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Effect of drought stress and phosphate solvent bacteria on yield and its components in maize (*Zea mays* L. var SC.704)

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Abstract

Field trials were carried out at the agricultural research college of Islamic Azad University of Firoozabad, Fars, Iran, during 2011-2012 growing season. These treatments were arranged in split plot design based on randomized complete block design in three replications. In recent study, water stress at different growth stages of maize was confirmed at three levels. The main factors were included S₁, S₂ and S₃, defining as control (normal irrigation), drought stress at silk emergence and at the end of pollination, respectively. Three fertilizer treatments including B₀: No bacterium (control), B₁: *Bacillus*+*Pseudomonas*, B₂: *Bacillus*+*Pseudomonas* with 50 percent of triple super phosphate were applied as secondary factors. Results revealed that drought stress levels had significant effects on ear diameter, kernels number per ear, 1000-kernel weight and grain yield. The lowest grain yield was obtained from maize under S₂ condition. Compared to the maize under normal irrigation condition, this represents a yield decrease of 41 %. It was evident that seed inoculums with phosphate solubilising bacterium with 50 percent of triple super phosphate treatment significantly affected the reduction of plant losses under drought stress condition and increased the total yield. Result illustrated that stress×bacterium interaction effects were significant for ear length, ear diameter and 1000-kernel weight.

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Introduction

Maize (*Zea mays* L.) is an important multipurpose cereal crop species (after wheat and rice), used as food, feed, fodder, fuel and in the manufacture of industrial product and is grown throughout a wide range of climates (Liu *et al.*, 2001). In Iran, it is grown on over 387 thousand hectare with a total production of 2949000 tons (FAO, 2009). Research points to the effect that this crop has high potential in the country by Producing 101000 ha and 804772 t ha⁻¹ in Fars province farms (FAO, 2009).

It should be pointed that among various constraints responsible for low grain yield, inadequate supply of water at its critical development stage and high sensitivity of different maize cultivars to water stress are of immense importance. The extent of the loss will depend on stage of growth and the length of time the crop is subjected to droughty conditions (Shakhathreh *et al.*, 2001).

Previous studies were illustrated that in maize, grain yield reduction caused by drought ranges from 10% to 76% depending on the severity and stage of occurrence. Drought stress coinciding with flowering delays silking and results in an increase in the anthesis-silking interval (Bolaños *et al.*, 1993); this usually associates with reductions in grain number and yield (Edmeades *et al.*, 1993). The effect of drought is usually perceived as a decrease in photosynthesis and growth (Mwanamwenge *et al.*, 1999).

Bacteria that colonize the rhizosphere and plant roots and enhance plant growth by any mechanism are referred to as plant growth-promoting rhizobacteria (PGPR). PGPR have been applied to various crops to enhance growth, seed emergence and crop yield and some have been commercialized (Herman *et al.*, 2008). PGPR have shown positive effects in plants on such parameters as germination rate, tolerance to drought, weight of shoots and roots, yield and plant growth (Kloepper *et al.*, 2004). Production of indole acetic acid (IAA) by *Pseudomonas* and its role in the development of root system is also evidenced (Patten

and Glick, 2002). For many *Pseudomonads*, production of metabolites such as antibiotics, siderophores and hydrogen cyanide (HCN) is the primary mechanism of biocontrol (Weller and Thomashow, 1993).

Plant growth promoting rhizobacteria (PGPR) enhance plant biometric parameters/yields and aid in biological control of soil borne pathogens, which has been intensively investigated by several workers. During the last couple of decades, a significant increase in growth and yield of agronomically important cereal crops and improved soil fertility, in response to inoculation with PGPR has been reported (Asghar *et al.*, 2002; Richardson *et al.*, 2009). Most of the work in this area has focused on strains of *Pseudomonas* and related genera. It should be noted that, the capacity of species of the bacterial genus *Azospirillum* to promote plant growth, and consequently enhance crop productivity, has been the subject of numerous research reports worldwide for the past 30 years (Bashan *et al.*, 2004).

Plant Growth Promoting Bacteria (PGPR) also help in solubilization of mineral phosphates and other nutrients, enhance resistance to stress, stabilize soil aggregates and improve soil structure and organic matter content (Al-Taweil *et al.*, 2009; Shah *et al.*, 2006). PGPR retain more soil organic N and other nutrients in the plant-soil system, thus reducing the need for fertilizer N and P and enhancing release of the nutrients (Hayat *et al.*, 2010; Baset *et al.*, 2010). Application of antagonistic fungi and plant growth-promoting rhizobacteria caused a significant ($p < 0.05$) increase in tomato growth (based on shoot dry weight), both with and without nematodes (Shah *et al.*, 2006).

It is well known that rhizosphere and soil microorganisms (PGPR) play an important role in maintaining crop and soil health through versatile mechanisms: nutrient cycling and uptake, suppression of plant pathogens, induction of resistance in plant host, direct stimulation of plant growth (Kloepper *et al.*, 2004).

The present study was therefore, mainly aimed to evaluate the drought tolerance of maize (var SC.704) during three growth stages, vegetative, reproduction and seed filling stages of growth and , In addition we analyzed the stimulating effect of Plant Growth Promoting Bacteria (PGPR) on maize growth and yield under drought stress.

Materials and methods

Trial site

Field trials were carried out at agricultural research college of Islamic Azad University of Firoozabad, Fars, Iran, during 2011-2012 growing season. The seeds were cultivated in a farmland with the following characterizations in Firoozabad: 3575 km² surface area; approximately 1560 meter high from sea region, (located at 53°, 20' N & 28°, 38S). Grains of the studied corn genotype (*Zea mays* L.) –var. SC.704 was obtained from the Agricultural Research Center, Karaj, Iran. The minimum and maximum cultivation temperatures were 21.1 and 42 c°, respectively. The annual raining was 270 mm by average and it had a relative humidity of 36%.

Management and layout of the field trials

These treatments were arranged in a split plot experiment based on randomized complete block design (RCBD) in three replications. In recent study, water stress at different growth stages of corn was confirmed at three levels. The main factors were included S₁, S₂ and S₃, defining as control (normal irrigation), drought stress at silk emergence stage and end of pollination stage of maize, respectively. Secondary factors were included three fertilizer treatments used as below:

B₀: No bacterium (control), B₁:

Bacillus+*Pseudomonas*, B₂: *Bacillus*+*Pseudomonas* with 50 percent of triple super phosphate Seeds of maize genotype (*Zea mays* L.) –var. SC.704 were treated with the *Bacillus* and *Pseudomonas* strains at approximately 10⁹ cfu/ml prior to planting. Phosphorus bio-fertilizers used in this experiment included phosphate-solubilising bacteria was *pseudomonas fluorescens* (CFU=107). Phosphate biofertilizer contained *Pantoea agglomerans* and

Pseudomonas putida bacteria which have phosphate enzymic activities and agglomerance bacteria has H⁺ activity, so the first one has efficiency in solving organic insoluble phosphate and the second in solving unsolvable phosphates. They efficiently raise the phosphate use of plants. After soaking the seeds in phosphate solving bacterial, amount of other chemical fertilizers was measured on soil science testing.

27 plots were established initially according to experimental design study. Thus each experimental plot area had a surface area of 30 m². With 6×5 dimensions and total area equals to 800 m². Each plot was consisted of five plant lines and six meter length. In addition, the distance between main plots was estimated three meters, whereas the plant distance on each row was 20 cm and the rows were 75 cm far from each other. The plant density was 66000 plant/ha. Fertilizer was used based on soil test. Also phosphorus chemical fertilizer was included triple super phosphate. The seeds and bio-fertilizers thoroughly mixed with hands. Then, spread the treated seeds over on plastic sheet and dry under shade.

Final harvest was made to study the characteristics of performance and the elements of the performance. Due to the importance of final performance in this research, the two meters of front and end of each row-leaving the margins- were allocated for samplings during growth and the two meters in the middle of each row were allocated to final performance. The final harvest was made after physiological growth of maize and stopping the irrigation. This stage could be identified by studying the formation of black layer in the joining points of seeds to the cobs. It should be noted that the base moisture in time of weighing the seeds was taken as 14%. In order to determine the process of growth and studying maize plant traits experiment during cultivation season, the first sample was taken 43 days after cultivation (July 7). Sampling was performed once per 14 days and the last sample was taken on September 15.

Measurement of traits

In this test, to determine the characteristics subject of study, five sampling were performed in destructive forms to determine traits. Process of sampling was as follows: From each experimental unit, one meter from the top and end of each plot and two marginal rows from the four cultivated rows were taken as margins and two plants were taken from plot from the two middle rows. The same procedure was made in next sampling too. In order to prevent samples from wilting, they were put in plastic bags immediately after picking them and were sent to the lab in very short time. Plant samples were sent to the laboratory instantaneously. They were clean thoroughly after being washed with ordinary and distilled water in the laboratory.

At the end of the experimental period, plants were fractionated into roots and shoots. The shoots and roots were quickly weighed separately for fresh weight (FW) determination. Freshly harvested roots and shoots were oven-dried at 70°C for 48 hours in

order to determine the dry weight (DW) and to follow some analysis.

Statistical analysis

Normality test was carried out by Minitab software (1998). Statistical calculations were performed using ANOVA appropriate with SAS (2001). Excel (2003) software was used for charts adjustments as well. It should be pointed out for means comparison we applied Duncan's multiple range test at 0.05 probability level by SAS (2001) and MSTAT-C (1990) softwares.

Results and Discussion*Ear length*

Variance analysis illustrated that stress treatments was not significant (Table 1, Fig. 1). Results also indicated that bacterium treatment had no significant effect on ear length (Table 1, Fig. 6). Interaction effect of stress×bacterium for ear length was significant at 1% probability level. The highest and lowest interaction effects were related to $S_1 \times B_2$ and $S_3 \times B_1$, respectively (Table 2).

Table 1. Mean squares of studied traits in maize.

SOV	df	Mean square (MS)				
		Ear length	Ear diameter	kernels number per ear	1000-kernel weight	Grain yield
Block	2	1.62 ^{ns}	0.27 ^{ns}	297.39 ^{ns}	111.02 ^{ns}	2809.43 ^{ns}
Stress	2	2.53 ^{ns}	2.26 ^{**}	24683.62 ^{**}	18220.48 ^{**}	346114.65 ^{**}
E (a)	4	0.44	0.11	497.32	118.85	4398.90
Bacterium	2	0.66 ^{ns}	0.38 ^{**}	1607.27 [*]	3605.61 ^{**}	53573.17 ^{**}
Stress × Bacterium	4	1.87 ^{**}	0.54 ^{**}	475.09 ^{ns}	247.63 ^{**}	2490.00 ^{ns}
E (b)	8	0.27	0.03	355.17	8.59	2220.07
CV%		2.51	1.56	2.91	1.18	6.13

ns, * and **: Not significant, significant at the 5% and 1% levels of probability, respectively.

SOV: Source of variation, df: degree of freedom.

In similar study reported that any decrease in irrigation intervals may directly alters factors such as ear width, ear length, leaf surface area, plant height and seed yield, therefore a 9-day period irrigation is considered as an ideal regimen (Bolanos *et al.*, 1996).

Ear diameter

In present study, variance analysis demonstrated that drought stress had significant effect on ear diameter at 1% probability level (Table 1). The highest and lowest ear diameter was belonged to control (normal irrigation) and drought stress at the end of pollination stage, respectively (Fig. 2). In maize, grain yield reduction caused by drought ranges from 10 to 76%

depending on the severity and stage of occurrence. Drought stress coinciding with flowering delays silking and results in an increase of anthesis-silking interval (Bolaños *et al.*, 1993); this usually associates with reduction in grain number and yield (Edmeades *et al.*, 1993). The other researcher showed that drought stress declined seed yield and its components (Reca *et al.*, 2001; Seghatoleslami *et al.*, 2008). Our

results showed that bacterium treatment was significant at 1% probability level. On the other hand, the highest and lowest ear diameter was belonged to B₀ and B₁, respectively (Fig. 7). Variance analysis indicated that interaction effect of stress×bacterium was significant at 1% probability level (Table 1). S₁×B₀ and S₃×B₁ interaction effects had the highest and lowest ear diameter, respectively (Table 2).

Table 2. Interaction effects of stress×genotype on studied traits in maize.

Interaction	Means				
	Ear length (cm)	Ear diameter (cm)	kernels number per ear	1000-kernel weight (g)	Grain yield (g/m ²)
S ₁ × B ₀	20.86 ab	15.67 a	695.09 a	263.75 c	866.45 a
S ₁ × B ₁	21.42 a	15.14 b	686.08 a	283.71 b	919.70 a
S ₁ × B ₂	21.50 a	15.03 b	717.19 a	313.84 a	1063.35 a
S ₂ × B ₀	20.58 ab	14.14 e	628.68 a	246.53 d	732.40 a
S ₂ × B ₁	20.97 ab	14.53 d	662.81 a	245.88 d	769.90 a
S ₂ × B ₂	21.08 ab	14.67 cd	652.93 a	287.47 b	886.84 a
S ₃ × B ₀	21.28 ab	14.89 bc	576.32 a	186.08 g	506.80 a
S ₃ × B ₁	19.03 c	13.81 f	598.11 a	199.99 f	565.19 a
S ₃ × B ₂	20.33 b	14.44 de	609.73 a	211.60 e	609.56 a

S₁: Non-stress condition, S₂: Drought stress at silk emergence stage, S₃: Drought stress at the end of pollination stage, B₀: No bacterium (control), B₁: *Bacillus*+*Pseudomonas*, B₂: *Bacillus*+*Pseudomonas* with 50 percent of triple super phosphate.

Kernels number per ear

The results demonstrate that drought stress was significantly affected on kernels number per ear. Researchers believe that kernel number decrease is due to tassel expression postponement (Pandey *et al.*, 2000). The highest and lowest kernels number per ear was belonged to control (normal irrigation) and drought stress at the end of pollination stage (Fig. 3). According to Krnak and Genkoglan (2003) and Calir (2004), the highest degree of sensitivity to dryness stress in maize life cycle is during floret grow and fertilization stages as far as dryness stress during floral stage causes non-synchronization of male and female organs creation in maize. The maize performance potentially has close relationship with accessibility to water. However, results exhibited that bacterium treatment had significant effect on number of kernel per ear (Table 1). On the other hand, the results demonstrated that the highest and lowest

kernels number per ear was belonged to B₂ and B₀ (Fig. 8). Researchers have confirmed an increase in access to phosphorus for the plant by phosphate solving microorganisms such as *Pseudomonas* (Suh *et al.*, 1995), *Bacillus* (Raj *et al.*, 1981), *Anterobacteri* and *Agrobacterium* and *aspergillum* (Varsha and Patel, 2000). It should be pointed that stress×bacterium interaction effect of kernels number per ear was not significant (Table 2).

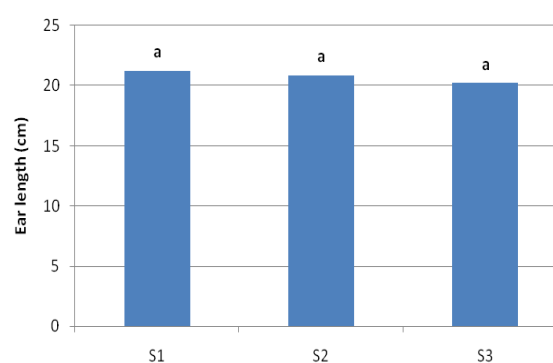


Fig. 1. Effect of drought stress on ear length maize.

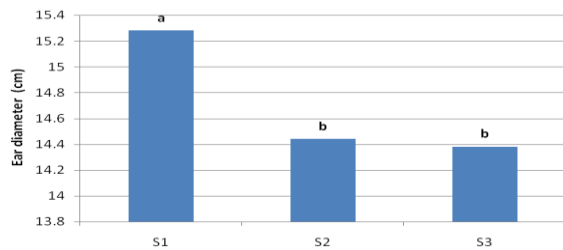


Fig. 2. Effect of drought stress on ear diameter in maize.

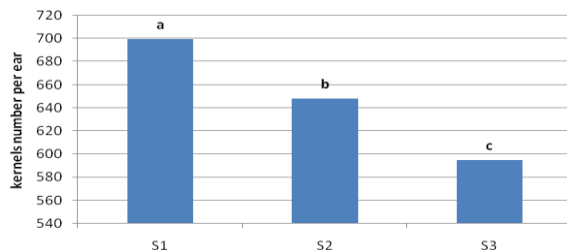


Fig. 3. Effect of drought stress on kernels number per ear in maize.

1000-kernel weight

Three main steps in plant growth in drought stress are considered as flowering, pollination and seed filling. Induced- water stress in pollination could cause kernel number decrease due to pollen deterioration or growth cessation of pollen tube in stigma (Santamaria *et al.*, 1990). However, the impact of water stress is more considerable in seed filling stage (Sremi *et al.*, 1994). Results indicated that drought stress significant impact on 1000-kernel weight (Table 1) whereas the maximum and minimum weight observed at S₁ and S₃, respectively (Fig. 4). In this study, bacterium treatment had significant effect on 1000- kernel weight (Table 1). The results illustrated that the highest and lowest 1000-kernel weight was related to B₂ and B₃, respectively (Fig. 9). Today, due to the pollutions caused by using chemical fertilizers, the impacts of biologic fertilizers and local foodstuffs sources in absorbing foods elements have been recognized and focused. By producing phosphate accessible for plant, it becomes possible to increase plants production and reproduction (Whiltelaw *et al.*, 1997). In addition, interaction effect of stress×bacterium on 1000-kernel weight was significant at (Table 1). S₁×B₂ and S₃×B₀ interaction effects had the highest and lowest 1000-kernel weight, respectively (Table 2).

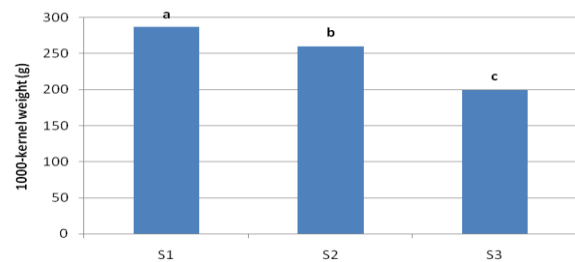


Fig. 4. Effect of drought stress on 1000-kernel weight in maize.

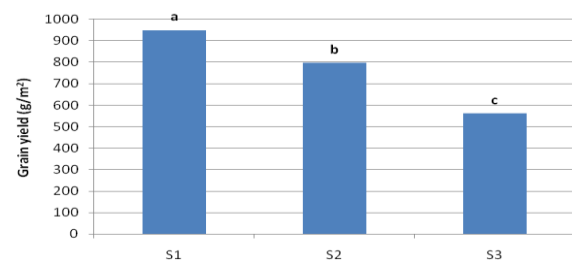


Fig. 5. Effect of drought stress on grain yield in maize.

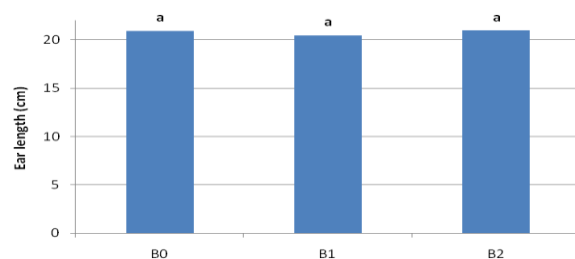


Fig. 6. Effect of bacterium on ear length in maize.

Grain yield

According to obtained results, drought stress level had significant effects on grain yield (Table 1). Previous studies indicate that drought stress before the onset of flowering, during and just after the end of flowering can results in 25, 50 and 21, respectively (Osborne *et al.*, 2002). In addition, induced-water stress in vegetative stage had the minor effect on seed yield while the major effect was observed regarding in reproductive stage (Rashidi, 2008). In one research, drought stress in flowering stage had a considerable impact on seed yield and results in 42 % reduction in plant yield. On the other hand water stress induces 15.8% and 12.5% decrease in yield through seed filling and at the beginning of flowering, respectively (Jaafari and Imani, 2004). However, the effect of drought stress before the onset of vegetative stage on yield altered from 15.8 to 22.1% (Cakir, 2004). The lowest grain yield was obtained from maize under S₃

compared to S₁ and S₂ conditions (Fig. 5). Compared to the maize under normal irrigation condition, this represents a yield decrease of 41 %. Our results concur partly with observations made by Choukan *et al.* (2007) who reported a decrease in total yield with increasing water deficit. Bacterium treatment had significantly effect on grain yield (Table 1). The results demonstrated that the highest and lowest seed yield was obtained regarding B₂ and B₀, respectively (Fig. 10). Wasule *et al.* (2002) mentioned that phosphate solvent bacteria and *Bradyrhizobium japonicum* application on soybeen increased significantly improved some characteristics such as nodulation, dry weight of nodules and plant dry weight. Interaction effect of stress×bacterium on grain yield was not significant (Table 1).

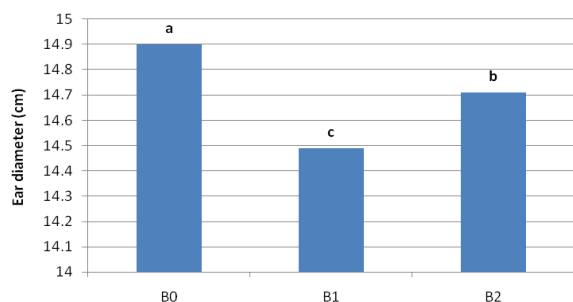


Fig. 7. Effect of bacterium on ear diameter in maize.

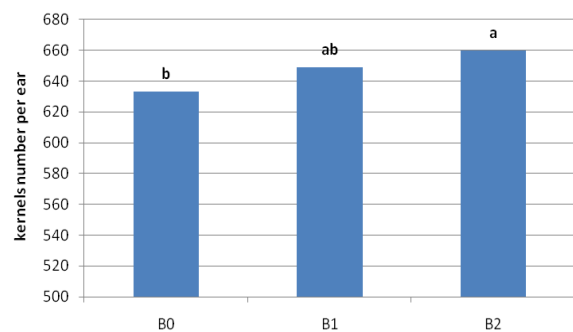


Fig. 8. Effect of bacterium on kernels number per ear in maize.

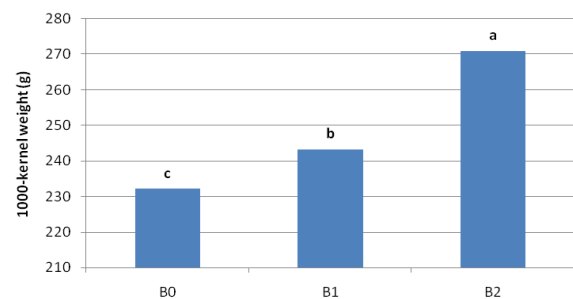


Fig. 9. Effect of bacterium on 1000- kernel weight in maize.

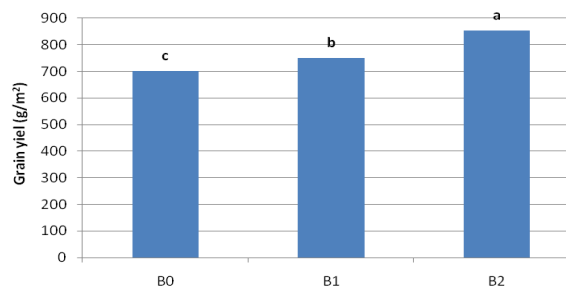


Fig. 10. Effect of bacterium on grain yield in maize.

Conclusion

Many researchers believe that amount of crop water use determine plant growth and development. Results of this study showed that drought stress caused decreases of yield and measured traits such as ear diameter, kernels number per ear, 1000-kernel weight and grain yield. Application of *Bacillus+Pseudomonas* with 50 percent of triple super phosphate has given the highest number of kernel per ear, 1000-kernel weight and grain yield. Also, our study showed that use of phosphate-solubilising could be improve plant growth and total yield of maize and thus reduction in use of chemical fertilizer and its costs.

It can be concluded that biological fertilizers with chemical fertilizers provides the plants requirements. Phosphate solubilizing bacterium with plant P supply cause root development and will increased plant resistance to water deficit. Water stress at critical growth stages can reduce yield and yield components and the impact of water stress on seed weight is at the beginning of reproductive growth. In drought conditions, yield reductions occur in the reproductive stage, which can reduce yield components.

The use of Plant Growth Promoting Bacteria (PGPR) as plant growth promoter or biofertilizer represents an attractive option for sustainable agriculture that it is due to their stimulating effects on plant growth, biomass production and their potential for increasing maize yield. However, further studies are needed in order to evaluate PGPR efficiency on other agricultural crops under greenhouse and field conditions.

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