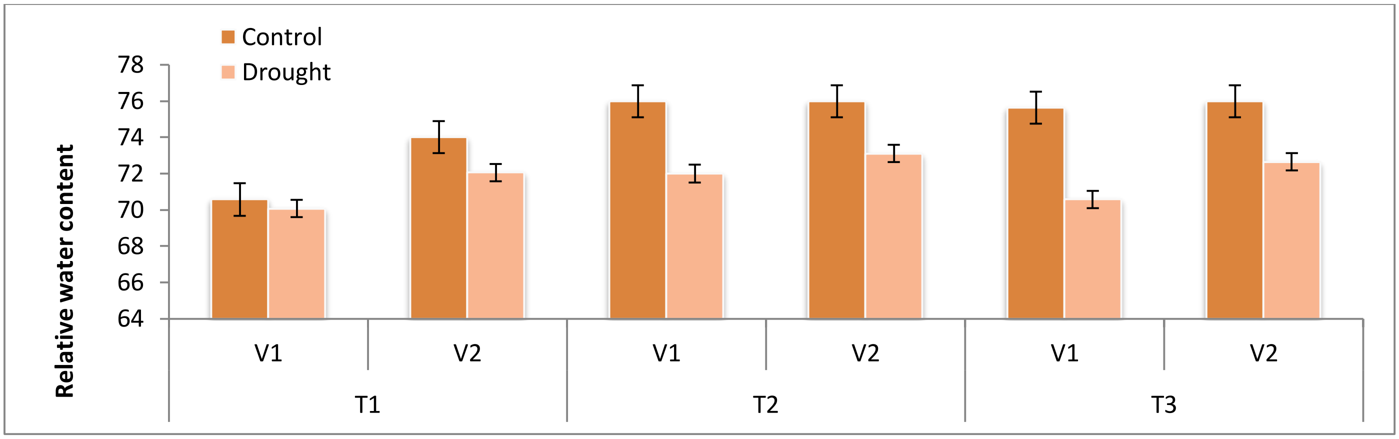

Figure 4.3: Mean values of selected Pea genotypes for Relative Water Content V1=climax, V2=Adventa selection, T1=0ppm Ferric oxide nanoparticles, T2=20ppm Ferric oxide nanoparticles, T3=50ppm Ferric oxide nanoparticles

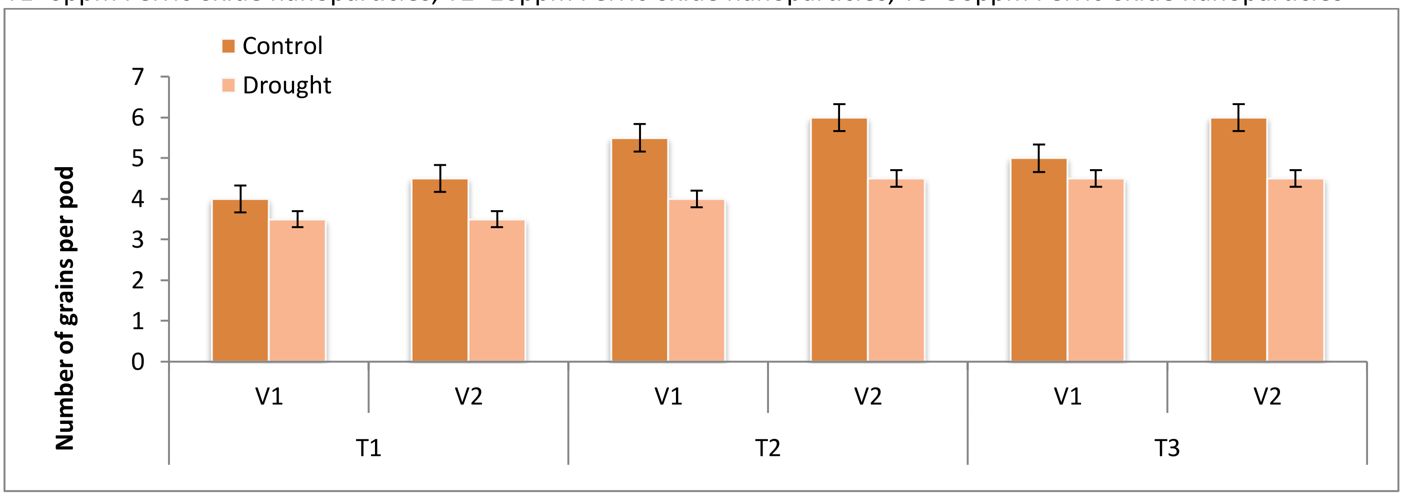

Figure4.4: Mean values of selected Pea genotypes for Number of grains per pod V1=climax, V2=Adventa selection, T1=0ppm Ferric oxide nanoparticles, T2=20ppm Ferric oxide nanoparticles, T3=50ppm Ferric oxide nanoparticles

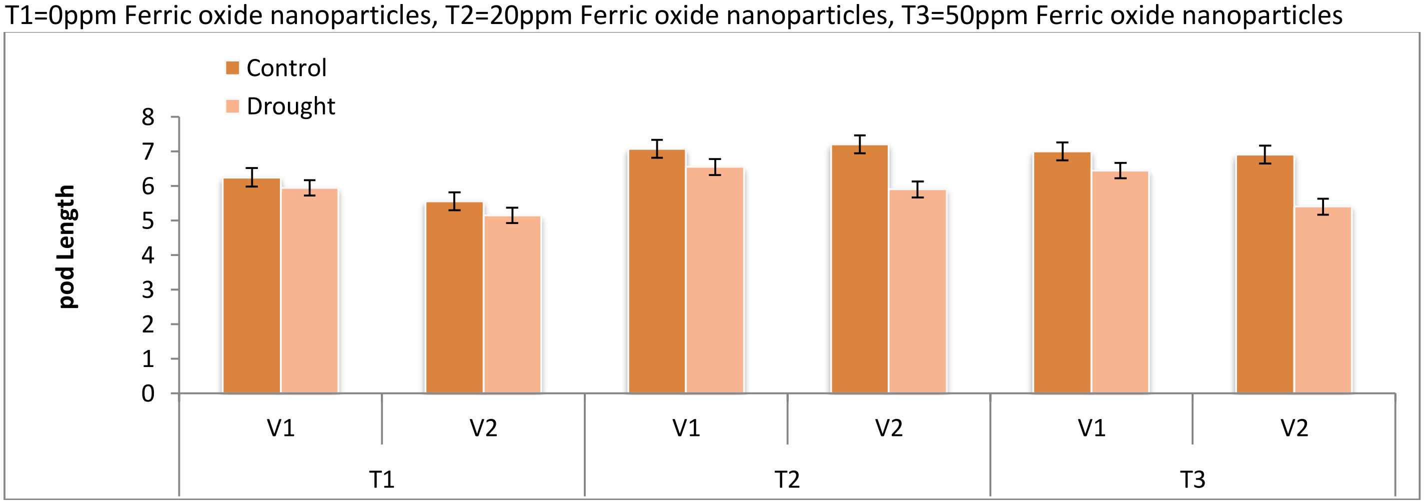

Figure 4.5: Mean values of selected Pea genotypes for Pod Length V1=climax, V2=Adventa selection, T1=0ppm Ferric oxide nanoparticles, T2=20ppm Ferric oxide nanoparticles, T3=50ppm Ferric oxide nanoparticles

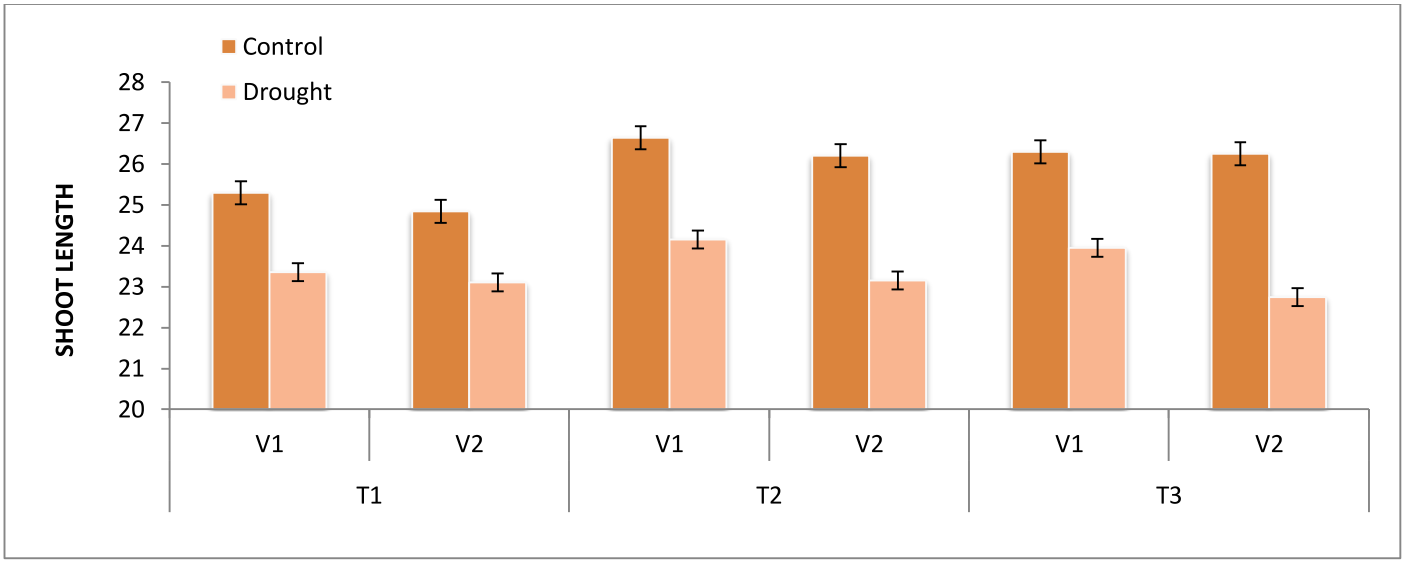


Figure 4.6: Mean values of selected Pea genotypes for Shoot Length V1=climax, V2=Adventa selection, T1=0ppm Ferric oxide nanoparticles, T2=20ppm Ferric oxide nanoparticles, T3=50ppm Ferric oxide nanoparticles

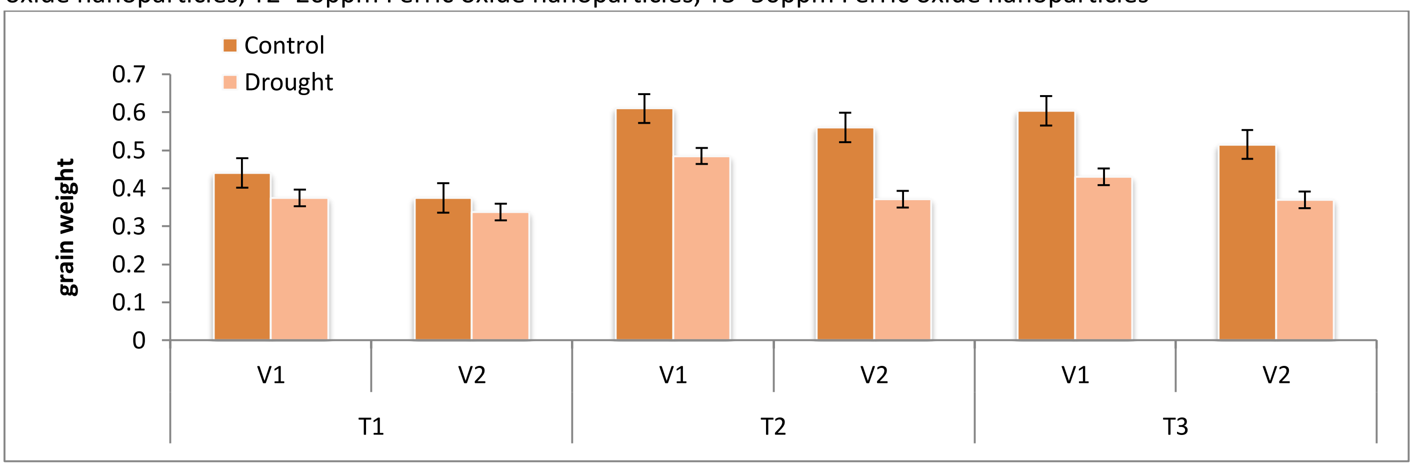


Figure 4.7: Mean values of selected Pea genotypes for Grain Weight V1=climax, V2=Adventa selection, T1=0ppm Ferric oxide nanoparticles, T2=20ppm Ferric oxide nanoparticles, T3=50ppm Ferric oxide nanoparticles

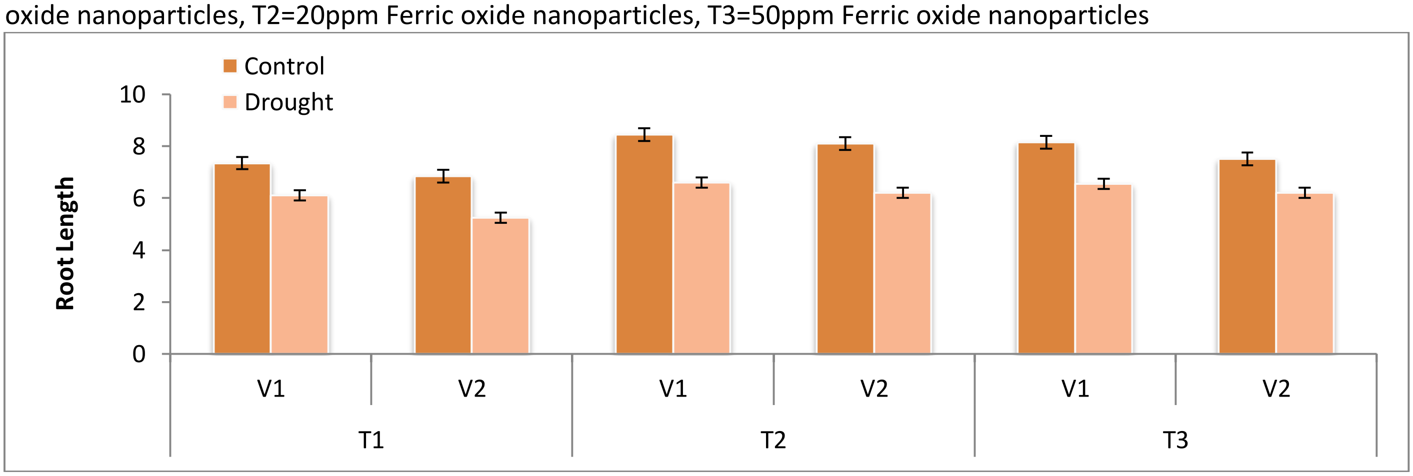


Figure 4.8: Mean values of selected Pea genotypes for Root Length V1=climax, V2=Adventa selection, T1=0ppm Ferric oxide nanoparticles, T2=20ppm Ferric oxide nanoparticles, T3=50ppm Ferric oxide nanoparticles

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